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Genetic diversity of *Rickettsia africae* isolates from *Amblyomma hebraeum* and blood from cattle in the Eastern Cape province of South Africa

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Abstract

Rickettsia africae is a re-emerging tick-borne pathogen causing African tick bite fever (ATBF) in humans. *Amblyomma variegatum* is the principal vector in most sub-Sahara African countries, whereas in South Africa it is *A. hebraeum*. Reports of high genetic heterogeneity among *R. africae* isolates in southern Africa have prompted the need for molecular investigations of isolates form South Africa. Therefore, this study aimed to determine the prevalence and genetic diversity of *R. africae* in *A. hebraeum* collected from cattle, grazing pasture, as well as from blood of cattle in the Eastern Cape Province of South Africa. *Amblyomma hebraeum* and blood from cattle were screened by PCR and the *gltA*, *ompA*, *ompB*, *sca4*, and *17kDa* genes were sequenced for *R. africae* from samples collected from Caquba in Port St. Johns along the coastal region in the Eastern Cape province of South Africa. The overall proportion of adult *A. hebraeum* that were positive for the *gltA* and *ompA* genes was 0.63 (108/180). The overall proportion of nymphs positive for the *gltA* and *ompA* genes was 0.62 (23/37) and 0.22 (20/90) from cattle blood. A positive *R. africae* infection was inferred by analysis of 26 sequences of the *ompA*, *gltA*, *ompB*, *17kDa* and *sca4* genes. Neighbour-joining and Maximum Likelihood analysis revealed that the study isolates were closely related to *R. africae* isolates from South Africa deposited in GenBank, forming a clade that was separate from north, east and west African strains. This study provides new information on the epidemiology and phylogeny of *R. africae* isolated from *A. hebraeum* ticks in the Eastern Cape province of South Africa. The heterogeneity observed between *R. africae* isolates from South Africa deposited in GenBank and *R. africae* isolates from Africa retrieved from Genbank highlight the importance of differentiation and tracking of the genetic

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Conflict of interests The authors declare that they have no competing interests.

Ethical approval Ethical considerations were fulfilled by obtaining approval of the study from the Animal Research Ethics Committee, University of KwaZulu Natal (Ref: AREC/056/017).

movement among *R. africae* isolates in southern Africa for the better characterisation of ATBF cases, especially in rural communities and travellers visiting the region.

Keywords

African tick-bite fever; *Amblyomma hebraeum* ; *Rickettsia africae* ; Spotted fever; Travel medicine

Introduction

Rickettsia africae is an obligate, intracellular bacterium belonging to the spotted fever group *Rickettsiae* (Raoult 1997). It is the etiological agent of African tick bite fever (ATBF), an emerging infectious disease endemic to sub-Saharan Africa (Freedman et al. 2006). Since the first case report in 1992 in a patient from Zimbabwe, ATBF has been detected throughout eastern, western and central Africa, and several countries in the Caribbean region and linked to its main tick vector, *Amblyomma variegatum* (Mutai et al. 2013; Maina et al. 2014; Yssouf et al. 2014). In South Africa, the main tick vector is *A. hebraeum*, and this tick determines the geographical distribution of *R. africae* in the country.

Reports suggest that ATBF is not uncommon in international travellers; however, it is less documented in resource-poor livestock farmers in South Africa and other southern African countries. Most cases which have been reported present non-specific signs and symptoms that resemble influenza or malaria resulting in case misdiagnosis and delays in the appropriate treatment (Jensenius et al. 2003; Chandler et al. 2008). In addition, livestock and farming practices play a major role in the transmission dynamics of *R. africae* to humans as seen in reports from ticks and cattle with a prevalence of up to 92.6% (Mutai et al. 2013; Yssouf et al. 2014).

The ecological plasticity of the vectors *A. variegatum* and *A. hebraeum* has been shown to influence the distribution of ATBF. The typical landscape in which *A. hebraeum* is found are wooded and bushed grasslands with a preference for semi-arid and humid areas but this tick cannot able survive in open grasslands (Petney et al. 1987; Nyangiwe et al. 2011; Yawa et al. 2018).

