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A REVIEW OF SOME OF THE HEALTH ISSUES OF CAPTIVE BLACK RHINOCEROSES (*DICEROS BICORNIS*)

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Abstract: In captivity, black rhinoceroses (*Diceros bicornis*) are beset by many disease syndromes not described in black rhinoceroses in the wild. Hemolytic anemia, hepatopathy, and ulcerative dermatopathy that lead to increased morbidity and mortality characterize these syndromes. It is uncertain whether these are separate disease syndromes with different etiologies or the same disease with different manifestations. This article offers a brief review of some of the health issues of concern for the captive black rhinoceros population and proposes some possible avenues of research for consideration.

Key words: Black rhinoceros, *Diceros bicornis*, hemolytic anemia, hepatopathy, ulcerative dermatopathy.

INTRODUCTION

More than 100,000 black rhinoceroses (*Diceros bicornis*) were living in the wild in Africa in the 1960s according to the International Union for the Conservation of Nature (IUCN).[10] By 1995, this population had declined to 2,410 (2002 IUCN Red List of Threatened Species, http://mercury.ornl.gov/metadata/nbii/html/ma/www.nbii.gov_metadata_mdata_Millennium_nbii_wdc_ma_d_2002redlist.html). One of the main threats to the wild population is illegal hunting for the international rhino horn trade. The horn is used for traditional Chinese medicine, primarily as a fever-reducer and for ornamental use, particularly for dagger (jambiya) handles.[10] Curtailing the depredation of wild black rhinos by antipoaching measures is a very expensive strategy, with long-term success jeopardized by declining budgets.[10] One of the justifications for captive populations is to serve as a reservoir, potentially providing a source of animals for reinforcement or re-establishment of wild populations.[35] Unfortunately, the captive population suffers its own threats to survival. Black rhinoceroses in captivity display unusual disease syndromes not described in black rhinoceroses in the wild. Hemolytic anemia, hepatopathy, and ulcerative dermatopathy that lead to increased morbidity and mortality characterize these syndromes. It is uncertain whether these are separate disease syndromes with different etiologies or the same disease with different manifestations. These syndromes not only pose a threat to the survival of black rhinos in captivity, but they could jeopardize the wild populations if captive black rhinos are used to supplement the wild populations and if the etiology of any of the syndromes proves to be transmissible.[35]

Until recently, the health of the captive black rhinoceros population in the United States has not been systematically reviewed. The lack of controlled investigations has forced zoo veterinarians in many instances to rely on case reports and anecdotal information in managing the health of these animals in captivity.

Hemolytic anemia

Hemolytic anemia syndrome is frequently cited as one of the leading causes of death in captive black rhinos.[25,28] Mortality is high with this syndrome; 47 instances of hemolytic anemia have been documented in 39 animals, with a 75% mortality rate.[39] The cause of the hemolytic anemia syndrome is not known. Earlier studies of black rhino red blood cells (RBCs) found no metabolic abnormalities to explain the hemolysis.[5,11,40] However, adenosine triphosphate (ATP) levels in black rhinoceros RBCs were found to be less than 5% of levels found in human erythrocytes.[40] In addition, black rhinoceros red cells have low catalase activity compared with humans.[36] In humans, hemolytic syndromes are often caused by enzyme defects in the erythrocyte, impairing the cellular ability to neutralize oxidants. The most common defect is a hereditary deficiency of glucose-6-phosphate dehydrogenase, which predisposes affected individuals to hemolysis secondary to oxidant stress, typically associated with drugs or foodstuffs.[36] This led some researchers to hypothesize that ATP deficiency could be responsible for hemolysis in black rhinoceroses under oxidant stress.[39] The researchers spec-

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ulated that ATP was diverted from its role in the phosphorylation of glucose to the hexose monophosphate shunt pathway (HMPS) to neutralize peroxides and reactive oxygen species.[39] Research showed that HMPS capacity of rhinoceros erythrocytes was independent of intracellular ATP concentrations.[41] Accordingly, the researchers concluded that limited HMPS capacity alone could not explain the hemolytic anemia syndrome seen in black rhinoceros.[41] This study did find that erythrocytes from three species of rhinoceros had HMPS glycolytic and recycling rates and responses to activators that were low when compared with those of human erythrocytes.[41] The researchers offered this as a contributing factor to the supposed high susceptibility of black rhinoceros erythrocytes to oxidant-induced hemolysis.[39] The study did not address, however, the question of why black rhinoceros erythrocytes are deficient in ATP and whether it was diverted to the HMPS to combat oxidant stress. It remains unknown if this is a pathologic condition or normal for this species. It remains undetermined whether the ATP deficiency observed in the erythrocytes is associated with the hemolytic anemia observed in captive black rhinoceros.

