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# Table of contents

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# Trypanosomosis and other co-infections in translocated black *(Diceros bicornis michaeli)* and white *(Ceratotherium simum simum)* rhinoceroses in Kenya

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**Abstract.** Recent efforts towards the conservation of endangered rhinoceroses in Kenya include re-introduction of the animals into regions where they occurred previously. These efforts have however been hampered by mortalities of translocated animals. The current study was undertaken to determine the cause of deaths amongst black rhinoceroses *(Diceros bicornis michaeli)* and white rhinoceros *(Ceratotherium simum simum),* which were re-introduced into Meru National Park. Out of 28 translocated rhinoceroses, 7 (3 white and 4 black) (25%), died 3 months post translocation. At post-mortem, the carcasses of these animals were emaciated and heavily infested with ticks of different species. Further studies were conducted to determine the occurrence of hemoparasites in two sick black rhinoceroses and one white rhino. The density of tsetse flies and species of ticks present at the locality were also determined. The sick black rhinoceros were found to harbour mixed infections of *T. congolense*, *T. simiae* spp, and *T. godfreyi*, *Theileria* spp. and microfilaria. One of the sick black rhinos which never recovered presented very low packed cell volume and total protein levels. The tick species observed in all rhinos included *Amblyomma, Rhipicephalus* and *Boophilus*. The total number of *Glossina pallidipes* Austen and *G. brevipalpis* Newstead flies trapped per trap per day were 395 and 25, respectively. The tsetse density was considered very high and species distribution overlapped in the sanctuary. The study shows that the rhinos could have died from a co-infection of various haemoparasites. Control of tsetse flies should be implemented and its effectiveness regularly evaluated before and after translocation of rhinoceroses into areas known to be endemic for trypanosomosis.

**Keywords:** Conservation; Kenya; Translocation; Trypanosomosis; Tsetse flies; Rhinoceros; Wildlife.

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## Introduction

The number of rhinoceroses in Africa, especially the black rhinos *(Diceros bicornis michaeli),* has decreased dramatically from more than 70,000 in 1970s to approximately 11,000 in 1994 (Brooks, 1994). Intensive efforts to conserve both eastern black *(Diceros*

*bicornis michaeli)* and southern white *(Ceratotherium simum simum)* rhinos in Kenya are based on managing small discreet sub-populations in highly protected sanctuaries (KWS, 1993). This has necessitated translocation of rhinos among sanctuaries to manage social stability, sex ratios, population sizes and genetic variation. In addition, rhinos are also being re-introduced to their historical range to seed new wild populations and to broaden the genetic base. For the latter purpose, 28 rhinos were captured and translocated to Meru National Park in January 2006 from Lake Nakuru and Nairobi National parks in Kenya. The animals were re-introduced to Meru Park to boost a population of one black rhino and thirty white rhinos introduced in 2002 and 2004, respectively. However, three months after translocation, the body condition of the animals began to deteriorate and some of them died. The purpose of this study was to establish the cause of the deaths with a goal of instituting remedial measures.

## Materials and methods

*Study area*

The Meru National Park (MNP) rhino sanctuary is an area of 38.8 km$^2$ (N 00° 15.125, E 038° 06.481) located on the western side of the park bordering a local community area. The sanctuary is separated from the community by a buffer fence ensuring that no livestock get into the sanctuary and no wildlife move into the community area. Both eastern black rhino and southern white rhino are kept in the sanctuary, but other wildlife from the park may move in and out of the sanctuary. Southern edge of the sanctuary is characterized by a thick forest, while the rest of the sanctuary is covered with thickets, bushes and grassland. The sanctuary is well watered with rivers Makutano, Kanjoo, Kathithi, Rujuwero and Kindani streaming across it.

*Study design*

Retrospective data on the translocation, morbidity and mortality of rhinos was obtained from the Rhino Management Team at MNP. A prospective study was also carried out on two subadult black rhinos considered ill as their body condition was deteriorating and combined with abnormal behavior (prolonged periods of recumbence, standing listlessly and anorexia). Therefore, we decided to treat and sample these two individuals and for comparison, a healthy sub-adult white rhino was also immobilized, treated and sampled. The rhinos were chemically immobilized using a mixture of etorphine hydrochloride (Norvatis, South Africa PTY Ltd.) and hyaluronidase (Kyron laboratories, South Africa). Blood samples were collected from the femoral vein into heparinised tubes and plain tubes. Whole blood was analysed by Polymerase Chain Reaction (PCR) using specific primers for trypanosomes according to procedures used by Njiru et al. (2004). Thin blood smears, fixed in methanol were stained in 10% Giemsa solution for 45 minutes and each smear was examined microscopically for haemoparasites. The rhinos were then treated with 100 mg of Diminazene di-aceturate (Trypan®, Troped, Germany) given intramuscular, 10 ml of Ivermectin (Ivomec®, Merial Ltd., Iselin NJ, USA) given subcutaneous and 12,000 mg of long acting oxytetracyline (Centervet® 20% LA, Norbrook Laboratories, Northern Ireland).

Before the rhinos were translocated into MNP, 80 pyrethroid-treated tsetse targets had been placed in different parts of the sanctuary. During the current study, tsetse density assessment within the sanctuary was undertaken by setting eight biconical traps baited with phenols and acetone in different parts of the sanctuary. The traps were set up in both forested and forest glades sections of the sanctuary. The traps were left for 24 hours and tsetse flies harvested, identified and counted. Data was entered and analyzed using MS Excel (Microsoft, USA) program.

## Results

Retrospective data revealed that seven rhinos (25% of translocated animals, three white and four black), aged less than five years, including a neonate, died during the post-release period of February 2006 to September 2007. Except the neonate, all the sub-adults presented a body wasting condition before death.

