14. Paglia, D. E., W. N. Valentine, R. E. Miller, M. Nakatani, and T. A. Brockway. 1986. Acute intravascular hemolysis in the black rhinoceros: erythrocyte enzymes and metabolic intermediates. Am. J. Vet. Res. 47: 1321–1325.

15. Paras, A. 1990. Veterinary Records, Chalpaltapec Park Zoo, Mexico City, Mexico.

16. Schenkel, R., and L. Schenkel-Hulliger. 1969. Ecology and behavior of the black rhinoceros (*Diceros bicornis* L.). Verlag Paul Parey, Hamburg, Germany. P. 26.

17. Valli, V. E. O. 1985. The hematopoietic system. *In*: Jubb, K. V. F., P. C. Kennedy, and N. Palmer (eds.), Pathology of Domestic Animals. Pp. 142–148.

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SEROLOGICAL EVIDENCE FOR *COWDRIA RUMINANTIUM* INFECTION IN FREE-RANGING BLACK (*DICEROS BICORNIS*) AND WHITE (*CERATOTHERIUM SIMUM*) RHINOCEROSES IN ZIMBABWE

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Abstract: Sera from 65 free-ranging Zimbabwean black rhinoceroses (*Diceros bicornis*) from the Chete safari area and the lower Zambezi Valley and 58 white rhinoceroses (*Ceratotherium simum*) from Hwange National Park were tested for antibodies to *Cowdria ruminantium* by monoclonal antibody-mediated competitive enzyme-linked immunosorbent assay. Eighteen of 32 (56.2%) black rhinoceroses from the lower Zambezi Valley were positive, whereas only one of 33 (0.03%) from the Chete safari area was positive. Mice inoculated with freshly collected rhinoceros blood from the Chete safari area did not seroconvert. In addition, 44 of 58 (75.9%) white rhinoceroses from Hwange National Park had antibodies to *Cowdria*. These findings signify a possible reservoir role for rhinoceroses in the epidemiology of heartwater disease. If the carrier status for *Cowdria* in rhinoceroses is further confirmed by isolation of the organism, spread of the disease into heartwater-free areas that harbor known *Amblyomma* tick vectors is conceivable and should be taken into consideration when rhinoceroses are translocated. The purpose of the study was to determine seropositive rates to *Cowdria ruminantium* in convenience samples of black and white rhinoceroses in the natural habitat where contact with domestic cattle is essentially nil.

Key words: Black rhinoceros, white rhinoceros, heartwater, *Cowdria ruminantium*, *Amblyomma* ticks, competitive ELISA.

INTRODUCTION

The critical predicament imposed upon the black rhinoceros because of poaching has prompted Zimbabwe's Department of National Parks and Wildlife Management (DNPWM) to take extreme measures to ensure survival of the species. Since 1986, over 400 black rhinoceroses (*Diceros bicornis*) have been translocated from the lower Zambezi Valley and the shores of Lake Kariba to safer locations within Zimbabwe and to other countries, including the USA and Germany.[2,7] Although efforts have been made to ensure that the animals are healthy prior to transport,[6,7] and conspicuous pathological conditions have been examined,[8–11] specific infectious disease investigations have been limited. The white rhinoceros (*Ceratotherium simum*), although at present less threatened with extinction, still experiences significant mortality in Zimbabwe because of poaching.

Heartwater disease caused by the rickettsia *Cowdria ruminantium* and transmitted by *Amblyomma* ticks is regarded as one of the most important tick-borne diseases of domestic ruminants throughout Africa south

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of the Sahara.[17] In addition to its occurrence in many African countries, the disease has also been demonstrated on several islands in the Caribbean.[18]

In Africa, a number of wild bovidae are susceptible to natural or experimental infection with *Cowdria ruminantium*. Naturally contracted heartwater has been diagnosed in eland (*Taurotragus oryx*)[20] and springbok (*Antidorcas marsupialis*).[13] Wild bovidae and cervidae exotic to sub-Saharan Africa, including Indian nilgai (*Boselaphus tragocamelus*), Barbary sheep (*Ammotragus lervia*), Himalayan tahr (*Hemitragus jemlahicus*), and fallow deer (*Dama dama*) in South Africa[20] and Java deer (*Cervus timorensis*) in Mauritius,[16] have been known to contract fatal heartwater.

