

# High Prevalence of *Rickettsia africae* Variants in *Amblyomma variegatum* Ticks from Domestic Mammals in Rural Western Kenya: Implications for Human Health

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## Abstract

Tick-borne spotted fever group (SFG) rickettsioses are emerging human diseases caused by obligate intracellular Gram-negative bacteria of the genus *Rickettsia*. Despite being important causes of systemic febrile illnesses in travelers returning from sub-Saharan Africa, little is known about the reservoir hosts of these pathogens. We conducted surveys for rickettsiae in domestic animals and ticks in a rural setting in western Kenya. Of the 100 serum specimens tested from each species of domestic ruminant 43% of goats, 23% of sheep, and 1% of cattle had immunoglobulin G (IgG) antibodies to the SFG rickettsiae. None of these sera were positive for IgG against typhus group rickettsiae. We detected *Rickettsia africae*-genotype DNA in 92.6% of adult *Amblyomma variegatum* ticks collected from domestic ruminants, but found no evidence of the pathogen in blood specimens from cattle, goats, or sheep. Sequencing of a subset of 21 rickettsia-positive ticks revealed *R. africae* variants in 95.2% (20/21) of ticks tested. Our findings show a high prevalence of *R. africae* variants in *A. variegatum* ticks in western Kenya, which may represent a low disease risk for humans. This may provide a possible explanation for the lack of African tick-bite fever cases among febrile patients in Kenya.

**Key Words:** *Amblyomma variegatum*—*Rickettsia africae*—Tick-borne spotted fever group—African tick-bite fever.

## Introduction

THE SPOTTED FEVER GROUP (SFG) *Rickettsia* comprises a diverse group of pathogenic bacteria that are largely known for causing spotted fevers in humans. In sub-Saharan Africa, the main pathogenic SFG rickettsiae include *R. conorii*, *R. africae*, *R. aeschlimannii*, *R. sibirica*, and *R. massiliae* (Parola 2006). SFG rickettsioses are second only to malaria as the most commonly diagnosed diseases in travelers returning from sub-Saharan Africa with systemic febrile illness (Freedman et al. 2006). African tick-bite fever (ATBF), caused by infection with *R. africae*, is the most prevalent tick-

borne rickettsiosis in sub-Saharan Africa. It is frequently diagnosed in travelers returning from rural sub-Saharan Africa (Fournier et al. 1998a, Jensenius et al. 2002, Jensenius et al. 2003b, Roch et al. 2008, Jensenius et al. 2009, Althaus et al. 2010), with attack rates of 100% in some outbreaks (Oostvogel et al. 2007). *R. africae* was identified as the cause of nearly all cases of tick-bite rickettsiosis in 376 patients who became ill after visiting sub-Saharan Africa (Raoult et al. 2001).

ATBF is an acute febrile illness characterized by headaches, myalgia, regional lymphadenitis, and inoculation eschars typically present in multiple sites (Jensenius et al. 2003a).

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Although usually considered a benign condition, ATBF may be complicated by severe neuropathy (Jacquemard et al. 1998, Jensenius et al. 2006, Auce et al. 2011) or acute myocarditis (Bellini et al. 2005). Elderly patients seem to be prone to more severe clinical manifestations (Roch et al. 2008).

*R. africae* is transmitted by ticks of the genus *Amblyomma* (Kelly et al. 1994, Kelly et al. 1996). Two *Amblyomma* tick species, *A. hebraeum* in southern Africa and *A. variegatum* elsewhere in sub-Saharan Africa, are recognized as the principal reservoir hosts and vectors of *R. africae* (Jensenius et al. 2003a). *R. africae* has been detected in these ticks by PCR across much of their range (Mediannikov et al. 2010a), extending as far as the West Indies where *A. variegatum* was introduced in the 1800s (Kelly 2006). Infection is maintained through transovarial and transstadial transmission in both *A. hebraeum* (Kelly and Mason 1991) and *A. variegatum* (Socolovschi et al. 2009), and as a result all life stages of the ticks may transmit infection to humans. The rates of infection in these ticks in endemic areas are typically high and may reach 100% (Parola et al. 2001, Nakao et al. 2013).

Adults of *A. hebraeum* and *A. variegatum* prefer large hosts such as cattle and large wild ruminants, although they also infest sheep and goats. The immature stages (larvae and nymphs) have a wider host-range, feeding on the same hosts as adults do, as well as on smaller mammals, birds, and reptiles (Walker et al. 2003). These nidifugous (exophilic) ticks display active host-finding behavior, with nymphs and adults in particular attacking hosts in an aggressive manner (Kelly and Mason 1991, Sone shine 1993). These ticks are known to attack humans, usually on the legs with attachment often behind the knee, in the groin, or in the axilla (Jensenius et al. 2003a).

Among domestic livestock, cattle serve as important hosts for both *A. hebraeum* and *A. variegatum*, and there is some evidence that they may play a role in the maintenance of human pathogenic rickettsiae (Kelly et al. 1991). A high proportion (80–100%) of cattle in endemic areas have serological evidence of exposure to SFG rickettsiae (Kelly et al. 1991, Parola et al. 1999, Kelly et al. 2010), and rickettsemia was detectable for at least 32 days following experimental infection of cattle (Kelly et al. 1991). High seroprevalence (87%) has also been reported in goats (Parola et al. 1999), although in another study seroprevalence rates in the three common domestic ruminants (cattle, goats, and sheep) reflected the host preference of the vector *A. variegatum*, with the highest prevalence in cattle and much lower prevalence in goats and sheep (Kelly et al. 2010).

In contrast to the number of reported cases of ATBF in travelers, reports of the disease in indigenous populations in affected areas of sub-Saharan Africa remain scarce. This is despite high seroprevalence to SFG rickettsiae in these populations: (16–55%) in eastern and southern Africa and (5–51%) in western and central Africa (Mediannikov et al. 2010b). In the coastal region of Cameroon, *R. africae* was identified in 6% of patients with acute febrile illness not caused by malaria or typhoid fever (Ndip et al. 2004). A study in rural Senegal found rickettsial DNA in a similar percentage (7%) of 134 febrile, nonmalarial patients (Mediannikov et al. 2010b); *R. felis* and *R. conorii*, and not *R. africae*, were identified.

