Molecular Detection of Ehrlichia chaffeensis in Amblyomma parvum Ticks, Argentina

To the Editor: Ehrlichia chaffeensis is an obligate intracellular bacterium in the family Anaplasmataceae. It is considered an emerging pathogen in the United States because it is the causative agent of human monocytotropic ehrlichiosis (1), a flu-like illness that can progress to severe multisystem disease and has a 2.7% case-fatality rate (2).

In Central and South America, human cases of ehrlichiosis with compatible serologic evidence have been reported in Venezuela, Brazil, Mexico, and Chile, although the bacterium has not been isolated (3). Recently, molecular evidence of E. chaffeensis infection was reported for a symptomatic 9-year-old child in Venezuela (4). In Argentina, antibodies reactive to E. chaffeensis, or an antigenically related Ehrlichia species, were detected in human serum samples during a serologic survey in Jujuy Province, where fatal cases of febrile illness were reported (5).

During November–December 2006, we collected ticks by dragging the vegetation and by examining mammal hosts, including humans, in semi-arid southern Chaco, Argentina, Moreno Department, Province of Santiago del Estero. Ticks, kept in 70% alcohol, were identified as Amblyomma parvum (n = 200), A. tigrinum (n = 26), and A. pseudoconcolor (n = 13). A sample of 70 A. parvum and 1 A. tigrinum ticks collected on domestic ruminants and canids were subjected to PCR and reverse line blot hybridization by using the TBD-RLB membrane (Isogen Life Science, Maarssen, the Netherlands) (6) to look for Anaplasma and Ehrlichia spp. DNA was extracted from individual ticks by using the DNeasy Blood and Tissue kit (QIAGEN Valencia, CA, USA); several negative controls (distilled water) for both DNA extraction and PCRs were run alongside the samples in random order throughout the experiments. Primers Ehr-R (5′-CGGATCCCAA GTTTGCCGGACCTTTTCTC-3′) (6) and Ehr-Fint (5′-GGCTCA GAACGAACGCTG-3′; Inst. Biotecnologia, Instituto Nacional de Tecnología Agropecuaria, unpub. data) were used to amplify a 500-bp fragment of the 16S gene of Anaplasma/Ehrlichia spp. PCR products were analyzed by reverse line blot hybridization, and 11.3% (95% confidence interval [CI] = 4.9–21.0) showed a positive signal to the specific E. chaffeensis probe: 8 A. parvum ticks collected from a dog (n = 1), a fox (Lycalopex gymnocercus, n = 1), goats (n = 2), and cattle (n = 4). No signals to other probes present in the membrane were recorded (A. phagocytophylum, A. marginale, A. centrale, A. ovis, E. ruminantium, E. sp. Omatjenne, E. canis). Further sequence analysis of 16S fragments confirmed the result, with our sequences showing 99.6% identity with the corresponding fragment of the E. chaffeensis strain Arkansas 16S gene (GenBank accession no. EU826516). To better characterize the positives samples, we then amplified variable-length PCR target (VLPT) of E. chaffeensis (7). PCR products of variable length were detected by conventional gel electrophoresis analysis (Figure). Distilled water and R. conorii DNA were used as negative controls, and E. chaffeensis DNA as the positive control. The finding was confirmed by sequence analysis (GenBank accession nos. EU826517 and EU826518).

In view of these positive results, another set of 108 specimens was tested by E. chaffeensis VLPT PCR: all the ticks collected on humans (80 A. parvum, 1 A. pseudoconcolor, and 4 A. tigrinum), 18 host-seeking A. parvum ticks, and 5 A. parvum ticks collected on armadillos of the genera Tolypeutes and Chaetophractus. E. chaffeensis was detected in A. parvum ticks only: 5 from humans (6.2%; 95% CI 2.1–14.0; Figure, panel A) and 3 from host-seeking ticks (16.7%; 95% CI 3.6–41.4). In total, E. chaffeensis was detected in 9.2% (95% CI 5.4–14.6) of tested A. parvum ticks in the study area. Of the 16 positive A. parvum, 5 were infesting humans.

Little is known about E. chaffeensis epidemiology in South America. In Brazil, wild marsh deer (Blastocerus dichotomus) are suspected to be its natural reservoir, but the tick involved in the transmission cycle is not known (8). In North America, E. chaffeensis sp. is maintained principally by the lone-star tick, A. americanum, and the white-tailed deer (Odocoileus virginianus) (2). However, the possibility of transmission by different ticks and infection among other hosts has been reported; specific antibodies to E. chaffeensis were detected in domestic and wild canids and goats (2), and recently experimental infection was demonstrated in cattle (9). We did find E. chaffeensis organisms in ticks collected on both wild and domestic animals, but the possible role of different mammals as reservoir hosts deserves further investigation. Moreover, the finding of polymorphic VLPT gene fragments in our sample indicates the circulation of E. chaffeensis genetic variants in the study area. VLPT repetitive sequences vary among isolates.
(7); however, it is not known whether genetic variants differ in pathogenicity or are correlated with geographic distribution or host range.