Subclinical infections have been reported in a variety of animals, including guinea fowl and tortoises, and some animals have been found refractory to infection.[15] Carrier status has been demonstrated in BALB/c laboratory mice.[19] Recent evidence indicates that the African buffalo remains a carrier of heartwater for long periods, and although clinical disease was not induced, ticks were able to acquire the organism 161 days after inoculation of host buffalo.[1] These findings indicate the potential significance of spread of heartwater through carrier animals.

In this study, a recently developed competitive ELISA[3] mediated by monoclonal antibodies to a conserved 32-kD *Cowdria* antigen was used to demonstrate specific anti-*Cowdria* antibodies in rhinoceroses.

MATERIALS AND METHODS

Blood samples were taken from chemically immobilized black rhinoceroses from the lower Zambezi Valley in 1988 and 1989[7] and from the Chete safari area on Lake Kariba in 1990 and 1991, prior to translocation. Samples were also collected from chemically immobilized white rhinoceroses in 1991, during a DNPWM-sponsored dehorning exercise aimed at impeding poaching in Hwange National Park. All blood samples were maintained at about 4°C until centrifuged for serum collection within 6 hr. Sera were maintained at −70°C until tested.

Sonicates of endothelial cells that were heavily infected with *Cowdria ruminantium* (Senegal isolate) were used as antigen in the cELISA.[3] Aliquots of 11.4 μg/ml were applied to microtiter plates in carbonate–bicarbonate buffer at pH 9.6 for 1 hr at 37°C and at 4°C overnight. Antigen-coated plates were washed with distilled water containing 0.05% Tween 20. After washing, 50-μl aliquots of test serum and monoclonal antibody 4F10B4 (dilution 1:100) were applied simultaneously and incubated for 1 hr at 37°C. Both were diluted in 1% milk/PBS/0.05% Tween 20. Rabbit anti-mouse antibody conjugated with horseradish peroxidase (Dakopatts, Denmark), diluted 1:600 in PBS containing 0.05% Tween 20 and 1% gelatin, was added and incubated for 1 hr at 37°C. After the plates were washed, 100 μl of ABTS substrate solution (Sigma Chemical Co., St. Louis, Missouri 63178, USA), freshly prepared as directed, was dispensed into each well. The optical density was measured at 405 nm after 30 min incubation.

All tests were performed in duplicate. Experimental calves were infected at Utrecht with *Cowdria ruminantium* (Senegal isolate) blood stabilate and subsequently treated with terramycin to obtain high-titer positive-control sera for use in the cELISA. Sera from six free-ranging black rhinoceroses from *Amblyomma*-free areas in Namibia were used as negative controls for the black rhinoceros samples, and sera from five captive white rhinoceroses born in captivity in the U.K. were used as negative controls for the white rhinoceros samples. Positive cases were defined as those with mean optical densities that differed by more than 3 SD from the means of the negative controls.

Blood samples were collected from 2-mo-old BALB/c mice by periorbital puncture prior to intraperitoneal injection with 2 ml fresh black rhinoceros blood that was collected during the 1991 capture exercise in the Chete safari area. The mice were observed daily for signs of disease, and after 1 mo blood samples were collected again. All samples were collected in capillary tubes, centrifuged prior to serum collection, and frozen at −20°C until tested for antibodies to *Cowdria ruminantium* by the indirect immunofluorescence (IFA) test.[4] Sera were applied in dilutions of 1:80 and 1:320 to slides coated with *Cowdria ruminantium* (Senegal isolate) and incubated for 30 min at room temperature. After washing in PBS (pH 7.4), FITC-labeled sheep anti-mouse immunoglobulins (Amersham, U.K.) diluted 1:80 were applied. After incubation for 30 min at room temperature and subsequent washing, fluorescence was observed with a Leitz fluorescence microscope at ×400 magnification. Hybridoma supernatant containing monoclonal antibody (4F10B4) to the Cr32 antigen[3] provided a positive control. Sera from unexposed mice were used as negative controls.

