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SEROSURVEY FOR SELECTED INFECTIOUS DISEASE AGENTS IN FREE-RANGING BLACK AND WHITE RHINOCEROS IN AFRICA

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ABSTRACT: Two hundred and eighty one serum samples collected from free-ranging black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros, in the Republic of South Africa (RSA), Namibia, and Kenya from 1987–97, were examined for antibody to 16 different infectious agents. Positive antibody titers were detected against Akabane (59.8%), bluetongue (55%), African horse sickness (27.9%), epizootic haemorrhagic disease of deer (19.4%), parainfluenza type 3 (25.3%), bovine herpes virus 1 (3.1%), equine herpes virus 1 (8.8%) and bovine viral diarrhea (1.2%) viruses, and four serovars of *Leptospira interrogans,* (ranging 1.2 to 8.8%). No antibody was detected against Rift Valley fever virus, encephalomyocarditis virus, *Brucella abortus,* and *Trypanosoma equiperdum*. Interspecies differences were detected for African horse sickness, epizootic haemorrhagic disease of deer and parainfluenza type 3 viruses. There appeared to be some geographic variation in the prevalence of antibody for African horse sickness, bluetongue, epizootic haemorrhagic disease of deer, parainfluenza type 3, equine herpes virus 1 and *Leptospira interrogans* serovar *bratislava.*

Key words: Free-ranging black and white rhinoceros, *Diceros bicornis, Ceratotherium simum,* infectious diseases, serosurveillance.

INTRODUCTION

The numbers of rhinoceros in Africa, particularly the black rhinoceros (*Diceros bicornis*), have decreased dramatically in recent years, from more than 70,000 at the beginning of the 1970's to approximately 11,000 in 1994 (Kemf and Jackson, 1994; Potter, 1994). As a consequence the rhino is now considered an endangered species. Conservation programs are ongoing to reintroduce and maintain populations of rhino in areas where they have either become or are becoming extinct.

Most of the free-ranging rhinoceros in the Republic of South Africa (RSA), Namibia, and Kenya live in highly protected areas such as national parks (NP) or commercial game parks (GP). Since they share these areas with other wildlife species, they are likely to be exposed to infectious and/or contageous agents (Fink, 1982). Further, the stresses exerted on rhinoceros during immobilization and capture, translocation, dehorning, and registration may have immunosuppressive effects on them, thereby increasing the risk of either primary infection by opportunistic pathogens or recrudescence of latent infections. Serological surveys have only been carried out on relatively small numbers of rhinoceros (Barnard, 1997; Anderson and Rowe, 1998). Furthermore, their susceptibility to infection and hence the role they play in the epidemiology of disease is virtually unknown. Because of the risk of introducing new pathogens into naive areas, it is important to know what pathogens rhinoceros have been exposed to and what they might be incubating before they are translocated.

The objectives of this study were to carry out a serological survey of 281 free-ranging black and white rhinoceros living in NP and GP in the RSA, Namibia, and Kenya and to determine the prevalence of antibody in these species to sixteen infectious agents that have been identified previously either serologically or by virus isolation in wild and/or domesticated species in Africa.

MATERIALS AND METHODS

Blood samples were collected between 1987 and 1997 from free-ranging rhinoceros that had been immobilized and captured for dehorning and/or translocation. Sera were decanted and

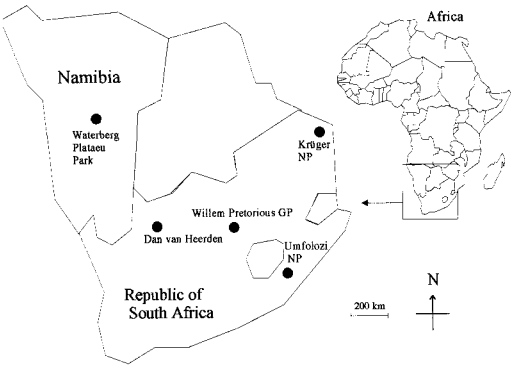

FIGURE 1. Rhino collection sites in southern Africa (●).

