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Detection of *Rickettsia parkeri* from within Piura, Peru, and the First Reported Presence of *Candidatus* Rickettsia andeanae in the Tick *Rhipicephalus sanguineus*

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Abstract

Domestic farm animals ($n = 145$) were sampled for the presence of ectoparasites in northwestern Peru during March, 2008. Ninety domestic animals (62%) were positive for the presence of an ectoparasite(s) and produced a total collection of the following: 728 ticks [*Amblyomma maculatum, Anocentor nitens, Rhipicephalus (Boophilus) microplus, Rhipicephalus sanguineus,* and *Otobius megnini*], 12 lice (*Haematopinus suis*), and 3 fleas (*Ctenocephalides felis*). A *Rickettsia* genus-specific qPCR assay was performed on nucleic acid preparations of the collected ectoparasites that resulted in 5% (37/743, 35 ticks and 2 fleas) of the ectoparasites positive for the presence of *Rickettsia*. DNA from the positive individual ticks was tested with 2 other qPCR assays for the presence of the *ompB* gene in *Candidatus* Rickettsia andeanae or *Rickettsia parkeri*. *Candidatus* R. andeanae was found in 25 *A. maculatum* ticks and in two *Rh. sanguineus* ticks, whereas *R. parkeri* was detected in 6 *A. maculatum* ticks. Two *A. maculatum* were co-infected with both *Candidatus* R. andeanae and *R. parkeri. Rickettsia felis* was detected in 2 fleas, *Ctenocephalides felis,* by multilocus sequence typing of the 17-kD antigen and *ompA* genes. These findings expand the geographic range of *R. parkeri* to include Peru as well as expand the natural arthropod vector of *Candidatus* R. andeanae to include *Rhipicephalus sanguineus*.

Key Words: *Candidatus* Rickettsia andeanae—*Rickettsia parkeri*—Ticks—Peru.

Introduction

Rickettsial diseases have re-emerged as a significant public health threat in South America (Labruna 2009). Evidence of the spotted fever group (SFG) rickettsial infections have been documented from diverse areas of Peru (Schoeler et al. 2005, Forshey et al. 2010), including a 2002 outbreak in Sapillica, Piura, Peru. Serological evidence from villagers during that outbreak showed that the prevalence of immunoglobulin M (IgM) antibody to SFG rickettsiae ranged from 10% to 19% among the human population (Blair et al. 2004a). In addition, a novel species of SFG rickettsiae, *Candidatus* Rickettsia andeanae, was detected from the ticks *Amblyomma maculatum* (Koch) and *Ixodes boliviensis* (Neumann) (Blair et al. 2004b). The purpose of this study was to detect and identify rickettsial agents from within the same area of Peru affected by the 2002 outbreak by collecting ectoparasites from domestic animals and testing for the presence of *Rickettsia*.

Materials and Methods

Sample collections occurred in March, 2008, at 17 sites within 3 districts in the Department of Piura, Peru: Paimas (04°37′49″S and 79°56′85″W), Suyo (04°51′42″S and 80°00′42″), and Tambogrande (04°44′14″L and 80°17′69″W). A variety of domestic animals common to the typical rural farm were examined for external parasites. All visible ectoparasites to the unaided eye were removed with fine forceps, placed in appropriate containers, and later identified to species using morphological keys of Aragão and Fonseca (1961)

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Table 1. Ectoparasites Collected from Domestic Animals

Species	Surveyed	Species of ectoparasites encountered: Rh. sanguineus	A. maculatum	A. nitens	Rh. (B.) micropilus	O. megnini	Ct. felis	H. suis	Total ectoparasites
Cow, *Bos taurus*	13	0	1	0	9	0	0	0	10
Dog, *Canis familiaris*	50	218	37	0	0	0	3	0	258
Goat, *Capra hircus*	9	0	0	0	0	0	0	0	0
Guinea pig, *Cavia porcellus*	2	0	0	0	0	0	0	0	0
Donkey, *Equus asinus*	26	0	11	156	0	0	0	0	167
Horse, *Equus caballus*	25	0	43	117	0	129	0	0	289
Cat, *Felis catus*	2	0	0	0	0	0	0	0	0
Chicken, *Gallus gallus*	1	0	0	0	0	0	0	0	0
Sheep, *Ovis aries*	5	0	0	0	0	0	0	0	0
Pig, *Sus domesticus*	12	3	3	1	0	0	0	12	19
Total	145	221	95	274	9	129	3	12	743

and Guglielmone and Viñabal (1994). Specimens were transported alive to the laboratory, where they were flash-frozen and maintained at −80°C until DNA extraction. Each specimen was surface-sterilized using sequential washes of sodium hypochlorite (0.05%), benzalkonium chloride (0.5%), ethanol (70%), and distilled sterile water. The specimens were then dried on filter paper and cut in half with a sterile scalpel. One half was used for DNA extraction and PCR analysis and the other half stored for future analysis. *Candidatus* R. andeanae and *R. parkeri* were detected by species-specific qPCR, whereas *R. felis* was found by multilocus sequence typing of the 17-kD antigen and *ompA* genes. DNA extraction, PCR, and sequencing methodologies were performed according to Jiang et al. (2005).