In clinical situations, phosphate supplementation has been advocated for therapeutic intervention in clinical cases of hypophosphatemia or to increase endogenous ATP concentrations in rhinoceros erythrocytes as a preventive measure.[39] Supplementation of dietary phosphate to black rhinoceroses with a variety of clinical disorders has resulted in increased erythrocyte ATP concentrations.[39] Hypophosphatemia is a well-recognized cause of hemolysis in several species.[1,33,53] Hypophosphatemia has been induced in cattle by reducing the phosphorus content in their feed after parturition.[34] In these cattle, hypophosphatemia occurred within 10 days of parturition, red blood cell levels of ATP decreased, and hemolytic anemia occurred. This study in cattle concluded that the most likely cause for the hemolytic anemia was impaired viability of the red blood cells resulting from the hypophosphatemia and decreased ATP levels.[34] Humans with severe hypophosphatemia have anemia, decreased red cell ATP and reduced 2,3-diphosphoglycerate.[16,20,23] Reduction of ATP to levels less than 15% of normal values caused cells to become spherical and to have increased osmotic fragility and shortened life spans.[16] These findings warrant further consideration of hypophosphatemia and ATP deficiency of black rhinoceros erythrocytes as a cause of hemolytic anemia.

Hypovitaminosis E has also been proposed as a possible cause of hemolytic anemia in captive black rhinoceros. Low circulating levels of vitamin E cause hemolysis in humans,[51] rats (*Rattus norvegicus*),[4] and horses (*Equus caballus*).[50] Researchers found lower circulating vitamin E levels in captive black rhinoceros when compared with free-ranging animals.[8,13] In addition, researchers demonstrated that native forage consumed by free-ranging black rhinoceroses was higher in vitamin E and fat than diets of captive black rhinos.[7,12] Supplementation of the diet fed to captive black rhinoceros with vitamin E was advocated.[42] Recently, a study of fat-soluble vitamins in blood and tissues of free-ranging and captive rhinoceroses found significantly higher levels of circulating vitamin E since 1990 in captive compared with free-ranging black rhinoceroses.[6] Researchers suggest that currently there might be oversupplementation in captive animals.[6] The relationship between hemolytic anemia and vitamin E in black rhinos is still unclear.

Leptospirosis infection has been suggested as the underlying etiology in at least nine cases of hemolytic anemia on the basis of serum titers, special tissue stains, and fluorescent antibody testing.[9,26] Definitive diagnosis of leptospirosis can only be made by bacteriologic culture, a difficult and often unsuccessful procedure. Microscopic agglutination testing is the most common method for detecting leptospirosis, although it requires subjective interpretation because strict criteria for serologic confirmation of active infection have not been established.[44] Diagnosis of disease is further complicated by the observation that animals can be infected with leptospires without developing clinical signs. Accordingly, detection of leptospires or antibodies to leptospires does not necessarily correspond to the finding of the causative agent of disease.

Necropsy reports of black rhinoceroses that have died in captivity cite hemosiderosis in various organs as a frequent finding. Until recently, this finding was attributed as possible evidence of previous hemolytic anemia. However, some research suggests an alternative hypothesis. Smith et al.[47] found that serum ferritin levels (a measure of stored iron) in captive black rhinoceros in the United States tend to increase with age or time in captivity. They also found that ferritin and hepatic iron stores were significantly higher in captive black rhinoceros compared with white rhinoceros.[47] The haptoglobin in the two species were comparable, indicating that hemolysis could not be the underlying cause of the increased iron found in captive black rhinoceros.[47] The authors suggested that changes in the diet associated with captivity could result in increased iron absorption. Further support for the idea that the hemosiderin seen in black rhinoceros is asso-

ciated with captivity was provided in a study that found hemosiderosis was not reported in free-ranging black rhinoceros but was present in captive animals, in increasing amounts with longer time in captivity.[21]