All positive ticks were *A. parvum*, a common tick of domestic animals that frequently feeds on humans in Argentina and Brazil and is considered a potential vector of zoonoses (10). In our study area, this tick species was by far the most abundant on humans (93.2%), and our results suggest its potential role as a vector of *E. chaffeensis*.

**Acknowledgments**

We thank William Nicholson for providing us with positive controls, J. Stephen Dumler for useful suggestions in molecular diagnostic tools, Paula Ruybal for technical assistance in RLB assay, Alberto Guglielmone for assistance in tick identification, and the families of ‘Uchi’ Escalada (Amama) and of ‘Negro’ Pereira (Trinidad) for helping us with tick collection.

This project received support from the National Institutes of Health Research Grant #R01 TW05836 funded by the Fogarty International Center and the National Institute of Environmental Health Sciences to U.D.K. and R.E.G. Additional funding came from Agencia Nacional de Promoción Científica y Técnica de Argentina and Universidad de Buenos Aires (to R.E.G.) and Epigenevac Project (FP6-2002-INCO-DEV-1, EU) and Agencia Nacional de Promoción Científica y Técnica de Argentina (to M.F.). R.E.G. and M.F. are members of Consejo Nacional de Investigaciones Científicas y Técnicas’ Researcher’s Career, P.N. has a CONICET fellowship.

Laura Tomassone, Pablo Núñez, Ricardo E. Gürtler, Leonardo A. Ceballos, Marcela M. Orozco, Uriel D. Kitron, and Marisa Farber

Author affiliations: Università di Torino, Torino, Italy (L. Tomassone); Instituto de Biotecnología, INTA, Castelar, Argentina (P. Núñez, M. Farber); Universidad de Buenos Aires, Buenos Aires, Argentina (R. Gürtler, L.A. Ceballos, M.M. Orozco); and Emory University, Atlanta, Georgia, USA (U.D. Kitron)

DOI: 10.3201/eid1412.080781

**References**

1. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. Clin Infect Dis. 2007;45(Suppl):S45–51. DOI: 10.1086/518146

2. Walker DH, Ismail N, Olano JP, McBride JW, Yu XJ, Feng HM. *Ehrlichia chaffeensis*: a prevalent, life-threatening, emerging pathogen. Trans Am Clin Climatol Assoc. 2004;115:375–84.

3. da Costa PS, Valle LM, Brigatte ME, Greco DB. More about human monocytotropic ehrlichiosis in Brazil: serological evidence of nine new cases. Braz J Infect Dis. 2006;10:7–10.

4. Martinez MC, Gutiérrez CN, Monger F, Ruiz J, Watts A, Mijares VM, et al. *Ehrlichia chaffeensis* in child, Venezuela. Emerg Infect Dis. 2008;14:519–20.

5. Ripoll CM, Remondegui CE, Ordonez G, Arazamendi R, Fusaro H, Hyman MJ, et al. Evidence of rickettsial spotted fever and ehrlichial infections in a subtropical territory of Jujuy, Argentina. Am J Trop Med Hyg. 1999;61:350–4.

6. Bekker CP, de Vos S, Taoufik A, Sparagano OA, Jongejan F. Simultaneous detection of *Anaplasmata* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. Vet Microbiol. 2002;89:223–38. DOI: 10.1016/S0378-1135(02)00179-7

7. Sumner JW, Childs JE, Paddock CD. Molecular cloning and characterization of the *Ehrlichia chaffeensis* variable-length PCR target: an antigen-expressing gene that exhibits interstrain variation. J Clin Microbiol. 1999;37:1447–53.

8. Machado RZ, Duarte JMB, DagNONE AN, Szabo MBJ. Detection of *Ehrlichia chaffeensis* in Brazilian marsh deer (*Blastocerus dichotomus*). Vet Parasitol. 2006;139:262–6. DOI: 10.1016/j.vetpar.2006.02.038

9. delos Santos JRC, Boughan K, Bremer WJ, Rizzo B, Schaefer JJ, Rikihisa Y, et al. Experimental infection of dairy calves with *Ehrlichia chaffeensis*. J Med Microbiol. 2007;56:1660–8. DOI: 10.1099/jmm.0.47427-0

10. Guglielmone AA, Beati L, Barros-Battesti DM, Labruna MB, Nava S, Venzal JM, et al. Ticks (*Ixodes* species) on humans in South America. Exp Appl Acarol. 2006;40:83–100. DOI: 10.1007/s10493-006-9027-0
Enzootic Angiostrongylia in Shenzhen, China

To the Editor: Angiostrongylus cantonensis is a zoonotic parasite that causes eosinophilic meningitis in humans after they ingest infective larvae in freshwater and terrestrial snails and slugs, paratenic hosts (such as freshwater fish, shrimps, frogs, and crabs), or contaminated vegetables. With the increase of income and living standards, and the pursuit of exotic and delicate foods, populations around the world have seen angiostrongylia become an important foodborne parasitic zoonosis (1–9).