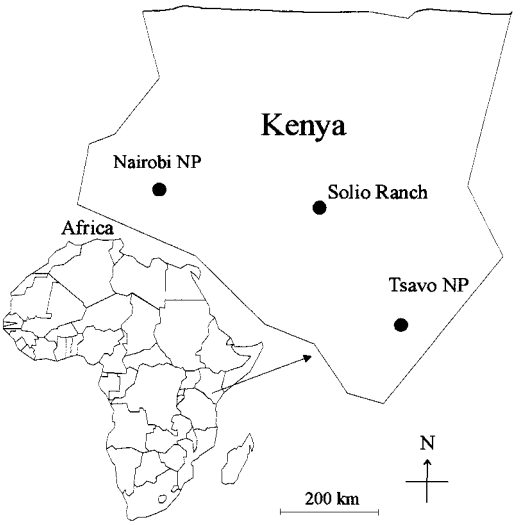

FIGURE 2. Rhino collection sites in Kenya (●).

stored at −20 C. The samples originated from 117 black and 164 white rhinoceros living in NP and GP in five geographically distinct regions in three African counties. These included three regions in the RSA: the Kruger National Park (KNP) in the Mpumalanga Province (24°00′S, 31°50′E; n = 120), the Willem Pretorius GP, and the Dan van Heerden GP in the Free State (FS) (27°00′S, 26°15′E and 28°40′S, 24°40′E, respectively; n = 13), the Umfolozi GP (28°20′S, 31°00′E) and Mkuzi GP (29°50′S, 30°20′E) in the Natal province (n = 50). Collections were also made at the Waterberg Plateau NP (20°21′S, 18°20′E) in Namibia (n = 32), and at the Solio Ranch, Tsavo NP and Nairobi NP in Kenya (2°23′S, 36°50′E; 2°50′S, 38°30′E; 1°18′S, 36°50′E, respectively; n = 66) (Figs. 1, 2). Where possible each serum sample was assayed for antibody against the 16 infectious agents.

A twofold dilution series of each serum was assayed by serogroup-specific competitive enzyme linked immunosorbant assay (ELISA) for the presence of antibody to African horse sickness (AHS) virus (Hamblin et al., 1992), bluetongue (BT) virus (Afshar et al., 1987), and epizootic haemorrhagic disease of deer (EHD) virus (Thevasagayam et al., 1996). End-point titers were calculated according to the method of Kärber (1931) and sera giving antibody titers ≥1/7.5 were considered positive.

Antibodies to Akabane virus were detected by liquid-phase ELISA (Hamblin et al., 1986). Sera were titrated in a twofold dilution series. End-point titers were calculated (Kärber, 1931) and sera giving antibody titers ≥1/30 were considered positive.

A twofold dilution series of each serum was titrated in virus neutralization tests for the detection of antibody to bovine herpesvirus 1 (BHV-1) (Frey and Liess, 1971; Jenny and Wessman, 1973). End-point titers were calculated using the method of Spearmann and Kärber (Finney, 1964) and sera giving antibody titers ≥1/4 were considered positive.

Sera were assayed for antibodies against Rift Valley fever (RVF) virus by indirect ELISA and indirect immunofluorescence. Antibody titers were recorded as the reciprocal of the last serum dilution giving an absorbance value >0.05 in the ELISA and the reciprocal of the last serum dilution of a twofold dilution series giving positive antigen staining in the indirect immunofluorescence test. Sera giving titers ≥1/100 and ≥1/16, respectively, were considered positive for ELISA and immunofluorescence.

Antibodies to parainfluenza type 3 (PI3) virus were detected using a hemagglutination inhibition test as described by Channok (1969). Sera were titrated in twofold dilution series and titers were expressed as the reciprocal of highest dilution showing hemagglutinating activity. Samples recording end-point titers ≥4 were considered positive.

The immunofluorescence assay (Mayr et al., 1977) was used to detect antibodies against equine herpes virus 1 (EHV-1). Serum titers were expressed as the reciprocal of the last serum dilution of a twofold dilution series giving a positive antigen staining. Samples with end-point titers ≥10 were considered positive.

