

Rickettsia parkeri in Uruguay

To the Editor: During 1990 in Uruguay, a rickettsiosis in the spotted fever group was presumptively diagnosed for 3 patients who had fever, an initial small maculopapulous lesion at the site of a tick bite on the scalp, and subsequent regional lymphadenopathy. Microimmunofluorescent serologic assay, with *Rickettsia conorii* as the sole antigen source, gave positive results for all patients, and these infections were presumptively identified as spotted fever caused by *R. conorii* (1). During 1993–1994, a total of 23 patients who had a history of tick bite, some of whom had exanthema and inoculation eschars, were identified from Canelones County, Uruguay. These patients had antibodies against *R. conorii*, according to microimmunofluorescence testing; however, *R. conorii* was again the sole antigen source used in the assay (2). Because 1 of the major limitations of serologic testing for diagnosis of rickettsioses is the cross-reactivity between different *Rickettsia* species, the association of *R. conorii* with the spotted fever group cases in Uruguay was considered inappropriate (3). In addition, *R. conorii* has never been found in the Western Hemisphere (3).

Amblyomma triste, a neotropical tick species with a variety of hosts, is the main tick species that feeds on humans in Uruguay and the primary candidate vector for tickborne

rickettsioses in that country (4). A recent investigation demonstrated DNA of *R. parkeri* in *A. triste* ticks collected from humans and animals, indicating that this rickettsia could be the pathogenic agent of spotted fever group rickettsioses in Uruguay (5). In the United States, where *A. maculatum* ticks infected with *R. parkeri* have been reported since the 1930s, the role of this rickettsial agent as a human pathogen was confirmed only recently (3). Our study is the first to isolate *R. parkeri* from *A. triste* collected in Uruguay and confirms the presence of this emerging pathogen in South America.

During September 2004, 78 adult flat ticks (25 males, 53 females) identified as *A. triste* were collected from vegetation in the suburban area of Toledo Chico (34°44'53"S, 56°06'19"W) in Canelones County, southern Uruguay. At the laboratory, the legs of live ticks were extirpated for DNA extraction, and the tick bodies were immediately frozen at –80°C. Each group of legs from 1 tick was subjected to DNA extraction by boiling at 100°C for 20 min as described (6). DNA extracted from each tick was tested by PCR by using primers CS-78 and CS-323 (Table), which targeted a 401-bp fragment of the citrate synthase gene (*gltA*) of possibly all *Rickettsia* species (7). For 2 ticks (1 male, 1 female) that had positive results with PCR testing, *Rickettsia* isolation in cell culture was attempted by using the shell vial technique with

the following modifications: Vero cells inoculated with tick body homogenate were incubated at 28°C; the level of infection of cells was monitored by Gimenez staining of scraped cells from the inoculated monolayer; and a rickettsial isolate was considered established after 3 passages, each reaching >90% of infected cells (7).

Rickettsiae were successfully isolated and established in Vero cell culture from the female tick. This isolate, designated as At5URG, has been deposited as a reference strain in the Rickettsial Collection of Faculty of Veterinary Medicine in the University of São Paulo. DNA extracted from infected cells of the third passage was tested by a battery of PCRs that used all primer pairs listed in the Table and targeted fragments of 3 rickettsial genes: *gltA*, *ompB*, and *ompA*. PCR products of expected size were obtained in all reactions and subjected to DNA sequencing as described (6). Fragments of 1,084, 775, and 491 nt of the *gltA*, *ompB*, and *ompA* genes, respectively, were obtained and showed 100% identity to the corresponding sequences available in GenBank (accession nos. U59732, AF123717, and U43802, respectively) for the Maculatum strain of *R. parkeri* from United States. Although isolation of *Rickettsia* from the male tick was unsuccessful, DNA extracted from remnants of the male and female ticks was tested by PCR (*ompA*, Table) and yielded product that after

Table. Primer pairs used for amplification of rickettsial genes

| Primer pairs | Genes and primers | Primer sequences (5'→3') | Fragment size (nucleotides) | Reference |
|--------------|-------------------|------------------------------|-----------------------------|-----------|
| <i>gltA</i> | | | | |
| 1 | CS-78 | GCAAGTATCGGTGAGGATGTAAT | 401 | 7 |
| | CS-323 | GCTTCCTTAAAATTCAATAAATCAGGAT | | 7 |
| 2 | CS-239 | GCTCTTCTCATCCTATGGCTATTAT | 834 | 7 |
| | CS-1069 | CAGGGTCTTCGTGCATTTCCTT | | 7 |
| <i>ompB</i> | | | | |
| 3 | 120-M59 | CCGCAGGGTTGGTAACTGC | 862 | 8 |
| | 120-807 | CCTTTTAGATTACCGCCTAA | | 8 |
| <i>ompA</i> | | | | |
| 4 | Rr190.70p | ATGGCGAATATTTCTCCAAAA | 530 | 9 |
| | Rr190.602n | AGTGCAGCATTCGCTCCCCCT | | 9 |

sequencing (491 nt) showed 100% identity to the *R. parkeri* sequence from GenBank (U43802).