In East Africa, *R. africae* was previously detected in 16% of adult *A. variegatum* ticks in southwestern Kenya (Macaluso et al. 2003). A fatal case of spotted fever rickettsiosis in

an expatriate in Kenya was attributed to *R. conorii* infection, the causative agent of Mediterranean spotted fever (MSF), on the basis of the severity of the disease (Rutherford et al. 2004), and a laboratory-confirmed case of MSF occurred in a Japanese traveler to Kenya (Yoshikawa et al. 2005). More recently, *R. felis*, the agent of flea-borne spotted fever, was detected in febrile patients in northeastern (Richards et al. 2010) and western (Maina et al. 2012) Kenya. We conducted serosurveillance for immunoglobulin G (IgG) antibodies to SFG and typhus group (TG) rickettsiae in domestic ruminants and surveyed for *R. africae* in *Amblyomma* ticks of domestic ruminants and ixodid ticks found on domestic dogs in a rural setting in western Kenya.

## Materials and Methods

### Study site

The study was conducted in the Asembo division of Rarieda (formerly Bondo) subcounty of Siaya County in western Kenya, in 2008–2010. This rural site on the eastern shore of Lake Victoria falls within a health and demographic surveillance system (HDSS) run by the Kenya Medical Research Institute (KEMRI) and US Centers for Disease Control and Prevention (CDC). The HDSS collects household demographic and socioeconomic data three times per year and includes information on the number of livestock owned (Adazu et al. 2005). The population undertakes agricultural production through a traditional mixed crop–livestock system and fishing. In this area, 44% of households own cattle (mean number owned was 1.84) and 43% own at least one sheep or goat (mean number owned was 2.12 [KEMRI/CDC HDSS data for 2008, unpublished]).

### Tick collection and domestic animals sampling

The Kenyan International Emerging Infections Program of KEMRI/CDC has conducted human population-based infectious disease surveillance (PBIDS) in Asembo since late 2005, with between 23,500 and 25,000 people under surveillance in 33 villages within the HDSS (Feikin et al. 2011). As part of a study on zoonotic diseases in the site, 300 livestock-owning compounds (LOC) were randomly selected from all known livestock-owning compounds in the 33 PBIDS villages, with the sampling frame of LOCs compiled from livestock census data collected by the HDSS. Samples were collected within a 5-km radius of Lwak hospital, the designated referral health facility for Asembo site located at –0.171388 South and 34.391388 East. Domestic ruminants (cattle, goats, and sheep) and dogs were sampled from January through May of 2009. Where present, cattle, goats, sheep, and dogs were randomly selected (up to a maximum of three per LOC) and sampled for ticks, by examining specific body sites known to harbor ticks (ear, dewlap, shoulder, belly, groin, udder, and perineum) and collecting one to two nonengorged adults of different genera per site. Ticks, pooled by individual host animal (one to six ticks per pool), were preserved in 70% ethanol and stored at –80°C.

*Amblyomma* ticks from domestic ruminants and all ticks obtained from dogs were subsequently retrieved for identification and testing. Identification was based on entomological keys by Walker et al. (2003), after which ticks were pooled based on species and host animal. After identification, ticks

were washed in molecular-grade water and mechanically disrupted using a bead mill (Qiagen TissueLyser LT). Genomic DNA was extracted using QIAamp blood and tissue kits (Qiagen, Valencia, CA) according to the manufacturer's instruction, using a final elution volume of 100  $\mu$ L.

Blood specimens from cattle, goats, and sheep were collected by venipuncture of the jugular vein into plain vacutainers for serum preparation and EDTA vacutainers for anti-coagulated blood (VWR International, West Chester, PA). Blood collection tubes were kept upright at ambient temperature for 15–30 min and then transported to the field laboratory on ice. The EDTA and the plain vacutainers were centrifuged to obtain the white cell fraction (buffy coat) and serum, respectively. DNA was extracted from buffy coat specimens using Qiagen blood and tissue kit (Qiagen), as per the manufacturer's instructions. Testing of samples was conducted as summarized in Figure 1.

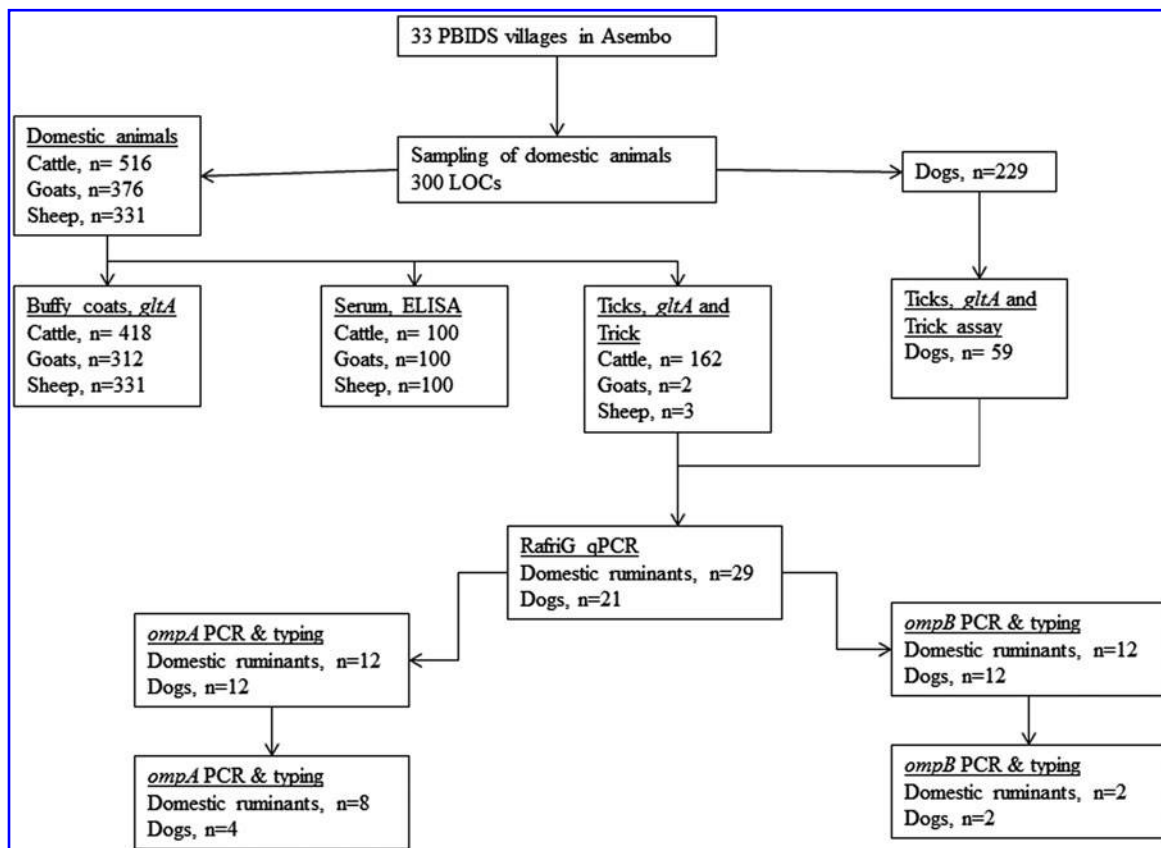
#### Serosurveillance for IgG antibodies

