

SPOTTED FEVER GROUP RICKETTSIAE IN TICKS FROM THE MASAI MARA REGION OF KENYA

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Abstract. We have identified for the first time *Rickettsia africae*, and the ticks that harbored them, in Kenya. A total of 5,325 ticks were collected from vegetation, livestock, and wild animals during two field trips to southwestern Kenya. Most were immature forms (85.2%) belonging to the genera *Amblyomma* or *Rhipicephalus*. The adults also included representatives from the genus *Boophilus*. Ticks were assessed for rickettsial DNA by a polymerase chain reaction (PCR) using primers for the spotted fever group (SFG)-specific rickettsial outer membrane protein A (*rompA*) gene, and positive amplicons were sequenced. While none of the immature ticks tested positive by PCR, 15.8% of the adult *Amblyomma variegatum* and less than 1% of the *Rhipicephalus* spp. were SFG positive. Sequences of amplified products were identified as *R. africae*. These findings extend the known range of *R. africae*.

INTRODUCTION

The application of molecular diagnostics has aided in the discovery of several new species of rickettsiae worldwide. In Africa, two species of spotted fever group (SFG) rickettsiae, *R. conorii* and *R. africae*, are known to cause human diseases. While *R. conorii*, the etiologic agent of Mediterranean spotted fever, is maintained in *Rhipicephalus* ticks, which seldom feed on humans, *R. africae*, the causative agent of African tick bite fever (ATBF), is maintained in *Amblyomma* ticks that readily feed on humans.

Pijper¹ first described ATBF in South Africa in the 1930s. The disease was characterized as mild, often without rash, and correlated with the bite of *Amblyomma*. The causative agent was isolated, and based on cross-protection studies in guinea pigs, was proposed as being different from *R. conorii*.² Subsequent studies aimed at confirming the existence of a new species were inconclusive.^{3–8} Eventually the rickettsiae were isolated from the blood of patients in Zimbabwe and the presence of a second tick-transmitted SFG rickettsiae in sub-Saharan Africa was verified and named *R. africae*.^{7,9}

Epidemiologic studies in Zimbabwe and other African countries have identified a strong geographic correlation between the prevalence of *Amblyomma hebraeum* ticks and the incidence of ATBF.⁷ Tick transmission of *R. africae* to humans has been reported in Zimbabwe, South Africa, Lesotho, and Swaziland, and seroprevalence studies have identified *R. africae*-specific antibodies in patients from Botswana, Mozambique, Tanzania, Ethiopia, and Djibouti.^{10,11} Although the presence of *R. africae* has now been documented along much of the east coast of Africa, its presence in ticks from Kenya, where there is evidence of human cases, has not yet been confirmed. Therefore, the purpose of this study was to identify rickettsiae in ticks collected in Kenya.

MATERIALS AND METHODS

Tick collection and processing. On two separate expeditions, in April and June 2001, ticks were collected from private and public land surrounding the Masai Mara game reserve (1°05'S, 35°13'E). The Masai Mara reserve is an extension of Tanzania's Serengeti National Park along Kenya's southern border with Tanzania. The reservation in Kenya covers approximately 1,672 km² and ranges in elevation from

1,500 to 2,100 meters above sea level. Tick collection methods included dragging, CO₂ traps (dry ice technique), and collection from livestock and other animals. Dragging and CO₂ traps were focused on open land frequently used for resting by feral animals and primitive campsites used by tourists visiting the area. Collection of adult ticks from livestock (cattle, goats, and sheep) was done with the permission of the owners, and care was taken to minimize discomfort for the animals. At the time of collection ticks were placed in 1.5-mL vials containing 70% ethanol, and labels identifying the date, location, and host (or collection site) were added to the vials. Ticks were transported to the University of Maryland, Baltimore where they were separated into pools based on collection site and life stage. Immature forms were further separated based on genus, and adult ticks were separated according to host, sex, and species. Preliminary adult tick identification was carried out at the University of Maryland, Baltimore, and comprehensive identification was conducted by D. E. Sonenshine (Old Dominion University, Norfolk, VA) and J. E. Keirans (United States National Tick Collection, Statesboro, GA).

