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Confirmation of spotted-fever group and typhus group rickettsiosis cases through a three-month hospital-based sentinel surveillance of acute undifferentiated febrile illness in the municipality of Santander de Quilichao, Cauca, Colombia

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Abstract

Introduction: Spotted fever group (SFG) and typhus group (TG) rickettsioses are important causes of acute undifferentiated febrile illness (AUFI). Its diagnosis is not routinely performed; thus, surveillance studies that attempt to identify active cases are highly important. The objective of this study was to conduct an AUFI sentinel surveillance in a region with high seropositivity rates in the Cauca Department, Colombia.

Materials and Methods: Patients with AUFI were recruited over three months in a local hospital in Santander de Quilichao municipality. Demographic and epidemiological data were also collected. Acute and convalescent blood samples were collected from each patient. Complete hemograms, liver function tests, and C-reactive protein (CRP) measurements were conducted. SFG and TG rickettsiosis was screened using an indirect immunofluorescence assay.

Results: A total of 22 febrile patients were included in this study. In six of them (27.3%), SFG rickettsiosis was detected; TG rickettsiosis was detected in four (18.2%) and three (13.6%) patients seroconverted to both *Rickettsia* spp. groups. Additionally, in fourteen patients (93.3%) and eleven patients (73.3%) patients had previous exposure to SFG and TG *Rickettsia* spp., respectively.

Discussion: Active circulation of SFG and TG rickettsioses cases was identified in rural areas of Santander de Quilichao, as well as previous exposure to *Rickettsia* spp., shedding light on a possible important novel endemic region for rickettsioses in Cauca.

Keywords: *Rickettsia*; rickettsiosis; spotted-fever group; typhus group; febrile patients; Colombia.

Confirmación de casos de rickettsiosis del grupo de las fiebres manchadas y del grupo del tifo mediante una vigilancia centinela de tres meses en el hospital para síndrome febril agudo indiferenciado en el municipio de Santander de Quilichao, Cauca, Colombia

Resumen

Introducción: Las rickettsiosis del grupo de las fiebres manchadas (GFM) y del grupo del tifo (GT) son causas importantes de síndrome febril agudo indiferenciado (SFAI). Su diagnóstico no se realiza de manera rutinaria, por lo que los estudios de vigilancia que intentan identificar casos activos son de gran importancia. Esta investigación tuvo como objetivo realizar una vigilancia centinela de SFAI en una región con altas tasas de seropositividad en el departamento del Cauca, Colombia.

Materiales y Métodos: Se reclutaron pacientes con SFAI durante tres meses en un hospital local del municipio de Santander de Quilichao. Se recolectaron datos demográficos y epidemiológicos. Se obtuvieron muestras agudas y convalecientes de cada paciente. Se realizó un hemograma completo, pruebas de función hepática y medición de proteína C reactiva. Las rickettsiosis del GFM y del GT se tamizaron mediante un ensayo de inmunofluorescencia indirecta.

Resultados: Se reclutaron un total de 22 pacientes febriles. En seis de ellos (27,3%) se detectó rickettsiosis del GFM; se detectó rickettsiosis del GT en cuatro (18,2%); y tres (13,6%) seroconvirtieron a ambos grupos de *Rickettsia* spp. Adicionalmente, en catorce pacientes (93,3%) y once pacientes (73,3%) se evidenció exposición previa a *Rickettsia* spp. del GFM y del GT.

Discusión: Se identificó circulación activa de casos de rickettsiosis del GFM y del GT en áreas rurales de Santander de Quilichao; así como exposición previa a *Rickettsia* spp., lo que arroja luces sobre una posible nueva región endémica importante para rickettsiosis en Cauca.

Palabras clave: Rickettsia; rickettsiosis; grupo de las fiebres manchadas; grupo del tifo; pacientes febriles; Colombia.

