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Original article

Genetic characterization of tick-borne pathogens in ticks infesting cattle and sheep from three South African provinces

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ABSTRACT

Ticks are involved in the transmission of many public health and veterinary important pathogens. Although tick-borne pathogens are widely distributed in South Africa, information on tick-pathogen relationship needs to be updated particularly using modern molecular techniques. This study used PCR and sequencing to confirm the identity of the tick species collected from cattle and sheep from KwaZulu-Natal, Free State and Eastern Cape. Furthermore, presence of *Babesia* spp., *Theileria* spp., *Anaplasma marginale*, *Rickettsia* spp., *Ehrlichia ruminantium* and *Coxiella burnetii* was detected from tick DNA using species-specific PCR or nested PCRs. The study samples consisted of 390 adult ticks (male and female) which were pooled according to species, host animal and sampling site (three ticks per pool) for DNA extraction. The PCR results revealed that out of 130 tick DNA pools, 30 (23.1%) were positive for at least one pathogen. The most frequent pathogen was *C. burnetii* (9.2%), followed by *Rickettsia* spp. (7.7%), *A. marginale* (3.8%), *T. mutans* (3.1%), *T. taurotragi* (2.3%) and *E. ruminantium* (1.5%). The highest prevalence of pathogens was observed in ticks collected from cattle in Eastern Cape (16/42) and the lowest was in ticks obtained from sheep in Free State (1/21). Infected ticks were identified as *Rhipicephalus evertsi evertsi* (n = 13), *R. appendiculatus* (n = 3), *R. decoloratus* (n = 7) and *Amblyomma hebraeum* (n = 7). Coinfection with two pathogens was found in 21% of pathogen-positive pools. Analysis of *Theileria taurotragi* 18S rRNA, *T. mutans* 18S rRNA, *C. burnetii* htpB, *Rickettsia* spp. gltA, *Rickettsia* spp. ompA, *E. ruminantium* pCS20 and *A. marginale* Msp5 sequences showed that the pathogens detected in this study were genetically related to isolates previously reported in Africa. These findings provide important information on distribution of ticks and tick-borne pathogens of ruminants and will contribute in the formulation of future control strategies in South Africa.

1. Introduction

Ticks carry and transmit a large number of pathogens including bacteria, viruses and protozoa. They are second only to mosquitoes in importance as vectors of disease agents causing great impact on human and animal health (Benelli, 2016; Mehlhorn et al., 2012; Sonenshine et al., 2002). Ticks have a wide range of hosts and their preferred hosts may differ depending on the tick species as well as the region. For many tick species, cattle are the favorite host, while sheep, dog and other small animals such as birds are alternative hosts (Moyo and Masika, 2009). Tick-borne diseases (TBDs) have a worldwide distribution. In South Africa, pathogens associated with ticks infesting cattle include agents of babesiosis, theileriosis, anaplasmosis, rickettsioses, heartwater and Q-fever.

Bovine babesiosis agents namely, *Babesia bovis* and *B. bigemina* are common in South Africa where their vectors are *Rhipicephalus microplus*, *R. decoloratus* and *R. evertsi evertsi* (Mtshali and Mtshali, 2013). Corridor disease, January disease and East Coast fever (ECF) are three recognized disease syndromes of *Theileria parva*-caused theileriosis. In South Africa, they are transmitted by *R. appendiculatus* and *R.*

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zambeziensis (Lawrence et al., 1983; Sibeko et al., 2011). Other pathogenic *Theileria* spp. are *T. taurotragi* and *T. mutans* transmitted by *R. appendiculatus* and *Amblyomma* ticks, respectively (Bishop et al., 2004). Bovine anaplasmosis is a hemolytic disease caused by infection with *Anaplasma marginale* in cattle and is in South Africa mainly transmitted by *R. decoloratus*, *R. microplus*, *R. simus*, *R. evertsi evertsi* and *Hyalomma rufipes* (De Waal, 2000; Kocan et al., 2000; Schmidt, 1937).

Tick-borne rickettsioses are caused by obligate intracellular bacteria belonging to the spotted fever group of the genus *Rickettsia*. Some *Rickettsia* species have been discovered in South Africa and the most common is *R. africae* which causes African tick bite fever in humans (Frean and Blumberg, 2007; Halajian et al., 2016; Mtshali et al., 2015). *R. africae*, transmitted by *Amblyomma hebraeum* and *Am. variegatum* (Jongejan and Uilenberg, 2004), is believed to be present in more than 30 African countries (Adjou Moumouni et al., 2016). Like rickettsioses, heartwater caused by *Ehrlichia ruminantium* is also transmitted by *Amblyomma* ticks and is endemic in most parts of South Africa (Mdladla et al., 2016). Q-fever is a worldwide zoonosis caused by *Coxiella burnetii* and appears to be widespread in South Africa (Mtshali et al., 2015). Cattle, sheep and goats are traditional sources of human infection (Cooper et al., 2011).