Some researchers speculate that there may be an increased availability of iron in the diet in captivity, perhaps because of decreased amounts of iron-binding substances (such as tannins, phytates, or polyphenols).[37] Mammals generally lack effective methods for excreting iron, and when challenged with dietary excess or reduced levels of competitors (competitive cationic minerals or luminal binding agents), iron deposition into tissues results.[49]

Ferritin is the protein-bound storage form of iron in the body. Iron in this form is vulnerable to attack by superoxide radicals and other free radicals, resulting in the reductive release of iron from ferritin. Although iron in the form of ferritin is harmless to the body, ''free iron'' in the form of reduced iron (FeII) is readily involved in oxidation–reduction reactions, resulting in the formation of hydroxyl radicals or the initiation of lipid peroxidation.[24] The production of superoxide radicals can trigger a cascade of events resulting in a chain reaction formation of hydroxyl radicals and hydrogen peroxide, leading to the depolymerization of polysaccharides, breakage of DNA strands, inactivation of enzymes, and lipid peroxidation.[24] In vivo, this cascade eventually terminates in a reaction with antioxidants, such as vitamin E. When the production of free radicals in the system exceeds the body's antioxidant capability, a condition of oxidative stress predominates.

Lipid peroxidation can also be caused by simple complexes of iron salts as well as nonheme iron proteins. Both ferric and ferrous iron, as well as free heme, hemoglobin, myoglobin, and cytochromes, are effective in causing lipid peroxidation.[15] Ferritin catalyzes hydroperoxide decomposition to an extent proportional to its iron saturation. Lipid peroxidation of erythrocyte membranes causes them to lose their ability to change shape and squeeze through capillaries and will eventually lead to hemolysis.[15]

Some researchers suggest, though currently proof is lacking, that iron overload in captive black rhinoceros plays a significant role in the pathology of many of their disease syndromes.[37] Hemosiderosis in other species is associated with disease, but none similar to those seen in black rhinoceros. In humans, iron overload is associated with hepatic, cardiac, and pancreatic dysfunction.[3] Liver fibrosis is a common pathologic finding in humans and other species with iron overload, including multiple species of lemurs and many avian species, but not often seen in black rhinoceros.[19,43,49] Hemolytic anemia is not associated with iron overload in other species.

Leukoencephalomalacia

Leukoencephalomalacia was diagnosed in four female captive black rhinoceroses from three different zoologic institutions.[18,27] Three of the animals were calves (3 wk, 2 mo, and 6 mo of age) and one was a 2-yr-old animal. Evaluation of the animals for known causes of encephalomalacia, including trauma, encephalitis, clostridial enterotoxemia, and toxins, failed to provide an etiologic diagnosis.[18,27] Thiamine deficiency, a common cause of polioencephalomalacia in domestic ruminants, was ruled out on the basis of the predominance of white matter necrosis in the affected rhinos, as well as the absence of similar signs in thiamine-deficient equines.[27]

Two of the animals, the 2-mo-old and 6-mo-old calves, died within 24 hr of the onset of neurologic signs.[27] The 2-yr-old animal developed severe neurologic signs 8 days after being transferred from one zoo to another.[27] The animal was hyperresponsive to different visual and auditory stimuli. At times, the animal appeared to respond to stimuli that were not apparent to people present in the area. The animal was comatose the following morning. A decision was made to euthanatize the animal after 48 hr of treatment without response. In the fourth case,[18] the clinical signs were first seen when the animal was 3 wk of age. Shortly after nursing and playing with its dam, the calf developed signs of head-pressing, continuous vocalizing, and circling to the left with a left-sided head tilt. The calf showed hypermetria, ptyalism, lip-smacking, and apparent blindness. The animal was hospitalized and given supportive care. Diagnostic tests, including computed tomography scanning, cerebrospinal fluid analysis, liver biopsy, and contrast portal venography, failed to indicate a diagnosis. In the 15 mo that followed the initial episode, the animal experienced 14 neurologic episodes. These included ataxia, left-sided circling, left-sided head tilt, head pressing, apparent blindness, and standing in a trance-like state for hours. At 16 mo of age, the animal became anorectic and severely ataxic. It became aggressive toward familiar staff and hyperexcited at any sudden movement. The animal also developed multifocal areas of dermal ulceration on the neck, back, and flanks. The animal was euthanatized.

