EPIDEMIOLOGY OF BLACK RHINOCEROSES (*Diceros bicornis*) IN CAPTIVITY IN THE UNITED STATES

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy

in the Graduate School of The Ohio State University

By

Patricia Marie Dennis, D.V.M, M.S.L, Diplomate A.C.Z.M.

The Ohio State University
2004

Dissertation Committee:

Dr. William J.A. Saville, Advisor

Dr. Evan S. Blumer

Dr. Julie Funk

Dr. Thomas E. Wittum

Approved by

Veterinary Preventive Medicine
Graduate Program

ABSTRACT

The black rhinoceros, endangered in the wild due to illegal hunting for its horn, also faces threats to its survival in captivity. A skewing of the natal sex ratio favoring males is a problem of increasing concern for the captive black rhino population. A retrospective case-control study was conducted to examine risk factors associated with the birth of a male calf. The outcome variable was the birth of a male calf. A generalized linear mixed model was constructed using a backward selection strategy. A skewed natal sex ratio was found in calves born to wild-born dams. Increased time in captivity was associated with an increased likelihood of a male calf, whereas the age of the dam between 12 and 19 years had a decreased likelihood of a male calf. No associations were found with the birth of male calves to captive-born dams.

A study including 296 of the 334 black rhinos (88.9%) ever in captivity in the United States in facilities accredited by the American Zoo and Aquarium Association (AZA) between the years 1930 and 2001 found that 35% of the animals have died without reproducing. This study also revealed that cases of primary hemolytic anemia peaked in the years 1976 -1980 and do not currently represent a major health problem to the captive population. This study identified cases of idiopathic hemorrhagic vasculopathy that occurred prior to 1995 and in animals living in regions other than Texas. Cholestatic hepatopathy, originally reported as toxic hepatopathy associated with

creosote exposure, was found in cases with no documented exposure to creosote. Additionally, several cases were identified that had lesions of both cholestatic hepatopathy and idiopathic hemorrhagic vasculopathy.

Survival analysis, using the Cox proportional hazards model, was performed to study the effects of disease parameters on survival. Several documented disease syndromes, idiopathic hemorrhagic vasculopathy and superficial necrolytic dermatopathy, were found to have adverse effects on survival. In addition, this study revealed previously undocumented health problems, including dental calculus and neurologic signs, that have negative impact on survival of the captive population. Housing animals in multiple institutions over time was associated with decreased survival.

ACKNOWLEDGMENTS

I would like to express appreciation to the International Rhino Foundation for funding this research and for recognizing the need for these studies. I also would like to thank the many people at the zoos that participated in this study, including keepers, curators, veterinarians, nutritionists and registrars. Without their knowledge and input this study would not have been possible.

I wish to express my sincere thanks and appreciation to the faculty, staff and graduate students of the Department of Veterinary Preventive Medicine. Dolores Fischer and Cindy Cheely were particularly helpful in teaching me the intricacies of the graduate school system and keeping me on track. I would like to give special thanks to Paivi Rajala-Schultz and Julie Funk for their strong support and advocacy for the graduate students in our department, and for creating new and challenging learning opportunities in veterinary epidemiology. Thanks to Alison Hurwitch and Holly Monke, classmates through all of those statistics and epidemiology courses across the river, you both made the classes far more enjoyable than I thought possible. I also appreciate the support and comraderie of fellow graduate students Cheyney Meadows and Audrey Torres. Our discussions of epidemiology and data analysis were an invaluable part of my education.

I am especially grateful for the guidance of my advisors and members of my graduate committee. I appreciate your careful balance between allowing me free-rein without providing enough rope for me to hang myself. I am grateful to Evan Blumer and

Eric Miller for their foresight in initiating this program, as well as for their support throughout the past four years. Julie Funk not only taught me about epidemiology and statistics, she shared her grantsmanship skills and knowledge. Tom Wittum introduced me to Popper, Hill, and Snow, among others, and led many a lively discussion on the philosophy of epidemiology. I am especially grateful for the support and guidance of Bill Saville, who always had faith that we would find interesting and important information through this work. Thanks for your tolerance of my skepticism and for teaching me the tools needed to continue further research in this field.

I would also like to acknowledge the assistance and friendship of Lindsey Long. I promise that the many long hours you spent entering white rhino and greater one-horned rhino data as a work-study student will not be in vain. I am grateful to Krista LaPerle and Bruce LeRoy for their support and friendship, as well as their mentoring skills. They both were always willing to listen to my musings on the possible etiologies of the various black rhino diseases, and often provided insight into possible mechanisms and biochemical pathways that I never would have considered.

Most importantly I would like to acknowledge the support of my family. I would not have completed my studies without them. Thanks to my husband, J. Larkin, for your tolerance and patience while I pursued this research, to my parents, Vincent and Patricia Dennis for the never-ending moral support, and to my children Caitlin and Phillip, for reminding me that there are many reasons to save the black rhinoceros.

VITA

1987	Bachelor of Science, Davidson College, Davidson, NC
1988	Masters of Studies in Law, Vermont Law School, S. Royalton, VT
1989-1991	Grants Administrator, Vermont Housing and Conservation Board, Montpelier, VT
1996	Doctor of Veterinary Medicine, NC State University, Raleigh, NC
1996-1997	Intern, Large Animal Medicine and Surgery, NC State University
1997-2000	Resident, Wildlife and Zoological Medicine, University of Florida
2000-present	Research Fellow, The Ohio State University

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FIELDS OF STUDY

Major Field: Veterinary Preventive Medicine

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

There were more than 100,000 black rhinoceros living in the wild in Africa in the 1960s.[1] By 1995, this population had declined to 2,410.[2] 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.[1] Curtailing the depredation of wild black rhinos by anti-poaching measures is a very expensive strategy, with long-term success jeopardized by declining budgets.[1] 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.[3] 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. To date there has been no systematic approach to the causation of any of these unusual syndromes. It is also 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.[3]

Demographically, the captive population also faces serious challenges. While the captive population has adequate effective founders to be sufficiently representative of the genetic diversity in the wild,[1] the captive breeding record is not good. Many of the wild-caught animals in captivity have never reproduced. In addition, there is an apparent skewing of the natal sex ratio favoring males. A skew of the sex ratio can have dire consequences in a captive management situation in which housing for these large animals is limited.

To date there has been no systematic review of the health of the captive black rhinoceros population in the United States. The lack of controlled investigations has forced zoo veterinarians to rely on case reports and anecdotal information in managing the health of these animals in captivity. The goal of this research is to describe the current health and reproductive status of the black rhinoceros in captivity in the United States. Analysis of this information should reveal risk factors associated with the increased morbidity and mortality seen in the captive population. Finally, analysis of the birth records in the captive population will determine the presence of a skewed natal sex ratio and risk factors associated with this pattern. First, however, it is important to briefly review the five purported major disease syndromes of captive black rhinoceros:

hemolytic anemia, leukoencephalomalacia, superficial necrolytic dermatitis, idiopathic hemorrhagic vasculopathy, and toxic hepatopathy.

1.2 Hemolytic anemia

Hemolytic anemia syndrome is frequently cited as one of the leading causes of death in captive black rhinos.[4] Mortality is high with this syndrome; 47 instances of hemolytic anemia have been documented in 39 animals, with a 75% mortality rate.[5] The cause of the hemolytic anemia syndrome is not known. Multiple studies of black rhino red blood cells (RBCs) found no metabolic abnormalities responsible for the hemolysis.[6-8] However, ATP levels in black rhinoceros RBCs were found to be less than 5% of levels found in human erythrocytes.[6] In addition, black rhinoceros red cells have low catalase activity as compared to humans.[9] 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 drugs or foodstuffs such as fava beans.[9] This led some researchers to hypothesize that ATP deficiency could be responsible for hemolysis in black rhinoceros under oxidant stress.[5] The researchers speculated 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.[5] Research by Paglia *et al.* showed that HMPS capacity of rhinoceros erythrocytes was independent of intracellular ATP concentrations.[10] Accordingly, they concluded that limited HMPS capacity alone could not explain the hemolytic anemia syndrome seen in black rhinoceros.[10] 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 to those of human erythrocytes.[10] Paglia *et al*. offered this as a contributing factor to the supposed high susceptibility of black rhinoceros erythrocytes to oxidant-induced hemolysis.[5] They, however, failed to address the question of why black rhinoceros erythrocytes are deficient in ATP, if it is not being diverted to the HMPS to combat oxidant stress? Is this a pathologic condition or normal for this species? Could ATP deficiency in the erythrocytes explain the hemolytic anemia seen in captive black rhinoceros?

In clinical situations phosphate supplementation has been advocated to increase endogenous ATP concentrations in rhinoceros erythrocytes as a preventive measure or for therapeutic intervention.[5] Supplementation of dietary phosphate to black rhinoceroses with a variety of clinical disorders has resulted in increased erythrocyte ATP concentrations.[5] Hypophosphatemia is a well-recognized cause of hemolysis in several species.[11-13] Hypophosphatemia has been induced in cattle by reducing the phosphorus content in their feed following parturition.[14] In these cattle, hypophosphatemia occurred within 10 days of parturition, red blood cell levels of ATP decreased, and hemolytic anemia occurred. This study 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.[14] Humans with severe hypophosphatemia have anemia, decreased red cell ATP and reduced 2,3-DPG.[15-17] 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.[17] These findings warrant further consideration of hypophosphatemia and ATP deficiency of black rhinoceros erythrocytes as a cause of hemolytic anemia.

1.2.1 Vitamin E

Hypovitaminosis E has also been proposed as a possible cause of hemolytic anemia in captive black rhinoceros.[18] Low circulating levels of vitamin E cause hemolysis in humans,[19] rats,[20] and horses.[21] Researchers found lower circulating vitamin E levels in captive black rhinoceroses as compared to free-ranging animals.[22-23] 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.[24-25] Supplementation of the diet fed to captive black rhinoceros with vitamin E was advocated.[26] Recently, a study of fat soluble vitamins in blood and tissues of free-ranging and captive rhinoceros found significantly higher levels of circulating vitamin E since 1990 in captive compared to free-ranging black rhinoceroses.[27] The researchers suggest that currently there might be oversupplementation in captive animals.[27] The relationship between hemolytic anemia and vitamin E in black rhinos is still unclear.

1.2.2 Leptospirosis

Leptospirosis infection has been suggested as the underlying etiology in at least nine cases of hemolytic anemia, based on serum titers, special tissue stains, and fluorescent antibody testing.[28-29] Definitive diagnosis of leptospirosis can only be made by bacteriologic culture, a difficult and often unsuccessful procedure. Microscopic

agglutination testing (MAT) 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.[30] Diagnosis of disease is further complicated by the observation that animals can be infected with leptospires without developing clinical signs. Accordingly, the detection of leptospires or antibodies to leptospires does not necessarily correspond to the finding of the causative agent of disease.

1.2.3 Iron overload

Necropsy reports of black rhinoceros that have died in captivity cite hemosiderosis in various organs as a frequent finding. Until recently, this finding was attributed as evidence of previous hemolytic anemia. However, some research suggests an alternative hypothesis. Smith *et al*. found that serum ferritin levels (a measure of stored iron) in captive black rhinoceroses in the United States tend to increase with age or time in captivity.[31] They also found that ferritin and hepatic iron stores were significantly higher in captive black rhinoceroses as compared to white rhinoceroses.[31] 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.[31] 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 rhinos is associated with captivity was provided in a study by Kock *et al*. This study found that hemosiderosis was not seen in free-ranging black rhinoceros but was present in captive animals, in increasing amounts with longer time in captivity.[32]

Some researchers speculate that there may be an increased availability of iron in the diet in captivity, perhaps due to decreased amounts of iron-binding substances (such as tannins, phytates or polyphenols).[33] 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.[34]

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. While 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.[35] 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.[35] 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 non-heme-iron proteins. Both ferric and ferrous iron, as well as free heme, hemoglobin, myoglobin, and cytochromes, are effective in causing lipid peroxidation.[36] 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.[36]

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.[33] 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.[37] Liver fibrosis is a common pathological finding in humans and other species with iron overload, including multiple species of lemurs and many avian species.[38-40] Hemolytic anemia is not associated with iron overload in other species.

1.3 Leukoencephalomalacia

Leukoencephalomalacia was diagnosed in four female captive black rhinoceros from three different zoological institutions.[41-42] Three of the animals were calves (three weeks, 2 months, and 6 months of age) and one was a two year old animal. Evaluation of the animals for known causes of encephalomalacia, including trauma, encephalitis, clostridial enterotoxemia, and toxins failed to provide an etiologic diagnosis.[41-42] Thiamine deficiency, a common cause of polioencephalomalacia in domestic ruminants, was ruled out based on the predominance of white matter necrosis in the affected rhinos, as well as the absence of similar signs in thiamine-deficient equines.[41]

Two of the animals, the 2-month old and 6-month old calves, died within 24 hours of onset of neurologic signs.[41] The 2-year old animal developed severe neurological signs 8 days after being transferred from one zoo to another.[41] 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 euthanize the animal after 48 hours of treatment without response. In the fourth case,[42] the clinical signs were first seen when the animal was 3 weeks 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 months that followed the initial episode, the animal experienced fourteen 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 months of age the animal became anorectic and severely ataxic. It became aggressive towards familiar staff and hyperexcited at any sudden movements. The animal also developed multifocal areas of dermal ulceration on the neck, back, and flanks. The animal was euthanized.

The first three cases of leukoencephalomalacia were very similar.[41] 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. Pre-mortem hematologic evaluations of the three animals were nondiagnostic, however all three animals had increased values for creatine

phosphokinase (CPK) and other muscle-related enzymes. Hyperglycemia was detected in the 2-month-old and the 6-month-old calf, with glucosuria identified with the 6-month-old calf. The 2-year-old animal was hyperresponsive to stimuli prior to 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 sixteen separate episodes of neurologic behavior exhibited.[42] This animal also was hyperresponsive to sudden movements and became aggressive prior to being euthanized.

One study considers the involvement of excessive maternal iron in the pathogenesis of leukoencephalomalacia in the black rhino calves.[43] The researchers compared serum iron, ferritin, and transferrin saturation from the families of the affected calves with other captive black rhinos and with wild 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 4 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 (18,900 $\pm$ 7,880 ng/ml) that were approximately five to ten times greater than the mean for the comparison population of captive adult females (2,864 $\pm$ 2,638 ng/ml). It is not clear in the report why the maximum ferritin concentrations rather than the mean ferritin concentrations were compared to mean concentrations in the comparison population. These captive adult females had serum ferritin concentrations that were twenty times higher than the mean of 6 wild black rhinoceroses (133 $\pm$ 68 ng/ml). Adult males and females in the families of the affected calves had serum iron concentrations of 463 $\pm$ 195 ug/dl, with transferrin saturation of 89 $\pm$ 12%. This was compared to 20 captive adult female black rhinos with serum iron

concentrations of 232 ± 84 ug/dl with transferrin saturation of 72 ± 21% and 12 captive adult male black rhinos with serum iron concentrations of 212 ± 33 ug/dl and transferrin saturation of 63 ± 18%. Six wild adult black rhinos had serum iron concentrations of 133 ± 46 ug/dl and transferrin saturation of 28 ± 6%. Based on 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 rhinoceroses. Based on this observation, they suggest that maternal iron overload may contribute to the development of congenital leukoencephalomalacia in captive black rhinoceroses.

