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# LEUKOENCEPHALOMALACIA IN A NEONATAL FEMALE BLACK RHINOCEROS (*DICEROS BICORNIS*): REPORT OF A FOURTH CASE

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*Abstract:* Acute neurologic signs were observed in a 3-wk-old female black rhinoceros (*Diceros bicornis*) born at the Denver Zoological Gardens. The neonate's condition deteriorated after the first 24 hr. Following physical examination, the calf was separated from its mother and transferred to the zoo hospital for intensive care, additional diagnostics, and hand raising. Standard hematologic tests, serologic tests (including bile acids), urinalyses, and *Leptospira* titers failed to establish a diagnosis. There was no detectable serum vitamin E at the neonatal examination, but 3 wk later at the onset of illness the levels were normal. After consultation with a veterinary neurologist, the rhinoceros was transported to the Colorado State University Veterinary Teaching Hospital for diagnostic tests, including computed tomography of the brain, lumbar spinal taps, and portal venography. Hypodense areas compatible with edema, ischemia, necrosis, or malacia were seen in each cerebral hemisphere. Hemorrhage was seen in each caudate nucleus. No portosystemic shunt was observed. After hospitalization for 15 mo, the animal's condition deteriorated and it was euthanized. Gross necropsy and histopathologic evaluation confirmed a diagnosis of leukoencephalomalacia. This is the fourth female black rhinoceros to be diagnosed with this condition.

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

## INTRODUCTION

Fatal encephalomalacia has been reported previously in three female black rhinoceroses (*Diceros bicornis*).[11] The animals became comatose after 1–7 days of illness. Two subsequently died, and one was euthanized. Laboratory data were not helpful in making the diagnosis, and the etiology in these three cases was unknown. In this paper, we report a case at the Denver Zoological Gardens (DZG) of a female black rhinoceros that survived for 15 mo following onset of clinical signs.

## CASE REPORT

A 30.2 kg female black rhinoceros born at DZG received a neonatal examination on day 2 after birth. Blood samples were obtained for a hemogram and serum chemistries and to confirm nursing through immunoglobulin status. DZG routine hoofstock neonatal treatments were administered.

At 3 wk of age, shortly after nursing and playing with its mother, the calf began vocalizing continuously, circling to the left with a left-sided head tilt, and head pressing. It exhibited hypermetria and ptyalism with lip-smacking and apparent blindness. The 41.4 kg calf was separated from the dam and examined. There was no evidence of trauma or omphalitis. Blood was collected for a hemogram and serologic analysis.

Initial therapy consisted of an i.v. infusion of lactated Ringer's solution (LRS) and 5% dextrose (150 ml) and 1,500 mg cef-

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triaxone (Hoffmann-La Roche, Nutley, New Jersey 07110, USA). The calf also received $0.9 \times 10^6$ units i.m. penicillin G benzathine and penicillin G procaine (G. C. Hanfords Manufacturing Co., Syracuse, New York 13201, USA), 5 ml i.m. vitamin B complex (Multi-B-Super, Phoenix Pharmaceutical, St. Joseph, Missouri 64506-0457, USA: 12.5 mg/ml thiamine, 2 mg/ml riboflavin, 12.5 mg/ml niacinamide, 5 mg/ml d-panthenol, 5 mg/ml pyridoxine, 0.2 ppm cobalt), 260 IU i.m. D-α-tocopherol (TechAmerica Veterinary Products, Kansas City, Missouri 64190, USA), 1,500 units i.m. equine origin tetanus antitoxin, and 1 ml i.m. tetanus toxoid (Super-Tet® with Havlogen®, Mobay Corp., Shawnee, Kansas 66201, USA) in the lumbar muscles.

There was no improvement overnight. Additional blood samples were obtained. Fluid and antibiotic therapies were repeated. The rhinoceros ceased nursing, so it was given 800 ml of a mixture of 50% skim milk, 50% lowfat milk, 3,300 units lactase (Lactaid® caplets, Lactaid, Pleasantville, New Jersey 08232, USA) and 5 g Gerval® protein powder (Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York 10965, USA) via stomach tube and returned to its mother.

Late that afternoon, the calf became severely depressed, weaker, and hypokalemic (K = 2.6 mEq/L).[8] It was transferred to the zoo hospital for intensive care and hand raising.