Genetic diversity studies of *R. africae* have focused mainly on isolates from *A. variegatum*, with little to no information on isolates from *A. hebraeum* from most localities (Maina et al. 2014). In addition, single genes have been used to determine the genetic diversity of the isolates not allowing discrimination at the genus and species level (Thu et al. 2019). As a result, the multiple genes approach has been recently recommended using the citrate synthase (*gltA*), outer membrane protein A (*ompA*), outer membrane protein B (*ompB*) and surface cell antigen 4 (*sca4*) genes (Fournier et al. 2003). The *ompA* and *gltA* genes were used for initial screening as they had a higher heterogeneity and sensitivity compared to the *16S rDNA* gene as reported by previous studies (Robinson et al. 2009). Thereafter the *sca4*, *ompB* and *17kDa* genes were used to further classify the *Rickettsia* spp. detected. This study determined the genetic diversity of *R. africae* in *A. hebraeum* and in *R. africae*-infected cattle blood.

Materials and methods

Study sites

Sampling of ticks was conducted at two sites in the Eastern Cape Province of South Africa. The first site was situated in Lucingweni in Mthatha (GQW4 + 97; 31°27′14.7° S, 28°45′20.5° E) and the second site was situated in Caquba in Port St. Johns (9FG7 + WF; 31°37′21.7° S, 29°27′49.3° E) (Fig. 1). Lucingweni is located inland with very sparse vegetation cover, whereas Caquba consisted of thick vegetation including shrubs. Caquba is located on the coast with a livestock-wildlife interface having the ideal conditions to sustain *A. hebraeum* populations. The climate at each site consisted of mild and tropical conditions with an average temperature of 17.5 and 20.3 °C at Lucingweni and Caquba, respectively. At both sites, extensive rearing of cattle, sheep and goats is practiced, and farmers are frequently in direct contact with their animals as well as with the dense pastures grazed by livestock.

Blood collection

Whole blood was collected from randomly selected cattle of mixed breeds at each location during routine acaricide application from at least 15 out of a total of 40 cattle monthly for a period of 7 months. Whole blood samples were collected in 10-mL ethylenediamine tetraacetic acid (EDTA) treated tubes from the tail vein whilst the animal was restrained in a cattle crush. All blood samples were transported on ice and kept in a refrigerator at 4 °C in the School of Life Sciences Parasitology Laboratory (University of KwaZulu-Natal, Durban, South Africa) until further analysis.

Tick collection

Ticks were collected manually from cattle from predilection sites (dewlap, udder/scrotum, fore/hind legs, perineum, and tail) using blunt-nose forceps. Questing ticks were collected at two quadrants grazed frequently by cattle using the drag sampling method (Daniels et al. 1998). Ticks were preserved in McCartney bottles with 70% ethanol until morphological and molecular analyses.

All ticks were identified morphologically under a stereomicroscope based on keys as described by Walker et al. (2003) and only *A. hebraeum* ticks were used for further analyses. Individual ticks were sterilized on the surface with 70% ethanol and rehydrated in distilled water. Ticks were dissected longitudinally with a sterile blade into two equal parts. One half of the sample was placed into a microcentrifuge tube and homogenized using glass microbeads in a Disruptor Gene bead-beater (Scientific Industries, Bohemia, NY, USA) to facilitate the release of pathogens and protein digestion and nucleic acid extractions. The other half of the sample was stored at 4 °C until further analysis.

Isolation of DNA from ticks and blood

Genomic DNA was extracted from 100 μL of blood following a previously described protocol (Bereczky et al. 2005) and from half the dissected ticks using the standard protocol for the ZYMO Quick-DNA Miniprep Plus Kit. The standard protocol was modified by

incubating ticks overnight at 56 °C to allow complete deproteinization. Purified DNA was stored at 4 °C until further use.

Confirmation of tick DNA

To confirm that the *Rickettsia* detected in the DNA extracts was that from ticks and not from cattle from which the tick was collected, the tick-specific 16S rRNA gene was amplified as described previously (Macaluso et al. 2003).

Polymerase chain reaction (PCR)

A single-step PCR assay targeting a 1,234 bp and 540 bp fragment of the *gltA* and *ompA* genes was carried out to detect SFG *Rickettsia* (Table 1). PCRs were carried out in a final volume of 25 μL comprising of 12.5 μL of OneTaq Quick-Load 2X Master Mix with standard buffer (NEB, Hitchin, UK), 5 μL of template DNA, 2 μL of forward and reverse primer (100 μM), and 4.5 μL of sterile water in an Applied Biosystems *Veriti* 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Samples that were positive for the *gltA* and *ompA* genes were further characterised by amplifying the *ompB*, *17kDa*, *sca4*, and 16S rRNA genes (Table 1). To determine the expected fragment size, the PCR product was separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide.