The authors of this paper fail to discuss the role of age in the development of iron overload in these animals. An earlier study by Smith *et al*.[31] showed increased iron accumulation in black rhinos as a function of time in captivity. In the report by Paglia *et al.*[43] the mean age of dams of affected calves was 17.5 years. The ferritin levels of these dams were compared to females whose mean age was 9.3 years. 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, is associated with the occurrence of leukoencephalomalacia in the calves.

Paglia *et al*[43] also found that a serum sample collected on the day of birth from one affected calf had a high ferritin concentration (14,590 ng/ml) whereas a male sibling of another affected calf had a serum ferritin concentration of 116 ng/ml on the day of birth. The affected calf had values within reference range for serum iron concentration (108 ug/dl) and transferrin saturation (27%). The dams of both the affected female calf and the male calf had increased serum ferritin concentrations prior to the births of the calves. The authors conclude that the high ferritin concentration in the affected calf may

have been a fetal response to maternal iron overload. They failed to discuss the possibility that ferritin, which is an acute phase inflammatory protein, may have been increased as part of an inflammatory response, not as a response to maternal iron overload. Based on the difference between the serum ferritin concentrations of a single affected female calf and the male sibling of an affected calf, the authors suggest that there is 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 may explain the reported male predominance in surviving live births among captive black rhinoceroses.

The authors refer to the leukoencephalomalacia as being a congenital problem. Upon reviewing the original descriptions of the four cases,[41-42] it seems that this disease is unlikely to be of congenital origin. The disease in each described case appears to have sudden onset, with the calves exhibiting normal behavior prior to the onset of clinical signs. The calves were affected at different ages, ranging from 3-weeks-old to 2-years-old. 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[42] 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.

1.4 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.[44] The syndrome consists of cutaneous lesions beginning as plaques, progressing to vesicles, and eventually to ulcers. The lesions are typically bilaterally symmetrical and seen on pressure points, at mucocutaneous junctions, coronary bands of the feet, along the lateral body wall and on the back.[44] 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.[44] The clinical presentation and pathologic findings in this disease resemble those of superficial necrolytic dermatitis in dogs and necrolytic erythema in humans.[44] In dogs, this syndrome is associated with a number of abnormalities, including glucagonomas (functional pancreatic islet cell neoplasms),[45] diabetes,[46] liver disease,[47] or poor diet.[48] In humans the syndrome is primarily associated with glucagonomas.[44] No similar associations have been found to date in black rhinos with this syndrome.

1.5 Idiopathic hemorrhagic vasculopathy syndrome

Idiopathic hemorrhagic vasculopathy syndrome is a recently described syndrome characterized by extensive regional swellings of the neck and limbs associated with acute, severe nonhemolytic anemia.[49] The swellings are due to 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.[50]

The report by Murray, et al[49] is a valiant effort on the part of the authors 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 at the same time to make diagnoses using tests that often have not been evaluated in the affected species.

The authors state that extensive serologic testing was done, in which serum was tested for equine, bovine, and exotic viruses but the report does not clarify what types of tests were used. It also appears that only one serological test was done per virus per animal, rather than serial testing looking for rising titers. No information is provided about the timing of the tests in the course of the disease. In addition, no information is provided with regard to the sensitivity and specificity of the various tests, in general, or particularly in reference to their use in the black rhino. Provided that the tests had been validated for use in the black rhino, and that the diagnostic test had a sufficiently high sensitivity, a negative result could be used to rule out the particular disease.[51] 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 legitimate test, negative titers can result from an animal's inability to mount an adequate immune response to the virus (due to underlying immunosuppression or compromise, improper nutrition, overwhelming viremia) or the test may have been done too early in the course of the disease, prior to the animal mounting an antibody response. Additional

forms of testing, particularly virus isolation, could have provided more insight into whether viruses play a role in this disease. While hindsight into these cases makes it seem obvious to suggest additional tests, the constraints faced by clinicians struggling to treat these cases with limited resources and few clues towards etiologies likely limited their ability to run all possible tests.

The observation that none of the rhinoceroses had a confirmed bacterial infection prior to onset of clinical signs, and 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, as does the fact that the animal's immune system likely is not at peak performance due to the underlying disease. Finding a *Streptococcus sp.* infection in any of these animals, particularly following the onset of clinical signs, would as likely be a secondary infection as an underlying cause of the disease.

Unable to find a single infectious agent as the likely cause, the authors raise the possibilities that this syndrome may be 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. It seems premature to rule out autoimmune disorders based on the negative results of these tests. Immune complex disease is currently being investigated as a possible etiology. While no circulating immune complexes were found in any of the affected animals, this could be due to insensitivity of the equine test used for detection, or that the immune complexes were not present in circulation at the time of detection. Immunohistochemistry studies that are currently in progress may 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 may 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. EPH, unfortunately, 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, while 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.

1.6 Toxic hepatopathy

There are several reports in the literature of deaths in black rhinos attributed to a toxic hepatopathy.[52-54] The first case report involved two black rhinos that had been at the same zoo for more than five years. The affected female had anemia and icterus of several months' duration prior to death and the affected male had anemia and ulcerative skin lesions scattered over its entire body.[52] 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.[52] 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, since creosote is known to cause liver and skin lesions in other animals.

The other two reports[53-54] 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 to the United States and Australia.[53] Two other animals became sick and were jaundiced, but recovered. Following 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.[54] 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 inappetance, 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 using the van den Bergh diazo methods.[54] Several animals had swellings of limbs. Fine needle aspiration of the swollen legs revealed the presence of blood.[54] Intrafascicular muscle hemorrhages and hematomata in the muscles and subcutaneous tissues were found on necropsy.[53-54] The animals that died in the United States had skin ulcerations, jaundice and lethargy prior to death. All animals were anemic and hypophosphatemic. All animals in both reports had access to creosote treated timber in the holding yards in Zimbabwe. While no definitive diagnosis was made in either report, creosote toxicity was implicated as the cause of death of these animals. While 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 occurred in Zimbabwe, why did many of the animals become ill in the United States or Australia? 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 due to creosote toxicity. While creosote has been shown to induce these lesions in some species,[53] toxicologic studies in mice found no adverse effect on the liver after being fed up to 462mg/kg/day of coal tar products for 185 days.[55] While 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 may be involved in the development of disease.

While the focus of these reports is on the hepatopathy seen in these animals, 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.[53] While the report by Schimdt *et al* did not describe any hematomas or swellings, the authors did find dermal blood vessels that were partially or completely occluded by endothelial proliferation.[52] These lesions all resemble the lesions seen with idiopathic hemorrhagic vasculopathy syndrome (*vide supra*), a new syndrome supposedly thought to have only recently occurred.

1.7 Natal sex ratio

The 2002 Rhinoceros SSP Masterplan[56] states that the skew towards males in the sex ratio of calves is a problem of major and increasing concern for the captive population in North America. A study of the possible determinants of skewed natal sex ratios in captive black (*Diceros bicornis*) and Indian (*Rhinoceros unicornis*) rhinoceros in North America found that the eastern subspecies of black rhinoceros in North America

had a skewed offspring sex ratio that favored males.[57] Some of the factors thought to influence the natal sex ratio include: maternal condition, age, and timing of insemination.

Trivers and Willard[58] suggested an hypothesis based on maternal condition to explain skewing of natal sex ratios. They hypothesized that natural selection favors parental ability to adjust the sex ratio of offspring produced based on the parental ability to invest in the offspring.[58] As adult females vary from the mean adult female condition, there is a tendency to bias the production of their young toward one sex or the other, whichever will have the greatest reproductive success. This hypothesis assumes that there is some tendency for the condition of the offspring at the end of parental investment to be maintained into adulthood. For polygynous species, the tendency is for females in good condition to produce male offspring, while females in poor condition tend to produce female offspring. In polygynous species, better adult condition affects the reproductive success of the male more than the female. The male in better condition can exclude other males from breeding, and sire many offspring himself, whereas a male in poor condition is not likely to sire any offspring since he will be driven away from the females by the better conditioned males. The female in good condition only shows moderate increase in reproductive success by producing one offspring. This hypothesis has been supported by studies in several species including several species of deer,[59, 60] sheep,[61] mice,[62] and several bird species.[63, 64]

A permutation of the maternal condition hypothesis is one that suggests maternal age influences the sex ratio. In sexually dimorphic large mammals, faster growth rates in males make them more susceptible to food shortages, both in utero and postnatally.[65] There is evidence in multiple species that male offspring are heavier at birth, born later,

and suckle more frequently and until a later age than female offspring.[57] Due to the high energy demands of male offspring, only mothers in good condition would be expected to have sufficient resources to produce surviving sons. The females of many large mammal species become reproductively active prior to achieving their adult body weight. As they reach the end of their reproductive life, their body condition diminishes. Thus, based on the maternal condition hypothesis, the sex ratio would be expected to be low in young and aging breeding females, and high in females of prime breeding age.[66]

Another suggested hypothesis to explain skewing of sex ratios is based on the timing of fertilization. This hypothesis proposes that males are produced when insemination occurs close to the time of ovulation, and females are produced when insemination occurs before or remote from the time of ovulation.[67] The rationale behind this proposal is that Y-bearing spermatozoa are more motile or better able to penetrate the membrane of the unfertilized egg, but do not survive as long as X-bearing spermatozoa.[67] Evidence supporting this hypothesis is found in several species of mammals, including white-tailed deer,[68] Barbary macaques,[69] hamsters,[70] and rats.[71]

All of these hypotheses are plausible with regard to the black rhinoceros. What remains to be determined is which, if any, hold true and how it affects reproduction in captivity for this endangered species.

Black rhinoceroses were brought into captivity to prevent their extinction. While captivity protects them from slaughter for their horns, they may have merely exchanged one threat for another. Successful management of the captive population requires a thorough understanding of the risk factors associated with morbidity and mortality. Without first understanding what has happened in the past, we cannot begin to improve

the situation for the future. What follows is a description of the current health and reproductive status of the black rhinoceros in captivity in the United States. From this information we have identified risk factors associated with health and reproductive problems in the captive population. Hopefully this information will lead to further research and management changes that will improve the health and welfare of these magnificent animals.

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CHAPTER 2

DESCRIPTIVE EPIDEMIOLOGY OF CAPTIVE BLACK RHINOCEROSES IN THE UNITED STATES

2.1 Introduction

There were more than 100,000 black rhinoceros living in the wild in Africa in the 1960s.[1] By 1995, this population had declined to 2,410. One of the justifications for captive populations is to provide a reservoir, potentially maintaining a source of animals for reinforcement or re-establishment of wild populations.[3] 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 idiopathic hemorrhagic vasculopathy that lead to increased morbidity and mortality characterize these syndromes. 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.[3]

Demographically, the captive population also faces serious challenges. While the captive population has adequate effective founders to be sufficiently representative of the genetic diversity in the wild,[1] the record of success for captive breeding is not good.

Many of the wild-caught animals have never reproduced in captivity. The purpose of this paper is to describe the current health and reproductive status of the black rhinoceros in captivity in the United States.

2.2 Methods

A survey was conducted of the American Zoo and Aquarium Association (AZA) accredited zoos with black rhinoceroses in their collections. A general survey was designed to gather information on the medical status of black rhinos in captivity. Because of the length of the survey, the need to gather information from multiple people at each institution, and the need to collect information from medical records, the survey was conducted on-site at each rhino-holding institution. The survey collected information on each individual black rhinoceros from entrance into captivity, either by birth or otherwise, until death or completion of the survey. Records of animals were reviewed for historical information, anesthetic events, preventive medical procedures, and clinical illness including signalment, diagnosis, and therapy prescribed. Hematology and biochemistry profiles were not reviewed as part of this survey. If this information was provided as part of the clinical notes in the medical record, then it was included in the survey information. All available necropsy records were reviewed for clinical diagnosis, gross post-mortem and histopathological findings, and any special tests that were performed. Information was collected as far back in time as was available at each institution. The time of the survey visit marked the final time point for data collection at each institution.

Standard descriptive statistics summarize the data. Frequency distributions of categorical variables were evaluated, and means, medians, standard deviations and ranges were calculated for continuous variables. Continuous variables were then categorized to facilitate analysis.

2.3 Results

This study surveyed 40 of the 43 (93%) facilities and included 296 of the 334 black rhinoceroses (88.9%) ever in captivity in the United States between the years 1930 and 2001 (based on the captive population through January 1, 2001). Table 2.1 describes the demographics of the study population. The population studied included two subspecies of black rhinoceros; *Diceros bicornis michaeli*, the eastern black rhino, and *Diceros bicornis minor*, the southern black rhino. The eastern black rhino has been kept in captivity in the United States for a longer period of time than the more recently imported southern subspecies. This is reflected in the fewer numbers of the southern subspecies in the study population. There are 97 wild-born and 199 captive-born animals in the population. Of these, 105 animals were alive during the time of the survey (June 2000 through Feb 2004) and 165 were dead. There were 26 stillbirths in the population; these are not included in the total of dead rhinos in the population.

Of the stillbirths, 6 were female, 15 were male, and there were 5 for which gender was not recorded. Most of the stillbirths were of the eastern subspecies (*Diceros bicornis michaeli*), with only one female, one male, and one unrecorded gender belonging to the southern subspecies (*Diceros bicornis minor*).

Table 2.2 illustrates some reproductive characteristics of the population. Of the wild-born black rhinos that have died, 43% (35/81) of them died without reproducing in captivity. Of the captive-born black rhinos that have died, 73% (61/84) of them did not reproduce. 77% (47/61) of these animals were less than 6 years of age at death. Black rhinos reach sexual maturity at approximately 6 years of age. Thus failure to reach sexual maturity prior to death is a major problem for the captive-born black rhinos. Only 22% (8/35) of the wild-born black rhinos that died were younger than 6 years of age, so failure to reach sexual maturity prior to death does not explain the lack of reproduction for the wild-born animals. Considering the entire captive black rhino population, 35% have died without reproducing (96/270).

Table 2.3 describes statistical information on the age of the wild-born and captive-born animals. Information is provided on a subset of the captive-born group, those older than 1.8 years. Captive-born animals less than 1.8 years of age were excluded to create an age-adjusted captive-born group that is more comparable with the wild-born animals, none of which was brought into the United States at an age younger than 1.8 years. The mean age of the living wild-born rhinos is significantly greater than that of the living captive-born group ($p < 0.0001$), even when compared to the captive-born >1.8 years of age ($p<0.0003$). The same is true for comparison of the mean age of those animals that have died (wildborn dead compared to captive-born dead $p<0.0001$; wildborn dead compared to captive-born dead >1.8 years old $p<0.0001$). However, direct comparison of the ages of these groups is complicated by the fact that members of the groups entered captivity at different times. Figure 2.1 portrays by decade when animals in the two groups entered captivity. Comparing the mean age of the wild-born group to that

of the captive-born animals greater than 1.8 years of age at the decade in which the animals entered captivity accounts for the differences in time in which the animals entered captivity. These comparisons are shown in Table 2.4. The mean age of live wild-born rhinos that were brought into captivity in the 1970s and 1980s is statistically greater than the mean age of live captive-born rhinos born in corresponding decades (1970 p= 0.03; 1980 p=0.04; one-tailed t-test with alternative hypothesis: $mean_{wild} > mean_{captive}$). Similarly, with regard to rhinos that have died, the wild-born rhinos brought into captivity in the 1980s and 1990s had a mean survival significantly greater than that of age-adjusted captive-born rhinos, born during those decades (1980 p=0.003, 1990 p=0.05; one-tailed t-test with alternative hypothesis: $mean_{wild} > mean_{captive}$). Looking at the two groups wild-born and captive-born separately over time reveals a decline in mean age over time for both groups. Table 2.5 summarizes the age of wild-born and captive-born groups by the decade they entered into captivity, by birth or otherwise. Table 2.6 summarizes the number of animals brought into captivity by decade. Among the black rhinos that died, there is a steady decline over time in the average survival for both the wild-born and captive-born animals. The mean survival of wild-born animals entering captivity in the 1930s was 34.56 $\pm$ 7.44 years, that decreased by the 1960s to 21.01 $\pm$ 10.13 years, and in the 1980s to 17.23 $\pm$ 3.18 years. A similar decline is seen in the captive-born group.

