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Black Rhinoceros (*Diceros bicornis*) Populations in Northwestern Namibia Are Apparently Not Infected with Piroplasms

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ABSTRACT: Babesiosis is a potentially fatal disease in black rhinoceroses. Blood specimens collected from black rhinoceroses from Etosha National Park ($n=29$) and Damaraland ($n=22$), Namibia, were subjected to polymerase chain reaction using *Theileria* and *Babesia* genus-specific primers and reverse line blot, with negative results. The animals were sparsely infested with ticks. In the absence of suitable prophylactic measures, naïve rhinoceroses would be at risk if translocated to *Babesia*-endemic areas.

Key words: *Babesia*, babesiosis, black rhinoceros, *Diceros bicornis*, Namibia, prophylaxis, *Theileria*.

Mortalities due to babesiosis were first reported in black or hook-lipped rhinoceroses (*Diceros bicornis*) during the 1960s in Kenya (Brocklesby, 1967; Mugera et al., 1967) and Tanzania (McCullough et al., 1969). Recently, *Babesia bicornis* was described from black rhinoceroses suffering from clinical babesiosis in both South Africa and Tanzania (Nijhof et al., 2003). In the same paper, *Theileria bicornis*, a piroplasm not known to cause disease, was also described from black rhinoceroses in South Africa.

Importantly, *B. bicornis* was found in five of 11 blood specimens collected from healthy black rhinoceroses in the Great Fish River Reserve Complex in Eastern Cape Province, South Africa (Nijhof et al., 2003). Seven of the animals were infected with *Theileria bicornis*, and one was infected with both *B. bicornis* and *T. bicornis*. This situation resembles endemic stability to babesiosis in cattle (Penzhorn, 2006). For the first few months, bovine calves are protected by passive immunity acquired from the dam's colostrum. If the calves are infected between the ages of about 3–9 mo, they develop a solid immunity without showing any clinical signs. Immunity will wane if the animal is immunocompromised. This stable situation requires a high prevalence of infection in cattle, as well as a large enough tick population to ensure that calves become infected during the critical period. Natural resistance against parasites in wildlife also cannot be ruled out. For example, *Theileria parva* infections are usually nonpathogenic in African buffaloes (*Syncerus caffer*), irrespective of prior exposure (Lawrence et al., 2004). Clinical babesiosis may be triggered in latent carrier animals by stress factors; in most of the black rhinoceros cases mentioned previously, animals died soon after capture, or during periods of nutritional or pregnancy-related stress, or during extreme climatic conditions.

Because of strict and effective conservation measures, black rhinoceros populations in northwestern Namibia are growing steadily. Translocation of individuals from these populations to strengthen or establish populations elsewhere is an ongoing process. If *Babesia*-naive animals are introduced into infected areas, however, they could become infected and develop clinical disease. It was therefore deemed prudent to investigate whether black rhinoceroses in these populations were subclinical carriers of piroplasms.

Specimens were collected from black rhinoceroses immobilized during routine management procedures during March and April 2006: 29 in western Etosha National Park and 22 in Damaraland. For security reasons, exact localities are not mentioned. The Etosha sample consisted of 10 males and 19 females; 13 of the animals were younger than 5 yr, whereas the oldest, a female, was estimated to be 20 yr old. The Damaraland sample consisted of 13 males and nine females; nine of the animals were <5 yr old.

Blood specimens were blotted on filter paper and dried. All visible ticks were collected from each animal and placed into separate vials containing 70% ethanol. The specimens were stored at the Etosha Ecological Institute until taken to the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, for processing.

DNA was extracted from blood spots using the QIAamp® DNA extraction kit (QIAGEN, Southern Cross Biotechnologies, Cape Town, South Africa) following the manufacturer's instructions. Extracted DNA was eluted in 100 μl of elution buffer and stored at 4 C until further analysis.