There were no trypanosomes observed in the blood smears. PCR analyses showed that two of the four dead black rhinos had trypanosome infection while none of the three dead white ones was infected (table 1).

One black rhino (R1) harboured a mixed infection of *Trypanosoma congolense* Forest, *T. godfreyi* and *T. simiae simiae.* The other black rhino (R2) was infected with *Trypanosoma congolense* Forest, *T. godfreyi, T. congolense* Savanna and *T. simiae* Tsavo. *Theileria* sp. piroplasms were observed in the thin blood smears from one black rhino (R1) and clinically healthy white rhino (R3).

All the rhinos had high infestation of ticks *(Amblyomma* spp., *Rhipicephalus* spp., and *Boophilus* spp.) mainly on the perianal area and around the teats. One black rhino (R2) presented a significantly low Packed Cell Volume (PCV) and total protein levels when compared with the other rhinos (table 2). This rhino (R2) eventually died, two weeks after medication while the other black rhino (R1) survived.

The species of tsetse flies identified were *Glossina pallidipes* Austen and *Glossina brevipalpis* Newstead. In a single trapping session, the total numbers of *G. pallidipes* flies per trap per day (FTD) were 395, while *G. brevipalpis* FTD were 25. The tsetse were widely distributed in the sanctuary.

**Table 1.** Parasites infecting three rhinos from Meru National Park, Kenya

| Rhino | Parasites | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Black rhino 1 (R1) | + | + | + | + |
| Black rhino 2 (R 2) | + | - | - | + |
| White rhino 3 (R 3) | - | + | + | + |

1 – Trypanosomes; 2 – *Theileria* spp; 3 – Microfilaria; 4 – Ticks.

## Discussion

Black rhino became nearly extinct in Kenya due to poaching and loss of suitable habitats (Muya and Oguge, 2000). Population recovery of the black rhino in particular has necessitated the establishment and protection of small discrete populations (KWS, 1993).

**Table 2.** Hematological and total protein values in three rhinos from Meru national Park, Kenya

| Rhino | Hematology | | | | | Total protein (g/dl) |
|---|---|---|---|---|---|---|
| | PCV | NO (%) | LO (%) | EO (%) | MO (%) | |
| Black rhino (R1) | 29 | 38 | 43 | 11 | 8 | 7.2 |
| Black rhino (R2) | 18** | 21 | 47 | 13 | 18 | 5.2*** |
| White rhino (R3) | 35 | 19 | 26 | 51 | 4 | 7.6 |

**Key:** ** = Low PCV; *** = Low protein levels; NO = neutrophils; LO = lymphocytes; EO = Eosinophils; MO = Monocytes; PCV = packed cell volume.

With about 500 individuals, Kenya has the largest population of free-ranging eastern black rhinos (KWS, 2003), but the species is still endangered. Although the population is slowly recovering, a sustained recovery remains a challenge. As such, factors that may cause death, or regulate population vital rates are of great concern (Hrabar and Du Toit, 2005) and are pertinent to the conservation plans for the species. In the present study, deaths of rhinos after release raised concerns that called for both retrospective assessments and investigations during morbidity. Except for the neonate, all the sub-adults presented a body wasting condition despite availability of food during the rainy season. The low haematological and protein values in the trypanosome infected sick rhinos (R1 and R2) (table 2) may be linked to the pathologic effects of various parasites including trypanosomes, *Theileria* spp. and *Babesia* spp. All these parasites have been shown to cause mortalities in rhinos (Jonyo, 1989; Taylor, 1986; Mihok et al., 1992; Nijhof et al., 2003). However, it is also important to note that the findings of this study were not conclusive and other conditions could have affected the rhinos.

In natural populations, wild animals are known to be trypanotorelant with no clinical signs until hosts are subjected to stress (captivity, translocation or drought) (Mbaya et al., 2009). Trypanosomosis in rhinos is characterized by very low parasite levels which are not easy to detect by direct microscopy (Mihok et al., 1992). Thus, molecular tools such as PCR have been recommended. It is argued that animals exposed to trypanosomes develop sufficient immunity against the parasites which inhibit

progression of infection to clinical disease (Black et al., 2001). This may be demonstrated by the low prevalence of clinical trypanosomosis in black rhinos within the tsetse fly infested Tsavo West National Park in Kenya (Mihok et al., 1992). Further, the black rhinos and white rhinos that were previously reintroduced in MNP were apparently healthy during the study. As such it is predictable that the reintroduced animals were immunologically naïve to trypanosomes or, in addition, they were immune suppressed. Since the animals were sourced from Lake Nakuru and Nairobi National Parks, areas with no recent reports of trypanosomes, these animals probably possessed low/no immunity to trypanosomes.

Before the rhinos were translocated into MNP, 80 pyrethroid-treated tsetse targets had been placed in different parts of the sanctuary. This control effort was expected to reduce tsetse density and the consequent trypanosome challenge. Effectiveness of the targets was not evaluated before translocation, and as per the FTD observed in the current study, the density of tsetse fly was regarded as high. It is possible that that the number of targets was insufficient or poorly maintained. Moreover, since the larger, more populated non-fenced section of the main park had no targets, it is likely that it was a tsetse reservoir. The high number of *G. pallidipes* in the sanctuary was significant as it is the main vector of various species of trypanosomes (Njiru et al., 2004).

Findings from this study suggested that MNP was still heavily infested with tsetse flies infected with various species of trypanosomes. Since translocation is a key to the recovery strategy and management of rhino metapopulations, it will continue to be practiced despite its distressful effects on the animals. However, since trypanosomes are lethal especially to stressed animals following translocation, tsetse fly control in rhino habitats is paramount to successful re-introductions and establishment of new populations of rhinos.

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