

Molecular detection of *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Coxiella burnetii*, *Ehrlichia chaffeensis* and *Rickettsia* spp. in ticks collected from wild animals in six provinces of South Africa

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ABSTRACT

Ticks carry and transmit various pathogens, which may be of veterinary and medical importance. There is limited information on the presence of tick-borne zoonotic pathogens in wild animals in southern Africa. The aim of our study was to detect five bacteria (*Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Coxiella burnetii*, *Ehrlichia chaffeensis* and *Rickettsia* spp.) in ticks collected from wild animals in six provinces of South Africa. A total of 2268 ticks (911 *Amblyomma*, 683 *Haemaphysalis*, 655 *Rhipicephalus*, eight *Hyalomma*, six *Ixodes* and five *Ornithodoros*) were used for DNA isolation. In total 870 samples of ticks pooled (adults individually, five nymphs per sample, and ten larvae per sample), with respect to tick genera, sex, developmental stage, year, season, and locality of sampling, were subjected to PCR. Bacterial infection was detected in 453 (52 %) samples, with single infection in 290 (33 %) and co-infection in 163 (18.7 %) samples. *Amblyomma*, and *Rhipicephalus* ticks were infected with diverse pathogens, whereas *Ixodes* and *Ornithodoros* ticks were generally infected with a single bacterium (*Anaplasma* or *Rickettsia*); whereas *Hyalomma* were negative. The highest prevalence was detected for *A. phagocytophilum* (32 %), followed by *E. chaffeensis* (21 %), *Rickettsia* spp. (17 %), *C. burnetii* (3 %), and *B. burgdorferi* s.l. (0.5 %). The results statistically differed in tick genus, sex and developmental stage, year of sampling, and provinces. The results of this study present new knowledge about five bacterial infections in ticks collected in six provinces of South Africa which may provide the basis for more extensive epidemiological research in this area.

1. Introduction

Ticks are ectoparasites of animals and humans that transmit a greater variety of pathogens (bacteria, protozoa, rickettsia, spirochaetes, and viruses) than any other arthropod vectors (Fuente et al., 2008). This means that they are the most important vectors of infectious pathogens affecting humans, livestock, companion animals and wildlife (Jongejan and Uilenberg, 2004). Djiman et al. (2024) did the systematic review and found a wide diversity of tick-borne bacteria, parasites, and viruses in sub-Saharan Africa, with 27 % of 159 identified pathogens being

zoonotic. In addition, they suggested that more attention should be given to the risk factors of infection from these zoonotic pathogens. In sub-Saharan Africa, most vectors that transmit human and livestock pathogens are native to the region and originate precisely from wild animals that harbour a diverse range of tick species. This level of diversity leads to a high potential for the emergence of novel tick-borne pathogens (M.B. Ledwaba et al., 2022). The main indigenous tick species affecting both domestic and wild animals in South Africa belong to the genera *Amblyomma*, *Hyalomma*, *Ixodes*, *Rhipicephalus*, with *Amblyomma hebraeum*, *Ixodes rubicundus*, *Rhipicephalus appendiculatus*

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and *Rhipicephalus decoloratus* being the most prevalent species (Horak et al., 2015, 2018; Guglielmone et al., 2023).

In most cases, domestic animals are regarded as the preferred hosts for ticks. Mtshali et al. (2016) tested ticks collected from ruminants in four South African provinces for zoonotic pathogens by PCR and DNA sequencing and detected *Anaplasma phagocytophilum*, *Coxiella burnetii*, *Ehrlichia ruminantium*, and *Rickettsia* species, but found no evidence of *Borrelia burgdorferi* s.l. The first report of *B. burgdorferi* s.l. was reported in ticks collected from domestic animals on communal farms in Eastern Cape, South Africa (Adelabu et al., 2019). However, a considerable number of ticks that are native to sub-Saharan Africa are natural parasites of wild animals, affecting livestock and humans, particularly at the wildlife-livestock-human interface. South Africa has various nature reserves and private game parks, containing many animals for conservation, wildlife trade, ecotourism and human consumption of bush meat. The high diversity of wild animals in game reserves makes them the potential sources of ticks (M.B. Ledwaba et al., 2022), however little is known about the role of wild animals as reservoirs of zoonotic pathogens (such as the bacteria *A. phagocytophilum*, *B. burgdorferi* s.l., *C. burnetii*, *Rickettsia* spp.) in South Africa (Mtshali et al., 2017). Studies on potential tick-borne zoonoses are under-represented and should be included in epidemiological surveys, especially in the light of climate change and other anthropogenic threats which might result in the emergence of novel tick-borne pathogens (M.B. Ledwaba et al., 2022). For that reason, the aim of this study was to detect selected pathogens (*A. phagocytophilum*, *B. burgdorferi* s.l., *C. burnetii*, *Ehrlichia chaffeensis* and *Rickettsia* spp.) by molecular methods in ticks collected from wild animals in South Africa.

2. Materials and methods

2.1. Sampling

The collection of ticks was done in six provinces in South Africa (Limpopo, Mpumalanga, Gauteng, North West, Free State, Northern Cape) (Fig. 1) during the years 2012–2019. Ticks, found both on surface hosts body or in a blood-feeding state, were collected from 156 animals of six orders (124 carnivora, 15 rodentia, 11 cetartiodactyla, three macroscelidea, two squamata, and one primates), found dead (natural death or road kills) or shot during hunting seasons. Ticks were identified to species level with a Nikon SMZ645 stereo microscope, using descriptions and illustrations from several publications e.g. Apanaskevich et al. (2007); Apanaskevich and Horak (2008), while Klompen et al. (2002), and Murrell and Barker (2003); Volzit and Keirans (2003); Walker et al. (2000) were used for verification of correct nomenclature. Some problematic ticks were also verified by comparing to identified tick specimens in the National Tick Collection of South Africa, housed in the Gertrud Theiler Tick Museum, ARC—Onderstepoort Veterinary Institute, South Africa.