The first three cases of leukoencephalomalacia were very similar.[27] The histopathologic lesions in

the brains included primary involvement of the cerebrum and lesser involvement of the diencephalon and midbrain. No significant lesions were found in the pons, medulla, or cerebellum of any of the three animals. Vascular changes, including focal necrotizing vasculitis and hemorrhage, were thought to be secondary to surrounding parenchymal necrosis. Premortem hematologic evaluations of the three animals were nondiagnostic; however, all three animals had increased values for creatine phosphokinase and other muscle-related enzymes. Hyperglycemia was detected in the 2-mo-old and 6-mo-old calves, with glucosuria identified in the 6-mo-old calf. The 2-yr-old animal was hyperresponsive to stimuli before becoming comatose. All three cases progressed rapidly to coma and death. The fourth case differed from the other three in that the course of disease was prolonged, with 16 separate episodes of neurologic behavior exhibited.[18] This animal also was hyperresponsive to sudden movements and became aggressive before being euthanatized.

One study considers the involvement of excessive maternal iron in the pathogenesis of leukoencephalomalacia in black rhinoceros calves.[38] The researchers compared serum iron, ferritin, and transferrin saturation from the families of the affected calves with other captive black rhinos and with free-living black rhinos. The researchers found that of the samples from 18 family members of the affected calves, 14 had serum ferritin concentrations higher than all but four of 46 other black rhinoceroses in captivity in the United States. The researchers also found that the dams of these calves had maximum serum ferritin concentrations of 42,468 + 17,706 pmol/L (18,900 + 7,880 ng/ml) that were approximately five to 10 times greater than the mean for the comparison population of captive adult females 6,435 + 5,928 pmol/L (2,864 + 2,638 ng/ml). It is not clear in the report why the maximum ferritin concentrations rather than the mean ferritin concentrations were compared with mean concentrations in the comparison population. These captive adult females had serum ferritin concentrations that were 20 times higher than the mean of six wild black rhinoceroses 299 + 153 pmol/L (133 + 68 ng/ml). Adult males and females in the families of the affected calves had serum iron concentrations of 83 + 35 μmol/L (463 + 195 μg/dl), with transferrin saturation of 89% + 12%. This was compared with 20 captive adult female black rhinos with serum iron concentrations of 42 + 15 μmol/L (232 + 84 μg/dl) with transferrin saturation of 72% + 21% and 12 captive adult male black rhinos with serum iron concentrations of 38 + 6 μmol/L (212 + 33 μg/dl) and transferrin saturation of 63% + 18%. Six wild adult black rhinos had serum iron concentrations of 24 + 8 μmol/L (133 + 46 μg/dl) and transferrin saturation of 28% + 6%. On the basis of these findings, the authors concluded that the families of the affected calves carried the greatest body burdens of iron found to date in captive black rhinoceros. On the basis of this observation, they suggest that maternal iron overload might contribute to the development of congenital leukoencephalomalacia in captive black rhinoceros.

The authors of this paper fail to discuss the role of age in the development of iron overload in these animals. An earlier study[47] showed increased iron accumulation in black rhinos as a function of time in captivity. In the report associating leukoencephalomalacia with iron overload,[38] the mean age of dams of affected calves was 17.5 yr. The ferritin levels of these dams were compared with females whose mean age was 9.3 yr. It is possible that the difference in age of the two groups explains the difference in iron accumulation and that age of the dam, not iron, might be associated with the occurrence of leukoencephalomalacia in the calves.

