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RESEARCH OPPORTUNITIES ON FREE-RANGING BLACK RHINOCEROS CAPTURED FOR RELOCATION AND COLLABORATION WITH CAPTIVE RHINO RESEARCH.

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Abstract: The 1986 African Rhinoceros Workshop in Cincinnati identified a number of promising areas for biomedical research to enhance the health and long term survival of black rhinoceros (*Diceros bicornis*). These included research into diseases in general, hemolytic anemia and hepatic disease, optimal vitamin and mineral levels, and genetics. Large scale capture and relocation efforts aimed at reducing poaching losses in the Zambezi Valley of Zimbabwe and smaller efforts in Namibia presented the opportunity to take biological samples from free-ranging black rhino for comparison with captive animals. In 1989 65 sets of samples were brought into the United States and distributed to a number of researchers associated with zoos, universities and government agencies. The methods of sampling free-ranging rhino are presented. The research projects are listed and briefly discussed.

History: The black rhinoceros is in imminent danger of extinction. Numbers have plummeted since the early 1970's to less than 4000. This huge drop is primarily due to rampant poaching of rhino for their horns, which are used for dagger handles in Yemen and for oriental medicines. Zimbabwe has the largest populations of free-ranging black rhino left in the world in the vast and largely unpopulated Zambezi Valley and Escarpment. The Zambezi River is not an effective barrier to poachers from Zambia. As a result Zimbabwe must support expensive and dangerous anti-poaching patrols to save existing rhinos. In addition rhino are captured for relocation to wildlife areas within Zimbabwe and some have been placed on breeding loan to zoos. In the summer of 1989 National Park's Capture Units captured and transported 10 black rhino to the United States and 2 to Germany. In 1990 efforts will intensify.

Capture: Black rhino are captured by darting them with etorphine (3mg/adult animal), or narcotics of similar potency (fentanyl or carfentanyl) and a sedative/tranquilizer such as xylazine (100mg/adult animal) from a helicopter or from the ground (Kock 1990a). The former is much easier, safer and a more efficient use of time, but helicopter time is difficult to obtain. A dose sufficient to produce standing deep sedation is preferred but not always possible. Hyaluronidase is often used as an adjunct to speed absorption. Average immobilization time is 13 minutes, ranging from 2-20 minutes. During this time the animals often travel considerable distances and a path to the animal must be cleared for the truck and sled. If respiration rates are noticeably depressed a respiratory stimulant, doxapram, may be given intravenously in the ear vein.

It may take 20 or 30 men to move the rhino from its initial position to a sled and to secure the animal. While the sled is being dragged to the truck and while the animal is in transit to the base camp, attention to vital signs and depth of narcosis is important. The ride back to camp may take several hours and is often very rough. To evaluate the effects of pursuit and capture blood samples have been taken at the time of capture and again when the rhino arrives at base camp. Surplus serum, plasma, and red and white cells from these blood samples have been sent to various researchers in the United States. A brief review of ongoing research efforts are found below.

When the animal arrives at base camp it is offloaded into a large square pen (boma) built of Mopane logs where a narcotic antagonist is given. Reversal and arousal usually take only a few minutes. The walls are thatched to reduce sensory input. In 1989 long acting tranquilizers (Trilafon) were used to help reduce aggression and self induced trauma. They also appear to speed the acceptance of native forage and artificial feeds. Stress, trauma and injury are the major causes of post capture mortality. As rhino calm down they are increasingly weaned from a natural cut browse diet to alfalfa cubes and pellets. The later are first placed near, then in crates that will eventually be used for shipping. Free-ranging rhino are allowed to adapt to captivity for at least two weeks before crating and shipping. It may take many men pulling on a large rope and a low dose of narcotic to load the rhino. In the 1960's rhino capture mortality rates ran up to 50%. Total mortality was reduced to about 14% by the late 1980's. Of 52 animals immobilized in Zimbabwe in 1988 none died during capture (Kock 1990a).

Research: Capture Stress: A very basic research problem is how to asses the physiologic effects of capture on rhino. This was done by sampling rhino immediately at capture, several hours later before reversal, and several days to a week or two later during relocation. The initial sample, taken at capture was used to help establish physiologic normals for 9 hematologic and 24 biochemical parameters (Kock 1990b). Kock classed rhino at capture as normal (56%) or stressed (44%) and the long term outcome was followed (Kock 1990c). When black rhino were sampled at capture, just before reversal and several days to weeks later, significant differences ($P < 0.05$) were seen in cortisol, CPK, LDH, AST, glucose, BUN, creatinine, PCV and WBC to name a few parameters (Kock 1990c). These changes were thought to be due to the effects of various stressors. Nine of 52 rhino (17%) died within two months from various causes, not directly the result of initial capture, including intraspecific aggression.