A twofold dilution series of each serum was assayed for antibody against four *Leptospira interrogans* serovars using the microagglutination test (Schonberg et al., 1984). Agglutination titers were recorded as the reciprocal of the highest dilution showing 50% antigen agglutination. Samples giving titers of ≥1/50 were considered positive.

Sera were assayed in a two fold dilution series for antibody against *Brucella abortus* and *Trypanosoma* spp. using the complement fixation test as described by Mayr et al. (1977). Titers were recorded as the highest dilution of serum showing a 50% hemolysis of the indicator system. Sera giving titers ≥1/40 were considered positive.

Associations between the seroprevalence recorded for the different geographic locations and for the different species of rhinoceros were determined using the Fisher's exact test (2-tailed) (Siegel, 1985). The significance level of $P \leq 0.05$ was adjusted for multiple tests using the Bonferroni correction (Sachs, 1997).

RESULTS

Table 1 shows the numbers of black and white rhinoceros sera tested from each region and includes the numbers of sera recorded positive for each pathogen. Significant interspecies differences were recorded for AHS and EHD viruses in KNP, Natal, Kenya, and Namibia ($P < 0.005$), and for PI3 virus in Natal ($P < 0.05$). Geographic differences were observed for five of the pathogens: AHS virus in the KNP and Natal were greater than Kenya ($P < 0.002$); BT virus in Natal and Kenya were greater than KNP ($P < 0.002$); EHD virus in Natal was greater than Kenya ($P < 0.002$); PI3 virus in KNP was greater than Kenya and Natal ($P < 0.002$); and *L. interrogans* serovar *bratislava* in Natal was greater than KNP ($P < 0.002$).

DISCUSSION

Information relating to the incidence of disease and/or the pathogenicity of specific agents is limited for most species of African wildlife. Further, in the absence of virus isolation the susceptibility of a species must remain speculative. Although many serological surveys of wildlife have been carried out, the data generated is generally of little or no value in determining their susceptibility. However, positive antibody reponses do show that a specific or closely related cross-reacting antigen is or has been present in an area and that animals have been exposed and have responded to that agent. The distribution and level of antibody may also give an indication of the possible susceptibility of a species, particularly if the amount of antibody is analogous to that detected in post-infected or convalescent domesticated species that are known to be susceptible.

The transmission of arthropod borne viruses is dependent on many factors which include location, season, climate, and abundance of vertebrate and invertebrate hosts.

Zebra (*Equus zebra burchelli*) are considered to be the natural hosts of AHS viruses but seldom show any clinical signs of disease (Davies and Otieno, 1977; Erasmus et al., 1978). These authors have also reported complement fixing antibody against AHS virus in free-living and experimentally inoculated African elephants (*Loxodonta africana*), again in the absence of clinical signs. The present study is the first report of AHS virus antibody in rhino, particularly the white rhino. The white rhino, like zebra, usually inhabit grassland areas and depending on the availability of food resources can establish local population densities of >5 individuals/km^2. The black rhinoceros, alternatively, are often solitary browsers living in areas less frequented by zebra.

Specific antibody against EHD viruses have been demonstrated in a number of domesticated and wild species from Africa (Thevasagayam, 1998) including black rhinoceros from Zimbabwe (C. Hamblin, unpubl. data). This is the first reported serological survey of rhino for antibody against EHD virus and the first record of antibody in both species.

Similarly, specific antibody against BT virus has been reported in several domesticated and wildlife species in Africa (Herniman et al., 1983; Hamblin et al., 1990; Davies and Walker, 1992) and also in rhinoceros from Zimbabwe (Anderson and Rowe, 1998). Contrary to the recent study carried out by Barnard (1997) using the agar gel immunodiffusion test, specific and often high antibody titers were recorded in 55% of the black and white rhinoceros

TABLE 1. Prevalence of antibody to sixteen pathogens in sera from free-ranging black and white African rhinoceros, collected between 1987 and 1997.

