EVIDENCE OF RICKETTSIAL SPOTTED FEVER AND EHRLICHIAL INFECTIONS IN A SUBTROPICAL TERRITORY OF JUJUY, ARGENTINA

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Abstract. Between November 1993 and March 1994, a cluster of pediatric patients with acute febrile illnesses associated with a rash was identified in Jujuy Province, Argentina. Immunohistochemical staining of tissues confirmed spotted fever rickettsial infection in a patient with fatal disease, and testing of serum of a patient convalescing from the illness by using an indirect immunofluorescence assay (IFA) demonstrated antibodies reactive with spotted fever group rickettsiae. A serosurvey was conducted among 16 households in proximity to the index case. Of 105 healthy subjects evaluated by IFA, 19 (18%) demonstrated antibodies reactive with rickettsiae or ehrlichiae: 4 had antibodies reactive with Rickettsia rickettsii, 15 with Ehrlichia chaffeensis, and 1 with R. typhi. Amblyomma cajennense, a known vector of R. rickettsii in South America, was collected from pets and horses in the area. These results are the first to document rickettsial spotted fever and ehrlichial infections in Argentina.

METHODS

Description of the area. Jujuy Province is situated in northwestern Argentina (Figure 1) and is a relatively warm (20–22°C) subtropical region located at 2,200 feet (670 meters) elevation. The average annual rainfall is 32 in (80 cm), and most precipitation occurs between December and March. The province has a stable population of approximately 560,000 inhabitants. The study area of Palma Sola and surroundings (which includes the town of Saladillo) has a population of approximately 2,000 persons. It is located in the Department of Santa Barbara, with a population of 15,540 persons. The terrain consists of partially forested rolling hills that support a diverse collection of birds, small, medium, and large mammals, as well as many domesticated animals including dogs, cattle, pigs, and horses. Adult A. cajennense have been collected from dogs and horses in the region.

Serosurvey. Healthy persons from 16 households in Saladillo were interviewed and a peripheral blood specimen was collected from each person. The interview included questions on prior tick bites and on illnesses at any time, specifically illnesses with signs and symptoms compatible with rickettsial spotted fever. Informed consent was obtained for the survey following guidelines of the U.S. Department of Health and Human Services and those of the Jujuy Provincial Ministry of Social Welfare for clinical studies.

Immunohistochemistry. Sections of formalin-fixed, paraffin-embedded tissues obtained at autopsy from a patient with fatal disease were evaluated for spotted fever rickettsial infection using an indirect immunofluorescence technique. Briefly, 3-μm sections were deparaffinized, rehydrated, and incubated in digestion buffer. The slides were incubated for 60 min at room temperature in hyperimmune rabbit anti-R. rickettsii antiserum diluted to 1/500. This polyclonal antibody reacts with spotted fever group rickettsiae (including R. rickettsii, R. akari, and R. conorii), but does not react with other rickettsial species. Slides were washed 3 times in buffer and incubated for 15 min with biotinylated anti-mouse and anti-rabbit IgG. Slides were then washed and incubated 15 min with alkaline phosphatase–conjugated streptavidin. The slides were rinsed and incubated in naphthol phosphate/fast red chromogen reagent and counterstained in Mayer’s hematoxylin.

Indirect immunofluorescence assay. Patient sera were evaluated for antibodies reactive with rickettsial and ehrlichial antigens by an IFA as previously described, with some modifications. Briefly, antigen slides were prepared from 10% suspensions of R. rickettsii, R. typhi, and Ehrlichia chaffeensis in 0.01 M phosphate-buffered saline (PBS),
RESULTS

Patients. The cluster of illnesses occurred between November 1993 and March 1994. All 6 patients were children 3–11 years of age. Five were male and 1 was female. All but 1 patient resided in Jujuy Province in the Departments of Santa Barbara or San Pedro. The exception was a boy who visited El Rey National Park in the adjacent Province of Salta. All case-patients reported tick bite in the 2-week period preceding their illnesses. Each patient presented with fever, headache, chills, and rash. Two of the 6 patients died. Two patients received chloramphenicol for their illness, including one patient who died. Routine aerobic and anaerobic blood cultures were performed from acute-phase whole blood of all children and no bacterial pathogens were isolated. Laboratory testing by serology or by immunohistochemical staining of tissues confirmed infection with spotted fever group rickettsiae for two patients.

