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POSTMORTEM FINDINGS IN TRANSLOCATED BLACK RHINOCEROSSES (DICEROS BICORNIS) IN ZIMBABWE

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Introduction

The black rhinoceros (*Diceros bicornis*) is severely threatened with extinction, having dropped from a total population in Africa of about 65 000 in 1970, to less than 4 000 at the present time (CUMMING, 1987). This decline has come about largely because of poaching practices. Zimbabwe has the largest remaining wild population of these animals, and during June and July of 1988, fifty were immobilised for relocation from the Zambezi Valley to other locations in Zimbabwe, where poaching threats would be minimal. Substantial data was collected on all fifty animals at capture, and follow-up information has been gathered since translocation.

While about 150 black rhinos are kept in captivity throughout the world in zoological gardens, they do not thrive in captivity (CUMMING, 1987). Death rates in most populations have been high, caused largely by a haemolytic syndrome, the aetiology and pathogenesis of which remain obscure at the present time (MILLER and BOEVER, 1982). Many animals show intermittent haemolysis with haemoglobinuria and anemia before a fatal haemolytic episode, although some die during an initial episode.

Materials and Methods

Thorough gross postmortem examinations were performed on all animals. Tissues were collected and fixed in 10 % phosphate buffered formalin prior to processing. The tissues were embedded in paraffin, cut at widths of six microns, and stained with haematoxylin and eosin stain. Some sections were also stained with Prussian blue stain for the identification of iron (haemosiderin).

Selected tissues were snap frozen and stored at -80 degrees centigrade. Frozen sections of liver and kidney were later stained with fluorescein-labelled antibodies to *Leptospira icterohaemorrhagiae*, serovar *icterohaemorrhagiae*; *L. pomona*, serovar *pomona*, *L. grippityphosa* serovar *grippityphosa*; *L. sejroe*, serovar *hardjo*, *L. sejroe*, serovar *sejroe*; and *L. sejroe*, serovar *sejroardi*, and examined under a fluorescent microscope. Frozen sections of oesophagus were stained with fluorescein-labelled antibodies to BVD and examined under a fluorescent microscope.

Characterisation of the streptococcal isolate was based on gram stain, haemolysis on bovine blood agar, catalase reaction, slide latex agglutination for Lancefield typing (Streptslide, Cambridge Biomedical Ltd.), and carbohydrate utilisation (OWAN, 1974).

Results

Case 1:
A female, 16 month-old animal was captured on 7-28-88 and relocated to the Karoi area on the following day. Weakness was noticed soon after relocation, and the animal died about a week later. It was noted to be an aggressive animal at the time of capture, having repeatedly charged the walls of its enclosure.

At postmortem examination, the animal was found to be in poor body condition, evidenced by protruding bony prominences and minimal visceral adipose. An unilateral mucopurulent nasal discharge was present, and a comminuted fracture of the nasal bone, adjacent to the rostral horn was found. The fractured bone was also covered with mucopurulent material, and histological sections demonstrated foci of necrosis, bacterial colonies, and inflammation. Tonsils of all peripheral and visceral lymph nodes examined were enlarged, owing to congestion, oedema, and hyperplasia. Moderate plasmacytosis was present in the spleen, and the liver was markedly congested. Approximately 40 bot fly larvae were present in the stomach, most in the sub-glandular region, and the caecum contained large numbers of *Oxyuris* sp. nematodes. Both adrenal cortices contained multiple 1-3 mm diameter calcified foci. Large numbers of *Streptococcus* sp., which were later characterised as *Streptococcus equisimilis* were recovered from the mucopurulent nasal discharge, tonsil, liver, and mesenteric lymph node.

