CHRONIC RECURRENT ANEMIA, MASSIVE PULMONARY AND SYSTEMIC MINERALIZATION, CHRONIC INTERSTITIAL NEPHRITIS AND MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS, AND HEMOSIDEROSIS WITH MYELOPHTHISIS IN A EUTHANATIZED BLACK RHINOCEROS

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Clinical presentation

On October 27, 1991 a 15 year old, male, black rhinoceros (*Diceros bicornis michaeli*) was found with approximately one third of its tail denuded of skin and hemorrhaging. No other animas shared the enclosure. The tail was observed for the next few days and apparently normal healing began. The animal, however, was reported to be somewhat lethargic. On November 1, 1991 the animal had developed multiple, 2 cm., raised areas within the dermis of subcutis, over the scapular regions. The areas were firm, with the overlying dermis able to slide easily over the subcutaneous lesions. The skin lesions were not warmer than the surrounding tissue and no erosion of epithelium was present. Blood was collected two days later with results presented in Table 1. Normal values for the Brookfield Zoo Clinical Pathology Laboratory are given in Table 4.

Due to the well documented black rhinoceros anemia syndrome and associated skin disease, a blood monitoring program was initiated to monitor the weekly progress of the animal (Tables 1-3). The animal had a history of becoming lethargic and/or lame each winter since the mid 1980's. Previous blood work had not been consistently performed. By February 27, 1992 the animal no longer showed signs of lethargy and skin lesions had recovered to minor scarred areas. The hematocrit had maintained a level of 31% (RBC > 3.0×10^6) for almost one month and there were no overt signs of illness.

For the next year and a half, the animal would have periods of normal activity alternating with bouts of lethargy. Periods of normal activity correlated directly with an increase in the hematocrit (RBC numbers). Episodes of apparent lameness which correlated with a low hematocrit were also observed. The typical hematocrit during bouts of anemia was a normocytic, normochromic, non-regenerative anemia. On only a few occasions were reticulocytes, basophilic stippling, or an increase in the CV seen. The leukogram remained within normal limits. As time passed however, there were indications of renal disease with increasing BUN and creatinine, along with shifts in the Ca:P ration (Tables 1-3). Multiple urinalyses showed no urinary blood loss, and direct fecal examination and fecal occult blood tests were uniformly negative.

With the onset of winter in 1992, the animal was lethargic and anorectic. On September 28, 1992, weekly blood analysis was again initiated (Table 2). Anemia had recurred, with a hematocrit of 27% (RBC = 2.89×10^6). Within one week, skin lesions reappeared. They consisted of hemorrhagic, open, moist, 1-2 cm erosions present on bony prominences such as the tuber coxae, trochanter major, carpus, and elbow. Cultures of open wounds produced variable growth of bacteria such as *Aeromonas hydrophila*. Leptospirosis titers revealed a titer of 1:200 for *Leptospira bratislava* and negative titers for *canicola, grippotyphosa, hardjo, pomona,* and *icterohaemorrhagiae* (Table 5). Due to the positive titer from December 8, 1992 a titer from previous stored serum was evaluated and found to be 1:400 for *Leptospira bratislava* on June 05, 1992 (Table 5). The animal was started on tetracycline, 5 grams, po,

q12h, along with supplementation of phosphorus (Wayne P-15) at 50 mg/kg of alfalfa fed. The dietary change resulted in a CA:P ratio of 1.23:1.0 whereas the standard diet is a ration of 3.73:1.0.

The rhinoceros' attitude continued to deteriorate and the skin condition worsened. Severe lameness developed in the right forelimb. A distended area on the caudal aspect of the right carpus appeared along with a 4/5 lameness. This swelling was sterilely tapped using a 14 gauge needle which yielded approximately 100 ml of a thick and clear, serosanguinous fluid. The fluid had few RBC's, rare lymphocytes, and scattered bacteria. The hematocrit continued to decrease with the initial hematocrit of 27% having dropped to 19% by March 23, 1993. Again, numerous attempts were made to determine the source of the blood loss by performing multiple urinalyses, occult blood tests on feces and direct examination of fecal material. The results were negative as with the previous bout of anemia the prior year.

Four, 6 mm, punch biopsies of the dermal swellings over scapular areas were obtained using local anesthesia with 20% lidocaine injected subcuticularly. Deep and superficial biopsies from each site of the raised skin plaques were obtained. The biopsies revealed that the deep dermis and subcutis contained regionally extensive areas of necrosis with collagen hyalinization surrounded by zones of mineralized cartilage. These lesions were not typical "ulcerative rhinoceros skin disease" but resembled equine nodular collagenolytic granuloma ("nodular necrobiosis").

On March 24, 1993 antifungal therapy was prophylactically initiated due to the history of fungal infections in compromised black rhinoceros'. Itraconizole was given, 1.5 gm, po, bid, for the duration of the tetracycline therapy. The animal's condition continued to deteriorate and by May 17, he could no longer walk more than a few feet without resting or collapsing. The hematocrit had dropped to 12% (RBC = 1.21×10^6). Due to the poor quality of life, unfavorable prognosis and prolonged course of the disease, euthanasia was performed on May 18, 1884.