A total of 2268 ticks (230 females, 958 males, 530 nymphs, and 550 larvae) belonging to six genera (911 *Amblyomma*, 683 *Haemaphysalis*, 655 *Rhipicephalus*, 8 *Hyalomma*, 6 *Ixodes*, and 5 *Ornithodoros*) and 22 species were included in the study. The complete list of ticks (their genera and species) is summarized in Table 1 suppl. Ticks were pooled into 870 samples (adults individually, five nymphs per sample, and ten larvae per sample), stratified by tick genus, sex, developmental stage, year and season of sampling, and province.



Fig. 1. Map of South Africa, showing six provinces (Limpopo, Mpumalanga, Free State, Northern Cape, Northwest, and Gauteng Province - gray coloured), where collection of ticks was performed.

2.2. Geographical and ecological characteristics of six provinces in south africa

South Africa can be topographically divided into an interior plateau which is separated from the coastal plain by the Great Escarpment stretching from north to south in the east of the country. In the largest part of the country, there is summer rainfall, usually from October to February, which diminishes from east to west. The south-western part of the country is a winter rainfall area. The Limpopo province is the most northern province of South Africa. While this province is also known as the Bushveld, the area to the east below the escarpment is known as the Lowveld. The province has a savanna biome with grassland biome on the escarpment edge. The savanna biome, the largest in Africa, is characterized by a grassy ground layer with a distinct upper layer of woody plants and almost every major geological and soil type occurs within this biome. The woodland and bushveld regions are mainly used for cattle farming, while the shrubveld areas are used for sheep and goat farming. The northern part of the Kruger National Park, a protected area known for its diverse wildlife, is situated in the eastern Lowveld of the province. Depending on the elevation, the province is mostly frost free. While the northern part of the Mpumalanga province to the east of the Limpopo province is also considered as part of the savanna biome, the southern parts fall under the grassland biome. The southern part of the Kruger National Park is situated on the eastern side of Mpumalanga, with the southern border less than 200 km north of Eswatini. Gauteng is the smallest but most populated and industrious province of South Africa. South of this province is known as the Highveld region and is categorized as grassland biome, while north is considered bushveld and falls under the savanna biome. Frost is common in the Highveld region. The Northwest province also falls in the grassland and savanna biomes. The south-eastern region is mainly grassland, while to the north and west the grassland is interspersed with trees and bushy shrubs. Agriculture mainly consists of cattle farming and maize production. The Free State province, is mainly grasslands and consists of single layer of grasses and the amount of cover is contingent on the annual summer rainfall. Trees are absent, except in a few localized habitats, while geophytes are often abundant. The grassland biome has an extremely high biodiversity and is used extensively for cattle farming and production of maize. The south-western part consists mainly of savanna and Nama Karoo biomes. Frost is common in this province. The Northern Cape province, is the largest but the most sparsely populated province. It includes six of South Africa's nine biomes, with the largest being the Nama Karoo biome, which is determined primarily by summer rainfall. This region consists of grassy dwarf shrubland used for mainly sheep and goats farming. The second largest biome in the northern region of the province is savanna used for beef cattle farming. The area west of the escarpment falls under the Succulent Karoo biome and covers a flat to slightly undulating plain with some hilly veld. It has low winter rainfall and is extremely dry during the summer months. The vegetation is mainly dwarf succulent shrubs and grasses are rare. This region is used

primarily for goat and sheep farming. No collections were made in the KwaZulu-Natal, Eastern Cape and Western Cape provinces.

2.3. Molecular methods

DNA was isolated from 870 tick pool samples by NucleoSpin Tissue (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. Isolated DNA was stored at -20°C prior to use. Selected pathogens (*A. phagocytophilum*, *B. burgdorferi* s.l., *C. burnetii*, *E. chaffeensis* and *Rickettsia* spp.) were detected in samples of ticks by a single PCR separately by pathogens. The primers and the conditions of PCR are summarised in Table 2. The positive controls for PCR were obtained from our previous research and included DNA obtained from samples positive for each of tested pathogens. The PCR amplicons (10 μL) were analysed by gel electrophoresis on 2 % agarose gels, containing Midori green fluorescent dye (NIPPON Genetics EUROPE, Düren, Germany). The gels were visualised on a UV transilluminator (BDL s.r.o., Turnov, Czech Republic).

2.4. Statistical analysis

The results were statistically analysed, with respect to several factors related to ticks (tick genera, sex, developmental stage, year, season and locality of sampling), as well as the factors related to the animals on which the ticks were found (host animal species, family and order). Data analysis was performed using the Pearson chi-square test of independence in contingency tables using STATISTICA Cz 12 (StatSoft, Inc., 2013). The Monte Carlo method implemented in IBM SPSS Statistics 29 was used if good approximation conditions were not met. We tested the null hypothesis that the prevalence of bacterial infections did not differ in groups according to the analysed factors. The differences were considered statistically significant if the p-value was ≤ 0.05 and statistically highly significant if the p-value was ≤ 0.001 . Statistical tests could not be performed if the number of ticks fell below 5 in any of the groups.

3. Results

Bacterial DNA was detected in 52 % (453/870) of pooled tick samples. Single infections were found in 33.3 % (290/870) of samples, whereas 18.7 % (163/870) of samples contained co-infections, including 16.8 % (146/870) with two pathogens and 1.9 % with three pathogens (Fig. 2, Table 3). The most frequently detected pathogen was *A. phagocytophilum* (32 %), followed by *E. chaffeensis* (21 %), *Rickettsia* spp. (17 %), *C. burnetii* (3 %) and *B. burgdorferi* s.l. (0.5 %) (Fig. 3).

Statistical evaluation revealed that the prevalence of *A. phagocytophilum* differed significantly among tick genera ($p < 0.0001$), with the highest infection rates observed in *Amblyomma* (48 %), *Rhipicephalus* (40 %). Significant differences were also found among years of sampling ($p = 0.0378$) and provinces ($p = 0.0415$). The

Table 2

Primers and PCR conditions used for detection of 5 bacteria (*Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Coxiella burnetii*, *Ehrlichia chaffeensis* and *Rickettsia* spp.) in ticks collected from wild animals in six provinces of South Africa.

