Fatal hemolytic anemia in the black rhinoceros: Case report and a survey

R. Eric Miller, DVM, and William J. Boever, DVM

FATAL HEMOLYTIC ANEMIA in the black rhinoceros *(Diceros bicornis)* has been attributed to leptospirosis,[1] babesiosis,[2-4] trypanosomiasis,[2,4] and unknown causes that temporarily responded to corticosteroid therapy.[5] In 1 report, hemolytic anemia developed in a black rhinoceros while being treated for tuberculosis.[6] Hemoglobinuria has been reported in the Indian rhinoceros *(Rhinoceros unicornis)* after the ingestion of plants of the Brassicae family.[7] In the present report, we describe a case of fatal hemolytic anemia in a black rhinoceros in which the cause was not determined. Also included are the results of a survey regarding the frequency of the problem in ZOOS.

Case History

A 9-year-old nulliparous female black rhinoceros was housed alone, and a male black rhinoceros and a female black rhinoceros were in an adjacent pen. The rhinoceros was in a concrete stall with access to an outdoor earthen yard and was fed a diet of timothy hay, grain supplement,[a] and fresh produce. On May 19, 1981, she was noticed to be passing port wine-colored urine. A urine sample was positive for hemoglobin/myoglobin, with no RBC in the sediment. Because there was no history of exertion that would precipitate myoglobinuria, the tentative diagnosis was hemoglobinuria. On May 20, the animal was anesthetized to obtain further diagnostic material. To induce anesthesia, 3 mg of etorphine hydrochloride[b] and 50 mg of xylazine[c] were administered IM via a dart pistol. Urine and blood samples were collected, and thin blood that failed to clot at venipuncture sites was noted. Ten milliliters of multiple vitamin B solution,[d] 200 ml of benzathine and procaine penicillin,[e] and 3 ml of a sodium selinite/vitamin E preparation[f] were administered IM. Anesthetic reversal was attempted with 6 mg of diprenorphine.[g] Cardiac and respiratory arrest ensued despite the administration of additional antagonist and emergency drugs.

This rhinoceros was 1 of 4 siblings (1 male and 3 females) born at the St Louis Zoo. One sister died of a similar syndrome in 1977, 1 had a decrease in PCV during an outbreak of hemolytic anemia at the Memphis Zoo but survived, and a 5-year-old brother has remained unaffected. The sire died suddenly in 1976. He was in excellent condition, and the cause of death could not be determined. His death did not appear to entail a hemolytic crisis.

Gross pathologic findings—The abdominal cavity contained approximately 250 ml of clear, dark-red peritoneal fluid. There were numerous petechiae in the renal parenchyma, particularly at the corticomedullary junction. The bladder contained clear, red urine and a normal mucosal surface. The liver was rust colored and friable. Extensive sectioning did not reveal any foci of necrosis or abscessation. Occasional ecchymoses were in the splenic capsule. Four healed 1-cm-diameter gastric ulcer scars were distributed along the margo plicatus. Stomach and rectal contents were dry but otherwise normal. Mucosal congestion and hemorrhage were noted in the distal portion of the duodenum and through the jejunum, the midjejunum being most affected. Whitish mucus was expressed from the uterine horns. The ovaries contained six 1- × 3-cm cysts.

Histopathologic findings—The most remarkable microscopic finding was extensive iron deposition in all major parenchymatous tissues. Diffuse iron pigment was noted in the liver, lungs, spleen, and lamina propria and submucosa of the small intestine. Mild hepatic erythrophagocytosis was noted. Examination of renal sections revealed tubular proteinaceous and RBC casts and mild multifocal hemorrhage in Bowman's capsule. Mild pulmonary edema and centrilobular hepatic fatty change and necrosis also were observed.

Laboratory findings—Results of blood analysis included a PCV, 14.8%; RBC, 1.35×10^6; hemoglobin, 6 g/100 ml; mean corpuscular volume, 106 μm^3 (measured value); mean corpuscular hemoglobins, 44.4 pg; and mean corpuscular hemoglobin concentration, 40.8%. Polychromasia was marked and occasional nucleated RBC were noted. Heinz bodies were not seen on smears, although new methylene blue

From the St Louis Zoological Park, St Louis MO 63110 (Miller, Boever); and the College of Veterinary Medicine, University of Missouri-Columbia, Columbia, MO 65211 (Miller, Boever).

