# Clinical Case Conference

## Hypergammaglobulinemia in a dog

Gina M. Michels, DVM; G. Daniel Boon, DVM; Brent D. Jones, DVM; Barry Puget, BS

An 11-year-old castrated male Poodle-type dog was admitted to the veterinary teaching hospital for repair of a left-sided perineal hernia. A right-sided perineal hernia repair and castration had been performed 3 weeks earlier. The dog had become lethargic at the time the right-sided hernia was diagnosed, but responded to a 5-day course of enrofloxacin (2 mg/kg of body weight, PO, q 12 h). At that time, the owner reported that the dog had a normal activity level and no history of dysuria or tenesmus. Results of a CBC, urinalysis, and serum biochemical analysis before the first hernia surgery had indicated a platelet count (231,000 thrombocytes/mm$^3$) within reference limits, normocytic normochromic nonregenerative anemia (PCV, 24%), high concentration of serum total proteins (8.7 g/dl) and globulins (5.8 g/dl), 3+/4+ proteinuria on urine dipstick, and concentrated urine (specific gravity, 1.051).

At the time of admission to the hospital for repair of the left-sided perineal hernia, physical examination findings were normal except for a small left-sided perineal hernia. Concentrations of serum total proteins and globulins had increased (11.0 g/dl and 7.9 g/dl, respectively). Although somewhat improved, a normocytic normochromic nonregenerative anemia persisted (PCV, 34%). Attempts to obtain a urine sample were unsuccessful. The albumin concentration and platelet count (205,000 thrombocytes/mm$^3$) continued to be within the reference range. The left-sided perineal hernia had stabilized without any noticeable clinical effect. The dog was admitted for evaluation of the hyperglobulinemia and anemia.

Protein electrophoresis revealed a narrow globulin peak suggestive of a monoclonal gammopathy. Protein electrophoresis results can be suggestive of a mono- or polyclonal gammopathy; however, it does not allow distinction between heavy-chain classes, in part because the $\gamma$ region on the protein electrophoretogram consists of immunoglobulins from all classes (IgG, A, M, D, and E).[1] Monoclonal gammopathy develops secondarily to proliferation of a single clone of plasma cells resulting in the production of immunoglobulins containing 1 heavy-chain class such as IgG, 1 heavy-chain subclass such as $IgG_1$, and 1 light-chain type, either $\kappa$ or $\lambda$.[1] Underlying causes for monoclonal gammopathy that have been described in dogs include lymphocytic leukemia, plasma cell leukemia, cutaneous amyloidosis, macroglobulinemia, idiopathic monoclonal gammopathy, ehrlichiosis, and multiple myeloma.[2-8] Lymphocytic leukemias are characterized by detection of lymphoblasts or a high number of mature lymphocytes in blood,[2] whereas plasma cell leukemia is associated with a predominance of plasma cells in blood and bone marrow.[3,4] Cutaneous amyloidosis is a histologic diagnosis and has been reported in 1 dog that had bleeding from the skin secondary to minor trauma.[5] Plasma cell and lymphocytic leukemias, as well as cutaneous amyloidosis, were ruled out for this dog on the basis of physical and clinicopathologic findings available at the time. Macroglobulinemia is characterized by an increased production of IgM,[6] and idiopathic or benign monoclonal gammopathy is a diagnosis of exclusion.[2] Normocytic normochromic nonregenerative anemia, hyperglobulinemia, bone marrow plasmacytosis, proteinuria, bacteriuria, normal platelet count, and hypergammaglobulinemia attributable to a monoclonal gammopathy have been associated with ehrlichiosis. Though fever, thrombocytopenia, and epistaxis are clinical findings that veterinarians often associate with canine ehrlichiosis, it is not uncommon for 1 or all of these signs to be absent.[5,7-11] A titer of 1:10 (the lowest detectable titer) or greater for *Ehrlichia canis*, however, is indicative of exposure to *E canis* and is presumptively diagnostic of ehrlichiosis.[12] In most cases of canine ehrlichiosis, protein electrophoresis indicates a polyclonal gammopathy. Some dogs, however, have a protein electrophoretogram suggestive of a monoclonal gammopathy similar to that seen with multiple myeloma.

