
PRELIMINARY EVALUATION OF SERUM PROTEIN ELECTROPHORESIS AS A DIAGNOSTIC TOOL IN THE BLACK RHINOCEROS (*Diceros bicornis*)

Jeffrey F. Gevanthor, DVM,^{1*} Lisa M. Tatum, DVM,² Sharon L. Deem, DVM, PhD,³ and Scott B. Citino, DVM⁴

¹Old Marple Veterinary Center, 820 West Springfield Road, Springfield, PA 19064 USA; ²University of Miami School of Medicine, Avian and Wildlife Laboratory, PO Box 016960 (R-46), Miami, FL 33101 USA; ³Wildlife Health Sciences, Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460 USA; ⁴White Oak Conservation Center, Animal Sciences Building, 3823 Owens Road, Yulee, FL 32097 USA

Abstract

Serum protein electrophoresis (SPE) is a valuable tool for the diagnosis of certain diseases in humans and animals.^{1,3-8,12} This technique could be particularly beneficial when applied to health monitoring in the black rhinoceros (*Diceros bicornis*), a species which is predisposed to a number of diseases in captivity, the etiologies of which are not yet fully understood.^{2,14-19} Serum protein fractionation varies widely between species, thus it is necessary to establish a reference range in order to interpret SPE for a given species.^{10,13,20} This study has two purposes. The first is to determine normal ranges and patterns of serum proteins separated by SPE in the black rhinoceros. The second is to make a preliminary evaluation of the diagnostic potential of SPE for this species.

Serum protein electrophoresis was performed on samples from both clinically healthy ($n = 38$) and clinically ill ($n = 16$) black rhinoceroses. Data gathered from healthy individuals were used to establish reference ranges for SPE. The absolute ranges for total protein, albumin, and gamma (γ) globulins in the black rhinoceros are similar to those of domestic mammals, however the albumin to globulin ratio and alpha (α) globulins tend to be lower while the beta (β) globulins tend to be higher in the black rhinoceros than in domestic mammals. Preliminary evaluation of data gathered from ill and subclinically ill individuals indicates that serum protein electrophoresis is helpful in the diagnosis of clinically inapparent disease in the black rhinoceros. Research toward identifying and characterizing electrophoretic patterns associated with subclinical and clinical disease in the black rhinoceros continues.

ACKNOWLEDGMENTS

The authors would like to thank Howard Gilman, John Lukas, Dave Thompson, Cyd Teare, and the staff of White Oak Conservation Center, and Carolyn Cray from the University of Miami, Division of Comparative Pathology, for making this research possible. The authors also thank Eric Miller and the staff of the Saint Louis Zoo, Dave Jessup of the California Department of Fish and Game, Robin Radcliffe and the staff of Fossil Rim Wildlife Center, Mitch Finnegan and the staff of the Oregon Zoo, Tom Alvarado and the staff of the Dallas Zoo, Chris Miller and the staff of the Miami Metrozoo, Peregrine Wolff, Michelle Miller, and the staff of Disney's Animal Kingdom, Nancy Lung and the staff of the Fort Worth Zoo, Ray Ball and the staff of Busch Gardens, Tampa, Nadine Lamberski and the staff of Riverbanks Zoological Park, and Roberta Wallace and the staff of the Milwaukee County Zoo for their time and effort in collecting and sending samples, Tom Foose of the International Rhino Foundation and The Wilds for providing studbook

information and current locations of black rhinoceroses, and Eric Lumis Shapiro of the Hospital of the University of Pennsylvania, Department of Anesthesiology, for reviewing this manuscript. Special thanks go to Lonnie McCaskill and Tortoise at White Oak Conservation Center for providing the inspiration to begin this work.