The epidemiology of babesiosis, theileriosis, anaplasmosis, rickettsioses, heartwater and Q fever have been investigated previously in South Africa (De Waal, 2000; Frean and Blumberg, 2007; Mtshali and Mtshali, 2013; Mdladla et al., 2016). However, the information on tick distribution and prevalence of the agents requires regular updates using modern, reliable, specific and sensitive molecular techniques. Therefore, this study was carried out with the objective of characterizing tick species collected from cattle and sheep in South Africa as well as identifying pathogens they are harbouring using DNA-based PCR and sequencing in order to gain understanding of their genetic diversity.

2. Materials and methods

2.1. Tick samples

A total of 390 adult ticks (male and female) were collected in three provinces of South Africa, namely, KwaZulu-Natal (KZN), Eastern Cape (EC) and Free State (FS) (Fig. 1). In KZN and EC the ticks were collected from cattle at uMsinga Mountain View diptank [S28° 41′43.1″ E 030° 16′14.6″] and Amathole District Municipality [S32° 48′ E27°01′49″], respectively. FS samples were collected from cattle in Hooningkloof farm [S28° 30. 666′ E 028° 42.701′] and from sheep in Seotlong Hotel and Agricultural School [S28° 35′ E 28° 50′]. The tick sampling was conducted with the consent of livestock owner, and care was taken to minimize animal discomfort. All procedures were performed in accordance with the ethical guidelines permitted by Obihiro University of Agriculture and Veterinary Medicine (approval no.: 1723).

2.2. DNA extraction

After microscopic identification based on entomological keys by Walker et al. (2003), the tick samples were pooled according to species, host animal and sampling site (three ticks per pool). Pooled ticks were used to obtain genomic DNA. Tick DNA was extracted using salting out method as described by Miller et al. (1988). The extracted DNA was dissolved in 100 μl of double distilled water and stored at −30 °C until further use.

2.3. Molecular characterization of ticks

For confirmation of tick species, tick DNA was amplified by PCR and sequencing of an approximately 360-bp fragment of tick mitochondrial 12S rDNA. Amplification was done with F1 (5′-AAACTAGGATTAGATACCCT-3′) and R1 (5′-AATGAGAGCGACGGGCGATGT -3′) primers (Beati and Keirans, 2001) and cycling conditions were: initial denaturation at 94 °C for 5 min; 5 cycles of denaturation at 94 °C for 15 s, annealing at 51 °C for 30 s and extension at 72 °C for 1 min, followed by 25 cycles of denaturation at 94 °C for 15 s, annealing at 53 °C for 30 s, and extension at 72 °C for 30 s, then final extension at 72 °C for 7 min. Each reaction was performed in a final volume of 12 μl containing 5 μl of 2x Ampidirect plus (Shimadzu, Japan), 0.075 μl of Ex Taq polymerase (Takara, Japan), 1 μl (10 μM) of each primer, 2 μl tick DNA template and 2.925 μl of double distilled water. The DNA of *Am. variegatum* ticks collected in Benin (Adjou Moumouni et al., 2016) and distilled water were used as positive and negative controls, respectively.

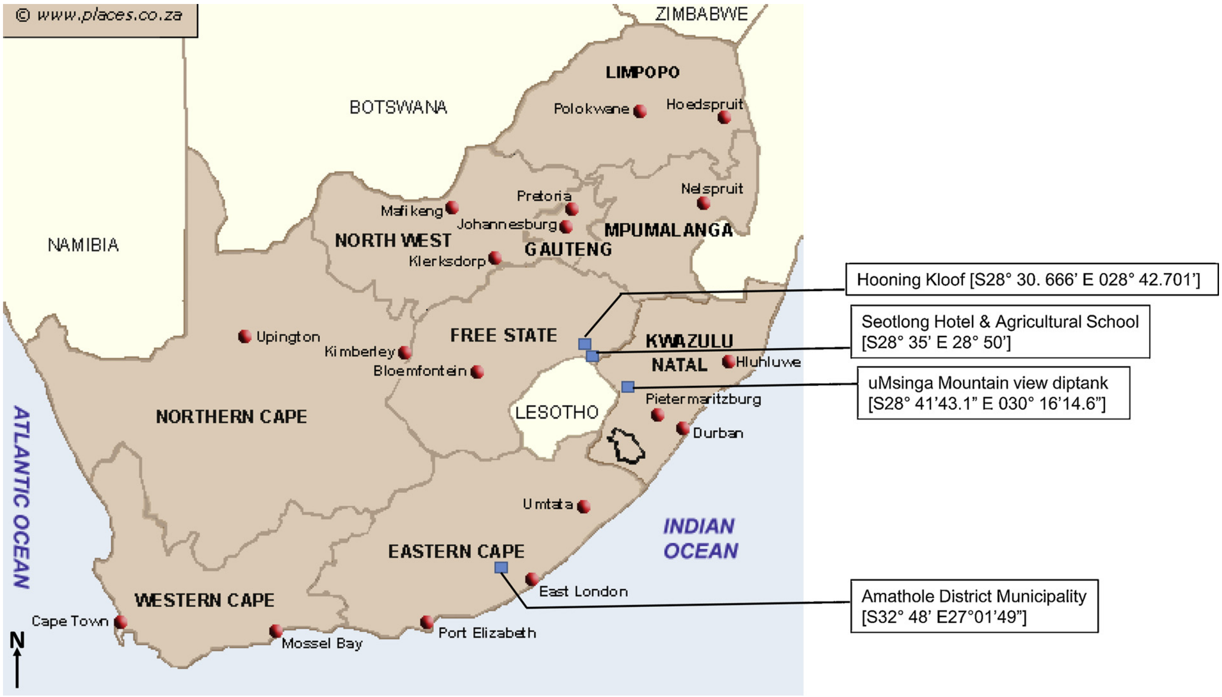

Fig. 1. Map of South African provinces. The squares show sampled areas.

