M. E. Fowler (ed.) Zoo and Wild Animal Medicine, 3rd ed. 1993

35 PERISSODACTYLIDS

HEMOLYTIC ANEMIA IN THE BLACK RHINOCEROS

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Hemolytic anemia has been a frequent and devastating disorder of the black rhinoceros (*Diceros bicornis*) in captivity. Currently, 44 hemolytic episodes have been identified in 36 black rhinoceroses in zoological parks in North America, Europe, and Japan. Almost 75% (23) of these animals died during their initial or subsequent hemolytic events. In one survey, hemolytic anemia accounted for 40% of all captive adult deaths in this species.[6] These fatalities are a disturbing factor in the maintenance of a species whose North American population currently numbers 80 animals, and whose wild population is undergoing a precipitous decline.

CLINICAL SIGNS

The intravascular destruction of red blood cells (RBCs) leads to the release of hemoglobin into the serum (hemoglobinemia; Fig. 35–1) and may result in its passage into the urine (hemoglobinuria). The latter often produces clear, dark red urine. In black rhinoceroses, hemoglobinuria is often the first clinical sign of hemolysis. At this stage, clinical signs may also include pale mucous membranes, generalized weakness, and muscle fasciculations. Frequently, cases progress to recumbency and death within 24 to 48 hours of the initial presentation.

Hematocrits from affected black rhinoceroses range from 4.5 to 43%, no doubt as a result of detection at varying stages of the hemolysis. Although reticulocytes have rarely been noted in blood smears collected during hemolysis or the recovery period, circulating nucleated RBCs have been observed (Fig. 35–2). This finding may correspond to the anemic horse, in which peripheral reticulocytes are rare, but circulating nucleated RBCs indicate a marked regenerative response. It also correlates with erythropoietic hyperplasia noted in bone marrow from rhinoceroses that died of hemolysis.

If a rhinoceros dies of hemolytic anemia, gross lesions at necropsy are minimal. Most notable are the presence of clear, dark red urine in the urinary tract and an orange-red discoloration of many parenchymal tissues. The latter results, at least in part, from high levels of iron in the tissues (greater than 3000 ppm in some cases; Fig. 35–3). Although the iron in these cases is presumably a product of heme breakdown, lesser but also elevated tissue iron levels have been noted in black rhinoceroses dying of nonhemolytic causes. The pattern of iron deposition suggests that a more chronic and subclinical stage of the hemolysis may exist. Additionally, histological examination often reveals centrolobular hepatic necrosis, most likely secondary to acute hypoxia.

EPIDEMIOLOGY

There is no apparent sex predilection nor seasonality for the hemolytic crises. The age of an affected black rhinoceros can range from 2 to 26 years (average, 10.7 years). Both captive-bred (19) and wild-caught (11) animals have been affected. Of possible interest is the difference between the average age of affected captive-bred (8.7 years) and wild-caught animals (14.7 years). Until recently, all affected black rhinoceroses have been of the East African subspecies (*Diceros bicornis michaeli*), but that may simply reflect the smaller numbers and shorter captive history of the southern subspecies (*D. bicornis minor*). Hemolytic anemia has not been reported in captive white (*Ceratotherium simum*) and great Indian (*Rhinoceros unicornis*) rhinoceroses, or in black rhinoc-

Figure 35–1. Hemoglobinemia in plasma from a 16-year-old rhinoceros. Hemoglobinuria was also noted. The hematocrit was 4.5%, and the animal died shortly after this sample was collected.

Figure 35–2. Peripheral blood smear from a 9-year-old black rhinoceros during a hemolytic crisis (hematocrit, 14.8%). Note the presence of a nucleated RBC (arrow) (Wright's stain).

eroses in the wild. Two possibilities may explain the latter observation: (1) the syndrome may not occur in wild animals; or (2) under field conditions, minimal clinical signs and gross lesions may be overlooked.