LITERATURE CITED

1. Altman N.H., G.D. Bossart, and C. Cray. 1994. Serum protein electrophoresis: a diagnostic tool you can use to your advantage. Univ. Miami Pathline Flyer 1(2):1-2.
2. Chaplin, H., A.C. Malecek, et. al. 1986. Acute intravascular hemolytic anemia in the black rhinoceros: hematologic and immuno-hematologic observations. Am. J. Vet. Res. 47(6):1313-1320.
3. Cray, C. Diagnostic use of protein electrophoresis in birds. In: Current Veterinary Therapy. (In press)
4. Cray, C., G. Bossart, and D. Harris. 1996. Plasma protein electrophoresis: an update. Proc. Annu. Conf. Assoc. Avian Vet., Pp. 97-100.
5. Cray, C., G. Bossart, and D. Harris. 1995. Plasma protein electrophoresis: principles and diagnosis of infectious disease. Proc. Annu. Conf. Assoc. Avian Vet., Pp. 55-59.
6. Cray, C. and L. Tatum. 1998. Applications of protein electrophoresis in avian diagnostics. J Avian Med. Surg. 12(1) (In press)
7. Deem, D.A., D.S. Traver, et. al. 1979. Agammaglobulinemia in a horse. J. Am. Vet. Med. Assoc. 175(5):469-472.
8. Jollif, C.A., Classification and interpretation of paragon serum protein electrophoresis patterns. Paragon systems reference manual. pp.1-26.
9. Kaneko, J.J. 1989. Clinical Biochemistry of Domestic Animals. Academic Press, Berkeley, California, Pp. 142-165, 888-896.
10. Keay, G. and D.L. Doxy. 1982. A comparison of serum protein electrophoretic patterns of young and adult animals. Vet. Res. Commun. 5(3):271-276.
11. Keren, D.F. High resolution electrophoresis in the detection of monoclonal gammopathies and other serum protein disorders. Paragon Æ Instruments booklet, Brea, California.
12. Lumeij, J.T. 1993. Avian plasma chemistry in health and disease. Proc. Annu. Conf. Assoc. Avian Vet., Pp. 20-26.
13. Margolin, T. 1995. Normal electrophoretic values in cockatiels (*Nymphicus hollandicus*) and factors affecting these values. Proc. Assoc. Avian Vet., Pp. 65-66.
14. Miller, R.E. 1991. Hemolytic anemia in the black rhinoceros. In: Fowler, M.E.(ed.). Zoo and Wild Animal Medicine. WB Saunders Company, Philadelphia, Pennsylvania, 35(3):455-458.
15. Munson, L. 1991 Mucosal and cutaneous ulcerative syndrome in the black rhinoceros (*Diceros bicornis*) Proc. Rhino. Conf., San Diego, Pp. 354-356.
16. Munson, L., L.W. Koehler, et. al. 1991. Vesicular and ulcerative dermatopathy resembling superficial necrolytic dermatitis in captive black rhinoceroses (*Diceros bicornis*). Vet. Pathol. 35:31-42.
17. Nelson, L. and M.E. Fowler. 1986. Rhinocerotidae. In: Fowler, M.E. (ed.). Zoo and Wild Animal Medicine. WB Saunders Company, Philadelphia, Pennsylvania, 50(2):934-938.
18. Paglia, D.E. and R.E. Miller. 1993. Erythrocytes of the black rhinoceros (*Diceros bicornis*): susceptibility to oxidant-induced haemolysis. Int. Zoo. Yb. 32:20-27.
19. Ramsay, E. and Z. Zainuddin. 1993. Infectious diseases of the rhinoceros and tapir. In: Fowler, M.E.(ed.). Zoo and Wild Animal Medicine. WB Saunders Company, Philadelphia, Pennsylvania. 35(3):459-466.
20. Riedarson, T.H. and J. McBain. 1995. Serum protein electrophoresis and *Aspergillus* antibody titers as an aid to diagnosis of aspergillosis in penguins. Proc. Annu. Conf. Avian Vet., Pp.61-64.

