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1. Dessauer HC: Blood chemistry of reptiles: Physiological and evolutionary aspects, in Gans C and Parsons TS (ed): *Biology of the Reptilia*, vol 3. New York, Academic Press, 1970, pp 1–72.
2. Thompson SW: *Selected Histochemical and Histopathological Methods*. Springfield, Ill, Charles C Thomas, Publisher, 1966, pp 1048–1049.
3. American Type Culture Collection, Rockville, Md.
4. Camin JH: Mite transmission of a haemorrhagic septicaemia in snakes. *J Parasitol* 34:345–354, 1948.
5. Buchanan RE, Gibbons NE: *Bergey's Manual of Determinative Bacteriology*, ed 8. Baltimore, Williams and Wilkins Co, 1974, pp 167–195.
6. Willcox RR: The epidemiology of the spirochetoses: A worldwide view, in Johnson RC (ed): *The Biology of the Parasitic Spirochetes*. New York, Academic Press, 1976, pp 133–155.
7. Roughgarden JN: Antimicrobial therapy of rat bite fever: A review. *Arch Intern Med* 116:39–54, 1965.

Hemolytic Anemia Suggestive of Leptospirosis in the Black Rhinoceros

AN 11-YEAR-OLD black female rhinoceros *(Diceros bicornis)* that had been born in the Denver Zoo became ill in May 1977. At that time, she began voiding dark red urine, became lethargic, and had a dramatic decrease in the amount of fecal output. After 5 days of nonspecific treatment, with little or no improvement, the animal was immobilized for examination. On the basis of an estimated body weight of 1,000 kg, a 1.25-mg dose of etorphine was administered intramuscularly, using a carbon dioxide-powered dart pistol. Rectal and vaginal palpations in addition to a routine physical examination were done in an attempt to eliminate a diagnosis of intestinal blockage or impending abortion. Blood was drawn for a complete blood count and blood chemical profile. A diagnosis was not made at this point, but an enema and supportive treatment were given. The only abnormal laboratory findings were in the hemogram, indicating severe anemia, though the source was undetermined. The PCV was 13%, the RBC count was 1.13 million/mm^3, and the hemoglobin was 4.1 g/dl.

The next day, the urine was clear, appetite was increased, and a large quantity of reddish-brown feces was passed. Recovery was gradual over the next few days. The rhinoceros became pregnant soon thereafter, giving birth to a healthy calf in November 1978. Other signs of illness were not evident until June 9, 1979, when she again began to pass red urine and demonstrated marked muscular weakness. In spite of supportive treatment, her condition rapidly deteriorated and she died within 24 hours.

Ten days later, a 9-year-old imported male rhinoceros passed a large quantity of red urine in his outside enclosure. While attempting to separate him from another rhinoceros, he fell over dead, which was only 2 hours after the passage of the red urine. Earlier signs of illness had not been noticed.

Postmortem findings for both rhinoceroses were essentially the same. Fortunately, the second rhinoceros to die was necropsied immediately, providing fresher tissue specimens for diagnostic studies. The most significant lesions on both animals were large quantities of red peritoneal and pericardial fluid, red edema throughout the omental and perirenal tissues, and orange discoloration of the pulmonary parenchyma. Bright red urine filled the urinary bladder. The blood of the second rhinoceros had a PCV of less than 5%. All of the findings indicated a hemolytic anemia of unknown origin.

When the first animal died, it was thought to be from a metabolic disorder, especially with the history of an episode of presumed hemolysis. With the death of the second animal, however, precautions were taken to prevent further deaths, now believed to be from an infectious agent.

The building was placed under quarantine, with strict sanitary precautions. Most of the building was scrubbed and disinfected daily with chlorhexidine. Rhinoceroses were not allowed outside access until all lots could be evaluated for potential sources of contamination. Though the black male rhinoceros

and a white female rhinoceros were separated both indoors and outdoors, a common shift pen and doorway were used when moving the animals in or out. This provided an opportunity for urine contamination despite concrete walls between the lots, which precluded animal-to-animal contact. Pest control for rodents and insects was increased. Food sources were inspected and analyzed for potential contaminants.