For the reverse line blot (RLB) hybridization assay, a 460- to 520-base pair fragment of the V4 variable region of the 18S rRNA gene was amplified by polymerase chain reaction (PCR) using the *Theileria* and *Babesia* genus-specific primers RLB F2 (5′-GAC ACA GGG AGG TAG TGA CAA G-3′) and biotin labelled RLB R2 (5′-Biotin-CTA AGA ATT TCA CCT CTG ACA GT-3′) (Nijhof et al., 2003, 2005). The PCR products were analyzed using the RLB hybridization technique, first described by Gubbels et al. (1999). The commercial TBD-RLB kit (Isogen Life Sciences, Ijsselstein, The Netherlands) was used following the manufacturer's instructions. The RLB membrane contained *Theileria* and *Babesia* genus- and species-specific probes, including *Babesia bicornis* (5′-TTG GTA AAT CGC CTT GGT C-3′) and *Theileria bicornis* (5′-GCG TTG TGG CTT TTT TCT G-3′). A plasmid control was used as an internal positive control (Matjila et al., 2005) to ensure that all *Babesia* species-specific probes were correctly bound to the RLB membrane, and functional. PCR grade water was included as negative control.

Ticks were identified using a stereomicroscope and a key (Walker et al., 2003). Specimens identified were compared with species descriptions in Hoogstraal (1956) and Walker et al. (2000).

The RLB is a versatile technique for the simultaneous detection of tick-borne protozoan parasites. Gubbels et al. (1999) determined the sensitivity of the assay at 0.000001% parasitemia, enabling detection of the carrier state of most parasites. In this study, there was no detectable hybridization with the *Babesia/Theileria* genus-specific or *Babesia* or *Theileria* species-specific probes that were present on the blot. Because we had a fairly large sample, we conclude that there is a high probability that these black rhinoceros populations do not harbor these piroplasms.

In Etosha National Park, the median overall tick load was 21, with a range from seven to 46. All animals were infested with *Hyalomma truncatum* (median, 15; range, 3–40), whereas *Hyalomma marginatum rufipes* was less abundant (median, 5; range, 0–11). In Damaraland, all animals were infested with *H. m. rufipes* (median, 6; range, 3–17). A single male *Rhipicephalus longiceps* was recovered from one rhinoceros in Damaraland, the first record from this host (Walker et al., 2000). The paucity of ticks found on black rhinoceroses in Namibia is in contrast to the large numbers and species diversity infesting this host in the more mesic eastern areas of South Africa, such as Eastern Cape and KwaZulu-Natal provinces, as well as in Zimbabwe (Knapp et al., 1997). In studies undertaken in South Africa, *H. m. rufipes* and *H. truncatum* populations peaked in the austral midsummer (January and February); however, with steep declines by March (Horak, 1982; Horak et al., 1991). *Amblyomma hebraeum* and *Haemaphysalis silacea* were recovered from a black rhinoceros from Addo Elephant National Park, Eastern Cape Province, where *B. bicornis* is known to occur (Knapp et al., 1997).

A similar situation may occur on black-faced impala (*Aepyceros melampus pe-*

tersi), which also occurs in arid northwestern Namibia: 26 animals captured for translocation were antibody negative for *Anaplasma* sp., a vector-borne rickettsial infection (Karesh et al., 1997). In contrast, all seven impalas sampled in the Machakos area of Kenya were seropositive for *Anaplasma* sp. (Ngeranwa et al., 2008).

Black rhinoceroses from Etosha National Park or Damaraland translocated to a *Babesia bicornis*-endemic area would be at risk to infection, and clinical signs may ensue. Under these circumstances, prophylactic administration of anti-*Babesia* compounds would be prudent. Diminazene aceturate has been administered to black rhinoceroses as prophylactic treatment against trypanosomosis at total doses varying from 1.5 to 10 g, with no apparent untoward effects (Mugera et al., 1967; McCullough et al., 1969). If the mean mass of an adult black rhinoceros is taken as approximately 850 kg, this translates into dosages from 1.8 mg/kg to 11.8 mg/kg. Subsequently, diminazene dosages of 2–3 mg/kg were injected by dart in the neck region of free-ranging black rhinoceroses, with apparent good effect where mortalities due to babesiosis had occurred (Fyumagwa et al., 2004).

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LITERATURE CITED

BROCKLESBY, D. W. 1967. A *Babesia* species of the black rhinoceros. Veterinary Record 80: 484.

FYUMAGWA, R. D., S. S. MKUMBO, AND P. V. D. B. MORKEL. 2004. Remote treatment of black rhinos against babesiosis in Ngorongoro Crater, Tanzania. Pachyderm 37: 80–83.

GUBBELS, J. M., A. P. DE VOS, M. VAN DER WEIDE, J. VISERAS, L. M. SCHOULS, E. DE VRIES, AND F. JONGEJAN. 1999. Simultaneous detection of bovine *Theileria* and *Babesia* species using reverse line blot hybridisation. Journal of Clinical Microbiology 37: 1782–1789.

