A 9-year-old nonpregnant, nonlactating doe Boer goat was examined because of a 2-day history of not being able to stand. Other than diarrhea associated with coccidiosis in the first year of life, the goat did not have a history of illness. The owner had obtained the goat at approximately 2 months of age. The goat lived with 3 other goats in the same pen on the same premise located on the coastal plains of North Carolina. The goats spent the majority of time in a barn, with access to a wooded 1-acre lot where they browsed. Routine deworming prophylaxis was verified by fecal egg counts. The goat was vaccinated for clostridial diseases, was fed 1 cup of 13.5% protein commercial goat pellets twice a day, and had free access to good quality coastal Bermuda hay.

At presentation for recumbency, the goat was non-weight bearing on the right forelimb and could stand only with assistance, but was unable to walk. Otherwise, the goat was bright, alert, responsive, and had a good appetite. Body condition score (5/5), body weight (65.5 kg), rectal temperature (39.2°C [102.5°F]), heart rate (80 beats per minute), respiratory rate (24 breaths per minute), mucous membrane color, and capillary refill time were normal. An abscess was present on the ventral aspect of the mammary gland.

To further assess the lameness, lateral, craniocaudal, and oblique radiographs of the right humerus were obtained, and a mildly comminuted, moderately proximo-caudally and medially displaced short oblique fracture of the proximal humeral diaphysis was identified (Fig 1). Marked soft tissue swelling was associated with the fracture. Mild rounding and blunting of the fracture margins without evidence of callus formation were observed and, consequently, some degree of chronicity (>7–10 days) was considered likely.

A CBC identified mild macrocytic normochromic anemia (PCV, 20%; reference range, 22–38%; mean cell volume [MCV], 26.4 fL; reference range, 16–24 fL; mean corpuscular hemoglobin concentration [MCHC],...
The total leukocyte count was 9,900/µL (reference range, 5,100–17,200/µL)² and characterized by a mild regenerative left shift (segmented neutrophils, 8,800/µL; reference range, 1,100–8,900/µL; band neutrophils, 100/µL; reference range, 0/µL).² Mild lymphopenia (lymphocytes, 900/µL; reference range, 1,200–10,500/µL)² and normal numbers of monocytes (100/µL; reference range, 0–300/µL).² Microscopic evaluation of a blood smear disclosed moderate anisocytosis, mild macrocytosis, and occasional basophilic stippling, suggestive of a regenerative response. Occasional eccentrocytes indicated oxidative damage, potentially related to the inflammatory illness. Approximately 1–2% of the segmented neutrophils contained single, approximately 2–3 x 2–3 µm, tightly packed, basophilic granular clusters of organisms within the cytoplasm, consistent with morulae (Fig 2). No other etiologic agents were observed. Platelet number estimate on the blood smear appeared adequate. Serum biochemistry abnormalities included mildly increased activities of AST (227 IU/L; reference range, 35.5–72.0 IU/L), CK (788 IU/L; reference range, 24.5–883 IU/L), and GGT (52 IU/L; reference range, 16.0–45 IU/L)² and decreased ALP activity (35 IU/L; reference range, 77–883 IU/L)².

The owners elected euthanasia because of the poor prognosis associated with the humeral fracture, and declined necropsy.

For molecular microbiological identification of granulocytic morulae, EDTA anti-coagulated blood was submitted to the Vector Borne Disease Diagnostic Laboratory at North Carolina State University College of Veterinary Medicine (VBDDL NCSU-CVM). In addition, a comprehensive polymerase chain reaction (PCR) panel, which includes detection of Anaplasma (A.)/Ehrlichia (E.) spp., Babesia spp., Bartonella spp., Mycoplasma (M.) spp. and Rickettsia spp., was performed. Briefly, DNA was extracted from 200 µL of whole blood. Negative extraction controls consisting of uninfected dog EDTA-whole blood were included. The absence of PCR inhibitors was demonstrated by the amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).³ The PCR conditions were previously described to amplify a 700-base pair (bp) fragment of the Mycoplasma 16S rRNA gene, a 430-bp fragment of the Anaplasma/Ehrlichia 16S rRNA gene, a 620-bp fragment of the Anaplasma/Ehrlichia GroEL gene, and a 304-bp fragment of the Ehrlichia sodB gene.⁴⁻⁷ Both the Anaplasma/Ehrlichia 16S rRNA and GroEL assays were run with a modified annealing temperature of 60°C. Positive (Candidatus Mycoplasma haematoparvum, Anaplasma platys or Ehrlichia chaffeensis plasmid DNA) and negative (RNase-free, molecular grade water, and a DNA extraction control) were included as controls in each assay. The PCR product visualization was performed using gel electrophoresis. Amplified DNA was sequenced directly,⁵ and alignments were compared with GenBank sequences.⁶ The PCR results from the comprehensive panel yielded positive amplicons for Anaplasma/Ehrlichia 16S rRNA and Mycoplasma 16S rRNA (Table 1). Additional PCR assays (GroEL and sodB) were performed to confirm the original Ehrlichia 16S rRNA results. Amplicons were sequenced and compared performed using BLAST against the GenBank database. Sequence identities for the partial Mycoplasma 16S rRNA, Ehrlichia 16S rRNA, GroEL, and sodB genes with respective GenBank accession numbers are listed in Table 2, all with 100% coverage. Additional Ehrlichia species-specific assays included Ehrlichia chaffeensis sodB, Panola Mountain Ehrlichia sp. sodB and gltA, and Ehrlichia muris dsb, and did not amplify DNA (Table 2). Based on these PCR results, co-infection with Ehrlichia ewingii and Mycoplasma sp. was diagnosed in this goat.