Species	Marker	Primer	Primer sequence 5' - 3'	Annealing ($^{\circ}\text{C}$)	Amplicon size (bp)	References
<i>Anaplasma phagocytophilum</i>	<i>msp4</i>	MSP4AP5 MSP4AP3	ATG AAT TAC AGA GAA TTG CTT GTA GG TTA ATT GAA AGC AAA TCT TGC TCC TAT G	60	849	De la Fuente et al., 2005
<i>Borrelia burgdorferi</i> s. l.	23 s RNA	Bo-bu-sl-23S-F Bo-bu-sl-23S-R	GAG TCT TAA AAG GGC GAT TTA GT CTT CAG CCT GGC CAT AAA TAG	60	73	Michelet et al., 2014
<i>Coxiella burnetii</i>	IS1111	Trans1 Trans2	TAT GTA TCC ACC GTA GCC AGT C CCC AAC ACC TCC TTA TTC	61	687	Hoover et al., 1992
<i>Ehrlichia chaffeensis</i>	16S RNA	ECH16S-17 ECH16S-97	GCG GCA AGC CTA ACA CAT G CCC GTC TGC CAC TAA CAA TTA TT	57	81	Massung et al., 2002
<i>Rickettsia</i> spp.	<i>gltA</i>	CS 78 CS 323	GCA AGT ATC GGT GAG GAT GTA AT GCT TCC TTA AAA TTC AAT AAA TCA GGA T	57	401	Labruna et al., 2004

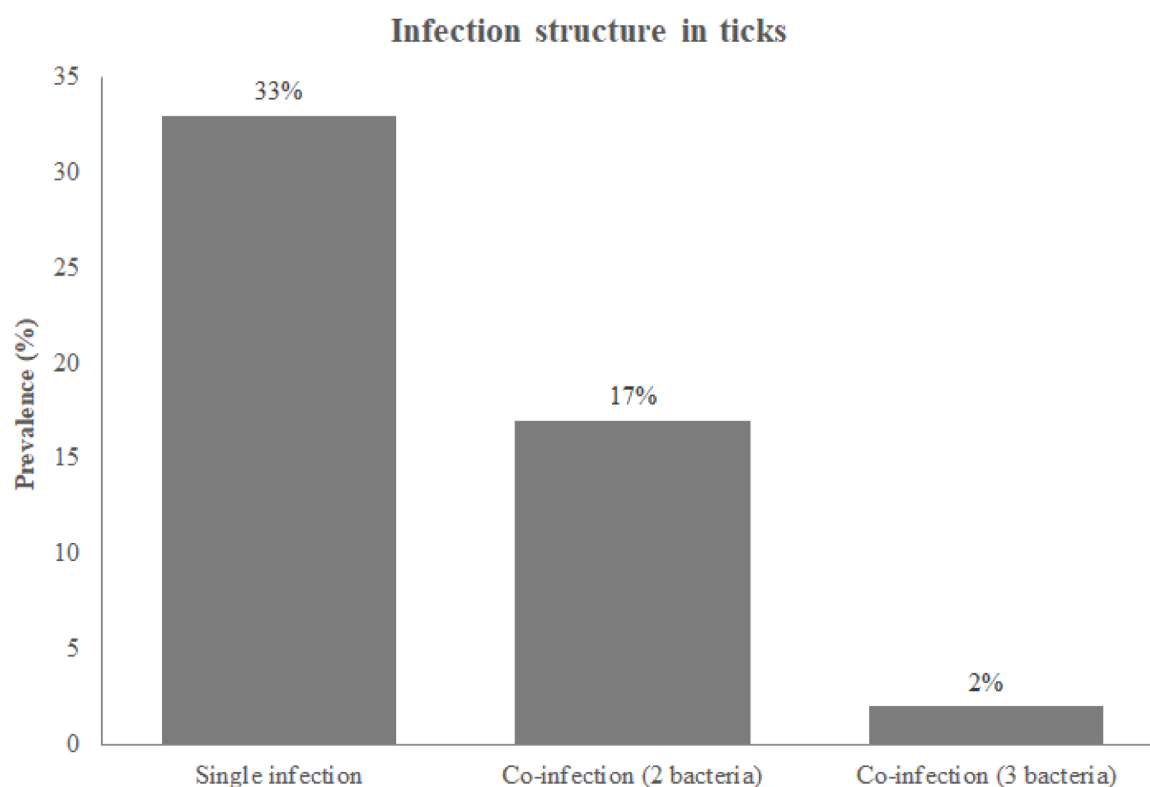


Fig. 2. Structure of bacterial infections in ticks collected from wild animals in six provinces of South Africa ($n = 870$ pooled samples) with single infection (33.3 %), double infection (16.8 %) and triple infection (1.9 %).

Table 3

Co-infection of tested bacteria (*Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Coxiella burnetii*, *Ehrlichia chaffeensis* and *Rickettsia* spp.) in ticks collected from wild animals in six provinces of South Africa.

Bacteria	Positive /total (%)
Single infection	290/870 (33.3 %)
<i>A. phagocytophilum</i>	134 (15.4 %)
<i>B. burgdorferi</i> s.l.	2 (0.2 %)
<i>C. burnetii</i>	12 (1.4 %)
<i>E. chaffeensis</i>	122 (14.0 %)
<i>Rickettsia</i> spp.	20 (2.3 %)
Co-infection (two bacteria)	146/870 (16.8 %)
<i>A. phagocytophilum</i> + <i>C. burnetii</i>	2 (0.2 %)
<i>A. phagocytophilum</i> + <i>E. chaffeensis</i>	34 (3.9 %)
<i>A. phagocytophilum</i> + <i>B. burgdorferi</i> s.l.	1 (0.1 %)
<i>C. burnetii</i> + <i>E. chaffeensis</i>	1 (0.1 %)
<i>Rickettsia</i> spp. + <i>A. phagocytophilum</i>	92 (10.6 %)
<i>Rickettsia</i> spp. + <i>E. chaffeensis</i>	16 (1.8 %)
Co-infection (three bacteria)	17/870 (1.9 %)
<i>A. phagocytophilum</i> + <i>E. chaffeensis</i> + <i>B. burgdorferi</i> s.l.	1 (0.1 %)
<i>Rickettsia</i> spp. + <i>A. phagocytophilum</i> + <i>C. burnetii</i>	8 (0.9 %)
<i>Rickettsia</i> spp. + <i>A. phagocytophilum</i> + <i>E. chaffeensis</i>	8 (0.9 %)
Total	453/870 (52.1 %)