Shenzhen municipality is situated in the most southern part of mainland People’s Republic of China between the northern latitudes of 22°27’ to 22°52’ and eastern longitudes of 113°46’ to 114°37’; it shares a border with the Hong Kong Special Administrative Region, China, in the south. The climate is subtropical, with an average annual temperature of 23.7 °C. The city is 1,952.84 km² and has a population of 10 million.

Since 2006, thirty-two sporadic cases of human eosinophilic meningitis caused by consumption of undercooked aquaculturated snails have been documented in Shenzhen (Shenzhen Center for Disease Control and Prevention, unpub. data). To identify the source of these infections and assess the risk for an outbreak of eosinophilic meningitis, we conducted a survey to investigate whether A. cantonensis occurs in wild rats and snails in Shenzhen.

To examine A. cantonensis infection in intermediate host snails, 302 terrestrial snails (Achatina fulica) were collected from 10 investigation sites across Shenzhen, and 314 freshwater snails (Pomacea canaliculata) were sampled from 6 investigation sites. We examined the snails for A. cantonensis larvae by using pepsin digestion standardized procedures (3). To survey the prevalence of adult A. cantonensis in definitive host rats, we collected 187 Rattus norvegicus rats and 121 R. flavivirgatus rats collected from 4 sites where positive snails positive for A. cantonensis were found. These rats were examined for the presence of adult A. cantonensis in their cardiopulmonary systems.

A. cantonensis larvae were found in 96 (15.6%) of 616 examined snails. Of these, P. canaliculata had an average infection rate of 20.7% (65/314), significantly higher than that of A. fulica (10.3%, 31/302), an indication that P. canaliculata may be the principal intermediate host for A. cantonensis in Shenzhen. A. cantonensis adults were recovered from the cardiopulmonary systems of 37 (12%) of 308 examined rats. Infection rate for R. norvegicus rats was 16.6% (31/187), significantly higher than that for R. flavivirgatus (4.9%, 6/121), an indication that R. norvegicus may be the principal definitive host for A. cantonensis in Shenzhen, possibly due to the rat’s preference for eating snails. Infection rates were higher for female rats (25.6% for R. norvegicus and 7.8% for R. flavivirgatus) than for male rats (8.9% for R. norvegicus, 2.9% for R. flavivirgatus), possibly because female rats eat more snails to supply proteins for reproduction. This report of enzootic A. cantonensis infection in wild rats and snails in Shenzhen demonstrates the existence of natural origins of infection with A. cantonensis for humans in this city.

Persons in Shenzhen eat raw or undercooked freshwater and terrestrial snails and slugs. This practice provides opportunities for infection with A. cantonensis, particularly given that P. canaliculata has been aquaculturated intensively for human consumption. The prevalence of A. cantonensis in wild rats and snails in Shenzhen poses substantial risk for future outbreaks of human eosinophilic meningitis. Moreover, public health officials, epidemiologists, researchers, clinical technicians, medical practitioners, parasitologists, and veterinarians, as well as the general public, should be aware of such risks, and integrated strategies should be taken to reduce or eliminate such risks.

Acknowledgment

We thank Alasdair Nisbet for his assistance in improving the manuscript.

Project support was provided in part by a grant from Shenzhen Municipal Bureau of Science and Technology (grant no. 2007079) to R.-L.Z. and a grant from the Program for Changjiang Scholars and Innovative Research Team in University (grant no. IRT0723) to X.-Q.Z.

Ren-Li Zhang, Mu-Xin Chen, Shi-Tong Gao, Yi-Jie Geng, Da-Na Huang, Jian-Ping Liu, Yuan-Liang Wu, and Xing-Quan Zhu

Author affiliations: Shenzhen Center for Disease Control and Prevention, Shenzhen, People’s Republic of China (R.-L. Zhang, M.-X. Chen, S.-T. Gao, Y.-J. Geng, D.-N. Huang, J.-P. Liu, Y.-L. Wu); and South China Agricultural University, Guangzhou, People’s Republic of China (M.-X. Chen, X.-Q. Zhu)

DOI: 10.3201/eid1412.080695

References

1. Alicata JE. The discovery of Angiostrongylus cantonensis as a cause of human eosinophilic meningitis. Parasitol Today. 1991;7:151–3. DOI: 10.1016/0169-4758(91)90285-V