The PCR products were electrophoresed on 1.5% agarose gels which were stained with ethidium bromide and visualized under UV light. All amplicons were extracted from gels using QIAquick Gel Extraction Kit (QIAGEN, Germany) and then sequenced.

2.4. Detection and characterization of tick-borne pathogens

To detect the presence of *B. bigemina*, *B. bovis*, *T. parva*, *T. taurotragi*, *T. mutans*, *A. marginale*, *Rickettsia* spp., *E. ruminantium* and *C. burnetii*, tick DNA pools were screened using previously established PCR assays. Partial sequence of *B. bigemina Rap1a*, *B. bovis SBP-4* (Terkawi et al., 2011), *T. parva, T. taurotragi* and *T. mutans 18S rRNA* (Georges et al., 2001; Simuunza et al., 2011), *A. marginale* major surface protein 5 (*Msp5*) (Ybañez et al., 2013), *Rickettsia* spp. outer membrane protein (*ompA*) (Roux et al., 1996), *Rickettsia* spp. citrate synthase (*gltA*) (Labruna et al., 2004), *E. ruminantium pCS20* (Farougou et al., 2012) and *C. burnetii htpB* genes (Reye et al., 2012) were amplified (Supplementary Table S1). PCR cycling conditions for all pathogens were the same as documented in referenced publications. The reaction mixtures and observation of PCR results were performed as described above. Amplicons were purified using QIAquick Gel Extraction Kit (QIAGEN, Germany) and used for sequencing.

2.5. Sequencing assays

All purified PCR products (Tick 12S rDNA and pathogen DNA amplicons) were cloned in pGEM-T Easy Vector and sequenced as described previously (Ringo et al., 2018) using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) and the ABI PRISM 3100 genetic analyzer (Applied Biosystems, USA). The GenBank accession numbers are shown in Tables 1 and 3.

2.6. BLASTn analysis, sequence alignment and phylogenetic analyses

The identities and similarities of sequenced DNAs were analyzed using BLASTn tool of NCBI GenBank database and Clustal X program. In addition, phylogenetic analyses were inferred using MEGA version 7.0 program and the neighbor-joining distance method (Kimura 2-parameter model). Bootstrap analysis was performed with 1000 replicates to estimate the confidence of branching patterns of the tree.

3. Results

3.1. Tick species identification

A total of 130 tick DNA pools were examined in this study. This includes 42, 27, 40 and 21 DNA pools obtained from ticks collected from EC cattle, KZN cattle, FS cattle and FS sheep, respectively. Thirty out of the 130 pools (23%) carried at least one pathogen. In detail, 16/42, 5/27, 8/40 and 1/21 DNA pools from EC, KZN, FS cattle and FS sheep, respectively, were found positive by the PCR assays. Preliminary morphological identification and species confirmation by molecular analysis of the mitochondrial 12S rDNA showed that pathogen-positive pools (n = 30) include four different species: *R. evertsi evertsi* (n = 13), *R. appendiculatus* (n = 3), *R. decoloratus* (n = 7) and *Am. hebraeum* (n = 7). The length of the obtained tick mitochondrial 12S rDNA sequences varied from 382 to 387 bp. A BLASTn analysis of the *R. evertsi evertsi*, *R. decoloratus*, *R. appendiculatus* and *Am. hebraeum* sequences in this study showed identity ranging from 97.9 to 100% with reference sequences from GenBank (Table 1).

Table 1
Results of tick species identification based on mitochondrial 12S rDNA gene partial sequence.

Tick 12S rDNA sequences		Blastn match		
Accession number	Length (bp)	Identity (%)	Accession number	Tick species
MH751454	383	99.2	DQ849229	*R. evertsi evertsi*
MH751455	383	99.4	DQ849229	*R. evertsi evertsi*
MH751456	383	99.2	DQ849229	*R. evertsi evertsi*
MH751457	384	99.5	DQ849203	*R. appendiculatus*
MH751458	385	98.7	KF569940	*R. decoloratus*
MH751459	387	97.9	KF569940	*R. decoloratus*
MH751460	382	100	AF150049	*Am. hebraeum*
MH751461	382	99.7	AF150049	*Am. hebraeum*

R.: *Rhipicephalus*, *Am.*: *Amblyomma*.