Immunoelectrophoresis by using monospecific antisera has demonstrated that the monoclonal spike observed in some cases of ehrlichiosis is a result of a restricted polyclonal gammopathy consisting of 1 heavy-chain class (IgG) and at least 4 subclasses ($IgG_{1-4}$), rather than a true monoclonal gammopathy in which there is a single heavy-chain class, subclass, and light-chain type.[1,13] Such monospecific antisera, however, are not readily available for veterinary use. Therefore, in the clinical situation, immunoelectrophoresis does not allow for differentiation of polyclonal and monoclonal gammopathies, because only isotype-specific heavy-chain class antibodies are used.[1] Diagnosis of multiple myeloma relies on demonstration of at least 3 of the following criteria: monoclonal gammopathy in serum or concentrated urine, radiographic evidence of a lytic bone lesion, Bence Jones proteins in urine, and > 5% plasma cells in bone marrow.[14]

From the Departments of Veterinary Medicine and Surgery (Michels, Jones), Veterinary Clinical Pathology (Boon), College of Veterinary Medicine, University of Missouri, Columbia, MO 65211, and the Department of Immunology (Puget), Veterinary Medicine Teaching Hospital, University of California, Davis, CA 95616.

Dr. Michels' present address is the Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907.

In the dog of this report, serum was obtained for determination of an *E canis* titer. While awaiting the result, further diagnostic tests were performed. During hospitalization, the dog had hematuria and severe pollakiuria. A voided urine sample was obtained for bacterial culturing and susceptibility testing. Two days after initiation of treatment with amoxicillin combined with clavulanic acid (22 mg/kg, PO, q 12 h for 21 days), an adequate volume of urine was available for collection and submission for Bence Jones protein analysis. Results of the Bence Jones protein analysis and the aerobic bacterial culture were negative. Anaerobic bacterial culturing resulted in growth of *Propionibacterium* sp (> 100,000 colony-forming units). The microbiologist performing the testing indicated that the organism usually is susceptible to amoxicillin with clavulanic acid.

Survey radiography of the vertebral column revealed an area of radiolucency of the dorsal spinous process of the fourth thoracic vertebra. This area was believed to either be a result of shadows from overlying tissue planes or an osteolytic lesion. Evaluation of the bone marrow biopsy specimen and aspirate obtained from the left humerus indicated mild plasmacytosis consistent with a nonneoplastic reaction to continuous antigenic stimulus.

The *E canis* titer was positive ($1:670\times10^6$). A presumptive diagnosis of ehrlichiosis was made. After the course of amoxicillin with clavulanic acid for the urinary tract infection was completed, doxycycline was administered (13 mg/kg, PO, q 24 h for 21 days). Four weeks after initiation of treatment, the serum total protein concentration was 9.4 g/dl, the globulin concentration was 6.0 g/dl, and the *E canis* titer was approximately $1:5\times10^6$. Results of immunoelectrophoresis, using γ-chain specific antibody, were consistent with an IgG monoclonal gammopathy. Radial immunodiffusion was used to quantitate the amount of immunoglobulin present in each immunoglobulin class. Results confirmed that the hyperglobulinemia was an IgG type [IgG, 93.0 mg/ml (reference range, 10.0 to 20.0 mg/ml); IgA, 0.18 mg/ml (reference range, 1.0 to 2.0 mg/ml); IgM, 0.6 mg/ml (reference range, 0.11 to 0.93)]. It has been demonstrated that the globulins contributing to hypergammaglobulinemia in dogs with ehrlichiosis do not correlate with the antibody to *E canis* that is detected by indirect fluorescent antibody.[13] Titers of indirect fluorescent antibody often persist for many months after resolution of hypergammaglobulinemia, following termination of infection by antibiotic treatment.[8,9,14] Buhles et al[15] suggested that levels of γ-globulin may be useful as a diagnostic aid in differentiating carrier dogs from dogs in which infection has been cleared. The response observed on protein electrophoresis following treatment with doxycycline provided additional support of *E canis* infection in the dog of this report.