Sequencing

PCR product purification and sequencing were performed on an ABI 3500XL genetic analyser at Inqaba Biotechnical Industries (Pretoria, South Africa) using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) according to the supplier's recommendations.

Phylogenetic analysis

Sequences obtained were aligned using the Clustal W algorithm and edited and trimmed using BioEdit v.7.0.9.0 (Hall 1999). The data set was converted into amino acids using MEGA v.7 to check for stop codons in the reading frame of the sequences (Kumar et al. 2016), the protein sequences were then used to guide the nucleotide alignment. The sequences were deposited into GenBank (Altschul et al. 1990) and the relevant accession numbers were retrieved (Table S1) (Benson et al. 2015).

Phylogenetic trees were constructed using the Neighbor-joining method in MEGA v.7 (Kumar et al. 2016) to elucidate the phylogenetic relationships between isolates from this study and the closest matches identified by homology searches downloaded from GenBank (Altschul et al. 1990). MEGA v.7 software was used to estimate the best substitution model for the Maximum Likelihood (ML) method. The ML tree was constructed using the Tamura-3-parameter model (T92) and ML tree values were transferred onto the Neighbor-joining tree.

Statistical analysis

Descriptive statistics were performed to summarize the tick and parasite prevalence data using Microsoft Excel (2016). Parameters included: number infected (NI) and total number

of ticks (N) from cattle and pastures. Prevalence (%) of tick species collected was calculated using the formula adapted from Thrusfield (1995): (total no. *A. hebraeum* infected with *R. africae*/total no. *A. hebraeum* screened for *R. africae*) × 100.

Results

Prevalence of tick species

Ticks were detected from two genera, *Amblyomma* and *Rhipicephalus*. In the *Amblyomma* genus, *A. hebraeum* was the only species detected. Prevalence in cattle was 56.5% (217/384) and from pastures it was 45.5% (293/645). In the *Rhipicephalus* genus, *R. micro-plus* and other unidentified *Rhipicephalus* spp. were detected. In cattle, a prevalence of 11.3% (43/384) was detected (Table S2) and 54.5% (352/645) from pastures. Cattle from Caquba were infested with adult and nymphal stages of *A. hebraeum* throughout the study period (Table 2).

Prevalence of *Rickettsia africae*

There was variation in the number of ticks screened for the presence of *R. africae* from both cattle and pasture which was dependent on the availability of ticks at the time of collection (Table 2). Prevalence in cattle blood was highest in the month of June 2019 (8/12; 66.7%). In August 2018 and January 2019 none of the blood samples screened were positive for infection. Of the 180 adult *A. hebraeum* collected from cattle, 107 (59.4%) were positive for *R. africae*, and out of a total of 37 nymphs collected from the same cattle, 23 (62.2%) were positive (Table 2). Nymphs were mostly abundant on cattle in August 2019 (a total of 20) and none was found during October 2018 and January 2019. From 12 nymphs collected from pastures, 5 (41.7%) were positive (Table 2.

Genetic characterization of isolates

In total 26 sequences were obtained and final sequence alignment lengths were trimmed for *ompA* (n = 9, 563 bp), *gltA* (n = 3, 946 bp), *ompB* (n = 5, 786 bp), *sca4* (n = 3, 1012 bp), *17kDa* (n = 3, 479 bp) and 16S rRNA (n = 3, 1004 bp) genes (Table S3). No stop codons were observed, and nucleotide sequences were subjected to a BLAST analysis for preliminary verification of their identity. All searches showed study sequences to be similar to those deposited in GenBank (Table 3) with sequence percent similarity in accordance with the guidelines of Fournier et al. (2003).