The authors thank Dr. Richard Evans, Ralston-Purina Co, St Louis, Mo; Dr. J. Thomas Lamont, Boston University Medical Center, Boston, Mass; Dr. A. B. Thiermann, National Animal Disease Center, Ames, Iowa; and the National Veterinary Services Laboratory, Ames, Iowa, for technical assistance.

[a]Omolene, Ralston-Purina Co, St Louis, Mo.
[b]M99, D-M Pharmaceuticals, Rockville, Md.
[c]Rompun, Haver-Lockhart Laboratories, Shawnee, Kan.
[d]B-complex 100 with B_{12} injection, Allemed, Fenton, Mo.
[e]Flocillin, Bristol Laboratories, Syracuse, NY.
[f]BoSe, Burns Biotec Laboratories Inc, Omaha, Neb.
[g]M50-50, D-M Pharmaceuticals, Rockville, Md.

stains were not performed. The WBC count was 13,400/ml with a left shift (54% segmented cells, 23% band cells, 12% lymphocytes, 9% monocytes, and 1% eosinophils).

A blood chemical profile was believed to be within normal limits, with the exception of total bilirubin of 2 mg/dl. Analysis for calcium, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, and total protein could not be done because of the severe hemolysis. The sera were clear, dark red.

Urinalysis revealed a specific gravity of 1.027, moderate fractionated RBC, and hemoglobinuria. Urine culture yielded a variety of bacterial species: *Staphylococcus* sp, *Corynebacterium* sp, hemolytic streptococci, *Escherichia coli,* and a *Neissiria/Moraxella*-type organism. Quantitative culture yielded 1,000 colonies/ml. Dark-field and phase-contrast microscopy failed to reveal any spirochetes in the urine.

Culture of the uterine mucus yielded *E coli* and hemolytic and nonhemolytic diplococci. Later attempts of aerobic bacterial culture from frozen specimens of kidney, liver, small intestine, and spleen yielded only common postmortem contaminants. Viral culture from the tissues was attempted at the US Department of Agriculture, National Animal Disease Laboratory, Ames, Iowa, in equine embryonic kidney, Vero-M, and BHK cell cultures and in embryonating chicken eggs. Growth or evidence of viral infection was not found. Electron microscopic examination of small intestinal contents also failed to reveal indications of viral infection. Specific attempts at isolation of bovine viral diarrhea, infectious bovine rhinotracheitis, and parainfluenza type 3 viruses were negative.

Leptospiral cultures were attempted from heparinized whole blood, from urine, and from pregnant guinea pigs that were injected intraperitoneally with blood or urine. Culture specimens were collected from these animals and their fetuses at slaughter, 10 days after inoculation. All cultures were negative for leptospiral growth after 6 weeks in bovine serum albumin polysorbate 80 (with 5-fluorouracil). Dark-field urine examination, tissue silver staining, and electron microscopy were all negative for leptospiral organisms. Titers for serovars of *Leptospirosa interrogans* were 1:320 for *icterohaemorrhagiae* and 1:80 for *ballum*.[h] Leptospiral titers were also determined at a 2nd laboratory, where values indicated no previous exposure.[i]

Examination of blood smears and tissue sections failed to reveal circulating or tissue forms of hemotropic parasites. Results of testing for anaplasmosis were negative.

Anaerobic cultures were not attempted at the time of death, but frozen gut tissue was assayed after 6 weeks for *Clostridium difficile* toxin. Tissue fluid was negative for specific cellular cytotoxicity and was nontoxic when injected intraperitoneally into mice.

Diagnosis of autoimmune conditions was hampered by a lack of appropriate globulins to perform a Coombs' test. Results of testing for antinuclear antibody and rheumatoid factors were negative.

Additional negative findings included those for equine infectious anemia (agar-gel immunodiffusion) and viral culture for bluetongue and epizootic hemorrhagic fever. Results of serotesting for bovine viral diarrhea, infectious bovine rhinotracheitis, parainfluenza type 3, and equine viral arteritis evidence were negative. Tissue radiography failed to reveal evidence of heavy metals. Hepatic copper content was 8 parts per million (ppm) (33 ppm in 1 clinically normal rhinoceros).[7] Hepatic iron content was 3,000 ppm.