Hemolytic Anemia: One of the more important disease syndromes in captive rhino has been termed "hemolytic anemia". It appears to be a fairly common cause of death and its origins are unknown. Red blood cell fragility, red cell parasites, leptospirosis, and vitamin E deficiency have been blamed.

Samples from apparently healthy free-ranging black rhino have helped to answer some of the questions about hemolytic anemia syndrome. Initial reports of excessive red blood cell fragility have not been reproducible.

Vitamin E levels in free-ranging black rhino are considerably higher than, up to ten times, those seen in captive rhino (Dierenfeld, 1988). Additionally, Namibian desert rhino living on very xeric plant species as well as Zambezi Valley rhino living in a relatively lush jungle show these same high levels (Dierenfeld, unpublished data, 1990). This suggests that supplementation of captive rhino diets with Vitamin E may be in order. The relationship between hemolytic anemia and Vitamin E levels is still speculative.

Leptospirosis: One organism that is known to cause intravascular hemolysis and hepatitis is leptospirosis. These organisms are usually passed between animals via urine contaminated water. Miller has implicated it in the deaths of at least three black rhino dying in zoos in the United States (Miller unpublished 1990). Free-ranging black rhino from the Zambezi Valley frequently have relatively high titers to a variety of leptospirosis serovars. Titers as high as 1:400 were seen in 9 of 37 (25%) in one group of animals tested (Bolin unpublished 1990). Namibian animals from a desert environment did not have significant titers. It would be logical that leptospiras are more common in some moist jungle environments than in desert environments, but the rodent fauna and other potential carriers may account for the differences between location. Leptospirosis may be a naturally occurring disease of black rhino in the Zambezi Valley but clinical cases have not yet been reported. The effects of leptospiras on free-ranging rhino and those destined for relocation need further investigation.

Hepadenoviruses: Dr. Mike Worley of the San Diego Zoo is testing serum and plasma from free-ranging rhino from Zimbabwe and Namibia for antibodies to and antigens cross reactive with hepatitis viruses. No data is available at this time.

Other Infectious Diseases: Dr. Ron Yedloutschnig of USDA APHIS Foreign Animal Disease Diagnostic Laboratory is testing black rhino serum for evidence of exposure to a number of dangerous diseases like African Horse Sickness, Foot and Mouth Disease, Rinderpest and others. No results are available at this time.

Baseline Physiology: Kock and his coworkers have documented many of the baseline metabolic and physiologic parameters of black rhino in Zimbabwe and changes that occur during capture, transport and confinement (Kock 1990b,c). Serum and plasma for additional trace element and metabolite levels were sent to Dr. U.S. Seal. No data is available from their work at this time.

may be given intravenously in the ear vein.

Genetics: Whenever wild populations decline sharply and particularly when captive breeding strategies may become part of species survival, genetic questions come to the forefront. The basic questions involve how much heterozygosity is present in natural populations, is there evidence of distinct sub-species, and how can existing heterozygosity (genetic diversity) best be preserved. Although pedigrees may be used to answer these questions for captive animals, they can seldom be applied to truly free-ranging animals. Patterns of electrophoretically separated proteins and, recently, DNA sequences may give researchers some idea as to how to answer these questions for black rhino.

When 7 blood proteins coding for 12 genetic loci from Zimbabwe and Namibian black rhino (19 individuals) were separated by electrophoretic means, no differences in patterns between populations and in fact no heterozygosity was found (May unpublished report 1990). These findings agree with those of Melnick who stated "one black rhino is - genetically speaking - pretty much like another". This preliminary data should not be over interpreted and should be supplemented by additional samples and by checking additional loci. At the present time frozen sets of white and red blood cells from free-ranging black rhino are being prepared in collaboration with Raoul du Toit, Zambezi Rhino Project, WWF, for shipment to IWVS. The proteins and DNA in these will be examined by Dr. Gary McCracken at the University of Tennessee.

Summary: The opportunity to sample significant numbers of endangered free-living black rhino is precious and will likely never come again. It is an opportunity not only to learn a great deal about the physiology, genetics and diseases of rhino in Namibia and Zimbabwe, but by cooperative efforts to better understand some of the problems of black rhino in captivity. Unfortunately two of the ten rhino captured and shipped to the United States in the summer of 1989 died within a few months of a poorly understood liver ailment. Until answers to some of the research problems posed at the 1986 Cincinnati workshop are found, zoos and game farms do not appear to be a completely safe haven for this beleaguered species. IWVS is willing to cooperate with any reasonable request for samples from free-ranging black rhino that may help answer significant health questions.

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The ejaculate was measured volumetrically, the oil recorded, sperm concentration was measured, and semen

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