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Short Communication

Spotted Fever Group Rickettsiae from Ticks Captured in Sudan

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SUMMARY: Ticks were collected from ruminants in various areas of Sudan in 1998 and 2000. Primer pairs of rickettsial citrate synthase gene (*gltA*) and a spotted fever group (SFG) rickettsial 190-kDa surface antigen gene (*rompA*), respectively, were used for identification. Polymerase chain reaction (PCR)-positive products were used for DNA sequencing. The *gltA* gene was detected in 55% of the ticks examined (57/104). Among the 57 ticks studied, 19 were positive for the *rompA* gene. Thus, 18% of the ticks examined were found to be infected with SFG rickettsiae. The nucleotide sequences of the amplified *rompA* gene fragment of *Hyalomma* spp. and *Amblyomma* spp. were similar to those of *Rickettsia aeschlimannii* and *Rickettsia africae*, respectively. In this study, we succeeded in detecting the SFG rickettsiae gene in ticks, and established that there were at least two species of SFG rickettsiae in field ticks in Sudan.

Spotted fever group (SFG) rickettsiae are transmitted to animals and humans through bites from infected ticks or mites (1). SFG rickettsiae consist of many pathogenic and nonpathogenic strains, and the diseases associated with each agent have been reported worldwide (2). Various ticks have been found to be vectors of SFG rickettsiae in Africa (2,3). *Rickettsia conorii*, which is prevalent in areas near the Mediterranean coast of Africa (1), is transmitted by dog ticks including *Rhipicephalus sanguineus* (4). *R. africae*, which causes African tick-bite fever, is transmitted by *Amblyomma* ticks (5-7). The disease is prevalent in South Africa and Zimbabwe (6,8,9), and agents have been detected from ticks in Ethiopia and the Central African Republic (10). In the Central African Republic, nonpathogenic strains such as *R. massiliae* have also been identified from *Rhipicephalus* spp. (10). Furthermore, *R. aeschlimannii* has been isolated in Morocco from *Hyalomma marginatum* (11). In Sudan, the presence of rickettsia-like organisms in *Hyalomma* ticks was first reported in the 1950s (12). However, little is known about the prevalence of rickettsiae in vector ticks carrying SFG rickettsiae in that country. The purpose of this study was to detect SFG rickettsial DNA in field ticks obtained in Sudan using polymerase chain reaction (PCR) and sequence analysis.

A total of 104 ticks were collected in various areas of Sudan (Fig. 1). Ticks were collected directly from camels in Gedaref and Kassala, from sheep in Khartoum, and from cattle in Juba. The ticks were identified as *Amblyomma lepidum*, *A. variegatum*, *Hyalomma dromedarii*, *H. marginatum*, and *H. truncatum*. Each tick was immersed in ethanol, and stored at −80°C awaiting further processing. For analysis, each tick was homogenized in sterilized phosphate-buffered saline, and DNA was extracted from the homogenate using a QIAamp tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. PCR amplification was performed as described by Regnery et al. (13), using oligonucleotide primer pairs, i.e., a rickettsial citrate synthase gene (*gltA*) primer pair (*Rp*CS.877p and *Rp*CS.1258n) and a SFG rickettsial 190-kDa surface antigen gene (*rompA*) primer pair (*Rr*190.70p and *Rr*190.602n). Amplifications were performed in a TP-3000 PCR thermal cycler (Takara Shuzo Co., Ltd.,

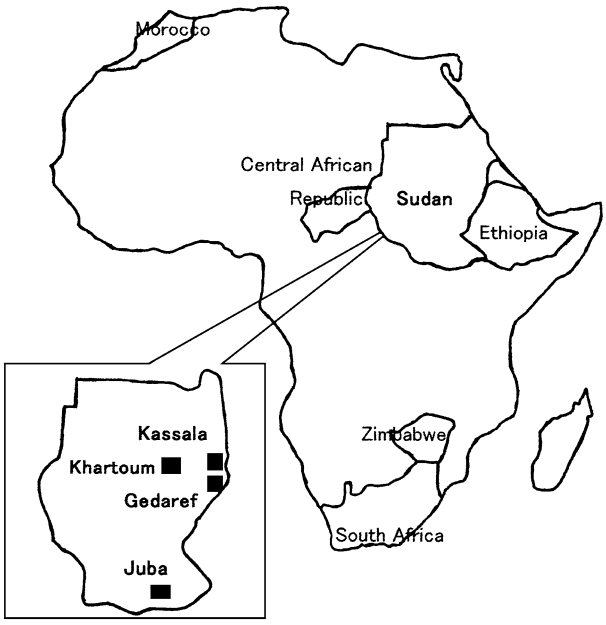

Fig. 1. Map of sampling sites (■) in Sudan.
Countries, including Sudan, mentioned in the text are also shown.