For the 16S rRNA gene, study samples collected from *A. hebraeum* adults from cattle (C1, C3 and C9) were found to be the most similar to a *R. conorii* isolate retrieved from GenBank with a similarity of 98.3%. This was the lowest similarity when compared to all other genes. For all other genes, isolates collected from *A. hebraeum* adults from cattle (C1, C3 and C9) were most similar to *R. africae* isolates retrieved from GenBank, with *sca4* having the lowest similarity (98.3–99.3%), followed by *glt*A (98.6–99.2%), *omp*B (98.7–99.8%), *omp*A (99.2–100%) and *17kDa* with 100% similarity across all sequences (Table 3). Based on the consensus across five genes (*glt*A, *omp*A, *omp*B, *sca4* and *17kDa*), sequences were identified as *R. africae* and all other positive samples were inferred to be *R. africae*.

Phylogenetic relationships between isolates

Rickettsia africae isolates from South Africa deposited in GenBank (C1, C3, C9, C12, C19, C20, C21 and BC1) formed a clade that was well supported by ML (65%) with *R. africae* isolates from the KwaZulu-Natal (MH751466.1) and Limpopo provinces (MG953294.1) retrieved from GenBank (Fig. 2). *Rickettsia africae* isolates from Kenya (AF548342.1) and Benin (KT633261.1) formed a strongly supported clade (94%), whereas *R. africae* isolates from the Carribean (GU247115.1), Madagascar (KJ645931.1) and east Africa (U83436.2) were found on the node of the tree with poor support. All isolates from the above regions except South Africa were isolated from *A. variegatum*. *Rickettsia africae* isolates from Egypt (HQ335137.1) and Lebanon (KY233233.1) formed a strongly supported clade (86%), and these were isolated from *Hyalomma* spp. From the *gltA* phylogenetic tree (Fig. S1), study isolates C1 and C9 from *A. hebraeum* formed a group that was supported by ML (65%) whereas sample C3 formed a monophyletic group with *R. africae* isolates from Africa that was poorly supported (Fig. 2). In the *ompB* tree (Fig. S2), study isolates C1 and R3 isolated from *A. hebraeum* and the rodent *Otomys irroratus* formed a group that was supported by ML (64%). *Rickettsia africae* isolates from Kenya (KF660532.1) and Sao Tome (MF667453.1) formed a group that was supported (55%); however, all other *R. africae* isolates from South Africa and Africa were situated on the node with no support.

Discussion

The presence of *A. hebraeum* in this study in the Eastern Cape province of South Africa lies within its reported geographical range, where it is present in the northern and eastern coastal regions of South Africa (Spickett 2013). Adult ticks were present throughout the study period in Caquba, and this was in accordance with previous studies where no clearly defined seasonal patterns were observed for the life cycle of *A. hebraeum* (Norval et al. 1991). However, the duration of this survey was too short to draw any inferences regarding the seasonality of *A. hebraeum* ticks from cattle and pastures.

The low nymph count from vegetation in our study is in accordance with previous studies that reported low counts of *A. variegatum* nymphs by drag sampling (Horak et al. 2011; Spickett et al. 1991). This is due to the difference in strategies of *Amblyomma* spp. from other ixodid ticks in which unfed nymphs prefer to quest from leaf litter in response to stimuli from hosts instead of questing on vegetation (Bryson et al. 2000; Horak et al. 2007).

This study detected a high prevalence of *R. africae* infection in *A. hebraeum* adults and nymphs from cattle in Caquba. Results are in agreement with those of other studies which have reported infection rates up to 92.6% in *A. variegatum* ticks from elsewhere in Africa (Mutai et al. 2013; Yssouf et al. 2014). Contrary to these findings, a previous study reported 20% prevalence of *R. africae* infection in *A. hebraeum* ticks, with *Rhiphichephalus* spp. having 30% prevalence (Mtshali et al. 2016). However, due to the scarcity of surveillance studies of *Rickettsia* spp. infection in *A. hebraeum* in South Africa, it is difficult to infer the significance of *R. africae* infections in our study to other regions of South Africa.

Prevalence of *R. africae* infection reported in this study was higher in *A. hebraeum* tissue samples than in blood from cattle. The low prevalence reported in this study could be an

indication that the infections were in the chronic phase where the rickettsemia was low (hence, low circulation/absence of the parasite in blood) or there could have been inhibitors which interfered with DNA extraction resulting in false negatives (Palmer et al. 1986; Tondella et al. 2002). A seroprevalence of 64.4% for *R. africae* from cattle was reported by Eisawi et al. (2017). This correlated with reports from the French West Indies where a seroprevalence of 55.3% for *R. africae* was reported in cattle (Parola et al. 1999). The wide distribution of *R. africae* with their respective tick vectors, mainly *A. variegatum*, is mainly attributed to the movement of the cattle host (Barré et al. 1995) and that immune cattle may serve as reservoirs of infection (Allsopp et al. 2004).