These researchers[38] also found that a serum sample collected on the day of birth from one affected calf had a high ferritin concentration of 32,784 pmol/L (14,590 ng/ml), whereas a male sibling of another affected calf had a serum ferritin concentration of 261 pmol/L (116 ng/ml) on the day of birth. The affected calf had values within reference range for serum iron concentration 19 μmol/L (108 μg/dl) and transferrin saturation (27%). The dams of both the affected female calf and the male calf had increased serum ferritin concentrations before the births of the calves. The authors conclude that the high ferritin concentration in the affected calf might have been a fetal response to maternal iron overload. They did not discuss the possibility that ferritin, which is an acute-phase inflammatory protein, might have been increased as part of an inflammatory response, not as a response to maternal iron overload. On the basis of the difference between the serum ferritin concentrations of a single affected female calf and the male sibling of an affected calf, the authors suggest a possible sex disparity in fetal response to maternal iron overload, possibly explaining the finding of leukoencephalopathy only in female calves. The authors go further and suggest that this possible discrepancy between the sexes could explain the reported male predominance in surviving live births among captive black rhinoceros.

The authors refer to leukoencephalomalacia as being a congenital problem. Upon reviewing the original descriptions of the four cases,[18,27] it seems that this disease is unlikely to be of congenital or-

igin. The disease in each described case appears to have sudden onset, with the calves exhibiting normal behavior before the onset of clinical signs. The calves were affected at different ages, ranging from 3 wk to 2 yr. One of the calves, in fact, was sent to another zoo, not a likely occurrence for an animal with a congenital abnormality. One of the case reports[18] notes the similarity between the leukoencephalomalacia seen in the black rhino calves and cerebral palsy seen in humans. One difference though, as pointed out in the report, is that cerebral palsy describes chronic, nonprogressive leukoencephalopathy present at birth in human infants. The black rhino calves gave no clinical indication of disease present at birth.

Superficial necrolytic dermatitis

Superficial necrolytic dermatitis (SND), formerly known as mucocutaneous ulcerative disease, has been diagnosed in at least 50% of the captive black rhinoceros population in the United States.[31] The syndrome consists of cutaneous lesions beginning as plaques, progressing to vesicles, and eventually to ulcers. The lesions are typically bilaterally symmetric and seen on pressure points, at mucocutaneous junctions, coronary bands of the feet, along the lateral body wall and on the back.[31] Most episodes are associated with stressful events or with concurrent disease. Hypophosphatemia is seen in some, but not all, of the cases. The skin lesions seen in SND are not inflammatory lesions but rather lesions of degeneration with parakeratosis without dermal inflammation.[31] The clinical presentation and pathologic findings in this disease resemble those of superficial necrolytic dermatitis in dogs and necrolytic erythema in humans.[31] In dogs, this syndrome is associated with a number of abnormalities, including glucagonomas (functional pancreatic islet cell neoplasms),[14] diabetes,[52] liver disease,[29] or poor diet.[48] In humans, the syndrome is primarily associated with glucagonomas.[31] No similar associations have been found to date in black rhinos with this syndrome.

Idiopathic hemorrhagic vasculopathy syndrome

Idiopathic hemorrhagic vasculopathy syndrome (IHVS) is a recently described syndrome characterized by extensive regional swellings of the neck and limbs associated with acute, severe, nonhemolytic anemia.[32] The swellings are the result of pooling of large quantities of blood in the subcutaneous and soft tissue regions of affected areas. Deep cutaneous biopsies were characterized by extensive vascular proliferation.[30]

In one report,[32] the authors attempt to draw some conclusions about a very nebulous syndrome seen in only a few black rhinos in captivity. As is often the case in zoo medicine, clinicians are faced with treating diseases of unknown origin in species about which little is known while trying to make diagnoses from the results of tests that often have not been evaluated in the affected species.

Extensive serologic testing was done in which serum was tested for equine, bovine, and exotic viruses. Unfortunately, as often happens when working with nondomestic species, validation of diagnostic tests for a specific species does not occur. Provided that the tests had been validated for use in the black rhino and that the diagnostic test had sufficiently high sensitivity, a negative result could be used to rule out a particular disease.[45] However, the likelihood of these conditions being met in this situation, especially for such a variety of diseases, is unlikely. Thus, the finding that results were negative, with a few exceptions, for these tests, provides little information on the underlying etiology of IHVS. Even with a validated test with high sensitivity, negative titers can result from an animal's inability to mount an adequate immune response to the virus (because of underlying immunosuppression or compromise, improper nutrition, overwhelming viremia), or the test might have been done too early in the course of the disease, before the animal mounted an antibody response. Additional forms of testing, particularly virus isolation, might have provided additional insight into whether viruses play a role in this disease. Although hindsight into these cases suggests additional testing, the constraints faced by clinicians struggling to treat these cases with limited resources, a lack of validated diagnostic tests, and few clues toward etiologies likely limited their ability to run all possible tests.

