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ENCEPHALOMALACIA IN THREE BLACK RHINOCEROSES (*DICEROS BICORNIS*)

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Abstract: Fatal encephalomalacia occurred in three female black rhinoceroses (*Diceros bicornis*) from three different zoos over a 9-yr period. Rhinoceroses affected included two calves (2 and 6 mo old) from the Denver and St. Louis Zoological Parks and a 2-yr-old from the Kansas City Zoo. Evaluation of the three rhinoceroses for known causes of encephalomalacia in domestic hoofstock failed to provide a definitive diagnosis.

Key words: Encephalomalacia, black rhinoceros, *Diceros bicornis.*

INTRODUCTION

Encephalomalacia describes softening of the brain that results from central nervous system (CNS) tissue necrosis.[7] Polioencephalomalacia results when gray matter necrosis predominates, and leucoencephalomalacia results when the affected tissue is white (myelinized) matter. Similar patterns of encephalomalacia may result from numerous insults to the CNS. Possible etiologies of encephalomalacia include trauma, encephalitis, clostridial enterotoxemia, metabolic diseases, and some neurological toxins.

A fatal encephalomalacia in which leucoencephalomalacia predominated is described in three black rhinoceroses housed at separate zoological institutions. Attempts to identify an etiology in these cases were unsuccessful.

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CASE REPORTS

The first case (#1) of encephalomalacia occurred in December 1979 in a 2-mo-old, zoo-born, female, dam-raised black rhinoceros calf at the Denver Zoological Gardens. The keepers noted acute onset of lethargy and depression and 18 hr later found the animal recumbent and comatose. Rectal temperature was below 34°C; despite supportive therapy with fluids, corticosteroids, and antibiotics, the animal died 3 hr later.

The second case of encephalomalacia (#2) occurred in April 1986 at the St. Louis Zoological Park in a 6-mo-old female calf that was zoo-born, dam-raised, and in the process of weaning herself. Keepers reported that she was moderately depressed late one afternoon after being outside in a yard in which the temperature was approximately 33°C. The calf was given 10 ml of a sulfadiazine–trimethoprim antibiotic (Tribrissen 48%, Coopers Animal Health, Kansas City, Missouri 64141, USA) i.m. and was sprayed with cold water in case of hyperthermia. Her condition remained the same after 6 hr, but 14 hr later, she was found recumbent and comatose. Her pupils were dilated and unresponsive to light, and rectal temperature was 41°C. Administration of i.v. fluids, corticosteroids, and antibiotics resulted in no improvement in her mental status, but her rectal temperature dropped

to 38°C. The calf died 24 hr after the initial onset of signs.

The third case (#3) occurred in January 1988 at the Kansas City Zoological Gardens where a 2-yr-old zoo-born female black rhinoceros had been transferred 8 days before developing severe neurological signs. She was given 100 mg haloperidol p.o. (Haldol, McNeil Pharmaceutical Co., Spring House, Pennsylvania 19477, USA) 72 and 48 hr prior to her arrival at Kansas City, which produced satisfactory tranquilization prior to shipment. The rhinoceros was loaded into a trailer after receiving 0.6 mg etorphine (M99, Lemmon Co., Sellersville, Pennsylvania 18960, USA) i.m. After 30 min, the effects of the etorphine were reversed with 0.6 mg diprenorphine (M50-50, Lemmon Co., Sellersville, Pennsylvania 18960, USA) i.m. Four days later (2 days after arrival in Kansas City), the rhinoceros showed a decreased appetite and mild depression, but she recovered and appeared normal over the next 2 days. However, on day 8 after her arrival, she was found in a highly agitated state with epistaxis from charging the stall walls. She was hyperresponsive to different visual and auditory stimuli and at times appeared to respond to stimuli that were not evident to those in attendance. The rhinoceros was given 20 mg of acepromazine (Acepromazine Maleate Injection, TechAmerica, Inc., Ellwood, Kansas 66024, USA) i.m. with no detectable effect. Three hours later, 280 mg azaperone (Stresnil, Pitman-Moore, Inc., Washington Crossing, New Jersey 62525, USA) was administered i.m., and within 1 hr the animal was sternally recumbent, but could still rise and charge the stall walls if provoked by even a slight stimulus. The following morning the animal was comatose. Treatment with antibiotics, corticosteroids, flunixin meglumine (Banamine, Schering Corp., Kenilworth, New Jersey 07033, USA), and 408 IU vitamin E and 13.14 mg sodium selenite via an injectable supplement (Bo-Se, Burns-Biotech Laboratories, Inc., Omaha, Nebraska 68127, USA) resulted in no improvement within 48 hr, and the rhinoceros was euthanized.