occurrence of *E. chaffeensis* varied by tick genus ($p = 0.0064$) and province ($p = 0.0098$), while *Rickettsia* spp. prevalence was associated with tick genus ($p < 0.0001$) and developmental stage ($p = 0.0106$). The prevalence of *C. burnetii* showed significant variation across tick genera ($p = 0.0157$) and years ($p = 0.0070$). In contrast, *B. burgdorferi* s.l. was detected at a very low prevalence (0.5 %) without significant variation among factors. No statistically significant effect of season on overall prevalence of pathogens was observed. The statistical results are summarised in Table 1 suppl. The host animal (order, family, species) from which the ticks were collected had also an effect on the prevalence of ticks to selected bacteria (Table 4 suppl.).

4. Discussion

The results of this study present new knowledge about five bacterial infections in ticks collected in six provinces of South Africa. These results can provide the basis for more extensive epidemiological research in this area, since there is a deficiency of information about the role of wild animals in South Africa as reservoirs of the above-mentioned tick-borne diseases.

Most studies in Africa have dealt with a tick morphology or pathogens in ticks collected from domestic animals. For example, Mtshali et al. (2017) examined by PCR 318 ticks (147 pools) collected from dogs and cats in South Africa and found a prevalence of 41 % for *C. burnetii*, 37 % for *Rickettsia* spp., 19 % for *E. canis*, and 18 % for *A. phagocytophilum*, while *B. burgdorferi* s.l. was not detected, similarly as in our study. Mtshali et al. (2016) examined by PCR 1 634 ticks collected from ruminants in four South African provinces, with the following prevalence: *E. ruminantium* (28 %), *Rickettsia* spp. (27 %), *A. phagocytophilum* (7 %), and *C. burnetii* (7 %), while *B. burgdorferi* s.l. was not detected. Olivieri et al. (2021) examined by PCR 339 ticks collected on wild and domestic animals from Kenya, with the following prevalence: *Rickettsia* spp. (5.3 %), *Anaplasma* sp. (2.1 %), and *Ehrlichia* sp. (0.9 %). *Rickettsia* spp. was more prevalent than *A. phagocytophilum* in all the above-mentioned studies, that is in contrast to our results where the highest prevalence was obtained for *A. phagocytophilum* (32 %), followed by *E. chaffeensis* (21 %), *Rickettsia* spp. (17 %), *C. burnetii* (3 %), and *B. burgdorferi* s.l. (0.5 %). Many areas in Africa have wildlife-livestock interfaces which allow ticks to feed on both wild and domestic hosts, increasing opportunities for multi-pathogen transmission (Makwarela et al., 2025).

The true prevalence of *A. phagocytophilum* in ticks from South Africa remains unclear, as data on wildlife are limited. *Anaplasma phagocytophilum* DNA was detected in vervet monkeys (*Chlorocebus pygerythrus*) and baboons (*Papio*) in Zambia (Nakayima et al., 2014), lions (*Panthera leo*), African wild cats (*Felis lybica*) and servals

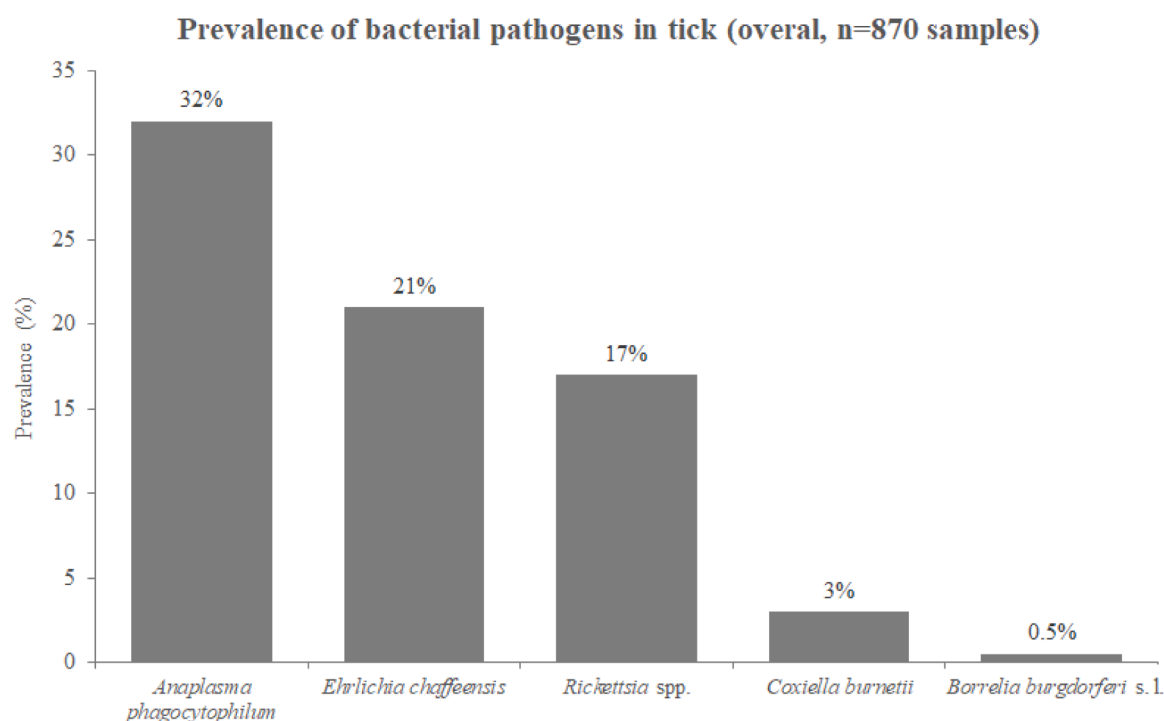


Fig. 3. Prevalence of five bacterial pathogens detected in ticks collected from wild animals in six provinces of South Africa. Values represent overall positivity across 870 pooled tick samples.