Identification of rickettsiae. Ticks were surface sterilized with sequential washes with 70% ethanol and stored in ethanol until used for isolation of genomic DNA (gDNA). Entire pools of whole, immature ticks were used for isolation of gDNA using the Wizard gDNA isolation kit (Promega, Madison, WI) according to the manufacturer's protocol. Individual adult ticks were dissected bilaterally and half of the tick was stored (according to the pool), while the other half was used for isolation of gDNA. For pools that were polymerase chain reaction (PCR) positive, gDNA was isolated from individual ticks (halves) and used as the template for another PCR.

All ticks were assessed for the presence of rickettsiae using a PCR that allowed for the amplification of a fragment of the rickettsial outer membrane protein A (*rompA*) gene encoding the SFG-specific 190-kD protein. The PCR amplification of the *rompA* gene fragment used primers Rr190.70p¹² and Rr190.701.¹³ The reaction mixture consisted of 2.0 μL of purified gDNA, 18 μL of PCR master mix (Roche, Mannheim, Germany), 1 μL each of forward and reverse primers, and 3 μL of sterile water. Negative and positive controls for the PCR were sterile water and *R. montanensis* (VR611), respectively. The reaction was carried out in a PCR thermal cycler (Hybaid, Franklin, MA) with the following thermal cycler

conditions: initial denaturation at 94°C for five minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for one minute, 72°C for two minutes, with an additional incubation period of 72°C for five minutes on the final cycle. The PCR products were visualized after electrophoresis on a 1% agarose gel stained with ethidium bromide, excised, and the DNA was recovered from the gel using a StrataPrep DNA extraction kit (Stratagene, La Jolla, CA) according to manufacturer's protocol. For sequencing, the purified *rompA* gene fragments were subcloned in TOPO TA cloning vector (Invitrogen, San Diego, CA) and were sequenced by the dye terminator method on a model 373 automated fluorescence sequencing system (Applied Biosystems, Foster City, CA). Sequence analysis was carried out with the MacVector software package, and the BLAST program was used for sequence comparisons (National Center for Biotechnology Information, Bethesda, MD). Sequencing of three clones was done to ensure sequence fidelity.

RESULTS

A total of 5,325 ticks were tested for rickettsial infection by the PCR. Of these, 85.2% were immature forms of either the genus *Amblyomma* (n = 2,867) or *Rhipicephalus* (n = 1,672). The remaining, adult ticks tested belonged to genera *Amblyomma* (n = 57), *Rhipicephalus* (n = 709), or *Boophilus* (n = 20). Immature forms were collected only during dragging and at the CO₂ trap sites. While few adults (n = 23) were collected these ways, the majority were recovered feeding on cattle, sheep, goats, and a white rhinoceros (*Ceratotherium simum*). Pools of ticks were made based on host and collection site and gDNA was extracted from each pool for PCR. None of the pools of immature ticks (n = 61) or of *Boophilus* (n = 3) were positive for SFG rickettsiae. Positive pools of *A. variegatum* (n = 3) and *Rhipicephalus appendiculatus* (n = 1) were identified from cattle and sheep. Saved individual tick halves from positive pools were then tested to determine prevalence (Table 1). Amplicons from positive individuals (n = 10) were cloned and sequenced. While three of the sequences were identical to those already reported for *R. africae* genotypes,¹⁴ seven amplicons were characterized by previously unreported base substitutions. Nucleotide deviation from reported examples ranged from one to eight bases, with

TABLE 1

Results of a rickettsial outer membrane protein A (*rompA*) gene polymerase chain reaction amplification for detection of rickettsial DNA in individual adult ticks collected from the Masai Mara region of Kenya

Tick species	Host	No. positive/no. examined
<i>Amblyomma variegatum</i>	Cow	7/26
	Sheep	2/4
	Rhinoceros	0/27
<i>Rhipicephalus appendiculatus</i>	Cow	1/257
	Sheep	0/169
	Goat	0/116
<i>Rhipicephalus evertsi</i>	Cow	0/47
	Sheep	0/52
	Goat	0/48
<i>Rhipicephalus pulchellus</i>	Cow	0/20
<i>Boophilus microplus</i>	Cow	0/20

an unusually high percentage resulting in amino acid substitutions. These variants are summarized in Table 2.

