HEMOLYTIC ANEMIA IN TWO BLACK RHINOS

E. Michael Douglass, D.V.M.
Memphis Zoo, Memphis, Tenn.

This past summer Memphis had the unpleasant experience of losing an adult breeding pair of black rhinos (*Diceros bicornis*) within a ten-day period from a disease process previously unrecorded in captive rhinos. Also, within the last three weeks a newborn white rhino (*Ceratotherium simum*) died which may be related to this same disease but is still under investigation at this time. The disease expressed itself by massive hemolysis of the red blood cells resulting in an acute death apparently due to anoxia of the tissues. After extensive investigations it appears that *Leptospira icterohemorrhagicae* is the causative agent of these deaths. Only one reference to leptospirosis in rhinos could be located and that occurred in a wild black rhino in Africa.(1) However, with the loss of these animals, it is apparent that leptospirosis is a potential hazard to any rhino population and should be considered in any disease process due to the multitude of manners in which the disease can express itself.

The first animal to die was an eleven-year-old female black born in Denver. The first signs of illness which may be related to her death occurred in May of 1977. At that time she began voiding a red-colored urine, became lethargic and had a dramatic decrease in fecal output. Treatment for this first few days was non-specific consisting of antibiotics, vitamins and steroids. With little improvement in four days, she was immobilized for examination. Blood was drawn for chemistries and a CBC. Rectal and vaginal palpations were performed in an attempt to eliminate intestinal blockage or abortion and a routine physical exam was performed. No diagnosis was made and she was given a water hose enema as a precaution along with further similar injections. The only significant finding from the blood was an anemia with HCT of 13%, RBC count of 1.13 million/mm^3, and Hgb of 4.1 gms. The next day the red urine ceased, she began eating and passed a large quantity of reddish-brown feces. Recovery was gradual over the next few days and she became pregnant soon thereafter, giving birth to a healthy male calf in November of 1978. No other signs of illness were present until June 9, 1979, when she began to pass red urine again and showed muscular weakness. Despite similar treatment she died that night.

Ten days later her mate, a nine-year-old male imported black, passed a large quantity of red urine about 2:30 P.M. and within two hours fell over dead before he could even be separated from his other mate. No other signs of illness were noticed prior to this and at this point no definitive diagnosis has yet been made on the first rhino to die.

Post-mortem examination of both rhinos was essentially the same. Fortunately, the second rhino to die was posted immediately which allowed much fresher examination and collection of tissues for diagnostic studies. The most significant lesions on both animals were red-colored peritoneal and pericardial fluid, red-colored edema throughout the tissues of the abdominal cavity, orange discoloration of the lung parenchyma and bright red-colored urine. The blood itself was the most dramatic being of watery consistency, dark, and having a hematocrit of less than five. All of these signs pointed towards a hemolytic anemia of as yet unknown origin but believed to be an infectious process. When the first animal died, it was thought to be possibly a metabolic disorder isolated to this one animal, especially with the history of the previous episode of presumed hemolysis. With the loss of the second animal, though, precautions were taken to hopefully prevent any further deaths.

The building was placed under a quarantine with strict sanitary precautions. Practically the entire building was scrubbed and disinfected daily. No animals were allowed outside access until their lots could be evaluated for potential sources of contamination. Pest control for rodents and insects was increased. Food sources were inspected and analyzed for potential contaminants. All animals in the building, including both rhinos and elephants, were started on oral prophylactic antibiotics. Blood was drawn on a daily basis from all animals that would allow this without immobilization or excessive physical restraint to hopefully allow early detection of a hemolytic process. Luckily, the remaining adult black female was docile enough to permit this procedure without any type of restraint, as were the elephants. The seven-month-old male could be bled with physical restraint but was not done daily. The white rhinos were sporadic on days they would allow blood sampling without restraint.

No significant variations from accepted normal hematocrit values were detected except on the remaining adult black. Her hematocrit initially was 30% and progressively declined to 22% over a period of several days causing considerable concern over her welfare especially since no diagnosis had yet

been made.