These data indicate that wild-born animals survive longer in captivity than captive-born animals. They also demonstrate that there is a decrease in the survival of both wild-born and captive-born animals over time.

Of the 199 animals born in captivity, there were 26 stillbirths and 22 animals that survived less than 1 year. Comparison of the mean age at time of calving of dams that had stillbirths (17.69 $\pm$ 9.44 years) to dams that had live calves (14.53 $\pm$ 6.67 years) showed no statistical difference in the mean ages of the dams (two-tailed t-test, p=0.110). Of the stillbirths, 15 were male, 6 were female, and 5 were unknown gender. It appears that increased age of the dam does not play a role in the occurrence of stillbirths in this population.

Review of the medical records of the captive population of black rhinos revealed some unusual clinical findings. These findings are outlined in Table 2.7. While bloodwork was not specifically reviewed as part of this study, if hematology or serum biochemistry abnormalities were mentioned in the clinical record of the animal, notation was made of this finding. Hypercalcemia was noted in the records of 20 animals, and hypophosphatemia was noted for 34 animals in this survey. Fifteen animals had moderate to severe dental calculus. Seven animals had teeth that were loose enough to extract manually. Ectopic mineralization was diagnosed on necropsy in 25 animals.

Skin lesions were noted on 57 animals in this study. The description of the lesions was consistent with superficial necrolytic dermatitis, but skin biopsies were not taken in all cases. Neurologic signs including confusion, odd behavior (such as unusual vocalization, appearing dazed, or uncharacteristic aggression), ataxia, tremors or convulsions were described in 37 animals. Jaundice was detected clinically on 12 animals, and hepatic lesions including hepatitis, hepatic necrosis or cholestasis, were found on necropsy of 34 animals.

Lameness of undetermined cause was diagnosed in 50 animals. Swellings of limbs or shoulders with no determined etiology were noted in 38 animals. Lesions consistent with idiopathic hemorrhagic vasculopathy, that is anemia, swelling of limbs or shoulders, and subcutaneous pooling of blood or serosanguinous fluid, were found in 20 animals. Necrosis or sloughing of the distal tail occurred in 13 animals, and 21 animals had loose horns. Neither the tail-sloughing or loose horns were directly attributed to trauma.

Figure 2.2 shows the occurrence over time of several major disease syndromes, including hemolytic anemia, idiopathic hemorrhagic vasculopathy syndrome (IHVS), hepatic cholestasis (also referred to as “toxic” hepatopathy), and soft tissue mineralization. The numbers of cases and the total number of animals in the captive population during the different time spans are listed in Table 2.8. The one case of hemolytic anemia that occurred in the time period 1991-1995 was associated with survival. The case that occurred in the time period 1996-2000 followed prolonged illness with the hemolytic crisis occurring as the terminal event. All of the earlier cases were episodes of primary hemolytic anemia and were fatal.

The cases of hepatic cholestasis had marked deposition of brownish-yellow pigment in the hepatocytes. Special staining for bilirubin indicated that the pigment was bile and not hemosiderin. Cases in which the pigment was not identified as bilirubin were not included as cases of hepatic cholestasis. The cases of idiopathic hemorrhagic vasculopathy syndrome (IHVS) were characterized by massive swelling of distal limbs or shoulders with swelling attributed to the pooling of blood in the subcutaneous and muscle tissues. There is a nonhemolytic anemia. Of the animals that died, the muscle tissues

were red/black edematous tissues with destruction of the normal muscle architecture caused by pressure created from the pooling of hemorrhagic fluid. Six animals had lesions consistent with both hepatic cholestasis / hepatopathy and IHVS. The clinical signs and lesions consistent with the two syndromes in these six animals are listed in Table 2.9.

Soft tissue or cardiovascular mineralization occurred in 25 animals. Animals with renal lesions significant enough to cause uremia and resultant metastatic calcification were excluded from classification into this group. There were 21 animals with cardiovascular mineralization and 13 had mineralization of internal organs other than the heart. The affected organs included lungs, spleen, skeletal muscle, skin, organs of the gastrointestinal tract, mammary gland, and testes. Nine animals had both cardiovascular and soft tissue mineralization. No difference was found in the mean age of the two groups ($P=0.38$, two-tailed t-test assuming unequal variances). There was a significant difference in the mean age of all animals with mineralization as compared to animals without mineralization ($P = 0.001$, two-tailed t-test assuming unequal variances). The ages of these groups are shown in Table 2.10.

2.4 Discussion

This survey reports on 88% of the black rhino population in captivity in AZA accredited zoos between 1930 – 2001. The major observations of this study are: 1) wild-born animals have greater survival in captivity than captive-born animals; 2) there is no difference in age of dams with stillbirths and dams with live offspring; 3) cases of

primary hemolytic anemia peaked in the years 1976 - 1980 and do not currently represent a major health problem; 4) idiopathic hemorrhagic vasculopathy has occurred in the captive black rhino population prior to 1995 and in animals living in regions other than Texas; 5) hepatic cholestasis in captive black rhinos may be associated with causes other than creosote toxicity; and 6) ectopic mineralization is increasing in occurrence over time in the captive population.

Captivity presents the population with its own unique threats to survival. Results of this study indicate that the average age of black rhinoceroses in captivity in the United States is declining over time. Additionally, the animals that were born in the wild and brought into captivity live longer than those that are born in captivity. This suggests that some factor of captive management may be affecting longevity in these animals, and that management is not improving over time.

Cases of hemolytic anemia appear to have peaked in the time period from 1976 to 1980. The two cases that occurred after 1990 were either ones in which the animal survived, or in which the hemolytic anemia occurred as a terminal event following a prolonged illness. Several possible causes of the hemolytic anemia seen in captive black rhinos have been investigated, including erythrocyte abnormalities,[1-4] leptospirosis,[5-6] vitamin E deficiency.[7] Changes in management practices including biannual vaccination with a five-way or six-way leptospiral bacterin[8] and supplementation of the diet with vitamin E[9] were advocated in the early 1990s as preventive measures against hemolytic anemia. Recently, a study of fat soluble vitamins in blood and tissues of free-ranging and captive rhinoceros found significantly higher levels of circulating vitamin E since 1990 in captive compared to free-ranging black rhinoceroses,[10] demonstrating the impact of

dietary vitamin E supplementation. These management changes coincide with the decline in primary cases of hemolytic anemia in captive black rhinos. Whether this is coincidence or effect is unclear.

Idiopathic hemorrhagic vasculopathy (IHVS) is a recently described disease of captive black rhinoceros,[11] characterized by acute swelling of the shoulders, neck, hips or limbs, a vasculopathy and acute non-hemolytic anemia. This study identified additional cases of IHVS, including cases that occurred prior to 1995, when the disease was first identified.[11] Cases were also identified from geographical regions outside of Texas, where 6 of the 7 reported cases originated.

Cholestatic hepatopathy was identified in 16 animals. This hepatopathy was characterized by marked deposition in the hepatocytes of a yellow-brown pigment identified as bile. The animals were jaundiced and many had bilirubinuria. Previously a similar hepatopathy was described in several reports,[12-14] including a report of animals recently exported from Zimbabwe. In these reports, the hepatopathy was identified as a toxic hepatopathy and was attributed to, but not definitively diagnosed as creosote toxicity. Three of the animals identified in this study with hepatopathy were part of the group imported from Zimbabwe in 1992 and thus were exposed to creosote in the holding pens in Zimbabwe. The two animals described by Schmidt *et al* are also included in this study. Other than with these five animals, creosote toxicity was not implicated in any of the cases identified in this study, although a telephone pole in the yard of one animal did test positive for phenol, a component of creosote. Investigation into other causes of hepatocellular cholestasis besides creosote toxicity that might be affecting captive black rhinos is needed.

In the previously published case reports, several animals were described as having swollen limbs, and fine needle aspiration of these swellings revealed only blood.[13] While the report by Schmidt *et al* did not describe any hematomas or swellings, the authors did find dermal blood vessels that were partially or completely occluded by endothelial proliferation.[12] These lesions all resemble the lesions seen with idiopathic hemorrhagic vasculopathy syndrome. In the current study, we identified six animals having clinical signs consistent with both idiopathic hemorrhagic vasculopathy and "toxic" hepatopathy. Interestingly, the report of Murray *et al* that first identified idiopathic hemorrhagic vasculopathy as a syndrome in captive black rhinos, noted that liver enzyme activities and bilirubin concentrations remained within reference ranges in affected animals.[11] It appears that some animals are affected in such a way that they suffer both syndromes concurrently, whereas not all animals experiencing one syndrome necessarily contract the other. Further research is required to elucidate risk factors associated with development of either or both syndromes. This study clarifies that idiopathic hemorrhagic vasculopathy is a newly recognized, not an emerging disease of captive black rhinos.

Ectopic calcification is the occurrence of inappropriate biomineralization of soft tissues.[14] With uremia or other metabolic abnormalities that cause a systemic mineral imbalance there is pronounced etopic calcification that is referred to as metastatic mineralization.[15] In the absence of a systemic mineral imbalance, ectopic calcification is commonly called dystrophic mineralization.[15] Tissue trauma, inflammation, and disease can all result in dystrophic mineralization.[14] Cardiovascular tissue, skin, kidney, and tendons are tissues often affected by dystrophic mineralization. The occurrence of ectopic mineralization appears to be rising over time. Hypercalcemia and hypophosphatemia

have also occurred in the captive black rhino population. In light of these findings, investigation into possible causes of mineral imbalance that would result in ectopic calcification in captive black rhinos is warranted.

This study reveals several impediments to the sustainability of the captive black rhinoceros population in the United States. The decline in survival of both the wild-born and captive-born animals over time and the decreased survival of captive-born as compared to wild-born animals indicate a problem in management of these animals in captivity. In addition, the loss of 35% of the population prior to their contributing to the genetic composition of the population is a significant loss.

An interesting question raised but unanswered by this study is whether some management change occurred in the 1980s that resulted in the decline of occurrence of primary hemolytic anemia and an increase in the occurrence of idiopathic hemorrhagic vasculopathy and ectopic mineralization. With the small number of cases it is difficult to determine whether this temporal trend is real, investigation into management changes that occurred during this time period could reveal additional information. Unfortunately, records of historic husbandry and nutrition protocols were not available at most of the zoos surveyed, so associations with management changes during this time could not be investigated.

