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Trypanosomiasis in Introduced Southern White Rhinoceros (*Ceratotherium simum simum*) Gifts to Ex Situ Habitat in Aitong, Kenya

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ABSTRACT: During the opening of diplomatic relations in the 1990s, South Africa gifted 20 southern white rhinoceros (*Ceratotherium simum simum*) to Kenya. The species is not indigenous to Kenya, and management of the introduction was not clearly addressed in the legislation. Responsibility was left to the private sector and local authorities. Ten of the animals were introduced to land contiguous with the Maasai Mara National Reserve, an area with tsetse–trypanosomiasis challenges, and with rare cases of human sleeping sickness. Mortalities had been previously documented when indigenous naïve black rhinoceros were introduced to areas with tsetse; hence there was no consensus on the management of this introduction. Feasibility was only explored once before with the introduction of two animals in a monitored and managed translocation from Lewa Downs, Laikipia in 1992–1994. Ultimately, Kenyan experts were co-opted to address risk after trypanosomiasis occurred in many animals. Unfortunately, this finding was followed by gradual mortalities of most rhinoceros with only a few being saved by removal to highland private sanctuaries. This event was complicated by many factors. Samples were only sporadically collected, and mainly from sick animals. With no clear responsibility by government agencies, a collaboration between veterinarians and researchers resulted in characterization of the disease challenge, and when invited, assessment of health status. Laboratory diagnostics revealed common and sometimes severe infections with *Trypanosoma brucei*, a normally infrequent trypanosome. Infection was associated with disturbances in erythropoiesis, especially anemia. Symptoms varied from sudden death associated with intestinal atony, to a semiparalyzed animal that was partially responsive to treatment for trypanosomes. This event should be used as a caution to future movements of this species that are planned or ongoing in Africa, for conservation or other purposes.

Key words: Ex situ introduction, Southern white rhinoceros, translocation, tsetse, trypanosomiasis, *Trypanosoma brucei*.

INTRODUCTION

African rhinoceros comprise two extant species that separated from a common ancestor about 3.3–4 million years ago (Moodley et al. 2020). The black rhinoceros (*Diceros bicornis*) is a browsing species living in bush and scrub habitats, whereas the white rhinoceros (*Ceratotherium simum*) is an obligate grazer restricted to grassland. In nature, African rhinoceros are relatively free of disease. The historical distribution of white rhinoceros is outside the tsetse belt (Rookmaaker and Antoine 2012); hence this species is assumed to have evolved in the near absence of trypanosomiasis. The tsetse belt is a contiguous geographic region running from West to East Africa in tropical latitudes (above and below the tropics of Capricorn and Cancer) where bush, wood, or forest predominate due to the climate. Africa held the now extinct, northern subspecies (*C. s. cottoni*), in grasslands of Sudan, eastern Democratic Republic of Congo and northwestern Uganda, along with the southern white rhinoceros (*C. s. simum*) in southern Africa.

The only reliable identification of a *Trypanosoma* sp. in a white rhinoceros hitherto was an ex situ animal infected with *Trypanosoma simiae* in Kenya (Mihok et al. 1994b). A report from

Zimbabwe is credible, but based only on circumstantial evidence (Taylor 1986). In contrast, black rhinoceros often harbor trypanosomes and naturally exist (or subspecies existed) in harmony with tsetse (*Glossina* spp.; Clausen 1981) in bush and wooded habitats where they browse across much of the tsetse belt. The importance of understanding these issues extends beyond Africa. Mortality attributed to trypanosomes has also occurred in Sumatran rhinoceros (*Dicerorhinus sumatrensis*; Mohamad et al. 2004; Andriansyah et al. 2008) although the trypanosome(s) detected appear to be a new species (Simon Reid pers. comm.). Researchers are also concerned about this issue for Javan rhinoceros (*Rhinoceros sondaicus*; Alvernita et al. 2016).

Trypanosome tolerance probably reflects innate immunity, rather than acquired immunity (Welburn et al. 2008), even if not exposed at birth. Hence, in well-managed and well-monitored translocations, naïve black rhinoceros can cope with trypanosomes (Mihok et al. 1992b). Many successful translocations in Kenya have confirmed this conclusion (Okita-Ouma 2004; Khayale et al. 2020). Impacts on black rhinoceros moved to areas with tsetse have mostly been subclinical, resulting from translocation stress and trypanosome exposure affecting erythrocyte dynamics (Kock et al. 1999). Disturbed erythropoiesis appears to be reversible, but with possible long-term effects such as hemosiderosis. This occurs rarely in free-ranging black rhinoceros (Kock et al. 1989), but is a concern in zoo animals (Kock et al. 1992a). Tsetse and trypanosomiasis in previous introductions of white rhinoceros to Kenya from Southern Africa was not an issue because sites selected were out of range of the parasite and fly on highland private cattle ranches, where the rhinos bred and thrived; six animals were introduced in 1965, 20 in the 1970s, and five in 1992 (Kenya Wildlife Service [KWS] 2007). The 1992 rhinoceros were the only ones apparently tested on arrival in Kenya in transit to Lewa Downs, Laikipia, Kenya. They were free of trypanosomes (parasitology, xenodiagnosis, mice; S. Mihok pers. comm.). Animals arriving from South Africa in 1992 after 2.5 wk at sea were sampled at the end of road travel from Mombasa to Nairobi (Table 1). All had atypical or abnormal low hemoglobin (Hb, 8.8–10.8 g/dL), normal or atypical low packed cell volume (PCV, 35–40%), and atypical low mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV; Table 1). Leukocyte profiles were also unremarkable except abnormal low neutrophils in two animals ($1.0–1.6 \times 10^3/\mu L$) resulting in a neutrophil:lymphocyte ratio (NLR) of 0.3–0.5. White rhinoceros from the KwaZulu-Natal, South Africa, are unlikely to be infected (Ledoka 2008), but trypanosomiasis does exist in that area (Gillingwater et al. 2010).

