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## *Coxiella burnetii* in Ticks, Argentina

**To the Editor:** The Gammaproteobacterium *Coxiella burnetii* is the causative agent of acute Q fever and chronic endocarditis in humans worldwide. It is transmitted primarily by aerosol route or by ingestion of fomites from infected animals, mostly from domestic ruminants (*1*). Although >40 tick species can be infected with *C. burnetii,* direct transmission of this agent to humans from infected ticks has never been properly documented. However, ticks may play a critical role in the transmission of *C. burnetii* among wild vertebrates (*1*). Only a few studies, mostly related to human clinical cases or seroepidemiogic evaluation of healthy animals, have reported *C. burnetii* in South America (*2–4*). However, to our knowledge, *C. burnetii* has never been reported in ticks in the continent.

During ecologic studies on *Amblyomma parvum* and *A. tigrinum* ticks in the Córdoba Province of Argentina, engorged nymphs were collected from the common yellow toothed cavy (the rodent *Galea musteloides*) (*5*,*6*). In the laboratory, engorged nymphs molted to adults (92 *A. tigrinum*, 13 *A. parvum*), which were individually submitted to the hemolymph test with Gimenez staining for detection of rickettsiae-like organisms (*7*). By the hemolymph test, 1 *A. tigrinum* female, and 2 *A. parvum* male ticks were found to contain red-stained rickettsiae-like structures. These 3 ticks were processed individually by the shell vial technique, with the purpose of isolating intracellular bacteria in Vero cell culture (*7*). Inoculated cells were always incubated at 28°C. Intracellular bacteria were successfully isolated from all 3 ticks and established in Vero cell culture, as demonstrated by Gimenez staining of infected cells from at least 10 subsequent passages, which all infected 100% of the cells (Figure, panel A). Infected Vero cells contained multiple vacuoles (Figure, panel B) that enclosed a seething mass of microorganisms (online Video; wwwnc.cdc.gov/EID/article/19/02/12-0362-F1.htm), compatible with *Coxiella* organisms. Such vacuoles were not seen in uninfected control Vero cells incubated under the same conditions as those of infected cells (Figure, panel C).

For molecular analyses, DNA from the infected cells of each of the 3 isolates was extracted by boiling at 100°C for 10 min; it yielded products of the expected size through PCR protocols selective for portions of 3 genes of the genus *Coxiella*: primers QR-FO (5′-ATTGAAGAGTTT GATTCTGG-3′) and QR-RO (5′-CG GCCTCCCGAAGGTTAG-3′) for the 16S rRNA gene (*8*); priers CAPI844F (5-ATTTAGTGGGTTTCGCG CAT-3′) and CAPI844R (5′-CAT CAGCATACGTTTCGGGAA-3′) for the *cap* gene (*9*); and primers Cox-F-pry2 (5′-TTATTTACCAAGTTCC GAGCCG-3′) and Cox-R-pry2 (5′ TTTATCCCGAGCAAATTCA ATTATGG-3′) for the *pyrG* gene (*9*). PCR products underwent DNA sequencing in an automatic sequencer (Applied Biosystems/Perkin Elmer, Foster City, CA, USA) according to the manufacturer`s protocol. We sequenced 1,386, 557, and 545 nt of the genes 16S rRNA, *cap*, and *pyrG*, respectively, which were identical to each other for each gene amplified from the 3 tick isolates. By BLAST analyses (www.ncbi.nlm.nih.gov/blast), these sequences were 99.9% (1,384/1,386 nt), 99.6% (556/558 nt), and 99.6% (452/454 nt) identical to the corresponding GenBank sequences of the North American *C. burnetii* genes 16S rRNA, *cap*, and *pyrG*, respectively (HM208383, CP001020, CP001020). Partial sequences (16S rRNA, *cap*, *pyrG*) from *C. burnetii* generated in this study were deposited into GenBank and assigned nucleotide accession nos. JQ740886–JQ740888, respectively.