DISCUSSION

Since its characterization, a number of cases of ATBF have been reported in travelers who had visited sub-Saharan Africa.^{10,11,15,16} The prevalence of ATBF appears to be high:¹⁷ an infection rate of nearly 30% was recorded in U.S. military training in Botswana¹⁸ and returning visitors to Kenya have been shown to have elevated levels of antibody to *R. africae*.¹⁹ However, little attention has been given to the epidemiology of rickettsial diseases in areas where wildlife, livestock, and humans mingle, as is common in much of Africa.

Our objective was to identify which SFG rickettsiae were present in Kenyan ticks. Masai Mara, the northern extension of the Serengeti plain, is a popular tourist destination and the suspected origin of several human cases of spotted fever, including one that was fatal (Rutherford J and others, unpublished data).

This is the first report of *R. africae* in ticks from Kenya. Because of transport and storage conditions we were unable to culture rickettsial organisms from the ticks, as has been done from ixodid ticks elsewhere in east Africa.¹⁴ Identification of the gene encoding the *rompA* protein by a PCR is the standard technique for preliminary identification of SFG rickettsiae. Restriction fragment length polymorphism analysis is one technique by which rickettsiae are often differentiated.¹³ Because of the relatively small number of positive samples, we chose sequence analysis. Our results are consistent with earlier findings for *R. africae* from *Amblyomma* ticks collected on cattle in Africa. Similar to Parola and others,¹⁴ we found genotypic variation in most of the samples we assessed. These genotypes were identified in ticks from cattle in the same herd, and possibly from the same individual cow.

It is interesting that an *R. africae* variant was identified in an *R. appendiculatus* tick. In the Central African Republic, 7.1% of *R. compositus* collected from cattle were positive for *R. africae*.²⁰ We collected *R. africae*-positive *A. variegatum* from the same herds of cattle in which the positive *R. appendiculatus* tick was collected. While the role of *Rhipicephalus* ticks in the transmission of *R. africae* is not clear, their infection may be dependent on coincidental transmission by other species, such as *A. variegatum*.

In conclusion, we report the first identification of *R. africae* in ticks collected in Kenya. With the presence of ATBF in much of sub-Saharan Africa, this information expands our knowledge of the pathogen's range into a topographically diverse area characterized by intimate human-animal interactions and active tourism. Besides the known reservoir/vector *A. variegatum*, we found an infected *Rhipicephalus* tick.

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TABLE 2

Comparison of nucleotide sequence of the rickettsial outer membrane protein A (*rompA*) gene fragment from infected ticks and to other published *Rickettsia africae* sequences*

Tick pool no. (tick no.)	GenBank accession no.	Identification	% Identities (query/subject)			
			<i>R. africae</i> U43790	Rav 1 AF311959	Rav 3 AF311960	Rav 9 AF311961
16 (4)†	AF 548342	RaR1/Kenya	98 (584/590)	99 (587/590)	99 (586/590)	99 (586/590)
27 (1)‡	AF 548338	AvR1/Kenya	99 (589/590)	99 (586/590)	99 (585/590)	99 (585/590)
27 (2,3)‡	ND§	–	100 (590/590)	99 (587/590)	99 (586/590)	99 (586/590)
28 (4,6)‡	AF 548339	AvR2/Kenya	98 (584/590)	99 (587/590)	99 (586/590)	99 (586/590)
28 (7)‡	AF 548340	AvR3/Kenya	98 (582/590)	99 (585/590)	98 (584/590)	98 (584/590)
45 (1)‡	ND§	–	99 (587/590)	100 (590/590)	99 (589/590)	99 (589/590)
98 (1,2)‡	AF 548341	AvR4/Kenya	99 (585/590)	99 (588/590)	99 (587/590)	99 (587/590)

* Amplification of a fragment of *rompA* (590 basepairs) from ticks collected in Kenya was done using gene-specific primers as described in the Materials and Methods. Sequences identified in this study were compared to deposited sequences for an *R. africae* type specimen and *R. africae* variants.¹⁴

† *Rhipicephalus appendiculatus*.

‡ *Amblyomma variegatum*.

§ ND = not done. Sequence not submitted to GenBank because it was identical to the already submitted sequence for *R. africae*.

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