Treatments consisted of penicillin G procaine ($0.9 \times 10^6$ IU i.m., s.i.d.), ceftriaxone (750 mg i.m., b.i.d.), a multiple vitamin tablet (One-a-day®, Geneva Pharmaceuticals, Broomfield, Colorado 80038, USA) (s.i.d.), DL-α-tocopherol acetate (400 IU p.o., s.i.d.), metoclopramide HCl (10 mg p.o., b.i.d.), sucralfate (1 g p.o., b.i.d.), and Zantac® (Glaxo Pharmaceuticals, Research Triangle Park, North Carolina 27709, USA) (300 mg p.o., b.i.d.). Antibiotics were administered for 7 days. The carafate and Zantac® were given for 6 days to prevent gastric ulceration. Metoclopramide was given three times to enhance gastric emptying and promote defecation, but this product may have contributed to depression and was discontinued. A solution of LRS (1,850 ml) and 5% dextrose with 44 mEq KCl was administered i.v. during the first 48 hr to correct hypokalemia.

A neurologic consultation 12 days following the initial episode revealed ataxia and postural placement deficits. A ventral strabismus was noted when the head and neck were extended. This strabismus is normal in large domestic animals but may be compatible with hydrocephalus in small animals.[10] The rhinoceros was transported twice (at 5 and 19 wk of age) to the Colorado State University Veterinary Teaching Hospital (CSU-VTH) for computed tomography (CT) scanning and cerebrospinal fluid (CSF) analysis. A liver biopsy and contrast portal venography (to evaluate for a portosystemic shunt) were also performed at 9 mo of age.

Fourteen aberrant neurologic episodes were observed during a 15-mo period following the initial episode, including ataxia, left-sided circling, left-sided head tilt, head pressing, apparent blindness, and standing in a trancelike state for hours. The rhinoceros never lost consciousness or fell, nor did it exhibit tonic-clonic limb activity of a generalized seizure.

Prior to its euthanasia and necropsy at 16 mo of age, the rhinoceros stopped feeding and became severely ataxic. It also became hyperexcited at any sudden movements and aggressive towards familiar staff and developed multifocal dermal ulceration involving the neck, back, and flanks.

**Laboratory findings**

The hemogram immediately following the initial neurologic episode showed a leukocytosis (WBC = 20,675/μl) with a mature neutrophilia (17,160/μl) and a monocytosis (1,241/μl), suggesting a chronic inflammatory process.[8] The hemogram was normal after 48 hr. The only serum chemistry abnormalities were several undetectable or low blood urea nitrogen values.[8]

Mean (±SD) blood ammonia level was 86.5 ± 19.7 μg/dl ($n$ = 6; range 61–111 μg/dl), which is similar to normal equine levels.[2] Mean bile acid level was 36.6 ± 23.1 μmol/L ($n$ = 7; range, 9.9–86.1 μmol/L). The mean from two normal DZG neonatal males rhinoceroses was 33.3 ± 10.1 μmol/L. Bile acids were not considered to be elevated. No urine or CSF abnormalities were found. Acute and convalescent titers (6 mo apart) were negative for five serovars of *Leptospira* (*L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomona*). Vitamin E was not detectable in serum during the neonatal examination but was 0.69 μg/ml 3 wk later at the onset of illness.

**Bacterial cultures**

Six weeks after the initial presentation, cultures of gastric contents aspirated via stomach tube showed *Salmonella enteritides* group B serotype Derby. The rhinoceros was treated with trimethoprim and sulfadiazine (Di-Trim®, Syntex Animal Health, West Des Moines, Iowa 50265, USA) initially i.m. and later p.o. at 30 mg/kg s.i.d. Subsequently, of 26 fecal cultures, 16 (62%) were positive for *S. enteritides.* The source of *Salmonella* was never identified.

**CT scans**

All CT scans were performed with a fourth generation scanner (Picker 600 SD, Picker International, Highland Heights, Ohio 44143, USA) while the animal was anesthetized with isoflurane (Aerrane®, Anaquest, Madison, Wisconsin 53713-2318, USA) administered first via mask and then via a 10-mm endotracheal tube.

The scans were made in the transverse plane using a 4-mm slice thickness with a 1-mm overlap. The first study was done at 5 wk of age, and the second was done at 19 wk of age and included contrast enhancement.

In the first study, two bilaterally symmetrical hypodense areas, approximately 15 mm thick, were present in the cerebrum and also in the thalamus (Fig. 1A). In addition, there were 8- × 6-mm bilaterally symmetrical hyperdense areas in the caudate nucleus (Fig. 1B). In the second study, the cerebral lesion was more pronounced, and the thalamic and caudate nucleus lesions were smaller and less dense. Dilation of the lateral and third ventricles occurred between the two studies. Hypodense areas on CT scans can be compatible with focal edema, ischemia, necrosis, or malacia. The lesions were too diffuse to be typical of inflammatory encephalitis. The hyperdense areas in the caudate nucleus were compatible with hemorrhage. The enlarging ventricles were probably secondary to the decreased size of each cerebral hemisphere produced by leukoencephalomalacia. Interpretation of the CT studies was difficult because of the unfamiliar cross-sectional anatomy of the normal black rhinoceros brain. The lesions in this case, unlike most brain lesions, were bilaterally symmetrical.

