TICK-BORNE RICKETTIOSIS IN GUADELOUPE, THE FRENCH WEST INDIES: ISOLATION OF RICKETTSIA AFRICA FROM AMBLYOMMA VARIEGATUM TICKS AND SEROSURVEY IN HUMANS, CATTLE, AND GOATS

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Abstract. Twenty-seven rickettsiae were isolated and/or detected from 100 Amblyomma variegatum ticks collected on Guadeloupe in the French West Indies. In this study, the polymerase chain reaction procedure appeared to be more sensitive in detecting rickettsiae in ticks than the shell-vial technique. Sequencing a portion of the outer membrane protein A—encoding gene showed that these rickettsiae appeared to be identical to Rickettsia aegypti, a member of the spotted fever group rickettsiae recently described as an agent of tick-bite fever occurring in sub-Saharan Africa. A high seroprevalence of antibodies to R. africae was demonstrated among mammals, particularly humans, cattle, and goats. These results and a recently reported case of an infection due to R. africae on Guadeloupe demonstrate that R. africae is present on this island. Although this disease has been underdiagnosed there, it may be frequent and may exist on other Caribbean islands where A. variegatum has propagated dramatically over recent years.

Bacteria of the genus Rickettsia are gram-negative intracellular bacilli associated with arthropods.1 Ticks are the main reservoir of these rickettsiae in which they are maintained by transstadial and transovarial transmission.2 Ticks may also act as vectors that infect humans or animals while feeding. The main clinical symptoms of the resultant human tick-borne rickettsioses include fever, headache, rash, local lymphadenopathy, and an inoculation eschar (tache noire).1

Over the past few years, the development of new cell culture isolation techniques and the use of molecular methods has allowed the isolation or the detection and characterization of many rickettsial strains from ticks and humans, and numerous emerging diseases, such as Japanese spotted fever, Astrakhan fever, or African tick-bite fever, have been described throughout the world.1 However, the only known tick-borne rickettsioses caused by the genus Rickettsia described in the New World is Rocky Mountain spotted fever, which is also one of the oldest recognized arthropod-borne diseases. It is caused by Rickettsia rickettsii and occurs in the United States, Canada, Central, and South America.1 Rickettsia rickettsii has been isolated from large numbers of ticks and its main vectors include Dermacentor andersoni, D. occidentalis, Amblyomma americanum, A. maculatum, Ixodes scapularis, I. pacificus, and I. cookei.1 Furthermore, numerous new rickettsial strains of unknown pathogenicity have been isolated or detected from arthropods, in particular ticks, in the United States and Canada.1 These must be considered as potential pathogens, particularly if their hosts are anthropophilic ticks that are likely to bite humans.

In the West Indies, tick-borne rickettsioses have never been properly described. In the 1960s, cases of human spotted fever were suspected on Guadeloupe in the French West Indies in tick-bitten patients.3 At the same time, rickettsial strains were isolated from A. variegatum ticks collected on Guadeloupe.4 Unfortunately, these strains were not identified and were lost.

Recently, we reported the first serologically documented case of a tick-borne rickettsiosis caused by R. aegypti on Guadeloupe.5 This rickettsia is the recently described agent of African tick-bite fever that occurs in sub-Saharan Africa and has been isolated or detected in Amblyomma ticks in Africa including A. variegatum.6

The aim of this work was to isolate and characterize rickettsiae from A. variegatum ticks collected on Guadeloupe and to establish the prevalence of antibodies against these isolates in humans, cattle, and goats in this country.

MATERIALS AND METHODS

Tick study. Tick sampling. In October 1997, 100 A. variegatum adult ticks were collected on the island of Guadeloupe. The study sites were the pastures situated around three towns: Saint-François (30 males and 10 females), Gosier (15 males and 5 females), and Lamentin (30 males and 10 females). Ticks were removed from cattle, identified according to standard taxonomic keys,7 and kept frozen at −80°C.