To the authors’ knowledge, ours is the first report describing naturally occurring E. ewingii infection in a goat that was also co-infected with a hemotropic Mycoplasma sp. Ehrlichia spp. are composed of a group of obligate intracellular bacteria that have a tropism for leukocytes. Ehrlichia ewingii is the causative agent of granulocytic ehrlichiosis in dogs and human ewingii ehrlichiosis.⁸⁻¹⁰ Based on serology using Ehrlichia sp.-specific peptides, E. ewingii is the most prevalent Ehrli-

Table 1. Peripheral blood polymerase chain reaction (PCR) panel results from a 9-year-old female Boer goat with a fracture of the right humerus and morulae identified within neutrophils on the CBC.

| Genus PCR Target | PCR Results |
|------------------|-------------|
| Anaplasma/Ehrlichia | Positive |
| Babesia | Negative |
| Bartonella | Negative |
| Mycoplasma | Positive |
| Rickettsia | Negative |
chiae spp. that infects dogs in the south central, south eastern and mid-Atlantic United States. This finding is consistent with the geographic distribution of the primary vector of *E. ewingii*, *Amblyomma americanum*, the lone star tick. Wildlife, particularly the white-tailed deer, are a major reservoir host for the maintenance of several *A. americanum*-associated pathogens including *E. ewingii*. Among domestic animals, dogs also may serve as a reservoir host for *A. americanum* because there is evidence of a chronic *E. ewingii* carrier status after acute infection.

Various *Anaplasma* and *Ehrlichia* spp. can infect domestic goats. *Anaplasma phagocytophilum* (referred to as tick-borne fever in Europe) and *Ehrlichia ruminantium* (heartwater disease, historically the organism was designated *Cowdria ruminantium*) are well described, clinically and economically relevant tick-transmitted diseases in Europe and Africa, respectively. The importance of other *Ehrlichia* spp. in goats is less clear. *Ehrlichia canis* and *Neorickettsia risticii* (formerly *Ehrlichia risticii*) were shown to infect goats by inoculation of infected blood. The recently discovered Panola Mountain *Ehrlichia* sp. was transmitted to goats by infected *A. americanum* ticks, and natural infection of goats with *E. chaffeensis* also has been reported.

Domestic goats serve as hosts for all life stages of *A. americanum*, and experimental infection of goats by *E. ewingii*-infected *A. americanum* ticks was demonstrated in 3 goats. However, to the authors’ knowledge, natural infection of goats with *E. ewingii* has not been reported previously. Clinical abnormalities in the experimentally *E. ewingii*-infected goats included mild pyrexia, lethargy, inappetence, serous nasal discharge, lameness, and coughing. None of these signs were noted in the goat of our report. Similar to our case, subclinical *E. ewingii* infection also has been reported in dogs. However, laboratory findings reported in this goat, including lymphopenia, neutrophilic left shift, decreased ALP activity, and increased GGT activity are changes reported in association with experimental *E. ewingii* and natural *A. phagocytophilum* infections in goats. Decreased ALP activity in goats with *Ehrlichia* sp. infection is a consistent and pathophysiologically interesting observation. It has been postulated that the activity of ALP, a zinc-dependent enzyme, is correlated with the plasma concentrations of zinc, which decrease during rickettsemic episodes as a result of the release of endogenous pyrogens and acute phase mediators. The clinical relevance of mildly increased GGT activity in this goat is unclear because no other laboratory data indicated cholestasis. The activity of SDH has not been a sensitive marker to screen for hepatic disease, but was not determined in this goat. Increased GGT activity also was related to altered hepatic metabolic activities associated with *A. phagocytophilum* infection in goats, and increased serum transaminase activities including GGT occur in association with *E. ewingii* ehrlichiosis in humans.

