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SEROSURVEY FOR SELECTED VIRAL AGENTS IN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*) IN KRUGER NATIONAL PARK, 2007

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Abstract: One hundred serum samples collected from free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park (KNP) during the 2007 capture season were selected for measurement of antibody levels to several different vector-borne viral agents. These infectious diseases were chosen to compare with an earlier serosurvey that had been conducted in KNP in rhinos during 1987–1997. Positive antibody titers were found against epizootic hemorrhagic disease (EHD) of deer (8%), Bluetongue (BT) (1%), and Rift Valley fever (RVF) (49%). However, none of the 100 animals tested had detected antibody levels to African horse sickness (AHS). These values were in sharp contrast to those measured in the 1987–1997 survey in KNP white rhinos (AHS 60%, EHD 30%, BT 37%, RVF 0%). Vector-borne viral infection prevalence in white rhinos in the same geographical location appears to vary over time and may be important for monitoring presence of pathogens in an ecosystem.

Key words: African horse sickness, Bluetongue, *Ceratotherium simum*, free-ranging white rhinoceros, Rift Valley fever.

INTRODUCTION

Conservation programs for African rhinoceros have resulted in most free-ranging animals being confined to protected areas such as national parks, game parks, or private sanctuaries and/or ranches. Management of these populations requires careful in situ regulation of the populations and translocations to supplement or establish new populations, or decrease the number of animals in confined areas. With the need to move free-ranging rhinos into new environments, there is an increasing concern for the potential introduction of diseases or risk to animals being presented with novel pathogens in new areas. However, complete understanding of the diseases of concern cannot be achieved without establishing what pathogens are present and what level of species exposure exists.

Serologic surveys of free-ranging rhinos have been limited.[2,4,6,7] These studies showed that antibody prevalence varied by geographic region and species. However, it is difficult to determine the significance of disease risk based on antibody titers without organism isolation, clinical, or pathologic signs. The objectives of this study were to perform an updated serologic survey of 100 free-ranging white rhinos in Kruger National Park (KNP) to several common vector-borne disease agents and compare these values with previous survey results to assess changes in prevalence.

MATERIALS AND METHODS

Blood was collected between February and August 2007 from white rhinos in KNP, Mpumalanga Province, South Africa (24°00′S, 31°00′E), that were immobilized for translocation. The surveyed population consisted of 55 males and 45 females, distributed in the following age categories: 5 calves with dams; 66 subadults (2.5–7 years of age), and 29 adults (older than 7 years). The samples collected in 1987–1997 were from subadult and adult white rhinos that were being translocated (sex ratio unknown). Because these animals were removed from KNP, it is unlikely that the same individuals were sampled in 2007. The animals were captured in the same geographic areas in the 2 studies. Approximately 35 ml of whole blood were collected from the auricular vein in ethylenediamine tetra-acetic acid (EDTA) and serum separator Vacutainers (Fisher Scientific, Suwanee, Georgia 30024, USA). Sera were decanted and stored at −20°C until analyzed.

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Table 1. 2007 Seropositive results from Kruger National Park white rhinoceros based on age category.[a]

Age category	No. samples	AHS I-ELISA	BT/EHD I-ELISA	BT C-ELISA	RVF I-ELISA
Calves (<2.5 yr)	5	0 (0%)	2 (40%)	0 (0%)	1 (20%)
Subadults (2.5–7 yr)	66	0 (0%)	1 (1.5%)	0 (0%)	29 (43.9%)
Adults (>7 yr)	29	0 (0%)	6 (20.6%)	1 (3.4%)	19 (65.5%)

[a] AHS, African horse sickness; I-ELISA, indirect enzyme linked immunosorbent assay; BT, Bluetongue; EHD, epizootic hemorrhagic disease; C-ELISA, competitive ELISA; RVF, Rift Valley fever.

Two-fold dilutions of each serum sample were tested by indirect enzyme linked immunosorbent assay (I-ELISA) for the presence of antibody to African horse sickness, Bluetongue (BT)–epizootic hemorrhagic disease (EHD) viruses, and Rift Valley fever (RVF) virus.[1,9,10] These assays were validated through laboratory trials at Onderstepoort Veterinary Institute (Pretoria, South Africa; Gerdes, pers. comm.). A commercial competitive ELISA was used to test any samples positive by I-ELISA to differentiate BT specific antibody.[1] The Fisher exact test was used for the statistical comparison between the two sets of antibody prevalences from 1987–1997 and 2007, and between adult and subadult rhino results in 2007. The significance level was set at $P < 0.05$.

RESULTS

Samples from 100 white rhinos were tested concurrently in the serologic assays to minimize any interassay variation. Overall, a higher seroprevalence was found in adult animals (Table 1). No antibodies to AHS were detected by I-ELISA in any of the 2007 white rhino samples. The BT–EHD I-ELISA had nine positive results, which ranged in value between 1:6000 and 1:14,000, with any dilution equal to or greater than 1:4500 considered positive in this assay. Because this test detects both BT and EHD antibodies, a confirmatory competitive ELISA (C-ELISA) was used to selectively detect true BT positive samples. Based on the selective nature of the C-ELISA, it was assumed that the other eight positive results were because of cross-reactivity with EHD. Forty-nine rhinos were positive for antibodies to RVF, with values that ranged from 1:11 to 1:98 (any result greater than 1:10 was considered positive). There were 19 seropositive adults, 29 subadults, and one calf.