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Introduction

Rickettsioses are a complex of vector-borne zoonotic infectious diseases caused by several pathogenic *Rickettsia* species[1]. Currently, *Rickettsia* spp. are classified into four groups: the spotted fever group (SFG) and the typhus group (TG), which are the most important for human health[2]. SFG rickettsioses are tick-borne diseases that develop as non-specific febrile illnesses, usually accompanied by a macular or maculopapular rash and sometimes by an inoculation eschar at the site of tick bite, the severity of which depends on the infecting species, with *Rickettsia rickettsii* being the most virulent one[3-5]. On the other hand, TG rickettsioses, which are caused by R. prowazekii and R. typhi, are also non-specific febrile illnesses caused by lice and fleas, respectively; murine typhus, caused by R. typhi, is usually mild, but epidemic typhus, which is caused by R. prowazekii, can be severe and fatal[3,6,7].

Rickettsioses are one of the etiologies of acute undifferentiated febrile illness (AUFI), a syndromic disease characterized by the presence of fever and non-specific symptomatology without any evident focus of infection of less than two weeks of duration which can be caused by a wide range of pathogens[8-10]. Although malaria and dengue fever are probably the main causes of AUFI in several tropical regions worldwide[8,11,12], other emerging and re-emerging etiologies are gaining strength, such as rickettsioses[13].

In recent years, rickettsioses have gained great importance as a more common cause of AUFI than previously thought in several tropical regions worldwide; however, its diagnosis has not been performed routinely in developing countries, since it is only performed in reference laboratories, and two different serum samples obtained from different times are required to confirm the etiology[14,15]. Although a single serum sample with a titer of at least 1:256 from a patient clinically epidemiologically compatible with the disease can be highly suggestive, it cannot confirm the disease[14,15]. Thus, surveillance studies attempting to identify cases of rickettsioses are highly important because this is the only way to identify regions in which human pathogenic *Rickettsia* species are actively circulating, and thus alert local health personnel to consider rickettsioses as a cause of AUFI in the region to be suspected[16,17].

In Colombia, despite the historical data from 1902 until 1986 that have confirmed the presence of rickettsioses in several regions throughout the country[18], to date, they do not form part of the notifiable diseases, and local health personnel are not aware of their importance[19]. More current data have allowed the identification of a few endemic regions throughout Colombia, and few isolated cases of rickettsioses have also been detected in some other regions[19-21]. These data confirm that rickettsioses are widely distributed throughout Colombia, and more importantly, reinforce and improve local health care guidelines, in addition to raising awareness among health personnel about the active circulation of rickettsioses in some specific regions where fatal cases due to rickettsioses occur[19,22].

The Cauca Department, located in the southwestern region of Colombia, is an important location where the proliferation of ticks and their infectious agents can occur due to its climatic and ecological conditions. In this region, human seroprevalence studies have shown 79%, 49%, and 17% seropositivity against *Rickettsia* spp. among the human population of El Tambo, La Sierra, and Rosas municipalities[23,24]. Additionally, further studies have demonstrated the presence of important tick species related to the transmission of rickettsial diseases, such as *Amblyomma patinoi* and other *Amblyomma* spp.[25,26]. Furthermore, we conducted a previous study among human population in eight rural areas from Cauca department, evidencing that Santander de Quilichao was the region with the highest seroprevalence against SFG *Rickettsia* spp. (unpublished data); moreover, data from Cauca's Health Secretary showed a high frequency of AUFI in Santander de Quilichao municipality.

Despite the evidence of previous exposure to *Rickettsia* spp. among the local population, to date, no studies have been conducted in this region to identify the importance of rickettsioses as one of the potential etiologies of AUFI, despite the high number of reported AUFI cases. Thus, to help fill this gap, we aimed to conduct an AUFI sentinel surveillance program in the municipality of Santander de Quilichao, Cauca Department, to identify active cases of rickettsioses.

Materials and Methods

Type of study

Cross-sectional sentinel surveillance study.