**Contrast portal venography and liver biopsy**

At 9 mo of age, the rhinoceros was transported to CSU-VTH for protal venography and liver biopsy. It was positioned in dorsal recumbency, and a 30-cm ventral midline incision was made through the skin and body wall. The surgeon was unable to elevate the jejunum through the incision for catheterization of a jejunal vein. Instead, the splenic vein was catheterized with an indwelling catheter for splenoportography.

The incision was closed completely around the catheter and the rhinoceros was transported to the radiology facility. Approximately 100 ml of tri-iodinated contrast material (Angiovist, Berlex laboratory, Wayne, New Jersey 07470, USA) was injected through the catheter over 5 sec. Nine radiographs were made at a rate of 1/sec. A normal portal vein and circulation pattern were identified on the study, thus the possibility of a portosystemic shunt was eliminated.

There was a mild diffuse hepatocellular swelling in a liver biopsy, which was interpreted as insignificant.

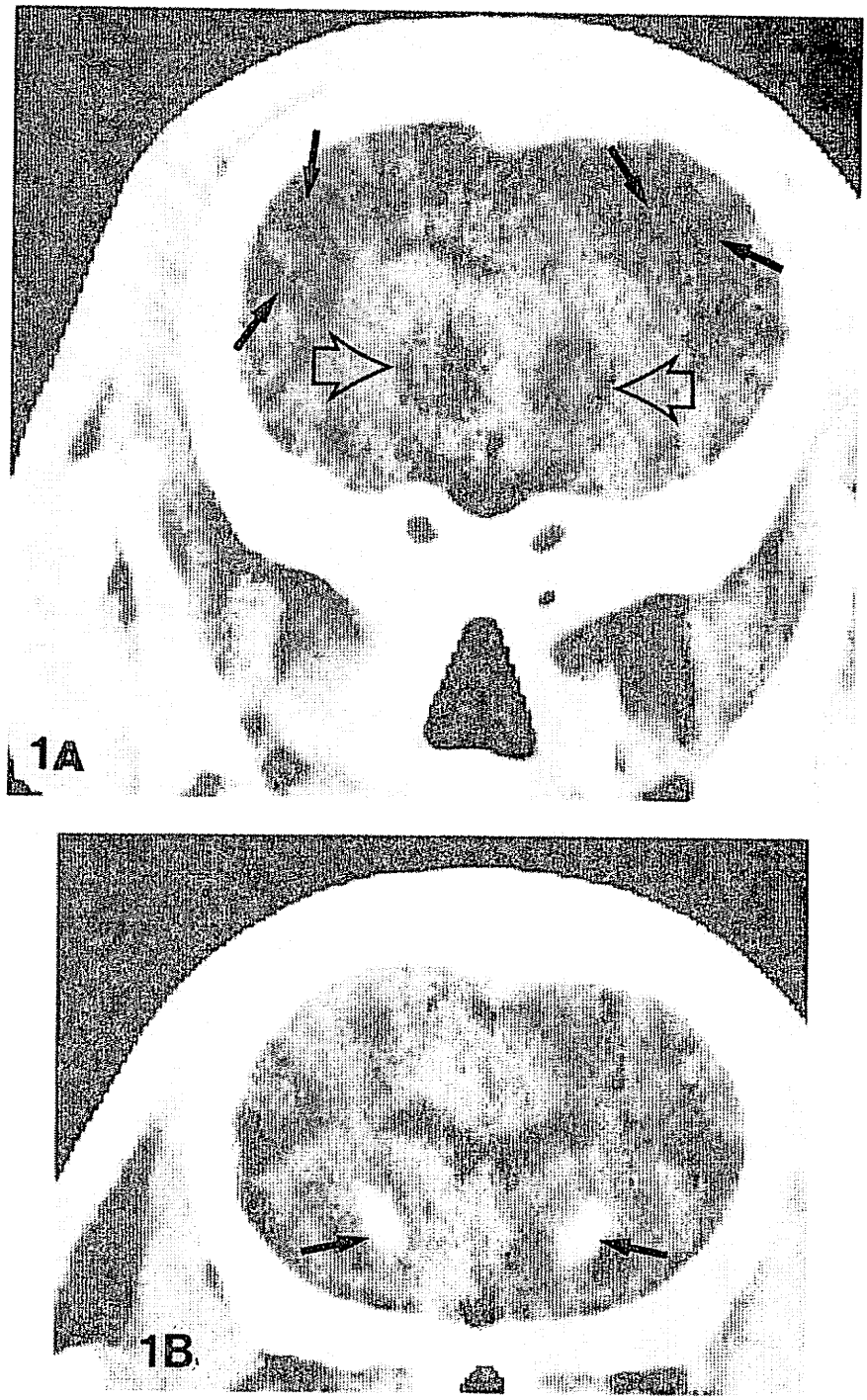