Case reports. An 11-year-old boy was admitted to Hospital de Niños in San Salvador de Jujuy on January 26, 1994, with 5 days of fever and myalgias. Two days before admission, the patient’s 7-year-old sister had died of an illness characterized by fever, nausea, vomiting, and a maculopapular rash. Tick bites preceded the illnesses of both siblings by 1 week. Examination of the boy revealed mild hepatospleno-megaly and muscle tenderness of the buttocks, lumbar region, and thighs. A complete blood cell count and sedimentation rate (ESR) were normal. Urinalysis showed proteinuria (1 gm/L). The blood urea nitrogen level was 28 mg/dl. Two days after admission, a petechial rash was noted on the patient’s trunk, palms, and soles. Two days later, the patient developed headache and the rash had evolved into diffuse hemorrhagic petechiae. The child subsequently developed meningismus, headache, obtundation, and generalized motor weakness. His white blood cell (WBC) count was 14.6 × 10⁶ cells/L on day 4 of hospitalization, with 82% neutrophils and 6% bands. Urinalysis showed proteinuria (1 g/L) and microscopic hematuria and scattered red blood cells (RBCs) (> 3 RBCs/high-power field). Cerebrospinal fluid (CSF) showed a glucose level of 56 mg%, 3 grams% of protein, and 20 × 10⁶ WBCs/L with 80% lymphocytes and 20% neutrophils. Cultures of CSF and blood for routine bacterial pathogens were negative. Chorioretinitis was noted on fundoscopic examination on day 7 of hospitalization. Chloramphenicol was started on day 8 of hospitalization but the patient’s condition continued to deteriorate. He died the following day of multiple organ failure, 14 days after the onset of symptoms. An autopsy showed necrotizing arteriolitis associated with microinfarcts in the skin (Figure 2), kidneys, liver, and spleen. Formalin-fixed, paraffin-embedded sections of spleen, liver, kidney, and skin demonstrated abundant spotted fever group rickettsial antigens by immunohistochemistry. Intensely staining coccobacilli and fragmented rickettsial antigens were present in the endothelial cells of precapillary arterioles of the dermis, in small interlobular vessels and arcuate arteries of the kidneys (Figure 3), and in small arteries in the liver and spleen. Rickettsial antigens were also visualized in vascular smooth muscle cells and in tissue macrophages and monocytes present in the predominantly mononuclear infiltrate involving the adventitia of small vessels in these tissues.

A 3-year old boy was admitted to the Dr. Oscar Orías Hospital in Ledesma, Jujuy Province on May 2, 1994, with a fever of 7–days duration, headache, malaise, vomiting, and a dry cough. The boy had reported a tick bite in the El Rey National Park in nearby Salta Province 2 weeks before admission. On admission, the patient was noted to have a measles-like rash. With the exception of rare and scattered mild
rhonchi, the remainder of the results of the physical examination were normal. The WBC count was $12 \times 10^9$ cells/L with 60% granulocytes, 31% lymphocytes, and 3% eosinophils. The ESR was 25 mm/hr. A presumptive diagnosis of atypical measles was established, and there was an apparent improvement of his illness on supportive therapy. Although the fever and skin rash persisted, he was released to the care of his parents. Three days later he became obtunded, and developed generalized edema and a diffuse ecchymotic rash involving the trunk and extremities (Figure 4). He was again admitted to the hospital. A physical examination showed fever, depressed sensorium, oliguria, and marked motor weakness of the left arm. The blood urea nitrogen level was mildly elevated (148 mg%). The CSF was clear and showed $15 \times 10^6$ WBC/L with 95% lymphocytes and a normal protein level. Blood and CSF cultures for routine bacterial pathogens were negative. Therapy with chloramphenicol was initiated. The patient became comatose and was mechanically ventilated for 5 days. He gradually improved and chloramphenicol was discontinued after 11 days of treatment. The rash resolved, but the patient’s motor and cognitive functions remained mildly impaired. The child was discharged two months later, with some recovery of the neurologic deficits. A serum sample collected 16 days following onset of symptoms demonstrated antibodies reactive with *Rickettsia rickettsii* by IFA (titer $= 256$) and by EIA. There was no evidence of antibodies reactive with *E. chaffeensis* or *R. typhi* by IFA.