(*Leptailurus serval*) in Zimbabwe (Kelly et al., 2014). In the case of *A. phagocytophilum*, there is not only one specific tick species being the most infected, that makes *A. phagocytophilum* to be the pathogen with wide spectrum of potential tick vector (Cossu et al., 2023). *Anaplasma phagocytophilum* was reported in 14 tick species belonging to five different Ixodid tick genera (*Amblyomma*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus*) and two Argasid tick genera (*Argas* and *Ornithodoros*) (Cossu et al., 2023).

Ehrlichiosis caused by *E. chaffeensis* is considered an emerging disease in human medicine, because of their relatively recent description and their steady increase of incidence throughout the world. In Africa the picture on ehrlichiosis is fragmented, however historic serologic studies and molecular detection of *Ehrlichia/Anaplasma* in ticks and animals suggest potential human exposure, but confirmed, contemporary human cases from South Africa are lacking. The emergence of ehrlichiosis as a human health problem is the result of complex interactions among humans, the environment, tick vectors and mammalian hosts (Esemu et al., 2011). White-tailed deer (*Odocoileus virginianus*) are the major reservoir host of *E. chaffeensis* in the U.S. Nevertheless, these bacteria were also detected by PCR (Paddock et al., 2007) across the globe in other deer species, such as the spotted deer (*axis*) in Japan (Kawahara et al., 2009) and Korea (Lee et al., 2009), as well as in numerous wild and domestic animals. *Ehrlichia chaffeensis* DNA was detected by PCR e.g., in coyotes (*Canis latrans*) (Kocan et al., 2000), ring-tailed lemurs (*Lemur catta*) and ruffed lemurs (*Varecia variegata*) (Williams et al., 2002). Adelabu et al. (2020) identified 1 200 tick species positive for *E. chaffeensis* and found the highest prevalence in tick *A. hebraeum* ($n = 335$, 27.9 %), followed by *R. appendiculatus* ($n = 274$, 22.8 %), *R. decoloratus* ($n = 224$, 18.7 %) and *R. evertsi evertsi* ($n = 200$, 16.7 %). In our study, we found similar results with *Amblyomma*, *Haemaphysalis*, and with *Rhipicephalus* being the only one tick species infected with *E. chaffeensis*.

African tick-bite fever caused by *Rickettsia africae* is considered as the second-most frequent febrile illness reported in travellers returning from sub-Saharan Africa, with the incidence of rickettsia infections being as high as 5.6 % (Freedman et al., 2006; Jensenius et al., 2009). African

tick-bite fever is generally transmitted by *Amblyomma* ticks, and its natural reservoir is wild rodents (Bogovic et al., 2016). *Rickettsia africae* DNA was detected by PCR in different tick species belonging to genera *Amblyomma*, *Hyalomma* and *Rhipicephalus* that were collected in several African countries e.g., in Mali, Senegal, Guinea, Liberia, Sudan, Democratic Republic of Congo, Cameroon, Nigeria, Niger, Kenya, Côte d'Ivoire and Burundi (Parola et al., 2013; Bogovic et al., 2016; Ehounoud et al., 2016). These reports are in accordance with our findings, since the DNA of *Rickettsia* spp. was detected in different genera of ticks, with the most infected being *Ixodes* (40 %), *Amblyomma* (29 %) and *Rhipicephalus* (28 %).

Coxiella burnetii was detected all over the African continent, with generally higher seropositivity in ticks in West African countries (Mediannikov et al., 2010). Although there are human-related studies (e.g., abattoir workers), the number of published, peer-reviewed studies with clinical human data (acute Q fever, confirmed by PCR, with epidemiologic follow-up) in South Africa appears very limited. The prevalence of *C. burnetii* in humans is thus unknown in South Africa, despite the fact that it was described as the most prevalent rickettsial infection prior to its reclassification (Frean and Blumberg, 2007). Humans can potentially be infected through various ways inclusive of tick bites (Porter et al., 2011). Many domestic and wild animal species are susceptible to infection, but infection most often remains asymptomatic. The main clinical signs are abortions observed in goats, sheep, cows and cats (Woldehiwet, 2004) and in gazelle (*Gazella gazella*) (Lloyd et al., 2010). Maurin and Raoult (1999) carried out the successful experimental transmission of *C. burnetii* from infected to uninfected guinea pigs (*Cavia porcellus*) via tick bite (*Ixodes holocyclus*, *Haemaphysalis bispinosa*, and *Rhipicephalus sanguineus*) and detected that this bacterium is probably not tied to one specific tick species. In our study, tick genera *Haemaphysalis* and *Rhipicephalus* were the only ones positive for *C. burnetii* in contrast to other genera (*Amblyomma*, *Hyalomma*, *Ixodes*, *Ornithodoros*) that were negative.

The prevalence of *B. burgdorferi* was not previously known in South Africa nor throughout Africa. In our study, this bacterium was detected in ticks in a very low grade 0.5 %. Given the absence of statistically

significant variation among the evaluated factors, we consider this finding to be isolated and we recommend further studies to confirm the presence of *B. burgdorferi* s.l. in this area. Similarly, low prevalence 1.67 % was detected e.g., in ticks *Rhipicephalus sanguineus* collected from dogs in Egypt (Elhelw et al., 2021), where domestic animals are highly infested with hard ticks. Although the incidence of *B. burgdorferi* infection in dogs and ticks was low, dogs can serve as sentinel carriers for these bacteria. The available data on *B. burgdorferi* transmission through tick bites are currently limited in Africa, suggesting that its occurrence may be underestimated.