Before the introduction of 10 animals to the Ol Choro Oirua Wildlife Conservancy (OWC) Aitong, adjacent to the Maasai Mara National Reserve (MMNR), Kenya in 1994, a trial introduction of two animals resident in the highlands of Laikipia (Lewa Downs, without tsetse) to the release area was conducted in 1992, accompanied by 6 months of tsetse suppression (Mihok et al. 1994b). The two rhinoceros remained healthy until they were returned to Laikipia 2 yr later, with information on trypanosome status obtained on three occasions. Of these two animals (*Chuma* and *Chekwai*), *Chuma* harbored a cryptic infection of *T. simiae* at the time of translocation from Lewa Downs (Mihok et al. 1994b) with several atypical or abnormal low hematological values; subsequent diagnoses are reported in following text. After 30 wk at Aitong, both animals had *T. brucei* infections with atypical low PCVs (34%) and atypical or abnormal low hemoglobin (9.9–11.1 g/dL), along with other anomalies. Animals were sampled again only at 2 yr; they were healthy and hematology was normal. During an exhaustive 2023 slide review, *Chekwai* was found to be harboring a cryptic *Nannomonas* (subgenus of *Trypanosoma*) parasite (a single parasite; Fig. 1K). The smear from *Chekwai* also had a few leukocytes with inclusions suggestive of either *Theileria* (Fig. 1K), or large granular lymphocytes. It is not known whether these animals received trypanocides after diagnosis of *T.*

TABLE 1. Hematological values for white rhinoceros (*Ceratotherium simum*) sampled in Kenya compared with free-ranging white rhinoceros in South Africa.

	Kenya residents[a] (*n*=3)		Aitong[b] (*n*=15–19)		South Africa[c] (*n*=80–142)		After shipment[d] (*n*=5)	
Parameter[e]	Mean	±95% CI[f]	Mean	±95% CI	Mean	±95% CI	Mean	±95% CI
WBC (10^9/L)	7.36	5.47	12.32	1.37	15.36	0.80	11.54	3.37
Neutrophils (10^9/L)	2.41	4.89	5.27	0.91	7.51	0.45	8.75	2.42
Band neutrophils (10^9/L)	—	—	—	—	0.01	0.01	—	—
Lymphocytes (10^9/L)	3.44	0.75	4.51	0.89	4.27	0.36	2.05	0.83
Monocytes (10^9/L)	0.32	0.46	1.45	0.47	1.86	0.19	0.65	0.28
Eosinophils (10^9/L)	1.17	0.56	1.07	0.34	2.33	0.23	0.08	0.09
Basophils (10^9/L)	0.04	0.16	0.02	0.02	0.02	0.01	0.01	0.03
Metarubricytes (10/L)	0.00	0.00	0.03	0.03	—	—	0.00	0.00
Neutrophil:lymphocyte ratio	0.70	1.31	1.71	1.06	1.76	—	4.38	0.60
RBC (10^{12}/L)	7.44	1.19	7.15	0.42	7.35	0.28	7.07	0.79
Hgb (g/dL)	13.10	2.17	11.73	1.07	14.94	0.57	10.12	1.01
PCV (%)	44.73	11.44	39.88	2.57	44.46	1.49	37.80	2.26
MCH (pg/cell)	17.63	4.73	16.47	1.39	20.72	0.91	14.28	0.65
MCHC (g/dL)	29.33	4.28	29.47	2.17	33.89	1.15	26.66	1.61
Platelets (10^9/L)	422	154	399	127	588	40	715	887
MCV (fl)	60.30	17.86	56.06	2.96	60.61	1.15	53.62	3.21
Mode erythrocyte size (fl)	61.00	2.52	61.55	3.05	—	—	64.32	3.65
Median erythrocyte size (fl)	67.30		70.14	3.20	—	—	70.04	3.38

[a] Kenyan residents (rhino introduced some years previous to the current introduced group): before transport while resident at Lewa Downs or Solio Ranch, Kenya.
[b] Aitong, Kenya: 9–60 wk after translocation except *Chuma*, which was included in this group as it had a *Trypanosoma simiae* infection acquired before translocation (Mihok et al. 1994b). *n*=19 except platelets with *n*=15.
[c] South Africa: free ranging animals in Table 1 of Miller et al. (2015).
[d] After sea shipment: South African animals after 2.5 wk at sea and 1 d transportation on the road within Kenya.
[e] WBC = white blood cell count, RBC = red blood cell count, Hgb = hemoglobin, PCV = packed cell volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume.
[f] 95% CI = 95% confidence interval.

brucei at 30 wk. Full information on these two animals is updated in following text.

