

## Outbreak of Rocky Mountain spotted fever in Córdoba, Colombia

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*Rocky Mountain spotted fever (RMSF) is a tick-borne disease caused by the obligate intracellular bacterium Rickettsia rickettsii. Although RMSF was first reported in Colombia in 1937, it remains a neglected disease. Herein, we describe the investigation of a large cluster of cases of spotted fever rickettsiosis in a new area of Colombia.*

Key words: *Rickettsia rickettsii* - outbreak - Colombia

*Rickettsia rickettsii* is a tick-borne intracellular bacterium that causes a febrile illness known as Rocky Mountain spotted fever (RMSF) in North America; this disease is known by different names according to its geographical location in Latin America (Walker 1989, 2007, Walker & Fishbein 1991, Horta et al. 2007). RMSF was first reported in Colombia in 1937 by Luis Patiño (Patino et al. 2006); it was named Tobia fever after the village where these cases occurred. The disease remained forgotten until 2003 when two fatal cases were identified and reported in Villeta, a locality near Tobia (Hidalgo et al. 2007a, b). A renewed interest in rickettsioses might have contributed to the identification of an outbreak of RMSF in 2005 among military personnel stationed at a remote base in Antioquia, a state bordering with Panama (Acosta et al. 2006). These reports defined the re-emergence of the disease in Colombia and alerted the systems of surveillance across the country. In this report, we describe the clinical and microbiological findings of a new outbreak of spotted fever rickettsiosis in a rural locality of the state of Córdoba on the northern coast of Colombia.

Between February-March of 2007, four fatal cases following a febrile syndrome of unknown aetiology were reported in the municipality of Los Córdoba, Córdoba, Colombia. Seven additional non-fatal cases were subsequently identified based on the following case definition: resident of the village of Los Córdoba presenting with fever  $\geq 38^{\circ}\text{C}$ , no documented origin of the fever (through either clinical or laboratory tests) and any of the following: headache, chills, malaise, myalgia, arthralgia, dizziness, fatigue, anorexia, nausea, vomiting, abdominal pain, diarrhoea or respiratory distress.

Los Córdoba is a municipality within the department of Córdoba near the Caribbean Sea on the northern coast of Colombia. This municipality has an altitude range of

20-100 m above sea level, is covered by tropical dry forest vegetation, has an average temperature of  $24^{\circ}\text{C}$  and receives between 1,000-2,000 mm<sup>3</sup> of rain per year.

The local laboratory first ruled out malaria with a thick smear. Indirect immunofluorescence antibody (IFA) tests for IgM against *R. rickettsii* and *Rickettsia typhi* (Focus Diagnostic, Cypress, CA, USA) were performed at University of Córdoba. Samples were also tested at The Colombian National Institute of Health (INS) for evidence of infection with *Leptospira* (E-LEP01M - *Leptospira* IgM Panbio diagnostic), dengue virus (E-DEN01M - Dengue IgM Capture Panbio), hepatitis A and B (Murex HBs-Ag Abbot Murex 9F80-01 and Murex anti-HAV IgM Abbot Murex 3M46-01) and spotted fever group *Rickettsia* (IFA for IgM and IgG). Tissues obtained from autopsies were analyzed at INS by reverse transcription polymerase chain reaction (PCR) for dengue and yellow fever virus and by immunohistochemistry for yellow fever (primary antibody dilution 1:1000, INS cat: v525-701-562), adenovirus (primary antibody dilution 1:125 Chemicon MAB805), influenza (primary antibody dilution 1:50 Chemicon AB1074F) and hepatitis B (primary antibody dilution 1:50 Novocastra NCL-HBsAg-2). Samples were also analyzed at the University of Texas Medical Branch for the detection of hantavirus (Caño Delgadito and Maporal by serology) and rickettsiae (by immunohistochemistry for spotted fever group rickettsiae in tissue samples). A case of spotted fever group rickettsiosis was identified as positive when the difference in anti-*Rickettsia* IgG titres between acute and convalescent sera was at least two-fold or when rickettsial antigen was detected by immunohistochemistry (Chapman et al. 2006). We used rabbit IgG anti-spotted fever group *Rickettsia* (which does not cross-react with typhus group *Rickettsia*) as the primary antibody, a biotinylated goat anti-rabbit IgG secondary antibody (Jackson Immunoresearch), an ABC system to detect the biotinylated antibody, and DAB to develop the peroxidase reaction (Vector Laboratories).