Infected Vero cell monolayers were fixed in a modified Karnovsky solution, stained with uranyl acetate and lead citrate, and examined in a transmission electron microscope according to standard procedures. Ultrastructurally, *Coxiella* organisms were identified by morphologic features within heavily infected Vero cells. The organisms possessed typical bacillary morphologic characteristics and were observed inside vacuoles (phagolysosomes) of different sizes, proportional to the

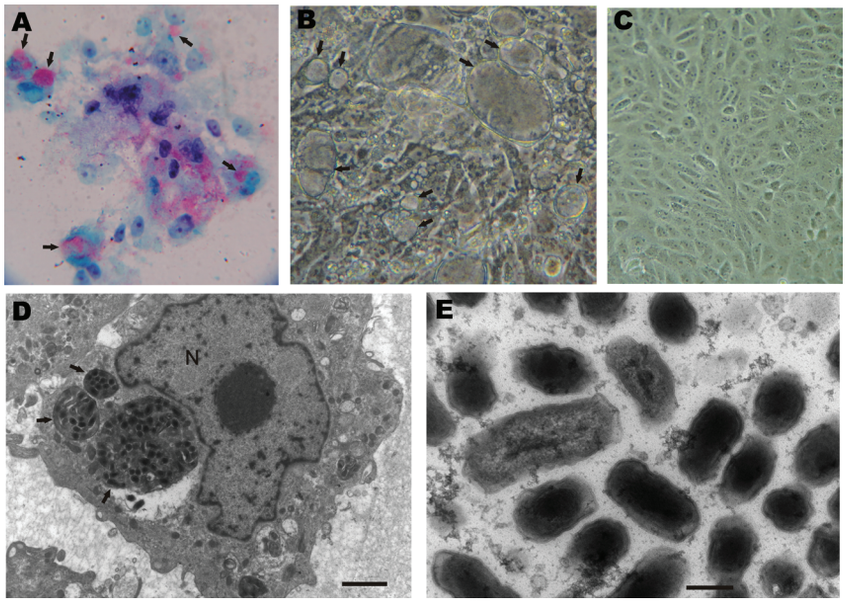


Figure. Vero cells inoculated with *Amblyomma* spp. tick extracts for isolation of rickettsiae. A) Rickettsiae-like organisms stained by Gimenez staining (original magnification ×400). B) Inoculated monolayer photographed under phase-contrast microscopy (original magnification ×400). C) Uninfected control monolayer under phase-contrast microscopy (original magnification ×400). D) Transmission electron microscopy of infected cells. Bar indicates 2 µm. E) Transmission electron microscopy image of intravacuolar bacteria. Bar indicates 250 nm. N, nucleolus. Arrows indicate vacuoles containing bacteria. A color version of this figure is available online (wwwnc.cdc.gov/EID/article/19/2/12-0362-F1.htm).

number of organisms (Figure, panels D, E). Intravacuolar organisms had a mean length of 0.55 ± 0.13 µm (range 0.42–0.85 µm) and a mean width of 0.25 ± 0.03 µm (range 0.22–0.32 µm).

Ticks negative for rickettsiae-like organisms by hemolymph testing were subjected individually to DNA extraction by the guanidine isothiocyanate-phenol technique (*10*) and screened for *Coxiella* spp. by PCR that targeted the *pyrG* gene, as described above. Although no *A. parvum* tick yielded amplicons, 40 *A. tigrinum* ticks yielded amplicons of the expected size for the *pyrG* gene. DNA sequences generated from these ticks were identical to the *pyrG* partial sequences obtained from the *C. burnetii* isolates mentioned above.

We found 41 (44.6%) of 92 ticks and 2 (15.4%) of 13 of the *A. tigrinum* and *A. parvum* adult ticks, respectively, to be infected by *C. burnetii.* Because these ticks were collected as engorged nymphs from wild rodents in a natural biome of Argentina, namely, the Chaco phytogeographic domain (*5*,*6*), our results indicate that *C. burnetii* is established in this part of the country where ticks possibly play an essential role in the enzootic cycle. Serologic evidence of *C. burnetii* infection has been found among goats and cattle in several areas of Argentina (3,4). Because free-ranging domestic cattle and goats are considered among the most likely hosts for *A. parvum* adult ticks in the Chaco domain (*6*), humans are likely being exposed to *C. burnetii* as well.