**Figure 1.** Transverse CT images of the brain of a 5-wk-old female black rhinoceros. **A.** Rostral thalamus level. Note 15-mm hypodense areas in the cerebral white matter (small closed arrows). Round hypodense areas (open arrows) are present in the thalamus. **B.** Caudate nuclei level. Symmetrical hyperdense areas (small closed arrows) are surrounded by a hypodense ring.

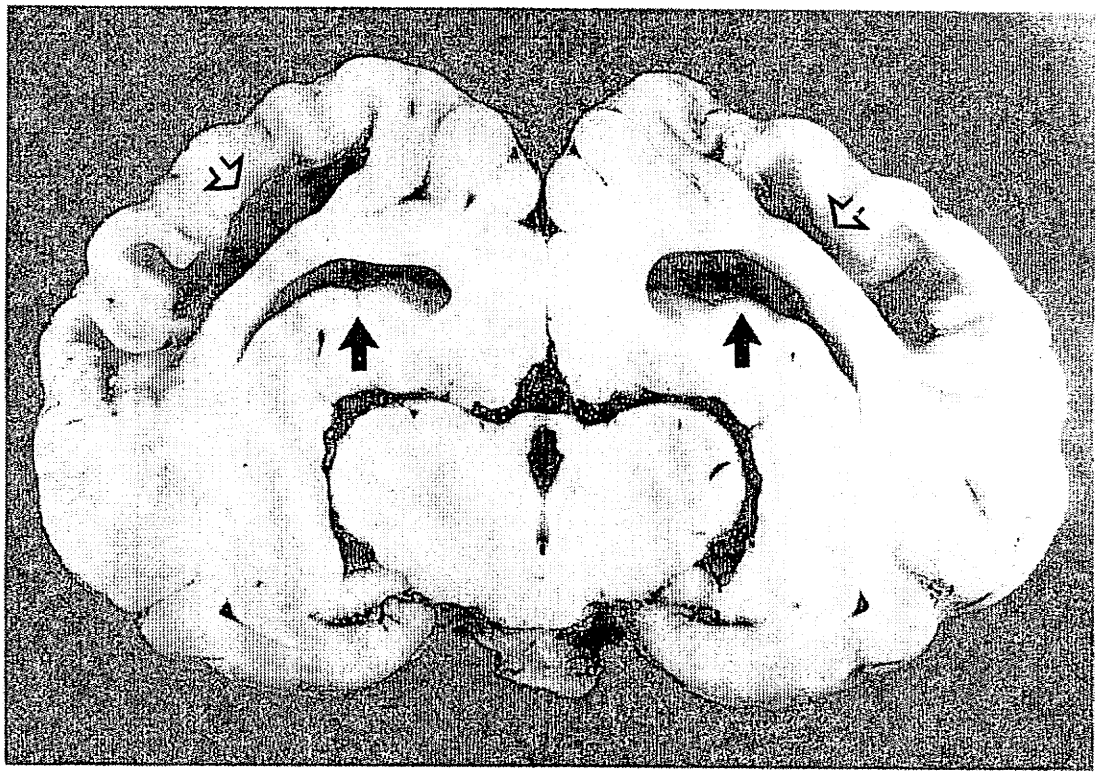

**Figure 2.** Midsagittal section of brain from a 16-mo-old female black rhinoceros with leukoencephalomalacia 463 days after the onset of clinical signs. Note the cavitations (open arrows) and the enlarged lateral ventricles (closed arrows). The cavitations correspond to the 15-mm bilaterally symmetrical hypodense areas in the CT scans from Figure 1A.

### Gross necropsy and histopathologic evaluation

At euthanasia, the rhinoceros was 16 mo old and weighed 207 kg. In addition to dermal ulcers, there were ulcers at the mucocutaneous junctions of the lips, in the nostrils, and in the esophageal portion of the stomach. Both cerebral hemispheres of the brain were grossly depressed on the surface. The brain floated when placed in 10% neutral buffered formalin. Cavitations in the brain were filled with fluid, presumably CSF (Fig. 2).

