

**HEALTH CONCERNS AND VETERINARY RESEARCH IN THE
NORTH AMERICAN BLACK RHINOCEROS (*Diceros bicornis*) POPULATION**

R. Eric Miller, DVM

St. Louis Zoological Park, Forest Park, St. Louis, Missouri 63110-1396
Veterinary Advisor, Black Rhinoceros SSP Committee

Under the auspices of the North American Species Survival Plan (SSP) for black rhinoceroses (*Diceros bicornis*), veterinary research to address the health needs of these animals in captivity is an active and ongoing effort. Research efforts have focused on a number of diseases that are limiting factors in the growth of the captive population. Hemolytic anemia is one example; in one survey of captive black rhinoceroses, it accounted for 40% of all adult deaths.^{14,15}

For comparative studies, obtaining tissue and sera from all species of captive and wild rhinoceroses remains a priority. Centralized North American storage banks have been established for formalinized tissues from black (Dr. Richard Montali, National Zoological Park) and white (Dr. Sylvie Gosselin, Cincinnati Zoological Gardens) rhinoceroses, and for frozen serum and tissue from both species (Dr. Eric Miller, St. Louis Zoological Park). Large volumes of blood required for these research endeavors have been obtained from the medial foreleg vein of anesthetized rhinoceroses.¹⁸ Following are summaries of ongoing and/or recently completed projects and their primary investigators:

1. Evaluation for Exposure to Leptospirosis

Researcher: Dr. Carole Bolin, National Animal Disease Center,
Ames, IA 50010, USA.

Although infection with *Leptospira interrogans* has been suggested as a cause of hemolytic anemia in the black rhinoceros,^{3,13,15} diagnosis has been based on microagglutination titers which can be difficult to interpret. Titer data from both captive and wild (Zimbabwe samples supplied by Drs. Michael and Nancy Kock, and David Jessup of International Veterinary Services, and Raoul du Toit of the WWF) are being compiled. Preliminary results suggest a "background" exposure in both populations. Recently a fluorescent antibody (FA) test for *L. interrogans* has become available. Liver samples from 4 black rhinoceroses that died during hemolytic crises were tested, and 3 of the 4 were positive.¹⁶ In one rhinoceros positive on FA, titers were negative; suggesting death occurred before the animal had time to mount an immune response. Additionally, spirochetes, suggestive of leptospiral infection, have been identified in the serum of a black rhinoceros undergoing a mild episode of hemolysis. It has been recommended that all captive black rhinoceroses be vaccinated with a bacterin that contains at least 5 serovars of *L. interrogans* (including *ictero-hemorrhagica* and *grippotyphosa*). Leptoform-5 (Norden Laboratories, Lincoln, NE 80809, USA) has been administered to black rhinoceroses at 6 month intervals and titer data suggest that their response to this regimen is comparable to that seen in domestic species.

Note: Although leptospiral infection may be indicated in 50%-75% of the fatal cases of hemolytic anemia, it is important to note that it has not been identified or suggested in all cases. Ongoing studies are attempting to identify other factors that may contribute to red blood cell (RBC) instability and allow a number of events (including leptospirosis) to "trigger" hemolysis (see #2 below).

2. Further Evaluation of Red Blood Cell Metabolism

Researcher: Dr. Donald Paglia, University of California - Los Angeles, Los Angeles,
CA 90024, USA.

A previous study that evaluated enzymes and metabolic intermediates of aerobic and anaerobic glycolysis, glutathione cycling and nucleotide metabolism in the black rhinoceros red blood cell (RBC) failed to identify a metabolic defect that would account for the hemolysis.²¹ In another study, black rhinoceros RBC did not appear unstable in either hydrogen peroxide or saline lysis tests.²⁴ However, in further studies at UCLA, it became evident that ATP levels in the black rhinoceros RBC are approximately 5%-20% of those in most other mammalian species. The impact of this finding is uncertain, but it raises the possibility that the black rhinoceros RBC may use alternative energy pathways. Dependent on the availability of funding, further research is planned to study additional aspects (eg, substrate utilization) of these cells.