Study area

This study was performed in the municipality of Santander de Quilichao (3°00′30″N, 76°29′02″W), located in the northern part of the Department of Cauca, Colombia. Santander de Quilichao municipality comprises a total area of 518 km2, which is distributed across 103 villages. Geographically, it is located 1071 m above sea level, 97 km from Popayán, which is the capital of the Cauca department, and 45 km from Cali, the capital of the Valle del Cauca department. Santander de Quilichao has an annual mean temperature of 26 °C and a relative humidity ranging from 63% to 99%. Its economic activity depends mainly on agriculture, principally coffee, sugarcane, and cassava production (https://santanderdequilichao-cauca.gov.co accessed on December 23rd, 2024). According to the 2018 national population and housing census of the "Departamento Administrativo Nacional de Estadística (DANE)", Santander de Quilichao has a total population of 96,032 inhabitants, of which 47,754 live in the urban area, 10,577 live in suburban settlements, and 37,701 live in rural areas (https://www.dane.gov.co accessed on December 23rd, 2024) (Figure 1).

Febrile patients' recruitment

A sentinel surveillance program on AUFI was conducted over three months (between March and May 2019) at the "Francisco de Paula Santander" Hospital, Santander de Quilichao, Cauca, Colombia. During this time, patients who met the fo-

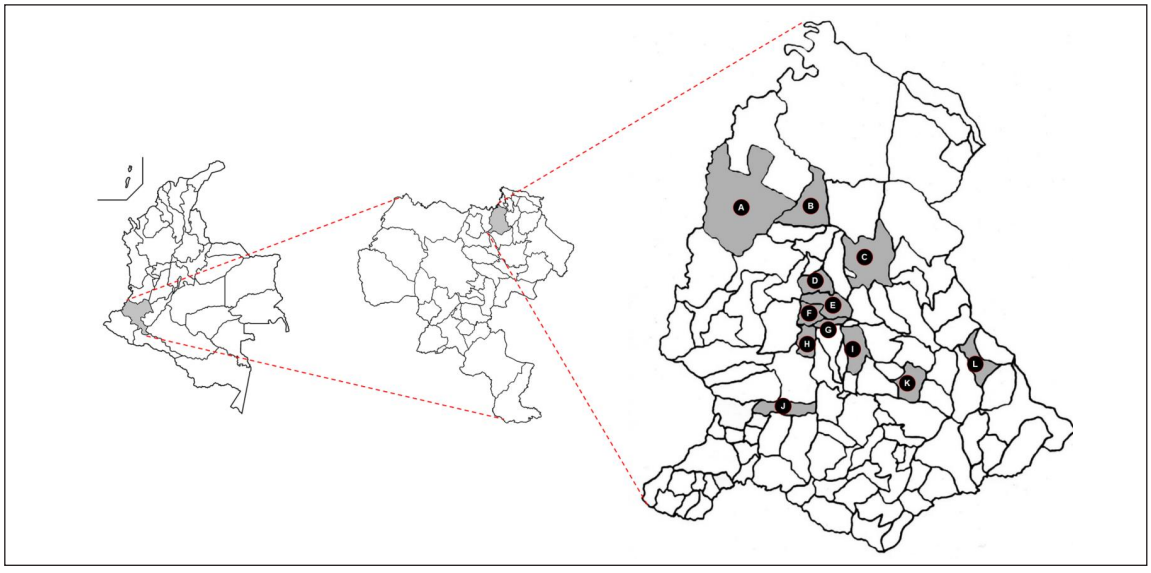

Figure 1. Map showing the location of Santander de Quilichao municipality. Left: Map of Colombia, where the Cauca department is marked in grey. Middle: Map of the Cauca department, where Santander de Quilichao is marked in gray. Right: Map of Santander de Quilichao municipality, where all the patients' villages of origin are marked in gray (A. Lomitas Arriba; B. Taminango; C. Cabecera Municipal; D. Santa Lucia; E. Alegrias; F. Quinamayo; G: Llano de Alegrias; H. El Arca; I. Dominguillo; J. Cachimbal; K. El Aguila; L: La Palomera).

llowing inclusion criteria were asked to form part of the study: be over 18-years-old; attend medical consultation to the emergency department of the recruiting hospital due to a documented febrile illness (body temperature ≥ 37.5 °C), no apparent focus of infection on physical examination or routine basic laboratory tests, and report a recent contact with ectoparasite vectors (ticks, fleas, and/or lice).