**Serosurvey.** A total of 105 sera were collected from residents of 16 households. None reported recent illnesses compatible with a spotted fever rickettsiosis and all residents described a history of tick bites or had seen ticks on pets or in their households. Forty of the subjects were children from...
2 to 14 years of age, and 65 subjects were 15 years of age or older. Serum samples were collected from 50 females and 55 males. Ninetene (18%) of the 105 subjects had serologic evidence of prior infection with a rickettsial agent (Table 1). Most (83%) of the seropositive subjects were clustered around the household of the fatal index case from the Department of Santa Barbara. Four (4%) were positive for antibodies to *R. rickettsii* by IFA, and 3 were confirmed by EIA. Fifteen (14%) were positive for antibodies to *E. chaffeensis* and 1 (1%) was positive for antibodies to *R. typhi*. One subject demonstrated IgG antibodies reactive with *R. typhi*, *R. rickettsii*, and *E. chaffeensis* at titers of 128, 64, and 64, respectively. Of the patients seropositive for *R. rickettsii*, 3 had titers of 64, and 1 had a titer of 256. Of the patients seropositive for *E. chaffeensis*, 6 had a titer of 64, 7 had a titer of 128, and 2 had titers of 256. The prevalence of antibodies reactive with *E. chaffeensis* was 14% and 14% among females and males, respectively. In 5 (31%) of 16 households, two or more family members demonstrated antibodies reactive with *R. rickettsii* or *E. chaffeensis*. There were no statistically significant differences in prevalence of antibody to *E. chaffeensis* among age groups (Table 1; \( \chi^2 = 2.0, \text{df} = 4, P > 0.73 \)) or between genders (\( \chi^2 \) with Yates’ correction = 0.04, \( \text{df} = 1, P > 0.84 \)).

**DISCUSSION**

Several lines of evidence suggest that the 1993–1994 cluster of febrile illnesses observed in these 6 children represented infection with rickettsia. Each patient presented with signs and symptoms clinically compatible with a spotted fever group rickettsiosis, and illnesses were uniformly preceded by tick bites. *Amblyomma cajennense*, a known vector of *R. rickettsii*, was collected from animals in the region. For two patients, spotted fever rickettsial infections were confirmed by laboratory tests. Archived tissues from the index case demonstrated spotted fever group rickettsial antigens in the endothelium and histopathology similar to that described for patients with Rocky Mountain spotted fever (RMSF), a disease caused by *R. rickettsii* in North America. Convalescent-phase serum of another patient showed diagnostic titers to *R. rickettsii* by IFA and EIA. The sibling of the index case from the Department of Santa Barbara showed a low (4%) prevalence of persons with antibody reactive with spotted fever group rickettsiae. In the same community, a surprisingly high (15%) number of persons demonstrated antibody reactive with *E. chaffeensis*. There were no statistically significant differences for prevalence of antehrlichial antibody by age group, suggesting that *E. chaffeensis* or an antigenically similar ehrlichial species occurs in northern Argentina and may have been recently introduced into Jujuy Province.

As far as we are aware, this is only the second report describing evidence of human infection with ehrlichiae in South America. A recent study in Venezuela reported diagnostic antibodies reactive with *E. chaffeensis* in 2 of 43 apparently healthy adults, and an *E. canis*-like bacterium was isolated from the blood of one of the seropositive individuals. It is not known whether the serologic responses observed among surveyed subjects in Argentina were caused by infection with *E. chaffeensis* or an antigenically related ehrlichial species.

This study clearly demonstrates that spotted fever group rickettsiae cause disease similar to RMSF in Argentina. Serologic data document that *E. chaffeensis*, or an antigenically related ehrlichial species, infects humans in Jujuy Province. Further studies are needed to determine whether infections with *Ehrlichia* are associated with human illness in northwestern Argentina and to confirm the vector(s) of rickettsial and ehrlichial species in the region.

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354 RIPOLL AND OTHERS

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