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Table 1. Ticks collected in Sudan, and *gltA* and *rompA* gene detection of the ticks and the sequence similarity of the *rompA* gene in SFG rickettsia

Tick species	Sampling sites	PCR-positive nos./nos. examined (positive rate %)		SFG rickettsia of the highest similarity (%) of that of the *rompA* gene examined[1)]
		gltA	*rompA*	
Amblyomma variegatum	Juba	10/10 (100)	7/10 (70)	*R. africae* (99.2)
	Kassala	1/2 (50)	0/1 (0)	
Amblyomma lepidum	Gedaref	8/10 (80)	6/8 (75)	*R. africae* (100.0)
Subtotal of *Amblyomma* spp.		19/22 (86)	13/19 (68)	
Hyalomma dromedarii	Kassala	15/34 (44)	1/15 (7)	*R. aeschlimannii* (99.6)
	Khartoum	0/5 (0)	0/0	
Hyalomma marginatum	Kassala	14/16 (88)	1/14 (7)	
Hyalomma truncatum	Kassala	9/27 (33)	4/9 (44)	*R. aeschlimannii* (99.6)
Subtotal of *Hyalomma* spp.		38/82 (46)	6/38 (16)	
Total		57/104 (55)	19/57 (33)	

[1)]: Four *rompA*-positive genes (one each from *A. variegatum*, *A. lepidum*, *H. dromedarii*, and *H. truncatum*) were sequenced, and the corresponding sequences of SFG rickettsiae, deposited in GenBank, were assessed using the multisequence alignment program CLUSTAL (version W).

Tokyo, Japan). The conditions of amplification were those described by Weller et al. (14). Amplified DNA was visualized on 1% agarose gel after electrophoresis (100 V for 30 min) of 5 μl of the amplified DNA. The gels were stained with ethidium bromide and examined using a UV transilluminator. The size of the PCR product was determined by comparison with DNA molecular weight marker V (Roche Diagnostics, Tokyo, Japan). Four out of the *rompA*-positive PCR products were purified by using a QIA Quick Gel Extraction Kit (QIAGEN), according to the manufacturer's instructions. Purified DNA was sequenced by using a BigDye Terminator Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems, Tokyo, Japan) with an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems). The obtained sequences, except for the primer regions, were aligned with the corresponding sequences of other rickettsiae strains deposited in GenBank using the multisequence alignment program CLUSTAL (version W).

In total, 104 ticks were examined for the presence of SFG rickettsiae using PCR (Table 1). The *gltA* gene was detected in every species of tick (55%: 57/104) in every location, except in *H. dromedarii* in Khartoum. Further, *gltA*-positive samples were used to amplify the *rompA* gene. The rate of *rompA*-positive gene identification in *Amblyomma* spp. ticks was higher (68%: 13 of 19) than that in *Hyalomma* spp. ticks (16%: 6 of 38). The nucleotide sequence of *A. variegatum* and *A. lepidum* was similar to that of *R. africae* (99.2 and 100%, respectively; the accession number of both fragments was U43790). On the other hand, the sequences of both *H. dromedari* and *H. truncatum* were most similar to *R. aeschlimannii* (99.6%; the accession number of both fragments was U43800).

We succeeded in detecting the SFG rickettsial gene in the ticks sampled here. Parola et al. (15) also detected the *R. africae* gene from *A. lepidum*. In addition, we demonstrated that the *R. africae* gene was most similar to that in *A. variegatum*. Furthermore, an *R. aeschlimannii*-like gene was detected in two species of *Hyalomma* ticks in this study. We first clarified that numerous SFG rickettsiae were harbored in several species of ticks in Sudan. The rates of SFG rickettsial infection, including *R. africae* and genotypically similar agents, in *A. variegatum* and *A. lepidum* were higher than in other tick species obserbed in this study. The *A. variegatum* spp. is closely associated with tick-borne diseases in Africa (1, 2), and this tick is one of the main vectors of African tick-bite fever caused by *R. africae* (6,7,10). Moreover, *A. lepidum* may play the same epidemiological role in Sudan, as the gene of this agent was previously detected (15), and was again identified in this study. The two species of ticks may therefore cause disease in Sudan, although no human case has been reported in that country. *R. aeschlimannii* was isolated from *Hyalomma* ticks in Morocco and Zimbabwe, but the pathogenicity of the agent was not known (11). We also detected a gene similar to that in *R. aeschlimannii* from two species of *Hyalomma* ticks in Sudan. This suggests that the agent, or a closely related one, is widely distributed in *Hyalomma* ticks in African. *Amblyomma* ticks had a higher rate of SFG rickettsial gene positivity than did *Hyalomma* ticks in this study. However, this result is inconclusive due to the small sample size of the present study. It remains difficult to compare the rate of SFG rickettsial infection among ticks in Sudan and other African countries. The prevalence of SFG rickettsiae in ticks has been demonstrated by hemolymph test (8,11), and a few reports have described the results of PCR analysis. Nucleotide sequence data have shown that at least two species of SFG rickettsiae in field ticks in Sudan.

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