A frequency analysis was conducted using percentages. Mortality rates were calculated as the cumulative incidence of disease or death among suspicious cases and

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the attack rate was calculated as confirmed cases among the population exposed. Twenty patients met the criteria of our case definition. Four of them died and the diagnosis was confirmed by immunohistochemistry (mortality rate of 36%). Seven other non-lethal cases were confirmed by IFA (Table). Thus, the attack rate was 1.25% (11 cases in a local population of 874 individuals). The most frequent symptoms, occurring among eight of the 11 confirmed cases, were fever, headache and myalgia. Rash was infrequent (18%). Among clinical laboratory test results, neutrophilia was the most frequent (60%), followed by leukocytosis (50%) and thrombocytopenia (40%).

One sample from a confirmed lethal case of rickettsiosis had IgM antibodies against dengue virus; IgM antibodies against *Leptospira* were found in five other sera samples. Analyses for hantavirus, hepatitis virus A and B, and yellow fever were all negative. The four fatal cases were clinically diagnosed as dengue and did not receive effective anti-rickettsial therapy; they all died between the 6-12th day of illness. The histopathological analysis of all visceral tissues from autopsies (stained with hematoxylin and eosin) showed vascular congestion, widespread non-occluding thrombi in small vessels and perivascular mononuclear cell infiltrates around small vessels. The immunohistochemical analysis for spotted fever group *Rickettsia* showed multifocal positivity in the endothelium of all vascular vessels. Antigen was also detected in some monocytes and hepatocytes.

RMSF is a zoonosis widely distributed throughout the Americas. It is present in endemic foci with sporadic, and often seasonal, outbreaks (Raoult & Roux 1997, Rizzo et al. 2004). The outbreak reported here occurred in an area that is approximately 190 km from the site of another outbreak that occurred in 2005. Interestingly, both outbreaks occurred during the dry season (February-April), suggesting that vector activity might be season dependent; this is an aspect that begs further investigation. The laboratory methods used here preclude the

exact identification of the species of *Rickettsia* involved because our investigation was limited to serological methods, which are cross-reactive for rickettsiae of the same group. Thus, we only know that a spotted fever group *Rickettsia* was involved. However, the high mortality of this outbreak is consistent with *R. rickettsii*. The clinical characteristics of the cases described here were not different from those previously described in other areas of Colombia endemic for RMSF (Acosta et al. 2006, Hidalgo et al. 2007a, b). The high mortality rate observed in Los Cordobas, 4/11 (36%) was associated with misdiagnosis and a lack of appropriate antibiotic treatment. These data highlight the importance of early clinical suspicion of RMSF and prompt initiation of appropriate anti-rickettsial therapy to prevent serious complications and mortality (Chapman et al. 2006). One limitation of our report is that the species of *Rickettsia* involved in the outbreak was not characterized with molecular methods. In the future, it will be important to survey ticks of this region for infection with rickettsiae and to identify the species of *Rickettsia* through sequencing of informative gene fragments amplified by PCR.

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TABLE

Antibodies titers against spotted fever group *Rickettsia* by indirect immunofluorescence assay (*Rickettsia rickettsii* used as antigen) in paired serum samples of confirmed non-lethal cases

Patient number	IgM titer		IgG titer	
	First sample	Second sample	First sample	Second sample
1	Negative	NT	Negative	1:512
2	Negative	NT	Negative	1:1024
3	1:64	1:1024	NT	NT
4	Negative	NT	1:256	1:1024
5	Negative	NT	Negative	1:128
6	1:128	1:512	NT	NT
7	Negative	NT	1:256	1:1024

NT: not tested.