Table 1. Results from cELISA for antibodies to *Cowdria ruminantium* in black (*Diceros bicornis*) and white (*Ceratotherium simum*) free-ranging rhinoceroses in Zimbabwe.

Location	Species	No. positive/total	% positive
Lower Zambezi Valley	Black rhinoceros	18/32[a]	56.2%
Chete safari area	Black rhinoceros	1/33[b]	0.03%
Hwange National Park	White rhinoceros	44/58	75.9%

[a] Mean optical density of negative control sera (n = 6) = 0.473 (SD = 0.093).
[b] Mean optical density of negative control sera (n = 6) = 0.488 (SD = 0.049).
[c] Mean optical density of negative control sera (n = 5) = 0.421 (SD = 0.036).

RESULTS

With the cELISA, 56.2% (18/32) of black rhinoceroses from the lower Zambezi Valley and 0.03% (1/33) from the Chete safari area were positive for antibodies to *Cowdria ruminantium* (Table 1). The mean optical density for the negative black rhinoceros controls (n = 6) for the Lower Zambezi Valley animals was 0.473 (SD = 0.093) and for those from the Chete safari area was 0.488 (SD = 0.049). Nearly 76% (44/58) of the white rhinoceroses from Hwange National Park were positive for antibodies to *Cowdria ruminantium* (Table 1). The mean optical density for the negative white rhinoceros controls (n = 5) was 0.421 (SD = 0.036).

None of the 20 mice inoculated with fresh blood from black rhinoceros from the Chete safari area showed any signs of disease. All mice were negative for antibodies to *Cowdria ruminantium* by IFA both before and 1 mo after inoculation.

DISCUSSION

Investigators have long suspected a reservoir for *Cowdria ruminantium* in African wildlife, given its extreme pathogenicity for imported domestic ruminant stock, intermittent pathogenicity for indigenous stock, and apparent nonpathogenicity in most indigenous wildlife species.[15,17] The high incidence of antibodies to *Cowdria ruminantium* in black rhinoceroses from the lower Zambezi Valley and white rhinoceroses from Hwange National Park may indicate a reservoir for *Cowdria* in these species. The low incidence of positive black rhinoceroses from the Chete safari area probably reflects differences in habitat and tick distribution. Twelve species of *Amblyomma* ticks are capable of transmitting *Cowdria ruminantium*; although *A. variegatum* is the most important vector on the African continent, *A. hebraeum* is more important in southern Africa and may also infest white rhinoceroses in Zimbabwe.[13] *Amblyomma sparsum* and *A. rhinocerotis* are commonly found on

free-ranging black rhinoceroses in Zimbabwe,[13] and although *A. sparsum* can transmit the disease under experimental conditions,[14] *A. rhinocerotis* has not been studied. Both species are commonly found infesting black rhinoceroses from the lower Zambezi Valley,[3] although only *A. sparsum* has been identified on animals from the Chete safari area (N. Kock, pers. obs.). This scarcity of ticks may explain the low incidence of antibodies in the animals from the Chete Safari area.

The results suggest that black and white rhinoceroses may carry *Cowdria ruminantium*, although confirmation by isolation of the organism from infected rhinoceros blood is required for positive proof. Although all rhinoceroses are treated with acaricides against ticks prior to translocation in Zimbabwe,[3] the possible presence of a carrier state means that spread of disease could occur if suitable vectors and hosts inhabit the translocation habitats. The testing of animals prior to translocation is important, especially before export to heartwater-free countries. It is not known if rhinoceroses are susceptible to the disease, which is another factor for consideration when decisions are made on translocation destinations for this endangered species.

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LITERATURE CITED

1. Andrew, H. R., and R. A. I. Norval. 1987. The carrier status of sheep, cattle and African buffalo recovered from heartwater. Vet. Parasitol. 34: 261–266.

2. Booth, V. F., and A. M. Coetzee. 1988. The capture and relocation of black and white rhinoceros in Zimbabwe. *In:* Nielsen, L., and R. D. Brown (eds.). Translocation of Wild Animals. Wisconsin Humane Society, Milwaukee, Wisconsin, and Caesar Kleberg Wildlife Research Institute, Kingsville, Texas. P. 333.

