

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

Patrick Kelly, Helene Lucas, Lorenza Beati\*, Charles Yowell†, Suman Mahan‡, and John Dame†

Ross University School of Veterinary Medicine, West Farm Road, Basseterre, St. Kitts and Nevis, West Indies. e-mail: pkelly@rossvet.edu.kn

**ABSTRACT:** We used PCRs with *ompA* 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 africanae*, the agent of African tick-bite fever. To determine if transmission of *R. africanae* occurred, we used *Rickettsia rickettsii* antigen in IFA tests and found positive titers ( $\geq 1/80$ ) 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. africanae* 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 africanae*, 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 africanae* 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. africanae* 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. africanae* 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: RML124495–124501, U.S. Virgin Islands: RML124502, St. Kitts: RML124504–124506). 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 (*ompA*) gene that encodes a 190-kD outer membrane protein (*rOmpA*) 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  $-20^{\circ}\text{C}$  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. africanae ompA* 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).

Received 28 May 2010; revised 17 July 2010, 21 July 2010; accepted 21 July 2010.

\*Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, Georgia 30460.

†Department of Infectious Diseases and Pathology, University of Florida, Gainesville, Florida 32608.

‡Global Clinical Development, Veterinary Medicine Research and Development, Pfizer Animal Health, Kalamazoo, Michigan 49001.

DOI: 10.1645/GE-2552.1

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

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

## ACKNOWLEDGMENTS

This project was funded by grant number 2006-34135-6930 from the U.S. Department of Agriculture to S.M. and J.B.D. through its program for Tropical and Subtropical Agricultural Research (T-STAR) and by Ross University. We thank the following for assistance with collection of samples: J. Mertins and J. Alfred (National Veterinary Services Laboratories, USDA, Iowa), R. Thomas (Ministry of Agriculture, Dominica), B. Louison (Ministry of Agriculture, Grenada), N. Waldron (Ministry of Agriculture, Montserrat), J. H. Urdaz, (Area Emergency Coordinator [AEC], Puerto Rico and U.S. Virgin Islands), T. Challenger, P. Bartlett, L. Henry (Ministry of Agriculture, St. Kitts and Nevis), and G. Joseph (Ministry of Agriculture, St. Lucia). *Rickettsia rickettsii* specimens for IFA were kindly supplied by Dr. G. Dasch, National Center for

Zoonotic, Vector-Borne, and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia.