Samples and data collection

Demographic, epidemiological, and clinical data were collected from all recruited febrile patients using a survey-type collection instrument that included genre, age, village of origin, animal ownership, ectoparasite (tick, flea, and lice) exposure, and clinical presentation. During emergency attendance, the first acute-phase serum sample and a whole-blood sample were collected from each patient. Fifteen days later, a domiciliary visit was conducted to each recruited patient's homeland to collect a second convalescent phase serum sample. All samples collected were kept at the local hospital laboratory, and monthly, they were sent to the "Bacteriología especial" laboratory at Sciences Faculty from the "Pontificia Universidad Javeriana", Bogotá D.C., Colombia, and stored at -80°C until further processing.

Routine clinical laboratory tests

A complete hemogram, liver function tests, and C-reactive protein measurements were conducted for all recruited febrile patients. Complete hemogram included red blood cell (RBCs), hemoglobin (Hb), hematocrit (Hct), white blood cell (WBCs), neutrophil (Neu), lymphocyte (Lymph), monocyte (Mono), eosinophil (Eos), basophil (Baso), and platelet (Plt) counts. Liver function tests included alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Spotted-fever and typhus group rickettsiosis detection

Screening for SFG and TG rickettsiosis was performed through indirect immunofluorescence assay (IFA) using the Rickettsia IFA IgG commercial kit (Focus Diagnostics, Cypress, CA, USA), which included both *R. rickettsia* and *R. typhi* antigenated slides to detect antibodies against SFG and TG *Rickettsia* spp., respectively. Anti-*Rickettsia* IgG titers were determined by antibody titration, beginning with 1:64 as initial titers, followed by two-fold serial dilutions until the disappearance of fluorescence. A case of SFG or TG rickettsiosis was confirmed when a four-fold seroconversion was observed between acute and convalescent serum samples. Samples in which antibodies against either SFG and/or TG rickettsiae were detected but without a four-fold seroconversion despite the specific antibody titer were only considered as evidence of previous exposure to the bacteria. IFA cannot determine specific species and can cross-react with different *Rickettsia* spp. from the same antigenic group.

Screening of Dengue virus and Leptospira

Furthermore, two common febrile pathogens, dengue virus (DENV) (*Orthoflavivirus denguei*) and *Leptospira* spp., were screened using serological methods. DENV screening was performed using the Bioline Dengue IgG/IgM rapid diagnostic test commercial kit (Abbott, Chicago, USA). *Leptospira* screening was performed using the Panbio Leptospira IgM ELISA commercial kit (Abbott, Chicago, USA). The results obtained from both the procedures were interpreted according to the manufacturer's instructions.

Ethical aspects

All patients who fulfilled the inclusion criteria and voluntarily agreed to participate in the study were asked to provide written signed informed consent. All procedures performed on fe-

brile patients and conservation of obtained biological samples were carried out following the norms established in resolution No. 8430 of 1993 of the Colombian Ministry of Health and the Declaration of Helsinki for ethical and medical research in human subjects. The study protocol and informed consent were approved by the Research and Ethics Committee of the Faculty of Science of the Pontificia Universidad Javeriana in an ordinary session in May 2016. All information provided was treated anonymously using numerical codes for each patient.