Results

A total of 145 domestic farm animals were surveyed, of which 90 animals were found to have ectoparasites (Table 1). From these 90 animals, 743 ectoparasites were collected: 728 ticks, 3 fleas, and 12 lice. Percentages of ectoparasites collected in relation to the domestic animals were the following: 38.9% on horses, 34.6% on dogs, 22.5% on donkeys, 2.7% on pigs, and 1.3% on cows. Cats, chickens, goats, guinea pig, and sheep did not yield any ectoparasites. SFG rickettsiae were detected in ticks and fleas from 22 domestic animals (11/50 dogs, 5/25 horses, 4/26 donkeys, 1/12 pigs, and 1/13 cows), resulting in 5% (37/743) of the total ectoparasite specimens positive for the presence of a SFG rickettsiae. Positive ectoparasites were 2 *Ctenocephalides felis* (Bouncé) fleas and 35 ticks: 34.7% (33/95) of the collected *Amblyomma maculatum* Koch and 0.9% (2/221) of the collected *Rhipicephalus sanguineus* (Latreille). No *Rickettsia* was found in the other 3 tick species collected, *Anocentor nitens* (Neumann), *Rhipicephalus (Boophilus) microplus* (Canestrini), *Otobius megnini* (Dugès), or in the 12 louse specimens, all identified as the hog louse *Haematopinus suis* (Linnaeus). *Candidatus* R. andeanae was found in 27 *A. maculatum* ticks (collected from 7 dogs, 5 horses, 4 donkeys, and 1 cow) and in 2 *Rh. sanguineus* ticks (from 2 dogs) (Table 2). *Rickettsia parkeri* was detected in 8 *A. maculatum* ticks (collected from 4 dogs, 3 horses, and 1 pig). *Rickettsia felis* was detected in the 2 positive fleas, *Ct. felis* (from 2 dogs). Two specimens of *A. maculatum* were found co-infected with *Candidatus* R. andeanae and *R. parkeri* (Table 2).

Discussion

In this study, we identified the presence of 3 species of SFG rickettsiae in ectoparasites collected in northwestern Peru. Two species of the SFG rickettsia, *Candidatus* R. andeanae and *R. felis*, were documented previously (Blair et al. 2004b). We now report the presence of *R. parkeri* in Peru. *R. parkeri* is commonly found in *A. maculatum* ticks in the southeastern United States (Paddock et al. 2010, Varela-Stokes et al. 2011), but an infected *A. maculatum* had not been documented in Peru until now. Two specimens of *A. maculatum* were detected with co-infections of *Candidatus* R. andeanae and *R. parkeri*, a mixed infection of the same tick with 2 species of *Rickettsia* that is not frequently reported in the literature. Also, our findings expand the number of tick species known to be infected with *Candidatus* R. andeanae to include *Rh. sanguineus.* We believe that this is the first report documenting detection of *R. parkeri* from within Peru and the first report of *Candidatus* R. andeanae from *Rh. sanguineus*.

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Table 2. Species of *Rickettsia* Sp. Found by Host And Site

Sites	*Meters above sea level*	*Geographic coordinates*	*Host code*	*Specie of ectoparasites*	Candidatus *R. andeanae*	R. parkeri	R. felis	Candidatus *R. andeanae* +R. parkeri
Paimas	550	04°37′49″L , 79°56′85″W	Dog-1	*A. maculatum*	1			
				A. maculatum		1		
				A. maculatum				1
			Pig-3	*A. maculatum*		1		
			Donkey-6	*A. maculatum*	1			
			Dog-17	*A. maculatum*		1		
				A. maculatum	1			
			Dog-19	*A. maculatum*		1		
				A. maculatum	1			
			Horse-35	*A. maculatum*	1			
				A. maculatum	1			
				A. maculatum	1			
				A. maculatum	1			
			Dog-38	*Ct. felis*			1	
			Dog-39	*Ct. felis*			1	
Jambur	558	04°36′50″L, 79°56′66″W	Donkey-22	*A. maculatum*	1			
			Horse-30	*A. maculatum*		1		
Guir-Guir	558	04°36′50″L, 79°56′66″W	Dog-46	*A. maculatum*	1			
				A. maculatum	1			
Corrales	480	04°37′79″L, 79°59′01″W	Horse-50	*A. maculatum*		1		
				A. maculatum	1			
			Horse-53	*A. maculatum*	1			
				A. maculatum	1			
				A. maculatum	1			
La Tina	422	04°24′22″L, 79°56′32″W	Dog-71	*A. maculatum*	1			
				A. maculatum	1			
			Cow-80	*A. maculatum*	1			
Sarayuyo	526	04°33′05″L, 79°56′08″W	Dog-104	*A. maculatum*	1			
				A. maculatum	1			
			Donkey-108	*A. maculatum*	1			
				A. maculatum	1			
			Donkey-109	*A. maculatum*	1			
Panam GP-VI	206	04°75′06″L, 80°29′12″W	Dog-124	*A. maculatum*	1			
				A. maculatum	1			
Partidor	225	04°73′ 68″L, 80°28′02″W	Horse-128	*A. maculatum*				1
Bolognesi	236	04°68′07″L, 80°25′11″W	Dog-129	*Rh. sanguineus*	1			
Los Laureles	463	04°62′12″ L, 79°01′60″W	Dog-136	*Rh. sanguineus*	1			
Total					27	6	2	2

Author Disclosure Statement

No competing financial interests exist.

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