# COMPARISON OF ANTI-PHOSPHOLIPID ANTIBODIES BETWEEN WILD AND CAPTIVE BLACK RHINOCEROS (DICEROS BICORNIS): IMPLICATIONS FOR HEALTH AND REPATRIATION

BALL RL[1], MORROW M[2], HOFMEYR M[3], BUSS P[3]

[1]Busch Gardens Tampa Bay, 3605 Bougainvillea Drive, Tampa, Fl, 33674; dr.ray.ball@buschgardens.com
[2]Warren Magnuson Clinical Center, National Institutes of Health, Bethesda, MD, [3]Veterinary Wildlife Services Kruger National Park Private Bag X402, Skukuza, 1350, South Africa

## Introduction

The antiphospholipid syndrome (APS) is defined as the occurrence of venous and arterial thrombosis, recurrent fetal losses, in the presence of the phospholipid antibodies (aPhL) (LEVINE et al, 2002). This is a broad definition in a syndrome that can affect virtually any body system. Deep venous thromboses (DVT) and pulmonary embolism (PE) are among the most common clinical presentations of APS. The aPhL proteins result in anti-coagulant activity but actually cause a hypercoaguable state in vivo. The pathogenesis of APS is quite simply thrombosis regardless of the organ system involved (ASHERSON et al, 1999; LEVINE et al, 2002).
Black rhinos in captivity have been plagued by a host of clinical entities. These include superficial necrolytic dermatitis (SND) (Munson et al, 1998), hemosiderosis (KOCK et al,1992; MURNANE et al, 1994), hemolytic (CHAPLIN et al,1986; MILLER and BOEVER, 1982; PAGLIA et al,1986) non-hemolytic (MURNANE et al, 1994; MURRAY et al 2000) anemia and recently the idiopathic hemorrhagic vasculopathy syndrome (IHVS) has been described in a group of black rhinos (MURRAY et al 2000). Comparisons between APS and black rhino syndromes may not be obvious at first but there may be some parallels (Table 1). Again the underlying pathogenesis for all the conditions may be thromboembolic events.

## Methods

A black rhino specific IgG-aPL ELISA has been developed and validated under the direction of Dr. Sylvia Pierangeli at the Moorehouse School of Medicine in Atlanta. A standard human assay (aPhL® ELISA Kit, Louisville APL Diagnostics, Inc., 3988 Flowers Rd. Ste. 620, Doraville, GA 30360, USA) was modified by substituting purified polyclonal black rhino Ig-G for the human Ig-G conjugate. Sera from wild black rhinoceros were collected during routine translocations in the Kruger National Park and from the serum bank stored there. Sera were stored in a -20°C freezer until assayed in March 2003. Captive samples were submitted directly from collaborating institutions in the USA, from the Species Survival Plan serum storage bank at the St. Louis Zoo, and from animals housed at Busch Gardens Tampa Bay.

## Results and discussion

To date 19/30 captive animals have tested positive for the presence of phospholipids antibodies. All 19 animals have some of the clinical signs associated with medical conditions in black rhinos. Of the

11 negative animals, 8 did not have any clinical signs. Several animals had increased titers with length in captivity. The age of positive animals ranged form threemonths to adult. Relatedness was not looked at because there is no reported correlation of the clinical syndromes seen in captivity to genetic predisposition. Wild caught rhinos have developed many of the problems as well. Twenty-one wild black rhinos tested at the Veterinary Science Services facility in the Kruger National Park all had negative titers. The wild rhinos were estimated to be between nine months of age to adult.
Antiphospholipid antibodies are also elevated in generalized inflammatory conditions. Comparing the two populations of black rhinoceros, it is apparent that there is some inflammatory process that triggers an exaggerated response to APS antibodies in captive black rhinoceros. The wild rhinoceros all exhibited some clinical manifestations of inflammation (tick loads, wounds, and keratitis) but still had negative APS titers. An obvious difference between the two populations is the diet. It is not believed that captive black rhinos have primary antiphospholipid syndrome, rather the increase in antibodies to APS serves as an indicator of a generalized inflammatory state that does not exist in the wild state. This generalized inflammatory state may be contributory to a depleted immune system, thus allowing infection with opportunistic infections. This chronic generalized inflammatory state may also contribute to other conditions such as hemosiderosis. Future work at Busch Gardens Tampa Bay will focus on evaluating diet hypersensitivity and the physical form of the diet as the inciting causes. Planned evaluations include food allergy profiles during feeding trials with a browser diet consisting of a low starch and high physically effective fiber.