Deaths in translocated rhinoceros within Kenya have been sporadically attributed to trypanosomiasis, but mortality diagnosis is often unclear (Obanda et al. 2011; Mutinda et al. 2012). White rhinoceros introduced to Ruma (22) and Meru (84) National Parks, Kenya, with tsetse (KWS 2022; Okello et al. 2023) appear to be coping with trypanosomiasis. This may be because of new practices in the prophylactic use of trypanocides during translocation and some local suppression of tsetse populations through use of insecticide-impregnated targets.

We describe the history and health outcomes of naïve white rhinoceroses from South Africa introduced ex situ to a tsetse–trypanosome infected zone, in OWC MMNR Kenya. Evidence of the exposure, infection, and pathologies of these rhinoceros, with respect to trypanosomiasis, prior to (in bomas) or during release is presented. Our goals were (1) to illustrate the need to develop feasibility and risk assessment protocols for a species introduced outside of its native habitat; (2) to present evidence that clear responsibilities for health of translocated animals is vital, perhaps through a greater degree of state control of introduced species for conservation purposes. This can ensure the activity is appropriate and risk free to both introduced and indigenous species as advised in IUCN reintroduction and translocation guidelines, and Kenya Wildlife

Service (KWS) white rhinoceros conservation strategies (IUCN/SSC 2013).

MATERIALS AND METHODS

The 1994 translocation to the Mara

This operation was developed by the private sector and focused on a gift of 20 southern white rhinoceros from South Africa: 10 to Lake Nakuru National Park (LNNP) and 10 to the OWC owned by the Maasai community. As this species was not indigenous to Kenya, private arrangements were possible, whilst regulations prohibited the release of exotic species onto state land; an exception was made for LNNP, which is fenced and is tsetse-free. Oversight by the KWS was restricted in OWC to security, and to veterinary support in case of emergencies. The OWC comprises unfenced hilly bushed grassland, contiguous with the MMNR, hosting many tourists and livestock, and with occasional reports of wildlife ill health (Mwanzia et al. 1995; Gakuya et al. 2012). After this transnational introduction, further translocations of individuals occurred locally either to remove animals from the OWC or replenish the OWC, from privately owned resident animals in Laikipia. The LNNP introduction was unremarkable.

For the transnational translocation, the rhinoceroses were airlifted; with most arriving on 25 September 1994 (Granier 1995). Animals were initially kept in a boma at Wanga Hill (1.101 S, 35.247 E; 1,850 m) with managed release for grazing (Fig. 2). All animals were released on 21 December 1994 followed by close management, monitoring, and security. Trypanosomiasis incidence in cattle grazing in the immediate area was high in 1993–1994: 20.9% by buffy coat examination (18.3% *Trypanosoma vivax*, 7.2% *Trypanosoma congolense*, 2.6% *T. brucei*, with some mixed infections) (Ndegwa 1997). Human sleeping sickness (HSS, *T. brucei rhodesiense*) occurs in this area but is rare (Auty et al. 2012b). Tsetse were mainly *Glossina pallidipes* (Ndegwa 1997) with *Glossina swynnertoni* only common in distant woodlands and *Acacia* habitat (Ndegwa et al. 2001). Despite high incidence in cattle, infection rates in sampled tsetse were low in 1993–1994 (1.2% infection overall, 10 *T. vivax*, three *T. congolense* of 1,105 tsetse dissected; Ndegwa 1997). This appears to be typical for the area (Makhulu et al. 2021). Before the translocation focused on in this report, a trial was undertaken in 1992 using two animals, progeny arising from an earlier translocation of this subspecies from South Africa to Kenya in the 1960s and kept on private land, Lewa Downs Ranch in Laikipia, Northern Kenya.

Sampling

The KWS sampled animals on request, hence sampling was neither random nor systematic. Blood was collected from auricular veins or the medial radial vein after immobilization with 2–4 mg of etorphine hydrochloride (Immobilon, Veta-Pharma Ltd., Leeds, UK) delivered by hypodermic dart (Palmer Capchur, Powder Springs, Georgia, USA), 3-mL dart, 30-mm barbed and beveled steel needle, delivered from the ground with the Palmer Capchur long range projector rifle. Blood was placed into vacutainer tubes (10-mL plain glass serum tubes (Vacutainer, Becton Dickinson, Wokingham, UK). Most animals were sampled in the boma so physical capture stress should have been minimal (Pohlin et al. 2020).

Hematology

Blood was kept in a cool box with ice packs during transport to maintain trypanosome viability (McOdimba 1990) with most samples analyzed the next day; longer delays are noted. Blood was processed at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, using an automated blood cell counter (Coulter Counter ZM with a 256 Channelyzer (Beckman Coulter, Brea, California, USA) in duplicate (EDTA and heparin tubes often both collected). For platelets, EDTA tubes were always used. Complete blood counts (CBC) were performed, and hemoglobin (Hgb) was determined after lysis of erythrocytes (Zap-oglobin, Beckman Coulter). Packed cell volume (PCV) was measured by microhematocrit, and a 200-cell differential count was performed manually on a Giemsa-stained blood smear with blood preserved in EDTA. Reticulocytes were not counted, as they are typically few (Miller and Buss 2015). This protocol differed from previous work, when samples were mostly processed on the same day and when samples were sometimes processed at KWS (Kock et al. 1995, 1999).