To assess previous exposure to rickettsiae, we examined a randomly selected subset of cattle, goat, and sheep sera (100 sera from each species) for SFG and TG rickettsiae-specific IgG. Serum specimens were screened at a dilution of 1:100 for IgG against SFG and TG rickettsiae, using an enzyme-linked immunosorbent assay (ELISA) as described by Graf et al. (2008). End point titers were determined for all specimens that were positive on screening.

#### Quantitative Real-time PCR assays

A quantitative real-time PCR (qPCR) assay that amplifies and detects a 74-bp segment of the citrate synthase (*gltA*) gene (Stenos et al. 2005) was used to screen nucleic acid preparations of blood samples from domestic ruminants and ticks for the presence of rickettsial DNA. Rickettsial DNA from ticks were further characterized using a tick-borne rickettsia-specific qPCR assay (Trick assay; Jiang et al. 2005a) that amplifies and detects a 128-bp fragment of the outer membrane protein B gene (*ompB*), and an *R. africae* (RafriG assay) genotype-specific qPCR assay using primers Raf1797F (TTGGAGCTAATAATAAACTCTTGAC), Raf1915R (GAATTGTACTGCACCGTTATTTCC), and Raf1879P (6-FAM-CGCGATGTTAATAGCAACATCACC GCCACTATCGCG-BHQ-1) that amplify and detect a 119-bp segment of the *ompB* gene.

The RafriG assay was performed on a Smart Cycler (Cepheid, Sunnyvale, CA) with the following cycling conditions: First hold at 50°C for 2 min, second hold at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 15 s and annealing and extension at 62°C for 30 s. Evaluation of the RafriG against a panel of closely related rickettsial ( $n=17$ ) and nonrelated bacterial ( $n=12$ ) DNA preparations (Jiang et al. 2012) showed the assay to be specific for the *R. africae* genotype (data not shown). Plasmid positive controls constructed with target sequences from Trick and RafriG assays were used at  $10^3$  copies per reaction. Molecular-grade water



**FIG. 1.** Framework for surveillance for rickettsiae in animals and ticks in Asembo collected in 2009 within a 5-km radius of Lwak hospital, located at  $-0.171388$  South and  $34.391388$  East. PBIDS, population-based infectious disease surveillance; ELISA, enzyme-linked immunosorbent assay.

was included at every sixth sample as an extraction control, and two negative controls (molecular-grade water) were included in every qPCR run. All negative controls were consistently negative.

#### Molecular identification of rickettsiae

To confirm the identity of the rickettsiae present in the ticks, standard and nested PCR together with sequencing of the specific rickettsial outer membrane protein genes *ompA* and *ompB* were done. Two segments of the *ompA* gene were amplified. The first segment used primers RompA1F (Jiang et al. 2005b) and Rr190.5044R (Fournier et al. 1998b), and the second segment targeted the 5' end of the *ompA* and used primers Rr190.70F and Rr190.701R, which amplify a 632-bp fragment (Fournier et al. 1998b). Molecular confirmation was also performed using the *ompB* gene with primers 120.607F (Roux and Raoult 2000) and ompB1902R (Jiang et al. 2005b). To further elucidate the extent of variability of the *R. africae* in the ticks, PCR and sequencing of a longer fragment of *ompB* was conducted in a subset of tick DNA preparations (four of 21) using previously reported primers (Table 1). The PCR products were detected using GelRed (Biotium, Inc, Hayward, CA) on 1% agarose gels.

Nested PCR products from the *ompA* and *ompB* assays were purified using QIAquick gel purification kit (Qiagen). A Big Dye Terminator v 3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) was used in subsequent sequencing reactions according to the manufacturer's instructions. Sequencing products were purified using Performa DTR Gel Filtration Cartridges (EdgeBiosystems, Gaithersburg, MD). Sequencing was done in ABI 3500 Genetic Analyzer (Applied Biosystems). The primers used for sequencing were the same as those for PCR. At least two reactions were done for each strand of DNA. Long contigs were generated using primers shown in Table 1 as well as RhoA4336F (Jiang et al. 2005b) for *ompA* to enable assemblage of a complete consensus. Sequences obtained in this study were aligned against *Rickettsia* sequences selected from different geographical regions, sourced from GenBank. Phylogenetic analyses were conducted using MEGA version 5 software, and BLAST searches

were managed on the National Center for Biotechnology Information website (<http://blast.ncbi.nlm.nih.gov>).

#### GenBank accession numbers

The sequences of the *R. africae* variants detected in the *A. variegatum* ticks have been deposited in GenBank with accession numbers KF660530, KF660531, KF660532, KF660533, KF660534, and KF660535.

