

St. Louis Zoo

September 1, 1991

Dear Rhinoceros Researcher:

Due to your interest in black rhinoceros research, please find enclosed a copy of this year's Black Rhinoceros Veterinary Research Update. In abstract form, this report describes projects that have been either active or initiated in the past year. For your interest, I have also enclosed a copy of the report of the Veterinary Working Group from the 1991 Rhinoceros Conference in San Diego, and a list of speakers and presentations in the Veterinary/Nutrition Session there.

Thank you for your past, present and future interest in the health of black rhinoceroses. If I can supply any further materials, or if you have any comments or suggestions, please feel free to contact me.

Sincerely,



R. Eric Miller, DVM
Associate Veterinarian

Saint Louis ZOO

Forest Park
Saint Louis, Missouri 63110
314-781-2900
Fax: 314-647-7805

Charles H. Hoessle
Director

Robert Hyland
President, Zoological Commission



BLACK RHINOCEROS VETERINARY RESEARCH UPDATE 1991

R. Eric Miller, DVM
Veterinary Advisor
Black Rhinoceros (Diceros bicornis) SSP Committee
Rhinoceros Taxon Advisory Group

Under the auspices of the SSP, animal health research in the black rhinoceros is an ongoing effort. This report will serve as an update to the 1990 veterinary report to the Black Rhinoceros SSP. Obtaining tissue and sera from all species of captive and wild rhinoceroses remains a priority. Central storage facilities exist for formalinized rhinoceros tissues (Dr. Richard Montali, National Zoological Park) and for frozen serum and tissue (Dr. Eric Miller, St. Louis Zoological Park). These banks have provided readily available sources of materials for comparative and retrospective studies. "Normal" values from wild black rhinoceroses (80+ animals) in Zimbabwe has been published by Dr. Michael Kock, Raoul du Toit, et. al. (1).

Four diseases in black rhinoceroses continue to be notable for their unusual nature and relatively high frequency of occurrence. Although hemolytic anemia has been the leading cause of death among captive animals (43 episodes of hemolysis noted in 34 rhinoceroses; 23 rhinoceroses died from their anemia), no deaths from "primary" hemolysis (not associated with other systemic disease) have been noted since 1986. It is too soon to determine the full significance of this, but it may be a hopeful sign that leptospirosis vaccination and dietary improvements have had some effect. Additionally, no new cases of encephalomalacia have been identified since 1988.

Fungal pneumonias (Aspergillus and less commonly phycomycetes) continue to be noted; at least 6 cases have been identified in black rhinoceroses in North America. Four occurred in black rhinoceroses receiving immunosuppressive therapy for other conditions and 2 cases were "spontaneous." The occurrence of these infections suggests an altered immunological response and has led to research on the immune status of black rhinoceroses (see Dr. Slavin's project below, Dr. Herron's project on the 1990 report). Last, but not least, is the occurrence of oral/skin ulcers. Twenty-six cases have been noted, ranging from mild skin ulcers to severe ulcerative lesions of the skin, mucosal junctions and gastrointestinal tract. Death may result from secondary complications. Dr.

Linda Munson is reviewing tissues from these cases (see description of her project below).

Prior to the 1991 International Rhinoceros Conference at the San Diego Zoo, Dr. David Jessup (International Wildlife Veterinary Services) organized a meeting of veterinarians active in rhinoceros medicine and research. Attendees represented zoo and wildlife veterinarians from the US, Great Britain, Zimbabwe and Namibia. The meeting presented an excellent opportunity for wildlife and zoo veterinarians to share clinical and research experiences and to identify areas of common interest and cooperation. A statement that resulted from this meeting and the Veterinary Session of the Rhinoceros Conference is attached. Two areas were identified that warrant further research: 1) immunological function (for reasons noted above) and 2) additional nutritional studies.

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

1. Project: T-Lymphocyte Stimulation Testing and Immunological Evaluation for Fungal Infections
Researchers: Dr. Raymond Slavin and Dr. Allan P. Knutsen, St. Louis University School of Medicine, St. Louis, MO 63104, and R. Eric Miller, DVM, St. Louis Zoological Park, St. Louis, MO 63110, USA.

Currently being designed, this project will employ various immunological tests to identify black rhinoceroses infected with Aspergillus sp. (primarily pneumonia as noted above) and to evaluate their response to fungal organisms. A more general immunological study will evaluate the response of black rhinoceros lymphocytes to in mitogen stimulation studies.