Serologic, toxicologic, and gross and histopathologic findings are presented below.

RESULTS

Hematology and serology

Results of the antemortem hemograms and serum chemistries are presented in Tables 1 and 2. In rhinoceros #3, blood was obtained on the first day of coma and 48 hr later immediately preceding euthanasia. Neutrophilia was noted in rhinoceroses #2 and #3, and there was an increase in band neutrophils in rhinoceros #1. All animals tested exhibited elevations of CPK, AST, and LDH. Rhinoceroses #1 and #2 were hyperglycemic; this was accompanied by glucosuria in rhinoceros #2. Rhinoceros #1 also had moderate elevations of BUN and creatinine values.

In rhinoceros #2, antemortem attempts at a lumbar spinal tap were unsuccessful, but CSF from a cisternal tap was obtained within 5 min of death. Blood contamination (30,000 RBC/ml) of the CSF was extensive enough to complicate interpretation of the sample results. However, the elevation of CSF total protein (285 g/dl) was considered significant.[11] There were only 30 WBC/μl (10% lymphocytes, 80% segmented cells, and 10% other cells). The results of aerobic, anaerobic, and viral cultures were considered noncontributory (Table 3). Other diagnostic tests showed no evidence of mycotoxin (rhinoceros #1, feed samples collected after the death of the calf), lead, copper, or arsenic poisoning (liver samples from #2 and #3), clostridial toxins (stomach contents from #2), rabies (brain and skin from #3), or hypomagnesemia (sera from #3). Serum alpha-tocopherol levels were 0.8 μg/ml in rhinoceros #2 and 0.04 μg/ml in rhinoceros #3. In the latter animal, sera levels increased to 0.9 μg/ml 48 hr after receiving the injectable alpha-tocopherol supplement. In rhinoceros #3, haloperidol levels assayed in serum collected 12 days after the administration

Table 1. Hemograms of three captive black rhinoceroses with encephalomalacia. Normal values are presented for comparison.

Parameter	Rhinoceros #1 (Denver) 12/26/79	Rhinoceros #2 (St. Louis) 4/27/86	Rhinoceros #3 (Kansas City) 1/25/88	Rhinoceros #3 (Kansas City) 1/27/88	Normal values[a]
WBC (μl)	5,500	21,400	10,800	6,900	9,700
Differential					
Segmented neutrophils (%)	53 (2,915)[b]	78 (16,692)	84 (9,072)	48 (3,312)	66 (6,400)
Band neutrophils (%)	14 (770)	1 (214)	11 (1,188)	31 (2,139)	3 (300)
Lymphocytes (%)	22 (1,210)	20 (4,280)	2 (216)	6 (44)	24 (2,300)
Monocytes (%)	4 (220)	1 (214)	3 (324)	14.5 (1,000)	5 (524)
Metamyelocytes (%)	7 (385)	—	—	—	—
RBC (10^6/ml)	—	8.2	6.21	6.19	5.2
PCV (%)	33	49.9	49	52	47.9
Hemoglobin (g/dl)	11.9	—	16.8	17.5	16.6

[a] See reference 6.
[b] Numbers in parentheses represent absolute values.

of the drug were <2.5 μg/ml, below the detection limit of the assay.

Pathology

Significant necropsy findings in rhinoceros #1 included cloudy meninges and hemorrhage in the cerebral sulci. The terminal colon contained watery green feces.

In rhinoceros #2, the external surface of the brain contained numerous pale yellow-green foci located primarily in the frontal lobes. Both cerebral hemispheres were dif-

Table 2. Clinical chemistry values from three black rhinoceroses with encephalomalacia. Normal values are presented for comparison.