#### Ethical review

The collection of specimens from animals was approved by the KEMRI Ethical Review Committee and CDC Animal Care and Use Committees (KEMRI SSC 1191 and 1562BRE-TBDX, respectively).

#### Results

Overall, 516 cattle, 376 goats, 331 sheep, and 229 dogs were examined in the 300 LOCs for the presence of ticks. Of these, 418 cattle, 312 goats, and 331 sheep had a serum and buffy coat specimen collected to look for evidence of rickettsial infection. Of the sera collected, a subset of 100 specimens each from cattle, sheep, and goats were randomly selected and tested for the presence of SFG- and TG-specific IgG; 1% of cattle, 43% of goat and 23% of sheep sera had IgG antibodies against SFG rickettsiae with end point titers ranging from 400 to 6400 for goats, 400 to 1600 for sheep, and < 100 for cattle. None of them were positive for antibodies against TG rickettsiae. In addition, none of the 418 cattle, 312 goats, and 331 sheep buffy coat specimens tested positive with the pan rickettsia qPCR screening assay.

For ticks collected from domestic ruminants, only *A. variegatum* ticks were assessed for the presence of rickettsiae. A total of 162 cattle (31.4%), two goats (0.5%), and three sheep (0.9%) carried *A. variegatum* ticks. In total, 263 adult *A. variegatum* were collected and pooled (one to five *A. variegatum*) by individual host (167 pools). DNA preparations were obtained from 162 tick pools of *A. variegatum* ticks from cattle, of which 79 (48.8%) consisted of a single individual tick. Of the 162 pools, 160 (98.8%) were positive for rickettsial DNA using the *gltA* pan-rickettsia qPCR assay whereas 150 (92.6%) were positive using the tick-borne rickettsiae-specific Trick assay. All of the Trick-positive pools selected for further testing were confirmed positive for the *R. africae* genotype using the RafriG qPCR assay. All *A. variegatum* ticks collected from goats and sheep (single tick per animal) were positive with the three assays. A total of 102 ticks were collected from 36 dogs, resulting in 59 pools of over five species of ticks summarized in Table 2.

#### Confirmation of rickettsiae in ticks by sequencing of *ompA* and *ompB* genes

Rickettsial DNA was amplified in 21/24 tick DNA preparations tested by PCR targeting *ompA* and *ompB*. The tick DNA preparations were from sheep ( $n=1$ ), cattle ( $n=11$ ), and dogs ( $n=12$ ). A 1364-bp sequence was obtained after assembling the sequences of the *ompA* (Romp1F, 190-5044R, RhoA4336F, and RompA4433R). BLAST searches showed that this sequence, obtained from a tick collected from a sheep (tick 25) was 100% identical with that of *R. africae* ESF-5 (accession no. CP001612) and *R. africae* ESF

TABLE 1. PRIMER USED FOR PCR AMPLIFICATION AND SEQUENCING THE LONG FRAGMENT OF THE *ompB* GENE

Fragment	Primer	Reference
I	M59F	Roux and Raoult 2000
	ompB1570R	Jiang et al. 2013
	120-607F <sup>a</sup>	Roux and Raoult 2000
	120-807R <sup>a</sup>	Roux and Raoult 2000
II	ompB1595F	Blair et al. 2004
	RompB3008R	Jiang et al. 2005b
	RompB2012F <sup>a</sup>	Jiang et al. 2005b
	RompB2553R <sup>a</sup>	Jiang et al. 2005b
III	120-2788F	Roux and Raoult 2000
	RompB4887R	Blair et al. 2004
	RompB3521F <sup>a</sup>	Jiang et al. 2005b
	RompB3637R <sup>a</sup>	Jiang et al. 2005b
	RompB4224F <sup>a</sup>	Jiang et al. 2005b

<sup>a</sup>Primers used for sequencing only.

TABLE 2. TICK SPECIES OBTAINED FROM DOGS IN ASEMBO

Tick species	No. positive with <i>gltA</i> , <i>Trick</i> , and <i>RafriG</i> assay	Total no. of ticks tested/pool
<i>Amblyomma variegatum</i>	6	10
<i>Rhipicephalus sanguineus</i>	0	15
<i>Rhipicephalus appendiculatus</i>	1	9
<i>Haemaphysalis leachi</i>	1	10
<i>Rhipicephalus decoloratus</i>	1	5
Unspecified <i>Rhipicephalus</i> spp.	3	10

2500 (accession no. U83436), whereas the sequences of the rest of the rickettsiae from tick DNA samples were 99% identical with *R. africana* ESF-5. The *ompA* sequences in this fragment contained a deletion in 6/21 specimens and a 4-bp deletion in 1/21 (tick 214) DNA extracts. Sequencing of the 5' end of the *ompA* gene that used primers Rr190.70F and Rr190.701R resulted in a 540- to 747-bp sequence summarized in Table 3. *Rickettsia africana*, *Rickettsia* sp. AvR4/Kenya, and some uncultured *Rickettsia* spp. were detected in the ticks. Unique among these sequences was a 173-bp insertion in four of 12 samples (tick 16, tick 56, tick 74, tick 226) assessed (Fig. 2). The insertion sequence was identical in all of the four *ompA* sequences except that the sequence of one tick extract (tick 74) had a 5-bp deletion.

A 1265-bp amplicon was produced using *ompB* nested PCR in 21 of 24 of the *gltA*-, *Trick*-, and *RafriG*-positive samples. A 1200-bp sequence was obtained after assembling the sequences of the *ompB* (Romp607F, RompB1007F, RompB1452R, and RompB1902R). BLAST searches showed that this sequence had 100% and 99% homology with *R. africana* ESF-5 (accession no. CP001612) in one of 21 (tick 25) and 20 of 21 DNA preparations, respectively (Fig. 3).

