A SURVEY FOR SPOTTED FEVER GROUP RICKETTSIAE AND EHRlichiae IN AMBLYOMMA VARIEGATUM FROM ST. KITTS AND NEVIS

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Abstract. Eighty-nine Amblyomma variegatum ticks were collected from the islands of St. Kitts and Nevis in the Caribbean and preserved in 70% ethanol or local rum. After being washed in sterile water, their DNA was extracted and analyzed by a polymerase chain reaction (PCR) for DNA of spotted fever group rickettsiae and ehrlichiae. None of the tested ticks was positive in a PCR assay using the primers 16S EHRD and 16S EHRR for the 16S rRNA gene of Ehrlichia spp. Forty-one percent of the A. variegatum (36 of 89 of which 34 [47%] of 72 were adult males, 2 [13%] of 16 were adult females, and 0 [0%] of 1 were nymphs) were positive in a PCR assay using the primer pair 190-70 and 190-701 for the outer membrane protein A (ompA) gene of spotted fever group rickettsiae. All PCR amplification products obtained had 100% sequence homology with Rickettsia africana, the agent of African tick-bite fever.

INTRODUCTION

Amblyomma variegatum was introduced from Africa into Guadeloupe in the 1800s and is now endemic on many islands in the Caribbean. The tick transmits important human and animal pathogens in Africa and its presence in the Caribbean poses a threat to people and animals in the region, including those on the American mainland. Of particular importance is Rickettsia africana, which has been found in high percentage of A. variegatum in various African countries. This spotted fever group rickettsia is the agent of African tick-bite fever, a generally mild but very common disease in Africans and tourists visiting the continent. In Africa, A. variegatum is also an important vector of Ehrlichia ruminantium, the etiologic agent of heartwater, which is one of the most devastating livestock diseases in sub-Saharan countries. Studies in the Caribbean have shown that R. africana is present in Guadeloupe, and C. ruminantium has been found in Guadeloupe, Marie Galante, and Antigua. To provide further information on rickettsiae in ticks in the Caribbean, we used a polymerase chain reaction (PCR) to detect these organisms in A. variegatum from the islands of St. Kitts and Nevis.

MATERIALS AND METHODS

Sampling and processing of ticks. Eighty-nine A. variegatum ticks collected from seven areas in St. Kitts in 1999 and from one site in Nevis in 2002 were used in the study. All ticks were identified on the basis of morphologic criteria. They were stored in 70% alcohol or local rum until processed for PCR studies.

Extraction of DNA and PCR amplification. Ticks were cut in half lengthways and half was washed overnight in sterile water on a mechanical shaker. The DNA of the washed tick portions was extracted into 80 μL of sterile water using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Aliquots of the DNA were used in PCRs with the primer pair 190-70 and 190-701, which amplifies a 632-basepair fragment of the outer membrane protein A (ompA) gene that encodes a 190-kD outer membrane protein (rOmpA) specific for the spotted fever group rickettsiae. The PCRs were carried out as previously described and incorporated negative (sterile water) and positive controls (DNA from R. conorii strain seven [Malish]. Tick DNA samples that were found positive were sequenced as previously described, using an ABI Prism 3100 automated fluorescent sequencer (Applied Biosystems, Foster City, CA). Sequences obtained were compared with those available in Genbank using the BLAST software. Aliquots of DNA were also used in a PCR using the primers 16SEHRD and 16SEHRR, which specifically amplify a 300-basepair fragment of the 16S rRNA gene of Ehrlichia species as previously described. Negative (sterile water) and positive (DNA from Anaplasma phagocytophilum strain Webster) controls were included in the assay.