3. Duncan, I. M. 1989. The use of flumethrin pour-on for de-ticking black rhinoceros (*Diceros bicornis*) prior to translocation in Zimbabwe. J. S. Afr. Vet. Assoc. 60: 195–197.

4. Jongejan, F. 1991. Protective immunity to heartwater (*Cowdria ruminantium* infection) is acquired after vaccination with in vitro-attenuated rickettsiae. Infect. Immun. 59: 729–731.

5. Jongejan, F., M. J. C. Thielemans, M. De Groot, P. J. S. Van Kooten, and B. A. M. Van Der Zeijst. 1991. Competitive enzyme-linked immunosorbent assay for heartwater using monoclonal antibodies to a *Cowdria ruminantium*-specific 32-kilodalton protein. Vet. Microbiol. 28: 199–211.

6. Kock, M. D., R. du Toit, D. Morton, N. Kock, and B. Paul. 1990. Baseline biological data collected from chemically immobilized free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. J. Zoo Wildl. Med. 21: 283–291.

7. Kock, M. D., M. La Grange, and R. du Toit. 1990. Chemical immobilization of free-ranging black rhinoceros (*Diceros bicornis*) using combinations of etorphine (M99), fentanyl, and xylazine. J. Zoo Wildl. Med. 21: 155–165.

8. Kock, N., C. Foggin, M. Kock, and R. Kock. 1993. Hemosiderosis in the black rhinoceros (*Diceros bicornis*): a comparison between free-ranging and recently captured, versus translocated, and captive animals. J. Zoo Wildl. Med.: in press.

9. Kock, N., C. Foggin, M. Kock, P. Trembath, and D. Jessup. 1991. Coronary artery aneurysms in two black rhinoceroses (*Diceros bicornis*) in Zimbabwe. J. Zoo Wildl. Med. 22: 355–358.

10. Kock, N., and M. Kock. 1990. Skin lesions in free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. J. Zoo Wildl. Med. 21: 447–452.

11. Kock, N., M. Kock, A. Pawandiwa, and T. Matambo. 1989. Postmortem findings in translocated black rhinoceroses (*Diceros bicornis*) in Zimbabwe. Proc. 31st Int. Congr. Dis. Zoo Wild Anim., Dortmund, Germany. Pp. 275–279.

12. Neitz, O. 1944. The susceptibility of the springbok (*Antidorcas marsupialis*) to heartwater. Onderstepoort J. Vet. Res. 20: 25–27.

13. Norval, R. A. I. 1983. The ticks of Zimbabwe. VII. The genus *Amblyomma*. Zimb. Vet. J. 14: 3–18.

14. Norval, R. A. I., and P. K. I. MacKenzie. 1981. The transmission of *Cowdria ruminantium* by *Amblyomma sparsum*. Vet. Parasitol. 8: 189–191.

15. Oberem, P. T., and J. D. Bezuidenhout. 1987. Heartwater in hosts other than domestic ruminants. Onderstepoort J. Vet. Res. 54: 271–275.

16. Poudelet, M., E. Poudelet, and N. Barre. 1982. Sensibilite d'un Cervide: *Cervus timorensis russa* a la cowdriose (heartwater). Rev. Elev. Med. Vet. Pays. Trop. 35: 23–26.

17. Uilenberg, G. 1983. Heartwater (*Cowdria ruminantium* infection): current status. Adv. Vet. Sci. Comp. Med. 27: 427–480.

18. Uilenberg, G., N. Barre, E. Camus, M. J. Burridge, and G. I. Garris. 1984. Heartwater in the Caribbean. Prev. Vet. Med. 2: 255–267.

19. Wassink, L. A., F. Jongejan, E. Gruys, and G. Uilenberg. 1990. Observations on mouse-infective stocks of *Cowdria ruminantium*: microscopical demonstration of the Kwanyanga stock in mouse tissue and the carrier status of the Senegal stock in mice. Res. Vet. Sci. 48: 389–390.

20. Young, E., and P. A. Basson. 1973. Heartwater in the eland. J. S. Afr. Vet. Assoc. 44: 185–186.

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