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Epidemiology of Spotted Fever Group Rickettsioses and Acute Undifferentiated Febrile Illness in Villeta, Colombia

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Abstract. Etiology of acute undifferentiated febrile syndrome (AUFS) is often unknown, leading to inaccurate diagnosis and treatment. Villeta town has been identified as an endemic area for spotted fever group (SFG) rickettsioses but little is known about possible amplifier hosts and other *Rickettsia* species different from *Rickettsia rickettsii*. Besides, few studies have approached other AUFS etiologies in the region. We investigated the role of dengue, leptospirosis, rickettsioses, human anaplasmosis, and Q fever as possible causes of AUFS in patients from Villeta. Sera specimens and ticks from animals as well as ticks from vegetation were studied for the presence of different *Rickettsia* spp. Among 104 sera from patients with AUFS, 16.4%, 24.0%, and 2.9% patients seroconverted to dengue, *Leptospira*, and SFG *Rickettsia*, respectively, with a case of probable coinfection or cross-reaction with *Anaplasma phagocytophilum*. None of the samples were reactive for *Coxiella burnetii*. Sera samples from 74 horses, 118 dogs, and 62 bovines were collected and showed 33.8%, 14.4%, and 50.0% of seroprevalence for SFG *Rickettsia*, respectively. A total of 1,287 ixodid ticks were collected from animals/vegetation and processed in pools for polymerase chain reaction. Among them, 1.7% was positive for *Rickettsia* genes, and *Rickettsia amblyommii*, *R. rickettsii*, and *Rickettsia* spp. were found. These results confirm the circulation of dengue, different SFG *Rickettsia* species and the relevance of other etiologies like leptospirosis and human anaplasmosis. Further studies must identify different epidemiological variables to establish proper surveillance and control programs.

INTRODUCTION

Acute undifferentiated febrile syndrome (AUFS) is defined as fever without a focus of infection on initial physical examination or in basic laboratory tests.[1] Etiologic nature of AUFS has been a challenge everywhere. Although AUFS is common in tropical regions, the specific etiology is often unknown, making accurate diagnosis, treatment, and surveillance very difficult. Villeta town in Colombia has been considered an endemic area for dengue, spotted fever group (SFG) rickettsioses, and recently, for chikungunya and Zika fever.[2,3] Since the first descriptions in 1937, high fatality rates of Rocky Mountain spotted fever (RMSF) have been reported in this region. In the beginning of the 21st century, fatal cases of RMSF and significant seroprevalences against SFG *Rickettsia* spp. in humans and domestic animals have been recorded.[3–7] The relationship and connection between vertebrate species, including humans, through the human–animal–ecosystem interface, could have an impact on human health and should be considered.[8] Nowadays, rickettsioses have generated a serious concern in public health because *Rickettsia* spp. are expanding their geographical distribution, hosts, and vectors leading to zoonotic processes that might affect humans.[9] Recent studies have confirmed the presence of *Rickettsia rickettsii* in *Amblyomma patinoi* ticks collected from cattle in Naranjal village, Villeta.[10] Despite this new evidence, since 2004, there are no new reported cases of SFG rickettsioses in this region. Several authors have confirmed the important role of domestic animals, mainly horses and dogs, as sentinels for monitoring the circulation of rickettsiae in urban areas.[8] A previous study in Villeta analyzed seroprevalence against *Rickettsia* spp. in dogs and horses as a first approach to the dynamics of the infection in domestic animals from this geographic area, showing values of 18.2% and 16.3%, respectively.[7] On the other hand, the role of the small wild mammals as amplifiers of rickettsioses has not been studied in the area. Besides, the circulating *Rickettsia* species in ticks and its possible relationship with the recent disease epidemiology for this area is unknown. In addition, no studies have evaluated the etiology of AUFS, except dengue and recent epidemiological surveys of chikungunya and Zika.[2]

The aim of this study was to approach the probable etiologies of AUFS. Since dengue can be misdiagnosed and/or overlapped with rickettsial syndromes,[11] we investigated (among others) the role of rickettsioses as possible causes of AUFS in patients with presumptive diagnostic of dengue in Villeta, including clinical characteristics, demographics, and epidemiology from a One Health perspective. In addition, sera specimens and ticks from animals as well as ticks from vegetation were studied for the presence of different *Rickettsia* spp.

