Idiopathic hemorrhagic vasculopathy syndrome in seven black rhinoceros

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➤ Idiopathic hemorrhagic vasculopathy syndrome (IHVS) is a newly described disorder of black rhinoceros.

➤ Idiopathic hemorrhagic vasculopathy syndrome should be considered a possible cause of non-hemolytic anemia in black rhinoceros.

➤ The cause of IHVS remains unknown, but an immune-mediated response to an infectious agent is suspected.

During the past 10 years, the leading cause of death in captive black rhinoceros has been acute, rapidly progressive hemolytic anemia.[1-3,a] This disorder appears as a massive hemolytic crisis that typically results in an overall mortality rate of 75%. However, since 1995, a syndrome has emerged in the North American black rhinoceros population characterized by extensive regional limb and neck swelling in conjunction with an acute, severe episode of nonhemolytic anemia. The extreme swelling and precipitous drop in Hct are thought to be caused by a small-vessel vasculopathy that results in hemorrhagic extravasation into soft tissues. Termed **idiopathic hemorrhagic vasculopathy syndrome (IHVS)** by an ad hoc workshop on the disorder, this condition appears in acute and chronic forms, with or without recurrent episodes, and is potentially fatal. Idiopathic hemorrhagic vasculopathy syndrome has many clinical and pathologic similarities to other cutaneous vasculopathies described in domestic animals but also has unique features that may define IHVS as a separate syndrome.[4,5] Despite extensive research, the cause of this vasculopathy remains unknown.

Seven cases of IHVS have been identified in black rhinoceros, 6 of which developed in rhinoceros from Texas. Three of the rhinoceros were male, and 4 were female. Four of the 7 were captive born, and 3 were captured in the wild. Six were Southern black rhinoceros (*Diceros bicornis minor*), and 1 was an Eastern black rhinoceros (*Diceros bicornis michaeli*). Five of the 7 rhinoceros survived an initial episode of IHVS, and 2

of these 5 survived a recurrent episode of IHVS. Two rhinoceros died during treatment of IHVS. Because the majority of IHVS cases developed in 1 subspecies of black rhinoceros in what appeared to be a geographic clustering of confirmed IHVS cases, infectious agents and noninfectious contributing factors have been considered and investigated. Nutritional, genetic, geographic, and environmental components were suggested and examined. Thorough case histories on all rhinoceros did not provide a definitive link between development of IHVS and recent vaccinations or administration of medications, and none of the affected rhinoceros were exposed to any known toxins. A firm connection between IHVS and any of these factors has not been identified, but epidemiologic review is ongoing.

Affected rhinoceros had histories of several days to several weeks of severe shoulder, neck, and limb swelling in conjunction with lameness, anorexia, various degrees and durations of lethargy, laminitis with associated sloughing of nails, respiratory stridor, oral ulcerations, and severe nonhemolytic anemia (Hct range, 9 to 13%; reference range, 24 to 45%). The low Hct values were paralleled by low hemoglobin concentrations, RBC count, and total protein concentrations. In the rhinoceros that survived, increased numbers of macrocytes and nucleated RBC on blood smears, along with the return to reference range values for Hct, provided the clearest indicators of a regenerative erythropoietic response, because rhinoceros characteristically do not develop reticulocytosis.[1] Leukograms were variable, with no consistent pattern of WBC counts among rhinoceros. Serum biochemical analyses revealed low serum albumin (mean, 1.31 g/dl; reference range, 2.7 ± 0.4 g/dl) and phosphorus (mean, 2.36 mg/dl; reference range, 4.8 ± 1.0 mg/dl) concentrations and high muscle enzyme activities (mean creatine phosphokinase, 1,068 U/L; reference range, 377 ± 303 U/L; mean lactate dehydrogenase, 1,473 U/L; reference range, 558 ± 389 U/L). Liver enzyme activity and bilirubin concentrations remained within reference ranges. Coagulation tests were performed in 4 rhinoceros, and results were considered typical for healthy rhinoceros (reference ranges: partial thromboplastin time, 22.6 to 33.7; prothrombin time, 10.5 to 13.0).

