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Case Report
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Naturally Occurring *Ehrlichia ewingii* and *Mycoplasma* sp. Co-Infection in a Goat

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Key words: Caprine; Ehrlichiosis; Tick-borne disease.

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,[a] 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.[1]

A CBC[b] 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],

Abbreviations:

A.	*Anaplasma*
ALP	alkaline phosphatase
AST	aspartate aminotransferase
Bp	base pairs
CK	creatinine kinase
C	celsius
DNA	deoxyribonucleic acid
E.	*Ehrlichia*
F	fahrenheit
GGT	gamma-glutamyl transferase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
Kg	kilogram
MCV	mean cell volume
MCHC	mean corpuscular hemoglobin concentration
M.	*Mycoplasma*
PCR	polymerase chain reaction
rRNA	ribosomal ribonucleic acid

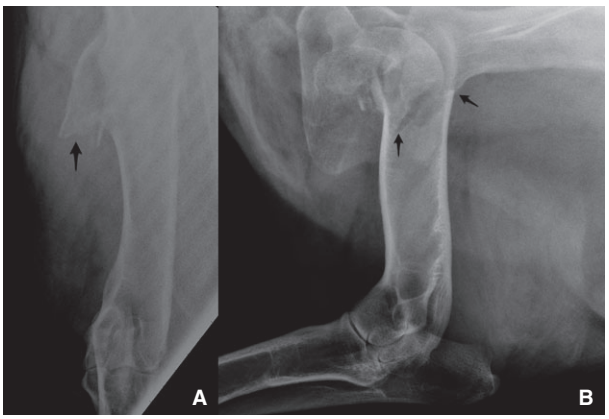

Fig 1. Craniocaudal (**A**) and lateral (**B**) radiographs of the right humerus of a 9-year-old female Boer goat presenting with non-weight bearing lameness. Radiographs show an oblique fracture of the proximal humeral diaphysis that is associated with marked soft tissue swelling. Very mild rounding and blunting of the fracture margins (arrows) without evidence of callus formation are consistent with a fracture of at least 7–10 days duration.

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34.3 d/dL; reference range, 30–37 g/dL). 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/uL; 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 × 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[c] included mildly increased activities of AST (227 IU/L; reference range, 35.5–72.0 IU/L[2]), CK (788 IU/L; reference range, 24.5–98 IU/L[2]), 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.[d] 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,[e] and alignments were compared with GenBank sequences.[f] 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 comparisons 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 canis p30*, *E. 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-*

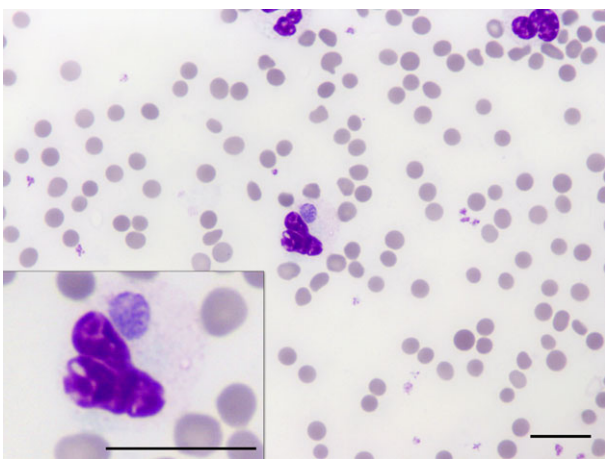

Fig 2. Blood smear from a 9-year-old female Boer goat. Mildly decreased RBC density, moderate anisocytosis, and mild macrocytosis indicate mild, likely regenerative anemia. The segmented neutrophil in the center (insert with higher magnification) contains a tightly packed, basophilic granular cluster of organisms (morula) in the cytoplasm, which was identified as *Ehrlichia ewingii* by polymerase chain reaction. No other etiologic agents are observed. Wright–Giemsa stain; bar = 10 μm; large image: 1,000× magnification field.

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

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* spp. *16S rRNA* from a white-tailed deer in North Carolina (KC512404)[4]
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 p30	N/A
E. chaffeensis sodB	N/A
Panola Mountain Ehrlichia sp. *sodB* and *gltA*	N/A
E. muris dsb	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.

chia spp. that infects dogs in the south central, south eastern and mid-atlantic United States.[11] This finding is consistent with the geographic distribution of the primary vector of *E. ewingii*, *Amblyomma americanum*, the lone star tick.[12] Wildlife, particularly the white-tailed deer, are a major reservoir host for the maintenance of several *A. americanum*-associated pathogens including *E. ewingii*.[12,13] 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.[12,14]

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.[15–18] 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.[19] 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.[20,21]

Domestic goats serve as hosts for all life stages of *A. americanum*,[22] and experimental infection of goats by *E. ewingii*-infected *A. americanum* ticks was demonstrated in 3 goats.[20] 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.[20] 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.[23] 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.[15–17,20] Decreased ALP activity in goats with *Ehrlichia* spp. 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.[15,17] The clinical relevance of mildly increased GGT activity in this goat is unclear because no other laboratory data indicated cholestasis. The activity of SDH would have 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,[15] and increased serum transaminase activities including GGT occur in association with *E. ewingii* ehrlichiosis in humans.[10]

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.[12] 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,[10] 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.[4] This finding suggests that white-tailed deer could be a reservoir host for both infections documented in this case, *E. ewingii* and *Mycoplasma* sp.[4,12,13] 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

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

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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