HOOGSTRAAL, H. 1956. African Ixodoidea. I. Ticks of the Sudan (with special reference to Equatoria Province and with preliminary reviews of the genera *Boophilus*, *Margaropus* and *Hyalomma*). Research Report NM 005.050.29.07. Department of the Navy, Bureau of Medicine and Surgery, Washington, D.C.

HORAK, I. G. 1982. Parasites of domestic and wild animals in South Africa. XV. The seasonal prevalence of ectoparasites on impala and cattle in the Northern Transvaal. Onderstepoort Journal of Veterinary Research 49: 85–93.

———, L. J. FOURIE, P. A. NOVELLIE, AND E. J. WILLIAMS. 1991. Parasites of domestic and wild animals in South Africa. XXVI. The mosaic of ixodid tick infestations on birds and mammals in the Mountain Zebra National Park. Onderstepoort Journal of Veterinary Research 58: 125–136.

KARESH, W. B., A. ROTHSTEIN, W. GREEN, H. O. REUTER, W. E. BRASELTON, A. TORRES, AND R. A. COOK. 1997. Health evaluation of black-faced impala (*Aepyceros melampus petersi*) using blood chemistry and serology. Journal of Zoo and Wildlife Medicine 28: 361–367.

KNAPP, S. E., R. C. KRECEK, I. G. HORAK, AND B. L. PENZHORN. 1997. Helminths and arthropods of black and white rhinoceroses in Southern Africa. Journal of Wildlife Diseases 33: 492–502.

LAWRENCE, J. A., B. D. PERRY, AND S. M. WILLIAMSON. 2004. Corridor disease. *In* Infectious diseases of livestock. 2nd Edition, J. A. W. Coetzer and R. C. Tustin (eds.). Oxford University Press, Cape Town, South Africa. pp. 468–471.

MATJILA, T. P., A. M. NIJHOF, A. TAOUFIK, D. HOUWERS, E. TESKE, B. L. PENZHORN, T. D. DE LANGE, AND F. JONGEJAN. 2005. Autochthonous canine babesiosis in The Netherlands. Veterinary Parasitology 131: 23–29.

MCCULLOGH, B., AND P. L. ACHARD. 1969. Mortalities associated with capture, translocation, trade and exhibition of black rhinoceroses. International Zoo Yearbook 9: 184–195.

MUGERA, G. M., AND J. G. WANDERA. 1967. Degenerative polymyopathies in East African domestic and wild animals. Veterinary Record 80: 410–413.

NGERANWA, J. J. N., S. P. SHOMPOLE, E. H. VENTOR, A. WAMBUGU, J. E. CRAFFORD, AND B. L. PENZHORN. 2008. Detection of *Anaplasma* antibodies in wildlife and domestic species in wildlife-livestock interface areas of Kenya by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. Onderstepoort Journal of Veterinary Research 75: 199–205.

NIJHOF, A., B. L. PENZHORN, G. LYNEN, J. O. MOLLEL, C. BEKKER, AND F. JONGEJAN. 2003. *Babesia bicornis* sp. n. and *Theileria bicornis* sp. n.: Tick-borne parasites associated with mortality in the black rhinoceros (*Diceros bicornis*). Journal of Clinical Microbiology 41: 2249–2254.

———, V. PILLAY, J. STEYL, L. PROZESKY, W. H. STOLTSZ, J. A. LAWRENCE, B. L. PENZHORN, AND F. JONGEJAN. 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. Journal of Clinical Microbiology 43: 5907–5911.

PENZHORN, B. L. 2006. Babesiosis of wild carnivores and ungulates. Veterinary Parasitology 138: 11–21.

WALKER, A. R., A. BOUATTOUR, J.-L. CAMICAS, A. ESTRADA-PEÑA, I. G. HORAK, A. A. LATIF, R. G. PEGRAM, AND P. RESTON. 2003. Ticks of domestic animals in Africa: A guide to identification of species. Bioscience Reports, Edinburgh, UK.

WALKER, J. B., J. E. KEIRANS, AND I. G. HORAK. 2000. The genus *Rhipicephalus* (Acari, Ixodidae): A guide to the brown ticks of the world. Cambridge University Press, Cambridge, UK, 655 pp.

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