Hematology summary results were compared to data for free-ranging white rhinoceros in South Africa (Miller et al. 2015). These data had been

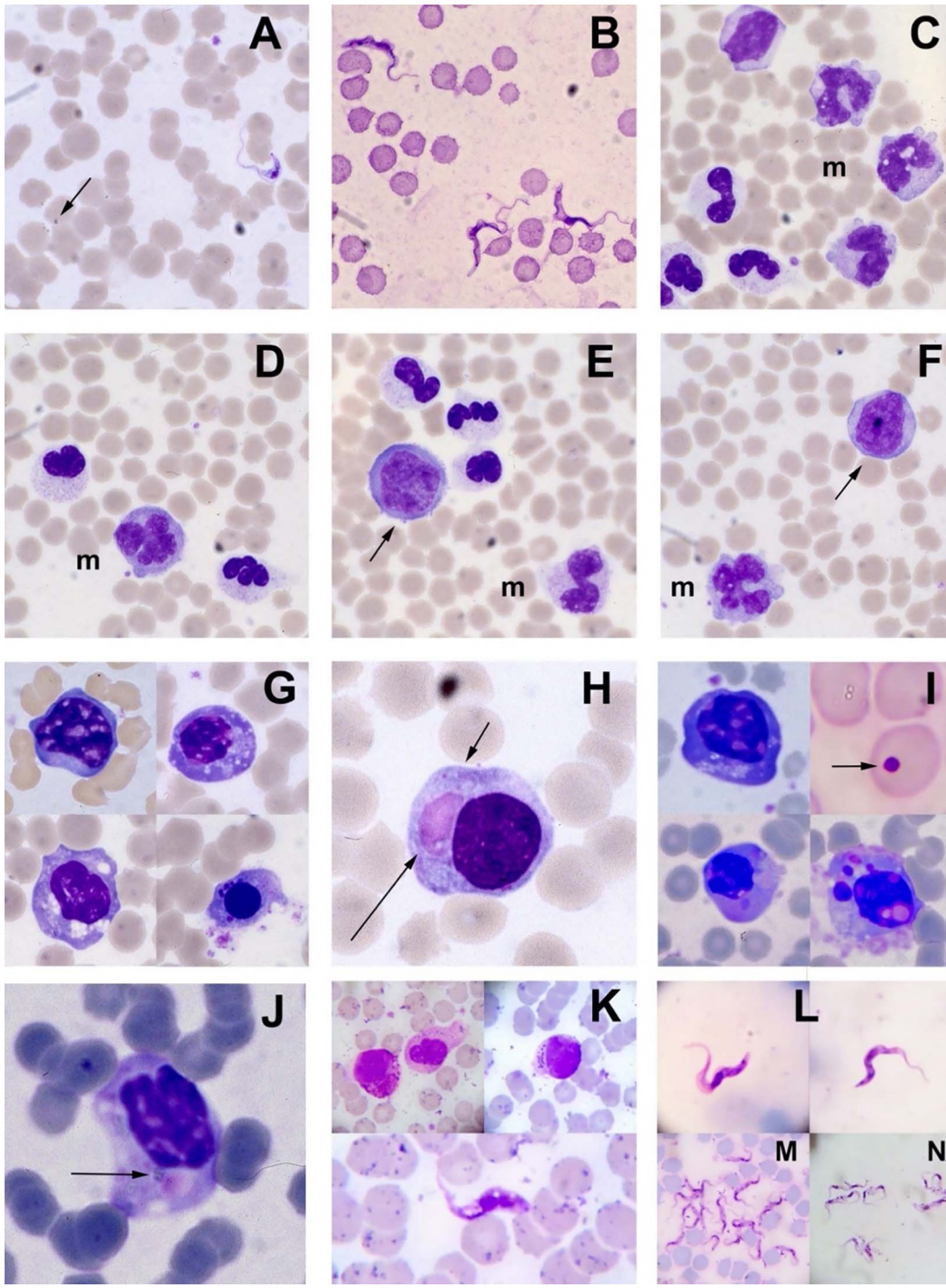

FIGURE 1. Trypanosomes and selected blood cells from differential smears taken from white rhinoceros (*Ceratotherium simum*) at Aitong, Kenya (A)–(K), and an unknown trypanosome found in a black rhinoceros (*Diceros bicornis*) at Ngulia, Kenya (L), compared with the two forms of *Trypanosoma simiae* (M, N). (A)–(G): white rhinoceros *KWS 663* with a *Trypanosoma brucei* infection at week 9 that died shortly afterwards, (H)–(J) white rhinoceros *Mweva* and *Mpekwa* at week 18. (K) White rhinoceros *Chekwai* at 2 yr, (L) black rhinoceros *Wairimu* 4 wk after translocation to Ngulia. Panels (A)–(F) are scans of Kodachrome color slides at 1,000×

collected under a wide variety of conditions and had robust sample sizes covering most of the parameters measured here. Individual values are referred to as abnormal when outside the 95% confidence interval (CI) of the mean, and atypical when outside the 75% CI. Similar data were consulted from white rhinoceros in bomas (Miller et al. 2022a, b), and at zoos (Trivedi et al. 2021). Comparisons added no further useful insights into the nature of our hematology observations. Interpretation of blood cells was based on Jain (1993) and Brooks et al. (2022), and blood smears examined from other wildlife.