3.2. Pathogens detected in the ticks and infection rates

C. burnetii, the most frequent pathogen, was detected in 12/130 pools (9.2%), all of which were identified as *R. evertsi evertsi*. *Rickettsia* spp. was present in 10/130 pools (7.7%). Six were positive for *Rickettsia* spp. gltA PCR, two were positive for *Rickettsia* spp. *ompA* gene, and two were positive for both PCRs. Positive ticks included *R. evertsi evertsi*, *R. decoloratus* and *Am. hebraeum*. *A. marginale* was detected in five tick pools (3.8%) which were all *R. decoloratus*. *T. mutans* was found in four *R. decoloratus* pools (3.1% of tested samples) whereas *T. taurotragi* occurred in three *R. appendiculatus* pools (2.3%). *E. ruminantium* (2/130; 1.5%) was the least frequent pathogen. It was detected in both *R. decoloratus* and *Am. hebraeum*. *Babesia* species and *T. parva* were not found in the ticks. The distribution of these pathogens in the ticks is presented in Table 2.

3.3. Analyses of tick-borne pathogen DNA sequences

Nested PCR products of *C. burnetii htpB*, *A. marginale Msp5*, *T. taurotragi 18S rRNA*, *T. mutans 18S rRNA*, and *E. ruminantium pCS20*; and PCR products of *Rickettsia* spp. *ompA* and *gltA* genes were cloned and sequenced. Two *C. burnetii htpB* gene sequences were identified. These sequences shared more than 99% identity with isolates deposited in the GenBank. One *Rickettsia* spp. gltA and one *Rickettsia* spp. ompA sequences were recovered from infected ticks and both gene sequences classified the *Rickettsia* spp. as *Rickettsia africae*. One *A. marginale* Msp5 sequence, which was 100% identical to previously published isolates, was obtained from infected ticks. Two sequence types were identified for *T. mutans* 18S rRNA whereas for *T. taurotragi* 18S rRNA, one sequence type was recovered from infected ticks. However, all the three sequences were closely related to previously published sequences. *E. ruminantium* pCS20 with 4 different sequences showed the highest genetic variation. None of the pCS20 sequences generated in this study was 100% identical to known genotypes. The results of the BLASTn analyses of the pathogen DNA sequences from this study are shown in Table 3.

3.4. Phylogenetic analyses

Phylogenetic analysis showed that *C. burnetii htpB* gene sequences located in the same clade with isolates obtained from ticks and mammalian tissues in various geographic areas (Fig. 2). The detected *Rickettsia* spp. belonged to the same clade with *R. africae* ESF 2500-1 strain and the *R. africae* found in *Am. variegatum* ticks from Benin. However, the *R. africae* ompA sequences of this study did not cluster with the one reported in *Hyalomma dromedarii* ticks from Egypt (Fig. 3). The *T. mutans* sequences of this study clustered with isolates recovered from cattle in Kenya, Uganda and Mozambique, but were distant from the ones detected in wild ungulates from South Africa and Kenya (Fig. 4). In *E. ruminantium* pCS20 phylogenetic tree, the sequences obtained from *Am. hebraeum* (MH751470, MH751471) formed a cluster while those obtained in *R. decoloratus* (MH751468, MH751469) were located in two separate clusters. One grouped with an isolate from Tanzanian cattle and the second was together with South Africa Ball Strain and the

Table 2
Infection rates and speciation of pathogens detected in cattle- and sheep-feeding ticks from 3 South African provinces.

Tick samples tested		Infected tick pools		
Source	No. of tick pool[a]	Number	Species (No)	Pathogens carried (No of positive)
KZN cattle	27	5	*Am. hebraeum* (5)	*Rickettsia* spp. (5)
EC cattle	42	16	*R. decoloratus* (1) *R. appendiculatus* (3) *R. evertsi evertsi* (12)	*T. mutans* + *A. marginale* (1) *T. taurotragi* (3) *C. burnetii* (10) *Rickettsia* spp.(1) *Rickettsia* spp. + *C. burnetii* (1)
FS cattle	40	8	*R. decoloratus* (6)	*A. marginale* (2) *T. mutans* (1) *T. mutans* + *A. marginale* (2) *E. ruminatium* + *Rickettsia* spp. (1)
			Am. hebraeum (2)	*Rickettsia* spp. (1) *E. ruminatium* + *Rickettsia* spp. (1)
FS sheep	21	1	*R. evertsi evertsi* (1)	*C. burnetii* (1)

KZN: KwaZulu Natal; EC:Eastern Cape, FS :Free State.
R.: *Rhipicephalus*, *Am.*: *Amblyomma*, *T.*: *Theileria*, *A.*: *Anaplasma*; *E.*: *Ehrlichia*, *C.*: *Coxiella*.
[a] Each pool consisted of 3 ticks of the same species collected from the same host animal and in the same sampling site.

FS342 strain (Fig. 5).

4. Discussion

Tick infestations are common in both cattle and sheep in Africa including South Africa. In this study, ticks collected from cattle or sheep in three provinces were examined using molecular techniques (PCR, sequencing and phylogenetics) to resolve the species and to detect microorganisms they are carrying in order to contribute to understanding tick-pathogen relationships and pathogen genetic diversities in South Africa. DNA of four bacteria and two protozoa of veterinary and/or public health significance, namely *C. burnetii*, *Rickettsia africae, A. marginale*, *E. ruminantium*, *T. mutans* and *T. taurotragi* was found in ticks identified as *R. evertsi evertsi*, *R. appendiculatus*, *R. decoloratus* and *Am. hebraeum*.