Phylogenetic analyses in this study showed that the *ompA* gene provided a strong intraspecific resolution among the various *R. africae* isolates compared to all other genes (Fig. 2). The formation of four major *R. africae* groups provides further support for the geographical heterogeneity within the *R. africae* lineages as previously reported (Kimita et al. 2016). A distinct cluster comprising of *R. africae* isolates from South Africa deposited in GenBank and *R. africae* isolates from South Africa retrieved from Genbank was observed which was separate from *R. africae* clusters from northern and western Africa, Antigua, Caribbean Islands and Madagascar retrieved from Genbank. This corresponds with the sequence alignment of *ompA* where a thymine (T) base pair substitution of *R. africae* isolates from Africa retrieved from GenBank had a cytosine (C) base pair at position 574 bp suggesting divergence of *R. africae* isolates from South Africa deposited in GenBank and *R. africae* isolates from Africa retrieved from GenBank (Table S3).

Sporadic reports in the Mediterranean showed a 4% prevalence of *R. africae* in *A. variegatum* from passerine birds further supporting this observation (Wallménius et al. 2014). In addition, the presence of isolates in St Kitts and Nevis and Antigua Islands is likely due to the translocation of infected cattle and/or ticks from Africa (Parola et al. 2005).

Phylogenetic trees based on the *gltA* and *ompB* genes resolved intraspecific variation among study samples (C1, C3 and C9) better than when based on *ompA*. In the *gltA* tree (Fig. S1), samples C1 and C9 from *A. hebraeum* ticks formed a clade that was supported (65%) in ML. In the *ompB* tree, *R. africae* isolate (R1) from *O. irroratus* tissue from this study formed a clade with *R. africae* sample C1 (64%) suggesting these samples are more closely related to each other than to *R. africae* sample C3. The observation of homogeneity among *R. africae* isolates from South Africa in this study and heterogeneity when compared to *R. africae* strains from Africa suggest a limited geographic range of *R. africae* in South African isolates. Results were not comparable across all genes as illustrated in the trees due to the paucity of *R. africae* isolates from Africa from GenBank and short sequence reads. As reported previously, PCR failure is common among the variable genes (*ompA*, *ompB* and *sca4*) for rickettsial classification (Nakao et al. 2013). This is attributed to nucleotide mismatches in the primer annealing sites and this results in poor sequence reads which result in incorrect phylogenetic inferences being made on isolates (Thu et al. 2019).

Conclusion

This study demonstrates that the use of a multiple gene approach to characterize *Rickettsia* spp. is necessary and that single gene trees can confound the true relationship between isolates. A 62% prevalence of *R. africae* infection provides one of the first epidemiological surveillance studies of *R. africae* from *A. hebraeum* from cattle in South Africa. The significance of these findings is important in the differentiation of *R. africae* strains, to allow the tracking of origin of strains and provide targeted treatments. This, paralleled with the growth in tourist visits to game reserves in South Africa, has led to *R. africae* being regarded as the most widely distributed of all human pathogenic SFG rickettsiae. Therefore, it is recommended that extensive epidemiological surveillance of *R. africae* is conducted in South Africa.

Additionally, the low genetic diversity shown by the *ompA, ompB* and *sca4* genes amongst study isolates indicates the need for new primers to be designed to cover longer regions of sequences to further characterise *R. africae* isolates throughout South Africa. Furthermore, it is recommended that patients displaying febrile illnesses should be routinely screened to accurately assess the incidence of *R. africae* in humans. Finally, studies should combine each member of the host, vector, and pathogen system to understand the combined effect of the evolutionary processes linking different species. This includes the screening of domestic and wild rodents for *R. africae*. Consideration of *A. hebraeum* dispersal and population dynamics in future population genetic studies will provide insights into the evolutionary forces driving species distribution, gene flow and host adaptations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