Some photos of parasites and cells are presented from the 1994 Aitong animals, and from rhinoceros *Chuma* and *Chekwai*, translocated on 19 August 1992 (Mihok et al. 1994b). Differential smears were also still available for review for two South African white rhinoceros that came to Kenya, along with several slides from translocated black rhinoceros (Kock et al. 1999). These slides were reexamined in 2023 to document unusual parasites or cells.

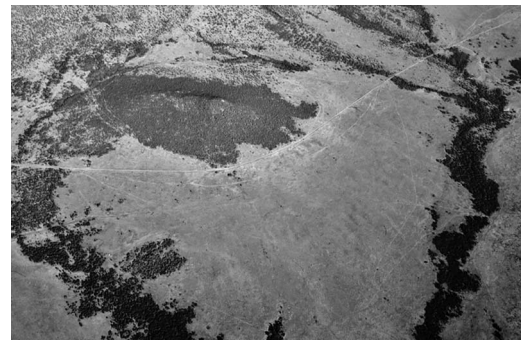

FIGURE 2. Aerial view of Wanga Hill at Aitong, Kenya on 16 March 1993, with north to the left. The rhinoceros were housed just off the top of the photograph. The topography and habitat view provides an insight into the presence of vector habitat atypical for southern white rhinoceros (*Ceratotherium simum simum*), which typically inhabit open grassland; the many thickets constitute a host risk in this context. Tsetse numbers were high in thickets and on the hill (~300 tsetse/trap/day in January 1992).

Parasitology

Diagnostics included examination of the buffy coat, wet, thin, and thick smears. Xenodiagnosis was performed for most samples using fresh and/or cryopreserved blood by feeding about 150 tsetse (*Glossina morsitans centralis* and *Glossina morsitans morsitans*) on rhinoceros blood. Infection was facilitated through the addition of goat blood and/or additives such as n-acetyleglucosamine and cholesterol (Mihok et al. 1992c, 1993; 1994a; Olubayo et al. 1994). Parasites that grew were sometimes passaged a second time in tsetse flies to verify identity. Isolation of *T. brucei* and *T. congolense* was routinely attempted by injecting 3–5 cyclophosphamide-immunosuppressed Balb/c mice (*Mus musculus*) with 0.5–1 mL of blood. Rats (*Rattus norvegicus*) were also occasionally used. Lastly, on just a few occasions when

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magnification from the 1990s. Panels (G)–(L) are handheld digital camera photos of differential smears photographed in 2023. m: monocyte. Object sizes can be estimated from erythrocytes which are about 6–8 μm in diameter given a similar mean corpuscular volume in rhinoceros and canine erythrocytes (Silva et al. 2017). Details: (A) *Trypanosoma brucei* in rhinoceros blood, comma-shaped *Theileria* spp. in erythrocyte (arrow); (B) *T. brucei* parasites in a mouse injected with rhinoceros blood; (C) three activated monocytes, a plain monocyte (m), and three neutrophils; (D) two neutrophils and a multilobed monocyte (m); (E) rare immature leukocyte (arrow), three neutrophils and a plain monocyte (m); (F) rare immature leukocyte (arrow) and multilobed monocyte (m); (G) three basophilic rubricytes and one flattened metarubricyte; (H) lymphocyte with an unusual magenta cytoplasmic inclusion (long arrow) and two small oval ringed inclusions with basophilic terminal spots (short arrow) in white rhinoceros (*Ceratotherium simum*) *Mweva*; (I) immature rubricytes from white rhinoceros *Mweva* and *Mpekwa* showing changes during maturation from a basophilic rubricyte to two polyorthocrhomatic rubricytes, one of which has a degenerating nucleus, the arrow in the top right of the image points to a micronucleus (very rare) in an erythrocyte from a South African white rhinoceros on arrival in Kenya; (J) uncertain mononuclear cell with small round inclusions containing basophilic spots (arrow) in *Mweva*; (K) basophilic inclusions resembling early life stages of *Theileria* in mononuclear cells, and a single unusual *Nannomonas* with morphology similar to *Trypanosoma conglense*, in white rhinoceros *Chekwai* 2 yr after translocation to Aitong—basophilic spots are stain granules and not piroplasms; (L) *Nannomonas* parasite with morphology similar to *Trypanosoma simiae* in black rhinoceros *Wairimu* 4 wk after translocation to Ngulia; (M) *T. simiae* in a pig (*Sus scrofa*); (N) *T. simiae* Tsavo trypomastigotes in bovine aortic endothelial cell culture at the time of first isolation from *Glossina pallidipes* at Ngulia.

parasites were detected but did not grow in rodents, an attempt was made to propagate them in rabbits (*Oryctolagus cuniculus*) and a pig (*Sus scrofa*).

Trypanosome identity was based on assessment of morphology, infectivity to rodents, location of mature infections in tsetse, and reactions with DNA probes (Mihok et al. 1992a). Probes for *Trypanosoma* (*T. brucei*), *Dutonella* (*T. vivax*), and three subtypes of *T. congolense* were available, along with probes for two types of *T. simiae* (Majiwa et al. 1993; Gibson et al. 2001). The human serum resistance gene could not be typed (Gibson et al. 2002), because PCR techniques were still in development (Desquesnes et al. 2022). No screening was done for *Trypanosoma godfreyi* or *Trypanosoma suis* as probes were not yet available (Hutchinson and Gibson 2015; Rodrigues et al. 2020).