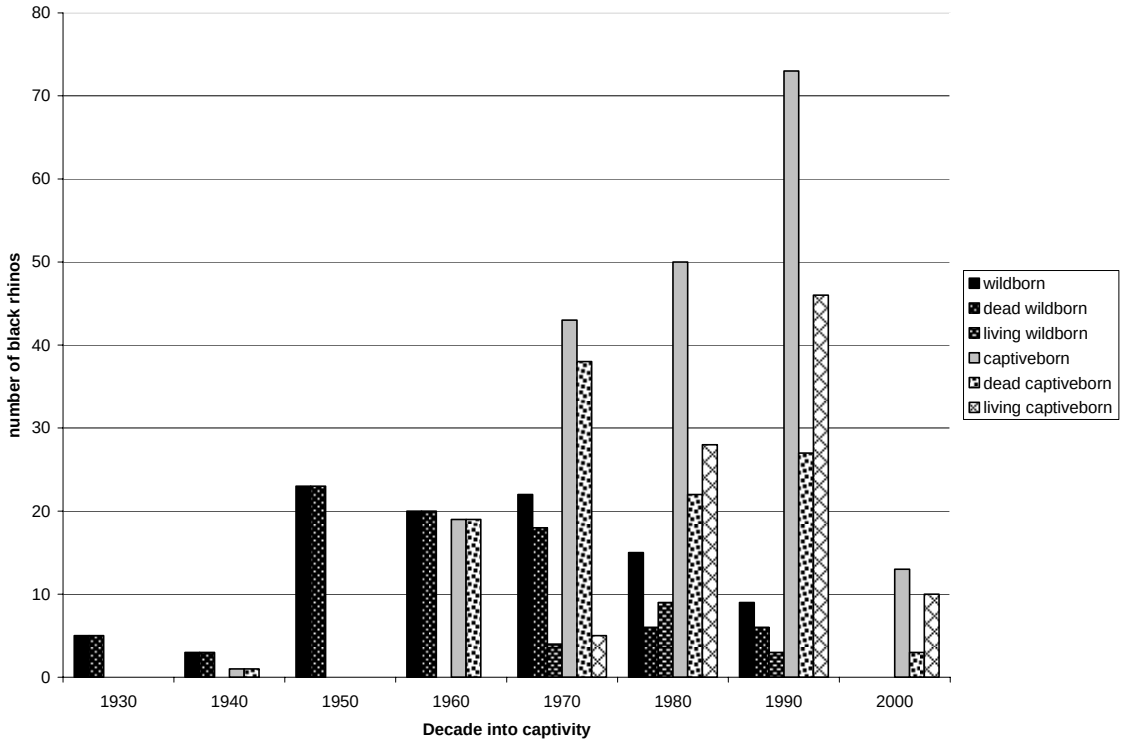

Figure 2.1 Decade into captivity for all black rhinos

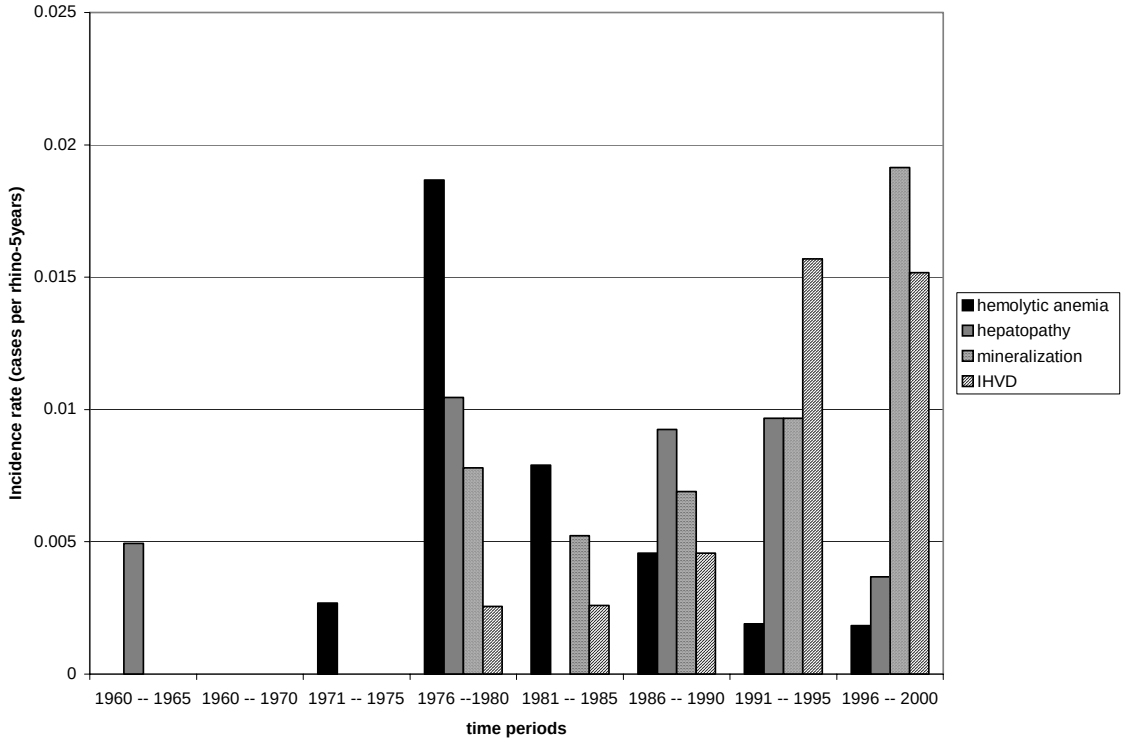

Figure 2.2 Incidence rate of disease syndromes over time

	n		n
Total animals	296	Total living	105
		wild-born	16
Diceros bicornis michaeli	244	captive-born	89
Diceros bicornis minor	52		
		Total dead	191
Total female	134	wild-born	81
Diceros bicornis michaeli	111	captive-born	84
Diceros bicornis minor	23	captive stillbirths	26
Total male	157	Stillbirths	26
Diceros bicornis michaeli	129	female	6
Diceros bicornis minor	28	male	15
		unrecorded gender	5
5 stillbirths of unrecorded gender			
Diceros bicornis michaeli	4	Total without offspring (excluding stillbirth animals)	149
Diceros bicornis minor	1		
		wild-born	35
Total wild-born	97	captive-born	114
Diceros bicornis michaeli	75		
Diceros bicornis minor	22	Total with offspring	121
		wild-born	62
Total captive-born	199	captive-born	59
Diceros bicornis michaeli	169		
Diceros bicornis minor	30		

Table 2.1 Descriptive statistics

	No offspring produced		***Offspring produced***	
	living	dead	living	dead
Diceros bicornis michaeli	35	88	38	60
Diceros bicornis minor	18	8	14	9
wildborn black rhinos	0	35	16	46
captive-born black rhinos	53	61	36	23

Age of black rhinos that have died without reproducing

	wild-born	captive-born
<6 years old	8	47
6 -- 10 years	3	6
>10 -- 18 years	8	7
>18 years	16	1

Table 2.2 Offspring produced by birth status and subspecies

	living wild-born	All living captive-born	living captive-born >1.8 years of age
N	16	89	79
Mean	19.94	9.53	10.64
standard deviation	8.02	6.42	5.96
Median	18.81	8.35	9.73
25 percentile	14.38	4.48	5.28
75 percentile	27.54	13.77	15.28
Range	6.59 -- 32.83	0.003 -- 24.90	2.51 -- 24.90

	dead wild-born	dead captive-born (excluding stillbirths)	Dead captive-born > 1.8 years of age
N	81	84	56
Mean	20.23	7.945	11.73
standard deviation	12	9.047	8.92
Median	19.42	3.12	9.77
25 percentile	11.03	0.48	3.12
75 percentile	27.78	13.6	16.68
Range	1.88 -- 49.40	0.0028 -- 32.61	1.83 -- 32.61

Mean age of living captive-born is significantly different from living wild-born as determined by t-test assuming unequal variances $P<0.0001$

Mean age of living captive-born >1.8yrs of age is significantly different from living wild-born $P<0.0003$

Mean age of dead captive-born and captive-born > 1.8yrs of age differ significantly from the mean age of dead wild-born ($P<0.0001$)

Table 2.3 Age in years of living and dead black rhinos by wild-born versus captive-born

t-Test: Two-Sample Assuming Unequal Variances			H1: uwild>ucaptive
decade	wildborn dead	captive born dead >1.8yrs	P-value (one-tailed) t-test
1960	21.01	17.92	0.20
1970	14.09	12.02	0.24
1980	17.23	9.99	0.003
1990	11.18	3.95	0.05
	wildborn living	captiveborn living	
1970	29.77	20.04	0.03
1980	19.10	15.72	0.04
1990	9.34	6.46	0.09

Table 2.4 Comparison of mean ages of wild-born and captive-born black rhinos

Dead

decade	wildborn dead	std dev	captiveborn dead (excluding stillbirths)	std dev	captive born dead > 1.8 yrs	std dev
1930	34.56	7.44				
1940	28.78	18.81				
1950	23.30	13.21				
1960	21.01	10.13	14.04	11.75	17.92	10.36
1970	14.09	9.98	8.00	8.58	12.02	8.34
1980	17.23	3.18	5.51	6.23	9.99	5.42
1990	11.18	8.62	2.84	2.43	3.95	2.05
2000			1.27	1.72		

Living

decade	wildborn living	std dev	captiveborn living	std dev
1930				
1940				
1950				
1960				
1970	29.77	3.13	20.04	7.75
1980	19.10	4.93	15.72	3.15
1990	9.34	2.40	6.46	2.71
2000			0.98	1.00

Table 2.5 Average age by decade brought into captivity

decade	Total wildborn	dead wildborn	living wildborn
1930	5	5	0
1940	3	3	0
1950	23	23	0
1960	20	20	0
1970	22	18	4
1980	15	6	9
1990	9	6	3
2000	0	0	0
Total	97	81	16

decade	Total captiveborn	dead captiveborn	living captiveborn	dead captiveborn >1.8 yrs old
1930	0	0	0	0
1940	1	1	0	1
1950	0	0	0	0
1960	17	17	0	14
1970	45	34	5	23
1980	59	17	28	9
1990	12	13	46	9
2000	13	2	10	1
Total	173	84	89	57

Table 2.6 Number of animals into captivity per decade (excluding stillbirths)

	Number affected
Unusual clinical findings	
Hypercalcemia	20
Hypophosphatemia	34
Diarrhea of unknown etiology	67
epistaxis	47
skin lesions	57
dental calculus	15
loose teeth	7
Anemia	37
necrosis or bleeding of tail	13
loose horn	21
Neurologic signs: confusion/odd behavior/ataxia/tremors or convulsions	37
Luxating patella	10
lameness of unknown etiology	50
swelling of limbs or shoulders	38
jaundice	12
IHVS-like lesions (anemia, swelling of limbs, subcutaneous pooling of blood)	20
Necropsy findings	
hemosiderin in multiple organs	48
Hepatic lesions (hepatitis, cholestasis or necrosis)	34
pneumonia	20
fungal disease	10
cystic or hyperplastic thyroids	20
Ectopic mineralization	25
Cardiac lesions (congenital defects, myocarditis, myocardial degeneration)	17
Muscle lesions (rhabdomyolysis, hemorrhage, necrosis)	11
serous atrophy of fat	11

Table 2.7 Unusual clinical and necropsy findings

year	hemolytic anemia	IHVS	cholestasis / "toxic" hepatopathy	ectopic mineralization	Total animals in population
1960 -- 1970	0	0	1	0	82
1971 -- 1975	1	0	0	0	86
1976 --1980	7	1	4	3	106
1981 -- 1985	3	1	0	2	98
1986 -- 1990	2	2	4	3	116
1991 -- 1995	1	8	5	5	137
1996 -- 2000	1	8	2	10	142
2001 -- 2004	0	0	0	3	84
Totals	15	20	16	26	

IHVS Cases Location	Number
Texas	11
Florida	3
Illinois	2
California	1
Colorado	1
Oklahoma	1

Table 2.8 Occurrence of disease syndromes by decade

International Studbook number	lesions consistent with IHVS	lesions consistent with cholestasis / hepatopathy
66	15-May-1986 swelling at left shoulder 24-Jun-1986 Swelling on right shoulder very prominent	urine bright yellow Tested negative for phenol. Wood from telephone pole in yard positive for phenol Icteric and bilirubinuric for 5 months prior to death 17-Oct-1986 Necropsy: bile duct hyperplasia; diffuse, severe intrahepatocellular bile stasis
367	31-Dec-1989 Right hind leg is very swollen 3-Jan-1990 Right hind leg is huge 8-Jan-1990 left front shoulder now swollen; right hind leg still very swollen	31-Dec-1989 urine rusty orange color 12-Jan-1990 urine yellow 31-Jan-1990 Necropsy: generalized icterus; hepatocytes stained golden-yellow by a yellow-brown pigment. Special stain showed pigment to be bile, not hemosiderin
433	8-Aug-1997 Both shoulders domed with swelling 12-Aug-1997 right rear leg now moderately swollen 3-Oct-1997 Aspiration of swelling of left leg yields 200ml of serosanguinous fluid. Aspiration of fluid does not decrease degree of swelling 2-Nov-1997 Necropsy: Muscular hematomas, compartmental syndrome / myositis Severe necrolyzing vasculitis Neovascularization and fibrosis of skin	19-Sept-1997 Urine flamingly yellow color 29-Oct-1997 bilirubinuric 2-Nov-1997 Necropsy: Icterus, Intracellular and intracanilicular cholestasis, individual hepatocellular necrosis

Table 2.9 Cases consistent with both IHVS and cholestasis / hepatopathy

Table 2.9 Continued

International Studbook number	lesions consistent with IHVS	lesions consistent with cholestasis / hepatopathy
469	27-June-1992 Necropsy: Extensive subcutaneous intramuscular hemorrhage of medial aspect of right foreleg, medial aspect of let front leg, and shoulders bilaterally between scapulae and rib cage; also on hind legs on caudal aspect of left hind leg and on lateral aspect of right hind leg	2-June-1992 Necropsy: Liver is green in color and friable on cut surface. There is a heavy deposition of brownish-yellow pigment in hepatocytes. Special staining of pigment is positive for bilirubin.
470	12-Jun-1992 Necropsy: skeletal muscle degeneration with hemorrhage and early vascularization. Muscle fibers swollen and granular and have lost their striation	12-Jun-1992 Necropsy: hepatocytes are dramatically laden with pigment. Special staining for bilirubin is positive.
471	3-Aug-1992 Right front leg is very swollen from shoulder to foot 9-Aug-1992 Right front leg still swollen 22-Aug-1992 Necropsy: hemorrhagic masses on pleura are resolving hemorrhages that appear to have occurred secondary to vasculitis	22-Aug-1992 Necropsy:Liver--severe intracellular bile stasis

Comparison of age of animals with either soft tissue or cardiovascular
mineralization to animals without mineralization

	mineralization	no mineralization
N	25	245
mean	21.17	12.02
std dev	12.27	10.25
median	20.60	9.78
25%	14.67	3.61
75%	27.86	18.00
range	0.047 -- 48.96	0.0028 --49.4

P = 0.001 two-tailed t-test assuming unequal variances

Comparison of age of animals with soft tissue mineralization
and animals with cardiovascular mineralization

	soft tissue	cardiovascular
n	13	21
mean	18.59	21.77
std dev	8.27	12.55
median	19.42	20.60
25%	14.67	14.67
75%	24.63	27.86
range	2.29 -- 30.23	0.047 --48.96

P = 0.38 two-tailed t-test assuming unequal variances

Table 2.10 Comparison of age of animals with and without ectopic mineralization

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CHAPTER 3

SKEWED NATAL SEX RATIO

3.1 Introduction

The 2002 Rhinoceros Species Survival Plan (SSP) Masterplan[56] states that the skew toward males in the sex ratio of calves is a problem of major and increasing concern for the captive population in North America. A study of the possible determinants of skewed natal sex ratios in captive black (*Diceros bicornis*) and Indian (*Rhinoceros unicornis*) rhinoceros in North America found that the eastern subspecies of black rhinoceros in North America had a skewed offspring sex ratio that favored males.[1] A skewed sex ratio favoring males creates difficulties in managing these large, solitary, long-lived animals in captivity. Unbalanced sex ratios can compromise use of limited space in zoos. This study investigates whether there is a skewing of the sex ratio in captivity and examines risk factors that could be associated with the skewed ratio.

3.2 Methods

3.2.1 Study Design

This study used a retrospective case-control design. Captive female black rhinoceroses housed in the United States that had given birth to at least one calf of known

gender were included in the study. Information on the black rhinoceroses was obtained from the 2001 International Studbook for the African Black Rhinoceros.[2] The outcome variable considered was the birth of a male calf.

3.2.2 Statistical Analysis

Standard descriptive statistics summarized the data. Frequency distributions of categorical variables were evaluated, and means, medians, standard deviations and ranges were calculated for continuous variables. Continuous variables were then categorized to facilitate analysis.

All independent variables were screened for simple association with the outcome variable by calculating chi-square tests of homogeneity, the odds ratios (OR), and associated 95% confidence intervals (95% CI). Variables were stratified by birth status of dam (whether wild-born or captive-born) in the model building process to control for possible confounding. Variables that met a critical alpha level of 0.25 on the initial screening were included in the model construction. A generalized linear mixed model was constructed using a backward selection strategy. A generalized estimating equation (GEE) was used to account for clustering at the level of the dam, given that many of the dams were multiparous. Development of the final multivariable model used a critical alpha level of 0.05 or a change in the parameter estimate greater than 20% as criteria to remain in the model.

3.3 Results

There were 62 female black rhinoceroses of which 34 females were wild-born and 28 were born in captivity. The wild-born females produced 133 calves included in the study, of which 55 were female and 78 were male. The captive-born females produced 64 offspring included in the study, of which 30 were female and 34 were male. The births of five calves were excluded from this study because the gender of the calves was not recorded. Three of the calves of unrecorded gender were stillbirths, and two were fetuses found at the deaths of the dams. The dams of four of these calves were captive-born and one was wild-born. The mean age of the wild-born dams is 23.56 $\pm$ 9.24 years and for the captive-born dams is 17.12 $\pm$ 6.83 years. The age at calving for all dams ranged from 4.82 to 36.92 years, with a mean of 14.35 years ($\pm$ 6.51 years). The mean age at calving for wild-born females was 15.38 $\pm$ 6.93 years, with a range of 5.08 to 36.92 years. The mean age at calving for the captive-born females was 12.23 $\pm$ 4.94 years, with a range of 4.82 to 27.66 years. Table 3.1 shows the mean age of dams at calving for female calves and male calves. The dams ranged in parity from primiparous to producing 13 offspring. The wild-born dams produced twice as many calves as the captive-born dams. Only 50% of the captive-born dams produced more than one calf, and only 32% produced three or more calves. Of the wild-born dams, 82% produced more than one calf, 50% produced 3 or more calves, and 32% produced 5 or more calves. The mean age at which captive-born dams gave birth was an average of 1.62 years younger per parity than that of the corresponding parity for wild-born dams. Table 3.2 shows the mean age per parity for the two groups, as well as the percentage of each group in each parity.