Antigens	KNP[b]		Natal		FS[c]	Kenya		Namibia		Prevalence estimates for total number of rhinoceros
	White	Black	White	Black	White	White	Black	White	Black	
AHS virus	49/81[a]	2/34	13/27	5/20	0/12	4/13	3/53	0/23	0/9	0.29
BT virus	31/83	12/33	24/29	18/21	7/13	10/13	36/47	9/23	2/9	0.55
EHD virus	24/81	1/34	14/29	5/20	0/13	1/12	4/52	3/23	1/9	0.19
Akabane virus	56/81	22/34	15/23	1/7	8/10	3/12	25/45	7/23	9/9	0.60
BHV-1	1/57	0/28	1/18	0/21	1/13	0/12	4/47	0/23	0/9	0.03
EHV-1	10/86	2/34	2/29	5/21	2/13	0/13	0/53	2/23	1/9	0.09
BVD virus	1/72	0/34	0/18	1/21	1/13	0/13	0/52	0/23	0/9	0.01
PI3 virus	22/51	11/14	2/29	8/20	0/13	2/13	5/53	5/23	2/9	0.25
EMC virus	0/66	0/18	0/29	0/20	0/13	0/9	0/44	0/23	0/9	0.00
RVF virus	0/85	0/34	0/29	0/21	0/13	0/9	0/49	0/23	0/9	0.00
B. abortis	0/79	0/34	0/29	0/21	0/13	0/13	0/48	0/23	0/9	0.00
Trypanosoma equiperdum	0/64	0/30	0/25	0/21	0/12	0/13	0/50	0/23	0/9	0.00
Leptospira interrogans serovars										
grippotyphosa	3/85	1/34	1/29	1/21	0/13	3/9	0/49	1/23	0/9	0.04
tarassovi	3/85	1/34	3/29	0/21	1/13	0/9	0/49	1/23	0/9	0.03
braislava	4/85	3/34	8/29	3/21	2/13	1/9	2/49	0/23	0/9	0.09
copenhageni	6/85	2/34	4/29	2/21	0/13	1/9	2/49	1/23	0/9	0.07

[a] Number positive/number samples tested.
[b] KNP = Kruger National Park.
[c] FS = Free State.

sera tested in this survey using the more sensitive competitive ELISA.

The predominance of low titers recorded against AHS, BT and EHD is probably a reflection of continual exposure to these agents. However, the high titers (≥1/60), albeit in small numbers of rhino sera are analogous to those recorded in post infected or convalescent equines and may suggest some virus replication.

Akabane virus is transmitted by mosquitos and vector *Culicoides* spp. midges; it causes abortion, premature birth and severe congenital abnormalities in newborn cattle, sheep and goats (Kurogi et al., 1997a, b; Narita et al., 1979). Neutralizing antibody to Akabane virus has been reported in African wildlife from several African territories (Al-Busaidy et al., 1987). The high prevalence (59.8%) and distribution of antibody titers against Akabane virus recorded here with sera from black and white rhino suggest virus replication and confirm the results of Barnard (1997) suggesting that these species might be susceptible to infection.

Antibody against BHV-1 is widespread in cattle and several species of wildlife throughout Africa (Swanepoel and Christie, 1972; Jesset and Rampton, 1975; Hedger and Hamblin, 1978). However, Pastoret et al. (1988) suggested that some of the wild species are probably susceptible to, and infected with BHV-1 cross-reacting herpes viruses that are species specific. Although this is the first record of antibody against BHV-1 in free-ranging rhinoceros, the low prevalence (3.1%) and low titers (1 in 6 to 1 in 28, mean 1 in 11) probably reflect cross-reactions rather than a susceptibility to BHV-1 infection. A relatively low prevalence (8.8%) of antibody was also detected against EHV-1. Like BHV-1, serological cross-reactions have been reported between equine herpes viruses, typically EHV-1 and EHV-4. Ludwig et al. (1983) reported that the glycoprotein B of EHV-1, which is expressed on the virus envelope and contains epitopes that are immunogenic and highly conserved, is responsible for these cross-reactions. However, in the absence of virus isolation, the susceptibility of rhinoceros to infection by either BHV-1, EHV-1 or a cross-reacting rhinoceros herpes viruses remains unclear.