The observation that none of the rhinoceroses had a confirmed bacterial infection before onset of clinical signs and that no single bacterial pathogen was consistently isolated from all seven animals suggests that a specific bacterial pathogen is not the underlying cause of IHVS. It is not surprising that bacteria were cultured from any of these animals, given the clinical signs associated with the disease. It would be more surprising if no bacteria had been cultured from animals with swellings associated with massive pooling of blood, laminitis with sloughing of nails and oral ulcerations. All of these characteristics provide wonderful breeding grounds for bacteria, and the animal's immune system likely was not at peak performance because of the underlying disease.

The authors raise the possibility that this syn-

drome is either an autoimmune disorder or an immune complex disease. The possibility of an autoimmune disorder is considered less likely by the authors because only one of two rhinos tested was positive for the cold agglutinin disease, two rhinos were negative for the Coombs test, and three rhinos tested for ANA titers were negative. None of these tests has been validated for use in black rhinos, and there are no black rhino–specific reagents for use with any of these tests. Because of the uncertainty of the results of these tests, it seems premature to rule out autoimmune disorders on the basis of the negative results of these tests.

Immune complex disease is currently being investigated as a possible etiology. Although no circulating immune complexes were found in any of the affected animals, this could be because of the insensitivity of the equine test used for detection or because the immune complexes were not present in circulation at the time of detection. Immunohistochemistry studies that are currently in progress might provide evidence suggestive of an underlying immune complex disease. This evidence, however, will not provide answers as to what invoked the formation of immune complexes that might have caused the disease. Unfortunately both autoimmune diseases and immune complex diseases are not well understood, and diagnosis of either type of disease is difficult, even in those species that have been well studied, such as humans. Diagnosis, especially with the additional goal of control or prevention, will be even more difficult in a much less understood species such as the black rhino.

The authors find similarities between equine purpura hemorrhagica (EPH) and IHVS. Unfortunately, EPH is not a well-understood disease in horses and, while thought to be caused by an allergic reaction to streptococcal or viral antigens, or possibly a suppurating wound, does not have a clearly understood etiology, much less a means of prevention. In addition, although parallels exist between the two syndromes, the histologic pattern of vascular proliferation and neovascularization seen in IHVS is not associated with EPH. However, the authors close with the suggestion that the hypothesis that IHVS is an immune-mediated response to an infectious agent is still plausible.

Toxic hepatopathy

Several reports in the literature attribute deaths in black rhinos to a toxic hepatopathy.[17,22,46] The first case report involved two black rhinos that had been at the same zoo for more than 5 yr. The affected female had anemia and jaundice of several months' duration before death, and the affected male had anemia and ulcerative skin lesions scattered over his entire body.[46] The liver lesions in both animals were similar, with most of the hepatocytes containing a green-brown pigment identified as bile. Histopathologic examination of the skin lesions of the male rhino showed endothelial proliferation that partly or completely blocked the vessel lumens of the dermal blood vessels at the base of most affected areas. The authors concluded that the hepatic lesions were suggestive of a toxic problem. The animals were housed in an enclosure that differed from neighboring white rhinoceros only by the inclusion of an area fenced with old telephone poles.[46] Material submitted for analysis from the telephone poles was lost at the receiving laboratory. No source of toxic material was found despite considerable environmental analysis. The authors speculate that creosote used to treat the telephone poles could have caused the lesions seen in these animals because creosote is known to cause liver and skin lesions in other animals.

The other two reports[17,22] refer to animals captured in Zimbabwe for exportation to the United States and Australia. One paper describes hepatopathy and death in two animals from a group of 20 black rhinos captured in December 1990 for exportation.[22] Two other animals became sick and were jaundiced, but recovered. After exportation, three animals sent to the United States and two sent to Australia died with liver lesions similar to those that died in Zimbabwe. The other paper reports about nine black rhinos captured in Zimbabwe in June 1992 for importation into Australia.[17] Of these animals, two adults died after developing a severe hepatopathy. The first death was an adult male that died during the quarantine period at Australia's high-security animal quarantine station on Cocos Island. The second death was an adult female that died in March 1993 after developing inappetence, skin eruptions, and jaundice. The necropsy findings closely resembled those of the male that died during quarantine on Cocos Island.

