Prevalence of Antibodies Against Spotted Fever Group Rickettsiae in a Rural Area of Colombia

Marylin Hidalgo, Ricardo Sánchez, Leonora Orejuela, Jorge Hernández, David H. Walker, and Gustavo Valbuena*

Grupo de Microbiología, Instituto Nacional de Salud, Bogotá, Colombia; Facultad de Medicina, Universidad Nacional de Colombia Bogotá, Colombia; Secretaria de Salud de Cundinamarca, Villeta, Colombia; Department of Pathology, University of Texas Medical Branch, Galveston, Texas

Abstract. We recently rediscovered Rocky Mountain spotted fever in Villeta, Colombia, near the same locality (Tobia) where it was first recognized in 1937. To have a better idea of the magnitude of this problem, sera from 392 randomly recruited healthy adults from Villeta were analyzed by indirect immunofluorescent antibody assay to detect IgG against *Rickettsia rickettsii* as antigen. The seropositivity rate for spotted fever group rickettsiae was 40.3%. We did not find any association between the presence of antibodies to spotted fever group rickettsiae and several demographic and epidemiologic variables, which could be a reflection of unique features of this area.

INTRODUCTION

Rickettsial spotted fever in South America was first described in 1931 in Sao Paulo, Brazil. Since then, serologic evidence of *Rickettsia rickettsii* infections has been documented in other Brazilian localities.1,2 In Colombia, Patiño and others3 described this disease for the first time in 1937; his group identified the etiologic agent (*R. rickettsii*) and described this entity as an epidemic disease with high mortality in a confined area, Tobia, state of Cundinamarca. After this initial description, no other cases were reported from Colombia in the international literature until recently, when we identified two fatal cases of spotted fever caused by *R. rickettsii*.4

The purpose of this study was to determine the prevalence of antibodies against spotted fever group rickettsiae (*R. rickettsii* as antigen) in rural inhabitants of Villeta, a small town near Tobia, Cundinamarca, Colombia, and its relationship with several demographic and epidemiologic variables.

MATERIALS AND METHODS

**Study area and design.** The study area, Villeta, Cundinamarca, Colombia (5°0’53” N, 74°28’29” W), is 842 m above sea level (Figure 1) and has an annual mean temperature of 26°C, with a relative humidity of 80–97%. According to the 1993 census report of the National Department of Statistics in Colombia (Departamento Nacional de Estadísticas [DANE]), the estimated population in the rural area is 16,781 people, distributed in 22 villages. The predominant economic activity is agriculture.

The sample size was obtained after considering the following factors: total number of the rural population (9,000 adults), geographical distribution of the population, a prevalence probability of 5–15%, a 15% rate of no response, and a confidence level of 95%.

**Samples.** Blood specimens from 423 persons were collected in January and February 2005. A structured questionnaire with the following information was obtained from all volunteers: age, sex, occupation, location of residence, medical history, and history of contact with domestic animals, rodents, and arthropods. Contact with the three different stages of the vector (larvae, nymph, adult) was assessed by showing resin-preserved tick specimens. In the statistical analysis, this information was analyzed as independent variables. Cases were rejected if they had a history of bleeding disorder, had fever at the time of interview, or had mental conditions that would preclude answering the questionnaire. For the analysis, cases were also rejected whenever the questionnaire was not completely filled and/or a serum sample was not obtained.

**Serology.** Serum samples were tested with two-fold serial dilutions (1:64 to 1:2,048) by indirect immunofluorescence assay (IFA) for the presence of IgG-specific antibodies using slides containing antigen of *R. rickettsii* (Sheila Smith strain). After incubation of the serum dilutions for 30 minutes at 37°C in a moist chamber, the slides were washed with phosphate-buffered saline (PBS). The human antibodies were detected with fluorescein (FITC)-conjugated, affinity-purified, goat anti-human IgG, Fc fragment-specific (Jackson Immuno-Research Laboratories, West Grove, PA) at a 1:800 dilution (as determined by checkerboard titration). The slides were incubated at 37°C for 30 minutes in a moist chamber and washed again as previously described. Evan blue (Sigma Chemical Company, St. Louis, MO) was added as a counterstain. Coverslips were mounted in glycerol-PBS (9:1) buffer, pH 9.0, and slides were examined using an ultraviolet microscope with filters for fluorescein. Positive and negative control sera were obtained from Focus Technologies (IF0112 and IF0115, respectively; Cypress, CA). Intense fluorescence of the rickettsiae within the Vero cells at 1.64 dilution was considered to be a positive reaction. In negative reactions, the cells were tinted red and did not display any fluorescence.

**Statistical analysis.** Prevalence of seropositivity and its association with the independent variables were evaluated using the software Stata 9.0 to account for the sampling design. Two-tailed statistical significance was set at α < 0.05. The association between seropositivity and independent variables was determined using logistic regression. To evaluate strength of association, odds ratios and their 95% confidence intervals were calculated.

* Address correspondence to Gustavo Valbuena, Department of Pathology, the University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0609. E-mail: gvaluen@utmb.edu
RESULTS

Of the 423 samples collected, 371 (87.7%) had all the requirements to be included in the study; the rest of the samples, 52, were rejected. IFA analysis showed that, of 371 tested sera, 149 (40.3%) contained IgG antibodies that reacted with R. rickettsii (95% CI, 0.36–0.45). Fifty-four (14.6%) showed an endpoint titer of 1:64, 68 (18.4%) showed a titer of 1:128, 22 (5.9%) showed a titer of 1:256, and 4 (1.1%) showed a titer of 1:512. The maximum titer observed was 1:1,024 in one sample (0.27%; Table 1).