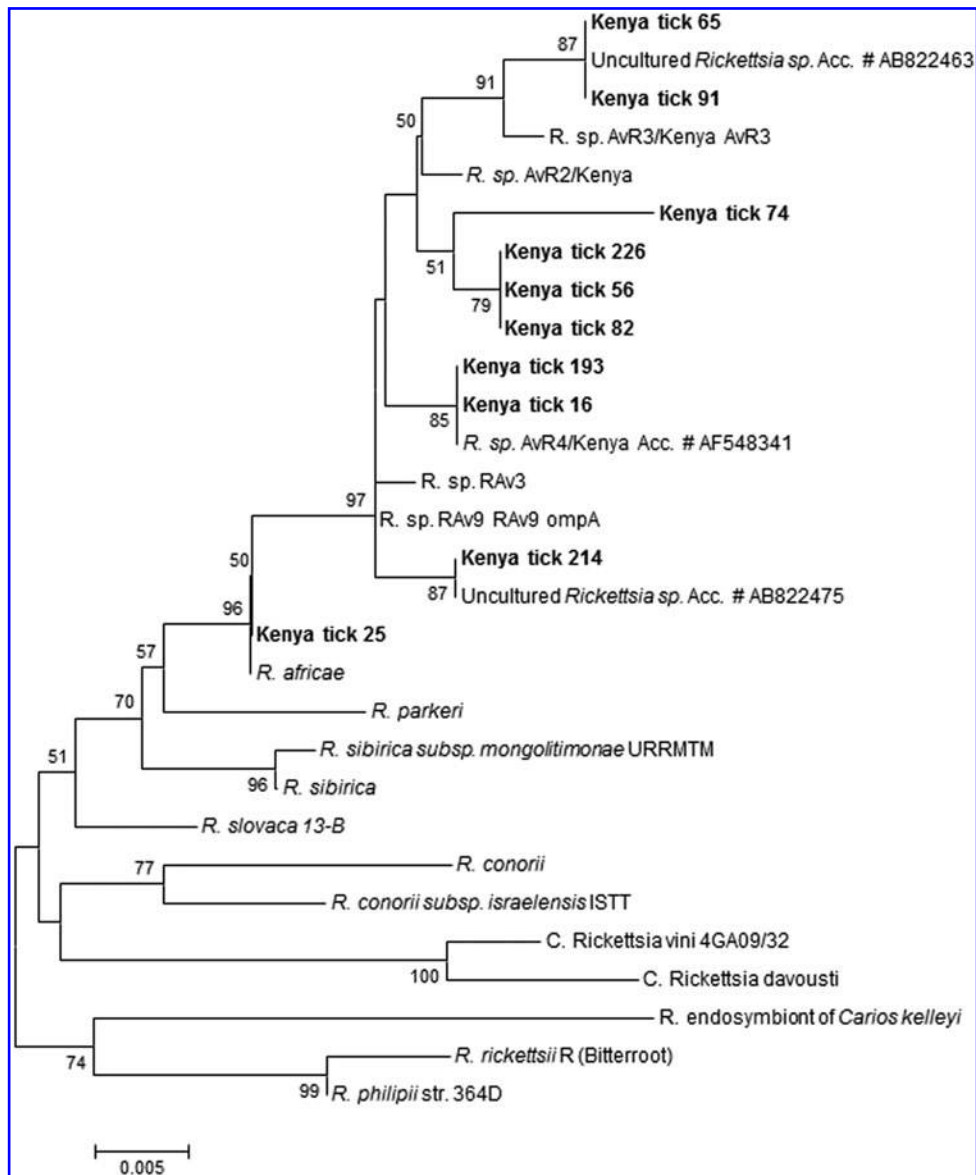
Four tick DNA preparations were selected for sequencing of a longer fragment of the *ompB* gene. A 4400- to 4867-bp sequence was generated in which the sequence from one of the four ticks (tick 25) was confirmed to be 100% identical to *R. africana* ESF-5 (accession no. CP001612). The other sequences (from tick DNA preparations 74, 214, and 226) had 99% homology with *R. africana* ESF-5. These sequences had a 4-base deletion at exactly the same site (Table 4) and 32–36 nucleotide substitutions. One sequence (tick 74) had an extra nucleotide deletion in a different site of its *ompB* gene.

**Discussion**

This report corroborates that of Macaluso et al. (2003, who found *R. africana* variants in ticks from Kenya. Diversity has also been reported among variants in *Amblyomma laculosum* ticks obtained from humans and a bird in New Caledonia (Eldin et al. 2011). Although the present study was conducted to determine the prevalence of *R. africana* in *A. variegatum* ticks of domestic animals, all tick species obtained from dogs were tested owing to their small numbers. *R. africana* variants have previously been detected in *A. variegatum* ticks collected in various countries in Africa, namely Niger, Mali, Sudan, Burundi, and Mauritania (Parola et al. 2001). On the basis of the nucleotide sequences of the *ompA* gene, two of 10

TABLE 3. THE SEQUENCE OF THE RICKETTSIAL OUTER MEMBRANE PROTEIN A GENE (*ompA*) FRAGMENT FOR TICKS IN WESTERN KENYA AND OTHER PUBLISHED RICKETTSIA AFRICAE VARIANTS

Tick DNA no.	Ticks/pool	Tick species	Sex	Host	% identity	Sequence length	Accession no.	<i>R. africana</i> CP001612	Insertion sequence
16	1	<i>A. variegatum</i>	Male	Cattle	100% <i>Rickettsia</i> sp. AvR4/Kenya	580 bp	AF548341	99% (575/580)	—
193	1	<i>H. leachi</i>	Male	Dog		564 bp		99% (559/564)	—
25	1	<i>V. variegatum</i>	Male	Sheep	100% <i>R. africana</i>	541 bp	CP001612	—	—
65	1	<i>V. variegatum</i>	Male	Cattle	100% uncultured <i>Rickettsia</i> sp. DF4 1	570 bp	AB822463	99% (561/570)	—
91	1	<i>V. variegatum</i>	Male	Cattle		570 bp		98% (554/563)	—
74	1	<i>V. variegatum</i>	Male	Cattle	99% <i>R. africana</i>	662 bp	CP001612	—	173 bp
214	1	<i>V. variegatum</i>	Male	Dog	100% uncultured <i>Rickettsia</i> sp. SM8 1	571 bp	AB822475	99% (565/571)	—
56	1	<i>V. variegatum</i>	Male	Cattle	99% <i>R. africana</i>	747 bp	CP001612	—	173 bp
82	1	<i>V. variegatum</i>	Male	Cattle	99% <i>Rickettsia</i> sp. AvR4/Kenya	654 bp	AF548341	—	173 bp
226	1	<i>V. variegatum</i>	Female	Dog	99% uncultured <i>Rickettsia</i> sp. SM8 1	739 bp	AB822475	—	173 bp
					99% uncultured <i>Rickettsia</i> sp. DF4 1		AB822463		