Finally, nine days after the death of the second animal, a presumptive daignosis of leptospirosis was made based on serology. This second animal tested out to have a 1:4000 titer to *Lepto. canicola* and a 1:8000 titer to *Lepto. ictero.* At the Diagnostic Laboratory of Tennessee anything over 1:500 is considered to indicate active infection. Unfortunately, the first animal to die could not be analyzed due to the combination of post-mortem degeneration and low serum quality as a result of the massive hemoglobinemia. At this point, all animals in the building were vaccinated with a double dose (10 cc) of the Beecham Leptomune 5 product, except for the juvenile male which was given a single 5 cc dose of vaccine.

In order to further define the causative agent, multiple other diagnostic techniques were performed. Hours were spent searching for red blood cell parasites, but none were found. This was made even more difficult by the absence of intact red blood cells in smears from the animals. Anaplasmosis was also eliminated on the basis of serology. Clostridia was eliminated on the basis of fluorescent antibody tests. Equine infectious anemia was eliminated on the basis of Coggin's test. A heavy metal screen from frozen liver eliminated several of the heavy metals. Separate testing on the liver eliminated copper poisoning. Cultures taken at the time of post-mortem examination revealed no growth considered significant to the hemolytic process. Other toxicities from food sources were eliminated on the basis of gross examination for toxic plants and by the fact that all feed is drawn from a central supply and no similar episodes occurred elsewhere in the Zoo. Autoimmune disease was ruled out mainly due to multiple animals being involved as were other metabolic disorders.

Histopathologic examination of the tissues confirmed the diagnosis of a hemolytic anemia by demonstrating marked to severe hemosiderosis in the spleen, liver, lymph nodes, kidney, lung and intestine. No specific etiologic agent could again be identified despite Gram's stains, fungal stains, acid-fast stains, or silver stains. Numerous silver stains on the kidney were attempted, but no leptospires could be identified.

Other attempts were also made to further identify the disease as due to leptospirosis in addition to the silver stains. Experimental animal inoculation in both a goat and sheep given 6 cc of whole rhino blood intravenously resulted in no positive seroconversion to lepto, and the animals remained healthy. Numerous urine cultures of the remaining animals resulted in no leptospirosis growth. Large quantities of free hemoglobin were found in the dead animal's urine, but no other significant abnormalities were detected, and no leptospires were identified. Blood from rats collected on the Zoo grounds did contain titers of less than 1:50 for *Lepto. ictero.* which is considered significant due to rodents are the most common carrier of leptospirosis. Attempts are now being made to culture lepto directly from the blood of the other animals in the building. Despite this lack of any confirmatory evidence, it is believed that leptospirosis is indeed the cause of the hemolysis and, according, to several pathologists, it is not uncommon when the diagnosis must be made strictly on serology. Rats do appear to be the source of infection in this case.

As mentioned earlier, at the time of vaccination blood was drawn for leptospirosis serology from animals that would allow this procedure that day. This included two elephants and two black rhinos. Titers from these animals were all negative except for one female Asian elephant that had a 1:500 titer to *Leptospira ictero.* Approximately six weeks after vaccination blood was again drawn for post-vaccinal titers to determine the effectiveness of this product in animals normally not vaccinated. At this time only one animal had a low titer, less than 1:250, which was a female Aftican elephant and happened to be the newest addition to the collection. The Asian elephant went from 1:500 to 1:8000 *ictero.* The female black rhino went from negative to 1:4000 *ictero.* A female white rhino which had no pre-vaccinal titer determined has a post-vaccinal titer of 1:2000 *ictero.* On the basis of this limited information it is believed that the vaccine is effective when the animals have prior antigenic exposure; in other words, antigenic memory is stimulated resulting in a dramatic increase in circulating antibodies as with other "booster" vaccinations. The African elephant with the low titer after vaccination has been revaccinated and is expected to show a more positive seroconversion when retested. The black rhino which was negative prior to vaccination and 1:4000 after vaccination is believed to have been incubating the disease at the time of initial testing. She was definitely exposed sharing the same stalls and lot with the animals that died. These titers have been performed by another laboratory with similar results, but the titers are in general lower. However, they are still considered of protective levels when extrapolated from the domestic animals.

In summary, the main two points to be illustrated here are:

1. Leptospirosis does appear to be a potential hazard to rhinos and possibly elephants as it is to other species of mammals and should be considered in the differential diagnosis of disease in these two species.
2. If faced with an outbreak of leptospirosis in these species, vaccination appears to be effective and should be considered as another preventative measure in disease control of zoo animals.

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