On three occasions we cultured fresh blood, using a kit for in vitro isolation (KIVI; Truc et al. 1997), from three of the white rhinoceros. Five animals with *T. brucei* infections and one without were screened for microfilaria (King'ori et al. 2024) using nucleopore membrane filtration (3 μm; GE Healthcare, Little Marlow, Buckinghamshire, UK) or sodium dodecyl sulfate lysis of 3–5 ml of blood (Da Silva et al. 2023). Five *T. brucei* isolates from different rhinoceros were also tested for sensitivity to human serum using the (blood incubation infectivity) test (Njagu 1998). Infectivity to rodents was neutralized by human serum, implying they were *T. brucei brucei* (Mihok et al. 1989). Lastly, as previously mentioned (Mihok et al. 1996), most rhinoceros were screened with a serum antigen-ELISA trypanosome test kit for bovines (Eisler et al. 1998).

Treatment

No prior treatment or hematology data were provided for animals on arrival. Sick animals were sometimes treated with one of the trypanocides diminazine aceturate (Berenil MSD Animal Health, Intervet Pty Ltd, Spartan, Republic of South Africa), isometamidium chloride (Samorin, Merial, Lyon, France), or melarsamine hydrochloride (Cymelarsan, Merial, Lyon, France). This was noted in case histories from KWS interventions, but animals also probably received undocumented treatments from local veterinarians. Clinical responsibilities were never clearly defined in this primarily private-sector activity. These trypanocides are not necessarily curative in rhinoceros (Clausen 1981).

RESULTS

Full hematology of all rhinoceros sampled in this study and in other studies for comparison is summarized in Table 1. Nearly all Aitong animals had active trypanosome infections, as per Table 2. Of the animals translocated to Aitong, 8/10 were sampled at different time points over 1 yr from arrival in OWC (week 1), either for general health screening or in response to sickness. Chronology of sampling and diagnostic events according to individual rhinoceros are presented with key hematological values (Table 2).

Early case histories

Week 9—A KWS veterinarian visited and sampled a sick animal (*KWS 663*) on 1 December 1994, considered by the handlers as behaviorally depressed. All animals were still in the boma with managed grazing. Tsetse would have been seasonally abundant at this time (Ndegwa 1997; Ndegwa et al. 2001). A second sample was taken on 4 December from KWS 663; the animal died 4 d later (Table 2). *Trypanosoma brucei* was detected in the buffy coat and on smears at low numbers (Fig. 1A) from the live animal. Leukocyte (white blood cell) counts were similar on both days (Table 2), normal but with atypical high monocyte count (3.6×10^9/L and 3.7×10^9/L, respectively). Neutrophils were finely granulated with many juvenile forms (Fig. 1C–E). Many monocytes were activated (large size, irregular outline, foamy cytoplasm, vacuolation; Fig. 1C), often with multilobular nuclei (Fig. 1D, F). Normal monocytes have simpler nuclear and cytoplasmic features (Fig. 1E). Two large immature cells with contrasting nucleoli were noted (Fig. 1E, F), possibly promonocytes. Between samples, neutrophil numbers doubled, and lymphocytes decreased dramatically (from 5.01×10^3/μL to 0.87×10^3/L), resulting in a very abnormal NLR of 10.9. Trypanosomes appeared on days 8–11 in 5/5 injected mice (Fig. 1B).

Disturbed erythropoiesis was reflected in the presence of metarubricytes (0.15×10^9/L and 0.05×10^9/L, in the first and second samples, respectively) and a few basophilic rubricytes (Fig. 1G), cells rarely seen in blood (Miller and Buss 2015). The PCV was normal to low and hemoglobin was atypically low (Table 2). Erythrocyte morphology was unremarkable (Fig. 1A–H) but with an increase in MCV to an abnormal high (rise from 61.5 fL to 70.1 fL from first to second sample). The red blood cell (RBC) count also decreased to an atypical low (6.34×10^{12}/L reduced to 5.42×10^{12}/L from first to second sample). Piroplasms (*Theileria* spp.) were present (Fig. 1A), but are a normal feature of rhinoceros hematology (Kock et al. 1999; Otiende et al. 2015); their presence does not apparently affect hematology (Govender et al. 2011). One mononuclear cell was found with inclusions resembling early life stages of *Theileria* (Fig. 1H). *Babesia bicornis* (Nijhof et al. 2003) was not detected.

Week 12—KWS sampled five animals on 20 and 21 December 1994 to assess their health, given concerns around the earlier death. This precautionary sampling confirmed subclinical infections: all harbored *T. brucei* infections with no other trypanosomes detected (Table 2). This was in contrast to the low prevalence of *T. brucei* in cattle and tsetse in the immediate area in 1993–1994 (Ndegwa 1997), typical of the region as a whole (Auty et al. 2016; Lord et al. 2020). Animals were then released to roam freely. Detection of infections in blood required significant effort as only a few parasites were seen in buffy coats (3/5 rhinoceros) and none were seen on blood smears, but parasites readily infected tsetse. No infections developed just in the proboscis, ruling out *T. vivax* (Mihok et al. 1992b), which is common in cattle and tsetse in the area. A representative example of diagnostics is provided in Figure 3 for *Mpekwa*.