**FIG. 2.** *ompA* partial gene sequences of rickettsiae detected in ticks obtained from domestic animals in western Kenya. The tree was inferred using the neighbor-joining method Bootstrap (1000 replicates) involving rickettsiae detected in this study and strains from GenBank.

sequences were identical to *Rickettsia* sp. AvR/4 Kenya (GenBank accession no. AF548341) that was detected in *A. variegatum* from Maasai Mara, Kenya (Macaluso et al. 2003). Uncultured *Rickettsia* spp. (GenBank accession nos. AB822463 and AB822475) (Nakao et al. 2013) were detected in three of 10 samples analyzed. However, *ompA* sequences of four of 10 tick DNA preparations were unique in that they had a 173-bp nucleotide insertion and a 5-nucleotide deletion in one of the sequences. In addition to nucleotide substitution, we report for the first time existence of a deletion mutation in the *ompA* and *ompB* genes of some *R. africae* variants. The percent divergence of the *ompA* and *ompB* sequences in the *R. africae* variants to the next closest accepted *Rickettsia* species, *R. africae*, was less than that required to define a new species (Fournier et al. 2003). Thus, we refer to these molecular isolates as *R. africae* variants as previously reported (Parola et al. 2001).

The variants described in this study have not been reported to be associated with ATBF, and we hypothesize that, *A. variegatum* infected with these variants represent a low risk for *R. africae* transmission to humans in Kenya. The above discussion suggests that *R. africae* variants may have reduced virulence due to host adaptation or possibly competitive exclusion that might account for the lack of ATBF in the indigenous populations. This kind of exclusion may be mediated by the host immune system (concomitant immunity) or by direct interference as shown for *R. peacockii*, where it has been shown to interfere with multiplication of the pathogenic *R. rickettsii* in *Dermacentor andersoni* ticks (Baldrige et al. 2004). Additionally, this is plausible given that no mixed results were found when assessing pooled samples in the present study with such a highly prevalent organism.

Supporting this notion, a study conducted in rural western Kenya, the region where these ticks were collected, found no



sequences analyzed, and only one of the four sequences conformed with the type strain (*R. africae* ESF-5). This suggests a high infection rate of the ticks with the variants, which might have reduced pathogenic potential for causing ATBF. Comparative analysis of clinical isolates with those from ticks warrants further investigation.

In the present survey, antibodies against SFG rickettsiae were detected in domestic ruminants—goats (43%), sheep (23%), and cattle (1%). The findings of this study confirm previous reports of the occurrence of SFG antibodies to rickettsiae in domestic ruminants (Philip et al. 1966, Kelly et al. 1991, Parola et al. 1999, Kelly et al. 2010). In contrast, however, the prevalence of SFG antibodies in cattle in our study were lower (1%) compared to what was reported in Zimbabwe (26–100%) (Kelly et al. 1991) and Guadeloupe ( $\geq 38.4\%$ ) (Parola et al. 1999). It is intriguing, however, that *A. variegatum* ticks containing the *R. africae* genotype were predominantly found on cattle, but only a small proportion of the cattle sera tested had antibodies to SFG rickettsiae.

Although antibodies reactive to *Rickettsia typhi* had been demonstrated using a complement fixation test in domestic ruminants in Egypt (Schmatz et al. 1978), our study did not find antibodies to TG rickettsia in these animals. This corroborates previous studies conducted in humans in eastern Africa where prevalence of TG rickettsia was found to be low (Prabhu et al. 2011, Maina et al. 2012). Likewise, there was no evidence of rickettsiemia in the ruminants from which these ticks were collected. This is likely to be associated with the high prevalence of an *R. africae* variant that may be an endosymbiont of *A. variegatum*. Insufficient numbers of rickettsiae in the blood of these animals coupled with the prevalent use of tetracyclines/antimicrobial drugs in these animals (Mitema et al. 2001) may explain the lack of detection by PCR method and the low prevalence of seroreactivity to SFG rickettsial antigens by ELISA. As preferred hosts of the tick vectors, they likely still play a pivotal role in the ecology of the pathogen through maintenance of tick populations, particularly in areas devoid of other large mammal species. Although this study recorded higher prevalence of IgG antibodies to SFG in goats (43%) and sheep (23%) than cattle (1%), only a small proportion of goats and sheep had *A. variegatum* ticks (2.9% and 1.5% in goats and sheep, respectively). These findings are consistent with those found in Ethiopia, where antibodies to SFG rickettsiae were significantly higher in goats and sheep than in cattle (Philip et al. 1966).

Whether these variants reported in this and other previous studies can cause human disease remains to be studied. A more comprehensive study is needed to elucidate whether translation of *ompA* and *ompB* to functional OmpA and OmpB do occur in the variants that have nucleotide deletions in the *ompA* and *ompB* genes. This study provides three important findings: First, antibody levels reported among livestock in this region appear unrelated to exposure to ticks infected with *R. africae*; second, a high tick infection rate was evident in the absence of *R. africae* infection; and finally, the variability observed among strains underscores the need to undertake comparative assessment of strains associated with clinical ATBF and those circulating within the larger tick population to decipher potential virulence markers.

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No competing financial interests exist.

