BLACK RHINOCEROS VETERINARY RESEARCH UPDATE 1992

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Under the auspices of the Rhinoceros Taxon Advisory Group (TAG) and the Black Rhinoceros SSP, veterinary research to address the many medical issues affecting this species remains an active and ongoing effort. Unfortunately, deaths from a number of diseases that are still not completely understood, most notably hemolytic anemia and oral/skin ulcers, continue to limit the growth of the captive black rhinoceros population.

Perhaps the most significant problem in the past year has been the deaths of 3 of 10 black rhinoceroses imported from Zimbabwe in April 1992. At necropsy, the animals marked biliary stasis in their livers was noted. Diagnostic tests and final diagnosis in these cases are still pending.

Additional cases of note include hemolytic anemia in a 27 year old female (studbook #121) at the Oklahoma City Zoo that survived a bout of acute hemolysis (PCV=18%) after antibiotic therapy and extensive IV phosphorous supple-mentation. A 4 month old calf at the Denver Zoo has apparently developed encephalomalacia as previously reported.[6] If confirmed, this would be the fifth case of encephalomalacia and again emphasizes the importance of collecting brain tissue from all rhinoceros necropsies.

On a more encouraging note, the interest spurred by these events has resulted in the initiation of several new research projects, most notably by Drs. Evan Blumer (nutritional studies in cooperation with Purina Mills and fecal steroid analyses) and Janet Stover (potential electron microscopic studies of liver and a bile acid study described below), and the augmentation of several preexisting studies.

Also on a positive note, Dr. Paglia's laboratory at UCLA has reported dramatic new findings in the metabolism of the black rhinoceros red blood cell (RBC) (see below and attachment).[8-10]

Several practical clinical suggestions have resulted, including a renewed emphasis on avoiding compounds known to cause hemolysis in human enzyme deficiencies and more aggressive treatment of acidosis and hypophosphatemia[1] in hemolytic black rhinoceroses.

Funding of rhinoceros research remains an ongoing problem. Until recently, Dr. Paglia's project had been funded by an NIH grant (which was not renewed). At the time of this report, his laboratory has obtained partial funding via a $5000 donation from the Cincinnati Zoo and $10,000 from an AAZPA Conservation Endowment Fund (CEF). A concerted effort from the Rhinoceros TAG is underway to support that project and several others that are currently in need of financial support. Included in this potential funding list are a dietary review of by a team assembled by Dr. Craig Thatcher at the Virginia-Maryland Regional College of Veterinary Medicine, a immunological and fungal studies by Dr. Slavin at St. Louis University, and support for Dr. Linda Munson's work on the ulcerative syndrome.

Lastly, a Rhinoceros Veterinary Bibliography, containing over 385 references, was compiled and distributed to veterinarians at all rhinoceros holding institutions in North America, rhinoceros coordinators worldwide, and researchers who have been active in rhinoceros health matters.[5]

Following is an updated list of animal health projects that have been initiated or active during the past year:

1. Project: Continued studies of red blood cell metabolism in rhinoceroses.

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

   Dr. Paglia's laboratory continues to document a marked deficiency of energy (ATP) in black rhinoceroses red blood cells (RBCs). Thus the compromised status of the RBCs apparently makes them susceptible to hemolysis "triggered" by a variety of "stresses" (eg, oxidant compounds such as drugs, infections such as leptospirosis).[8-10] Another promising breakthrough is the discovery that the enzyme catalase is nearly absent in black rhinoceros RBCs. In man, acatalasemia is associated with oral ulcers and in black rhinoceroses; this finding may link both that syndrome and hemolytic anemia. Interestingly, although the RBCs from the 2 white rhinoceroses tested to date were also energy deficient, they had catalase levels similar to those in normal human cells. This finding encourages further research to address the role of catalase deficiency in some of the unique disease problems of black rhinoceroses. A summary of these findings was distributed to veterinarians at rhinoceros holding institutions and will be presented at the AAZV meeting (copy enclosed).

2. Project: Comprehensive nutritional review of captive diets.

Researcher: Dr. Craig Thatcher, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24601, USA.

Initiation of this project is awaiting funding ($25,000). Dr. Thatcher has assembled a team of that includes nutritionists, an epidemiologist and an infectious disease specialist to assist in the review of captive rhinoceros diets. It is hoped that this project will provide a basis on which to build additional studies of specific nutritional factors.

3. Project: Evaluation of oral and skin ulcers

Researcher: Dr. Linda Munson, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901.

Dr. Munson continues to review tissues from biopsy and necropsy samples from these cases.[7] She has identified 32 cases. Histologically, the lesions have been characterized by ballooning degeneration and intraepithelial vesicle formation. Publication of her findings is pending.

4. Project: Leptospirosis evaluation by microagglutination titers and fluorescent antibody testing.

Researcher: Dr. Carole Bolin, National Veterinary Laboratories, Ames, IA 50010, USA.

