Polyarthritis in a Dog

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A 3-year-old, male Labrador retriever was presented with reluctance to move.

History. The dog began having difficulty getting in and out of the car 5 days previously. He also became lethargic and febrile and had a decreased appetite. Aside from thrombocytopenia, results from the CBC and clinical chemistry panel were within normal limits. The dog was treated with a glucocorticoid and intravenous lactated Ringer's solution containing cefazolin by the referring veterinarian. When clinical signs did not improve, the dog was referred to the University of Florida Veterinary Medical Teaching Hospital for evaluation.

Physical Examination. Findings included depression, weight loss, stiffness in the hind limbs, swollen stifle and hock joints, enlarged prescapular and popliteal lymph nodes, hair loss preceding the current clinical presentation, and tapeworms.

continues

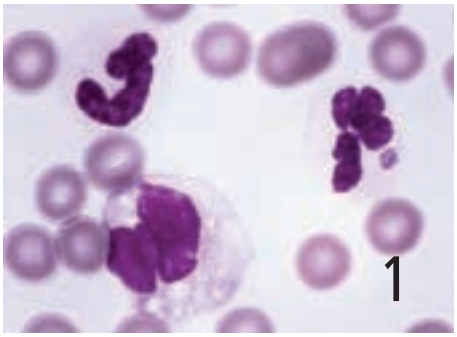
ASK YOURSELF . . .

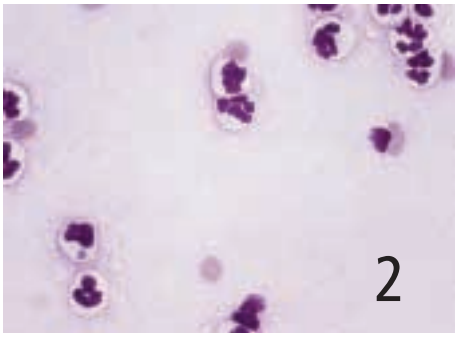
- Was this dog infected with *Ehrlichia canis*?
- Could it have been infected with more than one rickettsial organism?
- What is the most likely organism reported to infect dogs that would account for the polyarthritis and neutrophilic inclusions?
- What additional diagnostic tests might definitively identify the causative agents in this case?

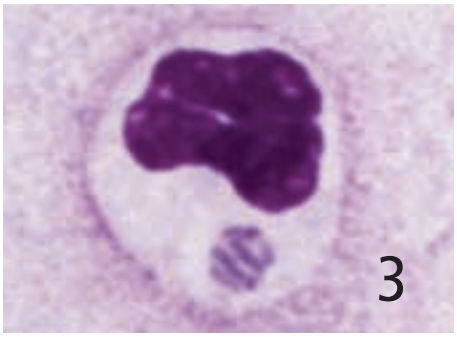
Laboratory Findings*

Variable	Result
CBC	Low-normal hematocrit (37%), thrombocytopenia (92×10^3/µl), monocytosis (2.1×10^3/µl), increased total plasma protein concentration (8.5 g/dl), hyperfibrinogenemia (500 mg/dl)
Stained blood film	Increased erythrocyte rouleaux formation and cytoplasmic inclusions in three neutrophils (**Figure 1**)
Serum chemistry analytes	Mildly increased alkaline phosphatase levels (probably the result of glucocorticoid treatments); slight increase in total globulin concentration
Urinalysis	Moderate bilirubinuria
Stained smears from aspirates of the right and left stifle joints	Total nucleated cell counts from both joints estimated at 50×10^3/µl; nondegenerate neutrophils (**Figure 2**) accounted for 80% to 90% of the nucleated cells present; lymphocytes and macrophages accounted for the remaining cells. Cytoplasmic inclusions were observed in a few neutrophils that had morphologic characteristics similar to those seen in blood neutrophils (**Figures 2 and 3**).
Serum IFA	Positive titers for *Ehrlichia canis* (1:160); *Ehrlichia equi* (now classified as *Anaplasma phagocytophilum*) (1:80); *Ehrlichia risticii* (now classified as *Neorickettsia risticii*) (1:20); *Ehrlichia sennetsu*, a human agent (now classified as *Neorickettsia sennetsu*) (1:10)

* Routine culture of joint fluid was negative; serologic tests were negative for *Rickettsia rickettsii*, *Borrelia burgdorferi*, and antinuclear antibody. Other findings on the CBC, serum chemistry, and urinalysis were unremarkable.