Reevaluation of *E canis* titer every 6 months and performance of serum protein electrophoresis every 6 to 8 weeks was recommended. The owner reported that the dog continued to have no abnormal clinical signs 8 months after initial diagnosis and did not return for further clinical evaluation. Follow-up radiography of the area of lucency was not performed. On further review of the initial radiographs, the radiologist believed that the area in question was a result of shadows from overlying tissue planes and was not clinically important.

## References


1. Kyle RA, Greipp PR. The laboratory investigation of monoclonal gammopathies. *Mayo Clin Proc* 1978;53:719–739.
2. Thompson JP. Immunologic diseases. In: Ettinger SJ, ed. *Textbook of veterinary internal medicine*. Vol 2. Philadelphia: WB Saunders Co, 1989;2297–2328.
3. Couto CG, Ruehl W, Muir S. Plasma cell leukemia and monoclonal (IgG) gammopathy in a dog. *J Am Vet Med Assoc* 1984;184:90–94.
4. Campbell KL, Latimer KS. Polysystemic manifestations of plasma cell myeloma in the dog: a case report and review. *J Am Anim Hosp Assoc* 1985;21:59–66.
5. Schwartzman RM. Cutaneous amyloidosis associated with a monoclonal gammopathy in a dog. *J Am Vet Med Assoc* 1984;185:102–104.
6. Yamaguchi RA. Immunoproliferative diseases. In: Morgan RV, ed. *Handbook of small animal practice*. New York: Churchill Livingstone Inc, 1988;861–866.
7. Breitschwerdt EB, Woody BJ, Zerbe CA, et al. Monoclonal gammopathy associated with naturally occurring canine ehrlichiosis. *J Vet Intern Med* 1987;1:2–9.
8. Perille AL, Matus RE. Canine ehrlichiosis in six dogs with persistently increased antibody titers. *J Vet Intern Med* 1991;5:195–198.
9. Codner EC, Roberts RE, Ainsworth AG. Atypical findings in 16 cases of canine ehrlichiosis. *J Am Vet Med Assoc* 1985;186:166–169.
10. Troy GC, Vulgamott JC, Turnwald GH. Canine ehrlichiosis: a retrospective study of 30 naturally occurring cases. *J Am Anim Hosp Assoc* 1980;16:181–187.
11. Kuehn NF, Gaunt SD. Clinical and hematologic findings in canine ehrlichiosis. *J Am Vet Med Assoc* 1985;186:355–358.
12. Woody BJ, Hoskins JD. Ehrlichial diseases of dogs. *Vet Clin North Am Small Anim Pract* 1991;21:75–98.
13. Weisiger RM, Ristic M, Huxsoll DL. Kinetics of antibody response to *Ehrlichia canis* assayed by the indirect fluorescent antibody method. *Am J Vet Res* 1975;36:689–694.
14. MacEwen EG, Hurvitz AI. Diagnosis and management of monoclonal gammopathies. *Vet Clin North Am Small Anim Pract* 1977;7:119–132.
15. Buhles WC, Huxsoll DL, Ristic M. Tropical canine pancytopenia: clinical, hematologic, and serologic response to *Ehrlichia canis* infection, tetracycline therapy, and challenge inoculation. *J Infect Dis* 1974;130:357–367.

*Commentary by*
*Johnny D. Hoskins, DVM, PhD,*
*Department of Veterinary Clinical Sciences,*
*School of Veterinary Medicine,*
*Louisiana State University,*
*Baton Rouge, La.*

The authors of this report remind us that *Ehrlichia canis* infection is still an important cause of monoclonal (or polyclonal) gammopathy in dogs.[1,2] Other causes of monoclonal gammopathy, as was mentioned in the report, include lymphocytic leukemia, multiple myeloma, plasma cell leukemia, cutaneous amyloidosis, idiopathic monoclonal gammopathy, and macroglobulinemia (listed in order of frequency of occurrence).[1] Without question, *E canis* infection is a frequent and important cause of hyperglobulinemia in dogs in the United States.