Treatment protocols varied, depending on severity of disease and discretion of the attending veterinarian. Because an infectious cause was suspected, all 7 rhinoceros were administered broad-spectrum antibiotics. Two rhinoceros received sulfamethoxazole-trimethoprim (15 mg/kg [6.81 mg/lb] of body weight, PO, q 12 h), 3 rhinoceros received cephalexin monohydrate (25 mg/kg [11.36 mg/lb], PO, q 12 h), 1 rhinoceros

received amoxicillin trihydrate (28 mg/kg [12.72 mg/lb], PO, q 12 h), and 1 rhinoceros received enrofloxacin (2.5 mg/kg, 1.14 mg/lb, PO, q 12 h). Five of the 7 rhinoceros received antibiotics early in the course of illness (on or before day 8), while the remaining 2 rhinoceros received antibiotics on or after day 17. All rhinoceros also received nonsteroidal anti-inflammatory drugs to provide symptomatic relief. Supportive care, including fluid and electrolyte administration, foot and nail care when necessary, care for pressure sores and draining areas, and nutritional supplementation was also instituted. Treatment with corticosteroid medications was initiated in 6 of 7 rhinoceros. One rhinoceros received 2 IM injections of dexamethasone (0.02 mg/kg [0.009 mg/lb]), and 2 other rhinoceros received a single IV injection of dexamethasone sodium phosphate (0.1 mg/kg [0.05 mg/lb]). The 3 remaining rhinoceros received more prolonged treatment with an initial IV injection of dexamethasone sodium phosphate (0.05 mg/kg [0.02 mg/lb]) or prednisolone sodium succinate (0.5 mg/kg [0.23 mg/lb]), followed by once-daily oral administration of prednisone (0.5 mg/kg [0.23 mg/lb]). In all rhinoceros, treatment with corticosteroids was initiated and modified in accordance with clinical signs and progression of disease. One rhinoceros had **dimethyl sulfoxide**[b] **(DMSO)** applied topically to alleviate swelling and inflammation locally, and 2 rhinoceros received DMSO topically and IV (0.4 mg/kg [0.18 mg/lb]) once for systemic anti-inflammatory effects. One rhinoceros was given furosemide (0.1 mg/kg [0.04 mg/lb], IM, q 12 h) twice to decrease edema and relieve respiratory stridor. One rhinoceros received a plasma transfusion for treatment of a suspected immune complex disease. One rhinoceros received erythropoietin[c] (50 U/kg [22.7 mg/lb], IM, q 72 h for 6 doses) to treat anemia and itraconazole[d] (3.2 mg/kg [1.45 mg/lb], PO, q 12 h for 28 days) as a prophylaxis against fungal pneumonia. In addition, veterinarians at all institutions administered phosphorus supplements orally in response to low serum phosphorus concentrations (low serum phosphorus concentration is known to reduce RBC ATP, potentially inducing acute hemolysis[3,6]).

Serologic testing was extensive; serum was tested for equine, bovine, and exotic viruses, including bluetongue virus, bovine viral diarrhea virus, epizootic hemorrhagic disease virus, equine infectious anemia virus, equine viral arteritis virus, malignant catarrhal fever virus, equine herpesvirus 1 and 4, vesicular stomatitis virus, foot-and-mouth disease virus, infectious bovine rhinotracheitis virus, bovine herpesvirus 2, African horse sickness virus, equine rhinovirus 1 and 2, eastern, western, and Venezuelan encephalitis viruses, and encephalomyocarditis virus. Serum was also tested for antibodies against *Ehrlichia risticii*, *Hemophilus* spp, and 15 leptospiral serovars. Results were negative, with a few exceptions. One rhinoceros had a positive **equine herpesvirus 1 (EHV-1)** titer. This rhinoceros was housed in a zoologic park at the same time zebras in the park had an outbreak of EHV-1. A high EHV-1 titer was detected in the affected rhinoceros 2 weeks after the onset of clinical signs in this animal. A high EVH-1 titer in conjunction with clinical

signs suggests that EHV may be implicated in the course of disease in this rhinoceros; also, this rhinoceros was seronegative for other pathogens. However, because all subsequently affected rhinoceros remained seronegative for EHV, the importance of this titer as it applied to the pathogenesis of IHVS was uncertain. A second rhinoceros had a low titer to eastern equine encephalitis virus, which was thought to be caused by a previous exposure, because this rhinoceros had not been vaccinated against encephalitis.