Age of dam at the birth of a calf was categorized into three groups. The categories were designed to reflect young, prime breeding age, and aging dams. There are varying estimates of the age at which black rhinoceroses first reproduce,[2,3] with an age at first conception reported to range between 3.8 and 12 years. Gestation length is 14 to 16 months.[3] Based on this information, we selected 0 to 12 years for the young age group. Atkinson used the range of 6 –20 years for prime breeding age and greater than 20 for ageing dams.[2] We altered these groupings somewhat to reflect our initial decision for the young group and to more approximately group animals into age tertiles. The categories were then adjusted to reflect the younger ages of the captive-born dams. For wild-born dams the age categories were: age at birth of calf less than or equal to 12 years, between 12 years and 19 years, and greater than 19 years. For captive-born dams the age categories were: less than or equal to 10 years, between 10 years and 14 years, and greater than 14 years. Time in captivity at birth of calf was also categorized into three groups for the wild-born dams: less than or equal to seven years; greater than 7 years and less than or equal to 12 years; and greater than 12 years, approximately reflecting tertiles of the time in captivity. Offspring were categorized by subspecies as either *Diceros bicornis minor* (southern black rhino) or *Diceros bicornis michaeli* (eastern black rhino). 17 female and 24 male southern black rhino calves were born (*D.b.minor*) and 68 female and 88 male eastern black rhino calves were born (*D.b.michaeli*). The dams were also categorized by housing: whether they had spent their entire time in captivity at one institution, two institutions, three institutions, or had been housed at more than three institutions. Dams were also categorized based on whether the calves were born before or after 1990. 54 female calves and 62 male calves were born before or in 1990, and 31

female calves and 50 male calves were born after 1990. A summary of the descriptive data is provided in Table 3.3.

Initially, variables were stratified by birth status of dam (whether wild-born or captive-born) in the model-building process to control for possible confounding. With the initial model building it became apparent that variables had widely different significance at the different strata. Separate models were built that only considered either the offspring born to wild-born dams or those born to captive-born dams. Independent variables were screened for simple association with the outcome variable of male calf.

Table 3.4 contains the results of the independent variables screened for simple association with the outcome variable of male calf for the wild-born dams and Table 3.5 shows the same for captive-born dams. Of the variables evaluated for the captive-born dams, none was significant. For the wild-born dams, the variables of time in captivity and birth of offspring before or after 1990 met the critical alpha level of 0.25 and were included in the model building. Neither age at birth of calf nor subspecies met the critical alpha level; however, these two variables were forced into the model to control for them in data analysis.

The final model is presented in Table 3.6 for wild-born dams. For the wild-born dams, the variable subspecies did not meet the final critical alpha level of 0.05; however, removal of the variable caused a greater than 20% change in the estimates of the other variables, so the variable subspecies was left in the model. Table 3.7 shows the final model for captive-born dams. Of the variables evaluated for the captive-born dams, none was significant.

Calves born to wild-born dams were almost 5 times more likely to be male if the dam had been in captivity for greater than 12 years (OR=4.85, 95% CI = 1.25 – 18.74), and almost 4 times more likely to be male if the dam had been in captivity between 7 and 12 years (OR 3.80, 95% CI = 1.14 – 12.74), as compared to the referent group of those dams in captivity less than or equal to 7 years. Age of the dam at the time of the calf's birth was also significant, with dams between the ages of 12 and 19 years having only a quarter the risk of having a male calf as those dams 12 years or younger (OR=0.24, 95% CI 0.09—0.63). The variable subspecies was not significant based on a 0.05 critical alpha level but was borderline at a p-value of 0.07 (OR=2.42, 95% CI= 0.93 – 6.32).

3.4 Discussion

This study confirms that there is a skewing of the natal sex ratio favoring male calves in the captive black rhinoceros population. The study extends this observation by demonstrating that the skewed ratio is found in calves born to wild-born dams, and that increased time in captivity was associated with an increased likelihood of a male calf, whereas the age of the dam between 12 and 19 years had a decreased likelihood of a male calf. The data also suggest that there may be a trend for the southern subspecies (*Diceros bicornis minor*) to be more likely to produce male calves as compared to the eastern subspecies (*Diceros bicornis michaeli*). No associations were found to be associated with the birth of male calves to captive-born dams.

Multiple theories attempting to explain the skewing of natal sex ratios have been published in the literature. Some of the factors thought to influence the natal sex ratio include: maternal condition, age, and timing of insemination.

Trivers and Willard[4] suggested an hypothesis based on maternal condition to explain skewing of natal sex ratios. They hypothesized that natural selection favors parental ability to adjust the sex ratio of offspring produced based on the parental ability to invest in the offspring.[4] As adult females vary from the mean adult female condition, there is a tendency to bias the production of their young toward one sex or the other, whichever will have the greatest reproductive success. This hypothesis assumes that there is some tendency for the condition of the offspring at the end of parental investment to be maintained into adulthood. For polygynous species, the tendency is for females in good condition to produce male offspring, while females in poor condition tend to produce female offspring. In polygynous species, better adult condition affects the reproductive success of the male more than the female. The male in better condition can exclude other males from breeding, and sire many offspring himself, whereas a male in poor condition is not likely to sire any offspring since he will be driven away from the females by the better conditioned males. The female in good condition only shows moderate increase in reproductive success by producing one offspring. This hypothesis has been supported by studies in several species including several species of deer,[5, 6] sheep,[7] mice,[8] and several bird species.[9, 10] The biological mechanism for skewing of natal sex ratios is unknown.

A permutation of the maternal condition hypothesis is one that suggests maternal age influences the sex ratio. In sexually dimorphic large mammals, faster growth rates in males make them more susceptible to food shortages, both in utero and postnatally.[11]

There is evidence in multiple species that male offspring are heavier at birth, born later, and suckle more frequently and until a later age than female offspring.[1] Due to the high energy demands of male offspring, only mothers in good condition would be expected to have sufficient resources to produce surviving sons. The females of many large mammal species become reproductively active prior to achieving their adult body weight. As they reach the end of their reproductive life, their body condition diminishes. Thus, based on the maternal condition hypothesis, the sex ratio would be expected to be low in young and aging breeding females, and high in females of prime breeding age.[12] It has been pointed out, however, that in some species, young or nulliparous dams are in superior body condition as compared to middle-aged or multiparous females, and thus the younger dams would be more likely to produce male offspring.[13]

Another suggested hypothesis to explain skewing of sex ratios is based on the timing of fertilization. This hypothesis proposes that males are produced when insemination occurs close to the time of ovulation, and females are produced when insemination occurs before or remote from the time of ovulation.[14] The rationale behind this proposal is that Y-bearing spermatozoa are more motile or better able to penetrate the membrane of the unfertilized egg, but do not survive as long as X-bearing spermatozoa.[14] Evidence supporting this hypothesis is found in several species of mammals, including white-tailed deer,[15] Barbary macaques,[16] hamsters,[17] and rats.[18]

While the data are not available in the current study to examine the third theory in relation to the skewed sex ratio in captive black rhinos, the first two theories warrant further consideration in light of the findings of this study. The finding that there is an increased likelihood of producing a male calf with greater time in captivity for the dam

supports the theory that increased body condition of the dam increases the likelihood of producing a male calf. The regular availability of food and its better quality, without the need to travel great distances to obtain it, results in increased body condition of captive animals as compared to those in the wild. There is a clear association between increased time in captivity and production of male calves by wild-born dams. While previous research suggests that the particular characteristic of captivity that influences the production of male calves is improved body condition of the dam, this cannot be determined by this study. Determination of this particular characteristic requires further research.

The influence of age on likelihood of having a male calf supports Trivers and Willard's hypothesis[4] that dams are more likely to produce one gender of offspring during prime breeding years, and the opposite gender during the early and late breeding years. The difference, however, is that these data suggest that the tendency is to produce female calves during the prime breeding years, and male calves in the early and late breeding years.

Inclusion of the predictor variables of age at birth of calf and time in captivity at birth of calf presents a challenge because of the possibility that these variables are highly correlated. That is, the older a captive animal the longer it has been in captivity. These two variables do not completely map each other because the wild-born dams were brought into captivity at varying ages, thus greater age does not necessarily equate to a greater time in captivity.

An interesting finding of this study is the presence of a skewed natal sex ratio only in the offspring of wild-born dams and not captive-born dams. This difference may

reflect the older age of the wild-born dam population. The wild-born dam population includes more animals that have entered into the older age group that produces more male calves, whereas more of the captive-born dams are still in the 12-19 year old bracket that tends to produce more female calves. It may be that as the captive-born dam population ages, a skewing of the natal sex ratio favoring males occurs.

It is also interesting to note that the wild-born dams produced twice as many calves as the captive-born dams. Although the captive-born dams are younger than the wild-born dams, younger age alone cannot explain the discrepancy in number of offspring between the two groups, especially considering that the mean age for both groups at the time of the first calf is approximately 9 years of age. An important management question raised by this study is why the captive-born dams are not producing as many calves as their wild-born counterparts? Breeding management of the two groups could not be examined as part of this study due to inavailability of historic husbandry information. It is possible that population management decisions are the controlling factors governing the discrepancy between wild-born and captive-born calf production. It may be that the wild-born dams, representing the founder population, were bred more often than captive-born dams, thus creating a significantly larger number of calves born to wild-born dams. While this factor was not considered in this study, further investigation to determine whether management decisions are the influencing variable in the greater calf production by wild-born dams is warranted.

The American Association of Zoos and Aquariums' Rhinoceros Advisory Group 3-year plan (2002) lists the development of methods for *in utero* sex determination of fetuses as a research priority for reproductive research on all rhinoceros species.

Presumably the development of these methods would enable the early termination of pregnancies with unwanted male calves. However, with increased time in captivity as a risk factor for male calf production in wild-born dams, this strategy may not prove that effective at correcting the skewed natal sex ratio in the captive black rhinoceros population. Directing efforts to identify the characteristic of captivity that is associated with this increased risk, identifying factors affecting decreased calf production by captive-born dams, and focusing breeding efforts on the dams in the age group of 12-19 years could better serve to correct the skewed sex ratio.

	wildborn dams		**captiveborn dams**	
	female calf	male calf	female calf	male calf
Age at calving				
n	55	78	30	34
mean	14.4	16.07	11.55	12.82
standard deviation	6.54	7.16	4.66	5.18
median	13.75	14.45	10.92	11.32
25% ile	9.37	10.9	8.52	9.36
75% ile	17.48	20.61	13.94	16.12
range	5.15 -- 35.64	5.08 -- 36.92	5.74 -- 27.66	4.82 -- 24.18

	wildborn dams	
time in captivity at calving	female calf	male calf
n	55	78
mean	10.3	12.35
standard deviation	7.43	7.99
median	8.55	9.67
25% ile	4.53	6.51
75% ile	13.68	17.42
range	0.36 -- 33.56	0.30 -- 34.83

Table 3.1 Age and time in captivity at calving for wild-born and captive-born dams

Wildborn	birthorder	n	percentage of dams	mean	Std dev	median	25%	75%	range
	1	34	100	8.85	2.96	8.18	6.83	10.08	5.08 -- 17.80
	2	28	82.35	12.62	3.61	12.12	9.73	14.18	6.79 -- 20.02
	3	23	67.65	15.8	4.32	15.89	12.82	17.51	8.89 -- 26.46
	4	18	52.94	17.79	4.52	17.2	14.47	19.71	10.76 -- 27.97
	5	11	32.35	20.36	5.19	20.24	17.22	25.69	12.71 -- 28.59
	6	5	14.71	22.23	5.84	22.82	19.56	24.03	14.44 -- 30.29
	7	4	11.76	24.94	6.98	24.21	19.75	30.13	17.46 -- 33.88
	8	4	11.76	26.97	7.78	26.64	20.51	33.44	18.97 -- 35.64
	9	2	5.88	28.73	11.58	28.73	20.54	36.92	20.54 -- 36.92
	10	1	2.94	24.58					
	11	1	2.94	26					
	12	1	2.94	27.5					
	13	1	2.94	29.23					

Captiveborn	birthorder	n	percentage of dams	mean	Std dev	median	25%	75%	range
	1	28	100	9.14	3.38	8.39	6.84	10.77	4.82 -- 20.54
	2	14	50.00	11.21	1.88	11.32	9.36	12.81	8.52 -- 14.32
	3	9	32.14	13.13	2.1	13.04	11.49	14.66	10.55 -- 16.12
	4	5	17.86	15.85	1.89	16.18	14.37	16.33	13.79 -- 18.58
	5	2	7.14	17.52	1.84	17.52	16.22	18.82	16.22 -- 18.82
	6	2	7.14	21.03	2.58	21.03	19.2	22.85	19.20 -- 22.85
	7	1	3.57	20.67					
	8	1	3.57	22.67					
	9	1	3.57	24.18					
	10	1	3.57	27.66					

Table 3.2 Age of dam at calving by birthorder of offspring

	Wildborn dams			**Captive-born dams**		
	female	male	Total	female	male	Total
Age of dam at calving						
>19 years	10	25	35	2	5	7
>12 and ≤ 19 years	25	23	48	8	11	19
>0 and ≤ 12 years	20	30	50	20	18	38
Years in captivity at calving						
>12 years	18	31	49	10	16	26
>7 and ≤ 12 years	14	25	39	19	16	35
>0 and ≤ 7 years	23	22	45	1	2	3
Subspecies of dam						
Diceros bicornis minor	13	20	33	4	4	8
Diceros bicornis michaeli	42	58	100	26	30	56
Housing: number of institutions						
1 institution	28	40	68	6	12	18
> 1 institution	27	38	65	24	22	46
Offspring born relative to 1990						
On or before 1990	38	46	84	16	16	32
After 1990	17	32	49	14	18	32
Generation						
Wildborn	55	78	133			
first generation captive born				23	23	46
2nd or 3rd generation captive born				7	11	18

Table 3.3 Initial variables considered in model-building by gender of calf

Variable	Estimate	Standard Error	95% Confidence Interval		P-Value	Odds Ratio	95% Confidence Interval	
age at birth of calf >19	0.512	0.488	-0.4446	1.4685	0.2942	1.67	0.64	4.34
12<age≤19	-0.4838	0.43	-1.3267	0.359	0.2606	0.62	0.27	1.43
0<age≤12	reference							
Diceros bicornis minor	0.1155	0.3553	-0.581	0.8119	0.7452	1.12	0.56	2.25
Diceros bicornis michaeli	reference							
housed at one institution	-0.1547	0.3889	-0.9169	0.6075	0.6907	0.86	0.40	1.84
housed at two different institutions	-0.3788	0.4322	-1.226	0.4684	0.3808	0.68	0.29	1.60
housed at 3 or more institutions	reference							
offspring born before or during 1990	-0.4605	0.3376	-1.1222	0.2011	0.1725	0.63	0.33	1.22
offspring born after 1990	reference							
time in captivity >12	0.5979	0.4405	-0.2653	1.4612	0.1746	1.82	0.77	4.31
7<time in captivity ≤ 12	0.6146	0.4863	-0.3386	1.5678	0.2064	1.85	0.71	4.80
0≤time in captivity≤7	reference							