Leukocyte profiles were near normal. The PCV was normal in 4/5 animals with an atypical low value only in *Mpekwa* (Table 2). Erythropoiesis was nevertheless clearly disturbed with several atypical or abnormal values in all five animals. The most consistent finding was an atypical low MCHC (24.0–27.8 g/dL); metarubricytes were also present in *Ndomba* (0.18×10^9/L) and *Mpekwa* (0.18×10^9/L).

Late case histories

Researchers assessed animals that were sick up to week 60 when monitoring by KWS and ICIPE ended. Symptoms included depression, inappetence, and in one case neurological signs. Animals were sampled as previously described and infections with *Trypanosoma brucei* continued to be the main finding, with a few unusual *Nannomonas* also present. *Mpekwa* was the only animal monitored after multiple treatments with trypanocides. Its PCV fell to 30% despite treatment prior to and on week 18. All erythrocyte indices were abnormal or atypical low values. This PCV was just lower than the lowest value in any rhinoceros sampled at ICIPE (31%), a black rhinoceros with *T. vivax* (Mihok et al. 1992b). Immature rubricytes were present on smears, some with nuclear remnants (Fig. 1I).

An adult male rhinoceros, *Mweva*, that died at week 60 had been sampled at week 18. Its PCV was normal but metarubricytes were present (0.15×10^3/μL) along with immature rubricytes (Fig 1I). Hemoglobin, MCH, and MCHC were atypically low, and platelets were abnormally low. An unusual leukocyte was noted with round inclusions (Fig. 1J), possibly *Ehrlichia* (Jalovecka et al. 2018) or *Theileria* sp. (Tajeri et al. 2021). No blood was taken postmortem.

Week 26, 35, 40—Two animals with infections of *T. brucei* at week 12 (*Ndomba*, *Mbuhinga*) were sampled again at week 26 and were still infected with *T. brucei*, along with *Lubisiani*, who was sampled for the first time. After several trypanocide treatments, *Mpekwa* was free of parasites at week 35 but remained ill; it was treated again and moved to LNNP. *Mbuhinga* (with previous *T. brucei*) harbored a *Nannomonas* parasite at week 35; this unusual parasite did not infect tsetse and did not react with any DNA probe. Three mice injected with blood also died within 1 wk, an unusual finding in similar routine work at ICIPE. Both *Mpekwa* and *Mbuhinga* had

TABLE 2. Key diagnostics for eight white rhinoceros (*Ceratotherium simum*) translocated from South Africa to Aitong, Kenya, in 1994, and final results for two individuals translocated within Kenya from Laikipia to Aitong in 1992.

Animal[a]	Weeks[b]	Status[c]	PCV[d] (%)	Hgb[d] (g/dL)	WBC[d] (10^9/L)	Diagnosis	Trypanosomes	Buffy coat	Differential smear	Growth in rodents	Xenodiagnosis	KIVI[t]	*Theileria*
						1994 translocations							
KWS 663[e]	9	Sick	39	11.5	14.9	*Trypanosoma brucei*	Patent infection	+[f]	+	Fast	nd[g]	nd	+
	9	Died[h]	38	12.0	14.4								
Ndomba	12	Boma	48	13.4	11.9	*T. brucei*	Patent infection	+	–[i]	Fast	*T. brucei*	nd	+
	26	Boma	38	12.9	16.7	*T. brucei*	Cryptic infection	–	–	None[j]	nd	nd	–
Sobokwe[k]	12	Boma	51	14.0	11.7	*T. brucei*	Cryptic infection	–	–	None[l]	*T. brucei*	nd	++
Dengeze	12	Boma	45	10.8	9.0	*T. brucei*	Patent infection	+	–	Slow	*T. brucei*	nd	++
Mbuhinga	12	Boma	48	12.7	10.2	*T. brucei*	Cryptic infection	–	–	Fast	*T. brucei*	nd	+
	26	Boma	43	13.9	10.5	*T. brucei*	Cryptic infection	–	–	Fast	nd	nd	–
	35	Free[m]	38	7.5	14.1	*Nannomonas*[n]	Patent infection	+	–	Mice died	–	*N. sp*	–
Mpekwa	12	Boma	37	9.7	14.0	*T. brucei*	Patent infection	+	–	Fast	*T. brucei*	nd	–
	18	Boma	30	7.4	13. 9	Treated[o,p]	–	–	–	None	–	nd	+
	35	Boma	36	10.1	17.7	–	–	–	–	None	–	–	–
Mweva	18	Boma[q]	44	11.1	8.6	–	–	–	–	None	–	nd	–
	40	Sick	44	17.4	11.3	*Nannomonas*[n]	Patent infection	+	–	None	–	–	+
	60	Died[r]	nr[s]	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Lubisiani	26	Boma	35	12.9	11.5	*T. brucei*	Cryptic infection	–	–	Fast	nd	nd	–
						1992 translocations							
Chuma	0	Arrival	36	10.7	5.3	*T. simiae*	Cryptic infection	–	–	None	*T. simiae*	nd	+
	30	Boma	34	11.1	15.1	*T. brucei*	Cryptic infection	–	–	Fast	*T. brucei*	nd	–
	110	Healthy	41	12.6	11.0	–	–	–	–	None	–	nd	+

TABLE 2. Continued.