*Ehrlichia canis* is transmitted among dogs primarily by the brown dog tick, *Rhipicephalus sanguineus*. Although acute, subclinical, and chronic phases of *E canis* infection have been identified experimentally, accurate clinical staging of naturally acquired infections is difficult, if not impossible. Initial complaints often are nonspecific and include lethargy, weight loss, and anorexia. Hemorrhagic tendencies, fever, lymphadenopathy, and swollen joints are the most frequently detected physical findings. Hematologic abnormalities identified most frequently include nonregenerative anemia and thrombocytopenia. As supported by this case, dogs infected with *E canis* may have normal platelet counts and be clinically ill. In dogs with bleeding problems, prolonged bleeding times usually are detected, but the activated coagulation time, one-stage prothrombin time, activated partial thromboplastin time, and fibrin degradation product measurements are within reference limits. The most frequent serum chemistry abnormalities are hyperproteinemia with hyperglobulinemia and high activities of alanine transaminase and alkaline phosphatase. Because of difficulty in identification of *E canis* morulae in blood or bone marrow smears, definitive diagnosis is based on serum indirect immunofluorescence, western immunoblotting, and polymerase chain reaction assays.[3] Assay results become positive 2 to 28 days after initial infection. Antibody titers usually persist until there is complete clearance of the *E canis* organisms by the host's immune response or as a result of appropriate antimicrobial treatment.

*Ehrlichia canis* infection may be successfully treated with tetracycline, oxytetracycline, doxycycline, minocycline, and chloramphenicol for at least 3 to 4 weeks. The effectiveness of enrofloxacin (10 mg/kg of body weight, PO) daily for 2 weeks is unclear; therapeutic trials are currently underway. With good response to medical treatment, clinical improvement can be seen within 24 to 48 hours, usually preceding hematologic improvement. Results of an immunofluorescent antibody assay become negative over 3 to 6 months if *E canis* organisms are completely eliminated.[4] Successfully treated dogs are susceptible to reinfection with *E canis*. Prevention of infection depends on eliminating ticks from dogs and the environment.

1. Breitschwerdt EB, Woody BJ, Zerbe CA, et al. Monoclonal gammopathy associated with naturally occurring canine ehrlichiosis. *J Vet Intern Med* 1987;1:2–9.

2. Hoskins JD, Barta O, Rothschmitt J. Serum hyperviscosity syndrome associated with *Ehrlichia canis* infection in a dog. *J Am Vet Med Assoc* 1983;183:1011–1012.

3. Iqbal Z, Chaichanasiriwithaya W, Rikihisa Y. Comparison of PCR with other tests for early diagnosis of canine ehrlichiosis. *J Clin Microbiol* 1994;32:1658–1662.

4. Iqbal Z, Rikihisa Y. Reisolation of *Ehrlichia canis* from blood and tissues of dogs after doxycycline treatment. *J Clin Microbiol* 1994; 32:1644–1649.

*Commentary by*
*Ibulaimu Kakoma, DVM, PhD,*
*Department of Veterinary Pathobiology,*
*College of Veterinary Medicine,*
*University of Illinois,*
*Urbana, Ill.*

Ehrlichiosis is one of the great imitators, perhaps next only to Lyme disease, a syndrome often included in the differential diagnosis of ehrlichiosis. Classically, canine ehrlichiosis is diagnosed on the basis of some or all of the following observations: a positive titer to *Ehrlichia canis* by indirect immunofluorescent antibody (IFA) assay, a thrombocytopenia that may be associated with coagulopathy, petechial or ecchymotic hemorrhages, or epistaxis; fever; a naso-ocular discharge that may be associated with ocular opacity; a gammopathy; response to tetracycline treatment; observation or isolation of an ehrlichia agent,[1-4] or a positive result in a polymerase chain reaction test for *E canis*.[5]

The circumstances surrounding this case are reminiscent of another case in which a healthy Belgian Tervuren was transported from Arizona to Chicago by road; after a series of exercises at a show, the dog became severely lethargic, developed a high fever and epistaxis, and had a specific IFA titer of 1:640 to *E canis*. The response to doxycycline was dramatic, and the dog recovered in less than a week.