The datasets supporting the findings of this article are included within the article and its additional files. The sequences generated in this study for *R.africae* for the 16SrRNA gene were deposited in the GenBank database under the accession numbers MN988998-MN989000. The sequences for *Rickettsia africae* for the *ompA* gene were deposited under the accession numbers MN972462, MT009455 and MT009349-MT009354. The sequences for *Rickettsia africae* for the *ompB* gene were deposited under the accession numbers MT150897-MT150899. The sequences for *Rickettsia africae* for the *gltA* gene were deposited under the accession numbers MT150894-MT150896. The sequences for *Rickettsia africae* for the *sca4* gene were deposited under the accession numbers MT150900-

MT150902. The sequences for *Rickettsia africae* for the *17kDa* gene were deposited under the accession numbers MT150903-MT150905.

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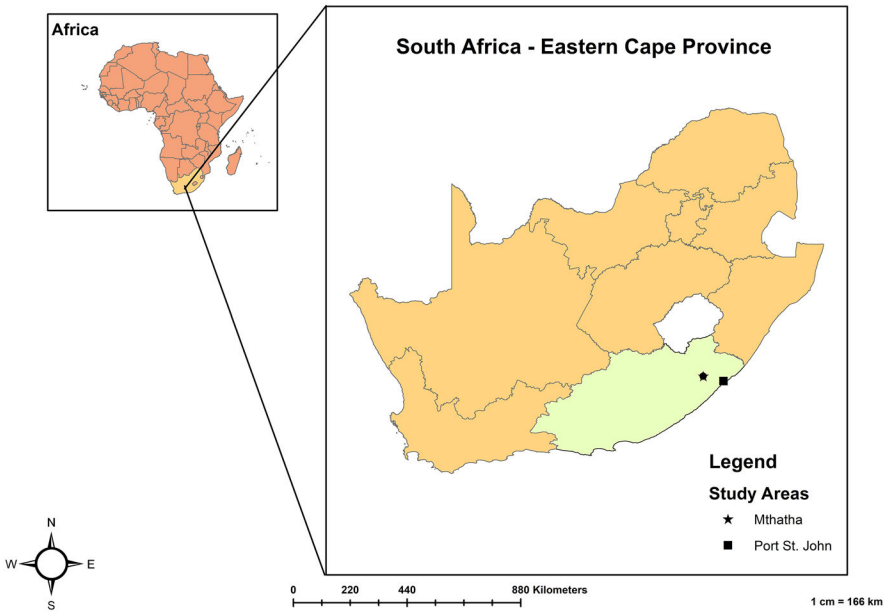

Fig. 1.

Location of sample collection sites (Mthatha and Port St Johns) in the Eastern Cape province, South Africa

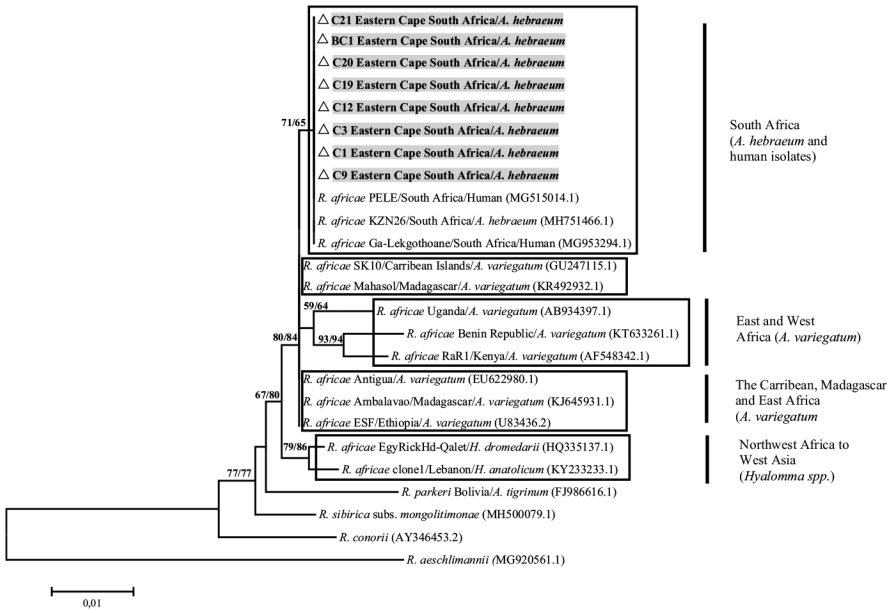

Fig. 2.