Results of serologic testing for exposure to 15 leptospiral serovars were negative in all rhinoceros tested, with a few exceptions. One rhinoceros had positive titers to 5 serotypes. These results were considered vaccine-induced, because this rhinoceros had been vaccinated with a 6-way leptospira vaccine[e] that contained antigens of each implicated serotype. A second rhinoceros had a positive titer to *Leptospira autumnalis* that did not increase or decrease during the course of disease. This result was attributed to previous exposure and was not thought to be related to the progression of IHVS.

Polymerase chain reaction analysis was performed on whole blood from 2 rhinoceros to test for evidence of *E risticii* and *E canis*; results were negative. Immunohistochemistry was also performed on tissue from 1 rhinoceros to test for *Rickettsia rickettsii*; results were negative. Electron microscopy performed on tissues from 1 rhinoceros did not reveal evidence of viral, rickettsial, or ehrlichial infection; special stains (Warthin-Starry) used to examine tissue from this rhinoceros did not reveal evidence of infection with *Bartonella* spp.

Results of bacteriologic cultures were more difficult to interpret. All 7 rhinoceros had wounds or bacterial infections of various organ systems throughout the course of disease, but none of the rhinoceros had a confirmed bacterial infection prior to onset of clinical signs. In addition, a single bacterial pathogen was not consistently isolated from all 7 rhinoceros. The most commonly isolated bacterial genus was *Streptococcus*, but the species varied among rhinoceros. A *Streptococcus* sp was isolated from an infected toe of 1 rhinoceros. The species was not specifically identified, but it most closely resembled *S equi* subspecies *zooepidemicus*. β-Hemolytic *Streptococcus* group B sp was isolated from a subcutaneous shoulder wound in another rhinoceros, and β-hemolytic *Streptococcus* sp was isolated from the lungs of a third rhinoceros. In a fourth rhinoceros, α-hemolytic *Streptococcus* sp and *S equisimilus* were isolated from nasal swab specimens. In each of these instances it was difficult, if not impossible, to determine whether these infections constituted a primary or a secondary event. Because the rhinoceros that received antibiotics earlier in the course of this illness recovered more quickly than the 2 rhinoceros that received antibiotics later, a bacterial component is suggested. The increased isolation of *Streptococcus* spp over other bacteria may have been important.

Deep skin biopsy specimens from the swollen limbs and mucosal biopsy specimens from the oral cavity were obtained from 6 of the 7 affected rhinoceros at

various stages of the disease, including the recovery period. During the early stages of IHVS, neutrophilic vasculitis in the upper portion of the dermis of the limbs and proliferative vasculitis in the oral mucosa were evident.

Complete necropsies were performed on the 2 rhinoceros that died. One rhinoceros died from purulent pneumonia secondary to transdiaphragmatic penetration of an ingested intestinal foreign body. The second rhinoceros that died had extensive necrotizing bacterial and fungal pneumonia. It has been reported that rhinoceros are extremely sensitive to the immunosuppressive effects of corticosteroids and are thought to be at greater risk of developing fungal pneumonia subsequent to corticosteroid administration.[7,8] In this instance, the rhinoceros with fungal pneumonia received long-term treatment with a low dose of prednisone administered orally (0.5 mg/kg [0.23 mg/lb]) to mitigate the effects of vasculitis, which may have predisposed this rhinoceros to the development of pneumonia. Therefore, it was not clear whether either rhinoceros would have survived an episode of IHVS. At necropsy, histologic evaluation of SC and intermuscular tissue taken from hemorrhagic areas in the limbs revealed numerous dilated thin-walled vessels filled with RBC, sequestered neutrophils, and thrombi. There was also extensive segmental atrophy of muscle tissue in these areas, suggestive of a compartmental effect. Recovery phase healing and repair were observed in both necropsied rhinoceros with phagocytosis of extravasated RBC, hemosiderosis, fibrosis, and extensive neocapillary formation.

