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

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## Abstract

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).<sup>5</sup> 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.<sup>1,5</sup> Black rhinoceros in captivity have been plagued by a host of clinical entities. These include superficial necrolytic dermatitis (SND),<sup>8</sup> hemosiderosis,<sup>4,9</sup> hemolytic,<sup>3,6,12</sup> non-hemolytic,<sup>9,10</sup> anemia and recently the idiopathic hemorrhagic vasculopathy syndrome (IHVS) has been described in a group of black rhinoceros.<sup>10</sup> Comparisons between APS and black rhinoceros syndromes may not be obvious at first but there may be some parallels.<sup>2</sup> Again the underlying pathogenesis for all the conditions may be thromboembolic events.

# Methods

A black rhinoceros-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<sup>®</sup> ELISA Kit, Louisville APL Diagnostics, Inc., 3988 Flowers Rd. Ste. 620, Doraville, Georgia 30360 USA) was modified by substituting purified polyclonal black rhinoceros Ig-G for the human Ig-G conjugate. A standard ELISA reader was then utilized at a wavelength of 405 nm to measure the optical density (O.D.) of the wells. Controls were established by pooling the strongest reactors as the positive control and pooling the lowest reactors as the negative control. Readings of greater than 0.6 were considered to have a significant level of aPhL antibodies and considered positive. The assay was species specific and did not react with domestic horses or white rhinoceros serum.

Sera from wild black rhinoceros were collected during routine translocations in the Kruger National Park and from other South Africa National Parks (SAND). Eleven of the wild rhinoceros were captured in the Hluhuwe-Imfolozi Wildlife Park. Sera were stored in a -20C

freezer until assayed. All the wild rhinoceros were estimated to be between 9 mo of age to adult. Captive samples were submitted directly from collaborating institutions in the USA, 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 19/31 captive animals have tested positive. All 19 animals have some of the clinical signs associated with medical conditions in black rhinoceros. Of the 11 negative animals, 8 did not have any clinical signs. Several animals had increased titers with length of time in captivity. The age of positive animals ranged from 3 mo to adult. Three tendencies where noted in the captive samples: positives with O.D. above the cutoff of 0.6, animals with moderate levels of between 0.4 and 0.6 O.D., and those below 0.4 O.D. The majority of captive animals fell in the high or low ends. Wild caught rhinoceros have developed many of the problems as well once brought into captivity and demonstrated rising levels of aPhL antibodies as well. All 32 wild black rhinoceros tested at the Veterinary Science Services facility in the Kruger National Park had negative titers. The majority of these animals interestingly had levels in the middle range of 0.4-0.6 O.D.

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. This inflammation is believed to be reflected in the middle range of O.D. seen in the wild rhinoceros. An obvious difference between the two populations is the diet. It is not believed that captive black rhinoceros 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.

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