The presence of *C. burnetii* in feeding ticks has previously been reported in South Africa (Mtshali et al., 2015) at an overall infection rate similar to this study. However, in the previous study, all ticks from EC were negative while in this study, cattle-feeding *R. evertsi evertsi* were found to be positive for *C. burnetii*. This result therefore demonstrates that the bacteria circulate in EC like in other South African provinces. In addition, reports from Senegal (Mediannikov et al., 2010), Nigeria (Reye et al., 2012) and Kenya (Koka et al., 2018; Ndeereh et al., 2017) also found that *R. evertsi evertsi* ticks harbor *C. burnetii*. These zoonotic bacteria have been reported in humans and animals throughout the African continent with well-known pathogenicity (Kelly et al., 1993; Schutte et al., 1976; Vanderburg et al., 2014). The fact that the *C. burnetii* in this study shared high genetic identity with Q-fever agents from several countries suggests that surveys in livestock are needed to clarify risk level and prevent human infection.

Analyses of gltA and ompA genes in all tick samples showed that *R. africae* was the only *Rickettsia* in the study area and was infecting three out of the four tick species identified. This is consistent with *R. africae* being widely distributed in Africa (Halajian et al., 2016; Kelly et al., 1996; Mtshali et al., 2015; Parola et al., 2013). In addition, the occurrence of this pathogen in *R. decoloratus, R. evertsi evertsi* and *Am. hebraeum* ticks corroborates previous reports (Halajian et al., 2016; Mtshali et al., 2015). Out of these three tick species only *Am. hebraeum* may pose a risk of acquiring *Rickettsia africae* after a tick bite as the principal vector of the agent and the only species that is known to attack humans (Horak et al., 2002). African tick bite fever caused by *R. africae* was previously reported in febrile travelers returning from South Africa (Fournier et al., 1998; Wieten et al., 2011). Therefore, our data reaffirm the need to educate travelers visiting South Africa about tick bite risks. The genetic identity between the South African *R. africae* sequences and those found in other African countries may be related to their characteristic of sharing the same tick hosts.

A. marginale, *T. mutans*, *T. taurotragi*, and *E. ruminantium*, all of

Table 3
Accession numbers of DNA sequences from this study deposited in GenBank.

DNA sequence				Closest Blastn match	
Pathogen	Target gene	Accession number	Length (bp)	Identity (%)	Accession number (country)
T. mutans	*18S rRNA*	MH751462 MH751463	259 259	99.6 100	KU206320 (Uganda) KU206320 (Uganda)
T. taurotragi	*18S rRNA*	MH751464	243	99.6	L19082 (South Africa)
A. marginale	*Msp5*	MH751465	195	100	KU042083 (Egypt)
Rickettsia spp.	*ompA* *gltA*	MH751466 MH751467	632 402	99.8 100	KT633262 (*R. africae;* Benin) JN043505 (*R. africae,* Liberia)
E. ruminantium	*pCS20*	MH751468 MH751469 MH751470 MH751471	279 279 279 279	99.6 98.9 98.6 98.9	AY236061 (Guadeloupe, Caribbean) AY236061 (Guadeloupe, Caribbean) AY236061 (Guadeloupe, Caribbean) AY236061 (Guadeloupe, Caribbean)
C. burnetii	*htpB*	MH751472 MH751473	325 325	99.1 99.7	CP014561 (Greece) CP014561 (Greece)