Table 3.4 Initial screening of variables for wild-born dams

Variable	Estimate	Standard Error	95% Confidence Interval			P-Value	Odds Ratio	95% Confidence Interval		
age at birth of calf >14	0.3674	0.5725	-0.7547	--	1.4896	0.52	1.44	0.47	--	4.44
10<age≤14	0.1532	0.6901	-1.1994	--	1.5058	0.82	1.17	0.30	--	4.51
0<age≤10	reference									
first generation captive-born	-0.4577	0.394	-1.2298	--	0.3145	0.25	0.63	0.29	--	1.37
second or third generation	reference									
Diceros bicornis minor	-0.1894	0.882	-1.918	--	1.5392	0.83	0.83	0.15	--	4.66
Diceros bicornis michaeli	reference									
housed at one institution	0.5171	0.8563	-1.1613	--	2.1955	0.55	1.68	0.31	--	8.98
housed at two different institutions	-0.321	0.9352	-2.154	--	1.5121	0.73	0.73	0.12	--	4.54
housed at 3 institutions	-0.0003	0.7699	-1.5093	--	1.5087	1.00	1.00	0.22	--	4.52
housed at > 3 institutions	reference									
offspring born before or during 1990	-0.1476	0.3172	-0.7693	--	0.474	0.64	0.86	0.46	--	1.61
offspring born after 1990	reference									

Table 3.5 Initial screening of variables for captive-born animals

Variable	Estimate	Standard Error	95% Confidence Interval			P-Value	Odds Ratio	95% Confidence Interval		
age at calving >19	-0.5014	0.6321	-1.7402	-	0.7374	0.4277	0.61	0.18	--	2.09
12<age≤19	-1.4189	0.4917	-2.3827	-	-0.4552	0.0039	0.24	0.09	--	0.63
0<age≤12	reference									
time in captivity >12	1.5788	0.6897	0.227	-	2.9305	0.0221	4.85	1.25	--	18.74
7<time in captivity ≤ 12	1.3358	0.6169	0.1267	-	2.5448	0.0304	3.80	1.14	--	12.74
0≤time in captivity≤7	reference									
Diceros bicornis minor	0.8835	0.4898	-0.0765	-	1.8435	0.0713	2.42	0.93	--	6.32
Diceros bicornis michaeli	reference									

Table 3.6 Final model for wild-born dams

	Estimate	Standard Error	95% Confidence Interval			P-Value
age at birth of calf >14	0.9351	0.7293	-0.4944	-	2.3646	0.1998
10<age≤14	0.2603	0.6812	-1.0748	-	1.5954	0.7024
0<age≤10	reference					
first generation captive-born	-0.4419	0.5978	-1.6135	-	0.7298	0.4598
second or third generation	reference					
Diceros bicornis minor	-0.0265	0.9554	-1.8991	-	1.8461	0.9779
Diceros bicornis michaeli	reference					
housed at one institution	0.637	0.6793	-0.6943	-	1.9684	0.3483
housed at two different institutions	-0.2318	0.8256	-1.85	-	1.3865	0.7789
housed at 3 institutions	0.0034	0.5932	-1.1593	-	1.1661	0.9954
housed at > 3 institutions	reference					
offspring born before or during 1990	-0.1925	0.5583	-1.2868	-	0.9018	0.7302
offspring born after 1990	reference					

Table 3.7 Final model for captive-born dams. No significant variables

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CHAPTER 4

SURVIVAL ANALYSIS OF BLACK RHINOCEROSES (*DICEROS BICORNIS*) IN CAPTIVITY IN THE UNITED STATES

4.1 Introduction

The captive black rhinoceros population in the United States is beset by multiple health problems not seen in the wild population. Hemolytic anemia, hepatopathy, and ulcerative dermatopathy that lead to increased morbidity and mortality characterize some of these disease syndromes. It is uncertain if these are separate disease syndromes with different etiologies or the same disease with different manifestations. To date there has been no systematic approach to examine risk factors associated with decreased survival in the captive population.

The purpose of this study is to identify disease-related risk factors associated with decreased survival in the captive black rhinoceros population in the United States. Identification of these risk factors can be used to direct research toward the factors limiting successful management of black rhinoceroses in captivity.

4.2 Methods

The data consisted of survey information collected on 270 black rhinoceroses housed in captivity in the United States in AZA accredited zoos between the years 1930 and 2001. Information on the animals was collected until the animal's death or

completion of the survey. The general survey was designed to gather information on the medical status of black rhinoceroses in captivity. The survey was conducted on-site at each rhino-holding institution. The survey collected information on each individual black rhinoceros from entrance into captivity, either by birth or otherwise, until death or completion of the survey. Records of animals were reviewed for historical information, anesthetic events, preventive medical procedures, and clinical illness including signalment, diagnosis, and therapy prescribed. Hematology and biochemistry profiles were included only if this information was provided as part of the clinical notes in the medical record. All available necropsy records were reviewed for clinical diagnosis, gross post-mortem and histopathological findings, and any special tests that were performed. Information was collected as far back in time as was available at each institution. The time of the survey visit marked the final time point for data collection at each institution.

This study surveyed 40 of the 43 (93%) facilities and included 296 of the 334 black rhinoceroses (88.9%) ever in captivity in the United States between the years 1930 and 2004 (based on the total captive population censused through January 1, 2001 according to the International Studbook for the African Black Rhinoceros[1]). The demographics of the population are described in Chapter 2.

Variables examined for their effect on survival included the animal's gender, subspecies, birthyear, whether the animal was born in the wild or captivity, and the number of institutions at which the animal was housed. Clinical findings that were examined included hypercalcemia, hypophosphatemia, jaundice, diarrhea of undetermined etiology, epistaxis, skin lesions, dental calculus, loose teeth, anemia, tail

sloughing or necrosis, loose horns, neurologic abnormalities including confusion, odd behavior (unusual vocalization, appearing dazed, or uncharacteristic aggression), ataxia, tremors or convulsions, luxating patella, lameness of undetermined etiology, swelling of limbs or shoulders, or clinical signs consistent with a diagnosis of idiopathic hemorrhagic vasculopathy syndrome. Cases of idiopathic hemorrhagic vasculopathy syndrome were characterized by massive swelling of limbs or shoulders with swelling attributed to the pooling of blood in the subcutaneous and muscle tissues. There is a non-hemolytic anemia. The description of skin lesions was consistent with superficial necrolytic dermatitis, but skin biopsies were not taken in all cases. Necropsy findings that were considered included the presence of hemosiderin in multiple organs, renal lesions including glomerulonephritis and glomerulosclerosis, hepatic lesions including hepatitis, hepatic necrosis, and cholestasis, pneumonia, fungal infections, thyroid cysts or hyperplasia, ectopic mineralization, cardiac lesions or abnormalities, muscle lesions including myositis, rhabdomyolysis or necrosis, and serous atrophy of fat.

Birthyear was categorized into four groups: before 1971, between 1971—1980, between 1981—1990, and after 1990. The number of institutions at which a particular animal was housed during its lifetime was categorized into three groups: 1 institution, 2 institutions, and more than 2 institutions.

4.3 Statistical analysis

Survival analysis, using the Cox proportional hazards model, was performed to study the effects of disease parameters on survival. The dependent variable was the age of the animal at time of death or censoring. A black rhino was considered censored if it

was alive at the time the survey, with censoring occurring on the date of the survey visit. Censoring means that the event (in this study, death) had not occurred by the time that the subject ceases to be observed. Data analysis was performed using a statistical software package (SAS 8.1, Cary, NC).

Time-dependent covariates were created for variables associated with clinical signs of disease and for movement to different institutions. Time-dependent covariates are those that can change in value over the course of the observation period. Inclusion of time-dependent variables assists in determining the effect of the covariate at different times during the study period. For covariates in which the variable could be detected either during the lifetime of the animal or at death, time-dependent covariates were created but only animals in which the variable was detected antemortem were considered to experience the event. When the variable was detected at necropsy, the animal was not considered to have the condition. This approach was used to minimize the chance of overestimating the hazard of death associated with experiencing the event.

Data were summarized by calculating standard descriptive statistics. Frequency distributions of categorical variables were evaluated. All independent variables were screened for their association with time to event (death). Variables that met a critical alpha level of 0.25 on the initial screening were included in the model construction. The significance of individual coefficients in the model was based on the Wald test. For covariates with multiple degrees of freedom, the log-likelihood ratio test was used to determine whether the covariate significantly improved the fit of the model. The Cox regression model was built using a backward selection strategy. Development of the final model used a critical alpha level of 0.05 or a change in the parameter estimate greater

than 20% as criteria to remain in the model. The proportional hazards assumption was tested at the level of the univariate and final model. The assumption of non-informative censoring was examined by fitting the model in 2 new situtations:[2] 1) all outcomes were set to failure (all censored observations experienced the event); and 2) all censored outcome times were extended to equal the maximum time under observation, that is, to the date of completion of the last survey. The model met the assumptions of proportional hazard and non-informative censoring.

4.4 Results

The number and frequencies of all independent variables evaluated for inclusion in this model are described in Table 4.1. A total of 270 black rhinos were included in this study, of these, 165 animals were dead and 105 were living at the time of censoring. The mean age of the black rhinos that died was 14.0 $\pm$ 12.24 years and the mean age of the living rhinos was 11.2 $\pm$ 7.63 years. A time to event curve is illustrated in Figure 4.1.

The proportional hazard assumption (that the hazard ratio is constant over time) was checked at the univariate and final model levels. The proportional hazards assumption was met at both the univariate and final model levels, as signified by a proportional hazards p-value >0.05. Meeting the proportional hazards assumption indicates that the hazard ratio of an increase in one unit of a particular covariate, while controlling for other covariates, is constant over time. The hazard can change over time, but the proportion remains constant. The hazard ratio represents the effect of one unit change in the covariate on the frequency of the outcome.[3]

Covariates that had a significant effect on time to death at the univariate level included birthyear, whether the animal had offspring, whether it was wild-born or captive-born, and the number of institutions at which the animal lived. Clinical findings that were significant at the univariate evaluation included hypercalcemia, hypophosphatemia, diarrhea of unknown etiology, epistaxis, skin lesions, dental calculus, tail necrosis or sloughing, neurologic signs, lameness, limb swelling, signs consistent with idiopathic hemorrhagic vasculopathy, and jaundice. Necropsy findings that met the initial alpha level cutoff of 0.25 included hemosiderin, hepatic lesions, cardiac lesions and muscle lesions. Table 4.1 indicates the covariates that met the initial critical alpha level cutoff and were included in the full model. A summary of the final model is provided in Table 4.2.

Gender did not have a significant effect on time to death at the univariate level, but was forced into the model because gender is a common confounding variable. Dental calculus was noted in 20 animals in this survey, but it was diagnosed at necropsy in five of these animals. To minimize the risk of overestimating the effect of this covariate on the hazard, only the animals in which dental calculus was diagnosed antemortem were considered to experience the event.

Hypercalcemia, hypophosphatemia, and dental calculus were all significant variables at the univariate level of evaluation, but dental calculus was the only one of these three variables to show a significant effect in the final model.

The final model indicates that there is a hazard ratio of 1.7 associated with being a captive-born animal, that is during any given risk period, death occurs in captive-born animals at 1.7 times the rate as in wild-born animals. Animals with skin lesions die at

almost 2.5 times (HR=2.45) the rate of those without skin lesions. Animals with neurologic signs of confusion, odd behavior, ataxia or tremors are 2.5 times as likely to die as those without these signs. Animals with clinical signs consistent with idiopathic hemorrhagic vasculopathy and those animals with muscle necrosis or rhabdomyolysis both have 3 times higher risk of dying than animals without these lesions. The greatest risk in this study was found to be associated with jaundice. Animals with jaundice have 75 times higher risk of dying than animals without jaundice. Having offspring is shown to provide a protective effect, with these animals having only one third the risk of dying as animals that have not reproduced. Gender did not show a significant effect on survival.

4.5 Discussion

This study identified several risk factors associated with decreased survival time, including the presence of skin lesions, dental calculus, neurologic signs, jaundice, and signs consistent with idiopathic hemorrhagic vasculopathy. Many of the disease-related predictors examined in this study were modeled as time-dependent covariates The covariates of skin lesions, dental calculus, neurologic signs, idiopathic hemorrhagic vasculopathy signs, and jaundice were all modeled as time-dependent covariates because dates of their first occurrence were available. The covariate of muscle lesions was modeled as a fixed covariate because these lesions were detected on necropsy examinations. . When modeling disease effects on survival time and treating the disease as a time-independent, or fixed, covariate, its effect is the same both before and after the occurrence of the disease.[4] Treating disease as a time-independent covariate considers the animal to either have the disease, or not, regardless of when the disease occurred. By

modeling the predictor as a time-dependent covariate, its effect is different before and after its occurrence. Necropsy findings were considered as fixed covariates because it was not possible to determine the time at which the lesions developed. Necropsy findings were considered for inclusion in this analysis in an effort to identify whether pathology of particular organ systems is associated with decreased survival. If significantly associated with decreased survival, this could warrant development of preventive medicine practices to routinely assess these systems, as well as research into more effective means of detecting pathology to allow intervention.

The hazard ratio associated with having offspring indicates that animals that do not have offspring are almost three times as likely to die during a given time period as animals that have offspring. (Hazard ratio = 0.335 for animals with offspring) This variable, however, may reflect an indirect measurement of age, in that animals that have reproduced have lived at least long enough to reach reproductive age.

The clinical description of plaques, vesicles and ulcers that characterize the skin lesions in this study is consistent with the syndrome of superficial necrolytic dermatitis described by Munson et al.[5] The finding that animals with skin lesions are 2.5 times more likely to die than those animals without these lesions emphasizes the significant effect that this disease has on the captive population. Munson et al consider superficial necrolytic dermatitis to be the most prevalent disease afflicting captive black rhinoseres, with approximately 50% of the population affected. Our study found 57 animals affected, or 21% of the study population.

Dental calculus in captive black rhinoceroses has not been reported in the literature. Clinical signs may include difficulty with mastication, weight loss, or the presence of masticated food boluses in the animal's enclosure. The presence of dental calculus is not easily detected on a visual oral exam in an awake animal, due to the narrow oral cavity of the animal and the typical location of the calculus on the buccal surface of the molars. Information from the present study suggest that more intense efforts to detect, understand and treat dental calculus might have beneficial effects on survival.

Neurologic signs of aberrant behavior and ataxia or tremors are associated with a 2.6 fold increase risk of mortality. Unfortunately the brain is often not examined in the necropsy of black rhinos so it is unknown whether these clinical signs are associated with specific lesions in the brain. Leukoencephalomalacia has been reported in four black rhinos.[6, 7] While somewhat far-reaching, it is tempting to speculate that these clinical signs are more moderate manifestations of a similar lesion.