Survey Findings

In an attempt to evaluate the frequency of hemoglobinuria/hemolytic anemia in the black rhinoceros, a questionnaire was mailed to 31 American, Canadian, British, and Irish zoos that owned black rhinoceroses. Responses were obtained from 20 institutions that had kept 98 black rhinoceroses at their facilities during the past 10 years. They reported 33 deaths, 25 of which occurred in animals greater than 1 year old. Eleven of the adult deaths (including the case at St Louis) were associated with hemoglobinuria/hemolytic anemia. In addition, contact with 2 continental European zoos produced reports of 4 additional cases (3 fatal) in those institutions. Of the 14 fatal cases, 12 cases were well-documented. In 2 cases, an association with "blood in the urine" was noted, but laboratory data were not available. Nine institutions were involved, 5 of which had lost 2 rhinoceroses each. Two had lost them in quick succession (1 day and 10 days apart), 2 pairs died 2 years apart, and the death of 1 pair was separated by 8 years. There were 7 males aged 2, 2.5, 3, 8, 9, 10, and 22 years, and 7 females aged 3, 8, 9, 10, 12, 13, and 18 years. Nine were born in a zoo and 5 were wild-caught. Deaths occurred in February (1), April (2), May (3), July (3), October (1), November (2), and December (2). In addition to the 2 affected siblings of our case, familial relationships were noted in 2 additional groups of affected black rhinoceroses.

Clinical signs preceded death by 2 to 48 hours, although 1 rhinoceros survived 4 days. Only 2 respondents indicated rhinoceroses had survived hemolytic episodes, although 1 of these rhinoceroses died of a recurrence 2 years later.[j] The 2nd survivor, a sister of our rhinoceros, developed a decreasing PCV in a hemolytic outbreak when 2 adults died. She had no signs of illness, and the PCV returned to normal after treatment with hematinics and antibiotics.[j] The 3rd rhinoceros lived for 4 years after her hemolytic crisis, but died of nonhemolytic anemia.

Antemortem laboratory data were available in 5 cases. Anemia was evident in each case, with PCV of 4.5%, 7%, 7.5%, 13%, and 14.8%. These values were reflected in low RBC and hemoglobin values. The WBC counts were normal (with the exception of 2 cases: 19,980 and 22,300 cells/mm^3), with moderate left shifts. Necropsy findings were available in 8 cases

[h]US Department of Agriculture, National Animal Disease Center, Ames, Iowa.

[i]University of Missouri-Columbia, Veterinary Medical Diagnostic Laboratory, Columbia, Mo.

[j]Douglass EM, Memphis Zoo, Memphis, Tenn: Personal communication, 1982.

and generally were compatible with those in our case. In most of the cases, there was iron pigment deposition in the gut, lungs, and liver, with centrilobular necrosis of the liver. Dark-red kidneys with hemorrhagic streaks were noted in 3 cases. Splenitis was identified in 1 case (a 3-year-old male), and anaerobic cultures were negative for *Clostridium* sp.

In most of the fatal cases, there was no evidence of common exposure to an infectious agent or toxin; however, titers for serovars of *L interrogans* were found in an 8-year-old female (*autumnalis* 1:400 and *canicola* 1:100) and in an 8-year-old male (*grippotyphosa* 1:6400). The death of 1 pair followed by 24 and 48 hours the accidental ingestion of diaphacinon (warfarin-like pesticide), but clinical signs and toxicologic findings failed to support a diagnosis of poisoning with the compound. In the same case, spiriform organisms were seen in silver-stained sections of kidney, but could not be identified. Definitive diagnosis in most of the cases remains undetermined.