The mode, timing, and duration of *E. ewingii* infection in this goat remains speculative. Morulae were visualized in the fall when *Amblyomma americanum* ticks are still active in North Carolina. Findings of neutrophilic morulae in blood smears occurs during the acute infection, or when a persistently infected reservoir host is severely stressed or treated with immunosuppressive drugs. Underlying immunosuppression is a known risk factor for *E. ewingii* infection in humans, but it is unclear if immunosuppression might have contributed to *E. ewingii* infection in this goat.

Another interesting aspect of this case was the co-infection with hemotropic *Mycoplasma* sp., which was not visualized on the blood smear. The amplified partial *Mycoplasma 16S rRNA* sequence was recently described to occur with high incidence in asymptomatic white-tailed deer in North Carolina. This finding suggests that white-tailed deer could be a reservoir host for both infections documented in this case, *E. ewingii* and *Mycoplasma* sp. Perhaps both organisms were transmitted by the same vector (*A. americanum*), and access to the wooded lot likely exposed the goat to infected ticks. Similar to the *E. ewingii* infection, the clinical relevance of the *Mycoplasma* sp. infection is not clear in the present case. Although blood smear evaluation has a low sensitivity for detection of hemotropic...

Table 2. Sequence identities for *Mycoplasma 16S rRNA* and *Ehrlichia* polymerase chain reaction (PCR) amplicons.

| Gene Target         | GenBank Sequence Comparisons |
|---------------------|------------------------------|
| *Mycoplasma 16S rRNA* | 100% (552 bp) identical to an uncultured *Mycoplasma* sp. 16S rRNA from a white-tailed deer in North Carolina (KC512404) |
| *Ehrlichia 16S rRNA* | 99% identical (366/368 bp) to *E. ewingii*, genotype Panola Mountain partial 16S rRNA gene from *Amblyomma americanum* (DQ365880) and *E. ewingii*, strain 95E9-TS from a dog from North Carolina with granulocytic ehrlichiosis (U96436.1) |
| *Ehrlichia GroEL*    | 100% identical (584 bp) to *E. ewingii* partial GroEL gene from a naturally infected human patient with ehrlichiosis (AF195273) |
| *Ehrlichia sodB*     | 100% identical (303 bp) to *E. ewingii* partial sodB gene from a naturally infected dog (KC778986) |
| E. canis p90         | N/A                          |
| E. chaffeensis sodB  | N/A                          |
| Panola Mountain     | N/A                          |
| *Ehrlichia* sp. sodB and gltA | N/A                      |
| E. muris db          | N/A                          |

bp, base pairs; *E., Ehrlichia*; N/A, not applicable, as no PCR amplicon was obtained for DNA sequencing.

Sequences were compared to the GenBank database using the Basic Local Alignment Search Tool.
**Mycoplasma** infection, failure to visualize organisms on the blood smear in this case suggests a low infectious burden in a chronically infected goat.24 To the authors’ knowledge, this case represents the first reported case of natural *E. ewingii* infection in a goat that was also co-infected with a hemotropic *Mycoplasma* sp., acquired in south eastern North America. The clinical relevance of naturally occurring *E. ewingii* infection in goats should be determined, and whether the goat is an incidental or a primary host should also be investigated. The prevalence of ticks infected with *E. ewingii* is increasing markedly in North Carolina,25 and *E. ewingii* can infect wildlife species, domestic animals, and also humans.10 Therefore, farm workers and owners of pet goats should be aware that pastures might contain infected ticks. Serological and molecular surveys of this pathogen in goats should be performed to identify the role of goats as a potential reservoir host and to determine the prevalence of infection in this species.

**Footnotes**

a CXDI-50G, Canon, USA, Inc., Lake Success, NY
b HemaTrue Hematology Analyzer, HESKA, Loveland, CO
c VetScan VS2, Abaxis, Union City, CA
d QIASymphony® DNA Mini Kit (192) (# 931236), Qiagen, Valencia, CA
e GENEWIZ, Inc., Research Triangle Park, Raleigh, NC
f AlignX softwareVector NTI® Advance Version 11.5, Invitrogen, Inc, Life Technologies, Thermo Fisher Scientific, Waltham, MA

**Acknowledgments**

Conflict of Interest Declaration: Authors disclose no conflict of interest. Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