3. Hemoglobin Stability and Polymorphism

Researcher: Dr Virgil Fairbanks, Mayo Clinic, Rochester, MN 55902, USA.

In a recent study, no evidence was found that an unstable hemoglobin was the cause of hemolytic anemia in black rhinoceroses.⁴ Though an earlier report had suggested the presence of an unstable hemoglobin in black rhinoceroses from Zimbabwe, the more recent study included captive animals of both the eastern and southern subspecies (*D. b. michaeli* and *minor*) and from normal and hemolytic animals. A marked beta chain hemoglobin polymorphism was noted in the black rhinoceroses, and may vary by subspecies, but it is probably of no significance to the etiology of the hemolysis.

4. Autoimmune Testing and Hematologic Evaluation of Black Rhinoceroses Undergoing Hemolysis.

Researcher: Dr. Hugh Chaplin, School of Medicine, Washington University, St. Louis, MO 63110, USA.

Autoimmune testing of additional black rhinoceroses undergoing hemolytic crises did not yield any further evidence that the syndrome is autoimmune in origin.^{1,14} Polyclonal antiglobulin reagents (Coombs reagents) specific to black rhinoceros whole sera and IgG were employed. Osmotic fragility, RBC membrane composition and hemoglobin electrophoretic patterns did not distinguish between normal and hemolytic rhinoceroses.¹

5. Complete Blood Counts and Sera Chemistries

Researcher: Dr. Stephen Stockham, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211, USA.

To reduce the variability between laboratory methods, a request was made that complete blood counts and serum chemistries from all rhinoceroses be submitted to a central laboratory. Preliminary evaluation reveals no unusual patterns. Similar testing has been performed on a wild population in Zimbabwe,¹⁰ and captive black rhinoceroses.^{1,8,9}

6. Serum Iron Levels and Iron Binding Proteins

Researcher: Dr. Joseph Smith, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA.

Due to the elevated tissue levels of iron noted at necropsy in many captive and recently captured black rhinoceroses,^{9,11} tissue iron levels from necropsy specimens and sera levels of iron and iron transporting proteins in living animals are being assayed. Liver and splenic tissue levels of nonheme iron have been elevated in several black rhinoceroses and any relationship to hemolytic anemia is being investigated.

7. **Cross-Matching of Black Rhinoceros Sera and Red Blood Cells**
Researcher: Dr. Ann Bowling, School of Veterinary Medicine, University of California, Davis, CA 95616, USA.

Subsequent to the necessity of administering whole blood to 2 black rhinoceroses in the US in 1990, this project was initiated to determine the compatibility of black rhinoceros blood for transfusion. Citrated samples are being cross-matched with sera samples from 17 black rhinoceroses.

8. **Encephalomalacia in Black Rhinoceroses**
Researcher: Dr. Eric Miller, St. Louis Zoological Park, St. Louis, MO 631109, USA.

Three cases of acute, fatal encephalomalacia have been identified and described in black rhinoceroses.¹⁷ An additional rhinoceros calf at the Denver Zoological Gardens may have survived an episode, but at times has been similar in appearance to "dummy" bovine calves. Though the histologic pattern resembles leucoencephalomalacia in horses, the etiology remains unknown. The vague symptoms and acute deaths in 2 of the 3 affected animals emphasize the importance of examining brain in all rhinoceros necropsies.

9. **Evaluation of Oral and Skin Ulcers**
Researcher: Dr. Linda Munson, Dept. Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901, USA.

Due to the occurrence of oral and/or skin ulcers in captive black rhinoceroses, ^{6, 7, 20} biopsy and postmortem tissues from all North American cases are being reviewed by Dr. Munson. Twenty-six cases have been noted in 14 US zoos and 14 cases have been examined histopathologically. Microscopically, the lesions appear as a primary epithelial degenerative disease with secondary ulceration. The cause of these lesions was not apparent from the histopathological examination. No evidence of *Stephanofilaria dinniki* infestation was noted in any case. Viral, nutritional, and toxic etiologies are being investigated. (See Dr. Munson's report at this meeting).