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MATERIALS AND METHODS

Study area. The study area was Villeta, Cundinamarca, Colombia (5°0′53″N, 74°28′29″W), located at 842 m above sea level and with an annual mean temperature of 26°C, a relative humidity varying between 80% and 97%, and a total area of 140 km^2. According to the 2014 data from the National Department of Statistics (DANE), the estimated population is 25,061 inhabitants, distributed in 22 villages. Agriculture and ecotourism are the predominant economic activities (www.villeta-cundinamarca.gov.co).

Patients. Patients included in the study were those who attended Salazar Hospital (Villeta) with presumptive diagnosis of dengue from October 2011 to March 2013. Sera specimens from acute and convalescent phase (> 15 days and < 2 months from the onset of symptoms) were obtained. Only those patients who had paired samples were included in the study. All of them accepted the informed consent that was approved by the ethical committee from Pontificia Universidad Javeriana.

Serological assays in humans. Sera samples were centrifuged and stored at –20°C for subsequent analyses. The following diagnostic tests were performed: 1) capture enzyme-linked immunosorbent assay (Panbio Diagnostic®, San Diego, CA) for the detection of IgM antibodies against dengue virus in the acute illness phase; 2) microagglutination test (MAT) for five *Leptospira* pathogenic serovars (Icterohaemorrhagiae, Hardjo, Pomona, Grippotyphosa, and Canicola) for the detection of IgG antibodies with a cutoff value of 1:25; 3) indirect immunofluorescence assay (IFA) for the detection of IgG antibodies against *R. rickettsii* (strain Taiaçu) and *Rickettsia amblyommii* (strain Ac37) with a cutoff value of 1:64[12]; 4) IFA using the commercial kit "*Rickettsia* IFA IgG" (Focus Diagnostics, Cypress, CA) for the detection of IgG antibodies of *Rickettsia typhi*, with a cutoff value of 1:64; 5) IFA using the commercial kit "*Anaplasma phagocytophilum* IFA IgG" (Focus Diagnostics) for the detection of IgG antibodies against *A. phagocytophilum*, with a cutoff value of 1:64; 6) IFA using the commercial kit "Q Fever IgG" (Focus Diagnostics) for the detection of IgG antibodies against *Coxiella burnetii* (phase I and II antigens), with a cutoff value of 1:64. Throughout the text, for IFA and MAT, seroconversion was defined as a difference of 4-fold titers between acute and convalescent phases or when a negative sample turned into positive.

Serum samples from domestic mammals. From November to December 2011, domestic animals (dogs, horses, and cattle) were sampled for sera. Sera samples were analyzed by in-house IFA identifying IgG antibodies against *R. rickettsii* (strain Taiaçu). Samples with IgG titers ≥ 64 were considered positive for *Rickettsia*.[7] Reactive sera (*R. rickettsii* antigen) obtained from a dog and a horse in the same town in 2008[7] and a reactive serum for SFG *Rickettsia* spp. (*Rickettsia africae* antigen) obtained from cattle from Caribbean Islands, were used as positive controls.

Ticks from domestic, wild mammals, and vegetation. From November to December 2011, ticks were removed with tweezers from the above-mentioned domestic animals and during July 2012, from wild mammals captured (by Sherman and Tomahawk traps) in 17 villages, previously selected for high seroprevalence against SFG *Rickettsia* in domestic animals and humans.[6,7] Ticks from vegetation were also captured by flagging and dragging methods. Collected specimens were kept in 70% ethanol and further classified by taxonomic keys.[13,14]

Molecular detection of *Rickettsia* species in ticks collected from Villeta. Ticks were grouped by species, stage, locality, and host as main criteria. Females were processed individually and males, nymphs, and larvae in pools of 2–4, 7–12, and 10–20 individuals, respectively. They were processed for DNA extraction using a modified protocol of the commercial kit DNeasy Blood and Tissue (QIAGEN Inc., Valencia, CA) with the addition of guanidine thiocyanate (DNAzol; Invitrogen™, Life Technologies Corp., Grand Island, NY).[15]