Despite extensive clinical research to determine the cause and pathophysiologic mechanisms of IHVS, a single etiologic agent has not been identified. An autoimmune disorder for IHVS is considered possible but unlikely. One of the 2 rhinoceros that were tested for cold agglutinin disease was considered to have positive results. The importance of this result is not clear, because this test has not been validated for use in rhinoceros. Similarly, a Coombs test was performed in 2 rhinoceros, and ANA titers were measured in 3 rhinoceros; all tests yielded negative results. Unfortunately, because species-specific reagents were not available for these tests, the results must be interpreted with caution.

An immune-mediated disorder, such as an immune complex disease, remains a possibility, as suggested by the vascular changes in the biopsy specimens and tissues obtained at necropsy. The most common mechanism associated with immune complex diseases is the deposition of immune complexes in vessel walls, resulting in activation of complement and infiltration of neutrophils and mononuclear cells.[9] However, preliminary attempts to identify circulating immune complexes in IHVS patients, using an equine C1q binding assay, were not successful. This may reflect an insensitivity of technique in detecting certain types of immune complexes, or it may indicate that the complexes are formed and then cleared at a rapid rate that precludes detection.[4,9,f] Immunohistochemistry studies to identify immune complexes deposited in affected vessels are currently in progress.

The most commonly diagnosed cutaneous vasculitis with a purported immunologic basis in large animals is **equine purpura hemorrhagica (EPH)**.[4,5,10] Equine purpura hemorrhagica is a sporadic, noncontagious disease that is believed to be caused by an allergic reaction to streptococcal or viral antigens or, rarely, a chronic suppurating wound. Clinical signs of EPH include demarcated areas of cutaneous edema on the head, body surface, ventral portion of the abdomen, or extremities. Skin sloughing, laminitis, cellulitis, pneumonia, and diarrhea are not uncommon sequelae. Diagnosis is usually made on the basis of history and clinical signs; detection of leukocytoclastic venulitis in skin biopsies adds support to this diagnosis. Treatment of EPH is directed at removing the antigenic stimulus, reducing the immune response and resultant vessel wall inflammation, and providing supportive care. The prognosis of EPH is fair with early and aggressive treatment; however, some cases of EPH are refractory to treatment and may result in death.[4,5]

The clinical signs, diagnostic testing, treatment protocols, and prognosis of IHVS in all affected rhinoceros were similar to that of EPH. In addition, the high incidence of streptococcal infections in animals with IHVS or EPH lends further support to the theory that the 2 syndromes may be related. However, the histologic pattern of vascular proliferation and neovascularization observed in IHVS, but not in EPH, may serve to distinguish these 2 syndromes. The relevance of this vascular change is still open to interpretation. These specific vascular lesions may identify a separate vascular component of IHVS that is directly related to an as yet undiagnosed infectious agent, or alternatively, be interpreted as a normal aspect of the healing process.[11] In either instance, the characterization of IHVS as a potential immune-mediated response to an infectious agent remains a plausible theory. Further research is warranted to help more clearly define IHVS.

[a]Miller RE, Chaplin H, Paglia ED, et al. Hemolytic anemia in the black rhinoceros—an update, in *Proceedings*. Annu Meet Am Assoc Zoo Vet 1986;7–8.
[b]DMSO, American Marketing Inc, Rising Sun, Ind.
[c]Epogen, Amgen Inc, Thousand Oaks, Calif.
[d]Sporonox, Janssen Pharmaceutica Inc, Titusville, NJ.
[e]BratiVac-6, Pfizer Animal Health, Exton, Pa.
[f]Dr. John Timoney, University of Kentucky, Lexington, KY: Personal communication, March, 1999.

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