All samples were obtained from adults. The median age was 48.9 years (range, 18–86 years). The median age of those with no detectable anti-Rickettsia IgG was 50.6 years, whereas the median age of persons with positive titers was 45.3 years (not significantly different, \( P = 0.095 \)). Women made up 58.3% of the population. Of those having a positive IFA test, 57.7% were women (the difference between men and women was not statistically significant). Among all the cases studied, 70.94% were exposed to ticks as indicated by a history of tick bites or observation of ticks on pets or in the households. As another indicator of exposure, 88.4% of the volunteers recognized the adult stage of ticks, but only 59.4% documented contact with other stages of ticks or the ability to identify adult ticks. According to this criteria, 68.5% of those with no detectable anti-Rickettsia IgG were exposed to ticks, whereas 74.5% of those with a positive IFA test were exposed (no statistically significant difference: \( P = 0.357 \)).

We did not find any association between the presence of antibodies and occupation (\( P = 0.8 \)), education level (\( P > 0.05 \)), building material of the house (\( P = 0.5 \)), ownership of domestic animals or free circulation of domestic animals in and out of the house (\( P = 0.6 \)), time of residence in the area (\( P > 0.05 \)), or number of people living in the house (\( P > 0.05 \)). Seropositivity was less frequent among those who reported previous contact with the nymph (OR, 0.53; 95% CI, 0.32–0.89) or larval stages (OR, 0.59; 95% CI, 0.20–1.66).

DISCUSSION

This study shows that the prevalence of antibodies against spotted fever group rickettsiae (R. rickettsii was used as antigen) in a rural area of Colombia (where lethal cases have recently been reported) was 40.3%. Previous studies have shown seroprevalence rates between 1.4% in Western Australia\(^7\) and 10.4% in Gag Island, Indonesia.\(^8\) In South America, antibodies against spotted fever group rickettsiae were detected in 4.2% of the samples in an area of Brazil that was considered endemic for rickettsiosis.\(^9\) In the subtropical territory of Jujuy, Argentina, a serosurvey of asymptomatic
subjects revealed antibodies reactive with spotted fever group rickettsiae in 4%,10 In Yucatan, Mexico, the seroprevalence was ~5%.11 Our reported seroprevalence is higher than any other published to date in South America (even in the endemic zones) and only compares to Croatia, where a seroprevalence of 43.7% was observed among suburban and rural populations.12

The studied population was mainly rural, with agriculture as their main economical activity. These activities are by themselves considered risk factors for tick parasitization, and therefore, for rickettsial infection. Thus, it is not surprising that most people reported contact with adult stages of ticks. We did not observe an association between seropositivity and several epidemiologic factors. Interestingly, the seroprevalence was lower in people reporting contact with the immature stages of ticks. This awareness may correlate with a tendency to remove the immature stages and thus decrease the chances of acquiring a rickettsial infection. Despite their continuous contact with ticks, people of this rural region of Colombia do not recognize every stage as a part of the natural life cycle of the same animal, considering every stage as a different species.

It should be emphasized that there is strong cross-reactivity among rickettsial species when using IFA as an antibody detection technique. This cross-reactivity has been previously reported for several rickettsial species13 and can overestimate the seroprevalence against a single species. Thus, in the future, we will study the possibility of the circulation of a different spotted fever group *Rickettsia*, other than *R. rickettsii*, such as *R. parkeri*, *R. amblyommii*, *R. akari*, or *R. felis*.

In conclusion, seroprevalence against spotted fever group rickettsiae in the rural area of Villeta, Cundinamarca, Colombia, is very high, identifying this area as an endemic zone for exposure to these rickettsiae. Appropriate public health interventions will be necessary to decrease the morbidity and mortality associated with these severe infectious diseases.

Received March 15, 2007. Accepted for publication May 14, 2007.

Financial support: This research was supported by Grant 1204-04-16332 from Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología Francisco José de Caldas, Colciencias to G. Valbuena.

Disclaimer: We do not have conflicts of interest related to this article.

Authors’ addresses: Marylin Hidalgo, Instituto Nacional de Salud, Grupo de Microbiología, AV Cll 26 No. 51-60, Bogotá, Colombia, Telephone: 57-1-2207700-446, E-mail: mhidalgo@ins.gov.co. Ricardo Sánchez, Universidad Nacional de Colombia, Facultad de Medicina, Ciudad Universitaria, Cra 30 No 45-03 Facultad de Medicina, Edificio 471 Oficina 202 Bogotá, Colombia, Telephone: 57-1-3165000-15117, E-mail: rsanchez@unal.edu.co. Leonora Orejuela, Departamento de Pathology, The University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0609, Telephone: 409-747-2464, Fax: 409-747-0762, E-mail: leorejue@utmb.edu. Jorge Hernández, Hospital Salazar, Villeta Calle 1 No 7-56, Villeta, Cundinamarca, Colombia, Telephone: 57-1-844-4118, E-mail: jhernandezvilleta@gmail.com. David H. Walker, Department of Pathology, The University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0609, Telephone: 409-772-3989, E-mail: dwalker@utmb.edu. Gustavo Valbuena, Department of Pathology, The University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0609, Telephone: 409-747-0763, Fax: 409-747-2429, E-mail: gvalbuen@utmb.edu.

Reprints requests: Gustavo Valbuena, Department of Pathology, The University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0609.

REFERENCES