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Case 2:

A female, 16 month-old animal was captured on 24-7-88 and relocated to the Karoi area on the following day. The animal developed lameness, with swelling and deviation of the right carpus soon after relocation, which persisted until its death on 17-8-88. Abscessation developed on the skin of the flank at the site of the dart wound, and just prior to death clinical dehydration was observed. The animal was noted to be quite aggressive at the time of capture, having chased members of the capture team before falling six feet off a cliff. At postmortem examination the animal was found to be in poor body condition. A chip fracture of the right, medial, proximal carpal bone was present and the adjacent soft tissue was oedematous and hyperaemic. The oedema and hyperaemia extended along the tendon sheaths and fasciae one third of the way up the medial aspect of the distal forelimb. Abscessation with cutaneous myiasis of the dart wound had occurred, with pus extending along fascial planes in the flank region. The nearby inguinal lymph node was enlarged, due largely to congestion and oedema. Acute congestion of the lungs, liver, and spleen was marked, and the cytoplasm of the subcapsular renal tubular epithelial cells was often scant in amount, having sloughed into the lumina. A few bot fly larvae were attached to the oesophageal mucosa, and about 40 were present in the stomach, most in the non-glandular region. Large numbers of *Streptococcus equisimilis* were recovered from the wound in the flank, but not from other sites.

Case 3:

A three year old, female black rhinoceros, which had been captured and translocated to the Pamuzinda Lodge, a 2000 hectare game ranch, in 1986, showed weakness and collapse just prior to death. Other antemortem signs of disease were not reported, but one other female died under similar circumstances a week before this animal succumbed. The two remaining animals on the property, both males, were also reported weak at about the same time as the first animal died, although they both apparently recovered. Investigation into the conditions on the game ranch revealed an almost complete absence of suitable forage for the rhinoceroses, and certainly more of them had actually invaded the lodge's veranda and eaten all of the potted plants.

Postmortem examination revealed an animal that looked to be in fair body condition outwardly, although serous atrophy of pericardial fat was present and internal adipose was scant. Fatigue, subcutaneous oedema, pulmonary congestion with patchy oedema, and moderate cerebral oedema were noted grossly. The most striking gross lesion, however, was the presence of punctate to quite large irregular foci of mucosal necrosis with pseudomembrane formation beginning in the mid-oesophageal region and extending to the cardia of the stomach. The glandular gastric mucosa also had a few blackly pigmented erosions, but no ulcers. Histologically, the oesophageal and gastric lesions were extremely bland, with minimal accompanying tissue reaction, although the submucosa was markedly oedematous and degeneration and necrosis of the muscular wall of the oesophagus were present beneath some of the ulcers. In addition, granulation tissue covered a few of the oesophageal lesions.

The spleen was extremely thin and meaty, thymus could not be located, and all lymph nodes were small. Lymphoid development was notably meager in all organs, although plasma cells were seen in some of the lymph nodes. Small to moderate amounts of haemosiderin, identified by Prussian blue staining, were present in the lung, lymph nodes, spleen, kidney, and adrenal gland, but deposition was excessive and diffuse throughout the liver. Fluorescent antibody testing of the liver and kidney of this animal for leptospiral antigens was negative for all of the six serovars used.

Discussion

Traumatic wounds, of which fractures were a part, were found in both of the two juveniles captured in 1988, and heavy growths of *Streptococcus equisimilis* were recovered from both animals. *S. equisimilis* is commonly isolated from horses, where it is considered a commensal organism of the respiratory tract. It is occasionally associated with respiratory infections in the horse and with arthritis and orchitis in swine (JURB et al., 1986). It has also been isolated from wounds, genital infections, and mastitis in equines (CARTER, 1984). Phylogenetically, rhinos and horses are both classified as perissodactylids, or odd-toed ungulates (FOWLER, 1986), perhaps not making the isolation of a typical equine bacterium surprising.

In the first case, the organism was isolated from visceral organs as well as from the fractured nasal bone. The status of the lymphoreticular system at the time of necropsy indicated an ongoing reaction to antigenic stimulation, most likely the result of the *S. equisimilis* gaining entrance to the body, which may have overwhelmed the animal terminally and resulted in death.