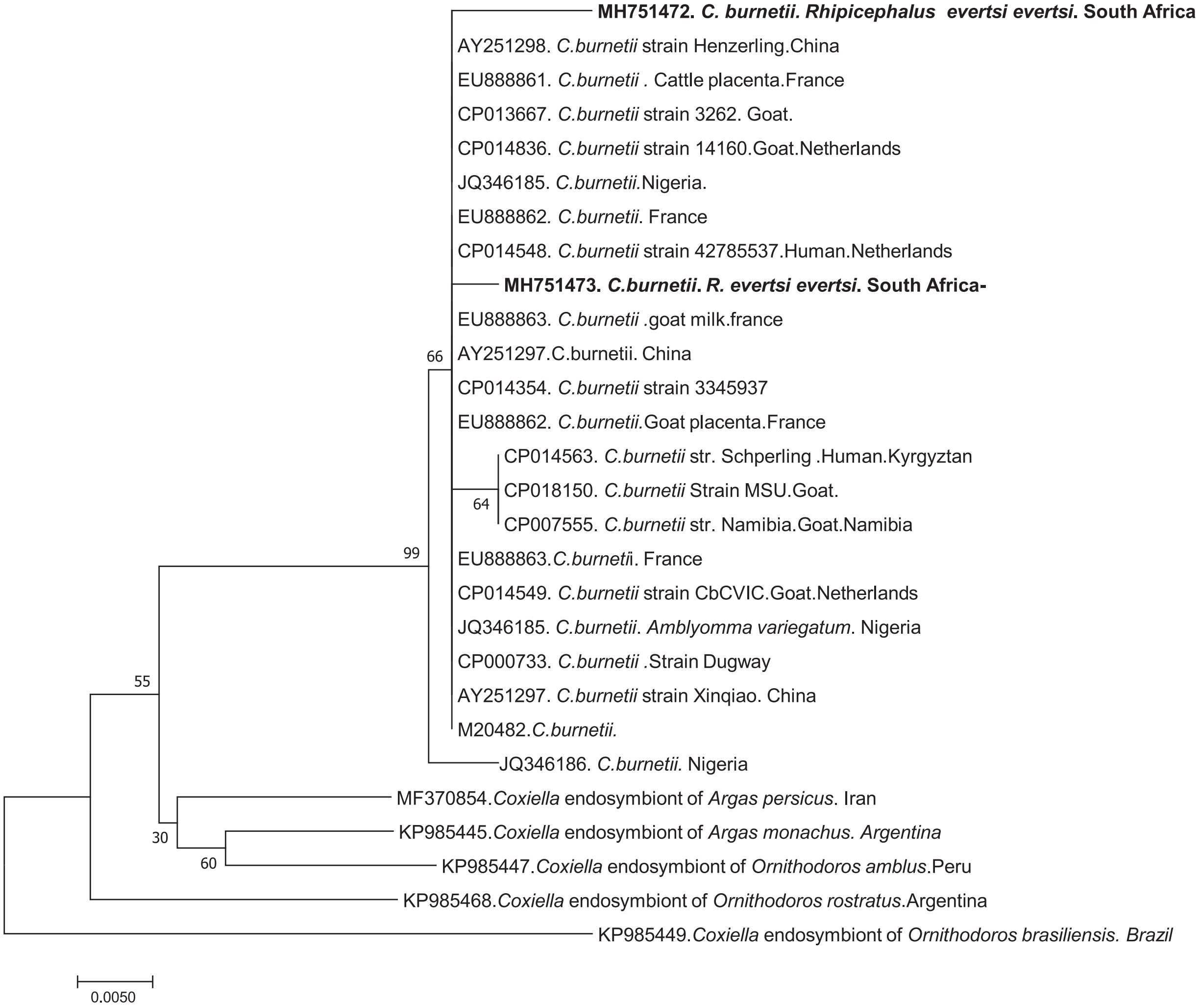

Fig. 2. Phylogenetic tree showing relationship between *C. burnetii* detected in South African ticks and specimen reported in other geographical areas. The tree was constructed based on *htpB* gene partial sequences, using MEGA ver.7 and neighbor-joining method. Sequences from this study are shown in bold font. The numbers at the nodes represent bootstrap values.

which are pathogens causing bovine diseases, were only found in ticks collected from cattle in EC and FS provinces. EC and FS provinces are located in the *A. marginale* epidemic areas of South Africa (Mtshali et al., 2007; Mutshembele et al., 2014) and therefore, the presence of the bacteria in these provinces was not surprising. Although previous studies reported infection in cattle, this study provides the first molecular evidence to the suspected importance of *R. decoloratus* in *A. marginale* transmission in the study areas.

T. mutans and *T. taurotragi* are benign *Theileria* which occasionally cause neurological disorders in infected cattle (Catalano et al., 2015; Moll et al., 1986; Moll and Lohding, 1984). Both pathogens were previously detected in cattle from KZN (Yusufmia et al., 2010), FS and Mpumalanga provinces (Berggoetz et al., 2014). The occurrence of *T. taurotragi* in cattle-feeding *R. appendiculatus* is in concordance with the vector role of this tick (Wesonga et al., 2010; Chumo et al., 1985) and indicates that EC is also a province where the pathogen is prevalent. The presence of *T. mutans* in *R. decoloratus* collected from EC and FS cattle reaffirms the ubiquity of this *Theileria* species in South Africa. Since *R. decoloratus* does not have a proven vector competence for *T. mutans*, the pathogen may have originated from the cattle that ticks were feeding on. This hypothesis is further supported by the location of isolates from this study in the phylogenetic tree.

E. ruminantium infection is widespread in South Africa (Mdladla et al., 2016). The low infection rate and the presence of *E. ruminantium* in *Am. hebraeum* and *R. decoloratus* feeding on FS cattle are in agreement with previous data (Allsopp, 2010; Mtshali et al., 2015). The absence of positive samples in the other study areas may be attributed to the sample size, the animal species from which the ticks were collected or / and difference of prevalence between areas. In the previous study, FS ticks were shown to have higher infection rates than those from KZN and EC (Mtshali et al., 2015), which can explain why the probability to find positive samples was higher in FS. Most of the ticks analyzed are cattle-feeding ticks and these ticks have been shown to be less likely to be infected than those collected from small ruminants (Mtshali et al., 2015).

Regarding the tick-pathogen relationship, the role of *Am. hebraeum* as the main vector of heartwater in southern Africa (Allsopp, 2010) explains our results. In the absence of any proven vectorial capacity, it is likely that *R. decoloratus* acquired *E. ruminantium* infection through feeding on *E. ruminantium*-infected hosts. Nevertheless, with a recent report showing the ability of *R. microplus* to transmit *E. ruminantium* (Biguezoton et al., 2016), studies on the vector competence of other *Boophilus/Rhipicephalus* ticks will be of interest to clarify the transmission routes of the heartwater agent.