RESULTS

None of the ticks was positive in PCRs using the primers specific for the 16S rRNA gene of Ehrlichia spp. In contrast, 41% (36 of 89) of the ticks were positive in PCRs using primers for the ompA gene of the spotted fever group rickettsiae. Adult male ticks showed the highest prevalence (34 of 72, 47%), with adult females (2 of 16, 13%) and nymphs (0 of 1, 0%) having lower prevalences. Positive ticks were found at all but one site (0 of 2 positive) on St. Kitts with prevalences ranging from 14% (1 of 7) to 71% (5 of 7). Of the A. variegatum collected from Nevis, 23% (5 of 22) were positive by the PCR. The sequences of all 36 PCR products obtained using the primer pairs 190-70 and 190-701 had 100% homology with R. africana (GenBank Accession number U43790).

DISCUSSION

Local experience suggests that E. ruminantium was never introduced onto St. Kitts or Nevis because clinical cases of heartwater have not been seen on the islands. Our finding that E. ruminantium DNA could not be detected in A. variegatum from the islands is consistent with these clinical observations. In a previous seroepidemiologic survey for heartwater in the West Indies using an indirect ELISA, reactive antibodies were found in 1.3% of ruminant livestock from St. Kitts and Nevis. The investigators surmised that the low percentage of positive sera and the absence of clinical cases on the islands strongly suggested that positive sera were probably due to non-specific cross-reactions between E. ruminantium and closely related Ehrlichia spp. Our study failed to provide further information on the organism responsible for these non-specific cross-reactions.
We did, however, show that a significant number of A. variegatum in St. Kitts and Nevis that harbored R. africae. Our findings are consistent with recent studies in which R. africae was identified in 27% of A. variegatum in Guadeloupe and in seven of 12 A. variegatum (58%) in Martinique (Philippe Parola (P.P.)). These data indicate that R. africae is probably widespread in A. variegatum in the Caribbean. In southern Africa, the major vector of R. africae is A. hebraeum and very high infection rates in ticks (up to 80%) have been reported. This is probably because the organism is transmitted both transovarially and trans-stadially in the ticks, and also because infections in cattle and goats, which are important hosts of A. hebraeum, result in prolonged rickettsemias.

This rickettsemia of long duration, although causing no clinical or laboratory signs of infection, is probably an important source of organisms for concurrently feeding ticks not yet infected with R. africae. There are no data on whether R. africae infections are transmitted trans-stadially and/or transovarially in A. variegatum.

We are unaware of any studies on the effects of preserving ticks or other biologic samples in rum and subsequent PCR analysis. Only one of our collections was preserved in rum and only 14% of the ticks in this sample were positive by the PCR (1 of 7 adult females). All female ticks tested were in various states of engorgement, and those preserved in 70% ethanol also had a low percentage of ticks positive by the PCR (11%, 1 of 9). There was a significantly lower number of PCR-positive female ticks than male ticks (2 of 16 versus 34 of 72; \( \chi^2 = 6.25, P < 0.025 \)). It would appear that PCR inhibitors present in the blood meal or a dilution effect of the blood meal on DNA extraction was the most likely cause of this difference in PCR results.

Rickettsia africana is a spotted fever group rickettsia that is the agent of African tick-bite fever, which is a common disease in people in Africa and in tourists visiting the continent. The disease is often mild or subclinical, but can be associated with fever, an influenza-like syndrome, one or multiple inoculation eschars particularly on the legs, regional lymphadenopathy, and a rash.14 Recent reports have provided serologic evidence of a clinical case of African tick-bite fever in a tourist returning from Guadeloupe, and serosurveys have shown high prevalences of reactive antibodies to R. africana in people (49%), cattle (81%), and goats (86%) on the island.5 On St. Kitts, an active eradication program over the past two years has been successful in eliminating A. variegatum from the island, but the ticks are still present in Nevis. Medical practitioners in Nevis and those treating people who have recently visited the island should have a high level of suspicion of African tick-bite fever in patients presenting with a history of tick bites and clinical signs of fever, headache, and multiple eschars. Also, transportation of infected ticks or animals rickettsemic with R. africana to the American mainland might enable R. africana to become established on this continent.

Received December 11, 2002. Accepted for publication February 26, 2003.

Acknowledgments: We thank Sheldon McMahon and Dr. Patricia Bartlett for providing us with the ticks, and Annick Abeille for her expert technical assistance.

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