Isolation of rickettsiae from ticks. Ticks were thawed, sterilized by immersion in iodinated alcohol for 10 min, rinsed with distilled water for 10 min, and dried on sterile filter paper under a laminar flow hood. Each tick was cut in half lengthways. Half of each tick was triturated in 1 ml of brain heart infusion (BioMerieux, Marcy L’Etoile, France) and the mixture was placed into a shell vial containing monolayers of L929 cells. The shell vials were centrifuged at 700 × g for 1 hr, after which the supernatant was replaced with 1 ml of Earle’s minimum essential medium (MEM) containing 4% fetal calf serum, 2 mM L-glutamine, gentamicin (200 µg/ml), cotrimoxazole (150 µg/ml), and amphotericin B (5 mg/100 ml). After four of incubation at 37°C in a CO2 incubator, the medium was changed and fresh MEM was added. After an additional four days of incubation, the L929 cells were scraped of the shell vial, applied to a microscope slide, and stained using the method of Gimenez to detect rickettsiae. Cells from infected shell vials were trypsinized...
and the isolated strains were subcultured in L929 cells by subsequent trypsinsizations as previously described.\textsuperscript{9,10}

\textit{Polymerase chain reaction (PCR).} The DNA of the remaining half of each tick was extracted, purified, and recovered into 80 μl of sterile water as previously described.\textsuperscript{9,10} The amplification of a 532-basepair fragment of the gene encoding for the 190-kD outer membrane protein A \textit{(rOmpA)} was performed using the previously described oligonucleotide primer pairs Rr 190.70p and Rr 190.602n \textit{(Bio-probe Systems, Montreuil-sous-Bois, France)}\textsuperscript{.10} A negative (distilled water) and a positive \textit{(R. conorii} DNA) control was included in each test. Polymerase chain reaction conditions were applied as previously described.\textsuperscript{10} The amplification products were subjected to electrophoresis on a 1% agarose gel, and the gel was stained with ethidium bromide and examined using ultraviolet transillumination. A DNA molecular weight marker (standard marker V; Boehringer-Mannheim, Mannheim, Germany) was used to estimate the size of the DNA fragments.

\textit{Sequencing a portion of the rOmpA gene.} Oligonucleotide fluorescent primers Rr 190.70p and Rr 190.602n were labeled at their 5' ends with fluorescein isothiocyanate (Eurolux\textsuperscript{3}IF, Seraing, Belgium). Sequencing reactions, amplification conditions, and electrophoresis of the sequenced products in an automated laser fluorescent DNA sequencer (Pharmacia LKB, Uppsala, Sweden) were used as previously described.\textsuperscript{11} The sequences were aligned with the corresponding sequences of the spotted fever group rickettsiae and similarity levels between pairs of sequences were determined as previously described.\textsuperscript{11}

\textit{Seroepidemiologic study. Human serum samples.} In 1997, 149 sera were obtained for blood chemistry studies from afebrile subjects living in Grande-Terre, Guadeloupe. Sera were collected from 54 men and 95 women between the ages of 18 and 70 years (mean age = 43 years old for men and 36 years old for women). Patients were invited to have their sera tested in a seroepidemiologic study; they were informed about the nature of the study and written informed consent was obtained.

\textit{Cattle and goat sera.} In 1994, 47 sera were obtained from cattle in Saint-François (29 sera), Moule (13 sera), and Grand-Bourg (5 sera), and 30 sera were obtained from goats in Saint-François (11 sera), Moule (10 sera), Lamentin (5 sera), and Baie-Mahault (4 sera). The sera were stored at −80°C prior to being tested.

\textit{Antigen preparation. Rickettsia conorii} (Moroccan strain, VR 141; American Type Culture Collection, Rockville, MD) and \textit{R. africae}\textsuperscript{e} were continuously grown in Vero cells and were purified by centrifugation through 25% sucrose in phosphate-buffered saline followed by Renografin \textit{(meclumine diatrizoate)} density gradient centrifugation as previously described.\textsuperscript{12}

\textit{Immunofluorescence (IF) assay.} An IF assay was performed as previously described.\textsuperscript{13} All sera found to be positive for total immunoglobulins were serially diluted (two-fold dilutions ranging from 1:50 to 1:1,600 and higher if needed) and the titers of IgG \textit{(Fluoline G, M; BioMérieux)} were determined.\textsuperscript{13}

\textit{Serum cross-absorption.} Three IF-positive human sera (randomly chosen between those with a titer ≥ 1:400) were absorbed with \textit{R. conorii} and \textit{R. africae} antigens.\textsuperscript{14} Sera were diluted 1:10 in an antigen suspension previously adjusted to a concentration of 2 mg/ml. The mixture was shaken for 24 hr at room temperature and then centrifuged at 10,000 ×g for 10 min. The supernatant was retained and tested by the IF assay as described above.

