Immune-mediated hemolytic anemia in a horse

Nat T. Messer IV, DVM, and Kristin Arnold, BVSc, MS

An 18-year-old 500-kg Quarter Horse gelding was referred to the Colorado State University Veterinary Teaching Hospital because of anemia, dark red urine, and signs of depression. One week prior to admission, minor skin lacerations were sutured, and the horse was given tetanus toxoid and treated with procaine penicillin G for 7 days. At admission, the horse appeared depressed, weak, and unresponsive to stimuli. Physical examination revealed tachycardia (84 beats/min) and pale and icteric mucous membranes. The spleen was large extending from the kidney caudally to the pelvic brim, with a thick, rounded caudal border. The PCV and total plasma protein concentration were 16% and 8.0 g/100 ml, respectively. Marked clumping of RBC was noticed on a blood smear, and when the cells were suspended in 0.9% NaCl solution, they failed to disperse. The WBC differential indicated neutrophilic leukocytosis, with a mild left shift, lymphopenia, and eosinopenia. Spherocytes were first seen on day 5 of hospitalization. Spherocytes and anisocytes persisted throughout the course of the disease. Dark red urine and hyperbilirubinemia coincided with the declines in PCV.

The result of the agar-gel immunodiffusion test for equine infectious anemia was negative on days 1, 32, and 93. Bacteriologic culturing of urine yielded no growth, and dark-field microscopy of urine did not reveal evidence of *Leptospira* spp. Examination of a biopsy specimen of liver failed to reveal any abnormalities. Results of Coombs antiglobulin test[a] were positive at all dilutions on days 1 and 93 of hospitalization. Cytologic examination of a bone marrow aspirate revealed marked erythroid hyperplasia, M:E ratio of less than 1:10, and greater than normal iron stores. A tentative diagnosis of immune-mediated hemolytic anemia (IHA) was made.

Initial treatment included IV administration of 20 L of a balanced electrolyte solution,[b] 8 L of blood from a compatible donor, and dexamethasone[c] (0.25 mg/kg of body weight, q 12 h). An increase in PCV and decrease in dark red urine was detected between days 2 and 5 of hospitalization. On days 7 to 15, the PCV decreased and dark red urine developed, indicating further hemolysis. On day 11, signs of severe laminitis were observed. Administration of dexamethasone was discontinued, and the horse was treated with phenylbutazone[d] (4.4 mg/kg, q 24 h, IV), sodium heparin[e] (1.1 mg/kg, q 12 h, SC), cyclophosphamide[f] (1.1 mg/kg, q 24 h, IM), and azathioprine[g] (1.1 mg/kg, q 24 h, IM). Heparin was discontinued after 5 days. Treatment with phenylbutazone, cyclophosphamide, and azathioprine was discontinued after 10 days because of development of leukopenia and hypoproteinemia. Two months after admission, the PCV, WBC, and total plasma protein concentration had returned to normal and remained so until the horse was euthanatized for an unrelated cause.

Necropsy did not reveal lesions consistent with infection or neoplasia that would have precipitated secondary (IHA). Major pathologic abnormalities included marked hemosiderosis of liver and kidney and extramedullary hematopoiesis in the spleen.

In horses, development of IHA may be idiopathic (primary) or secondary to systemic bacterial or viral infections, neoplasia, or drug administration.[1] Acute hemolytic anemia in horses also has been associated with piroplasmosis, leptospirosis, equine infectious anemia, acute hepatic insufficiency, and certain toxins,[2] none of which was involved in this case. Most reported cases in horses have been secondary IHA.[3-9] In addition to being classified as idiopathic or secondary, IHA also may be classified on the basis of the type of autoantibody

From the Departments of Clinical Sciences (Messer) and Pathology (Arnold), College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523. Dr. Messer's present address is Department of Medicine and Surgery Equine Center at Middlebush Farm, Hwy 63 South, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211.

[a]Antiequine IgG and Complement Antisera, Miles Laboratories, Elkhart, Ind.

[b]Multisol-R, CEVA Laboratories, Inc, Overland Park, Kan.