*Tab. 1: Comparisons within organ systems between APS and Black Rhino Syndromes.*

| **System** | **APS** | **Black Rhino Syndrome** |
|---|---|---|
| Skin/Digits | Cutaneous necrosis, livedo reticularis | Superficial necrolytic dermatitis, laminar necrosis, IVHS |
| Pulmonary | Pulmonary embolism | IVHS, hemosiderosis |
| Cardiovascular | Valvular lesions | Valvular hemosiderosis |
| Reproductive | Embryonic/fetal loss | Embryonic/fetal loss |
| Neurological | Embolic stroke | Encephalomalacia |

## References


*ASHERSON RA, CERVERA R, LIE JT (1999): The Antiphospholipid Syndromes. Systemic Lupus Erythematosus. New York, London. The Academic Press, 829 - 869.*

*CHAPLIN H Jr, MALECEK AC, MILLER RE, BELL CE, GRAY LS, HUNTER VL (1986): Acute intravascular hemolytic anemia in the black rhinoceros: hematologic and immunohematologic observations. Am J Vet Res **47**, 1313 - 20.*

*KOCK N, FOGGIN C, KOCK M, KOCK R (1992): Hemosiderosis in the black rhinoceros (Diceros bicornis): comparison of free-ranging and recently captured with translocated and captive animals. J Zoo Wildl **23**, 230 - 234.*

*LEVINE JS, BRANCH DW, RAUCH J (2002): The Antiphospholipid Syndrome. N. Engl. J. Med., Vol. **346,** 752 - 763.*

*MILLER RE, BOEVER WJ (1982): Fatal hemolytic anemia in the black rhinoceros: case report and a survey. J Am Vet Med Assoc **181**, 1228 -1231.*

*MILLER RE, CAMBRE RC, DE LAHUNTA A, BRANNIAN RE, SPRAKER TR, JOHNSON C, BOEVER WJ (1990): Encephalomalacia in three black rhinoceros (Diceros bicornis). J Zoo Wildl **21**, 192 -*

*199.*

*MUNSON L, KOERLER JW, WILKINSON JE, MILLER RE (1998): Vesicular and ulcerative dermatopathy resembling superficial necrolytic dermatitis in captiveblack rhinoceroses (Diceros bicornis). Vet Pathol **35**, 31 - 42.*

*MURNANE RD, RAVERTY SA, BRIGGS M, PHILLIPS LG (1994): Chronic recurrent anemia, massive pulmonary and systemic mineralization, chronic interstitial nephritis and membranoproliferative glomerulonephritis, and hemosiderosis with myelophthisis in a euthanized black rhinoceros (Diceros bicornis). Proceedings American Association of Zoo Veterinarians. 325 - 331.*

*MURRAY S, LUNG NP, ALVARADO TP, GAMBLE KC, MILLER MA, PAGLIA DE, MONTALI RJ 2000): Idiopathic hemorrhagic vasculopathy syndrome in seven black rhinoceros. J Am Vet Med Assoc **216**, 194, 230 -233.*

*NANCE M (1998): Clinical management of severe necrotic laminar disease in an eastern black rhinoceros (Diceros bicornis michaeli) associated with anundetermined etiology. Proceedings AAZV and AAWV Joint Conference..208 - 211.*

*PAGLIA DE, VALENTINE WN, MILLER RE, NAKATANI M, BROCKWAY RA (1986): Acute intravascular hemolysis in the black rhinoceros: erythrocyte enzymes and metabolic intermediates. Am J Vet Res. **47**, 1321 - 1325.*