DNA extracts were used for molecular detection of *Rickettsia* spp. by conventional and nested polymerase chain reaction (PCR) assays, ensuring 150 ng/μL of DNA per reaction. An initial PCR targeting 16S rRNA mitochondrial tick gene (as internal control) was performed.[16] Positive pools were further tested for rickettsial infection using *ompA* (seminested assay with primers Rr190.70p-Rr190.701n/Rr190.602n)[17,18] and *ompB* (nested assay with primers rompB OF-rompB OR/rompB SFG IF-rompB SFG IR)[19] as PCR target genes. DNA of *Rickettsia slovaca* strain S14ab (from the Center of Rickettsiosis and Arthropod-borne Diseases, CIBIR, La Rioja-Spain) and water were used as positive and negative controls, respectively. All PCR products with expected sizes (532 and 420 base pairs for partial *ompA* and *ompB* genes, respectively) were sequenced, and nucleotide sequences were compared with GenBank data by BLAST analysis. Minimum infection rates for positive pools were calculated as the percentage of the ratio between the number of pools positive for SFG *Rickettsia*, and the total number of individual ticks included in the specific sample.

Data analysis. Official Colombian report documents for dengue from patients included in the study were evaluated. Gender, age, village, symptoms, laboratory analysis (hematocrit, platelets, and leukocytes), were analyzed.

RESULTS

AUFS cases from Salazar Hospital. A total of 104 paired samples (31%) were obtained from 335 patients who attended Salazar Hospital with presumptive diagnoses of dengue during the inclusion period. Seventeen of 104 patients (16.4%) presented only IgM positive for dengue in the acute illness phase. Twenty-five (24%) presented unique seroconversion to one or more pathogenic serovars of *Leptospira*. Individually, the Icterohaemorrhagiae serovar was the most representative one (15 patients). Three patients (2.9%) showed unique seroconversion to SFG *Rickettsia* (one patient for *R. rickettsii* [< 1:64/1:128] and two for *R. amblyommii* [1:64/1:256 and < 1:64/1:128]).

We detected probable coinfection or cross-reaction in 17/104 (16.4%) for dengue/leptospirosis, 6/104 (5.7%) leptospirosis/SFG rickettsioses, 5/104 (4.8%) leptospirosis/SFG rickettsioses/dengue, and 1/104 (0.9%) for SFG rickettsioses/dengue. Likewise, a probable case of human anaplasmosis/dengue coinfection was detected in one sample (0.9%). Curiously, 70/104 (67.3%) and 7/104 patients (6.7%) presented seropositivity to SFG *Rickettsia* (*R. rickettsii* and/or *R. amblyommii*) and *A. phagocytophilum,* respectively, in at least one of the two sera specimens (in

acute or convalescent phase), without evidence of seroconversion in paired samples.

None of the paired samples were reactive for *C. burnetii* (phase I and II antigens) or for *R. typhi*. Finally, for 29/104 samples (27.8%), all the tests were negative. No patients developed clinical complications or death.

A total of 88 Official Colombian report documents for dengue were evaluated from the 104 patients with paired serum samples. For each etiology, we analyzed all patients that had shown unique probable diagnosis for dengue, leptospirosis, and rickettsioses ($N = 45$) and sociodemographic features like gender, age, and area of origin (Table 1). The most common symptoms for the three etiologies were fever, myalgia, headache, arthralgia, and retro-orbital pain. Specifically, two-thirds of patients with SFG rickettsioses (66.67%) showed rash (Table 2). Patients with unique seroconversion for SFG *Rickettsia* presented the following clinical signs: fever, myalgia, nonpurulent conjunctival injection, vomit, and headache, for the *R. rickettsii*-seropositive case; and fever, myalgia, arthralgia, retro-orbital pain, abdominal pain, rash, and headache, for both *R. amblyommii*-seropositive cases.

According to the laboratory results, the initial average hematocrit was 37.2% ± 12.0 for dengue, 39.7% ± 10.2 for leptospirosis, and 31% ± 16.5 for SFG rickettsioses (normal range: 39–50); the average leukocyte counts were 3,282.3 ± 714.3, 4,420 ± 2,626.3 and 3,300 ± 1,473.0 cells/mm^3 (normal range: 4,600–10,600) and platelet counts were 134,352 ± 26,542, 124,300 ± 34,563.6, and 154,666 ± 17,214 cells/mm^3 (normal range: 160,000–380,000), respectively.