At most institutions, single deaths from hemolysis have occurred with apparently normal and unaffected black rhinoceroses in the same or nearby enclosures. However, two black rhinoceroses at the Toronto Zoo and three at the Memphis Zoo were affected within 1- and 10-day periods, respectively. In those cases, the pattern suggested a common agent and leptospirosis was indicated in the Memphis "outbreak."[3]

Familial groupings of affected rhinoceroses have been noted at the Denver, Frankfurt (Germany), and St. Louis Zoos. However, these families are unrelated to each other and account for only 10 of the 36 affected black rhinoceroses. Inbreeding does not appear to be a factor in these families or in the general captive black rhinoceros population.

POSSIBLE CAUSES AND DIAGNOSIS

Investigations to determine the cause of the hemolysis have been based on known causes of hemolysis in domestic animals and humans. In most rhinoceroses, the cause has not yet been identified definitively. However, infection with serovars of the spirochete bacterium *Leptospirosa interrogans* has been suggested in at least nine cases on the basis of titers, special tissue stains, and fluorescent antibody (FA) testing.[3,7,11]

Prior to the advent of the FA test, diagnosis of leptospiral infection was complicated by deficiencies in available methods. Titers, the most common method of diagnosis, may vary among laboratories, and often paired titers are not possible because of the peracute and fatal nature of hemolysis in rhinoceroses. At two zoos, convalescent titers in rhinoceroses surviving hemolysis have been elevated (1:8000 to *Leptospira icterohaemorrhagiae* and 1:12,800 to *L. grippotyphosa*), although surviving animals at other institutions have not experienced a titer change. Titers indicative of exposure to leptospirosis have also been noted in sera from wild black rhinoceroses in Zimbabwe.

Additionally, leptospiral organisms are difficult to grow in culture. Special stains (e.g., silver) can be applied to tissue from suspect animals, but infection with low numbers of organisms may be missed. The availability of the FA test has provided a much more reliable method of diagnosis. Frozen (−75° C) liver tissue from four adult rhinoceroses that died of hemolytic anemia was tested, and three animals were positive.[7] Concurrent titers in two of these three positive animals were negative. Most likely, death occurred before sufficient time had elapsed for mounting an immunological response. Preliminary results indicate that black rhinoceroses vaccinated biannually with leptospiral bacterins mount immune responses equivalent to those in domestic species.

Although perhaps most hemolytic cases are linked to infection with *Leptospira interrogans*, not all the cases are associated with this organism. The clinical similarities among hemolytic black rhinoceroses emphasize efforts to identify a single "common denominator" for this syndrome, a basic defect that would allow a number of factors (including leptospirosis) to trigger a peracute hemolytic crisis. To that end, a dual approach to the study of black rhinoceros RBCs was chosen: (1) evaluation of its metabolism; and (2) investigation of its structural and immunological parameters.

The study of RBC metabolism is warranted because of the association of RBC enzymatic deficiencies and hemolytic syndromes in humans. In studies of aerobic glycolysis, glutathione cycling, and nucleotide metabolism in black rhinoceros RBCs, no abnormalities were noted that would account for the hemolysis.[9] However, ATP levels in black rhinoceros RBCs were only 5 to 20% of the levels measured in most other mammalian species. Decreased energy resources could predispose these cells to lysis. Additionally, when compared to the red blood cells of humans and white rhinoceroses, black rhinoceros RBCs are deficient in catalase activity (acatalasemia).[10] Further investigations are designed to evaluate this finding and determine whether an alternative energy pathway exists for black rhinoceros RBCs and to evaluate

Figure 35–3. Liver tissue from a black rhinoceros that died of hemolytic anemia (H & E stain). Hepatic iron levels were 3000 ppm. Iron deposits (confirmed by special stains) are noted by arrows.

Few treatments have been reported to be successful in ameliorating the condition. This may be partly caused by the severity of the anemia when many cases are presented. Several rhinoceroses have apparently responded to corticosteroid therapy. However, three rhinoceroses on this therapy (for other conditions) developed fungal pneumonia. Because of this and the possibility of a link with leptospirosis, immunosuppressive therapy should be used with extreme caution and only for a short time.