\textit{Western blotting.} For the Western blot, purified \textit{R. conorii} and \textit{R. africae} antigens were solubilized, electrophoresed, and transferred as previously described.\textsuperscript{15,16} Untreated and absorbed sera were tested by Western blotting as previously described.\textsuperscript{15,16} Antibodies reacting against antigens of low molecular weight were specific for the lipopolysaccharide of the spotted fever group rickettsiae including \textit{R. africae} and \textit{R. conorii}. Serologic specificity towards the spotted fever group rickettsia species was determined by the relative IgG reactivity to species-specific antigens in the 110–145-kD region.\textsuperscript{17}

\textbf{RESULTS}

\textit{Detection and identification of Rickettsiae from \textit{A. variegatum} ticks.} Isolation of Rickettsiae from ticks using the shell-vial technique. Thirteen rickettsiae isolates were recovered from \textit{A. variegatum} ticks using the shell-vial technique (Table 1). The isolates were subcultured onto L929 cells. No cytopathogenic effect was observed. Staining using the method of Gimenez\textsuperscript{9} revealed small, intracellular, rod-shaped bacteria.

\textit{Amplification by the PCR.} Rickettsial DNA was detected in the 13 ticks from which rickettsial strains were obtained using the shell-vial technique. Fourteen additional rickettsial-positive ticks were detected by the PCR procedure (Table 1). The sensitivity of the detection of rickettsiae in ticks was higher using the PCR procedure than when using the shell-vial technique (27% versus 13%; \textit{P} < 0.02). No amplification was obtained with any of the negative controls. The amplification results were visualized on an ethidium bromide–stained 1% agarose gel and had an approximate size of 530 basepairs.

\textit{Sequencing a portion of the rOmpA gene.} A 532-basepair sequence of PCR-derived fragments of the rOmpA gene was generated from the 27 PCR products. Comparison of these sequences with the corresponding sequences of the spotted

\begin{table}
\centering
\caption{Isolation and detection of rickettsial strains in \textit{Amblyomma variegatum} ticks collected on Guadeloupe}
\begin{tabular}{lccc}
\hline
 & Shell-vial & PCR\textsuperscript{*} & Total \\
 & technique & & & \\
 & & & & \\
St. François & & & & \\
10 males & 0 & 1 & 7/40 \\
30 females & 2 & 6\textdagger & \\
Lamentin & & & & \\
10 males & 0 & 8 & 7/40 \\
30 females & 3 & 3\textdagger & \\
Gosier & & & & \\
5 males & 4 & 4\textdagger & 9/20 \\
15 females & 4 & 5\textdagger & \\
Total & 13 & 27 & 27\% \\
\hline
\end{tabular}

\textsuperscript{*}PCR \textsuperscript{\textdagger} polymerase chain reaction.

\textsuperscript{\textdagger} Including the ticks from which strains were isolated using the shell-vial technique.
\end{table}
fever group rickettsiae revealed a 100% similarity between all the sequences and the corresponding sequence of *R. africae*.

**Human sera. Immunofluorescence assay.** Antibodies against *R. africae* were detected in 49% of the human sera at a titer $\geq 1:50$, in 43.6% at titer $\geq 1:100$, in 27.5% at a titer $\geq 1:200$, and in 8% at a titer $\geq 1:400$. The seroprevalence was significantly higher in male than in female subjects, 66% versus 39% at a titer $\geq 1:50$ ($P = 0.001$) and 55.5% versus 43.6% at a titer $\geq 1:100$ ($P = 0.03$), respectively. The prevalence of positive sera was similar using *R. conorii* antigens. However, the titers against *R. conorii* were higher in most cases when compared with those obtained using *R. conorii* antigens. The differences in titers were usually a two-fold dilution in 52% of the positive sera and a four-fold dilution in 33% of the positive sera. The titers using both antigens were equal in 15% of the cases. Thus, at a titer $\geq 1:100$, 43.6% of the sera were positive against *R. af-

**Cross-absorption and Western blotting.** After cross-absorption, the three randomly chosen IF-positive sera were retested by both immunofluorescence and Western blot. When absorption was performed with *R. africae*, it resulted in the disappearance of homologous and heterologous antibodies, but when it was performed with *R. conorii*, only homologous antibodies disappeared (Figure 1), indicating that antibodies were specific for *R. africae*.

