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HEPATOPATHY IN TWO BLACK RHINOCEROSES (*DICEROS BICORNIS*) IN ZIMBABWE: CREOSOTE TOXICOSIS?

Nancy D. Kock, D.V.M., M.S., Michael D. Kock, B.Vet.Med., M.R.C.V.S., M.P.V.M., and K. B. Young, F.Z.I.M.L.S.

Abstract: Four of 20 black rhinoceroses (*Diceros bicornis*) became lethargic, anorectic, anemic, and jaundiced, with elevations in serum bilirubin, after being moved into creosote-treated holding pens (bomas). One of these animals died, and a second became moribund and was euthanized. Both had oral and gastric ulcers, widespread hemorrhages and hematomata, and uniformly swollen, intensely green livers, containing excessive intrahepatic bilirubin. The remaining two animals made full clinical recoveries, and additional cases were not seen in Zimbabwe, although three of these animals died with similar liver lesions after final translocation to the USA, and two with similar liver lesions died after translocation to Australia. This report describes clinical, hematologic, and pathological findings in two black rhinoceroses that were confined in wooden pens that had been treated with creosote, noting similarities described in creosote toxicosis in other animals.

Key words: Black rhinoceros, *Diceros bicornis*, hemolysis, hepatopathy, creosote.

INTRODUCTION

Creosote is not a single compound, but rather a mixture, primarily of coal tar derivatives, which is toxic to fungi and insects and often used to preserve wood.[1] Cresols in creosote are particularly toxic to pigs, causing acute periacinar hepatic necrosis, and chronically associated with jaundice, ascites, and anemia.[3] Cresols are also corrosive and have resulted in stillbirths.[1] Cresol toxicosis has been suspected in the past in black rhinoceroses with liver disease and skin lesions.[2,8]

MATERIALS AND METHODS

Twenty black rhinoceroses were translocated from the Chete safari area (17°20′ S, 27°35′ E) to a newly built holding facility near Harare, Zimbabwe, in December 1990. All animals were deemed healthy upon arrival, based upon routine blood examinations,[5] and adjusted well to captivity. The bomas were constructed with poles that had been recently treated with creosote, and while this was a concern at the time, there was no choice but to move the animals into them. The animals were fed on a mixture of fresh browse and commercial horse cubes (Agrifoods, Harare, Zimbabwe), standard diet for black rhinoceroses in confinement in Zimbabwe, and had fresh water available at all times.

About a month after moving into the bomas, one adult bull (9103) began passing dark brown urine, became lethargic, partially anorectic, and lame with marked swelling of the right foreleg. The animal was immobilized for examination and blood collection. A week later, two other males and a female that were partially off feed were similarly handled. Two of these (9107, 9114) gradually improved and apparently recovered, while the third, another bull (9120), died about a week later, with two swollen limbs. The first animal (9103) declined in condition, became moribund, and was euthanized. Both were necropsied within 6 hr of death, tissues were fixed in 10% buffered formalin, cut at 6 μm, and stained with hematoxylin and eosin. Sections of liver were

From the Departments of Paraclinical (N. Kock) and Clinical (Young) Studies, Faculty of Veterinary Science, University of Zimbabwe, Box MP167, Mount Pleasant, Harare, Zimbabwe; Department of National Parks and Wildlife Management (DNPWM) (M. Kock), Box 8365, Causeway, Harare, Zimbabwe; and International Wildlife Veterinary Services (IWVS) (M. and N. Kock), 1850 North Main Street, Salinas, California, USA.

also stained with Perl's iron stain and Fouchet stain for bile pigments, as well as subjected to diazo methods to distinguish between conjugated and unconjugated bilirubin.[9] Heart blood and liver from both animals were submitted for bacteriologic culture. No new cases were recognized in Zimbabwe, but three others from this group that were translocated to the USA (Miller, pers. comm., 1992), and two that were translocated to Australia (Hartley and Blyde, pers. comm., 1993) died with findings similar to those described in this study.

Because muscles from the affected limbs were darkly discolored, resembling clostridial lesions in cattle, 5-g portions were homogenized with 10 ml of normal saline and injected into Balb/c mice by intraperitoneal injection, at 0.2 ml per mouse. The mice were observed daily for 2 wk, afterward.

Complete blood counts (CBC) were done on ethylenediaminetetraacetic acid (EDTA)-preserved whole blood, and serum was assayed for total protein, albumin, globulin, creatine kinase, aspartate transaminase (AST), alanine transaminase, lactic dehydrogenase, alkaline phosphatase, gamma-glutamyl transaminase, bilirubin, creatinine, and blood urea nitrogen using an Electro-Nucleonics Biochemical Analyzer (Altaire, Electro-Nucleonics International Ltd., Adriaan van Bergenstraat 202-208, 4811 S.W. Breda, The Netherlands). Quality control of Altaire procedures used Gemcal Electro-Nucleonics reference. Ciba-Corning (Ciba-Corning Diagnostic Corp., Irvine, California 92714, USA) normal and abnormal assay sera were used as controls.