Because of the possibility of leptospiral infection, antibiotic therapy is indicated. Injectable penicillins and aminoglycosides may be used for the treatment of acute leptospirosis, although for both the administration of large doses by injection may complicate prolonged therapy. Tetracycline therapy may be more practical for animals that survive and can be treated in their feed. Injectable supplementation with vitamin E (E-Se, Bo-Se, Schering Animal Health) is also advisable because of its low level in captive animals. Although specific pharmacokinetic studies for these and other drugs have not been carried out in the black rhinoceros, it appears reasonable to base therapeutics on those used in horses. Black rhinoceros weights are usually estimated, but an "average" male at the St. Louis Zoo weighed 1000 kg. Institution of phosphorus supplementation therapy should be considered. Additional supportive therapy (fluids, warming) should be instituted, as needed.

If the rhinoceros continues to deteriorate, blood transfusion can be considered as a treatment of last resort. Transfusion with 16 liters of whole blood (collected from two adult animals) was attempted in a 3-year-old hemolytic rhinoceros. The hematocrit rose from 12.4 to 18%. Major and minor crossmatches were compatible but, within 3 days, the hematocrit had decreased to 9.8%. It is not known whether this was caused by continued hemolysis or a transfusion reaction. Further studies are in progress to determine the compatibility of black rhinoceros blood for intraspecific transfusion.

If the rhinoceros dies, formalinized tissues should be saved and consideration given to saving tissues in glutaraldehyde for electron microscopy. Standard aerobic and leptospiral cultures should be submitted. Additionally frozen liver, kidney, spleen, and other appropriate tissues should be saved for further evaluation.

Perhaps the most difficult question facing those treating hemolytic black rhinoceroses is that of prevention. Although challenge studies have not been (and are not likely to be) performed, biannual vaccination with a five-way leptospiral bacterin (Leptoferm-5, Norden Laboratories) is recommended. Evaluation of diets and improved nutrition, including vitamin E supplementation, may improve the overall health status of these animals, and possibly increase their resistance to anemia. Because more plant species (particularly of browse) are offered to captive animals, care should be taken to avoid red maple (*Acer rubrum*), onions, and *Brassica* species (e.g., kale, rape, cabbage) that are associated with hemolytic anemia in horses and other domestic species. Vital to the prevention of hemolysis and other diseases of undetermined cause in the black rhinoceros is the development of a data base of baseline values from captive and wild rhinoceroses. To that end, the Black Rhinoceros SSP Committee encourages the continued cooperation and participation of rhinoceros-holding institutions in the blood and tissue collection protocol for all rhinoceros species.

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other intracellular systems that may counter oxidative stresses.

Autoimmune hemolytic anemia (AIHA) was evaluated using Coombs' reagents (an anti–black rhinoceros whole serum and a more specific anti-IgG developed for the black rhinoceros), but testing of normal and affected black rhinoceroses with these reagents did not indicate an immune basis for the hemolysis.[1] Additionally, isopropanol and heat stability tests found no evidence that the hemolytic events are the result of an unstable hemoglobin.[4]

Another area of investigation is the nutritional status of captive black rhinoceroses. Although nearly an exclusive browser in the wild, feeds more closely resembling those for a grazer often predominate in captive diets for this species. Marked decreases in serum α-tocopherol (vitamin E) levels have been noted in captive animals when compared to wild animals in Zimbabwe (0.18 versus 0.77 μg/ml).[2] The significance of vitamin E in black rhinoceros hemolysis is unknown, but it does play a role in the stability of cellular membranes, including those of the RBC. Vitamin E–responsive hemolytic anemias have been reported in primates and rats, and a decrease in in vitro erythrocyte stability has been noted in vitamin E–deficient horses.