These procedures enabled the identification of *R. parkeri* in 2.56% of the *A. triste* ticks from Uruguay. Previous findings of *R. parkeri* DNA in *A. triste* ticks from Uruguay (5) are corroborated by our isolation of a Uruguayan strain of *R. parkeri* in cell culture. The only other country where *R. parkeri* has been previously reported is the United States, where it is associated with *A. maculatum* ticks and is the causative agent of an emerging rickettsiosis (3). As *A. maculatum* and *A. triste* are established in at least 12 other Latin American countries (10), the distribution of *R. parkeri* in the Americas is likely continental. Finally, our results corroborate recent reports (3,5) that suggest *R. parkeri* is the causative agent of previously reported cases of rickettsiosis in Uruguay.

This study was financially supported by Foundation of Support to the Research of the State of São Paulo (FAPESP).

Richard C. Pacheco,*
José M. Venzal,†
Leonardo J. Richtzenhain,*
and Marcelo B. Labruna*

*University of São Paulo, São Paulo, SP, Brazil; and †University of La República, Montevideo, Uruguay

5. Venzal JM, Portillo A, Estrada-Peña A, Castro O, Cabrera PA, Oteo JA. *Rickettsia parkeri* in *Amblyomma triste* from Uruguay. *Emerg Infect Dis.* 2004;10:1493–5.
6. Horta MC, Pinter A, Cortez A, Soares RM, Gennari SM, Schumaker TTS, et al. *Rickettsia felis* (Rickettsiales: Rickettsiaceae) in *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) in the State of São Paulo, Brazil. *Arq Bras Med Vet Zoot* 2005;57:321–5.
7. Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A, et al. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the State of São Paulo, Brazil, where Brazilian spotted fever is endemic. *J Clin Microbiol.* 2004;42:90–8.
8. Roux V, Raoult D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer membrane protein rOmpB (*ompB*). *Int J Syst Evol Microbiol.* 2000;50:1449–55.
9. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol.* 1991;173:1576–89.
10. Guglielmone AA, Estrada-Peña A, Keirans JE, Robbins RG. Ticks (Acari: Ixodida) of the Neotropical Zoogeographic Region. International Consortium on Ticks and Tick-borne Diseases, Atlanta, Houten, The Netherlands; 2003.

Address for correspondence: Marcelo B. Labruna, Laboratório de Doenças Parasitárias, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva 87, São Paulo, SP, Brazil 05508-270; email: labruna@usp.br

Influenza-related Death Rates for Pregnant Women

To the Editor: Articles about influenza in the January 2006 issue of *Emerging Infectious Diseases* discussed a pandemic possibly as profound in its effect as the 1918–19 pandemic, when attack rates were >20% worldwide and death rates were 1%–2%. Then, as when subsequent virus antigenic shifts have occurred, all age groups were affected. Governments are now preparing contingency plans against the effects of an expected further antigenic shift.

However, insufficient consideration may have been given to how, in the absence of effective prophylaxis against a novel strain of influenza virus, to avoid deaths on the scale seen in the fall and winter of 1918–19. In particular, the vulnerability of pregnant women and their offspring appears to have been forgotten. Bland reported on pregnant influenza patients in Philadelphia and elsewhere in the fall of 1918; of 337, 155 died (1). Harris obtained by questionnaire from obstetricians medical histories of 1,350 pregnant patients in Maryland and in 4 large US cities (2). Pneumonia developed in half (678) of these patients and 365 died. Death rates from pneumonia were >40% for every month of pregnancy; fetal loss

References

1. Conti-Diaz IA, Rubio I, Somma Moreira RE, Perez Bormida G. Rickettsioses cutaneo ganglionar por *Rickettsia conorii* en el Uruguay. *Rev Inst Med Trop Sao Paulo.* 1990;32:313–8.
2. Diaz IA. Rickettsioses caused by *Rickettsia conorii* in Uruguay. *Ann N Y Acad Sci.* 2003;990:264–6.
3. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev.* 2005;18:719–56.
4. Venzal JM, Guglielmone AA, Estrada Peña A, Cabrera PA, Castro O. Ticks (*Ixodida: Ixodidae*) parasitising humans in Uruguay. *Ann Trop Med Parasitol.* 2003;97:769–72.

Correction: Vol. 12, No. 10

In Human Prion Disease and Relative Risk Associated with Chronic Wasting Disease by Samantha MaWhinney et al., an error occurred in the list of references. Missing from the list is reference no. 36: Belay ED, Maddox RA, Gambetti P, Schonberger LB. Monitoring the occurrence of emerging forms of Creutzfeldt-Jakob disease in the United States. *Neurology.* 2003;60:176–81.

The corrected list of references appears in the online article at <http://www.cdc.gov/ncidod/EID/vol12no10/06-0019.htm>

We regret any confusion this error may have caused.