RESULTS

Clinical examination of the four animals revealed jaundice that varied from mild to extreme, and blood examinations of the two that died indicated anemia, elevated AST, and bilirubinemia, the result of elevations in both unconjugated and conjugated bilirubin (Table 1). Other measures were within normal published values.[5] Fine needle aspiration of the swollen legs revealed only the presence of blood.

Postmortem findings were similar for both animals that died, and included jaundice, widespread and often extensive hemorrhage, swollen and pigmented livers, oral and gastric ulcers, and myeloid hyperplasia. Conjunctiva, fat, mucous membranes, fascia, and joint surfaces were all pale to deep yellow. Ecchymotic hemorrhages were found throughout the alimentary canal, endocardium, and mesentery. Hematomata were found in the lungs, muscular wall of the urinary bladder, middle ear, and, along with interfascicular muscle hemorrhages, accounted for the swollen extremities. Ulcers covered with fresh blood were found on the surfaces of the tongue, gingiva, and nonglandular stomach. Underlying reasons for the ulceration (i.e., vasculitis) were not evident histologically.

The livers were enlarged with rounded edges, slightly friable, and intensely green. Abundant granular, golden to light brown pigment was visible in hepatocytes in sections stained with hematoxylin and eosin. This was negative for iron with Perl's stain, largely negative for bile pigments with Fouchet stain, but strongly positive for pure bilirubin using van den Bergh diazo methods.[9] In addition, darker, coarser pigment identified as hemosiderin with Perl's iron stain was present in Kupffer cells. Other significant lesions were not observed.

Significant organisms were neither seen on direct smear nor recovered from either heart blood or liver. None of the mice injected with muscle homogenate died or showed any signs of disease.

DISCUSSION

The extensive intrahepatic accumulation of bilirubin in these rhinoceroses was an unusual finding, inconsistent with the well-recognized hemolytic disease[6,7] as well as with other reported pathological conditions in black rhinoceroses. It likely represented an inability to process bile and did not appear to have resulted in direct hepatocyte

Table 1. Abnormal hematologic and biochemical measures for black rhinoceroses (*Diceros bicornis*) with hemolysis, pigmentary hepatopathy, hemorrhage, and ulceration, possibly associated with creosote toxicosis. All other measures were within normal limits.[a]

Measure	Animal number				Normal range
	9103[b]	9107	9114	9120[b]	
RBC ($\times 10^6/\mu l$)	2.9	5.9	4.4	2.1	3.6–7.2
Hemoglobin (g/dl)	9.2	18.6	16.9	7.1	10.8–20.6
Hematocrit (%)	25	52	39	18	29–54
AST (IU/dl)	323	42	197	145	22–132
Bilirubin (mg/dl)	23.80	0.58	0.50	13.92	0.12–1.54
Conjugated	7.36	ND[c]	ND	4.44	
Unconjugated	16.44	ND	ND	9.48	
Hemoglobinuria	+	ND	ND	ND	

[a] Animals that died.
[c] ND = not done.

damage, but rather caused progressive hepatic dysfunction, given the protracted clinical disease. As the jaundice resulted from elevations in both conjugated and unconjugated bilirubin, both hepatic injury as well as hemolysis may have contributed. The amount of hemorrhage discovered at necropsy was not felt to be of sufficient quantity to have resulted in the degree of anemia present in either of the animals, making it possible that hemolysis was also a factor. The terminal hemorrhages were consistent with coagulation defects, the result of trauma imposed upon reduced ability to clot. The middle ear, subcutis, skeletal muscle, lung, and muscular wall of the urinary bladder are all likely locations for hemorrhage with coagulation defects, and while the causes for coagulopathies are numerous, hepatic dysfunction is a well-recognized cause.

More than 2 yr after the construction of the bomas in Harare, a strong smell of creosote continues to linger in the area, and visitors still come away with the smell on their clothes. These 20 rhinoceroses were constantly exposed to this compound in the bomas, by inhalation, possibly ingestion due to contamination of food and water, as well as direct skin contact through rubbing on the walls. The absence of underlying causes for the ulcers found in these rhinoceroses suggests some direct injury, possibly the ingestion of a corrosive substance, given the location (oral cavity and stomach). Anorexia, weight loss, dermatitis, ventral edema, alopecia, and responsive anemia with jaundice and hepatic hemosiderosis and bilirubin retention have been found in chronically intoxicated pigs exposed to creosote,[3] as well as in horses exposed to dioxin, which may be a contaminant of creosote.[4] While the implications for creosote toxicosis in these cases are circumstantial at this stage, the similarities between the signs in these rhinoceroses and other animals intoxicated by compounds contained in creosote make it prudent to suggest that black rhinoceroses be kept from exposure to these wood preservatives in the future.

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