Necropsy Results

Gross examination revealed a moderately severely emaciated animal, with healing, cutaneous, ulcerations over pressure point areas. Two, large, subcutaneous, fibrous encapsulated masses were present on the elbow which internally contained gritty, caseous material. The lungs had thickened septa, numerous, 1-2 cm and occasionally larger, cavitations, and the lungs were gritty on section. Cytological smears of a lung cavitation revealed numerous fungal hyphae and spores. Numerous, large vessels and the heart valves had multiple, firm, gritty, intimal to medial plaques, and the cardiac vasculature was enlarged and tortuous. The kidneys had numerous, usually military but up to 1 cm, cyst, and poor demarcation of corticomedullary junctions. There was generalized lymph node enlargement, and cystic thyroid glands. Mild gastric ulcerations were noted, and femoral, humeral, and ischial bone marrow was dark brown and gelatinous.

Histologically, the lungs had multifocal, moderate sized to massive areas of alveolar septal calcification, bronchiolar and alveolar duct, mucosal to sometimes mural, mineralization, and associated with scant to moderate granulomatous infiltrate. Numerous cavitations were also present which centrally contained abundant necrotic debris and degenerate inflammatory cells, mineralized debris, and outer fibrotic and calcified walls. One large area of acute necrosis of parenchyma, with calcification of large amounts of necrotic tissue, abundant deposition of fibrin, and severe infiltrate of neutrophils, macrophages, giant cells and lymphocytes was also present. Interlobular septa and pleura were moderately to markedly fibrotic. The glandular, gastric, mucosal had moderate to severe, multifocal calcification, and large regions of muscularis were effaced by irregular, disorganized, hypercellular, cavitated, and often calcified collagen. Vasculature throughout most organs often exhibited intimal to medial calcification. Heart valves were similarly mineralized, as were adjacent myocytes, and surrounding myocardium had moderate, interstitial fibrosis. The subcutaneous masses consisted of miltilobular, fibrous-encapsulated,

necrotic and mineralized debris, with scant granulomatous infiltrate. Moderate to moderately severe, random, and often occlusive calcification of renal tubules was present, and the kidneys also exhibited moderately severe, fibrosing, granulomatous interstitial nephritis with moderate obliteration of parenchyma. Remaining glomeruli exhibited marked enlargement and increase in cellularity and mesangium, and also often were sclerotic or had adhesions to thickened Bowman's capsules. The hematopoietic component of the bone marrow was nearly obliterated by dense sheets of hemosiderin-laden macrophages. Remaining hematopoietic cells consisted primarily of progenitor and maturing erythroid cells, although myelopoiesis and thrombopoiesis was also present. There also was moderate fibrosis between the abundant, bony trabeculae. Moderate to large numbers of macrophages distended with hemosiderin were also present in the lamina propria of the small intestine and colon, the liver, adrenal glands, lymph nodes, and spleen. Also, lymph nodes were usually moderately reactive, there was moderate, diffuse, hyperplasia of adrenal cortical zonas fasiculata and reticularis, moderate, granulomatous hepatitis with biliary hyperplasia and centrilobular, intrahepatocellular, biliary stasis, cystic hyperplasia of the thyroid glands with a focal area of dysplasia, and moderate lipofuscin deposition in brain stem neurons. *Aspergillus* sp. was cultured from one of the pulmonary cavitations, and was considered to be an opportunistic invader.

Interpretations

The most profound histologic lesions were the massive pulmonary and systemic vascular mineralization, the obliteration of bone marrow hematopoietic cells by hemosiderin-laden macrophages and fibrosis, the iron storage in various organs, and the interstitial nephritis and membranoproliferative glomerulonephritis. The severe and recurrent anemia probably led to the iron deposition in multiple organs and bone marrow. The hematopoietic cells exhibited an erythroid "shift", but if the sections of marrow examined were representative, there was inadequate tissue remaining to respond to the high demand. Erythropoietin production by the kidney was also probably moderately to markedly reduced. Additionally, an iron storage disease or primary bone marrow abnormality cannot be entirely ruled out, but is less likely. The cause of the tissue mineralization is also speculative. However, as the BUN was chronically elevated, and there were severe, chronic renal lesions, the most likely cause was uremia-induced tissue damage with resultant dystrophic calcification. The cause of the chronic interstitial nephritis and membranoproliferative glomerulonephritis also was speculative, as is usually the case even in well-characterized domestic species. The positive titer to *Leptospira bratislava* suggests the possibility of primary renal leptospirosis, but the chronicity of the lesions obscured the primary pathogenesis. The adrenal gland hyperplasia was secondary to chronic disease, and other lesions were either secondary or clinically insignificant. In summary, the tissue calcification, iron storage, interstitial nephritis and membranoproliferative glomerulonephritis, and hemosiderosis with myelophthisis were the most significant changes. Further, the marrow changes, anemia, and iron storage were likely all related, and the mineralization was probably directly related to renal lesions with resultant uremia-induced tissue damage.