Jaundice is a predictor with an extremely high hazard ratio as compared to the other covariates in this model. A hepatopathy with intrahepatocellular cholestasis has been reported in association with creosote exposure in animals transported from Zimbabwe to Australia and the United States.[8, 9] There is also a case report from a zoo in the United States of two jaundiced black rhinos that died and intrahepatocellular cholestasis was found on necropsy.[10] These animals had possible exposure to creosote via a treated telephone pole in their yard. These are the only reports of mortality of jaundiced animals, and all of them were associated with possible creosote exposure and toxicity. Our study found additional cases of jaundice in captive black rhinos, and these were not

associated with creosote exposure. Given the high risk of mortality seen with the development of this clinical sign, research into the underlying pathology is warranted. Further investigation into the role that hemosiderin plays in the development of hepatic lesions is also needed. Hemosiderosis is associated with jaundice and hepatic fibrosis in humans, both in neonatal intrahepatic cholestasis[11] and in alcoholics with alcoholic liver disease.[12] The accumulation of hemosiderin in multiple organs, while frequently seen on necropsy of captive black rhinos, was not significantly associated with time to death in this model. While this variable may have dropped out of the model because it truly has no effect on survival, another possibility is that the study had insufficient power to detect the effect. While we were able to include information on 88% of the entire captive population of black rhinos in the United States in the study, not all of the medical records were complete for all animals

The finding of this study with the most serious implications for management of captive black rhinoceroses is that death is 1.7 times as likely to occur in captive-born animals as in wild-born animals. This strongly suggests that some aspect of captivity has a detrimental effect on the survival of black rhinos.

In summary, a study that includes information on 88% of the black rhinoceros population in the United States between 1930 – 2001 shows some disturbing threats to sustainability of the captive population. Several documented disease syndromes, IHVD and superficial necrolytic dermatopathy, have an adverse effect on survival. In addition, this study reveals previously undocumented health problems, including dental calculus and neurologic signs, which have negative impact on the survival of the captive population.

The most alarming finding, however, is the almost 2-fold increase in the likelihood of death for captive-born black rhinos compared to wild-born animals. Further investigation to uncover the adverse characteristics of captivity that are threatening the captive black rhinos is needed.

Variable	All Number	All Percentage	Dead Number	Dead Percentage	Censored Number	Censored Percentage	univariate level screening p-value	
Female	128	47.4	87	52.7	41	39	0.7381	
Male	142	52.6	78	47.3	64	61		
Diceros bicornis michaeli	221	81.6	148	89.7	73	69.5	0.3172	
Diceros bicornis minor	49	18.2	17	10.3	32	30.5		
							0.0037	*
<1970	74	27.4	73	44.2	1	1		
1971 -- 1980	66	24.4	54	32.7	12	11.4		
1981 -- 1990	70	25.9	27	16.4	43	41		
>1990	60	22.2	11	6.7	49	46.7		
No offspring	149	55.2	96	28.1	53	50.5	<0.0001	*
offspring	121	44.8	69	71.9	52	49.5		
wild-born	97	35.9	81	49.1	16	15.2	0.0006	*
captive-born	173	64.1	84	50.9	89	84.8		

* indicates a variable that met the critical alpha level of >0.25 at the univariate screening and was included in the initial model

Table 4.1 Summary of all categorical covariates for all black rhinos, as well as by event status

Table 4.1 Continued

Variable	All Number	All Percentage	Dead Number	Dead Percentage	Censored Number	Censored Percentage	univariate level screening p-value	
hypercalcemia absent	250	92.6	154	92.9	96	91.4	<0.0001	*
hypercalcemia present	20	7.4	11	7.1	9	8.6		
hypophosphatemia absent	236	87.4	152	91.4	84	80	<0.0001	
hypophosphatemia present	34	12.6	13	8.6	21	20		
diarrhea absent	203	75.2	135	77.8	68	64.8	0.0011	*
diarrhea present	67	24.8	30	22.2	37	35.2		
epistaxis absent	223	82.6	137	79.6	86	81.9	<0.0001	*
epistaxis present	47	17.4	28	20.4	19	18.1		
skin lesions absent	213	78.9	129	72.1	84	80	<0.0001	*
skin lesions present	57	21.1	36	27.9	21	20		
dental calculus absent	255	94.4	156	94.2	99	94.3	0.0002	*
dental calculus present	15	5.6	9	5.8	6	5.7		
Institutions							0.013	*
housed at only 1 institution	126	46.7	89	53.9	37	35.2		
housed at 2 institutions*	100	37	54	32.7	46	43.8		
housed at 3 institutions*	44	16.3	22	13.3	22	21		

* indicates a variable that met the critical alpha level of >0.25 at the univariate screening and was included in the initial model

Table 4.1 Continued

Variable	All Number	All Percentage	Dead Number	Dead Percentage	Censored Number	Censored Percentage	univariate level screening p-value	
lameness absent	220	81.5	142	83.8	78	74.3	0.0262	*
lameness present	50	18.5	23	16.2	27	25.7		
limb swelling absent	232	85.9	140	82.1	92	87.6	<0.0001	*
limb swelling present	38	14.1	25	17.9	13	12.4		
hemosiderin absent	222	82.2	117	59	105	100	0.2336	*
hemosiderin present	48	17.8	48	41	0	0		
IHVD signs absent	252	93.3	153	92.2	99	94.3	<0.0001	*
IHVD signs present	18	6.7	12	7.8	6	5.7		
jaundice absent	258	95.6	154	92.9	104	99	<0.0001	*
jaundice present	12	4.4	11	7.1	1	1		
hepatic lesions absent	254	94.1	149	89.3	105	100	0.179	*
hepatic lesions present	16	5.9	16	10.7	0	0		
tail necrosis absent	257	95.2	159	96.2	98	93.3	0.0158	*
tail necrosis present	13	4.8	6	3.8	7	6.7		

* indicates a variable that met the critical alpha level of >0.25 at the univariate screening and was included in the initial model

Table 4.1 Continued

Variable	All Number	All Percentage	Dead Number	Dead Percentage	Censored Number	Censored Percentage	univariate level screening p-value	
neurologic signs absent	233	86.3	140	82.1	93	88.6	<0.0001	*
neurologic signs present	37	13.7	25	17.9	12	11.4		
loose teeth absent	263	97.4	159	96.4	104	99.1	0.5153	
loose teeth present	7	2.6	6	3.6	1	0.9		
anemia absent	233	86.3	135	81.8	98	93.3	0.5998	
anemia present	37	13.7	30	18.2	7	6.7		
loose horn absent	249	92.2	160	97	89	84.7	0.4132	
	21	7.8	5	3	16	15.3		
luxating patella absent	260	96.3	160	97	100	95.2	0.6073	*
luxating patella present	10	3.7	5	3	5	4.8		
cardiac lesions absent	253	93.7	148	88.5	105	100	0.0659	*
cardiac lesions present	17	6.3	17	11.5	0	0		
muscle lesions absent	259	95.9	154	92.9	105	100	0.0009	
muscle lesions present	11	4.1	11	7.1	0	0		

* indicates a variable that met the critical alpha level of >0.25 at the univariate screening and was included in the initial model

Table 4.1 Continued

	All		Dead		Censored		univariate level screening
Variable	Number	Percentage	Number	Percentage	Number	Percentage	p-value
renal lesions absent	235	87	130	78.8	105	100	0.6112
renal lesions present	35	13	35	21.2	0	0	
pneumonia absent	250	92.6	145	87.9	105	100	0.6357
pneumonia present	20	7.4	20	12.1	0	0	
fungal infection absent	260	96.3	155	93.9	105	100	0.9596
fungal infection present	10	3.7	10	6.1	0	0	
thyroid cyst absent	250	92.6	145	87.9	105	100	0.3063
thyroid cyst present	20	7.4	20	12.1	0	0	
serous atrophy of fat absent	259	95.9	154	93.3	105	100	0.3084
serous atrophy of fat present	11	4.1	11	6.7	0	0	

* indicates a variable that met the critical alpha level of >0.25 at the univariate screening and was included in the initial model

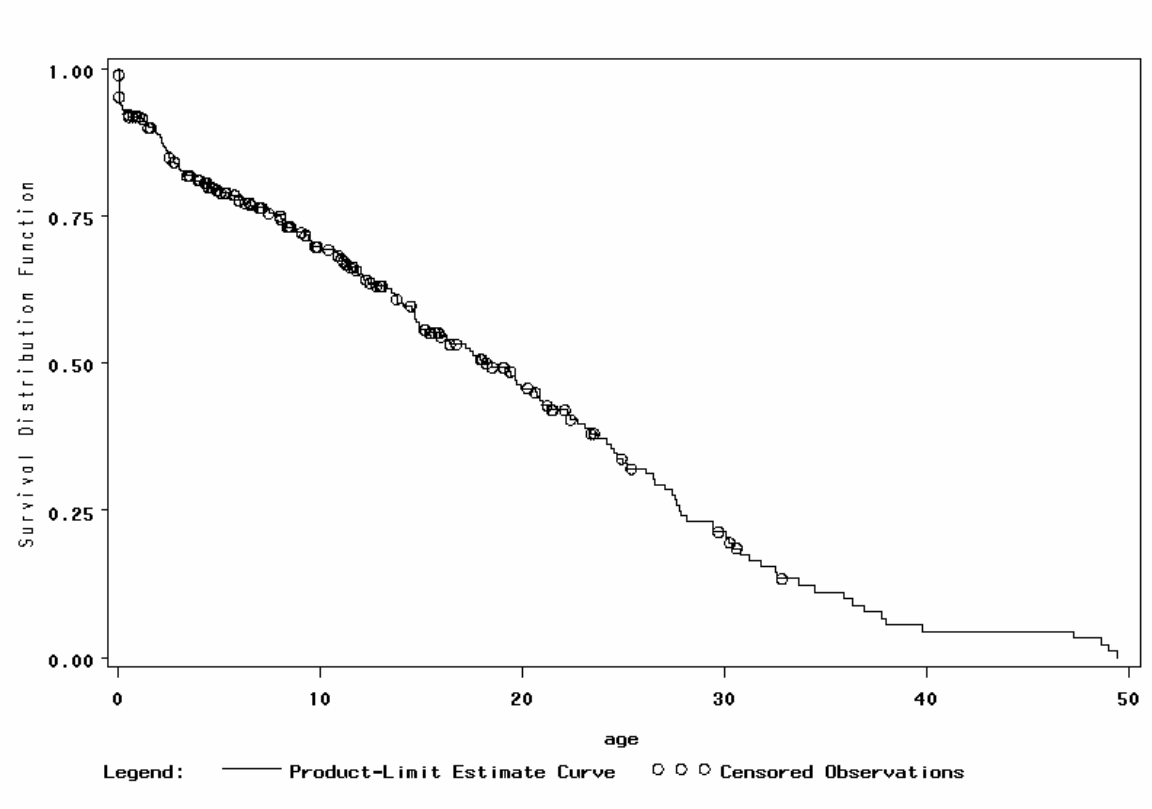

Figure 4.1. Time to event curve showing survival time in the captive black rhinoceros population in the United States between 1930—2004. Censored animals are those that were alive at the end of the study period.

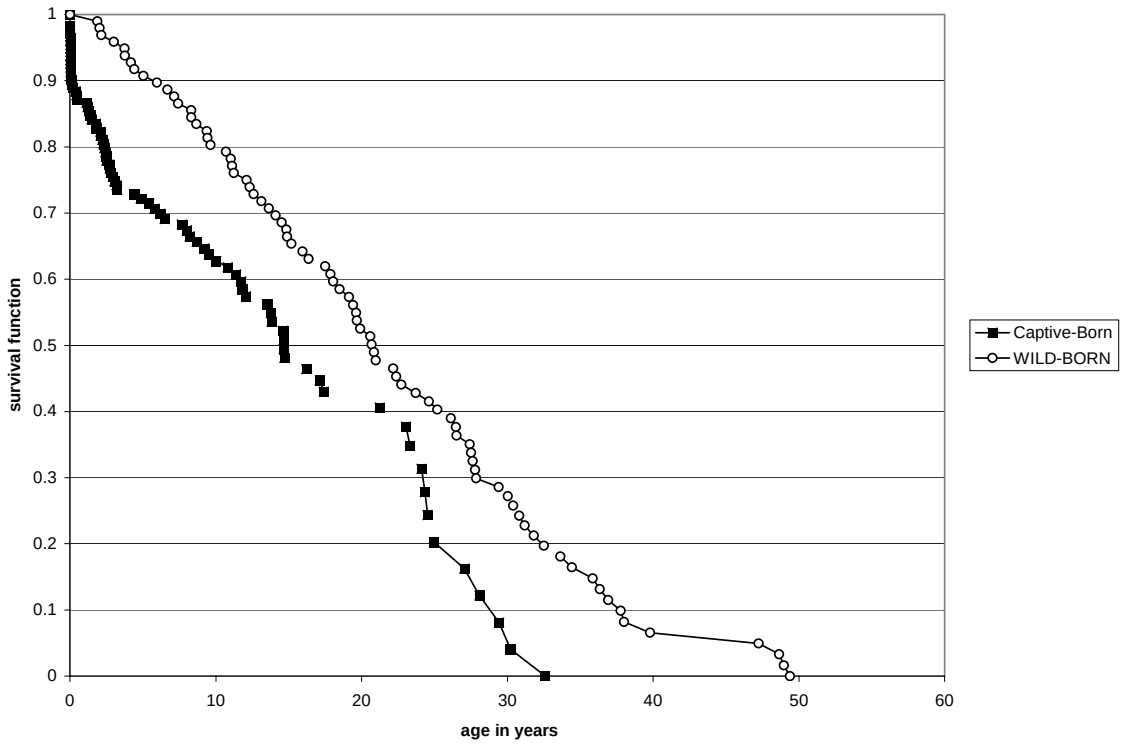

Figure 4.2 Time to event curve showing survival time for all black rhinos included in the survey, by birth status

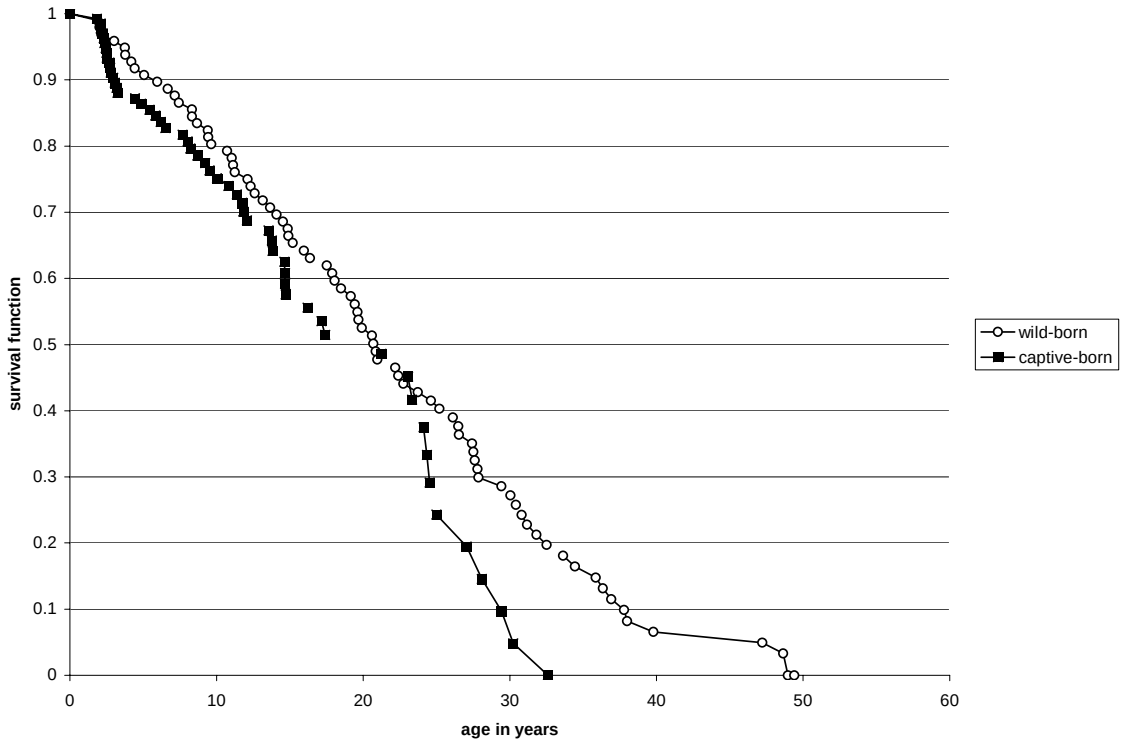

Figure 4.3 Time to event curve showing survival time for black rhinos greater than 1.8 years of age, by birth status