Although the lesions apparently respond to corticosteroids, several animals have developed *Aspergillus sp. pneumonias* while on this immunosuppressive therapy. (See below).

10. **Fungal Pneumonia in Black Rhinoceroses**
Researcher: Dr. Scott Citino, National Zoological Park, Washington, DC 20008, USA.

Fungal pneumonia caused by *Aspergillus sp.* has been noted in at least 5 captive black rhinoceroses. In mammals, pneumonia caused by this fungus is most often associated with marked depression of the immune system. Three of the 5 affected rhinoceroses were on immunosuppressive therapy with steroids for oral ulcers (see above). However, 2 of the cases were apparently spontaneous. The occurrence of fungal pneumonia in these captive animals warrants further investigation of their immunocompetence.

11. **Immunological Evaluation by Serum Electrophoresis and Macrophage Morphology**
Researcher: Dr. Alan Herron, School of Medicine, University of Miami, Miami, FL 33136, USA.

Electron Microscopic morphology of macrophages will be compared between normal and hemolytic black rhinoceroses and white rhinoceroses. Serum electrophoretic patterns will also be studied in order to obtain normal and comparative immunological data.

12. Evaluation for Hepadnavirus

Researcher: Dr. Mike Worley, Zoological Society of San Diego, San Diego, CA 92103, USA.

Rhinoceros serum samples have been evaluated for the presence of antibodies to hepatitis B-like virus. In both captive and free-ranging (Zambezi Valley) black rhinoceroses, serum antibodies have been detected that cross react with hepatitis B surface and core antigens. A higher prevalence of serum antibodies was noted in clinically normal animals in contact with rhinoceroses undergoing hemolytic anemia. Additional studies employing Western immunoblotting techniques and viral DNA amplification using polymerase chain reaction are in progress.

13. Vitamin E (Alpha-Tocopherol) Evaluation

Researcher: Dr Ellen Dierenfeld, New York Zoological Society, Bronx, NY 10460, USA.

Due to the numerous reports of low sera levels of alpha-tocopherol in captive black rhinoceroses, evaluation of the vitamin E status of the captive black rhinoceros population continues. Evaluation of sera vitamin E levels from wild-caught animals in the Zambezi Valley of Zimbabwe found higher levels than those in the North American population.^{2,5} However, data from a wild-caught Kenyan population suggest lower levels in that group. Currently multiple studies are in progress to determine the most appropriate methods of oral supplementation.

Author's note: As more plant species (particularly of browse) are offered to captive rhinoceroses, care should be taken to avoid red maple (*Acer rubrum*), onion, and members of the *Brassica* family (eg, kale, rape, cabbage) that are associated with hemolytic anemia in horses and other domestic species. Hemoglobinuria has been reported in Greater Asian rhinoceroses (*Rhinoceros unicornis*) after the feeding of kale.⁹

Diseases reported in black rhinoceros are not limited to the preceding list. Several past deaths²² of black rhinoceroses and two recent ones (a newly imported animal and an aged individual) have been caused by liver failure of unknown origin. Bile duct proliferation and hepatic necrosis have been noted in others.⁹ Recently, an aged female died with hepatocellular carcinoma. Encephalomyocarditis virus has caused the death of two black rhinoceroses in Florida. Several excellent reviews discuss the general disease problems of this species.^{9,12,19,23}

Of critical importance is the use of comparative data between the black and other rhinoceros species. For example, nutritional factors may play an important role as black rhinoceroses are almost exclusively browsers, but in captivity are often fed feedstuffs more appropriate to a grazing species, eg, white rhinoceroses. These comparisons also need to be continued for an additional step, evaluating values of captive versus wild black rhinoceroses. A number of factors may alter the "baseline normal" for the captive population, and thus the wild animals may be the best opportunity for realistic comparisons.

In summary, understanding disease factors is of major importance to the successful maintenance of black rhinoceroses in captivity. To paraphrase Winston Churchill, veterinary considerations in this species are often "a riddle wrapped in a mystery inside an enigma." In addressing this challenge, research efforts into these diseases are slowly unwrapping the mystery.

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