Hypophosphatemia may warrant further investigation. At the Frankfurt Zoo, two female black rhinoceroses developed postparturient hemoglobinuria, a condition in cattle associated with hypophosphatemia (unfortunately, serum phosphorus levels were not available from the rhinoceroses). Although serum phosphorus levels have been normal in most black rhinoceroses undergoing acute hemolysis and in the general black rhinoceros population, low serum phosphorus levels have been noted in several animals during hemolysis. It has been suggested that hypophosphatemia may be involved in the chronic anemia of black rhinoceroses with skin and oral ulcers.[5]

Even when previous test results have been negative, evaluation for known causes of hemolysis should continue in future cases. Infections with *Ehrlichia* and *Babesia* sp. have not been identified in hemolytic rhinoceroses, although they have been noted in newly captured black rhinoceroses. Attempts to isolate the viruses of equine infectious anemia (EIA) and equine viral arteritis, as well as isolation of toxins from *Clostridium* sp., have yielded negative results. Based on hepatic copper levels, copper toxicity also does not appear to be a factor.

TREATMENT

The clinical approach to a black rhinoceros in a hemolytic crisis requires astute clinical judgment on the part of the attending veterinarian. Anesthesia itself is a risk in a severely anemic animal but, without proper restraint, diagnostic evaluation and many treatments are difficult, if not impossible. Reduction in the anesthetic dose may be advisable in the anemic animal.

In a docile or depressed animal, small amounts of blood can be obtained from the ear vein. If the rhinoceros is sedated, markedly depressed, or possibly restrained in a chute, large amounts (liters) of blood can be obtained from the medial radial vein of the foreleg (Fig. 35–4).[8] A hematocrit and preferably a complete blood count are of vital importance in monitoring the severity of the anemia. A hematocrit tube should be spun immediately, because hemolysis may continue in the collected sample. If available, additional blood should be submitted for a blood chemistry profile, frozen sera saved, and diagnostic evaluations performed as described above. A detailed blood and tissue collection protocol for such testing has been designed and distributed to holding institutions by the Research, Nutritional, and Veterinary Advisors of the Black Rhinoceros Species Survival Plan (SSP) Committee.

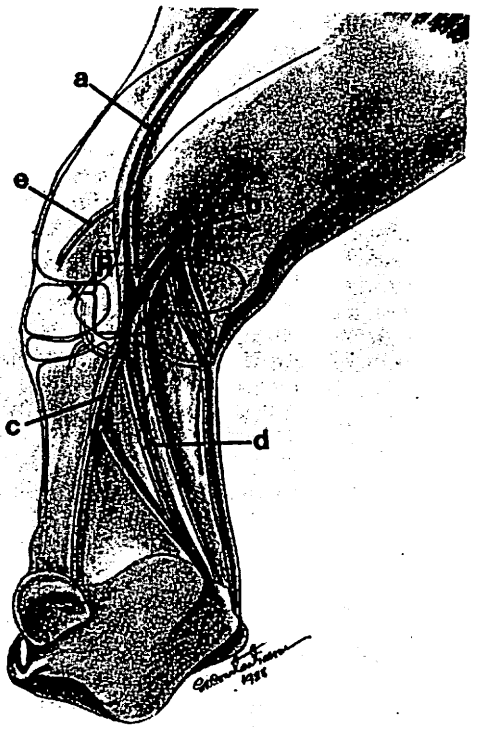

Figure 35–4. Medial view of the right forelimb of a black rhinoceros (a, cephalic vein; b, radial vein; c, superficial branches of the radial vein; d, palmar (deep) branch of the radial vein; e, accessory cephalic vein; R, distal medial aspect of the radial bone; X, suggested site for venipuncture). (From Miller RE, McClure RC, Constantinescu G, et al: A clinical note on the vascular anatomy of the black rhinoceros (*Diceros bicornis*) forelimb. J Zoo Wildl Med 20:229, 1989.)

When possible, a complete urinalysis should be performed. A urine test dipstick can be used to make a "field" evaluation for the presence of hemoglobin in the urine (an examination of the urine sediment can rule out hematuria). The dipstick may detect hemoglobinuria even when present in quantities insufficient to cause discoloration. If the possibility of myopathy exists, hemoglobin should be differentiated from myoglobin, because both react on the dipstick.