The brain had extensive cavitating necrosis of the white matter of each cerebral hemisphere, which was especially prominent in the centrum semiovale. This necrosis extended into numerous corona radiata and sometimes into the adjacent cerebral cortex. Occasionally at the bottom of a sulcus, the necrosis extended through the cortex to the leptomeninges. Rarely, a few laminae of normal neocortex were isolated between superficial and deep layers of necrotic cortex. Cavitating necrosis occurred in the lentiform nucleus and the adjacent internal and external capsules. Additionally, there were areas of cavitation of the thalamus, putamen, globus pallidus, pyriform lobe, and amygdala. Within the cavitations were blood vessels ranging from capillaries to the occasional large artery. On the borders of the zone of cerebral necrosis and in the lateral thalamus in an area unrelated to a cavitating lesion, the degeneration spared cell bodies and axons and appeared to be primarily demyelinating in nature.

Cellular reaction was minimal. There was some neuropile edema, with occasional lymphocytes and macrophages and numer-

ous reactive fibrous astrocytes. A few small foci of mineralization were present in the cerebral cortex. The CT scan lesions corresponded closely with the locations and severity of the lesions seen at necropsy. There was no evidence of viral etiology on sections submitted for electron microscopy.

## DISCUSSION

Leukoencephalomalacia has been reported in humans and domestic animals. In humans, cerebral palsy describes a group of chronic nonprogressive diseases present at birth. Major risk factors are abnormal pregnancy, premature birth, low birth weight, and prenatal or perinatal ischemia.[16] Periventricular leukomalacia in humans is most commonly associated with premature infants and affects the centrum semiovale and auditory and optic radiations.[7,13] Several leukodystrophies described in humans have a genetic etiology, and many are autosomal recessive.[14,15]

The etiology for leukoencephalomalacia has been reported in several domestic species, including mycotoxicosis from moldy corn or grain in the horse, nigropallidal encephalomalacia in the horse from yellow star thistle or Russian knapweed intoxication (limited to the gray matter), *Clostridium perfringens* type D enterotoxemia and congenital swayback in sheep and goats, and a nutritional encephalomalacia in poultry thought to be caused by vitamin E deficiency.[1,4,5,9,17,18]

During the last trimester of pregnancy, the dam of this rhinoceros had been knocked on her back into the exhibit moat by the sire and struggled violently for 20 min before righting herself. The possible effects of this episode on blood circulation to the fetal brain are unknown. The 15–16-mo gestation period was thought to be normal.[6] Parturition was not observed, but there was no evidence of dystocia. The birth weight of this neonate (30.2 kg) was low compared with the average of 36 kg for 10 North American calves.[12] The calf also had poor weight gain (0.71 kg/day over 7 days, compared with four DZG calves (1.21 ± 0.02 kg/day over 5–12 days). The significance of the low birth weight and poor weight gain are unknown.

The history of the affected calf closely parallels that of a 3-wk-old female black rhinoceros that was accidentally traumatized by its mother.[3] CT scanning of that calf revealed a small focal intracerebral hemorrhage that was not surgically treatable. The calf was euthanized when 2 yr old for poor weight gain, dysphagia, and mild proprioceptive deficits. Necropsy showed mildly dilated ventricles and necrosis and cavitation of the cerebral white matter. In that case, the clinical signs were directly associated with trauma and did not progress.

All known cases of leukoencephalomalacia have involved neonatal or juvenile females. The possible presence of an inherited genetic defect deserves serious consideration. The possible presence of an intrinsic metabolic abnormality should also be explored because both glia and myelin were involved. A lethal genetic trait would be of grave concern in such a small captive population. Frozen sections of brain and blood for karyotyping should be preserved in all future cases of leukoencephalomalacia to permit investigation of a possible genetic etiology.

## CONCLUSIONS

In retrospect, CT scanning was predictive of leukoencephalomalacia in this case. The hypodense areas seen on the CT scan corresponded to the extensive cavitations seen at necropsy. Our initial reluctance to accept a diagnosis solely from the CT scan stemmed from lack of familiarity with the normal CT anatomy of a rhinoceros of this age and the possibility that the symmetrical nature of the lesions might represent a normal pattern. Magnetic resonance imaging might be a more reliable modality for diagnosing leukoencephalomalacia because of greater soft tissue detail and contrast.

The etiology of leukoencephalomalacia in captive black rhinoceros remains un-

known. There is no treatment for this disease, and it has been universally fatal.

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