All animals in the building, including 2 remaining rhinoceroses and 2 elephants, were started on an oral prophylactic regimen of chlortetracycline. Blood was drawn on a daily basis from all animals that would allow venipuncture without immobilization or excessive physical restraint. One of the remaining rhinoceroses, a female, was docile enough to permit this procedure without any type of restraint, as were the elephants. For the other rhinoceros, a 7-month-old male, a blood sample could be obtained with physical restraint, but this was not done daily. The black male rhinoceros and the white female rhinoceros sporadically permitted blood sampling without restraint. The blood sampling was done for 30 days. Variations greater than 5% of accepted normal PCV values were not detected except in the remaining female black rhinoceros. Her PCV initially was 30%, and it progressively declined to 22% over a period of several days, causing considerable concern over her welfare, especially since a diagnosis had not been made.

Nine days after the death of the second rhinoceros, a presumptive diagnosis of leptospirosis was made, on the basis of microscopic agglutination-lysis titers. The serovars analyzed were *canicola, grippotyphosa, hardjo, icterohaemorrhagiae,* and *pomona.* This second animal had a 1:4,000 titer to *canicola* and a 1:8,000 titer to *icterohaemorrhagiae.* (Unfortunately, the first rhinoceros to die could not be tested because of the combination of postmortem degeneration of the blood and low serum quality, as a result of the massive hemolysis.) At this point, all animals in the building were vaccinated with a double dose (10 ml) of a 5-serovar bovine leptospirosis vaccine,[1] except for the juvenile male rhinoceros, which was given a single 5-ml dose of the vaccine.

In order to rule out other causative agents, many other diagnostic techniques were performed on the second dead rhinoceros. Extensive searching for red blood cell parasites was conducted, but none was found. Anaplasmosis was eliminated on the basis of the card test; *Clostridium haemolyticum* infection was eliminated on the basis of fluorescent antibody testing; and equine infectious anemia was eliminated on the basis of the Coggins' test. A heavy metal "screen" from frozen liver eliminated several of the heavy metals (mercury, arsenic, bismuth, and antimony), and separate testing of liver tissue eliminated copper poisoning. Cultures of blood, urine, and peritoneal fluid taken at the time of the postmortem examination yielded no growth considered significant to the hemolytic process. The possibility of a toxin from the food sources was eliminated by gross examination for toxic plants and by the facts that all feed is drawn from a central supply and no similar episodes of illness occurred elsewhere in the zoo. Autoimmune disease and other metabolic disorders were ruled out mainly due to involvement of more than 1 rhinoceros.

When histologic examinations of both animals were complete, the diagnosis of hemolytic anemia was confirmed. Marked to severe hemosiderosis was evident in the spleen, liver, lungs, lymph nodes, adrenal glands, kidneys, and intestines. Specific etiologic agents could not be identified despite bacterial, fungal, acid-fast, or silver stains. Examination of numerous silver-stained sections from kidney failed to reveal any leptospires.

Other attempts were made to confirm the identity of the etiologic agent. Whole rhinoceros blood (6 ml) from the second rhinoceros was inoculated IV in a sheep and a goat, with no seroconversion for leptospirosis, and the animals remained healthy. Numerous cultures of urine from the remaining animals resulted in no *Leptospira* growth.

Attempts were made to culture *Leptospira* directly from the blood of the remaining animals, but there was no growth. Blood from rats *(Rattus norvegicus)* collected on the zoo grounds had titers of approximately 1:25 for serovar *icterohaemorrhagiae,* which we considered significant since rats are considered to be the most common carriers of *Leptospira.* Despite the lack of any confirmatory evidence, we believe that leptospirosis was indeed the cause of the hemolysis. Paired serum samples were not possible because the only

blood obtained from the rhinoceros was at death. Diagnosis in these circumstances must depend on interpretation of results from a single specimen.[2] Rats would provide a likely source of infection in this case.