2. Project: Nutritional studies

Researchers: Dr. Craig Thatcher, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24601, USA, R. Eric Miller, DVM, St. Louis Zoological Park, St. Louis, MO 63110, USA.

See Nutritional Report to the Black Rhinoceros SSP Committee.

3. Project: Evaluation of oral and skin ulcers

Researcher: Dr. Linda Munson, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901-1076, USA.

Due to the occurrence of oral and/or skin ulcers in captive black rhinoceroses (3), biopsy and postmortem tissues from all cases are being reviewed by Dr. Munson. Twenty-six captive black rhinoceroses in the US have had mucosal and/or cutaneous

ulcers; 3 cases have been noted in the past year. Most of the rhinoceroses have had recurrent ulcers. Microscopically, the oral and skin lesions appear as chronic ulcers, though, as of yet no single histologic pattern has emerged. In these captive rhinoceroses there has been no evidence of the dirofilarid parasite Stephanofilaria dinniki. The etiology remains unknown. Dr. Munson is preparing a paper describing the ulcerative "syndrome."

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

Researcher: Dr. Carol Bolin, National Veterinary Services Laboratory, Ames, IA 50010, USA

On the basis of fluorescent antibody (FA) tests, infection with Leptospirosis interrogans has been noted in 3 of 4 cases of fatal hemolytic anemia in black rhinoceroses (2). Additionally, in the past year, another FA+ case was noted in a female that died in 1990 at the Cincinnati Zoo with severe skin ulcers and anemia. The relationship of L. interrogans infection with disease in this animal is unclear. Currently titer data from captive and wild (Zimbabwe samples supplied by Drs. Michael and Nancy Kock and David Jessup and Raoul du Toit, Namibian samples supplied by Dr. Peter Morkel and Louis Geldenhuys) are being submitted for publication. Of interest is evidence of exposure to varying serovars (strains) of L. interrogans in different areas of Zimbabwe and Namibia (no evidence of exposure in 3 rhinoceroses from the latter's arid habitat). The presence of infection with L. interrogans in some of the hemolytic cases and the titer data continue to support the previous recommendation that all black rhinoceroses be vaccinated biannually with a bacterin that contains at least 5 serovars of L. interrogans including icterohaemorrhagiae and grippotyphosa. Leptoferm-5 (Norden Laboratories, Lincoln, NE 80809, USA) is recommended. Opportunistic postvaccinal sampling of black rhinoceros continues to demonstrate responses in microagglutination titers that would be considered appropriate and protective in domestic species.

Note: Though 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 in all cases. Ongoing studies are attempting to identify other factors that may contribute to red blood cell instability.

5. Project: Further evaluation of red blood cell metabolism.

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

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 (4). Further analysis of rhinoceros RBC metabolism and substrate usage is ongoing at UCLA as heparinized blood samples become available. Funding sources need to be identified to maintain and continue this project.

6. Project: Aspergillus sp. pneumonia in black rhinoceroses.

Researcher: Dr. Scott Citino, Metro Miami Zoo, Miami, FL 33176, USA and Dr. Eric Miller, St. Louis Zoological Park, St. Louis, MO 63110, USA.

Fungal pneumonia caused by Aspergillus sp. has been noted in at least 6 captive black rhinoceroses. At least 4 of the 6 affected animals were on immunosuppressive therapy for ulcers (see Dr. Munson's project above). However, 2 of the cases were apparently spontaneous. The occurrence of fungal pneumonia in captive black rhinoceroses warrants further studies regarding their immunocompetence (see Dr. Slavin's project).

7. 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

Red blood cells (citrate samples) from 9 black rhinoceroses have been cross-matched with sera from 18 black rhinoceroses. In agglutination testing, weak to moderate reactions have been observed in 13 of the 18 sera samples. One sera sample has produced weak lytic reactions against 7 of the 8 animals tested. Interpreted in light of experience in domestic animals, no evidence has been found that would suggest a clinically obvious problem being defined by these tests. However, it is tempting to speculate that a pattern is emerging from these reactions which may define one or more naturally occurring anti-red cell antibodies. Hopefully, further samples will help in interpreting these observations.

