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Natural Infection of Domestic Goats with *Ehrlichia chaffeensis*

VIVIEN G. DUGAN,[1] SUSAN E. LITTLE,[1]* DAVID E. STALLKNECHT,[2] AND ASHLEY D. BEALL[1]

Department of Medical Microbiology and Parasitology[1] and Southeastern Cooperative Wildlife Disease Study,[2] College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602

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Thirty-eight domestic goats from an area of *Ehrlichia chaffeensis* endemicity were tested for antibodies reactive to *E. chaffeensis* and for *E. chaffeensis*-specific 16S rRNA gene fragments by an indirect fluorescent antibody test and a nested PCR assay, respectively. Twenty-eight of 38 (73.7%) goats had antibodies reactive to *E. chaffeensis* (≥1:128), and 6 of 38 (15.8%) goats were positive by diagnostic PCR; *E. chaffeensis* was isolated in cell culture from one goat. Our data indicate that goats in areas of endemicity are naturally exposed to and infected with *E. chaffeensis*.

Human monocytic ehrlichiosis is a newly described tick-borne disease caused by *Ehrlichia chaffeensis*. The disease presents as a nonspecific, febrile, flu-like illness; characteristic clinical pathology findings include thrombocytopenia and leukopenia (1). Since human monocytic ehrlichiosis was first detected in 1986, approximately 750 cases have been reported to the Centers for Disease Control and Prevention (13), with most occurring in the southeastern and south-central United States (15). Previous research has demonstrated that *E. chaffeensis* is maintained in nature through a cycle involving white-tailed deer, *Odocoileus virginianus*, as the cardinal reservoir host and the lone star tick, *Amblyomma americanum*, as the primary vector (5, 9, 10). Additional studies have shown that dogs are also naturally infected with *E. chaffeensis* in areas of endemicity (4). Because domestic goats are parasitized by the lone star tick (6), we evaluated domestic goats from Clarke County, Georgia, an area of *E. chaffeensis* endemicity (9), for exposure to this organism.

Blood samples were collected from 38 domestic goats from an established herd pastured at a farm maintained by the College of Veterinary Medicine, University of Georgia (Athens). Serum samples were tested for antibodies reactive to *E. chaffeensis* by indirect fluorescent antibody assay (IFA) as previously described (2). Serum or plasma samples initially were tested at 1:64 and 1:128 dilutions, with positive samples further tested in twofold serum dilutions to an endpoint. A comparative IFA performed on serum and plasma samples from the same goat confirmed that plasma samples are viable substitutes for serum samples in our system.

For PCR, DNA was extracted from 100 μl of EDTA-anticoagulated whole blood by using the USB Isolation Kit (Amersham Life Science) or the GFX Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech) following the manufacturers' protocol for fast DNA extraction. The final pellet was resuspended in 50 μl of molecular-biology-grade water, and 5 μl was tested by nested PCR as previously described (8) for the presence of the *E. chaffeensis* 16S rRNA gene (rDNA), by using primers ECC and ECB in a primary reaction followed by primers HE1 and HE3 in a secondary reaction. DNA extraction, DNA amplification, and analysis of the results were performed in different laboratories to prevent contamination. A negative control was included in each step of the assay; a positive control was included with each set of reactions.

Culture isolation was performed with blood from 10 goats as previously described (3). Briefly, 7 to 10 ml of EDTA-anticoagulated blood was transferred into a sterile 50-ml centrifuge tube containing 25 ml of ACE lysing buffer (150 mM NH_4Cl, 0.7 mM KH_2PO_4, 3 mM EDTA-Na_2), and the tube was gently inverted. After 5 min at room temperature, the suspension was centrifuged at 160 × *g* for 5 min, the supernatant was removed, and the leukocyte fraction was washed in ACE lysing buffer. After a second centrifugation, the pellet was suspended in 1 ml of minimal essential medium supplemented with 5% fetal bovine serum. Confluent cultures of DH82 canine macrophages in a 12.5-cm^2 flask with 5 ml of minimal essential medium were inoculated with the suspended cells. After 72 h the supernatant was decanted, and 5 ml of fresh minimal essential medium was added. Fresh medium was added to each culture twice weekly. Cultures were tested by direct fluorescent antibody assay (FA) as previously described (2) if cytopathic effect was observed or approximately 60 days following inoculation if cytopathic effect was not observed. Nucleic acid was extracted from FA-positive culture material by using InstaGene purification matrix (Bio-Rad; Hercules, Calif.) according to the manufacturer's directions and was tested by nested PCR as described above. Representative amplicons from blood and from cell culture material were sequenced as previously described (7).

Twenty-eight of the 38 (73.7%) goats had antibodies reactive to *E. chaffeensis* at a titer of ≥128, with a maximum titer of 2,048. The overall geometric mean titer (14) for positive reciprocal titers was 453. On PCR assay, 6 of 38 (15.8%) samples showed evidence of 16S rDNA characteristic of *E. chaffeensis*. *E. chaffeensis* was successfully isolated from one goat on two separate occasions; the interval between isolations was 40 days. The sequence of the 16S rDNA fragment amplified from goat blood and from cell culture was identical to previously published sequences of *E. chaffeensis*.

These data provide serologic and PCR evidence and cell culture confirmation that domestic goats in an area of *E. chaffeensis* endemicity are naturally exposed to and infected with *E. chaffeensis*. To date, white-tailed deer are the only confirmed natural reservoir host of *E. chaffeensis* proven capable of infecting ticks (5, 9). Previous serological surveys of wildlife also have demonstrated *E. chaffeensis*-reactive antibodies in raccoons (*Procyon lotor*) and opossums (*Didelphis virginianus*) (10). In contrast, *E. chaffeensis*-reactive antibodies have not been found in wild rodent populations (11) and immuno-

* Corresponding author. Mailing address: Department of Medical Microbiology and Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, GA 30602. Phone: (706) 542-8447. Fax: (706) 542-0059. E-mail: slittle@calc.vet.uga.edu.

competent rodents are difficult to infect experimentally (12). Although both serologic and PCR evidence of *E. chaffeensis* in dogs has been reported (4), to our knowledge this organism has not been isolated in cell culture from a naturally infected dog. Thus, this work is the first report of isolation of *E. chaffeensis* from a naturally infected vertebrate other than a deer. Further characterization of *E. chaffeensis* infection dynamics in domestic goats, including tick transmission studies, may provide a model reservoir host for use in long-term studies.

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