Seroprevalence against SFG *Rickettsia* in domestic mammals. Sera samples were collected from 74 horses, 118 dogs, and 62 cows. Among the sera samples from domestic animals, 25/74 from horses (33.8%), 17/118 from dogs (14.4%), and 31/62 from cattle (50%) showed titers equal to or higher than 64, and three of them reached titers of 8,192 (Table 3).

Molecular detection of *Rickettsia* species in ticks. A total of 516 ticks were collected from domestic animals and were identified as follows: *Dermacentor nitens* and *Amblyomma cajennense* sensu lato (s.l.) from horses; *Rhipicephalus sanguineus* s.l., *A. cajennense* s.l., and *Amblyomma ovale* from dogs; and *Rhipicephalus microplus* and *A. cajennense* s.l. from cattle (Table 4). Besides, 13 wild mammals (five *Didelphis marsupialis*, three *Marmosa robinsoni*, three *Mus musculus*, one *Rattus rattus*, and one *Sigmodon hirsutus*) were captured and tick samples were only collected from *D. marsupialis* and classified as *Ixodes luciae* and *Ixodes* spp. (Table 4). A total of 744 ticks were obtained from vegetation and identified as *R. microplus*, *Amblyomma* sp., *A. cajennense* s.l., and *Dermacentor* sp. (Table 4).

As it is showed in Table 5, ticks were grouped into 446 pools. Among them, 358 pools were positive for the 16S rRNA PCR and further screened for *Rickettsia* spp. (*ompA* and *ompB* genes). *Rickettsia* spp. was found in 6/358 pools (1.7%). *Rickettsia amblyommii* (GenBank accession no. KJ433807) was detected in larvae of *R. microplus* (pool M841). In addition, *R. rickettsii* (GenBank accession nos. KJ433802, KJ433806, and KJ433805, respectively) was found in male adults of *D. nitens* (pool M181), in nymphs of *A. cajennense* s.l. (pool M827), and in larvae of *Amblyomma* sp. (pool M822). Moreover, *Rickettsia* spp. were detected in male adults of *A. cajennense* s.l. (pool M235) and a female specimen of *R. microplus* (pool M196), showing 94.9% identity with *Rickettsia conorii* (GenBank accession no. KJ433804) and 99.7% identity with *Rickettsia monacensis* (GenBank accession no. KJ433803), respectively, as highest identities with validly published *Rickettsia* species (Table 5).

TABLE 1

Sociodemographic features of patients with acute febrile syndrome by etiology attended in Villeta town (October 2011–March 2013)

Variables	Dengue *n* (%)	Leptospirosis *n* (%)	SFG rickettsioses *n* (%)
Gender			
Male	9 (53)	11 (44)	2 (66.7)
Female	8 (47)	14 (56)	1 (33.3)
Age (years)			
< 10	6 (35.3)	6 (24)	1 (33.3)
10–20	7 (41.2)	5 (20)	–
21–30	1 (5.9)	5 (20)	1 (33.3)
31–40	3 (17.6)	4 (16)	–
41–50	–	3 (12)	–
51–60	–	1 (4)	–
> 60	–	1 (4)	1 (33.3)
Area			
Urban	11 (64.7)	14 (56)	2 (66.7)
Rural	4 (23.5)	2 (8)	1 (33.3)
Undetermined	2 (11.8)	9 (36)	–
No. of patients	17 (100)	25 (100)	3 (100)

SFG = spotted fever group.