Neighbour joining phylogenetic tree based on the *ompA* gene (563 bp) of *Rickettsia africae* strains from Genbank and study samples from the Eastern Cape, South Africa (T92 + G model. Bootstrap values indicated show NJ/ML values. Taxon labels show the species name strain/origin/source of isolate. Isolates shaded in grey are from this study

Table 1

Primers used for the characterisation of *Rickettsia* spp

Gene	Primer name	PCR	Size (bp)	Sequences (5′–3′)
gltA	gltAF	Single	1234	ACCTATACTTAAAGCAAGTATYGGT
	gltAR			TCTAGGTCTGCTGATTTTTTGTTCA
ompA	ompAF	Single	540	ATGGCGAATATTTCTCCAAAA
	ompAR			GTTCCGTTAATGGCAGCATCT
17kDa	17kDaF	Semi-nested	450	AATGAGTTTTATACTTTACAAAATTCTAAAAACCA
	17kDaR			CATTGTTCGTCAGGTTGGCG
	17kDaF2			GCTCTTGCAACTTCTATGTT
16S rRNA	16S1	Single	1482	TAAGGAGGTAATCCAGCC
	16S-1			CCTGGCTCAGAACGAA
Sca4	Sca4R	Semi-nested	2700	ATGAGTAAAGACGGTAACCT
	Sca4F			TCAGCGTTGTGGAGGGGAAG
	Sca4R			TTCAGTAGAAGATTTAGT
ompB	ompBF	Semi-nested	444	ACATKGTTATACARAGTGYTAATGC
	ompBR			CCGTCATTTCCAATAACTAACTC
	ompBR2			SGTTAACTTKACCGYTTATAACTGT

Table 2

Number of *Ambyomma hebraeum* nymphs and/or adults (from cattle and pasture) and cattle blood samples screened (N) and infected (NI) by PCR using the *gltA* and *ompA* genes for *Rickettsiae africae* in Caquba in the Eastern Cape province of South Africa

Month of collection	Prevalence in cattle blood	Prevalence in *A. hebraeum* from cattle		Prevalence in *A. hebraeum* from pastures
	N (NI)	Nymphs N (NI)	Adults N (NI)	Nymphs N (NI)
July 2018	21 (4)	8 (6)	17 (8)	0
Aug 2018	12 (0)	20 (11)	43 (26)	0
Sept 2018	14 (3)	2 (0)	25 (23)	7 (2)
Oct 2018	9 (3)	0	35 (10)	5 (3)
Jan 2019	15 (0)	0	26 (15)	0
March 2019	7 (2)	6 (5)	29 (21)	0
June 2019	12 (8)	1 (1)	5 (4)	0
Total	90 (20)	37 (23)	180 (107)	12 (5)

Table 3

Characterisation of *Rickettsia* spp. isolates from *Ambyomma hebraeum* and blood from cattle, plus guidelines for minimum similarity (%) by Fournier et al. (2003)

Sample ID	**% identity to closest *Rickettsia* spp. (i.e., *R. africae* in all instances) by BLAST search (GenBank accession number)**				
	gltA	***ompA***	***ompB***	***Sca4***	***17kDa***
Minimum % similarity (≥)	99.9	98.8	99.2	99.3	No guidelines for this gene
C1	99.20 (CP001612.1)	99.82 (MH751466.1)	98.69 (KU721071.1)	99.62 (AF151724.2)	100 (CP001612.1)
C3	99.02 (CP001612.1)	100 (MH751466.1)	99.52 (KU721071.1)	98.31 (CP001612.1)	100 (CP001612.1)
C9	98.58 (CP001612.1)	100 (MH751466.1)	99.52 (KU721071.1)	98.31 (CP001612.1)	100 (CP001612.1)
BC1	–	99.81 (MH751466.1)	–	–	–
C12	–	99.49 (MH751466.1)	–	–	–
C19	–	99.66 (MH751466.1)	–	–	–
C20	–	99.16 (MH751466.1)	–	–	–
C21	–	98.99 (MH751466.1)	–	–	–
R1	–	–	99.75 (KU721071.1)	–	–
R3	–	–	99.75 (KU721071)	–	–

‘–’ No sequencing results were obtained for this sample