In both reports, all animals that died had similar liver lesions. The livers were enlarged, friable, and intensely green. Granular pigments in the hepatocytes were strongly positive for bilirubin according to the van den Bergh diazo methods.[17] Several animals had swellings of limbs. Fine needle aspiration of the swollen legs revealed the presence of blood.[17] Intrafascicular muscle hemorrhages and hematomata in the muscles and subcutaneous tissues were found on necropsy.[17,22] The animals that died in the United States had skin ulcerations, jaundice, and lethargy before death. All animals were anemic and hypophosphatemic. All animals in both reports had

access to creosote-treated timber in the holding yards in Zimbabwe. Although no definitive diagnosis was made in either report, creosote toxicity was implicated as the cause of death of these animals. Although there is an association with creosote, the timing of the clinical onset of disease does not fit well with that of an acute exposure to creosote. If the exposure did occur in Zimbabwe, it is noteworthy that many of the animals became ill in the United States or Australia rather than in Zimbabwe, the site of their proposed exposure. In fact, one of the animals did not become ill until after release from quarantine in Australia, almost a year after the exposure to creosote in Zimbabwe. The lesions seen on necropsy suggest an acute insult to the liver. The timing of their occurrence in these animals does not fit with the diagnosis of an acute toxic hepatopathy from creosote toxicity. Although creosote has been shown to induce these lesions in some species,[22] toxicologic studies in mice found no adverse effect on the liver after being fed up to 462 mg/kg per day of coal tar products for 185 days.[2] Although there is an association between exposure to creosote and the development of lesions in the affected animals, the onset of clinical signs suggests that other factors could be involved in the development of disease.

The focus of these reports is on the hepatopathy seen in these animals, but another interesting lesion is the hematomas found in the musculature and subcutaneous tissues. Several animals were described as having swollen limbs, and fine needle aspiration of these swellings revealed only blood.[22] One report[46] did not describe any hematomas or swellings, but the authors did find dermal blood vessels that were partially or completely occluded by endothelial proliferation.[46] These lesions all resemble the lesions seen with idiopathic hemorrhagic vasculopathy syndrome (vide supra), a new syndrome supposedly thought to have only recently occurred.

Black rhinoceroses were brought into captivity to prevent their extinction. Although captivity protects them from slaughter for their horns, they could merely have exchanged one threat for another. Successful management of the captive population requires a thorough understanding of the risk factors associated with morbidity and mortality. It is important to understand previously reported disease syndromes so improvements can be made for present and future captive rhinos.

Further research is needed to determine whether certain temporal associations with disease occurrence signify causality. Associations exist between a decrease in the incidence of primary hemolytic anemia and management changes, including vaccination of black rhinoceroses against leptospirosis and supplementation of the diet with vitamin E; cholestatic hepatopathy and exposure to creosote; and maternal iron load and leukoencephalomalacia in offspring. In studies of these associations, it is critical that other variables be controlled in that they could influence the outcome and cloud the vision of the current disease process. With regard to leukoencephalomalacia, it is necessary to elucidate whether maternal iron load is the real problem rather than maternal age. It is necessary that all variables that might influence this outcome be examined. It is also critical that proper tissues are collected, for it would be difficult to evaluate leukoencephalomalacia in the population when so often the brain is not collected as part of a necropsy procedure on black rhinoceros. It is as important to gather information on the unaffected population as it is to know the specific details of affected animals, for it is by comparing these two groups that differences can be identified and possible risk factors that could influence the occurrence of disease be determined.

Further research is also needed to investigate the many questions generated by earlier research. Some of this research will require the development of black rhino–specific reagents and diagnostic tests or the validation of existing tests for use with black rhinoceros. Controlled investigations are needed to elucidate the complex pathophysiology of these disease syndromes and to determine appropriate means of treatment and of prevention.

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