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# Epizootic Hemorrhagic Disease in Brocket Deer, Brazil

**To the Editor:** In February 2008, a 2-year-old female gray brocket deer (*Mazama gouazoubira*) at Pomerode Zoo in Santa Catarina, Brazil, exhibited sublingual swelling, drooling, lethargy, prostration, glossitis, slight cyanosis, and blood on the perineum. The animal showed progressive anorexia, ataxia, dyspnea, marked cyanosis, hypothermia (34.5°C), and severe anemia (hematocrit 4.6%). The deer died 5 days after the first clinical signs. Necropsy indicated that the gray brocket deer had pulmonary congestion and edema, mild renal congestion, and renal focal necrosis. Some intestinal vessels along the serosa presented with hyalinization of the walls with mild leukocytic infiltrate of neutrophils featuring vasculitis.

Seventeen days after this first case, a 1-year-old male pygmy brocket deer (*Mazama nana*) from the same zoo suddenly died. Findings on necropsy were cyanosis and petechiae in the oral mucosa, tongue, and gastrointestinal mucosa. There was bloody intestinal content, petechiae in the mucosa of the urinary bladder, and also petechiae and ecchymoses in the pericardium and epicardium. The spleen was contracted; lymph nodes and kidney medullae were hemorrhagic. The lungs showed congestion and petechiae, and the airways had a frothy fluid content. Histopathologic lesions included mild diffuse congestion in the pygmy brocket deer's kidneys and extensive subendocardial hemorrhage.

To identify the suspected disease agent (members of the species *Bluetongue virus* or *Epizootic hemorrhagic disease virus*), we performed virus isolation and reverse transcription PCR on tissues (heart, liver, lung, and bowel) from the pygmy brocket deer. (Virus isolation was not carried out on specimens from the gray brocket deer because brain tissue samples were inadequate and results of PCR were negative for epizootic hemorrhagic disease virus [EHDV] or bluetongue virus [BTV].) Infection with EHDV and BTV was first diagnosed by virus isolation. Pooled specimens from spleen-liver and heart tissue were inoculated in embryonated chicken eggs and thereafter in BHK-21 cells (*1*). Chicken embryos inoculated with tissues of the pygmy brocket deer died from 1 to 6 days after inoculation. All embryos showed swelling and hemorrhage throughout the skin and extensive areas of hemorrhage in the brain and heart. Cytopathic effects started 48 hours postinoculation in the BHK-21 monolayer. An indirect immunoperoxidase test, using anti-BTV/EHDV polyclonal antiserum of porcine origin (VMRD Inc., Pullman, WA, USA) and direct fluorescence assay using an anti-BTV monoclonal antiserum fluorescein conjugate (VMRD) were performed for virus identification (*1*). The isolates were then identified as EHDV.

To confirm the EHDV serogroup, we performed reverse transcription PCR. A fragment of ≈260 bp, which encodes the partial NS3 gene of EHDV, was detected from pooled tissue and the BHK-21 monolayer with cytopathic effect (*2*). No amplification was obtained in the S10 gene PCR for BTV (*3*). An amplified fragment was sequenced and identified as strain LDVA (GenBank accession no. GU014478). The phylogenetic relationship was assessed by using the neighbor-joining Mega.5 (*4*) method with the BTV sequence as an outgroup. The phylogenetic tree shows that the partial sequence of the S10 gene segregates EHDV serogroup into 2 clusters, with LDVA (GU014478) grouping together with North American EHDV samples (Figure).

EHDV and BTV (*Reoviridae: Orbivirus*) are involved in outbreaks of hemorrhagic disease, which occur mainly in late summer and early autumn in various parts of the world, probably because of the increase in the *Culicoides* population, the biologic vectors of these viruses. Clinical signs of EHDV and BTV infections are indistinguishable in deer and are characterized by severe depression, respiratory distress, anorexia, and blood-tinged oral and nasal discharge. Gross lesions are mainly edema and hemorrhage caused by vascular injury (*5*).

Clinical reports of EHDV infection have occurred in Australia, Asia, Africa, and North America (*6*). In Brazil, serologic research in marsh deer (*Blastocerus dichotomus)* populations in São Paulo and Mato Grosso do Sul revealed that 74% of animals were seropositive for EHDV,