Serological surveys in Africa have demonstrated antibody against PI3 in cattle (Provost et al., 1967; Kalunda, 1970) and several species of wildlife (Hamblin and Hedger, 1978) including rhino (Erasmus and Boshoff, 1967). Our results confirm the presence of PI3 virus antibody in rhinoceros (26.7%). The virus is best transmitted by close contact, infecting the respiratory tract, often in the absence of clinical signs. However, when associated with other pathogens it can cause severe clinical disease particularly in stressed animals (Reisinger et al., 1959). Since rhino captured for translocation are likely to be stressed and are usually kept in close contact while in confinement, the possibility of transmission and cross-infection is increased.

Atang and Plowright (1969) reported the isolation of BVD virus from a captive giraffe (*Giraffa camelopardalis*) in Kenya and also cited the isolation of BVD from a sick buffalo (*Syncerus caffer*) shot in the Central African Republic, which was apparently involved in an outbreak of disease that affected warthog (*Phacocoerus aethiopicus*) and eland (*Taurotragus oryx*). Antibody against BVD virus has been shown to be widespread in domestic and wild species throughout Africa (Hamblin and Hedger, 1979; Nettleton, 1990), although the prevalence of antibody appears to vary for different countries, particularly for wildlife species (Hamblin and Hedger, 1979; Soine et al., 1992). In cattle, a high prevalence of antibody often correlates with the presence of persistently infected animals within the herd and spread is most successfully achieved by close contact with these persistently infected animals (Roeder and Drew, 1984). The low to zero prevalence and the low titers of BVD virus antibody detected in the rhino sera tested

here might suggest either lack of exposure, a coincidental exposure to the virus or a cross-reaction with an unidentified virus.

The results from these studies help confirm the absence of antibody to EMC virus in free-ranging rhinoceros. However, deaths of captive rhino due to EMC virus have been reported by Gaskin et al. (1980). These were probably due to contact with infectious rodents. Consideration should therefore be given to maintaining rodent-free containment facilities when animals are being held prior to movement.

Epizootics of RVF virus are thought to occur in cycles and it has been suggested that wild species may act as maintenance hosts during the silent years. Anderson and Rowe (1998) reported positive RVF virus antibody reactions in sera from black (14.5%) and white (8.3%) rhinoceros in Zimbabwe. The last recorded outbreaks of RVF in South Africa were 1974 to 1976 (Barnard and Botha, 1977), in Namibia 1984 (Anonymous, 1992) and in Kenya 1997 (Sall et al., 1998). The absence of antibody in the rhinoceros sera tested here helps confirm that RVF virus is not currently present in RSA and Namibia. The absence of antibody in sera from the 58 Kenyan rhinoceros may be due to a number of factors which include the sample size, their origin and their current location in relation to the recent outbreaks of RVF.

Several wildlife species are considered to be susceptible to brucellosis and may act as reservoirs of infection for domesticated animals (Berman, 1981). The absence of antibody to *B. abortus* in the sera of both species of rhinoceros, at this time, might suggest that either they are not susceptible or have not been exposed.

Antibody against different *Leptospira* serovars have been reported in African wildlife including free-ranging and captive rhinoceros (Jessup et al., 1992; Miller, 1993; Anderson and Rowe, 1998). Although infections are usually considered inapparent, Miller (1993) has suggested *Leptospira* spp. as the possible causal agent of hemolytic anemia in captive black rhinoceros. Results from the present study show positive antibody reactions against the four serovars of *Leptospira interrogans* tested, ranging 3.3 to 8.5%, and are in agreement with previously published data of Jessup et al. (1992).

Trypanosoma equiperdum is a sexually transmitted parasite that is considered to be specific for equines. Although rhinoceros are related, albeit distantly, to the family *Equidae* and have been reported to be susceptible to infection with *T. brucei* spp. (McCulloch and Achard, 1969; Clausen, 1981), no antibody to *T. equiperdum* was detected in the rhino sampled here. Hence the susceptibility of these species to *T. equiperdum* remains unclear.

In conclusion, the results presented here show that both species of rhino have been exposed and have responded serologically to a number of agents. Further studies need to be carried out to determine the role of rhino in the epidemiology of different pathogens so that appropriate quarantine and test procedures can be implemented thereby minimizing the risk of translocating animals that may be incubating infectious agents.

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