The lack of life-threatening lesions in the second animal, and the inability to recover pathogens from visceral organs probably rule out all but peracute infectious processes as causes of death. This animal most likely succumbed to shock secondary to dehydration and electrolyte imbalances. The clinical signs and subtle histological lesions support this.

The oesophageal ulceration in the third animal was an interesting finding. Caustic agents are capable of producing lesions of this sort, but one would expect to find similar lesions on the muzzle and in the mouth and cranial oesophagus if such an agent had been ingested. Gastric reflux can also create this type of lesion, but one would then expect to find similar ulcers in the non-glandular stomach as well, and the lesions in this region were minor. Although atypical in their mid-oesophageal location, the lesions are similar to those of BVD in cattle (JURB et al., 1985). Fluorescent antibody testing on frozen oesophageal sections, however, were negative for BVD antigens. The possibility that these ulcers were caused by botfly larvae is suggested by their presence in the two juvenile animals examined, although they were not found in this animal, either attached to the mucosa or free in the ingesta. Information on the use of anthelmintics on this ranch was not available, but they have been used on black rhinoceroses in Zimbabwe, and it is possible that the ulcers represent old sites of attachment. The surprising lack of tissue reaction to these lesions may have indicated an inability to respond due to the severe and widespread lymphoid depletion.

The widespread haemosiderosis in this third case is suggestive of a syndrome commonly reported in captive rhinoceroses, and suggests haemolysis (MILLER, 1982). The firstnamed author of this paper has examined tissues from nine other black rhinoceroses, four from zoological gardens and five from animals dying after capture and relocation in Zimbabwe in previous years. While the tissues from the captive animals regularly showed widespread haemosiderosis, only one of these other free ranging animals had this lesion, one that died in confinement five weeks after capture.

The haemolytic syndrome of captive animals has been under investigation for some time although its cause has not been elucidated (MILLER, 1987). Leptospirosis has been incriminated in some cases (DOUGLASS, 1980; MILLER, 1987), based largely on serological titres. While serology was not performed on this animal, leptospiral antigens could not be demonstrated in the tissues examined using fluorescent antibodies. Haemoparasites, including *Trypanosoma* sp., *Babesia* sp., and *Theileria* sp. have also been suspected causes for haemolysis (McCULLOCH and ACHARD, 1969), based upon parent infections in wild-caught animals that subsequently died after capture. However, evidence directly relating these infections to death is inconclusive, and it may be that these organisms are harboured in free-ranging animals without clinical significance. Vitamin E levels have been found to be significantly lower in captive rhinoceroses than in free ranging ones, another possible factor involved in their susceptibility to this syndrome (DIERKENFELD et al., 1988). Recent haematological studies have suggested an inherent haemoglobin abnormality, possibly associated with Heinz body formation and haemolysis under a variety of conditions which place the red blood cells under oxidant stress (PAUL et al., 1988). The animal described in this paper was undoubtedly under nutritional stress, given both the history of inadequate forage available on the game ranch, as well as the gross evidence for malnutrition (serous atrophy of fat), perhaps lending credence to this last hypothesis.

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Summary

Postmortem findings in translocated black rhinoceroses (*Diceros bicornis*) in Zimbabwe
Of fifty black rhinoceroses captured for relocation from the Zambezi Valley in Zimbabwe during June and July of 1988, eight (16 %) have been reported dead, and of those, two have had thorough postmortem examinations performed. Both had wounds from which large numbers of *Streptococcus equisimilis* were recovered, and *S. equisimilis* was probably directly responsible for death in one of the animals. A third animal, captured and translocated in 1986, was also necropsied. Severe oesophageal ulceration, widespread haemosiderosis and lymphoid depletion were the major findings.