At the time of vaccination, blood was drawn for leptospirosis serology from all animals that allowed this procedure that day. This included 2 elephants and 2 black rhinoceroses. These animals were all seronegative except for 1 Asian elephant that had a 1:500 titer to *icterohaemorrhagiae*. Approximately 6 weeks after vaccination, blood was obtained for determination of *Leptospira* titers to determine the effectiveness of this product in animals normally not vaccinated. At this time, a low titer (1:250) for serovar *icterohaemorrhagiae* was found in only 1 animal, that happened to be the newest addition to the collection. The Asian elephant went from 1:500 to 1:8,000 for *icterohaemorrhagiae*. One black rhinoceros that had been seronegative had a titer of 1:4,000 for *icterohaemorrhagiae*. A female white rhinoceros that had no prevaccination titer determined had a postvaccination titer of 1:2,000 for *icterohaemorrhagiae*. On the basis of this limited information, it was believed that the vaccine was effective. Postvaccination titers obtained by 2 other laboratories were generally lower, however.

Within the same time frame of these investigations, 3 animals died from acute septicemia within the first few days of life. They included 1 Rocky Mountain goat and 2 bonteboks; all 3 were housed in a barn on the opposite end of the zoo. Serologic determinations again indicated leptospirosis, with titers of 1:500, 1:8,000, and 1:8,000, respectively. Since it appeared that the disease was present over the entire zoo grounds, the entire hooved stock collection was vaccinated against leptospirosis, using the bovine dosage (5 ml). Whenever the opportunity became available to obtain blood samples, *Leptospira* titers were obtained on these animals also. All titers were above 1:250.

Though leptospires have been commonly incriminated as pathogens among domestic animals, they are much less often isolated in exotic animals, possibly because of failure to test for the disease. In the rhinoceros, only 1 case has been documented and that was in a wild black rhinoceros.[3] Apparently, these animals were the first captive rhinoceroses reported with the disease.—*E. Michael Douglass, DVM, Memphis Zoo and Aquarium, Memphis, TN 38112, and Raymond E. Plue, DVM, C. E. Kord Animal Disease Laboratory, Melrose Station, Nashville, TN 37204.*

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1. Leptomune 5, Beecham Laboratories, Bristol, Tenn.
2. Blood DC, Henderson JA, Radostits OM: *Veterinary Medicine,* ed 5. Philadelphia, Lea & Febiger, 1979, p 569.
3. Schenkel R, Schenkel-Hulliger L: *Ecology and Behavior of the Black Rhinoceros (Diceros bicornis): A Field Study.* Hamburg and Berlin, Verlag, Paul Parey, 1969, p 103.

Glioblastoma Multiforme in a Budgerigar

A 3-YEAR-OLD male budgerigar (*Melopsittacus undulatus*) was examined because of progressive weakness and loss of coordination. The owners first recognized the loss of coordination 5 months earlier, when the bird began to fall from its perch. In the month prior to admission, falling from the perch was accompanied by tremors of the wings and rigidity of the legs.

The bird was thin. Heart rate, respiration rate, and body temperature were normal. Neurologic examination revealed normal head posture, normal strength in the wings and legs, and normal spinal reflexes. A resting tremor, indicative of basal ganglion dysfunction, was noticed. There was a bilateral menace deficit and the pupils were widely dilated. There was only minimal constriction of the pupils to light, and the light responses were symmetric. The tentative diagnosis was diencephalic space occupying disease. The bird died 2 hours after the onset of supportive therapy.

Gross lesions were not seen at necropsy. Microscopically, lesions were seen in the diencephalon, mesencephalon, and preoptic regions of the brain. A well-

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