In contrast with previous study in the same area (Mtshali et al., 2015), the *E. ruminantium* pCS20 ribonuclease region sequences generated were not conserved. Differences between our report and the previous ones may be due to the use of different set of primers. The

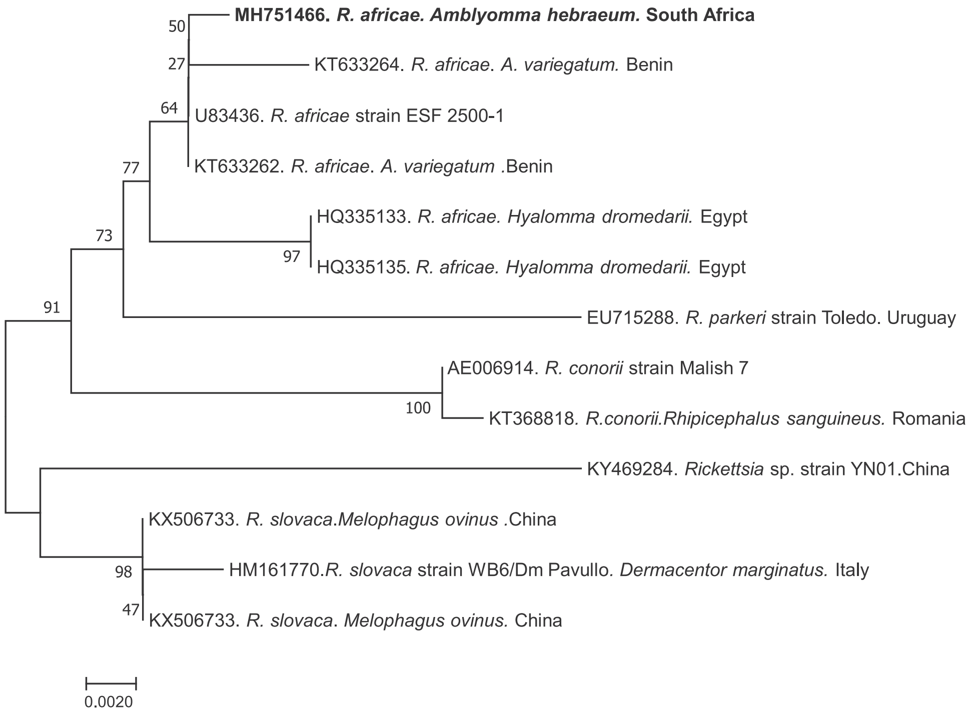

Fig. 3. Phylogenetic tree showing the relationship between *Rickettsia africae* detected in South African ticks and other SFG *Rickettsia* spp., based on partial sequences of *ompA* gene. The tree was constructed in MEGA ver.7 using neighbor-joining method. Sequence from this study is shown in bold font. The numbers at the nodes represent bootstrap values.