Table 1. Serum protein electrophoresis: protein fractions and components.^{9,11}

Protein fraction	Major protein constituents		
Albumin	Albumin		
α_1 Globulins	α_1 -Acid glycoprotein α_1 -Antichymotrypsin	α_1 -Antithrombin III α_1 -Antitrypsin	α_1 -Lipoprotein ^a
α_2 Globulins	α_2 -Globulin α_2 -Lipoprotein ^b	α_2 -Macroglobulin Ceruloplasmin	Haptoglobin Protein C
β Globulins	Amyloid A β_2 -Lipoprotein ^c C3 Complement Transferrin	C4 Complement C-Reactive protein Ferritin	Hemopexin Plasminogen
γ Globulins	Immunoglobulin A Immunoglobulin E	Immunoglobulin G Immunoglobulin M	

^aHigh density lipoprotein (HDL).^bVery low density lipoprotein (VLDL).^cLow density lipoprotein (LDL).**Table 2.** Differential diagnoses of serum protein alterations.^{9,11}

Protein fraction	Differential diagnoses ^a	
Albumin	Increase:	Dehydration
	Decrease:	Hepatic disease, gastrointestinal disease, renal disease, internal parasites, overhydration, malnutrition, blood or plasma loss
α_1 Globulins	Increase:	Acute inflammatory disease, pregnancy
	Decrease:	Hepatic disease, pulmonary disease, nephrotic syndrome
α_2 Globulins	Increase:	Acute inflammatory disease, nephrotic syndrome, hepatic disease, diabetes mellitus, hypothyroidism
β Globulins	Increase:	Acute hepatitis, chronic active hepatitis, nephrotic syndrome, suppurative dermatopathy, anemias
	Decrease:	Autoimmune disease
γ Globulins	Increase:	Chronic inflammatory disease, immune mediated disease, infectious disease, suppurative disease, connective tissue disease, multiple myeloma, lymphosarcoma
	Decrease:	Immune deficiency diseases

^a Evaluation of serum protein status should include assessment of the albumin to globulin ratio (A:G).

Table 3. Reference ranges for SPE in the black rhinoceros ($n = 33$).

Protein fraction	Quantity (g/dl)			Percent of total protein (%)		
	Range	Mean	SD	Range	Mean	SD
Total protein	6.00-8.40	7.37	0.71	N/A	N/A	N/A
A:G ratio	0.31-0.79	0.53	0.13	N/A	N/A	N/A
α Globulins	1.79-3.41	2.49	0.37	23.6-44.1	33.5	5.20
α_1 Globulins	0.09-0.27	0.16	0.05	1.2-4.7	2.3	0.84
α_2 Globulins	0.23-0.68	0.45	0.11	3.9-9.2	6.0	1.31
β Globulins	1.69-3.33	2.51	0.38	26.9-43.8	34.3	5.05
γ Globulins	1.08-2.58	1.76	0.43	15.4-34.4	23.8	4.58

Table 4. SPE reference ranges (g/dl) for the black rhinoceros compared to domestic animals.

Protein fraction	Black rhinoceros ^{a,b}	Horse ^c	Cow ^c	Dog ^c	Cat ^c
Total protein	6.00-8.40	5.20-7.90	6.74-7.46	5.40-7.10	5.40-7.80
A:G ratio	0.31-0.78	0.62-1.46	0.84-0.94	0.59-1.11	0.45-1.19
Albumin	1.79-3.02	2.60-3.70	3.03-3.55	2.60-3.30	2.10-3.30
α Globulins	0.33-1.00	0.37-2.01	0.75-0.88	0.50-1.60	0.50-1.60
α_1 Globulins	0.10-0.32	0.06-0.70		0.20-0.50	0.20-0.50
α_2 Globulins	0.23-0.68	0.31-1.31		0.30-1.10	0.30-1.10
β Globulins	1.81-3.33	0.69-2.47	0.80-1.12	1.30-2.70	1.30-2.70
γ Globulins	1.08-2.58	0.55-1.90	1.69-2.25	0.90-2.20	1.70-4.40

^aReference ranges for the black rhinoceros are repeated from Table 4 for ease of comparison.

^bRhinoceros values determined by agarose gel SPE; domestic animal values by cellulose acetate SPE.⁹