TABLE 2

More frequent symptoms presented in dengue fever, leptospirosis, and SFG rickettsioses from patients attended in Villeta town (October 2011–March 2013)

Symptoms	Dengue fever (%)	Leptospirosis (%)	SFG rickettsioses (%)
Fever	16/17 (94.12)	25/25 (100)	3/3 (100)
Myalgia	13/17 (76.5)	18/25 (72)	3/3 (100)
Headache	11/17 (64.7)	15/25 (60)	3/3 (100)
Arthralgia	10/17 (58.8)	15/25 (60)	1/3 (33.3)
Retro-orbital pain	9/17 (52.9)	13/25 (52)	1/3 (33.3)
Rash	2/17 (11.8)	7/25 (28)	2/3 (66.7)
Vomit	4/17 (23.5)	8/25 (32)	1/3 (33.3)
Abdominal pain	3/17 (17.6)	8/25 (32)	1/3 (33.3)
Red eye illness	3/17 (17.6)	4/25 (16)	1/3 (33.3)
Diarrhea	3/17 (17.6)	4/25 (16)	ND
Jaundice	2/17 (11.8)	ND	ND
Tachycardia	1/17 (5.8)	3/25 (12)	1/3 (33.3)
Hypotension	1/17 (5.8)	ND	ND

ND = no data; SFG = spotted fever group.

DISCUSSION

The probable etiology of AUFS for patients who attended Salazar Hospital from October 2011 to March 2013 was mainly leptospirosis, with 24% of seroconversion, especially against the Icterohaemorrhagiae serovar (60%). In a national study that showed the epidemiology of human leptospirosis from 2007 to 2011, the Icterohaemorrhagiae serovar represented 7.56% of the circulation.[20] Our study evidences the circulation of *Leptospira* serovars and indicates that leptospirosis, a common worldwide zoonotic disease with a predominant presence in tropical regions,[21] is one of the main causes of AUFS in Villeta. Our results of possible etiologies of AUFS are comparable with those from a recent study in the region of Urabá (Antioquia),[22]

TABLE 3

Seropositive dogs, horses, and cattle from Villeta town against *Rickettsia rickettsii* antigen by IFA (IgG titer ≥ 64); November–December 2011

	Horses		Dogs		Cattle	
Village	No. positive /no. tested	Maximum titer	No. positive /no. tested	Maximum titer	No. positive /no. tested	Maximum titer
Alto de Paja	1/2	64	0/5	–	–	–
Alto de Torres	0/3	–	0/5	–	–	–
Bagazal	0/1	–	0/9	–	2/3	64
Balsal	1/3	128	0/2	–	–	–
Chapaima	3/8	64	4/5	4,096	1/4	64
Chorrillo	1/4	64	1/8	64	0/5	–
Cune	0/5	–	1/5	8,192	3/4	64
El Puente	1/4	512	0/6	–	–	–
Ilo Grande	1/6	64	0/6	–	–	–
La Bolsa	3/4	64	0/2	–	0/1	–
La Esmeralda	–	–	0/5	–	0/2	–
La Mazata	0/1	–	1/3	64	–	–
Maní	1/1	128	0/3	–	7/8	64
Mave	1/5	64	3/8	64	0/1	–
Naranjal	2/4	128	1/3	512	6/12	64
Payandé	2/2	64	0/4	–	–	–
Potrero Grande	–	–	1/4	64	2/2	64
Quebrada Honda	–	–	0/4	–	–	–
Río Dulce	–	–	1/3	64	1/1	64
Salitre Blanco	0/3	–	2/11	8,192	2/9	64
Salitre Negro	2/7	128	0/1	–	0/2	–
San Isidro	1/4	128	0/2	–	7/8	128
Urban area	5/7	8,192	2/14	64	–	–
Total (%)	25/74 (33.8)		17/118 (14.4)		31/62 (50.0)	

IFA = immunofluorescence assay.

where *R. rickettsii* cases were confirmed.[23,24] In Antioquia, dengue was probably the main cause of AUFS (37.3%) with higher seroprevalence than the one found herein (16.4%). In addition, percentages of *Rickettsia* infection (2.7%) and coinfection of SFG rickettsiosis/dengue (0.5%) were comparable to those found in our study (2.9% and 0.9%, respectively).[22]

Herein, the continuous circulation of SFG *Rickettsia* spp. in humans and in domestic animals (cattle, horses, and dogs) from Villeta has been confirmed, showing 67.3%, 50.0%, 33.8%, and 14.4% of seroprevalence, respectively. These data suggest that pathogenic and nonpathogenic SFG *Rickettsia* species may cause human diseases or asymptomatic infections in the area. Also, the finding of three patients with unique SFG *Rickettsia* seroconversion is noteworthy; those with *R. amblyommii* seroconversion presented clinical signs previously reported from probable cases of *R. amblyommii* rickettsiosis (i.e., fever, headache, myalgia, and rash)[25]; unfortunately it was not possible to confirm (with molecular or Western Immunoblotting tests) the specific SFG *Rickettsia* species involved in these patients.