All previous reports involving the above-mentioned bacteria indicate that ticks of genera *Amblyomma*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Ornithodoros* and *Rhipicephalus* are the indigenous tick species harboured by wild animals in South Africa. Our results correspond with this statement, since we identified the same tick species. Almost 50 % (91/191) of *A. hebraeum* ticks, the most predominant tick species, were infected with *Anaplasma* sp. The second most predominant tick species *R. sinus* was associated with a 40 % (112/278) detection rate of *Anaplasma* sp. *Amblyomma*, *Rhipicephalus* ticks were infected with diverse pathogens, whereas *Ixodes* and *Ornithodoros* ticks were generally infected with a single bacterium (*Rickettsia* spp. or *Anaplasma*).

There is insufficient information on co-infection in ticks from Africa. In our study, we detected 18.7 % of tick samples with co-infection (two bacteria in 16.8 % and three bacteria in 2 % of samples). The most frequent co-infections involved two bacteria (*Rickettsia* spp. and *A. phagocytophilum*) or three bacteria (*Rickettsia* spp., *A. phagocytophilum*, and *E. chaffeensis*/*C. burnetii*). Kim et al. (2018) detected co-infection in Tanzania only in 5.7 % of ticks. Understanding of tick co-infections in Africa reflects the tick–host–pathogen ecology on the continent. In Africa, there are conditions that support high diversity of tick species (hard ticks, e.g. genera *Amblyomma*, *Rhipicephalus*, and *Hyalomma* among others) feeding on an equally broad range of domestic animals, wildlife, and in some cases humans (Gabriel et al., 2025). Additionally, many areas in Africa have wildlife-livestock interfaces (buffer zones, grazing in savannahs, game reserves adjacent to farming lands) which allow ticks to feed on both wild and domestic hosts, increasing opportunities for multi-pathogen transmission (Makwarela et al., 2025).

Jongejan and Uilenberg (2004) reported that the abundance of ticks and tick-borne pathogens are determined by both biotic and abiotic factors, such as the presence of hosts, rainfall, humidity and temperature. Moreover, the increasing geographical range and incidence of animal reservoir hosts and tick vectors has resulted in the emergence of new infections in new habitats globally. Wikel (2018) indicated that any compromise on the factors that have a direct or indirect effect on ticks will lead to changes in the distribution and prevalence of tick populations. In our study, we detected that the prevalence of ticks to selected bacteria differed in tick genus/species, sex, developmental stage, province, year of sampling and animal host from which the ticks were collected, while seasons did not affect the results. According to Ellis and Ramankutty (2008), more than 70 % of the earth's dry land has been altered mostly by replacement with infrastructure or farming. Climate change and other factors could create better conditions for ticks to live in throughout the year. Thus, the continuous identification of novel tick-borne pathogens results in a constant change in the epidemiology and diversity of known vector-borne diseases (Paddock et al., 2016).

The results of this study present original knowledge about the prevalence of selected bacteria (especially *Rickettsia* spp., *Coxiella*, and *Borrelia burgdorferi* s.l.) in ticks collected from wild animals from six provinces of South Africa, which can provide the basis for more intensive epidemiological research in South Africa. Regardless of these findings, it is clear that ticks play an important role in maintaining the viable pathogens of veterinary and medical importance in nature. Considering climate change and other anthropogenic threats that result in the emergence of tick-borne pathogens, it is important to raise awareness of

these tick-borne diseases and their impact on human health.

5. Institutional review board statement

Permission for collection of road-killed animals was received from the Limpopo Department of Economic Development, Environment & Tourism (LEDET) (permit number ZA/LP/87,586) and Department of Agriculture and Rural Development, Gauteng Provincial Government (permit number CPGF-0136).

Author statement

- Each author on a publication has made a substantial contribution to the work and takes responsibility for its contents
- All authors meet the criteria for authorship, have approved the final version, and agree to be accountable for the integrity of the work
- The authors have no conflicts of interest or funding sources

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CRediT authorship contribution statement

Eva Bártová: Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Ali Halajian:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Marie Budíková:** Validation, Software. **Alena Žáková:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis. **Heloise Heyne:** Writing – review & editing, Investigation.

Declaration of competing interest

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2026.107976.

Data availability

Data will be made available on request.

References

- Adelabu, O.A., Iweriebor, B.C., Okoh, A.I., Obi, L.C., 2020. Phylogenetic profiling for zoonotic *Ehrlichia* spp. from ixodid ticks in the Eastern Cape, South Africa. *Transbound. Emerg. Dis.* 67 (3), 1247–1256.