Zusammenfassung

Sektionsbefunde bei umgesetzten schwarzen Nashörnern (*Diceros bicornis*) in Simbabwe
Von fünfzig im Sambesital wegen einer Umsetzung im Juni - Juli 1988 eingefangenen schwarzen Nashörnern (*Diceros bicornis*) waren acht Tiere (16 %) verstorben. Bei zwei dieser Tiere wurde eine Sektion durchgeführt. Beide Tiere wiesen Wunden auf, aus denen Streptokokken isoliert werden konnten. Bei einem Tier muß die Infektion mit *Streptococcus equisimilis* als direkte Todesursache angesehen werden. Ein drittes Tier, das 1986 eingefangen und umgesetzt worden war, wurde ebenfalls seziiert. Es zeigte Ulzerationen im Ösophagus, eine Hämösiderose und einen Lymphozytenschwund.

Résumé

Résultats des dissections chez des rhinocéros noirs (*Diceros bicornis*) au Simbabwe
Huit des rhinocéros noirs (16 %) sur les cinquante capturés dans la vallée du Sambési à cause d'un déplacement sont morts. Sur deux de ces animaux a été effectuée une dissection. Les deux animaux en question ont présenté des blessures desquelles ont été isolés des streptocoques. L'infection avec *Streptococcus equisimilis* déterminée sur un animal a dû être considérée comme cause mortelle directe. Un troisième animal, capturé en 1986 et déplacé, est également passé à la dissection. Il a présenté des ulcérations dans la région de l'œsophage, une hémosidérose et une régression des lymphocytes.

Резюме

Секционные исследования переселенных черных носорогов (*Diceros bicornis*)
Из 50 пойманных для переселения в долине Замбабве в июне-июле 1988 года черных носорогов 8 животных (16%) погибло. На двух из них проведены секционные исследования. Непосредственной причиной смерти одного животного была инфекция, вызванная *Streptococcus equisimilis*. У обоих животных обнаружены раны, из которых были выделены стрептококки. 3 животное, пойманное в 1986 году, так же было переселено и погибло. При секционном исследовании найдена ульцирация в озофагусе, гемоцидероз и снижение числа лимфоцитов.

References

- CARTER, G.P. (1984): Diagnostic Procedures in Veterinary Bacteriology and Mycology. Illinois: Chas. C., Thomas Publishers.
- COWAN, S.T. (1974): Cowan and Steel's Manual for the Identification of Medical Bacteria. Cambridge: Cambridge Univ. Press.
- CUMMING, D. (1987): Small population management of black rhinos. *Pachyderm* 2, 12 - 19.
- DIERENFELD, E.D., Du TOIT, R., and E.R. MILLER (1988): Vitamin E in captive and wild black rhinoceros (*Diceros bicornis*). *J. Wild. Dis.* 24, 547 - 550.
- DOUGLASS, E.M., PLEU, R.E., and C.E. KORD (1980): Haemolytic anemia suggestive of Leptospirosis in the black rhinoceros. *J. Am. Vet. Med. Ass.* 177, 921 - 923.
- FOWLER, M.E. (1986): Zoo and Wild Animal Medicine. London: W.B. Saunders Co.

- WHE, K.V.F., KENNEDY, P.C., and N. PALMER (1985): Pathology of Domestic Animals. London: Acad. Press, Inc.
- MULLOCH, B., and P.L. ACHARD (1969): Mortalities associated with the capture, translocation, trade, and exhibition of black rhinoceroses. Internat. Zoo Yearb. 9, 184 - 195.
- WILKINSON, E.E., and W.J. BOEVER (1982): Fatal haemolytic anemia in the black rhinoceros: Case report and a survey. J. Am. Vet. Med. Ass. 181, 1228 - 1231.
- WILKINSON, E.E. (1987): Haemolytic anemia in the black rhino. Pachyderm 9, 26 - 28.
- WILKINSON, E., Du TOIT, R., LLOYD, S., and A. MANDISODZA (1988): Haematological studies on wild black rhinoceros (*Diceros bicornis*)-evidence of an unstable haemoglobin. J. Zool., London 214, 399 - 405.

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