In addition, this study represents the second report of probable exposition to *A. phagocytophilum* in Colombia,[26] based on the probable case of infection defined by seroconversion and the seroprevalence found in our study (6.7%), which is lower than that reported in Cordoba.[26] Although *A. phagocytophilum* is typically transmitted by *Ixodes* ticks,[27] which did not represent a huge concern in Latin America,[28] this bacterium has been recently detected in *A. cajennense* s.l.[29] This tick species is present in our area but the finding of *A. phagocytophilum* in *Amblyomma* ticks does not confirm their role as vectors. Besides, to confirm the circulating *Anaplasma* species and its vector,

TABLE 4

Number and species of ticks collected from animals and vegetation in Villeta town (November–December 2011 and July 2012)

		No. of individuals					
Species of ticks	Source	M	F	N	L	Total	*Rickettsia* infection*
Amblyomma cajennense s.l.	Horses	35	26	7	1	69	–
	Dogs	3	–	–	–	3	–
	Cattle	18	19	1	–	38	Positive
	Vegetation	2	2	158	–	162	Positive
Amblyomma ovale	Dogs	1	–	–	–	1	–
Amblyomma sp.	Vegetation	–	–	–	222	222	Positive
Dermacentor nitens	Horses	61	120	50	10	241	Positive
Dermacentor sp.	Vegetation	–	–	–	64	64	–
Ixodes luciae	*Didelphis marsupialis*	14	11	–	–	25	–
Ixodes sp.	*D. marsupialis*	–	2	–	–	2	–
Rhipicephalus microplus	Cattle	9	31	2	–	42	Positive
	Vegetation	–	–	–	296	296	Positive
Rhipicephalus sanguineus s.l.	Dogs	66	47	9	–	122	–

F = female adults; L = larvae; M = male adults; N = nymphs.

* Positive for *Rickettsia ompA* or *ompB* genes.

TABLE 5
Tick pools evaluated for the presence of *Rickettsia* spp. in Villeta town (November–December 2011, and July 2012)

Village	Tick species (source)	Positive pools*/total of pools	Total of ticks (% MIR)	BLAST analysis
Chapaima	*Amblyomma cajennense* s.l. (DA)	0/5		
	Dermacentor nitens (DA)	0/81		
	Rhipicephalus sanguineus s.l. (DA)	0/3		
	Rhipicephalus microplus (DA)	0/1		
Cune	*A. cajennense* s.l. (DA)	0/7		
	D. nitens (DA)	0/11		
	R. sanguineus s.l. (DA)	0/1		
	R. microplus (DA)	0/22		
Mani	*R. microplus* (V)	1/26	229 (0.4)	*Rickettsia amblyommii*†
Naranjal	*A. cajennense* s.l. (DA)	1/43	43 (2.3)	*Rickettsia* sp.‡
	A. cajennense s.l. (V)	1/32	51 (2.0)	*R. rickettsii*§
	Amblyomma ovale (DA)	0/1		
	Amblyomma sp. (V)	1/23	104 (1.0)	*R. rickettsii*¶
	Dermacentor sp. (V)	0/8		
	Ixodes luciae (WA)	0/16		
	Ixodes sp. (WA)	0/2		
	R. sanguineus (DA)	0/1		
Urban area	*A. cajennense* s.l. (DA)	0/12		
	D. nitens (DA)	0/58		
	R. sanguineus s.l. (DA)	0/69		
Salitre Blanco	*A. cajennense* s.l. (DA)	0/5		
	D. nitens (DA)	1/4	10 (10)	*R. rickettsii*‖
	R. microplus (DA)	1/15	16 (6.3)	*Rickettsia* sp.**