Results

A total of 22 febrile patients were recruited during the three-month study period. Most of the recruited patients were women (72.7% [16/22]); 36.4% (8/22) were middle-aged adults (30-45 years old) and older adults (above 45 years old), and the remaining 27.2% (6/22) were young adults (18-29 years old). Twenty-one patients were from 12 small towns located in the municipality of Santander de Quilichao (Figure 1), and the remaining patient was from Gargantillas town, Toribío municipality. All patients owned at least one domestic animal species and dogs were the most common, as all patients (100% [22/22]) reported having at least one dog specimen. Cats were the second most common companion animal identified in 45.6% (10/22) of the patients; other animals included domestic equines, bovines, chickens, and cuckatoos. Exposure to ectoparasite vectors through domestic animals´ infestation of the homeland has also been reported in febrile patients, with ticks being the most common (72.7% [16/22]), followed by fleas (54.5% [12/22]); none of the patients reported to have been exposed to lice. All the patients presented with fever, headache, and malaise, and 81.8% (18/22) presented with arthralgia. None of the patients had fatal outcomes, but 18.2% (4/22) required hospitalization. The additional data are shown in Table 1.

A complete hemogram, measurement of liver enzymes, and C-reactive protein (CRP) levels identified some alterations in some laboratory parameters among recruited febrile patients. CRP was measured in 14 patients, and in 13 of them (92.9% [13/14]), values were above the normal limits (CRP <10 mg/L). All hepatic enzymes were measured in all 22 recruited febrile patients, detecting decreased ALT values in eight patients (36.4% [8/22]) (normal limits 7 – 56 U/L), elevated ALP in three (13.6% [3/22]) (normal limits 44 – 147 U/L), and elevated AST in two (9.1% [2/22]) (normal limits 8 – 38 U/L). Regarding the parameters on complete hemogram: RBCs count was decreased in two patients (9.1% [2/22]) (normal limits: males 4,35 to 5,65 million cells/uL; females 3,92 to 5,13 million cells/uL); Hb was decreased in six (27.3% [6/22]) (normal limits: males 13,8 to 17,2 g/dL; females 12,1 to 15,1 g/dL); Hct was decreased in four (18.2% [4/22]) (normal limits: males 40 to 50%; females 36 to 48%); Plt was elevated in two (9.1% [2/22]) and decreased in one (4.5% [1/22]) (normal limits 150 to 450 thousand cells/uL); and WBCs were elevated in six (27.3% [6/22]) and decreased in one (4.5% [1/22]) (normal limits 4,5 to 11 thousand cells/uL) (Table 1).

Among all recruited febrile patients, in six of them (27.3% [6/22]), SFG rickettsiosis was detected in whom a four-fold seroconversion was observed, which confirmed the diagnosis (Patients No. 5, 10, 12, 15, 16, and 18), and TG rickettsiosis was detected in four patients (18.2% [4/22]), which also had a four-fold seroconversion against TG *Rickettsia* spp. (Patients No. 5, 8, 15 and 16); three of these patients (13.6% [3/22]) seroconverted to both SFG and TG *Rickettsia* spp. (Patient Nos. 5, 15, and 16). Additionally, IgM anti-Leptospira spp. antibodies were detected in two of these patients (Patients No. 5 and 18), and in one Patient No (Patient No. 12) IgG antibodies anti-DENV were also detected. Furthermore, in 14 of the 15 (93.3% [14/15]) remaining febrile patients, previous exposure to SFG *Rickettsia* spp. was detected with IgG antibody titers ranging from 1:64 to 1:512, and in 11 patients (73.3% [11/15]), seropositivity was observed in TG *Rickettsia* spp. with antibody titers ranging between 1:64 and 1:256. IgM antibodies against *Leptospira* spp. were also detected in four of these patients (26.7% [4/15]), as well as IgG antibodies against DENV in six patients who did not seroconvert to either SFG or TG *Rickettsia* spp. (40% [6/15]). More information regarding each patient's specific results is shown in Table 2.

Discussion

Rickettsial diseases are recognized as important causes of AUFI in several regions worldwide. Unfortunately, in some other regions, these diseases remain underrecognized despite the morbidity, mortality, and economic losses that they cause, mainly in poor disadvantaged populations[27,28]. The identification of confirmed cases of rickettsiosis is an important contribution to public health, since with this data, local medical personnel and surveillance systems can be alerted and prepared for possible outbreaks of the disease[27,29]. Thus, to contribute to the knowledge of the distribution of rickettsial diseases, we report confirmed cases of SFG and TG rickettsioses among patients with AUFI from rural areas of Santander de Quilichao municipality, Cauca Department, Colombia. Three of them had seroconversion to both *Rickettsia* groups, suggesting a possible co-infection or, more rarely, a cross-reaction serological result.