8. Project: Complete blood counts and serum chemistries.

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

Because of variability between laboratory methods, a request was made that complete blood counts and serum chemistries from all rhinoceroses be submitted to a central laboratory. To date, 35 samples have been received from 15 black and 6 white rhinoceroses. Data are currently being reviewed.

9. Project: Serum iron levels and iron binding proteins

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

Due to the elevated tissue levels of iron noted at necropsy in many black rhinoceroses, additional tissue iron levels from necropsies and serum levels of iron and iron transporting proteins in living animals are being assayed. Thirty-seven sera samples (29 black, 7 white, and 1 Indian), and 17 liver and/or splenic samples (14 black, 3 white) samples have been evaluated. When the data from black rhinoceroses are compared to the white rhinoceroses included in the study, they do not appear to differ significantly. Further analysis is underway to determine if initial impressions that black rhinoceroses accumulate iron in the liver and spleen as they age, and if the serum iron and TIBC of adult black rhinoceroses are higher than that of younger animals or white rhinoceroses.

10. Project: Evaluation for hepadnavirus.

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

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

BLACK RHINOCEROS DEATHS IN NORTH AMERICA

1991

<u>STDBK #</u> <u>NAME</u>	<u>SEX</u>	<u>DOB</u>	<u>DOD</u>	<u>CAUSE OF DEATH</u>
239 Nanyuki SD-WAP	F	15OCT76	13JUN91	Ruptured liver, trauma
2066 No Name Bentsen	M	20JUL91	20JUL91	Weak, possibly premature

BIBLIOGRAPHY

1. Kock, MD, R du Toit, D Morton, N Kock, B Paul: Baseline biological data collected from chemically immobilized free-ranging black rhinoceroses (Diceros bicornis) in Zimbabwe. J. Zoo Wildl. Med. 21: 283-291, 1990.
2. Miller, RE, CA Bolin: Evaluation of leptospirosis in black rhinoceroses (Diceros bicornis) by microscopic agglutination and fluorescent antibody testing. Proc. Am. Assoc. Zoo Vet., pp. 161-162, 1988.
3. Ott, JE, SE McDonald, PT Robinson, FH Wright: Ulcerative stomatitis in a black rhinoceros (Diceros bicornis). Proc. Am. Assoc. Zoo Vet., pp. 68-71, 1982.
4. Paglia, DE, WN Valentine, RE Miller, M Nakatani, RA Brockway: Acute intravascular anemia in the black rhinoceros (Diceros bicornis) - II. Erythrocytic enzymes and intermediates. Am. J. Vet. Res. 47: 1321-1325, 1986.

Respectfully submitted,

R. Eric Miller, DVM
Associate Veterinarian
St. Louis Zoological Park

September 1, 1991

INTERNATIONAL RHINOCEROS CONFERENCE - SAN DIEGO 1991
VETERINARY WORKING GROUP/SESSION REPORT

In view of the role that health and nutritional problems have had in the maintenance of captive rhinoceros populations (eg, as a limiting factor in the growth of the captive black rhinoceros population), and that they have presented concerns in wild populations and their translocations, the following points for consideration and action are recommended:

1. Continued investigation of health problems in wild and captive rhinoceroses. New and continued research should be organized and encouraged in the following areas:

- All morbidity and mortality data from captive, and where possible, wild populations should be compiled and reviewed annually under the auspices of the regional species management plans and national wildlife programs, and those regional data reviewed under the auspices of the IUCN/CBSG Rhinoceros Action Plan. Such studies should include evaluation of post-capture and post-translocation mortalities.

- Further investigation of the incidence and prevention of management related disease, trauma and infertility.

- Additionally, monitoring the fertility of all rhinoceros populations with particular attention to Indian rhinoceroses and abortion rates in black rhinoceroses should be emphasized.

- Enhancement of baseline data ("normal" values) from free-ranging and captive rhinoceroses of all species is of critical importance to all fields of research.

- Epidemiology of health problems in captive and wild rhinoceros populations and comparison of patterns in each. Such research should include seroprevalence surveys for infectious diseases and evaluation of internal and external parasites and their significance to rhinoceros health.

- Continued sharing and refinement of immobilization regimens between wildlife and zoo veterinarians should take place. Narcotic agents (etorphine and carfentanil) are the primary drugs used for immobilization, and further investigations are needed to establish preferable supplemental tranquilizers, particularly long-acting neuroleptic agents.