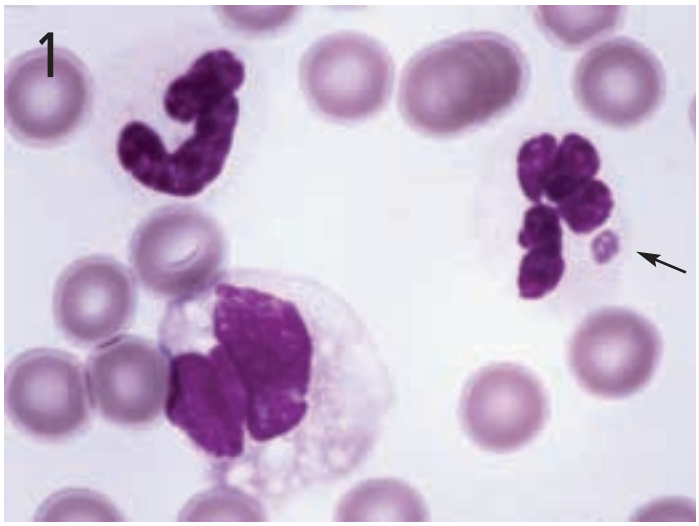


Serum and blood samples were submitted to the Centers for Disease Control and Prevention for further evaluation. Repeated testing by CDC revealed high serum IFA titers for both *E. canis* (1:4096 and 1:8192) and *E. chaffeensis* (1:8192 and 16,384). *E. chaffeensis* was initially recognized to cause disease in humans, but it can also infect dogs. Attempts were made to amplify *Ehrlichia* DNA for the 16S rRNA gene from blood using a nested PCR with a specific panel of primers. Neither *E. canis* nor *E. chaffeensis* could be identified, but a primer specific for *E. ewingii* generated a 300 base pair segment that when sequenced was identical to that published for *E. ewingii*.

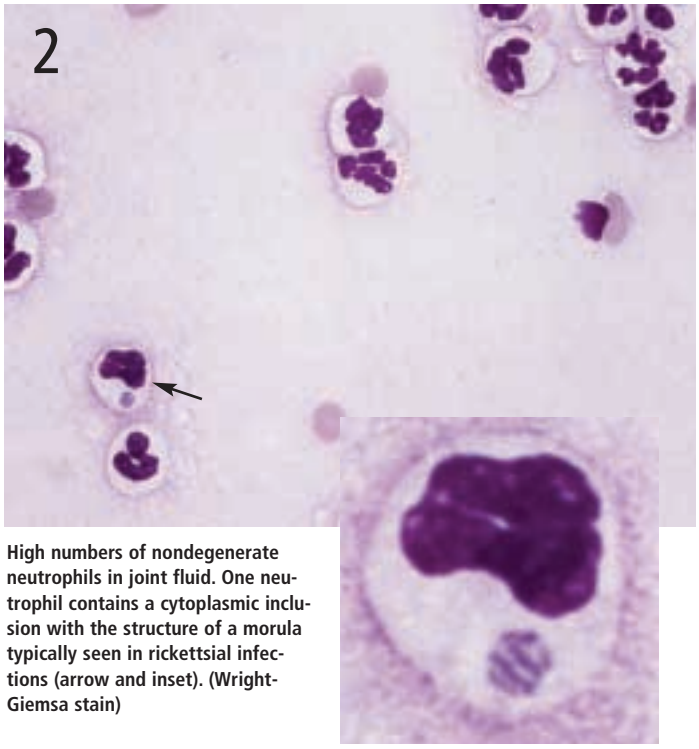
Diagnosis. *Ehrlichia ewingii* infection

DID YOU ANSWER . . .

- Possibly, but not necessarily. Serologic cross-reactivity occurs between genetically related rickettsial organisms. *Ehrlichia canis* and *E. chaffeensis* are especially closely related organisms that cannot be differentiated using conventional IFA tests. Although the highest titers were for these organisms, it is difficult to attribute the presentation in this case solely to *E. canis* or *E. chaffeensis* infections, because both agents infect mononuclear phagocytes, and morulae were seen in neutrophils.
- Probably. Dogs and tick vectors that spread rickettsial disease may be infected with several rickettsial species (as well as other infectious organisms) simultaneously. Although *E. canis* and *E. chaffeensis* were not identified by PCR, the IFA titers determined by the Centers for Disease Control are higher than one might expect for a cross-reaction with *E. ewingii*. Consequently, this dog could also be chronically infected with *E. canis* or *E. chaffeensis* but not have sufficient numbers of organisms in blood to be identified by PCR.
- Although arthritis has been reported in dogs believed to have been infected with *E. canis*, this diagnosis was based on serum IFA tests and not on genetic identification of the organism involved. Infections with other rickettsial agents, including *E. ewingii*, might also result in a positive *E. canis* titer, or the dogs might have had concurrent infections with more than one organism. Morulae have been identified in neutrophils of dogs infected with two rickettsial organisms, *A. phagocytophilum* (formerly *E. equi* [horses], *E. phagocytophila* [ruminants], and human granulocytic ehrlichiosis agent) and *E. ewingii*. Polyarthritis has been associated with *E. ewingii* infections, but not with *A. phagocytophilum* infections. Based on these considerations, an *E. ewingii* infection was suspected, but a specific serologic assay for *E. ewingii* was not available.
- PCR-based assays that can identify specific infectious organisms based on their genetic composition. The 16S rRNA gene is currently the most widely studied gene for bacterial identification. In this case, *E. ewingii* infection was proven by sequencing a portion of the 16S rRNA gene amplified by PCR.



Blood film containing two neutrophils and a monocyte. A cytoplasmic inclusion is present in one neutrophil (arrow) with the structure of a morula typically seen in rickettsial infections. (Wright-Giemsa stain)



High numbers of nondegenerate neutrophils in joint fluid. One neutrophil contains a cytoplasmic inclusion with the structure of a morula typically seen in rickettsial infections (arrow and inset). (Wright-Giemsa stain)

See Aids & Resources, back page, for references, contacts, and appendices.