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 aeshlimannii 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. aeshlimannii 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 (RpCS.877p and RpCS.1258n) and a SFG rickettsial 190-kDa surface antigen gene (rompA) primer pair (Rr190.70p and Rr190.602n). Amplifications were performed in a TP-3000 PCR thermal cycler (Takara Shuzo Co., Ltd., Japan).
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 most 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 species of SFG rickettsiae, deposited in GenBank, were assessed using the multisequence alignment program CLUSTAL (version W).

**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

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Sampling sites</th>
<th>PCR-positive nos./nos. examined (positive rate %)</th>
<th>SFG rickettsia of the highest similarity (%) of that of the rompA gene examined†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. variegatum</td>
<td>Juba</td>
<td>10/10 (100)</td>
<td>7/10 (70)</td>
</tr>
<tr>
<td></td>
<td>Kassala</td>
<td>1/2 (50)</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>A. lepidum</td>
<td>Gedaref</td>
<td>8/10 (80)</td>
<td>6/8 (75)</td>
</tr>
<tr>
<td>Subtotal of Amblyomma sp.</td>
<td></td>
<td>19/22 (86)</td>
<td>13/19 (68)</td>
</tr>
<tr>
<td>H. dromedarii</td>
<td>Kassala</td>
<td>15/34 (44)</td>
<td>1/15 (7)</td>
</tr>
<tr>
<td></td>
<td>Khartoum</td>
<td>0/5 (0)</td>
<td></td>
</tr>
<tr>
<td>H. marginatum</td>
<td>Kassala</td>
<td>14/16 (88)</td>
<td>1/14 (7)</td>
</tr>
<tr>
<td>H. truncatum</td>
<td>Kassala</td>
<td>9/27 (33)</td>
<td>4/9 (44)</td>
</tr>
<tr>
<td>Subtotal of Hyalomma spp.</td>
<td></td>
<td>38/82 (46)</td>
<td>6/38 (16)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>57/104 (55)</td>
<td>19/57 (33)</td>
</tr>
</tbody>
</table>

†: 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).

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