- Metabolic consequences of anesthesia and the stresses associated with capture and the sequelae to both should be assessed.

- Studies to address the immune status of wild and captive rhinoceroses and the role that immunology may play in several of their diseases, eg, fungal pneumonia of black rhinoceroses, should be initiated.

- Nutritional research should include a general review of the feeding practices used in all species in captivity with particular attention to establishing minimal requirements. Basic nutritional evaluations should focus attention on the nutrition of both wild and captive populations, and the resultant comparisons. Research to establish effective dietary supplementation with alpha-tocopherol should be encouraged.

- In black rhinoceroses, further research should be designed to evaluate the following diseases and syndromes:

Hemolytic anemia - Current recommendations for the prevention of acute hemolytic anemia include the vaccination of captive animals with a bacterin containing 5 leptospiral serovars. Research to identify an underlying cause for the hemolysis should continue.

Oral/skin ulcers - Ongoing efforts to identify the pathophysiology of the ulcers should be encouraged.

Iron metabolism - Further evaluation due to the accumulation of hepatic iron in captive and newly captured black rhinoceroses.

Fungal pneumonia
Encephalomalacia

2. In conjunction with the above proposals, identification of additional funding sources to support health research in rhinoceroses is vital.
3. Continued maintenance and enhanced participation in regional biomaterial banks (tissue, sera, urine, etc.) with samples from both captive and wild rhinoceroses of all available species is vital to future comparative studies.
4. Continued and enhanced collection of genetic samples from anesthetized rhinoceroses whenever possible should continue to be encouraged.
5. Communication between veterinarians working with both wild and captive rhinoceroses should be continued and enhanced through future meetings. Special effort should be given to the maintenance of continuous medical histories for rhinoceroses translocated from the wild to captivity.

In summary, there should be veterinary participation in the management of captive and wild rhinoceros populations. This participation should be an integral part of a multidisciplinary approach to their care, and is particularly relevant to their capture and translocation. Such efforts will contribute to the long term survival of both in situ and ex situ rhinoceros populations.

Submitted by : R. Eric Miller, DVM
Veterinary Working Group/Session Chair

1991 RHINOCEROS CONFERENCE
VETERINARY AND NUTRITIONAL MANAGEMENT SESSION

1. Dr. Michael Kock
"Capture and Translocation of Black Rhinoceroses in Zimbabwe,
Capture and Management Modifications to Reduce Stress and
Mortalities"

Department of National Parks and Wildlife Management
PO Box 8365
Causeway
Harare
Zimbabwe

2. Dr. David Jessup
"Health Data Gained from Black Rhinoceroses Immobilized for
Relocation"

International Wildlife Veterinary Services
7945 Amalfi Way
Four Oaks, CA 95628

3. Louis Geldenhuys
"Black Rhinoceros Translocation in Namibia"

Etosha Wildlife Institute
Namibia Department of Nature Conservation
Private Bag 13306
Windhoek 9000
Namibia

4. Dr. Peter Morkel
"Dehorning of Wild Black Rhinoceroses in Namibia"

Etosha Wildlife Institute
Namibia Department of Nature Conservation
Private Bag 13306
Windhoek 9000
Namibia

5. Dr. Eric Miller
"Health Concerns in Captive Rhinoceroses"

St. Louis Zoological Park
1 Government Drive
Forest Park
St. Louis, MO 63110-1396

6. Dr. Richard Kock
"Veterinary Management of Rhinoceroses in Zoos"

Zoological Society of London
Whipsnade Park
Whipsnade, Bedfordshire
Great Britain

7. Dr. Christopher Furley
"Diseases and Management of Black and Sumatran Rhinoceroses at
the Howletts and Port Lympne Zoos"

c/o Howletts and Port Lympne Estates Ltd.
Port Lympne
Lympne nr Hythe
Kent CT21 4PD
Great Britain

8. Dr. Richard J. Montali
"Pathological Findings in Diseases of Captive Rhinoceroses"

National Zoological Park
3000 Connecticut Ave.
Washington, DC 20008

9. Dr. Linda Munson
"Pathological Findings in Oral and Skin Ulcers in Black
Rhinoceroses"

College of Veterinary Medicine
University of Tennessee
Knoxville, TN 37901