Parameter	Rhinoceros #1 (Denver)	Rhinoceros #2 (St. Louis)	Rhinoceros #3 (Kansas City) 1/25/88	Rhinoceros #3 (Kansas City) 1/27/88	Normal values[a]
BUN (mg/dl)	>60	20	17	28	14.3
Creatinine (mg/dl)	7.2	1.9	1.1	0.7	1.3
Glucose (mg/dl)	234	380	126	127	61
Total protein (g/dl)	6.1		8.9	9.7	9
Globulin (g/dl)	2.9		5.8	6.8	5.8
Potassium (mEq/L)	2.6	5.5	4.0	4.2	4.9
Sodium (mEq/L)	126	128	134	137	137
Chloride (mEq/L)		94	102	105	96.2
Calcium (mg/dl)	11.8	18.1	10.4	10.1	12.3
Magnesium (mEq/L)				2.3	
Phosphorus (mg/dl)	>10		4.8	6.4	3.9
Uric acid (mg/dl)	7.1		1.3		0.9
Cholesterol (mg/dl)	140		57	47	76.7
Total bilirubin (mg/dl)	0.8		0.3	0.3	0.6
Alkaline phosphatase (IU/L)	>70		87		88.5
AST (SGOT) (IU/L)	>225		1,560	3,535	132
ALT (SGPT) (IU/L)	78	15	191	118	8.7
GGT (IU/L)			17	66	
CPK (IU/L)	>1,000	5,620	128,560		
LDH (IU/L)	>350		2,660		384

[a] See reference 6.

Table 3. Central nervous system culture results from three captive black rhinoceroses with encephalomalacia.

Test	Rhinoceros #1 (Denver)	Rhinoceros #2 (St. Louis)	Rhinoceros #3 (Kansas City)
Aerobic bacteria	Meninges, brain stem: light growth *Escherichia coli* (nonhemolytic)	CSF[a]: rare staphylococci and streptococci	Brain: *Streptococcus* (beta-hemolytic) and occasional *Pseudomonas* spp. and *Escherichia coli* (nonhemolytic)
Anaerobic bacteria	ND[b]	CSF: neg.	Brain: *Bacteroides melaninogenicus*
Cryptococcus	ND	CSF: neg.	ND
Viral cultures for malignant catarrhal fever and equine herpesvirus-1	ND	CSF: neg.	Brain: neg.
Viral culture in bovine embryonic lung, fetal aoudad kidney, bovine turbinate, equine skin fibroblast, and black rhinoceros skin fibroblast cell cultures	ND	CSF[c]: no cytotoxic or cytopathic effects observed after 8 weekly passages	Brain: no cytotoxic or cytopathic effects observed after 8 weekly passages

[a] Cerebrospinal fluid.
[b] Not done.
[c] Tests performed on sample stored at room temperature for 72 hr.

ficult to section transversely because they were extremely soft, especially in the regions of the internal capsule and corona radiata, which had a gray to brown discoloration. Similar softening and discoloration were also present in the diencephalon and midbrain. There was also marked edema of the stomach wall and numerous nonhemorrhagic punctate ulcers in the mucosa of the squamous portion of the nonglandular stomach. The stomach contents had a yeastlike odor. There were mucosal hemorrhages at the tips of the jejunal folds.

Gross lesions from rhinoceros #3 included subdural hemorrhage with approximately 30 ml of blood in the subdural space. Hemorrhage was also noted in the nasal sinuses, with bruising of the overlying subcutaneous tissues. Punctate ulcers were present in the glandular portion of the stomach.

Microscopic findings in the brains of the three rhinoceroses were remarkably similar with primary involvement of the cerebrum and lesser involvement of the diencephalon and midbrain. No significant changes were observed in the pons, medulla, or cerebellum of any animal. Cervical segments of the spinal cord and sciatic nerve from rhinoceros #1 were normal; however, in the brain there was cavitation in the centrum semiovale and corona radiata with involvement of both gray and white matter (Fig. 1). In all three rhinoceroses, the white matter showed cavitation and a widely separated pale-staining neuropil attributed to edema and sometimes hemorrhage. In some areas, only spongiform change was evident, but in others, the parenchyma was heavily infiltrated with neutrophils, accompanied by a focal necrotizing vasculitis and scattered hemorrhages. The vascular changes appeared to be secondary to the extensive surrounding parenchymal necrosis. Segments of the internal capsule had extensive vacuolation, resulting in a spongiform appearance. In some areas, gray matter was involved; however, these changes appeared to