Covariate	DF	Coefficient	Standard Error	Hazard Ratio	95% CI lower limit	95% CI upper limit	p-value
hypercalcemia	1	0.82995	0.39738	2.293	1.052	4.997	0.0368
skin lesions	1	0.76863	0.23673	2.157	1.356	3.43	0.0012
dental calculus	1	0.8221	0.40672	2.275	1.025	5.049	0.0433
neurologic signs	1	0.96219	0.27513	2.617	1.526	4.488	0.0005
IHVD signs	1	1.23044	0.3482	3.423	1.73	6.773	0.0004
jaundice	1	3.96602	0.5659	52.774	17.407	159.999	<.0001
cardiac lesions	1	0.57139	0.27622	1.771	1.03	3.043	0.0386

Table 4.2 Summary of final model for clinical signs and necropsy findings

Covariate	DF	Coefficient	Standard Error	Hazard Ratio	95% CI lower limit	95% CI upper limit	p-value
offspring	1	-0.98473	0.17082	0.374	0.267	0.522	<.0001
gender	1	-0.2135	0.1618	0.808	0.588	1.109	0.187
captive-born	1	0.39545	0.19478	1.485	1.014	2.175	0.0423
2 institutions	1	0.66513	0.19619	1.945	1.324	2.857	0.0007
> 2 institutions	1	0.62214	0.28004	1.863	1.076	3.225	0.0263

Table 4.3 Summary of final model for all black rhinos included in survey

Covariate	DF	Coefficient	Standard Error	Hazard Ratio	95% CI lower limit	95% CI upper limit	p-value
offspring	1	-0.74417	0.18342	0.475	0.332	0.681	<.0001
gender	1	-0.10028	0.17805	0.905	0.638	1.282	0.5733
2 institutions	1	0.58025	0.1968	1.786	1.215	2.627	0.0032
> 2 institutions	1	0.72464	0.25847	2.064	1.244	3.425	0.0051

Table 4.4 Summary of final model for black rhinos greater than 1.8 years of age

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CHAPTER 5

CONCLUSIONS

5.1. Unusual disease syndromes have plagued the captive black rhinoceros population in the United States. These syndromes have resulted in increased morbidity and mortality in the captive population.

5.1.1. Cases of hemolytic anemia peaked in the time period of 1976 – 1980. Early cases were associated with acute hemolysis and death. More recent cases were either nonfatal or secondary to other diseases. The decrease in the rate of disease occurrence coincides with dietary vitamin E supplementation and vaccination against leptospirosis. Investigation into other management changes that occurred coincident with the decrease in disease rate is warranted.

5.1.2. Idiopathic hemorrhagic vasculopathy (IHVD), originally thought to be a newly emerging disease in captive black rhinos, has occurred prior to 1995. While cases were originally thought to be limited to Texas, cases consistent with the diagnosis of IHVD have occurred in Florida, Illinois, California, Colorado, and Oklahoma.

5.1.3. Hepatocellular cholestasis, previously associated with creosote exposure, has been identified in animals with no known exposure to creosote.

5.1.4. Additionally, hepatocellular cholestasis was seen in six animals with concurrent signs consistent with idiopathic hemorrhagic vasculopathy. Further research is needed to determine whether these syndromes share a similar or the same pathophysiology.

5.1.5. Ectopic mineralization, or the inappropriate biomineralization of soft tissues, has been identified in multiple animals. Ectopic mineralization is associated with multiple etiologies, including systemic mineral imbalance, tissue trauma, inflammation, or disease. The incidence rate is increasing over time in captive black rhinos. Hypercalcemia and dental calculus, both possible indicators of systemic mineral imbalance, are associated with decreased survival in the captive black rhino population. All of these factors raise the question of whether systemic mineral imbalance plays a role in the pathophysiology of the disease syndromes of the captive black rhino.

5.1.6. There appears to be a temporal trend suggesting a decline in the incidence of hemolytic anemia and an increase in the incidence of ectopic mineralization and idiopathic hemorrhagic vasculopathy. While these temporal fluctuations in the incidence of various disease syndromes raise the question of a possible association with changes in management, the lack of available historic information regarding husbandry and nutrition precludes direct investigation of such an association.

5.2. Wild-born dams produced twice as many calves as captive-born dams. Only 50% of captive-born dams produced more than one calf compared to 82% of wild-born dams producing more than one calf. Historic information was not available to determine whether the decreased calf production from captive-born dams was due to breeding decisions made as part of the management of the captive black rhino population.

5.3. There is a skewing of the sex ratio favoring male calves of calves born to wild-born dams. Calves are five times as likely to be male if the wild-born dam has been in captivity for more than 12 years. Calves are four times as likely to be male if the wild-born dam has been in captivity between seven and twelve years. Wild-born dams between the ages of 12 and 19 are four times as likely to have a female calve as are dams 12 years old or younger. No variables were significantly associated with the production of male calves by captive-born dams.

5.4. Survival analysis revealed several variables associated with decreased survival time in the captive black rhino population.

5.4.1. The variable of having offspring was associated with a hazard ratio of 0.374. It is likely that is variable reflects an indirect measurement of age, in that animals that have reproduced have lived at least long enough to reach reproductive age.

5.4.2. The decreased survival associated with captive-born animals compared to wild-born animals appears to be due to deaths of animals less than 1.8 years of age. When all animals younger than 1.8 years of age are excluded from analysis, there is no statistically significant difference in the survival of wild-born and captive-born animals. Since no wild-born animals were brought into captivity at an age younger than 1.8 years, there is no influence of deaths of wild-born animals of this age and younger on survival time for the captive population.

5.4.3. Being housed at more than one institution during an animal's time in captivity is associated with a three-fold increase in the likelihood of death in a given time period as compared to animals housed only at one institution. Further research is needed to determine what characteristics of multiple housing are associated with decreased survival.

5.4.4. Two previously described disease syndromes are associated with decreased survival time. Animals with skin lesions consistent with superficial necrolytic dermatitis are 2.2 times more likely to die than those animals without lesions. Animals with clinical signs consistent with idiopathic hemorrhagic vasculopathy have 3.4 times greater risk of dying than animals without these lesions.

5.4.5. Decreased survival time is also associated with previously undescribed risk factors, including hypercalcemia, dental calculus, neurologic signs,

jaundice, and cardiac lesions. More intense efforts to detect, understand and treat these lesions might have beneficial effects on survival for captive black rhinos.

5.5. Many variables could not be adequately assessed in this study due to lack of or incomplete availability of information concerning many aspects of husbandry and nutrition. Material was difficult or impossible to find on historic husbandry practices for many animals. While this study attempted to collect all historic information on each animal, there were incomplete records for many animals. There was also wide variation in record keeping and in diagnostic approaches over time and among institutions. Recognizing the significant influence that husbandry and nutrition have on animal health, it is vital that this information become incorporated as a routine part of medical record keeping.

5.6. It is important to acknowledge the limitations of this study when interpreting the results. It is possible that the lack of significance attributed to some of the variables considered in this study was due to inability to adequately measure the parameters, rather than a lack of significance to the health of the animals. Certain parameters, particularly those variables considered from necropsy findings, may actually show significance if we could assess them adequately ante-mortem. Further research in the development of advanced diagnostic techniques will assist in the improved detection of disease and better management of the health of the captive population. This study presents many avenues for future research. This

study, while identifying factors associated with decreased survival in black rhino population in captivity, is only a starting point in understanding the factors influencing the health of captive black rhinoceroses.

APPENDIX

Institution Data Sheet

Date:
Zoological institution:
Contacts:

Institutional information

Total number of animals:

Species of rhinoceros:

Numbers of each species:

Diceros bicornis: Ceratotherium simum: Rhinoceros unicornis:

Does this facility have a rhino restraint chute? Yes ☐ No ☐
Describe:

When was it installed?

How often is it used?
Once a week ☐ Once a month ☐ every 3 months ☐ every 6 months ☐ Once a year ☐

Biosecurity

Preshipment exam requirements:

Quarantine protocol:

Routine testing

Fecal parasite screen frequency:

Once a month ☐ Every 3 months ☐ Every 6 months ☐ Annually ☐

Not done routinely ☐

Bloodwork frequency:

Once a month ☐ Every 3 months ☐ Every 6 months ☐ Annually ☐

Not done routinely ☐

Are animals conditioned for routine venipuncture?

Yes ☐ No ☐

Are sick animals isolated or do they remain in normal enclosures?

Isolated ☐ Remain in normal enclosure ☐

What species of wildlife are the rhinos exposed to?

Birds ☐ Deer ☐ Oppossum ☐ Raccoons ☐ Fox ☐

Skunks ☐ Bats ☐ Mice ☐ Rats ☐ Other:

Are there any procedures to control pest species?

Yes ☐ No ☐

Trapping ☐ Poisoning ☐ Other ☐ Describe:

Spraying of rhinos for insects

Yes ☐ No ☐

What collection animals are the rhinos exposed to?

Where is food for rhinos stored?

Is there exposure of food to wildlife or pest species?

Yes ☐ No ☐ Describe:

Is there treatment of enclosures for pests?

Yes ☐ No ☐

Trapping ☐ Poisoning ☐ Other ☐ Describe:

Is there treatment of food storage areas for pests?

Yes ☐ No ☐

Trapping ☐ Poisoning ☐ Other ☐ Describe:

Species Data Sheet

Date:

Species:

Zoological institution:

Housing

Enclosure type

Winter:

Amount of time indoors:

Flooring:

Concrete ☐ Dirt ☐ Grass ☐ Other ☐ Describe:

Summer:

Amount of time indoors:

Flooring:

Concrete ☐ Dirt ☐ Grass ☐ Other ☐ Describe:

Is there a wallow present?

Yes ☐ No ☐

During what months is the wallow used?

Is there a pool present?

Yes ☐ No ☐

Can the animals submerge completely in the pool?

Yes ☐ No ☐

During what months is the pool used?

Ventilation system:

Cleaning:
How?

Are disinfectants used?

Yes ☐ No ☐

How often?

Daily ☐ Weekly ☐ Monthly ☐

Disposal system for waste:

On grounds ☐ off grounds ☐

Housed singly or with other animals?

Singly ☐ With same species ☐ With other species ☐

What other species?

How many animals?

Diet

	Type	Amount	Frequency Fed
Hay			
Pellet			
Browse			
Produce			
Supplements			

Fed singly or with others?

Singly ☐ With same species ☐ Mixed species ☐

Substrate on which animal is fed

Concrete ☐ Dirt ☐ Grass ☐ Other ☐ Describe:

Are feed analyses performed?

Yes ☐ No ☐

Dates of analyses?
Results?

Access to pasture?

Yes ☐ No ☐

What type of grass(es):
Describe:

Access to browse?

Yes ☐ No ☐

What type of browse:
Describe:

How is water provided?

Demand waterer ☐ Trough ☐ Pond ☐ Stream ☐ Other ☐ Describe:

What is the source of water?

Well water ☐ Surface water ☐ City water ☐ Other ☐ Describe:

Has a water analysis been performed?

Yes ☐ No ☐

When?
Dates:

Results?

Individual animal data sheet

Animal #:

Sex:

Date of birth:

Species:

Zoological institution:

Origin

Subspecies

Captive-born ☐ wild-caught ☐

If wild-caught, where?

When?

Sire:

Captive-born ☐ wild-caught ☐

If wild-caught, where?

When?

Dam:

Captive-born ☐ wild-caught ☐

If wild-caught, where?

When?

How long has the animal been at this institution?

Date of arrival:

Previous institutions:

Where?

Dates of transfer to different intstitutions:

Anesthesia

Sedation

Dates of sedation:

Why was the animal sedated?

What drugs and doses were used?

How long was the animal sedated?

Immobilization

Dates of immobilization:

Why was the animal anesthetized?

What drugs and doses were used?

Duration of anesthesia

Describe the induction

Smooth ☐ Rough ☐ Prolonged ☐ Other ☐ Describe:

Describe the recovery

Smooth ☐ Rough ☐ Prolonged ☐ Other ☐ Describe:

How was the animal monitored?

Temperature ☐ Heart Rate ☐ Resp rate ☐ pulse oximetry ☐ ECG ☐

Blood gases ☐ blood pressure ☐

arterial ☐ direct ☐

venous ☐ indirect ☐

Other ☐ Describe:

Describe any anesthetic complications

Preventative medicine

Vaccinations

Dates of vaccinations:

Type:

Frequency:

Every 6 months ☐ Annually ☐ Every two years ☐ Other ☐ Describe:

Any adverse reactions to vaccination?

Yes ☐ No ☐

When? (dates of reactions and time post-injection)

Describe:

Routine testing

Fecal parasite screen:

Yes ☐ No ☐

Once a month ☐ Every 3 months ☐ Every 6 months ☐ Annually ☐

Bloodwork:

Yes ☐ No ☐

Once a month ☐ Every 3 months ☐ Every 6 months ☐ Annually ☐

Deworming

Type of anthelmintic

Frequency

Once a month ☐ Every 3 months ☐ Every 6 months ☐ Annually ☐

As indicated by fecal egg count ☐ Other ☐ Describe:

How is it administered?

Reproductive history

How are animals paired?

Pair ☐ Herd with breeder male ☐ Herd with multiple males ☐

Housed together continuously ☐ Together only for breeding ☐ Other ☐

Describe:

Routine reproductive evaluation

Yes ☐ No ☐

Ultrasound ☐ plasma hormones ☐ fecal hormones ☐ semen collection ☐

Offspring

Animal Stud book #:

Date of birth:

Sex:

Complications/Problems

Life changes

Note time of any of these events and describe

- Weaning
- Introduction to another animal
- Separation from another animal
- Change in diet
- Restraint

- Moved to another enclosure within institution
  How was the animal moved?

Walked to nearby enclosure ☐ Conditioned to crate ☐ Caught in crate w/o conditioning ☐

Sedated and crated ☐ Immobilized ☐ Other ☐

Describe:

- Breeding
- Calving

History of illness

Illness:

Date first diagnosed:

Diagnostic test results:

Treatment:

Results:

Resolution of problem:

Death

Date:

Definitive diagnosis:

Clinical diagnosis:

Post-mortem findings
Gross necropsy findings:

Histopathology findings:

Special tests:

Tissues saved:

Who were tissues sent to?

Who did the necropsy?

Who read the histopath?

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