In Colombia, from 1902 to 1986, many studies have pointed out the presence of rickettsioses in several regions throughout the country [18]. Since then, only a few studies have been performed to identify four endemic regions: The Tobia valley in the Cundinamarca department, specifically the Villeta municipality[10,30,31], the northern part of the Caldas department[32-34]; the Urabá antioqueño region[10,22,35,36], and the Caribbean region of the Córdoba department[37,38]. It is highly probable that active cases of rickettsioses also occur in other regions of Colombia; however, due to the lack of interest, these remain misdiagnosed and unrecognized.

In Cauca, historical data identified rickettsioses in Florencia, Puerto Tejada, and Silvia municipalities[18]. No additional studies have been performed to date in which active cases

Table 1. Demographic, epidemiological, clinical, and laboratory data of recruited febrile patients.

Patient	Genre	Age	Village	Animal owner	Tick exposure	Flea exposure	Lice exposure	Clinical presentation	Laboratory parameters
1	F	28	Lomitas Arriba	Dog, Horse	No	No	No	Fever, cough, headache, arthralgia, malaise	↑CRP, ↑Mono, ↑Eos, ↓ALT, ↓AST
2	F	72	Dominguillo	Dog	No	No	No	Fever, nausea, vomiting, yellow urine, headache, malaise, cough	↑CRP, ↑Plt, ↑WBCs, ↑Mono, ↓ALT, ↓Hb, ↓Hct
3	M	59	Alegrias	Dog, Cat, Cow, Horse	Yes	No	No	Fever, headache, malaise, abdominal and back pain, arthralgia	↑CRP, ↑ALP, ↑AST
4	F	38	ND	Dog	No	No	No	Fever, malaise, headache, myalgia, arthralgia, rhinorrhea, yellow urine, abdominal and back pain	↑CRP, ↑Plt, ↑WBCs, ↑Neut, ↑Mono
5	M	29	ND	Dog	No	No	No	Fever, malaise, headache, myalgia, nausea, arthralgia, sore throat	↑CRP, ↑WBCs, ↑Neut, ↑Mono
6	F	36	Quinamayo	Dog, Cat, Chicken	Yes	Yes	No	Fever, headache, arthralgia, malaise, sore throat	Within normal parameters range
7	F	36	El Arca	Dog	Yes	Yes	No	Fever, headache, arthralgia, malaise, tachycardia	↓ALT, ↓AST
8	M	38	Quinamayo	Dog, Cat	Yes	Yes	No	Fever, headache, malaise	↓ALT, ↓AST, ↓Plt
9	M	61	Llano de Alegrias	Dog, Cat	Yes	Yes	No	Fever, headache, malaise, back pain	↑Eos, ↓ALT
10	F	27	Cabecera municipal	Dog	Yes	Yes	No	Fever, malaise, headache, chest and back pain	↑CRP, ↑Mono, ↓Hb
11	F	58	Llano de Alegrias	Dog, Cat	Yes	Yes	No	Fever, headache, arthralgia, malaise, back pain, sore throat	Within normal parameters range
12	F	43	ND	Dog, Cat	Yes	Yes	No	Fever, headache, arthralgia, malaise	↑CRP
13	F	30	Cachimbal	Dog, Cat	Yes	No	No	Fever, malaise, headache, diarrhea, arthralgia, back pain	↑CRP, ↓Lymph
14	F	44	ND	Dog	Yes	No	No	Fever, headache, arthralgia, malaise, diarrhea, abdominal pain, nausea, vomiting	↑CRP, ↑ALP, ↑AST, ↓Hb, ↓Hct
15	F	40	Gargantillas*	Dog, Cat	Yes	Yes	No	Fever, headache, arthralgia, malaise, chest and back pain, cough	↑RBCs, ↑Hb, ↑Hct, ↑Mono, ↓ALT
16	M	48	Taminango	Dog	Yes	Yes	No	Fever, headache, malaise, nausea, arthralgia, dizziness	↑Mono, ↓ALT, ↓AST, ↓RBCs, ↓Hb, ↓Hct
17	F	18	ND	Dog	No	No	No	Fever, rash, headache, arthralgia, malaise	↑CRP, ↓Hb, ↓WBCs, ↓Neut, ↓Lymph
18	F	62	El Aguila	Dog	Yes	Yes	No	Fever, malaise, headache, arthralgia, abdominal and back pain, dysuria, oliguria	↑CRP, ↑ALP, ↑WBCs, ↑Lymph, ↑Mono
19	M	29	ND	Dog	No	No	No	Fever, abdominal pain, malaise, diarrhea, nausea, headache, arthralgia, dizziness	↑CRP, ↑WBCs, ↑Neut, ↑Mono
20	F	52	La Palomera	Dog, Cat, Cockatoo	Yes	Yes	No	Fever, headache, arthralgia, malaise, sore throat	↓ALT
21	F	19	Santa Lucia	Dog	Yes	Yes	No	Fever, malaise, headache, arthralgia, eye pain	↑Hb, ↓Lymph
22	F	70	ND	Dog, Cat	Yes	No	No	Fever, malaise, headache, arthralgia, abdominal pain	↑CRP, ↑WBCs, ↑Mono, ↓RBCs, ↓Hb, ↓Hct, ↓Plt