**Cattle and goat sera. Cattle sera.** Antibodies against *R. africae* were detected in 80.8% of the cattle sera at a titer $\geq 1:50$ (Saint-François = 82.7%, Moule = 69.2%, and Grand-Bourg = 100%) and in 55.3% at a titer $\geq 1:100$ (Saint-François = 65.5%, Moule = 38.4%, and Grand-Bourg = 40%). The prevalence of positive sera was similar using *R. conorii* antigens. However, when compared with those obtained using *R. conorii* antigens, the titers against *R. africae* were higher in 77% of the positive sera and equal in 23% of the positive sera. The differences in titers were usually a single two-fold dilution (72%) or a four-fold one (5%). Thus, 55.3% of the sera in cattle were positive against *R. africae* at a titer $\geq 1:100$ compared with 34% for the same sera against *R. conorii* antigens ($P = 0.04$).

**Goat sera.** Antibodies against *R. africace* were detected in 86.6% of the sera at a titer $\geq 1:50$ (Saint-François = 90.9%, Moule = 80%, Lamentin = 100%, and Baie-Mahault = 75%) and in 63.3% at a titer $\geq 1:100$ (Saint-François = 63.6%, Moule = 60%, Lamentin = 60%, and Baie-Mahault = 50%). Although the same sera also reacted with *R. conorii* antigen, the titers against *R. africace* were higher in 65% of the positive sera. The differences in titers were usually a single two-fold dilution (54%) or a four-fold one (11%). The titers were equal among 35% of the positive sera.

**DISCUSSION**

*Amblyomma variegatum* (Figure 2) is an African tick species that is distributed throughout western sub-Sahelian Africa, the horn of Africa, and eastern and central Africa. In southeastern Africa, this tick is replaced by *A. hebraeum*. *Amblyomma variegatum* is established in a wide spectrum of habitats in different climatic zones and has a wide range of hosts. Thus, this tick has all the characteristics required for expansion and colonization throughout Africa and elsewhere.

*Amblyomma variegatum* was introduced into the West Indies during the 18th or 19th centuries on cattle shipped from Senegal to Guadeloupe. Over the past 50 years, it has propagated and invaded more than 15 islands, including Martinique, Dominica, St. Lucia, St. Vincent, Barbados, and Puerto Rico. In the Caribbean, cattle and goats are the main hosts of adults, whereas immature forms may parasitize a large variety of hosts, including birds. Although livestock movements have been implicated in the explanation of the propagation of the tick in the West Indies, Barré and others.
The dispersal of hosts carrying ticks is one factor of dissemination of tick-borne diseases, particularly rickettsioses. Amblyomma variegatum is a vector of Cowdria ruminantium, the agent of heartwater, a rickettsial disease that affects ruminants in sub-Saharan Africa. In the 1980s, this disease was reported in the West Indies.

Rickettsia africae is the recently described agent of African tick-bite fever, a spotted fever rickettsiosis occurring in sub-Saharan Africa. Although this rickettsia was initially isolated from ticks including A. variegatum and A. hebraeum, the first human case due to R. africae was reported in 1992. Subsequently, several cases have been reported among travelers returning from either Zimbabwe or South Africa who presented with this rickettsiosis as an emerging disease. The incubation period is about six days. It is a mild disease with signs including headache, fever, eschar at the tick-bite site (often multiple because Amblyomma ticks readily feed on humans), and regional lymphadenopathy. The rash is frequently absent or very transient and may be vesicular. In fact, this disease had been described by Piiper in the 1930s in patients traveling in rural areas following contact with cattle ticks, particularly, Amblyomma spp. and wild animals. He isolated the causative rickettsia and demonstrated that it was different from R. conorii, the agent of the potentially severe Mediterranean spotted fever transmitted by the dog tick Rhipicephalus sanguineus. Unfortunately, the isolate was lost and R. conorii remained the only recognized agent of tick-bite fever in Africa.

In the 1960s, Morel reported two cases of spotted fever in patients living in Guadeloupe and suspected A. variegatum, the only species to attack and bite humans in large numbers on Guadeloupe, to be the vector of both diseases. The patient had antibodies to R. conorii, but a nonspecific serologic technique was used. At the same time, a rickettsial strain was obtained from A. variegatum collected on Guadeloupe and was shown to be a less virulent strain of R. conorii. Unfortunately, as with the isolates of Piiper in South Africa, the strain was not properly identified and was lost. Recently, we reported the first serologically documented case of R. africae infection on Guadeloupe. The patient was bitten by a tick and presented a few days later with an erythema of the right groin associated with a low-grade fever and an erythematous nodular lesion at the bite site.