- Adelabu, O., Iweriebor, B.C., Okon, A.I., Obi, L.C., 2019. Evidence of pathogenic zoonotic tick-borne *Rickettsia* and *Borrelia* spp. in some communal farms in the eastern cape province. *S. Afr. Res. Sq.* <https://doi.org/10.21203/rs.2.17600/v1>.
- Apanaskevich, D.A., Horak, I.G., Camicas, J.-L., 2007. Redescription of *Hyalomma* (*Euhyalomma*) *truncatum* and the closely related species, *H. (E.) albiparmatum* and *H. (E.) nitidum* (Acari: ixodidae). *Exp. Appl. Acarol.* 44, 115–136.
- Apanaskevich, D.A., Horak, I.G., Camicas, J.-L., 2007. Redescription of *Haemaphysalis* (*Rhipistoma*) *elliptica* (Koch 1844), an old taxon of the *Haemaphysalis* (*Rhipistoma*) *leachi* group from East and southern Africa, and of *Haemaphysalis* (*Rhipistoma*) *leachi* (Audouin, 1826) (Ixodida, Ixodidae) Onderstepoort. *J. Vet. Res.* 74, 181–208.
- Bogovic, P., Lotric-Furlan, S., Korva, M., Avsic-Zupanc, T., 2016. African tick-bite fever in traveler returning to Slovenia from Uganda. *Emerg. Infect. Dis* 22 (10), 1848–1849.
- Cossu, C.A., Collins, N.E., Oosthuizen, M.C., Menandro, M.L., Bhoora, R.V., Vorster, I., Cassini, R., Stoltz, H., Quan, M., van Heerden, H., 2023. Distribution and prevalence of anaplasmataceae, rickettsiaceae and coxiellaceae in African ticks: a systematic review and meta-analysis. *Microorganisms* 11 (3), 714, 9.
- De La Fuente, J., Lew, A., Lutz, H., Meli, M.L., Hofmann-Lehmann, R., Shkap, V., Molad, T., Mangold, A.J., Almazán, C., Naranjo, V., Gortázar, C., Torina, A., Caracappa, S., García-Pérez, A.L., Barral, M., Oporto, B., Ceci, L., Carelli, G., Blouin, E.F., Kocan, K.M., 2005. Genetic diversity of *Anaplasma* species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. *Anim. Health Res. Rev.* 6, 75–89.
- Dijman, T.A., Biguezoton, A.S., Saegerman, C., 2024. Tick-borne diseases in Sub-Saharan Africa: a systematic review of pathogens, research focus, and implications for public health. *Pathogens* 13 (8), 697.
- Ehounoud, C.B., Yao, K.P., Dahmani, M., Achi, Y.L., Amanzougaghene, N., N'Douba, A. K., N'Guessan, J.D., Raoult, D., Fenollar, F., Mediannikov, O., 2016. Multiple pathogens including potential new species in tick vectors in Côte d'Ivoire. *PLoS Negl. Trop. Dis.* 10 (1), e0004367.
- Ellis, E.C., Ramankutty, N., 2008. Putting people in the map: anthropogenic biomes of the world. *Front. Ecol. Env.* 6, 439–447.
- Elhelw, R., Elhariri, M., Hamza, D., Abuwarda, M., Ismael, E., Farag, H., 2021. Evidence of the presence of *Borrelia burgdorferi* in dogs and associated ticks in Egypt. *BMC Vet. Res.* 17 (1), 49.
- Esemu, S.N., Ndip, L.M., Ndip, R.N., 2011. *Ehrlichia* species, probable emerging human pathogens in sub-Saharan Africa: environmental exacerbation. *Rev. Env. Health* 26 (4), 269–279.
- Frean, J., Blumberg, L., 2007. Tick bite fever and Q fever – A South African perspective. *S. Afr. Med. J.* 97, 1198–1202.
- Freedman, D.O., Weld, L.H., Kozarsky, P.E., Fisk, T., Robins, R., von Sonnenburg, F., Keystone, J.S., Pandey, P., Cetron, M.S., 2006. Spectrum of disease and relation to place of exposure among ill returned travelers. *N. Engl. J. Med.* 354, 119–130.
- Fuente, J., Estrada-Peña, A., Venzal, J.M., Sonenshine, K.A., Overview, D.E., 2008. Ticks as vectors of pathogens that cause disease in humans and animals. *Front. Biosci.* 13, 6938–6946.
- Gabriel, A.N.A., Wang, X.Y., Zu, G.Y., Zhen, P.Y., Jamil, L., Shen, S.J., Li, C., Theoneste, N., Zhao, L., Cao, W.C., 2025. Geospatial and modelling analyses reveal diverse tick and tick-associated microbes in the East African Community. *Infect. Dis. Poverty.* 14, 39.
- Guglielmone, A.A., Nava, S., Robbins, R.G., 2023. Geographic distribution of the hard ticks (Acari: ixodida: ixodidae) of the world by countries and territories. *Zootaxa* 5251 (1), 1–274.
- Horak, I.G., Heyne, H., Williams, R., Gallivan, G.J., Spickett, A.M., Bezuidenhout, J.D., Estrada-Peña, A., 2018. The ixodid ticks (Acari: ixodidae) of Southern Africa. Springer International Publishing AG, Basel, Switzerland, p. 676.
- Horak, I.G., Jordaan, A.J., Nel, P.J., Van Heerden, J., Heyne, H., Van Dalen, E.M., 2015. Distribution of endemic and introduced tick species in free state province, South Africa. *J S Afr Vet Assoc* 86, 1255.
- Hoover, T.A., Vodkin, M.H., Williams, J.C., 1992. A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. *J. Bacteriol* 174 (17), 5540–5548.
- Jensenius, M., Davis, X., von Sonnenburg, F., Schwartz, E., Keystone, J.S., Leder, K., Parola, P., 2009. Multicenter geospatial analysis of rickettsial diseases in international travelers, 1996–2008. *Emerg. Infect. Dis* 15 (11), 1791–1798.
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitology* 129, 3–14.
- Kawahara, M., Tajima, T., Torii, H., Yabutani, M., Ishii, J., Harasawa, M., Isogai, E., Rikihisa, Y., 2009. *Ehrlichia chaffeensis* infection of sika deer. *Jpn. Emerg. Infect. Dis.* 15, 1991–1993.
- Kelly, P., Marabini, L., Dutlow, K., Zhang, J., Loftis, A., Wang, C., 2014. Molecular detection of tick-borne pathogens in captive wild felids. *Zimb. Paras. Vectors* 7, 514.
- Kim, T.Y., Kwak, Y.S., Kim, J.Y., Nam, S.H., Lee, I.Y., Mduma, S., Keyyu, J., Fyumagwa, R., Yong, T.S., 2018. Prevalence of tick-borne pathogens from ticks collected from cattle and wild animals in Tanzania in 2012. *Korean J. Parasitol.* 56 (3), 305–308.
- Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., McBride, J.W., Pinter, A., Popov, V., Gennari, S.M., Walker, D.H., 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. *J. Clin. Microbiol* 42, 90–98.
- Ledwaba, M.B., Nozipho, K., Tembe, D., Onyiche, T.E., Chaisi, M.E., 2022. Distribution and prevalence of ticks and tick-borne pathogens of wild animals in South Africa: a systematic review. *Curr. Res. Parasitol. Vector-Borne Dis.* 2, 1–14.
- Lee, M., Yu, D., Yoon, J., Li, Y., Lee, J., Park, J., 2009. Natural coinfection of *Ehrlichia chaffeensis* and *Anaplasma bovis* in a deer in South Korea. *J. Vet. Med. Sci.* 71, 101–103.
- Lloyd, C., Stidworthy, M.F., Ulrich, W., 2010. *Coxiella burnetii* abortion in captive dama gazelle (*Gazella dama*) in the United Arab Emirates. *J. Zoo. Wildl. Med.* 41, 83–89.
- Kocan, A.A., Levesque, G.C., Whitworth, L.C., Murphy, G.L., Ewing, S.A., Barker, R.W., 2000. Naturally occurring *Ehrlichia chaffeensis* infection in coyotes from Oklahoma. *Emerg. Infect. Dis* 6 (5), 477–480.
- Klomp, H., Dobson, S.J., Barker, S.C., 2002. A new subfamily, Bothriocrotinae n. subfam., for the genus *Bothriocroton*, Keirans, King & Sharrad, 1994 status amend. (Ixodida, Ixodidae), and the synonymy of *Aponomma* Neumann, 1899 with *Amblyomma* Koch, 1844. *Syst. Parasitol* 53, 101–107.
- Makwavela, T.G., Seoraj-Pillai, N., Nangammi, T.C., 2025. Distribution and prevalence of ticks and tick-borne pathogens at the wildlife-livestock interface in Africa: a systematic review. *Vet. Sci.* 12 (4), 364.
- Massung, R.F., Lee, K., Mauel, M., Gusa, A., 2002. Characterization of the rRNA genes of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*. *DNA Cell. Biol.* 21 (8), 587–596.
- Maurin, M., Raoult, D., 1999. Q fever. *Clin. Microbiol. Rev.* 12, 518–553.
- Mediannikov, O., Fenollar, F., Socolovschi, C., Diatta, G., Bassene, H., Molez, J.F., Sokhna, C.H., Trape, J.F., Raoult, D., 2010. *Coxiella burnetii* in humans and ticks in rural Senegal. *PLoS. Negl. Trop. Dis* 4, 654.
- Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., Bodker, R., Fach, P., Moutailler, S., 2014. High-throughput screening of tick-borne pathogens in Europe. *Front. Cell. Infect. Microbiol.* 4, 103.
- Mtshali, K., Khumalo, Z.T.H., Nakao, R., Grab, D.J., Sugimoto, C.H., Thekiso, O.M.M., 2016. Molecular detection of zoonotic tick-borne pathogens from ticks collected from ruminants in four South African provinces. *J. Vet. Med. Sci.* 77, 1573–1579.
- Mtshali, K., Nakao, R., Sugimoto, C., Thekiso, O., 2017. Occurrence of *Coxiella burnetii*, *Ehrlichia canis*, *Rickettsia* species and *Anaplasma phagocytophilum* - like bacterium in ticks collected from dogs and cats in South Africa. *J. S. Afr. Vet. Assoc.* 88, 1390.
- Murrell, A., Barker, S.C., 2003. Synonymy of *Boophilus curtipis*, 1891 with *Rhipicephalus* Koch, 1844 (Acari: ixodidae). *Syst. Parasitol* 56, 169–172.
- Nakayima, J., Hayashida, K., Nakao, R., Ishii, A., Ogawa, H., Nakamura, I., Moonga, L., Hango, B.M., Mweene, A.S., Thomas, Y., Orba, Y., Sawa, H., Sugimoto, C., 2014. Detection and characterization of zoonotic pathogens of free-ranging non-human primates from Zambia. *Paras. Vect.* 7, 490.
- Olivieri, E., Kariuki, E., Floriano, A.M., Castelli, M., Tafesse, Y.M., Magoga, G., Kumsa, B., Montagna, M., Sassara, D., 2021. Multi-country investigation of the diversity and associated microorganisms isolated from tick species from domestic animals, wildlife and vegetation in selected african countries. *Exp. Appl. Acarol.* 83, 427–448.
- Paddock, C.D., Lane, R.S., Staples, J.E., Labruna, M.B., 2016. Changing paradigms for tick-borne diseases in the Americas. *Glob. Health Impacts Vector-borne Dis.* A8.
- Paddock, C.D., Yabsley, M.J., 2007. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. *Curr. Top. Microbiol. Immunol.* 315, 289–324.
- Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abad, M.Y., Stenos, J., Bitam, I., Fournier, P.E., Raoult, D., 2013. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin. Microbiol. Rev.* 26 (4), 657–702.
- Porter, S.R., Czaplinski, G., Mainil, J., Guattéo, R., Saegerman, C., 2011. Q-fever: current state of knowledge and perspectives of research of a neglected zoonosis. *Int J Microbiol.* 248418.
- StatSoft, Inc, 2013. STATISTICA (data analysis software system), version 12. www.statsoft.com.
- Volz, O.V., Keirans, J.E., 2003. A review of African *Amblyomma* species (Acari, Ixodida, Ixodidae). *Acarina* 11, 135–214.
- Walker, J.B., Keirans, J.E., Horak, I.G., 2000. The genus *Rhipicephalus* (Acari: ixodidae): a guide to the brown ticks of the world. Cambridge University Press, Cambridge, p. 643.
- Wikel, S., 2018. Ticks and tick-borne infections: complex ecology, agents, and host interactions. *Vet. Sci.* 5, 60.
- Williams, C.V., Van Steenhouse, J.L., Bradley, J.M., Hancock, S.I., Hegarty, B.C., Breitschwerdt, E.B., 2002. Naturally occurring *Ehrlichia chaffeensis* infection in two prosimian primate species: ringtailed lemurs (*Lemur catta*) and ruffed lemurs (*Varecia variegata*). *Emerg. Infect. Dis* 8, 1497–1500.
- Woldehiwet, Z., 2004. Q fever (coxiellosis): epidemiology and pathogenesis. *Res. Vet. Sci.* 77 (2), 93–100.