There are numerous hypotheses to explain the recurrent anemia syndrome of captive black rhinoceros' including erythrocyte ATP deficiency and acatalasemia, nutritional deficiencies, exposure to toxins, hemoglobinopathies, immune mediated conditions, and leptospirosis. Failure to consistently demonstrate any one of these changes in affected animals suggests a complex, multifactorial pathogenesis, or a yet undefined mechanism. Additionally, there is a lack of thorough ante- and postmortem descriptions of individuals succumbing to this condition. The complex and intriguing findings in this case underline the need for thorough examination of all organ systems in black rhinoceros' that die or are euthanized due to profound, recurrent anemia, and the need for in-depth, scientific investigation of this syndrome. Further, investigation as to the cause of death of free-ranging black rhinoceros', and the characterization of typical geriatric lesions in wild animals would also probably elucidate upon the pathogenesis of this important condition.

Table 1. Selected blood values*, 1991.

	11/3	11/7	11/11	11/23	11/30	12/8	12/14	12/26
WBC	9.0	8.6	8.2	8.8	8.2	9.0	8.5	9.5
RBC	2.83	2.98	3.05	2.79	2.58	2.76	3.15	2.87
HCT	27.0	25.0	26.0	26.0	26.0	24.0	30.0	26.0
MCV	95	84	85	93	100	87	95	90
MCHC	34	39	38	35	32	37	37	35
36BUN	12	10	13	17	30	13	14	14
CRT	1.3	0.8	1.2	1.4	1.2	1.3	0.5	1.3

*WBC = white blood cells x 10^3/ul, RBC=red blood cells x 10^6/ul, HCT – hematocrit in volume percentage, MCV=mean corpuscular volume in femtoliters, MCHC=mean corpuscular hemoglobin concentration in g/dl, BUN=blood urea nitrogen in mg/dl, CRT=serum creatinine in mg/dl, CA=serum calcium in mg/dl, PHOS= serum phosphorus in mg/dl.

Table 2. Selected blood values*, 1992.

	1/10	1/28	2/6	2/13	2/26	6/5	9/28	10/9
WBC	9.1	8.7	9.3	9.7	9.5	2.2	9.0	9.0
RBC	2.86	2.92	3.33	3.08	3.11	3.43	2.89	2.56
HCT	27.0	29.0	31.0	31.0	31.0	34.0	27.0	25.0
MCV	94	99	93	100	100	99	93	98
MCHC	37	36	37	35	35	34	39	40
BUN	15	17	16	16	17	19		11
CRT	1.2	13	1.3	1.3	1.5	1.6		1.0
CA								8.7
PHOS								3.8

Table 2 (Continued). Selected blood values*, 1992.

	11/17	12/8	12/27
WBC	5.7	9.1	15.5
RBC	2.58	2.75	2.82
HCT	25.0	25.0	26.0
MCV	97	90	92
MCHC	34	37	37
BUN	15	14	16
CRT	0.9	1.5	1.6
CA		12.3	12.1
PHOS		6.2	5.3

Table 3. Selected blood values*, 1993.

	1/11	2/4	3/8	3/21	3/23	4/5	4/15	5/3
WBC	3.1	8.5	13.1	8.4	8.1	7.4	5.7	6.9
RBC	2.90	2.51	2.37	2.34	2.04	1.88	1.63	1.50
HCT	27.0	22.0	22.0	18.0	19.0	18.0	17.0	13.0
MCV	93	88	92	77	93	96	104	87
MCHC	37	40	44	44	38	38	34	41
BUN	20	14	26	27	28	31	25	27
CRT	1.8	1.8	1.5	2.4	2.4	2.1	1.9	1.7
CA		12.6	11.7		11.4	8.9	11.6	14.2
PHOS		6.6	6.9		7.5	6.5	9.1	4.0

Table 4. Normal blood values* for the black rhinoceros.

	Mean	S.D.	Minimum	Maximum
WBC	8.677	2.836	6.200	15.50
RBC	4.735	0.979	2.510	6.130
HCT	37.75	7.85	22.00	55.00
MCV	80.96	11.97	46.32	95.51
MCHC	36.75	1.90	33.64	40.00
BUN	10	2.78	7.000	15.00
CRT	1.073	0.042	1.040	1.120
CA	12.80	0.42	12.37	13.60
PHOS	4.500	0.923	3.700	6.600

Table 5. Results of Leptospirosis titers, State of Illinois Department of Agriculture Laboratory.

	2/26/92	6/5/92	12/8/92
L. canicola	negative	negative	negative
L. grio	negative	negative	negative
L. ictero.	negative	negative	negative
L. hardjo	negative	negative	negative
L. pomona	negative	negative	negative
L. bratislava	positive @ 1:200	positive @ 1:400	positive @ 1:200