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## References

- Adazu K, Lindblade KA, Rosen DH, Odhiambo F, et al. Health and demographic surveillance in rural western Kenya: a platform for evaluating interventions to reduce morbidity and mortality from infectious diseases. *Am J Trop Med Hyg* 2005; 73:1151–1158.
- Althaus F, Greub G, Raoult D, Genton B. African tick-bite fever: A new entity in the differential diagnosis of multiple eschars in travelers. Description of five cases imported from South Africa to Switzerland. *Int J Infect Dis* 2010; 14 (Suppl 3):e274–e276.
- Auce P, Rajakulendran S, Nesbitt A, Chinn R, et al. Internuclear ophthalmoplegia following African tick bite fever. *J Neurol Neurosurg* 2011; 82:681.
- Baldrige, GD, Burkhardt NY, Simser JA, Kurti TJ, et al. Sequence and expression analysis of the *ompA* gene of *Rickettsia peacockii*, an endosymbiont of the Rocky Mountain wood tick, *Dermacentor andersoni*. *Appl Environ Microbiol* 2004; 70:6628–6636.
- Bellini C, Monti M, Potin M, Ave AD, et al. Cardiac involvement in a patient with clinical and serological evidence of African tick-bite fever. *BMC Infect Dis* 2005; 5:90.
- Blair PJ, Jiang J, Schoeler GB, Moron C, et al. Characterization of spotted fever group rickettsiae in flea and tick specimens from northern Peru. *J Clin Microbiol* 2004; 42:4961–4967.
- Blanc G, Ngwamidiba M, Ogata H, Fournier PE, et al. Molecular Evolution of rickettsia surface antigens: evidence of positive selection. *Mol Biol Evol* 2005;22:2073–2083.
- Cohen GL, Blumberg LS, Karstaedt AS. Tick bite fever in black South Africans—a rare disease? *J Infect* 1996; 32:235–237.
- Eldin C, Mediannikov O, Davoust B, Cabre O, et al. Emergence of *Rickettsia africae*, Oceania. *Emerg Infect Dis* 2011;17:100–102.
- Feikin DR, Olack B, Bigogo GM, Audi A, et al. The burden of common infectious disease syndromes at the clinic and household level from population-based surveillance in rural and urban Kenya. *PLoS One* 2011; 6:e16085.
- Feisheim RF, Kurti TJ, Munderloh UG. Genome sequence of the endosymbiont *Rickettsia peacockii* and comparison with virulent *Rickettsia rickettsii*: Identification of virulence Factors. *PLoS One* 2009; 4:e8361.