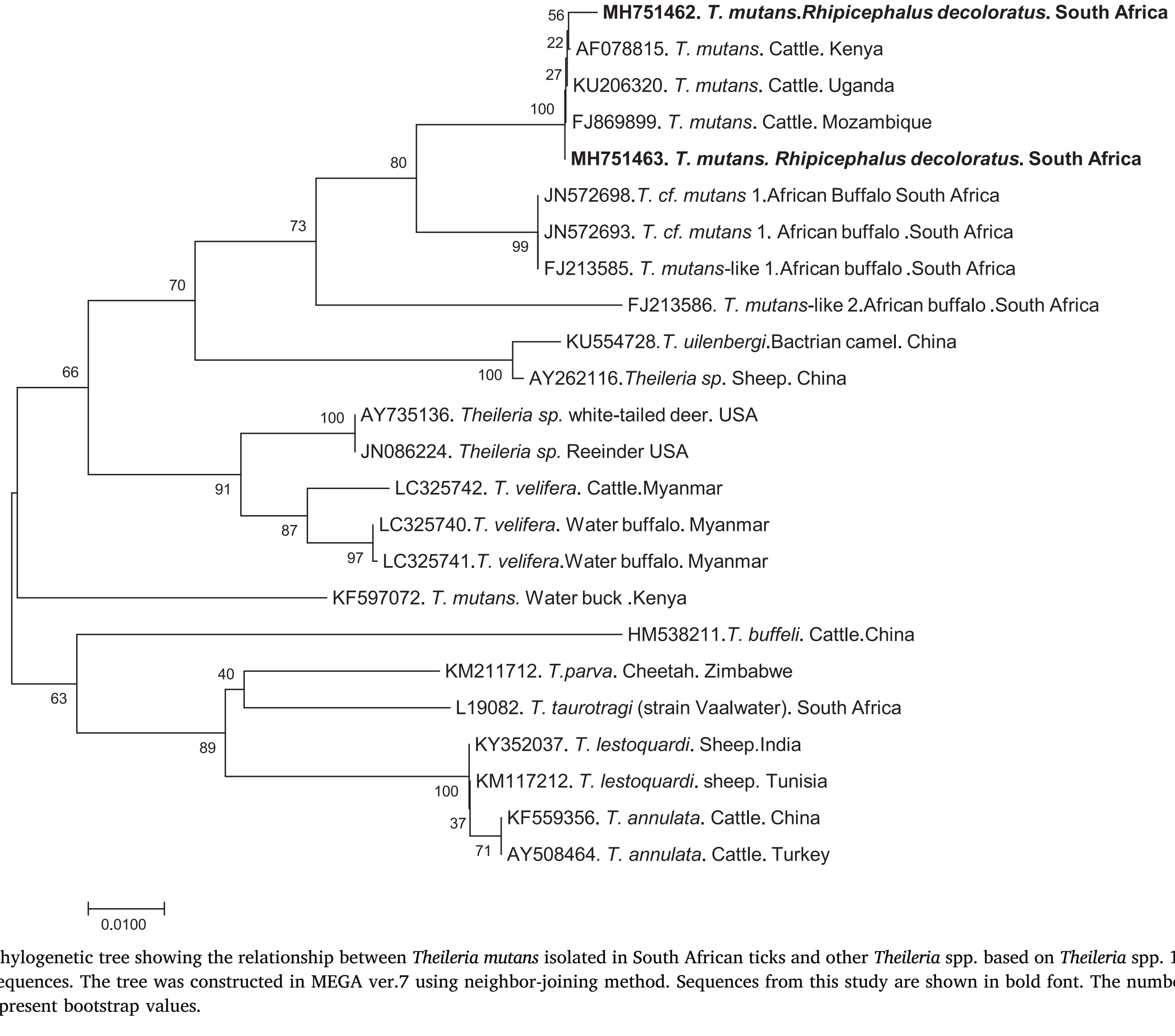

Fig. 4. Phylogenetic tree showing the relationship between *Theileria mutans* isolated in South African ticks and other *Theileria* spp. based on *Theileria* spp. 18S rRNA partial sequences. The tree was constructed in MEGA ver.7 using neighbor-joining method. Sequences from this study are shown in bold font. The numbers at the nodes represent bootstrap values.

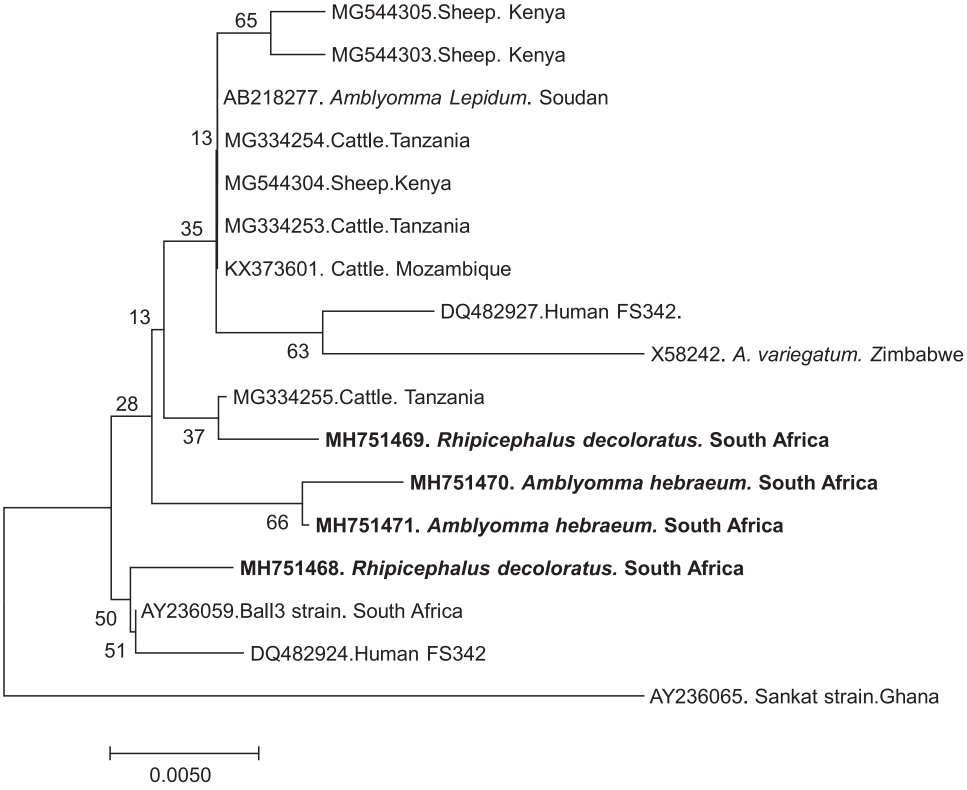

Fig. 5. Phylogenetic tree of *Ehrlichia ruminantium* detected in South African ticks based on partial pCS20 region nucleic acid sequences. The tree was constructed in MEGA ver.7 using neighbor-joining method. Sequences from this study are shown in bold font. The numbers at the nodes represent bootstrap values.

genetic diversity observed is in accordance with the presence of several *E. ruminantium* genotypes in South Africa and the occurrence of genetic recombination between stocks (Allsopp, 2010; Allsopp and Allsopp, 2007). This result suggests that several genotypes are circulating in the FS province and this should be taken into account while devising heartwater control strategies.

In conclusion, this study investigated the distribution and genetic diversity of important tick-borne pathogens and the tick species harboring them in three South African provinces. The findings enable a better understanding of tick-pathogen relationships and epidemiology of tick-borne pathogens and are expected to contribute to the development of ticks and tick-borne diseases control strategies.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.04.008.

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