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RICKETTSIA AFRICAE IN *AMBLYOMMA VARIEGATUM* AND DOMESTIC RUMINANTS ON EIGHT CARIBBEAN ISLANDS

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ABSTRACT: We used PCRs with *omp*A primers to determine if spotted fever group rickettsiae occurred in *Amblyomma variegatum* from 6 Caribbean islands. Positive amplicons were obtained from ticks from the U.S. Virgin Islands (9/18; 50%), Dominica (39/171; 30%), Montserrat (2/5; 40%), Nevis (17/34; 50%), St. Kitts (46/227; 20%), and St. Lucia (1/14; 7%). Sequences for a convenience sample of reaction products obtained from *A. variegatum* on St. Kitts (7), American Virgin Islands (4), Montserrat (2), and St. Lucia (1) were 100% homologous with that of *Rickettsia africae*, the agent of African tick-bite fever. To determine if transmission of *R. africae* occurred, we used *Rickettsia rickettsii* antigen in IFA tests and found positive titers (≥1/80) with sera from cattle, goats, and sheep from Dominica (24/95 [25%], 2/136 [2%], 0/58 [0%]), Nevis (12/45 [27%], 5/157 [3%], 0/90 [0%]), St. Kitts (2/43 [5%], 1/25 [4%), 1/35 [3%]), and St. Lucia (6/184 [3%] cattle), respectively. No seropositive animals were found in Grenada (0/4, 0/98/, 0/86), Montserrat (0/12, 0/26, 0/52), or Puerto Rico (0/80 cattle). Our study indicates that *R. africae* and African tick-bite fever are widespread in the Caribbean.

Amblyomma variegatum, the tropical bont tick, is a major vector of important human and veterinary pathogens. The tick occurs widely in Africa and was introduced into the Caribbean in the early 1800s (Uilenberg et al., 1984). Initially, it was confined to Guadeloupe, Antigua, and Marie Galante, but in 1948, it invaded Martinique and subsequently spread to 14 other islands (Barre et al., 1995). Despite various eradication programs (Rose-Rosette et al., 1998; Pegram et al., 2004), *A. variegatum* is still widespread in the Caribbean and continues to be a significant threat to human health because it is the vector of *Rickettsia africae*, a spotted fever group (SFG) rickettsia that is the agent of African tick-bite fever (ATBF) (Kelly et al., 1996). This is a common and widespread disease in Africa, which is of particular note for international travelers (Jensenius et al., 2003). *Rickettsia africae* is transmitted transovarially and transtadially by *Amblyomma hebraeum* (Kelly and Mason, 1991) and probably also by *A. variegatum*, which are commonly infected with the organism in Africa (16–75%) (Ndip et al., 2004). Limited data from St. Kitts (Kelly et al., 2002) and Antigua (Robinson et al., 2009) suggest that *A. variegatum* might also be commonly infected with *R. africae* in the Caribbean and that infections might be common in local people (Parola et al., 1999) and tourists (Raoult et al., 2001).

To provide further information on the distribution of *R. africae* in the Caribbean, we studied infections in ticks and domestic livestock on 8 islands.

MATERIALS AND METHODS

Ticks and DNA extraction

Amblyomma variegatum (506 adults) were collected from animals on 6 islands in 2007–2009 (Table I) and identified at the U.S. National Tick Collection (by L.B.), where voucher specimens were deposited (St. Lucia: RML124488–124492, Montserrat: RML124493–124494, Dominica: RML 124495–124501, U.S. Virgin Islands: RML124502, St. Kitts: RML 124504–124506). They were stored in 70% ethanol before DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, California) according to the manufacturer's instructions.

PCR

Aliquots of the DNA were used in PCRs with the primer pair 190-70 and 190-701, which amplifies a 632-base-pair fragment of the outer membrane protein A (*omp*A) gene that encodes a 190-kD outer membrane protein (*rOmp*A) specific for the spotted fever group rickettsiae (Fournier et al., 1998). The PCRs were carried out as previously described (Fournier et al., 1998) and incorporated negative (sterile water) and positive controls (DNA from *Rickettsia conorii* strain 7 [Malish]). Tick DNA samples that were found to be positive were sequenced at the High-Throughput Genomics Unit, Department of Genomic Sciences, University of Washington, Seattle, Washington.

Serology

For serology, whole blood was collected into EDTA from convenience samples of domestic ruminants on 8 Caribbean islands. Approval for the study was obtained from the IACUC of Ross University School of Veterinary Medicine, St. Kitts. After centrifugation, plasma was separated and stored at −20 C until thawed and used for indirect fluorescent antibody (IFA) tests as described previously (Kelly et al., 1991) to detect antibodies reactive with *Rickettsia rickettsii*.

RESULTS

PCR

Positive PCR results were obtained for *Amblyomma variegatum* from each of the 6 islands on which the tick was collected (Table I). Overall, 83 of the 309 (21%) ticks tested were positive; the highest prevalence occurred in Nevis and the U.S. Virgin Islands, and the lowest prevalence was found in St. Lucia. Sequences of convenience samples of positive ticks from St. Kitts (7), American Virgin Islands (4), Montserrat (2), and St. Lucia (1) were all identical to one another (GenBank accession number GU247115) and to other recognized *R. africae omp*A gene sequences submitted to GenBank by Fournier et al. (1998) and Robinson et al. (2009) (GenBank accession numbers: EU622980; U83436).