Dr. Bolin's laboratory continues to perform micro-agglutination titers on sera and fluorescent antibody (FA) testing on rhinoceros tissue samples. FA tests on the two rhinoceros deaths at Fossil Rim were negative. A paper summarizing the results from both captive and translocated rhinoceroses in Zimbabwe is in press.[2]

Titers from the recent importation of 10 black rhinoceroses from Zimbabwe found evidence of exposure in all animals to the serovar *Leptospirosis interrogans* serovar *bratislava*. Due to these findings, a revised leptospiral vaccination protocol has been recommended that includes this serovar. We are now recommending biannual vaccination with a 6-way serovar (*canicola, grippotyphosa, hardjo, icterohemorrhagiae, pomona,* and *bratislava*). This vaccine is commercially available as Brativac, a swine product from Norden Pharmaceuticals (Lincoln, NE, USA) that comes in 10 or 50 dose units.

5. Project: Evaluation of bile acid levels and their effect on platelet function in black and white rhinoceroses.

Researchers: Dr. Roger Clemens, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610-0103, Dr. Janet Stover, White Oak Plantation, Yulee, FL 32907.

Bile acid levels appear to be elevated in several black rhinoceroses tested. Interference of the test with other compounds in the blood is being evaluated. In the horse, elevated levels of bile acids have been associated with increased red blood cell fragility and altered platelet function. Results from normal and abnormal black and white rhinoceroses are pending.

6. Project: Complete blood counts and serum chemistries.
Researcher: Dr. Steven Stockham, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211.

Testing of blood and sera samples continues. These values will further establish "normal" values in the captive black rhinoceros population, and provide comparative information with data previously published from free-ranging black rhinoceroses.[3]

7. Project: Evaluation of iron levels and metabolism.

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

Dr. Smith is continuing to measure iron and iron binding proteins in samples submitted to Kansas State. In general, his quantitative measurements indicate increased levels of hepatic iron in captive black rhinoceroses and that sera iron and TIBC of black rhinoceroses are higher in older than younger animals, and are higher than in white rhinoceroses. A publication summarizing his data is pending. Of related interest is a recent publication that suggests that the accumulation of hepatic iron may be a function of the length of time spent in captivity.[3]

8. Project: Evaluation for hepadnovirus.

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

This study continues to evaluate rhinoceros sera samples for antibodies to hepatitis-B like virus. Additional testing is in progress in an attempt to more definitively identify viral isolates.

9. Project: Cross-matching of black rhinoceros sera and red blood cells.

Researcher: Dr. Ann Bowling, School of Veterinary Medicine, University of California, Davis, CA 95616.

Citrated samples of whole blood from 20+ black rhinoceroses have been cross-matched with sera from 18 other black rhinoceroses. Although the pattern emerging from these reactions may define one or more naturally occurring anti-red blood cell antibodies, no evidence has been found that would suggest a clinically obvious problem with transfusions being defined by these tests.. No obvious patterns or differences in reactivity were noted between the Eastern and Southern subspecies (*D. b. michaeli* and *D. b. minor*).

10. Project: Evaluation of the immune response in rhinoceroses with particular emphasis on aspergillosis infections.

Researchers: Drs. Raymond Slavin and Alan P. Knutsen, St. Louis University School of Medicine, St. Louis, MO 63104, USA.

The general immune response in the rhinoceros will be determined by comparing anti-leptospiral antibodies in rhinoceroses affected with aspergillosis pneumonia and unaffected individuals. Cellular immunity will be measured by *in vitro* lymphocyte response to phytohemagglutinin. The specific response to aspergillus will be determined by detection of precipitating antibodies; measurement of IgG anti-aspergillus antibody in the serum, and *in vitro* lymphocyte proliferative response to aspergillus. This project is awaiting funding ($5000).

Respectfully submitted,

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-OVER FOR MORTALITIES/REFERENCES-

BLACK RHINOCEROS DEATHS IN NORTH AMERICA 1992

STDBK # LOCATION	SEX	DOB	DOD	CAUSE OF DEATH
74/San Francisco	M	1JAN54	7NOV91	Ruptured hepatic tumor
2064/ Denver	F	10APR90	30JAN92	Euthanized due to encephalomalacia
2078/Fos. Rim	F	Adult	12JUN92	Hepatic bile stasis, dystocia, oral/skin ulcers, anemia, imported from Zimbabwe 22APR92
2077/Fos. Rim	M	Adult	27JUN92	Hepatic bile stasis, oral/skin ulcers, imported from Zimbabwe 22APR92
#188/ Columbus	F	1970	25JUL92	Metritis? Pending histology.
/Wh. Oak	M	Adult	22AUG92	Hepatic bile stasis, oral ulcers, thoracic hemorrhage, imported from Zimbabwe 22APR92

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