When the two serologic survey data were compared by using the Fisher exact test, there were statistically significant differences in the seroprevalence values to AHS, EHD, BT, and RVF between 1987–1997 and 2007 ($P < 0.05$) (Table 2).[7] AHS antibody decreased from 60% to 0% between the two periods. Significant decreases in the percentage of antibody positive samples to EHD (30% and 8%, respectively) and BT (37% and 1%) between the survey periods were also observed. The only increase in number of antibody-positive samples over time was seen with RVF (0%–49%). Interestingly, of the five calves included in the 2007 samples, two were seropositive in the I-ELISA for BT–EHD, and one was seropositive to RVF. When seroprevalence was compared between adults and subadults, samples from adults were more likely to be positive to EHD, BT, and RVF, although only the differences in the BT–EHD I-ELISA were significant different at $P < 0.05$ (Table 1). It is difficult to compare seroprevalence in calves because of the lower sample number and possibility of maternal antibody transfer.

DISCUSSION

Serosurveys have limitations in the information that can be extrapolated from them, especially when much of the information about the sample population is unknown. Although it does not necessarily provide knowledge about disease prevalence, the presence of antibody does indicate that an individual has been exposed to a specific pathogen (or cross-reacting antigen) and mounted an immune response. Vector-borne

Table 2. Serologically positive samples to various viral pathogens.[a]

Sampling period	AHS I-ELISA	BT/EHD I-ELISA	BT C-ELISA	RVF I-ELISA
1987–1997	49/81 (60.5%)	24/81 (29.6%)	31/83 (37.5%)	0/85 (0%)
2007	0/100 (0%)	9/100 (9%)	1/100 (1%)	49/100 (49%)

[a] AHS, African horse sickness; I-ELISA, indirect enzyme linked immunosorbent assay; BT, Bluetongue; EHD, epizootic hemorrhagic disease; C-ELISA, competitive ELISA; RVF, Rift Valley fever.

pathogens are dependent on a variety of environmental and host factors that affect infection and development of disease. These include periods of drought, seasonal rains, maintenance of the pathogen in the environment, the presence of appropriate vectors, immunologic status, and other host factors such as body condition and concurrent diseases. Changes in any of these factors could account for the variation seen in results between the periods surveyed. It is difficult to determine all the variations that occurred during 1987–1997 that could have impacted pathogen prevalence and host immunity. Implementation of periodic serosurveillance may detect trends in pathogen patterns over time and provide clues to changes in vector, host, and potential disease patterns.

When examining specific antibody prevalence, the interaction of maintenance or reservoir host species, vectors, and rhinos in a particular ecosystem is important in interpreting values. Most of the rhinos captured in 2007 were relatively young (71/100 were younger than 7 years of age) and appeared healthy on examination in the field. The absence of antibody to AHS was unexpected because the previous seroprevalence was relatively high (60%).

The zebra (*Equus zebra burchelli)* is considered the natural host for AHS viruses and usually found in the same habitat as the white rhino. A 1993 study of AHS in zebra in KNP showed that almost 100% of foals become infected by the time they turn 1 year of age. This was attributed to increased susceptibility during the peak *Culicoides* season.[3] The predominant vectors in South Africa are *Culicoides imicola* (which breeds in damp top soil) and *Culicoides bolitinos* (which breeds in ruminant dung). The presence of large buffalo herds and other antelope along with seasonal rainfall facilitates endemic AHS virus. Therefore, an epidemiologic evaluation would be valuable to determine possible correlation of rhino and zebra AHS seroprevalences along with *Culicoides* species abundance and distribution.

Antibodies to Bluetongue and EHD viruses may cross-react in certain serologic assays. In previous surveys, KNP white rhinos had relatively high antibody prevalence to BT and EHD viruses (37% and 30%, respectively), although there have been no reported cases of disease.[7] Bluetongue and EHD can cause morbidity and even fatal disease in a variety of domestic and wildlife species.[8] BT virus and EHD virus are also transmitted by the same vectors as AHSV. Understanding the susceptibility and pathogenesis of infection in rhinos is relevant because BT testing is often required for international transport and would aid in interpretation of serologic results.

RVF virus outbreaks occur in cycles. It is hypothesized that the virus is maintained in ruminants between outbreaks.[5,10] The mosquito vector breeds in water pools; drinking at waterholes may increase risk of transmission. A survey of Kenyan wildlife demonstrated antibody titers in animals born during the interepidemic period.[6] Black rhino, along with kudu, impala, buffalo, and waterbuck, had a relatively high antibody prevalence (>15%). The high number of samples from KNP white rhinos that were seropositive (49%) in 2007 suggests that they may have been exposed recently, because the majority of animals was younger than 7 years of age. Because none of the white rhinos from KNP tested during the 1987–1997 survey had antibodies, this exposure probably occurred during the interim, between 1997 and 2007. In 1999, a small RVF outbreak occurred among buffalo in a boma in KNP, which suggests that the virus was moving through the park during this period (Gerdes, pers. comm.). RVF was also reported in South Africa in 2007.[5] Epidemiologic trace-back of any outbreaks would allow an estimation of period of exposure.

In summary, the serosurvey performed in KNP on white rhinos in 2007 demonstrates that this population has been exposed to a number of vector-borne viral agents and responded by producing antibodies. By comparing the results to previous surveys, trends in pathogen exposure may start to emerge, which facilitates epidemiologic analyses. Studies such as this one can lead to better understanding of potential pathogen exposure risks associated with introduction, re-emergence, and other population management strategies.

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