- Fournier P-E, Roux V, Caumes E, Donzel M, et al. Outbreak of *Rickettsia africana* infections in participants of an adventure race in South Africa. *Clin Infect Dis* 1998a; 27:316–323.
- Fournier P-E, Roux V, Raoult D. Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. *Int J Syst Bacteriol* 1998b; 48:839–849.
- Fournier P-E, Dumler S, Greub G, Zhang J, et al. Gene sequence-based criteria for identification of new *Rickettsia* isolates and description of *Rickettsia heilongjiangensis* sp. nov. *J Clin Microbiol* 2003; 41:5456–5465.
- Frean J, Blumberg L. Tick bite fever and Q fever—a South African perspective. *S Afr Med J* 2007; 97:1198–1202.
- Freedman DO, Weld LH, Kozarsky PE, Fisk T, et al. Spectrum of disease and relation to place of exposure among ill returned travelers. *N Engl J Med* 2006; 354:119–130.
- Graf PCF, Chretien JP, Ung L, Gaydos JC, et al. Prevalence of seropositivity to spotted fever group rickettsiae and *Anaplasma phagocytophilum* in a large, demographically diverse US sample. *Clin Infect Dis* 2008; 40:70–77.
- Jacquemard R, Pretorius A-M, van der Ryst E, Venter A, et al. Severe encephalopathies in children with antibodies reactive with *Rickettsia africana*. *S Afr Med J* 1998; 88:1432.
- Jensenius M, Hoel T, Raoult D, Fournier PE, et al. Seroprevalence of *Rickettsia africana* infection in Norwegian travelers to rural Africa. *Scand J Infect Dis* 2002; 34:93–96.
- Jensenius M, Fournier P-E, Kelly P, Myrvang B, et al. African tick bite fever. *Lancet Infect Dis* 2003a; 3:557–564.
- Jensenius M, Fournier P-E, Vene S, Hoel T, et al. African tick bite fever in travelers to rural sub-Equatorial Africa. *Clin Infect Dis* 2003b; 36:1411–1417.
- Jensenius M, Fournier P-E, Fladby T, Hellum KB, et al. Subacute neuropathy in patients with African tick bite fever. *Scand J Infect Dis* 2006; 38:114–118.
- Jensenius M, Davis X, von Sonnenburg F, Schwartz E, et al. Multicenter GeoSentinel analysis of rickettsial diseases in international travelers, 1996–2008. *Emerg Infect Dis* 2009; 15:1791–1798.
- Jiang J, Chan T, Temenek JJ, Dasch GA, et al. Development of a quantitative real-time polymerase chain reaction assay specific for *Orientia tsutsugamushi*. *Am J Trop Med Hyg* 2004; 70:351–356.
- Jiang J, Blair PJ, Felices V, Moron C, et al. Phylogenetic analysis of a novel molecular isolate of spotted fever group rickettsiae from northern Peru: *Candidatus* Rickettsia andeanae. *Ann NY Acad Sci* 2005b; 1063:337–342.
- Jiang J, Maina AN, Knobel DL, Cleaveland S, et al. Molecular detection of *Rickettsia felis* and *Candidatus* Rickettsia asemboensis in fleas from human habitats, Asembo, Kenya. *Vector Borne Zoonotic Dis* 2013; 13:550–558.
- Jiang J, Sangkasuwan V, Lerdtusnee K, Sukwit S, et al. Human infection with *Rickettsia honei*, Thailand. *Emerg Infect Dis* 2005a; 11:1473–1475.
- Jiang J, Stromdahl EY, Miller M, Richards AL. Detection of *Rickettsia parkeri* and *Rickettsia andeanae* in *Amblyomma maculatum* Gulf Coast ticks collected from humans, USA. *Vector Borne Zoonotic Dis* 2012; 12:175–182.
- Kelly P, Lucas H, Beati L, Yowell C, et al. *Rickettsia africana* in *Amblyomma variegatum* and domestic ruminants on eight Caribbean islands. *J Parasitol* 2010; 96:1086–1088.
- Kelly PJ. *Rickettsia africana* in the West Indies. *Emerg Infect Dis* 2006; 12:224–226.
- Kelly PJ, Mason PR. Transmission of a spotted fever group rickettsia by *Amblyomma hebraeum* (Acari: Ixodidae). *J Med Entomol* 1991b; 28:598–600.
- Kelly PJ, Mason PR, Manning T, Slater S. Role of cattle in the epidemiology of tick-bite fever in Zimbabwe. *J Clin Microbiol* 1991a; 29:256–259.
- Kelly PJ, Beati L, Matthewman LA, Mason PR, et al. A new pathogenic spotted fever group rickettsia from Africa. *J Trop Med Hyg* 1994; 97:129–137.
- Kelly PJ, Beati L, Mason PR, Matthewman LA, et al. *Rickettsia africana* sp nov, the etiological agent of African tick bite fever. *Int J Syst Bacteriol* 1996; 46:611–614.
- Macaluso JD, Davis J, Alam U, Korman A, et al. Spotted fever group rickettsiae in ticks from the Masai Mara region of Kenya. *Am J Trop Med Hyg* 2003; 68:551–553.
- Maina AN, Knobel DL, Jiang J, Halliday JEB, et al. *Rickettsia felis* infection in febrile patients from a region with high seroprevalence of rickettsial infections, western Kenya, 2007–2010. *Emerg Infect Dis* 2012; 18:328–331.
- Mediannikov O, Trape JF, Diatta G, Parola P, et al. *Rickettsia africana* in western Africa. *Emerg Infect Dis* 2010a; 16:571–573.
- Mediannikov O, Diatta G, Fenollar F, Sokhna C, et al. Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. *PLoS Negl Trop Dis* 2010b; 4:e821.
- Mitema ES, Kikvi GM, Wegener HC, Stohr K. An assessment of antimicrobial consumption in food producing animals in Kenya. *J Vet Pharmacol Ther* 2001; 24:385–390.
- Nakao R, Qiu Y, Igarashi M, Magona JW, Zhou L, et al. High prevalence of spotted fever group rickettsiae in *Amblyomma variegatum* from Uganda and their identification using sizes of intergenic spacers. *Ticks Tick Borne Dis* 2013; 31:506–514.
- Ndip LM, Fokam EB, Bouyer DH, Ndip RN, et al. Detection of *Rickettsia africana* in patients and ticks along the coastal region of Cameroon. *Am J Trop Med Hyg* 2004; 71:363–366.
- Oostvogel PM, van Doornum GJ, Ferreira R, Vink J, et al. African tick-bite fever in travelers, Swaziland. *Emerg Infect Dis* 2007; 13:353–355.
- Parola P. Rickettsioses in sub-Saharan Africa. *Ann NY Acad Sci* 2006; 1078:42–47.
- Parola P, Vestris G, Martinez D, Brochier B, et al. Tick-borne rickettsiosis in Guadeloupe, the French West Indies: Isolation of *Rickettsia africana* from *Amblyomma variegatum* ticks and serosurvey in humans, cattle, and goats. *Am J Trop Med Hyg* 1999; 60:888–893.
- Parola P, Inokuma H, Camica JL, Brouqui P, et al. Detection and identification of spotted fever group rickettsiae and *Ehrlichiae* in African ticks. *Emerg Infect Dis* 2001; 7:1014–1017.
- Philip CB, Hoogstraal H, Reiss-Gutfreund R, Clifford CM. Evidence of rickettsial disease agents in ticks from Ethiopian cattle. *Bull Wild Hlth Org* 1996; 35:127–131.
- Prabhu M, Nicholson WL, Roche AJ, Kersh GJ, et al. Q fever, spotted fever group, and typhus group rickettsioses among hospitalized febrile patients in northern Tanzania. *Clin Infect Dis* 2011; 53:e8–e15.
- Raoult D, Fournier PE, Fenollar F, Jensenius M, et al. *Rickettsia africana*, a tick-borne pathogen in travelers to sub-Saharan Africa. *N Engl J Med* 2001; 344:1504–1510.
- Richards AL, Jiang J, Omulo S, Dare R, et al. Human infection with *Rickettsia felis*, Kenya. *Emerg Infect Dis* 2010; 16:1081–1086.
- Roch N, Epaulard O, Pelloux I, Pavese P, et al. African tick bite fever in elderly patients: 8 cases in French tourists returning from South Africa. *Clin Infect Dis* 2008; 47:e28–e35.
- Roux V, Raoult D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer membrane protein rompB (*ompB*). *Int J Syst Evol Microbiol* 2000; 50:1449–1455.

- Rutherford JS, Macaluso KR, Smith N, Zaki SR, et al. Fatal spotted fever rickettsiosis, Kenya. *Emerg Infect Dis* 2004; 10:910–913.
- Schmatz HD, Krauss H, Viertel P, Ismail AS, et al. Seropidemiological investigations in domestic ruminants from Egypt, Somalia and Jordan for the demonstration of complement fixing antibodies against *Rickettsia* and *Chlamydia*. *Acta Trop* 1978; 35:101–111.
- Socolovschi C, Huynh TP, Davoust B, Gomez J, et al. Trans-ovarial and trans-stadial transmission of *Rickettsia africae* in *Amblyomma variegatum* ticks. *Clin Microbiol Infect* 2009; 15(Suppl 2):317–318.
- Sonenshine DE. Ecology of non-nidicolous ticks. In: Sonenshine DE, ed. *Biology of Ticks*, vol 2. New York: Oxford University Press, 1993:3–65.
- Stenos J, Graves SR, Unsworth NB. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group rickettsiae. *Am J Trop Med Hyg* 2005; 73:1083–1085.
- Walker AR, Bouattour A, Camicas JL, Estrada-Pena A, et al. Ticks of domestic animals in Africa: A guide to identification of species. Edinburgh: Bioscience Reports, 2003:221.
- Yoshikawa H, Kimura M, Ogawa M, Rolain J, et al. Laboratory-confirmed Mediterranean spotted fever in a Japanese traveler to Kenya. *Am J Trop Med Hyg* 2005; 73:1086–1089.

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