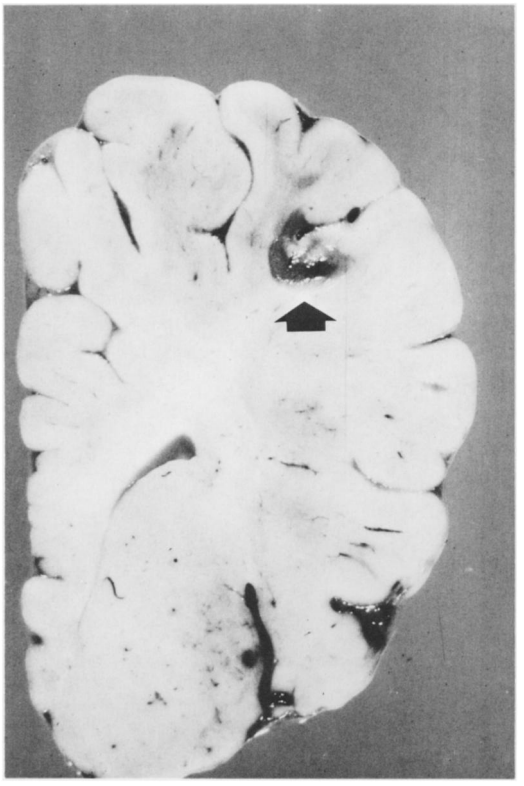

Figure 1. Transverse section of the prosencephalon from a 2-mo-old captive black rhinoceros (#1) with encephalomalacia. Note discolored corona radiata of gyri with cavitation of cerebral cortex and adjacent white matter at bottom of dorsolateral sulcus (arrow).

be secondary to the lesions in the subcortical white matter (Figs. 1, 2).

The cerebrocortical lesions in the three rhinoceroses were most severe in the middle and deep laminae and in some areas consisted of spongiform change with large empty perineural and perivascular spaces assumed to represent swollen glial processes. Affected neurons were shrunken, with an occasional pyknotic nucleus and eosinophilic cytoplasm evident. Some of the cortical lesions were packed with neutrophils and occasionally contained numerous proliferating blood vessels with hyperplastic endothelial cells often associated with neurons that appeared ischemic. Usually there was sparing of the outer molecular layer. Macrophages and mineralization were rarely a part of the cortical lesion proper but occurred in the lentiform nucleus accompanied by severe necrosis and hemorrhage. Although a few vessels in necrotic cortical areas were degenerate, primary vasculitis or thrombosis was not evident. Gram stains (Brown and Bren) for bacteria and methenamine silver (Gomori) for fungi were negative. In all three cases, the lesions were categorized as extensive acute encephalomalacia with leucoencephalomalacia predominating.

Histologic examination of the gastrointestinal tracts showed mild epithelial necrosis of the tips of intestinal villi in rhinoceros #1 and noninflammatory erosions of the gastric mucosa in rhinoceroses #2 and #3. In rhinoceros #3, superficial intestinal fibrosis was noted in some of the gastric ulcers, which may have represented healing attempts. Extensive transmural edema was noted in the stomach wall of rhinoceros #2. Additional notable histologic changes in rhinoceros #1 included granular degeneration of skeletal muscle and moderate renal tubular nephrosis.

DISCUSSION

Although the rhinoceroses were at different zoos when they died with encephalomalacia, the clinical and pathologic similarities are suggestive of a common etiology. The encephalomalacic lesions in the three animals varied only with the increased presence of neutrophils and vascular necrosis in rhinoceroses #2 and #3. This probably represented an inflammatory response to a more advanced stage of the same lesion as in rhinoceros #1. Although blood contamination of the CSF sample from rhinoceros #2 was considered to be iatrogenic, the significant protein elevation noted probably resulted from the necrosis and vascular leakage into the CNS.

Although blood evaluations were generally nondiagnostic, all cases exhibited elevated values for CPK and muscle-related enzymes. Histologic muscle changes were evident only in rhinoceros #1, but the ele-