Discussion

In domestic animals, hemolytic anemia has been attributed to a variety of causes. In our case, several factors suggest a duration of blood loss longer than the period of clinical hemoglobinuria. The possible increased mean corpuscular volume,[8] evidence of increased gut resorption of iron, and the presence of nucleated RBC suggested attempts at regeneration longer than the 36 hours the crisis was clinically evident. The presence of lipofuscin and hemosiderin pigments in adult rhinoceroses has been noted.[7] Although the clinical significance of these pigments is uncertain, they were reported as having accumulated in hepatocytes of 2 black rhinoceroses with toxic liver conditions and nonhemolytic anemia, possibly related to coal tar exposure.[9] In the hemolytic syndrome, the pigment accumulation may reflect a more chronic stage of the anemia.

Although leptospirosis was not confirmed in our case, it should remain a strong consideration in any similar case that may be encountered. The clinical signs were similar to those expressed in leptospirosis of other large animal species, and the evidence in a previous report of hemolytic anemia in the black rhinoceros was suggestive of leptospirosis.[1] Leptospirosis has been described in wild populations of the black rhinoceros.[10] The inability to provide paired titers in acute cases hampers diagnosis and makes single high titers difficult to interpret. The range of values in our case could coincide with acute infection, but additional diagnostic work failed to provide any support for the diagnosis.

Clostridial infection was considered because of its association with hemolytic syndromes in cattle and in other animal species; however, reports of clostridial infections in the black rhinoceros do not include *Clostridium haemolyticum* as the causative agent.[11,12] Foci of hepatic necrosis, as in bacillary hemoglobinuria, were not evident in our case. Tissue fluid was negative for specific cellular cytotoxicity and was nontoxic when injected intraperitoneally into mice, although degraded toxins may have been missed in the delay (6 weeks) in the performance of the test. Also, the absence of marked intestinal necrosis would not support a diagnosis of clostridial enteritis.

Fatal episodes of babesiosis[2,4] and trypanosomiasis[2,4] have been reported in wild black rhinoceroses. McCulloch and Achard[2] reported a decrease in postcapture deaths with prophylactic medication, although in unstressed animals, infections of *Babesia* sp, *Trypanosoma* sp, and *Theileria* sp may be of little clinical significance. Tissue and blood evaluation failed to yield any indication of similar infections in our case.

Autoimmune hemolytic anemia, particularly the evaluation for synthesis of reagents for the Coombs' test, deserves greater consideration in the differential diagnosis. In other species, autoimmune hemolytic anemia may be manifested solely as a hemolytic syndrome or may be found in association with other disorders.[13,14] In a previously reported case, an affected rhinoceros temporarily responded to corticosteroid therapy,[5] and the clinical history of the present case leaves this as a valid possibility. In the present case, we did the antinuclear antibody and rheumatoid factor tests because of their lesser specificity for a given species. Although the results were negative, the available data did not allow confirmation or denial of autoimmune hemolytic anemia.

Heinz bodies have been reported as a common finding in the white rhinoceros.[8] Kale, rape, onions, phenothiazines, and a variety of oxidants may act on the RBC to precipitate hemoglobin in the form of Heinz bodies and lead to early RBC destruction. Kale has caused nonfatal hemoglobinuria in the Indian rhinoceros.[7] Heinz bodies were not seen in our smears, although new methylene blue stains were not included. Heinz bodies may become scarce when older cells are lysed and are replaced by newer, more resilient cells (such as might be found in acute hemolysis).

Cells deficient in glucose-6-phosphate dehydrogenase are more susceptible to oxidant stress. Such a defect has been described in man and may serve an adaptive function in areas of widespread trypanosomal infections, although a similar deficiency has not been described in domestic or exotic animals.[14] Another enzyme deficiency, pyruvate kinase, is capable of causing hemolytic anemia in the Basenji dog.[14]

Diet evaluation in our case failed to reveal an oxidant stress. For example, processed feeds in Missouri do not contain propylene glycol as a preservative. Inadequate removal of disinfectants[k,l] could provide a source of chronic oxidant exposure. It is interesting to note that hemolytic anemia developed in a rhinoceros on isoniazid therapy, and that is an occasional side effect of the drug in man.[6] The death of 2 rhinoceroses following exposure to diaphacinon may be coincidental or could indicate a common inducement for the onset of hemolysis.