* From Tacuello municipality. ALP Alkaline Phosphatase; ALT Alanine Aminotransferase; AST Aspartate Aminotransferase; Baso Basophils; CRP C-Reactive Protein; Eos Eosinophils; Hb Hemoglobin; Hct Hematocrit; Lymph Lymphocytes; Mono Monocytes; ND No Data; Neut Neutrophils; Plt Platelets; RBCs Red Blod Cells; WBCs White Blood Cells.

Table 2. Results for *Rickettsia* spp., *Leptospira* spp and Dengue virus screening.

Patient	SFG *Rickettsia* spp. IgG IFA		TG *Rickettsia* spp. IgG IFA		*Leptospira* IgM ELISA	DENV IgM/IgG ELISA
	Acute sample	Convalescent sample	Acute sample	Convalescent sample		
1	1:256	1:256	Negative	Negative	Negative	Negative
2	1:256	1:256	1:64	1:64	Negative	Negative
3	1:128	1:128	1:64	1:64	Positive	Negative
4	1:512	1:512	Negative	Negative	Negative	IgG
5	**1:128**	**1:512**	**1:64**	**1:256**	Positive	Negative
6	1:256	1:256	1:64	1:64	Negative	Negative
7	1:512	1:512	1:256	1:256	Positive	IgG
8	1:512	1:512	**1:64**	**1:256**	Negative	Negative
9	1:64	1:64	1:64	1:64	Negative	Negative
10	**1:256**	**1:1024**	1:256	1:256	Negative	Negative
11	1:512	1:512	1:64	1:64	Negative	IgG
12	**1:256**	**1:1024**	1:256	1:64	Negative	IgG
13	1:128	1:128	Negative	Negative	Negative	IgG
14	1:256	1:256	1:256	1:256	Positive	Negative
15	**1:256**	**1:1024**	**1:256**	**1:1024**	Negative	Negative
16	**1:256**	**1:1024**	**1:256**	**1:1024**	Negative	Negative
17	Negative	Negative	Negative	Negative	Negative	Negative
18	**Negative**	**1:512**	Negative	1:128	Positive	Negative
19	1:256	1:256	1:256	1:256	Negative	Negative
20	1:256	1:256	1:128	1:128	Positive	Negative
21	1:128	1:128	1:128	1:128	Negative	IgG
22	1:256	1:256	1:256	1:256	Negative	IgG