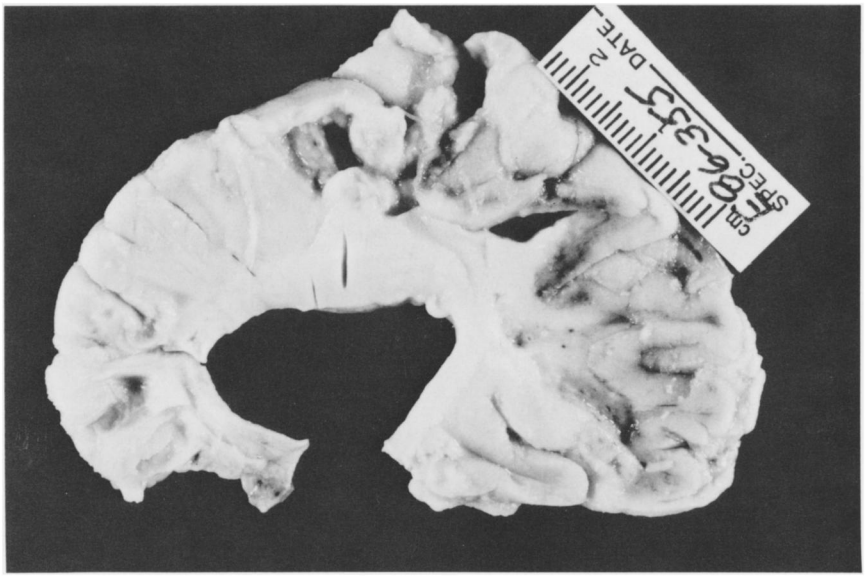

Figure 2. Transverse section of cerebrum of a 6-mo-old captive black rhinoceros (#2) with encephalomalacia. Note extensive necrosis of white matter and adjacent cerebral cortex with cavitation.

vated values in rhinoceroses #1 and #2 may have resulted from lateral recumbency, and in rhinoceros #3, from the marked degree of self-trauma. Rhinoceroses #1 and #2 exhibited elevated serum glucose, also reflected in the glucosuria of #2. Though these values could reflect the hyperglycemia of stress or possibly a response to endotoxic shock, a definitive cause for the elevations remains uncertain.

The origin of the hyperexcitable state in rhinoceros #3 remains unknown. Although that animal had received haloperidol 9 days prior to the onset of signs and the drug may cause extrapyramidal side effects in humans and some hoofstock species,[5] serum haloperidol levels were below the detection limits of the assay, and structural CNS changes have not been noted with the use of this drug (E. Piperno, pers. comm.).[1,12,14]

The histologic pattern of encephalomalacia was most similar to leucoencephalomalacia in horses that ingest food contaminated with the mold *Fusarium moniliforme*.[3,10,15] A sample of rhinoceros feed from Denver contained no evidence of mold toxin, but the sample was collected after the death of the animal. Thus, an exposure preceding death could have been missed in the sampling interval. Also with regard to rhinoceros #1, the possibility that a mold toxin could have been passed through the milk remains uncertain; however, the similarities of the pattern and extent of the white matter lesions in these three animals to mold-induced encephalomalacia of horses keeps this differential as a leading possibility.

Vitamin E deficiency has been associated with degenerative spinal cord lesions in domestic and wild equids,[8–10] and vitamin E levels in captive black rhinoceroses have been shown to be 4–5-fold lower than those of a wild Zimbabwe population.[2] However, the serum level of rhinoceros #2 was one of the highest levels measured in the captive population. In addition, the CNS lesions of vitamin E deficiency in mammals are not characterized by necrosis, and in the horse, lesions have been limited to the spinal cord and brain stem.[9]

Polioencephalomalacia associated with thiamine deficiency as described in domestic ruminants[7] was also considered as a cause of the rhinoceros encephalomalacia but was

deemed unlikely because of the predominance of white matter necrosis in our cases as well as the absence of a similar syndrome in thiamine-deficient equines. Other noninfectious causes of encephalopathy in domestic ungulates were also deemed unlikely on the basis of histology, pattern of distribution, or direct assay, including heavy metal toxicosis, an intrinsic enzyme defect,[4] and salt toxicosis (water deprivation).[13]

Focal asymmetrical encephalomalacia, the lesion of edema disease in swine, and enterotoxemia in sheep were also considered, but the pattern of the edema and necrosis in these rhinoceroses differed from that in sheep.[7] Gut edema was noted in rhinoceros #2, but mouse inoculation studies with gut contents from that animal were negative for clostridial toxins.

Other possible infections causing CNS necrosis such as *Hemophilus somnus, Listeria monocytogenes,* malignant catarrhal fever, equine herpes I, or equine togaviruses were ruled out on the basis of the lack of a primary inflammatory response or other hallmarks such as inclusion bodies. Attempts at viral and aerobic cultures and cytotoxicity studies in rhinoceroses #2 and #3 also failed to provide evidence of these agents.

CONCLUSIONS

Three captive black rhinoceroses died of encephalomalacia. Though several known etiologies for leucoencephalomalacia and polioencephalomalacia in domestic equids and ruminants were investigated, none clearly described all of the clinical and pathologic findings in these cases. The exact etiology for the encephalomalacia remains unknown. The onset of the disease in all three cases was noted as mild to moderate depression with progression to coma. Coma was preceded by a period of hyperexcitability in rhinoceros #3. The clinical course in all three animals was rapid and fatal. These findings emphasize the importance of examining both brain and spinal cord in all future rhinoceros deaths.

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