#### LITERATURE CITED

- BARRE, N., G. I. GARRIS, G. BOREL, AND E. CAMUS. 1988. Hosts and population dynamics of *Amblyomma variegatum* (Acari: Ixodidae) on Guadeloupe, French West Indies. *Journal of Medical Entomology* **25**: 111–115.
- , ———, AND D. CAMUS. 1995. Propagation of the tick *Amblyomma variegatum* in the Caribbean. *Revue Scientifique Technique Office International Epizootic* **14**: 841–855.
- FOURNIER, P.-E., V. ROUX, AND D. RAOULT. 1998. Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein *rOmpA*. *International Journal of Systematic Bacteriology* **48**: 839–849.
- GODDARD, J. 2003. Experimental infection of Lone Star ticks, *Amblyomma americanum* (L.), with *Rickettsia parkeri* and exposure of Guinea pigs to the agent. *Journal of Medical Entomology* **40**: 686–689.
- JENSENIUS, M., P.-E. FOURNIER, P. KELLY, B. MYRVANG, AND D. RAOULT. 2003. African tick bite fever. *Lancet Infectious Diseases* **3**: 557–564.
- KELLY, P. J. 2006. *Rickettsia africae* in the West Indies. *Emerging Infectious Diseases* **12**: 224–226.
- , L. BEATI, P. R. MASON, L. A. MATTHEWMAN, V. ROUX, AND D. RAOULT. 1996. *Rickettsia africae* sp. nov., the etiological agent of African tick-bite fever. *International Journal of Systematic Bacteriology* **46**: 611–614.
- , P.-E. FOURNIER, P. PAROLA, AND D. RAOULT. 2003. A survey for spotted fever group rickettsiae and ehrlichiae in *Amblyomma variegatum* from St. Kitts and Nevis. *American Journal of Tropical Medicine and Hygiene* **69**: 58–59.
- , AND P. R. MASON. 1991. Transmission of a spotted fever group rickettsia by *Amblyomma hebraeum* (Acari; Ixodidae). *Journal of Medical Entomology* **28**: 598–600.
- , ———, L. MATTHEWMAN, AND D. RAOULT. 1991. Seroepidemiology of spotted fever group rickettsial infections in humans in Zimbabwe. *Journal of Tropical Medicine and Hygiene* **94**: 304–309.
- NDIP, L. M., E. B. FOKAM, D. H. BOUYER, R. N. NDIP, V. TITANJI, AND D. H. WALKER. 2004. Detection of *Rickettsia africae* in patients and ticks along the coastal region of Cameroon. *American Journal of Tropical Medicine and Hygiene* **71**: 363–366.
- PAROLA, P., J. ATTALI, AND D. RAOULT. 2003. First detection of *Rickettsia africae* on Martinique, in the French West Indies. *Annals of Tropical Medicine and Parasitology* **97**: 535–537.
- , G. VESTRIS, D. MARTINEZ, B. BROCHIER, V. ROUX, AND D. RAOULT. 1999. Tick-borne rickettiosis in Guadeloupe, the French West Indies: Isolation of *Rickettsia africae* from *Amblyomma variegatum* ticks and serosurvey in humans, cattle, and goats. *American Journal of Tropical Medicine and Hygiene* **60**: 888–893.
- PEGRAM, R. G., AND C. EDDY. 2002. Progress towards the eradication of *Amblyomma variegatum* from the Caribbean. *Experimental and Applied Acarology* **28**: 273–281.
- , L. INDAR, C. EDDI, AND J. GEORGE. 2004. The Caribbean *Amblyomma* Program: Some ecologic factors affecting its success. *Annals of the New York Academy of Science* **1026**: 302–311.
- RAOULT, D., P. E. FOURNIER, F. FENOLLAR, M. JENSENIUS, T. PRIOE, AND J. J. DE PINA. 2001. *Rickettsia africae*, a tick-borne pathogen in travelers to sub-Saharan Africa. *New England Journal of Medicine* **344**: 1504–1510.
- ROBINSON, J. B., M. E. EREMEEVA, P. E. OLSON, S. A. THORNTON, M. J. MEDINA, J. W. SUMMER, AND G. A. DASCH. 2009. New approaches to detection and identification of *Rickettsia africae* and *Ehrlichia ruminantium* in *Amblyomma variegatum* (Acari: Ixodidae) ticks from the Caribbean. *Journal of Medical Entomology* **46**: 942–951.
- ROCH, N., O. EPAULARD, I. PELLOUX, P. PAVESSE, J. P. BRION, D. RAOULT, AND M. MAURIN. 2008. African tick bite fever in elderly patients: 8 cases in French tourists returning from South Africa. *Clinical Infectious Diseases* **47**: e28–e35.
- ROSE-ROSETTE, F., N. BARRE, AND P. FOURGEAUD. 1998. Successes and failures in the tropical bont tick eradication campaigns in the French Antilles. *Annals of the New York Academy of Science* **29**: 349–354.
- UILENBERG, G., N. BARRE, E. CAMUS, M. J. BURRIDGE, AND G. I. GARRIS. 1984. Heartwater in the Caribbean. *Preventive Veterinary Medicine* **2**: 255–267.
- VACHIERY, N., H. JEFFERY, R. PEGRAM, R. APRELON, V. PINARELLO, R. L. KANDASSAMY, M. RALINIAINA, S. MOLIA, H. SAVAGE, R. ALEXANDER, ET AL. 2008. *Amblyomma variegatum* ticks and heartwater on three Caribbean Islands. *Annals of the New York Academy of Science* **1149**: 191–195.