**References**

1. Thrall DE. Textbook of Veterinary Diagnostic Radiology. St. Louis, Missouri: Elsevier Health Sciences; 2013.
2. Stevens JB, Anderson KL, Correa MT, et al. Hematologic, blood gas, blood chemistry and serum mineral values for a sample of clinically healthy adult goats. Vet Clin Pathol 1994;23:19–24.
3. Birkenheuer AJ, Levy MG, Breitschwerdt EB. Development and evaluation of a seminested PCR for detection and differentiation of *Babesia gibsoni* (Asian genotype) and *B. canis* DNA in canine blood samples. J Clin Microbiol 2003;41:4172–4177.
4. Maggi RG, Chitwood MC, Kennedy-Stoskopf S, et al. Novel hemotropic *Mycoplasma* species in white-tailed deer (*Odocoileus virginianus*). Comp Immunol Microbiol Infect Dis 2013;36:607–611.
5. Eddelstone SM, Diniz PP, Neer TM, et al. Doxycycline clearance of experimentally induced chronic *Ehrlichia canis* infection in dogs. J Vet Intern Med 2007;21:1237–1242.
6. Barber RM, Li Q, Diniz PP, et al. Evaluation of brain tissue or cerebrospinal fluid with broadly reactive polymerase chain reaction for *Ehrlichia, Anaplasm*, Spotted Fever Group *Rickettsia*, *Bartonella*, and *Borrelia* species in canine neurological diseases (109 cases). J Vet Intern Med 2010;24:372–378.
7. Qurollo BA, Riggins D, Comyn A, et al. Development and validation of a sensitive and specific sodB-based quantitative PCR assay for molecular detection of *Ehrlichia* species. J Clin Microbiol 2014;52:4030–4032.
8. Ewing SA, Roberson WR, Buckner RG, et al. A new strain of *Ehrlichia canis*. J Am Vet Med Assoc 1971;159:1771–1774.
9. Anderson BE, Greene CE, Jones DC, et al. *Ehrlichia ewingii* sp. nov., the etiologic agent of canine granulocytic ehrlichiosis. Int J Syst Bacteriol 1992;42:299–302.
10. Dumler JS, Madigan JE, Pusterla N, et al. *Ehrlichioses* in humans: Epidemiology, clinical presentation, diagnosis, and treatment. Clin Infect Dis 2007;45:S45–S51.
11. Qurollo BA, Chandrashekar R, Hegarty BC, et al. A serological survey of tick-borne pathogens in dogs in North America and the Caribbean as assessed by *Anaplasm phagocytophilum*, *A. platys*, *Ehrlichia canis*, *E. chaffeensis*, and *Borrella burgdorferi* species-specific peptides. Infect Ecol Epidemiol 2014;204.
12. Goddard J, Varela-Stokes AS. Role of the lone star tick, *Amblyomma americanum* (L.), in human and animal diseases. Vet Parasitol 2009;160:1–12.
13. Paddock CD, Yabsley MJ. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*–associated zoonoses in the United States. Curr Top Microbiol Immunol 2007;315:289–324.
14. Starkey LA, Barrett AW, Beall MJ, et al. Persistent *Ehrlichia ewingii* infection in dogs after natural tick infestation. J Vet Intern Med 2015;29:552–555.
15. Watson AD, van Duin CT, Knoppert NW, et al. Effect of tick-borne fever on liver and kidney function in dwarf-crooss goats. Br Vet J 1988;144:581–589.
16. Gokce HI, Woldehiwet Z. Differential haematological effects of tick-borne fever in sheep and goats. Zentralbl Veterinarmed B 1999;46:105–115.
17. Gokce HI, Woldehiwet Z. The effects of *Ehrlichia* (*Cytocetes*) *phagocytophila* on the clinical chemistry of sheep and goats. Zentralbl Veterinarmed B 1999;46:93–103.
18. Stuen S, Longbottom D. Treatment and control of ehrlymial and rickettsial infections in sheep and goats. Vet Clin North Am Food Anim Pract 2011;27:213–233.
19. Pennisi MG. Infection of small ruminants with *Ehrlichia* spp. Sicily. Parasitologia 1999;41(Suppl 1):85–88.
20. Lofis AD, Levin ML, Spurlock JP, Two USA *Ehrlichia* spp. cause febrile illness in goats. Vet Microbiol 2008;130:398–402.
21. Dugan VG, Little SE, Stallknecht DE, et al. Natural infection of domestic goats with *Ehrlichia chaffeensis*. J Clin Microbiol 2000;38:448–449.
22. Liebisch A. General review of the tick species which parasitize sheep and goats worldwide. Parasitologia 1997;39:123–129.
23. Goodman RA, Hawkins EC, Olby NJ, et al. Molecular identification of *Ehrlichia ewingii* infection in dogs: 15 cases (1997–2001). J Am Vet Med Assoc 2003;222:1102–1107.
24. Messick JB. Hemotropic mycoplasmas (hemoplasmas): A review and new insights into pathogenic potential. Vet Clin Pathol 2004;33:2–13.
25. Lee S, Kakumanu ML, Ponnusamy L, et al. Prevalence of *Rickettsiales* in ticks removed from the skin of outdoor workers in North Carolina. Parasit Vectors 2014;7:607.