This report is the first description of the presence of R. africae on Guadeloupe and in the New World. These results were obtained using the shell-vial technique, an established method that detected 13 rickettsial strains, in combination with a modern and easy-to-perform technique, the PCR procedure, which detected 14 additional rickettsia-positive ticks. Moreover, this is the first time that the PCR procedure has been shown to be a more sensitive technique for the detection of rickettsiae in ticks in comparison with the shell-vial technique combined with Gimenez staining. The use of antibiotics in the culture medium cannot explain such a difference because those used were not bacteriostatic. Although the microimmunofluorescence test remains the reference method for the identification of new rickettsiae, the advent of molecular methods has recently enabled the development of useful and rapid methods, such as sequence analysis of PCR products, for the identification of rickettsiae. Identification strategies based on recognition of sequences within the gene encoding for the 16S rRNA gene, the citrate synthase-encoding gene, the rOmpB-encoding gene, the 17-kD protein-encoding gene, or the 190-kD protein RmpA as used in this work, have been described.

We demonstrated in previous studies that an IgG titer ≥ 1:64 is the best cut-off point for detecting R. conorii in endemic areas. In this work, sera were screened from a titer of 1:50 and were considered positive at a titer of 1:100. Although the IF assay is the reference method for detecting antibodies against rickettsiae, cross-reactivity may exist between the members of each group. This was illustrated in this work since cross-reactions were noted in all positive sera. However, the difference between the titers suggested the presence of antibodies against R. africae. Cross-absorption led us to the conclusion that antibodies were directed against R. africae in each serum sample tested. This is a useful technique for the detection of antibodies that are cross-reactive within the rickettsial groups, especially if verified by Western blot. However, the major limitation of this technique is the large amount of antigen needed. Thus, absorption was used only for three randomly chosen human sera.

The prevalence of human antibodies against R. africae reported in this work was much higher than the prevalence of antibodies against spotted fever group rickettsiae reported elsewhere, except in Africa. Recently, Tissot-Dupont and others demonstrated that the presence of Amblyomma ticks in Africa was correlated with a high seroprevalence of antibodies to spotted fever group rickettsiae. These findings may be explained by two factors that condition human exposure to rickettsiae. The first is the rate of infected ticks. In this work, we have demonstrated that 27% of the A. variegatum ticks collected on Guadeloupe harbored R. africae.
Such a high prevalence of infected ticks has to date only been reported in *Amblyomma* collected in Africa.\textsuperscript{45,47,48} The second factor is the capability of ticks to bite humans. *Amblyomma hebraeum* is implicated in outbreaks of African tick-bite fever in Rhodesian troops during the Zimbabwean War of Independence\textsuperscript{49} and in United States troops deployed in Botswana.\textsuperscript{51} Infection among tick-bitten French competitors returning from an adventure race in the Republic of South Africa have also been recently described.\textsuperscript{52} Our results suggest that infection due to *R. africae* may be frequent in Guadeloupe. The fact that the prevalence of human antibodies against *R. africae* was significantly higher in men than in women may be explained by a higher exposure to *A. variagatum* bites in men working in pastures than in women.

The serosurvey of cattle and goats on Guadeloupe demonstrated a high prevalence of antibodies against *R. africae*. As with the serosurvey of humans, our results can be compared with those of the serosurveys conducted among cattle in southern Zimbabwe in which 90% of the sera were positive.\textsuperscript{53} Furthermore, Kelly and others reported experimental infection of cattle with rickettsiae isolated from *A. hebraeum*. Only mild clinical symptoms were produced and a seroconversion was noted. Although the role of cattle as a potential reservoir has been discussed,\textsuperscript{54} the main reservoir of spotted fever group rickettsia is their tick hosts, in which infection is maintained through a transovarial and transstadial transmission.\textsuperscript{1,2} This has been specifically demonstrated for *R. africae* in *A. hebraeum* ticks.\textsuperscript{55}

The present work and a recently reported case\textsuperscript{5} demonstrate a concentration of *R. africae* infections in Guadeloupe. Because infection with *R. africae* is mild, it had been underdiagnosed there. We would like to alert physicians to the presence of African tick-bite fever on Guadeloupe and possibly on all the Caribbean islands where *A. variagatum* ticks are prevalent. Although diagnosis can be established by serology, only a few cases have been reported from African countries lacking the necessary facilities. Documented cases in the West Indies will lead to a better description of this emerging rickettsial disease.

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