Without a data base of RBC enzymatic and structural values in normal and in affected black rhinoceroses, it is impossible to evaluate the possibility

[k]Clori-Lee Bleach, ISIS Foods Inc, Kansas City, Mo.
[l]Roccal-D, Winthrop Laboratories, Division of Sterling Drug, New York, NY.

of such a defect in individuals or in the species. If such a defect were found, the hemolytic incidents could represent a general response to a variety of infections and chemical challenges present in captivity.

Further evaluation of the source and of any familial relationships of affected rhinoceroses may aid in the determination of any heritability of the syndrome. The horizontal relationship of siblings in 2 instances and the vertical relationship of a grandmother-daughter-granddaughter grouping should be noted. The results of our survey do not define a clear signalment or seasonal incidence for the hemolytic episodes, as was suggested by data available from a previous survey.[15] In that survey, 4 of 7 cases were fatal. Fatalities in our survey represented 14 of 17 hemolytic episodes.

We would recommend that the diagnostic workup of any future cases of this syndrome should include additional tests for RBC fragility, Coombs' test (if reagent can be synthesized, but if not, a major crossmatch of affected sera), fluorescent antibody for leptospirosis as reliable conjugates become available, new methylene blue staining, bone marrow evaluation, anaerobic culture of any suspect clostridial lesions, protein electrophoresis, and perhaps evaluation of glucose-6-phosphate dehydrogenase and other related RBC enzymes. Evaluation of fibrin/fibrinogen degradation products and clotting factors would help determine whether failure of blood to clot at venipuncture sites was a primary clotting defect or, more likely, a component of disseminated intravascular coagulation in an agonal patient. Obviously, for many of these tests, priority must be given to the establishment of values in unaffected rhinoceroses. Hopefully, such a study will establish a data base from which to pursue the etiology of the hemolytic syndrome in the black rhinoceros.

References

1. Douglas EM, Plue RE, Kord CE: Hemolytic anemia suggestive of leptospirosis in the black rhinoceros. *J Am Vet Med Assoc* 177:921–923, 1980.
2. McCulloch B, Achard PL: Mortalities associated with capture trade, translocation and exhibition of black rhinoceroses. *Int Zoo Yearb* 9:184–195, 1969.
3. Brocklesby DW: A *Babesia* sp of the black rhinoceros. *Vet Rec* 80:484, 1967.
4. Silberman MS, Fulton RB: Medical problems of captive and wild rhinoceros—A review of the literature and personal experience. *J Zoo Anim Med* 10:6–16, 1979.
5. Klos D. Heinz-Georg, Lang EM: *Handbook of Zoo Medicine.* New York, Von Nostrand Reinhold Co, 1982, pp 194–204.
6. Mann PC, Brush M, Janssen DC, et al: Clinicopathologic correlations of tuberculosis in large zoo mammals. *J Am Vet Med Assoc* 179:1123–1129, 1981.
7. Jones DM: The husbandry and veterinary care of captive rhinoceroses. *Int Zoo Yearb* 19:242–249, 1979.
8. Hawkey CM: *Comparative Mammalian Haemotology.* London, Heinemann Medical Books Ltd, 1975, p 155.
9. Schmidt RE, Toft JD, Eason RL, et al: Possible toxic liver degeneration in black rhinoceroses *(Diceros bicornis). J Zoo Anim Med* 13:3–10, 1982.
10. Schenkel R, Schenkel-Hulliger L: *Ecology and Behavior of the Black Rhinoceros (Diceros bicornis).* Berlin, Verlag Paul Paneg, 1969, p 103.
11. Mitra SC: The management of animals in the Calcutta Zoological Gardens. *Bombay Nat Hist Soc J* 8:254–272, 1893–1894.
12. Chaffee PS: Report on the death of a rhinoceros. *J Small Anim Pract* 9:133–134, 1968.
13. Schalm OW, Jain NC, Carroll EJ: *Veterinary Hematology,* ed 3. Philadelphia, Lea & Febiger, 1975, pp 642–650.
14. Schall WD, Perman V: Diseases of the red blood cells, in Ettinger SJ (ed): *Textbook of Veterinary Internal Medicine.* Philadelphia, WB Saunders Co, 1975, pp 1616–1618.
15. Beggs T: Haemoglobinuria in the black rhino. *Br Vet Zool Soc Newsletter and Summary of Papers,* 1981, p 3.