The dog of this report had signs that fulfilled some criteria for the diagnosis of ehrlichiosis, such as anemia, seropositivity, a gammopathy associated with plasmacytosis, and a good response to doxycycline. Recently, however, canine ehrlichiosis has been complicated by

the emergence of atypical canine ehrlichiosis caused by *E risticii*.[5] The disease presents with a negative *E canis* titer and a positive *E risticii* titer, which may coincide with fever and various abnormalities, but often has no abnormal effects on platelet counts. The management approach to infection with *E risticii* is identical to that for classical ehrlichiosis. It is important for clinicians and clients to know that a negative IFA test result when using *E canis* antigen does not rule out ehrlichiosis until an additional test for *E risticii* has been performed.

Many of the diagnostic criteria for ehrlichiosis (eg, IFA) with a cutoff point of 1:10,[5] as referred to by the authors of this report, have, unfortunately, not been universally adopted. A number of laboratories now use 1:40 as a cutoff for positivity and use this dilution for routine screening. In my experience, 1:40 is verifiable by other tests, such as western immunoblot,[3,7] whereas 1:10 is not consistently reproducible. In my opinion, paired serum samples should be adopted to assess the trend and significance of the seropositivity. In addition, a multicenter comparison of IFA titers should be considered to facilitate standardization. Until then, however, it is important to reflect on what diagnostic significance should be accorded to a positive IFA test result, especially when there are no clinical signs consistent with ehrlichiosis. There are dogs that have no other clinical signs except for a 1:40 titer for *E risticii* or *E canis*, and owners want recommendations. Our laboratory recommends that chemotherapeutic intervention be considered in those cases with signs consistent with the disease. The owner should be advised, however, that the presence of antibody is a genuine index of current or previous exposure to the ehrlichia agent. More infection-specific tests, such as polymerase chain reaction, and easier methods for in vitro cultivation need to be developed. We need to revisit issues such as whether antibiotic treatment completely eliminates ehrlichial organisms or whether subclinical ehrlichiosis can flare up as a result of predisposing factors such as stress or other cofactors.

1. Huxsoll DL. The historical background and global importance of ehrlichiosis. In: Williams JC, Kakoma I, eds. *Ehrlichiosis*. Dordrecht, The Netherlands: Kluwer Academic Publications, 1990;1–8.

2. Nyindo MBA, Holland CJ, Kakoma I. Current status of in vitro cultivation of Ehrlichiae. In: Williams JC, Kakoma I, eds. *Ehrlichiosis*. Dordrecht, The Netherlands: Kluwer Academic Publications, 1990;9–21.

3. Rikihisa Y. The tribe *Ehrlichieae* and ehrlichial diseases. *Clin Microbiol Rev* 1991;4:286–308.

4. Breitschwerdt EB, Woody BJ, Zerbe CA, et al. Monoclonal gammopathy associated with naturally occurring canine ehrlichiosis. *J Vet Intern Med* 1987;1:2–9.

5. Kakoma I, Hansen RD, Anderson BE, et al. Cultural, molecular, and immunological characterization of the etiologic agent for atypical canine ehrlichiosis. *J Clin Microbiol* 1994;32:170–175.

6. Weisiger RM, Ristic M, Huxsoll DL. Kinetics of antibody response to *Ehrlichia canis* assayed by the indirect fluorescent antibody method. *Am J Vet Res* 1975;36:689–694.

7. Nyindo M, Kakoma I, Hansen R. Antigenic analysis of four species of the genus ehrlichia by use of protein immunoblot. *Am J Vet Res* 1991;52:1225–1230.