Antibody titers in bold refer to cases in which seroconversion was evidenced.
DENV Dengue Virus; ELISA Enzyme Linked Immunosorbent Assay; IFA Indirect Immunofluorescence Assay; IgG Immunoglobulin G; IgM Immunoglobulin M; SFG Spotted Fever Group; TG Typhus Group.

of SFG and TG rickettsioses among rural inhabitants from Santander de Quilichao municipality have been identified. Previous exposure to *Rickettsia* spp. in the region has only been observed in three municipalities: El Tambo, La Sierra, and Rosas[23,24,39]. Specifically, in Santander de Quilichao, the only available data among the human population to date is the record of seropositivity against *Orientia* spp. in 12.8% of rural inhabitants from the two villages[40]. In the present study, antibodies against SFG and TG *Rickettsia* spp. were detected in more than 80% of the recruited patients, suggesting that a large number of inhabitants from the region are exposed to *Rickettsia* spp.

Two additional studies have been performed in the region on fleas and ticks collected from domestic animals to identify pathogenic *Rickettsia* species hosts[26,41]; however, none of the recognized human pathogenic species have been detected. It is highly probable that pathogenic *Rickettsia* spp. could infect other hosts, such as synanthropic rodents or wild animal species and their ectoparasites, for which data in the regions remain unavailable.

For several years, rickettsial diseases have been ignored and misdiagnosed as other etiologies of AUFI[13,42]. Furthermore, they are considered rare and exotic vector-borne diseases, only transcendental for poor and marginalized populations, and are of little relevance to public health priorities[17]. Unfortunately, laboratory diagnosis of rickettsioses poses several difficulties, such as the requirement of a fluorescence microscope that is unavailable in rural areas owing to its high cost, leading to underreporting of cases and impacting the true and real importance of rickettsioses for public health[16]. However, owing to climate change, overpopulation, and changes in land-use patterns, rickettsial diseases have gained immense importance, and in some countries, they are mandatory notifications[27,43,44]. Unfortunately, in Colombia, they still do not form part of the compulsory reporting of diseases despite efforts to identify active cases of these diseases in several regions[19]. Thus, research studies focused on the identification of confirmed cases are still necessary because this is the only way to determine whether AUFI in some regions could be caused by these pathogens until their inclusion within the notifiable diseases for the national local surveillance system.

In conclusion, the present study describes the presence of confirmed active cases of SFG and TG rickettsioses among patients with AUFI from rural areas of the municipality of Santander de Quilichao in Cauca department. Previous exposure to both SFG and TG *Rickettsia* spp has also been identified. These data reinforce the importance of rickettsial diseases as etiologies of AUFI in the region and shed light on a possible important novel endemic region for these diseases in the department of Cauca. Further studies are needed to establish the specific pathogenic species related to these cases and determine the seroprevalence among local populations to determine the burden of disease.

Ethical considerations

The present study was supported by call No. 744 of 2016 for research projects of Science, Technology and Innovation in Health from Minciencias through grant number 120374455209, and by call No. 727 of 2015 for national doctoral students from the same institution.

Protection of persons. All the management and procedures performed on febrile patients, and conservation of biological samples obtained from them were carried out following the norms established in the resolution No. 8430 of 1993 of the Colombian Ministry of Health and the declaration of Helsinki for ethical and medical research in human subjects, available https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/

Protection of vulnerable populations. Do not apply

Confidentiality. The protocol and informed consent were approved by the Research and Ethics Committee of the Faculty of Science of the Pontificia Universidad Javeriana in an ordinary session from May 2016. Each recruited patient provided written informed consent.

Privacy. All the information provided was treated anonymously using numerical codes for each recruited patient.

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Conflict of interests. The authors declare no conflicts of interest. All authors have read and approved the submitted manuscript. The authors declare that the article has not received prior publication and is not under consideration for publication elsewhere. On behalf of all the co-authors, the corresponding author shall bear full responsibility for submission.

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