DA = domestic animals; MIR = minimum infection rate (no. of positive pools/total no. of individual ticks) × 100; V = Vegetation; WA = wild animals.
* Positive pools for *ompA* or *ompB*.
† Pool "M841": 15 larvae from vegetation positive for *ompA* gene.
‡ Pool "M235": four males from cattle positive for *ompA* gene (94.9% identity with *Rickettsia conorii*).
§ Pool "M827": 10 nymphs from vegetation positive for *ompA* gene.
¶ Pool "M822": 15 larvae from vegetation positive for *ompA* gene.
‖ Pool "M181": three males from a horse positive for *ompB* gene.
** Pool "M196": one female from a cow positive for *ompB* gene (99.7% identity with *Rickettsia monacensis*).

further studies must attempt to isolate it from vertebrate and/or invertebrate hosts.

Herein, *A. cajennense* s.l. was parasitizing the three species of domestic mammals sampled (horses, dogs, and cattle) with the highest levels of infestation in cattle (47.5%). The multihost feeding habits and the anthropophilic behavior of this tick[28,30] represent a high risk of exposition to SFG *Rickettsia* for the human population of Villeta, even more considering that this species has been incriminated as vector of *R. rickettsii* in the region.[4,10] Furthermore, the tick species *A. ovale* and *I. luciae* have been recorded for the first time in this geographical area of Colombia. Previous studies documented the presence of *I. luciae* parasitizing *D. marsupialis* in the Orinoquia region,[31] and *A. ovale* parasitizing dogs in Sucre and Urabá (Antioquia).[32,33]

Molecular evidence of SFG *Rickettsia* spp. in ticks from animals has been previously reported in Colombia.[33–36] In this study, *R. amblyommii* was found in *R. microplus* from vegetation and *R. rickettsii* in *D. nitens* from a horse and also in *A. cajennense* s.l. and *Amblyomma* sp. from vegetation. In the same region, *A. cajennense* s.l. harboring *R. amblyommii* and *D. nitens* infected with *R. rickettsii* as well as the isolation of *R. rickettsii* (strain Villeta) from *A. patinoi* have been previously published.[10,36,37] The detection of *R. microplus* infected with *R. amblyommii* has been previously reported in Panama.[38] The cofeeding phenomenon could explain the association of *R. rickettsii* with *D. nitens* since this tick species and *A. cajennense* s.l. are common parasites of horses in Latin America.[13,39]

Furthermore, our study shows the first detection of SFG *Rickettsia* spp. in cattle from this country, and it is the second report from South America.[40] Specifically, *A. cajennense* s.l. and *R. microplus* removed from cows were found to be infected with *Rickettsia* spp. that showed the highest identities with *R. conorii* (94.9%) and *R. monacensis* (99.7%), respectively. New rickettsial genotypes related to *R. monacensis* had been found in other Colombian areas[34] or in neighboring countries such as Ecuador.[41] Further studies are necessary for a better characterization of these bacteria.

Despite the high lethality caused by *R. rickettsii* 80 years ago,[3] the occurrence of human fatal cases of RMSF in 2003 and 2004,[5] the high rates of seroprevalence for SFG *Rickettsia* spp. in humans and domestic mammals,[5–7] and the recent isolation of *R. rickettsii* from *A. patinoi* ticks,[10] there are no new reports of human cases caused by *R. rickettsii* in this endemic region of Villeta (Cundinamarca). This situation could be explained by misdiagnosis of this illness due to lack of clinical suspicions and of compulsory notification in Colombia, by possible cross-immune protection caused by less pathogenic rickettsiae,[42–44] and by ecological transformations which modulate the epidemiological pattern of the disease.[45] To better understand the latter scenario, further research must identify the vertebrate hosts which sustain the natural cycle of these pathogens (mainly as amplifier hosts) and comprehend the related epidemiological determinants and the effects of environmental changes on the tick–pathogen–vertebrate interface as has been studied for similar diseases.[46]

The results presented herein confirm the relevance of rickettsioses as a differential diagnosis in patients with AUFS and the importance of the clinical suspicion and laboratory confirmation of different etiologies with an early treatment. Nonetheless, other diseases (e.g., leptospirosis, anaplasmosis, and dengue), their interrelationships, and other epidemiological variables should be further studied in the region.

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