Serology

Positive IFA results were obtained for 44 of the 403 (10%) cattle examined and 7/442 (2%) of the goats, but none of the sheep (0/301) (Table I).

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TABLE I. Results of PCR analysis of *Amblyomma variegatum* for *Rickettsia africae* using primers for *omp*A and indirect fluorescent antibody testing for antibodies reactive with *Rickettsia rickettsii* in sera from domestic animals on 8 Caribbean Islands.

Island	Results of	
	*omp*A PCR	*R. rickettsii* IFA
Dominica		
A. variegatum	8/92 (9%)	
Cattle		24/95 (25%)
Goats		2/136 (1%)
Sheep		0/58 (0%)
Grenada		
A. variegatum	0/0 (0%)	
Cattle		0/4 (0%)
Goats		0/98 (0%)
Sheep		0/86 (0%)
Montserrat		
A. variegatum	2/5 (40%)	
Cattle		0/12 (0%)
Goats		0/26 (0%)
Sheep		0/52 (0%)
Nevis		
A. variegatum	17/34 (50%)	
Cattle		12/45 (27%)
Goats		5/157 (3%)
Sheep		0/90 (0%)
Puerto Rico		
A. variegatum	0/0 (0%)	
Cattle		0/80 (0%)
Goats		Not done
Sheep		Not done
St. Kitts		
A. variegatum	46/227 (20%)	
Cattle		2/43 (5%)
Goats		1/25 (4%)
Sheep		1/35 (3%)
St. Lucia		
A. variegatum	1/14 (7%)	
Cattle		6/184 (3%)
Goats		Not done
Sheep		Not done
U.S. Virgin Islands		
A. variegatum	9/18 (50%)	
Cattle		Not done
Goats		Not done
Sheep		Not done

DISCUSSION

The number of ticks we obtained from the various islands was consistent with the most recent reports on the distribution of *A. variegatum* in the Caribbean (Pegram and Eddy, 2002; Pegram et al., 2004). No ticks were obtained from Grenada or Puerto Rico, which are regarded as free of *A. variegatum*. Only relatively few ticks were obtained from islands that have hot spots (Montserrat, Dominica, U.S. Virgin Islands) or are partially infested (St. Lucia). Large numbers of ticks, however, were obtained on St. Kitts and Nevis, where infestations are island-wide.

The prevalences of infection of *A. variegatum* with *R. africae* found here are similar to those described previously in Guadeloupe (27%) (Parola et al., 1999), Antigua (62%) (Robinson et al., 2009), St. Kitts (41%) (Kelly et al., 2003), and Martinique (56%) (Parola et al., 2003). Such high prevalences are not unexpected because it is likely, as in the case of *A. hebraeum* (Kelly and Mason, 1991) and other *Amblyomma* (Goddard, 2003), that there is transovarial transmission of *R. africae* in *A. variegatum* and that the tick is the reservoir for infection.

We found relatively low prevalences of antibodies to *R. rickettsii* in the domestic ruminants we studied. Highest prevalences were in cattle and lowest prevalences were in sheep, which reflect the host preference of *A. variegatum* (Barre et al., 1988). Although serology does not enable one to determine the SFG rickettsia responsible for infection, to date, *R. africae* is the only SFG rickettsiae shown to be present in ticks that feed on domestic ruminants in the Caribbean. No SFG rickettsiae have been found in the other tick species commonly found on domestic ruminants in the Caribbean, i.e., *Rhipicephalus* (*Boophilus*) *microplus*, in studies carried out on St. Kitts (Kelly, 2006), Martinique (Parola et al., 2003), and Antigua (Robinson et al., 2009). Higher seroprevalences have been reported in Guadeloupean cattle (81%) and goats (87%) (Parola et al., 1999), but *A. variegatum* is very common on this island, with 36% of herds being infested (Vachiery et al., 2008). Only 1 human serosurvey has been performed in the Caribbean, and a high prevalence of antibodies to *R. africae* (49%) was found in local people from Guadeloupe (Parola et al., 1999).

There is accumulating evidence that *A. variegatum* is commonly infected with *R. africae* in the Caribbean, and this suggests that ATBF might be a significant problem in the area. Although originally considered to be a mild and self-limiting disease, ATBF has recently been associated with more severe clinical signs and prolonged recovery, especially in elderly patients (Roch et al., 2008). Health workers in the Caribbean and those dealing with tourists returning from the region must maintain a high level of suspicion for ATBF in their patients presenting with a history of tick bites and clinical signs of fever, headache, and multiple eschars (Jensenius et al., 2003). Further, vigilance is required to prevent transportation of *A. variegatum* or rickettsemic animals to the American mainlands, because this might enable *R. africae* to become established in these areas. Recent programs to eradicate *A. variegatum* from the Caribbean have met with mixed success (Rose-Rosette et al., 1998; Pegram et al., 2004). The potential impact of *R. africae* on the health of indigenous people and tourists in the West Indies and its potential introduction into the Americas support renewed efforts to eradicate *A. variegatum* from the region.

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