[c]Azium, Schering Corp, Kenilworth, NJ.

[d]Butazolidin, Coopers Animal Health, Kansas City, Kan.

[e]Heparin, Elkin-Sinn Inc, Cherry Hill, NJ.

[f]Cytoxan, Mead Johnson, Evansville, Ind.

[g]Imuran, Burroughs Wellcome Co, Research Triangle Park, NC.

causing the agglutination of RBC.[10] Immunoglobulin G (warm agglutinin) usually lacks complement-fixing ability or fails to fix complement in concentrations sufficient to cause lysis of RBC.[11] Immunoglobulin M (cold agglutinin) does fix complement in sufficient quantities to cause intravascular hemolysis[12] as well as cause intense autoagglutination when blood is stored at 4 C.[11]

In human beings, 3 classes of IHA are identifiable.[13] In class I (saline-acting autoagglutinins), agglutination can still be observed when a drop of blood is mixed with an equal volume of physiologic saline solution on a glass slide.[12] In class III, which is the most common form of IHA in dogs, RBC are coated with immunoglobulin of insufficient quantity, valence, or avidity to cause direct hemagglutination or lysis[12]; therefore, cell destruction occurs primarily in the reticuloendothelial system by phagocytosis of the opsonized RBC.[12] There may be pigmenturia, but rarely is there hemoglobinemia.[13]

The horse of the present report was believed to have had idiopathic IHA, because no underlying cause was found. Using the human classification system, the anemia in this horse had features of class I (saline-acting autoagglutinins) and class III (phagocytosis within the reticuloendothelial system). The leukopenia that developed during treatment was assumed to be induced by the chemotherapeutic agents, and the hypoproteinemia was presumed to be induced by phenylbutazone used to treat the laminitis. The use of chemotherapeutic agents was necessary because of the lack of response to corticosteroids.

1. Harvey JW. Canine hemolytic anemias. *J Am Vet Med Assoc* 1980;176:970–974.

2. MacLachlan NJ, Divers TJ. Hemolytic anemia and fibronoid change of renal vessels in a horse. *J Am Vet Med Assoc* 1982;181:716–717.

3. Schalm OW, Carlson GP. The blood and blood forming organs. In: Mansmann RA, McAllister ES, eds. *Equine medicine and surgery*. 3rd ed. Santa Barbara, Calif: American Veterinary Publications, 1982;408–409.

4. Farrelly BT, Collins JD, Collins SM. Autoimmune hemolytic anemia in a horse. *Ir Vet J* 1966;20:42–45.

5. Andeson LJ. Idiopathic autoimmune hemolytic anemia in a horse. *N Z Vet J* 1974;22:102–105.

6. Collins JD. Autoimmune hemolytic anemia in the horse, in *Proceedings. 1st Int Symp Equine Hematol* 1975;342–348.

7. Lokhorst HM, Breukink HJ. Autoimmune hemolytic anemia in two horses. *Tijdschr Diergeneenskd* 1975;100:752–757.

8. Reef VB. *Clostridium perfringens* cellulitis and immune-mediated hemolytic anemia in a horse. *J Am Vet Med Assoc* 1983;182:251–254.

9. Morris DD, Vaala WE, Sartin E. Protein-losing enteropathy in a yearling filly with subclinical DIC and autoimmune hemolytic disease. *Compend Contin Educ Pract Vet* 1982; 4:542–546.

10. Tizard I. *Veterinary immunology*. 2nd ed. Philadelphia: WB Saunders Co, 1982;319–320.

11. Schalm OW, Jain NC, Carroll EJ. *Veterinary hematology*. 3rd ed. Philadelphia: Lea & Febiger, 1975;642–645.

12. Werner LL. Immunologic diseases affecting internal organ systems. In: Ettinger SJ, ed. *Textbook of veterinary internal medicine*. 2nd ed. Philadelphia: WB Saunders Co, 1983;2163–2167.

13. Halliwell REW. Autoimmune disease in the dog. *Adv Vet Sci Comp Med* 1978;22:221–263.