Animal[a]	Weeks[b]	Status[c]	PCV[d] (%)	Hgb[d] (g/dL)	WBC[d] (10^9/L)	Diagnosis	Trypanosomes	Buffy coat	Differential smear	Growth in rodents	Xenodiagnosis	KIVI[t]	*Theileria*
Chekwai	0	Arrival	45	12.7	9. 9	–	–	–	–	None	–	nd	+
	30	Boma	34	9.9	13.0	*T. brucei*	Cryptic infection	–	–	Fast	*T. brucei*	nd	–
	110	Healthy	41	12.9	11.6	*Nannomonas*[n]	Single parasite	–	+	None	–	nd	+

[a] The individual animal names were kept in the text and table to be consistent with histories kept, as opposed to animal reference numbers, which varied with samples in all except one individual (*KWS 663*). These names and number ID used are italicized in the text.
[b] Weeks since translocation.
[c] Status of animal: sampled sick, dead, or in boma alive.
[d] PCV = packed cell volume, Hgb = hemoglobin, WBC = white blood cell count.
[e] Seven days before death.
[f] + = positive.
[g] nd = not done.
[h] Four days before death.
[i] – = negative.
[j] Blood was 3 d old and only one mouse injected.
[k] Odd result mice.
[l] Blood was 1 d old and four mice injected.
[m] Free = animal released out of boma into Ol Choro Oirua Wildlife Conservancy, Aitong, Kenya.
[n] Unknown species.
[o] This animal had received trypanocides recently and was negative for trypanosome infection.
[p] Free roaming before moved to Nakuru.
[q] Free roaming.
[r] Hemosiderosis.
[s] nr = no hematology record retained.
[t] KIVI = kit for in vitro isolation.

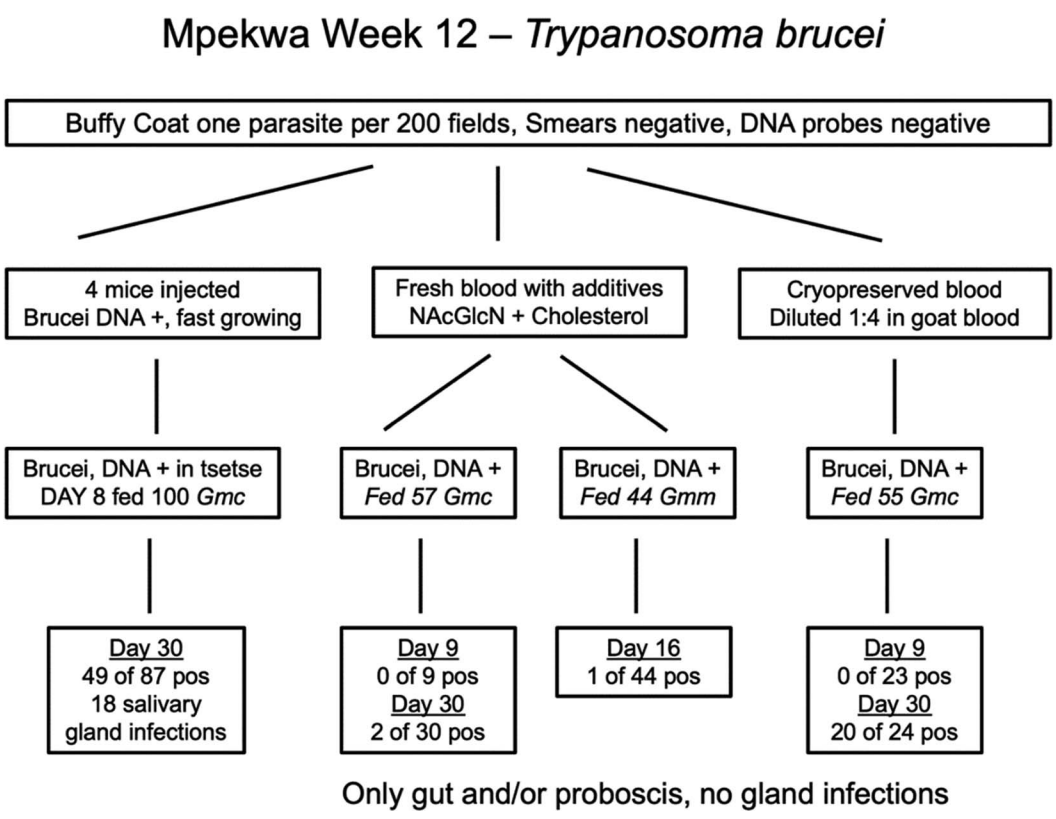

FIGURE 3. Schematic of diagnostics performed on white rhinoceros (*Ceratotherium simum*) *Mpekwa* on December 20, 1994, with a patent infection of *Trypanosoma brucei* at very low parasitemia. Blood was handled in three different ways. On the left pathway, blood was injected into mice and then mouse blood was fed to tsetse on day 8; identity was confirmed in mice and tsetse with DNA probes. Other blood was fed to different tsetse in three ways, either fresh or after cryopreservation. Only *T. brucei* was detected with DNA probes for several gut infections that were blotted. Salivary gland infections were common only in parasites passaged through mice; tsetse on day 9 were negative on microscopic examination of guts (*Gmc*: *Glossina morsitans centralis*, *Gmm*: *G. m. morsitans*).

atypical or abnormal hematological values. At week 40, *Mweva* was sampled again and also harbored a *Nannomonas* parasite, which might have been missed at week 18; this parasite did not grow in mice or tsetse or react with any DNA probe. Hematology was normal with the important exception of atypical high Hgb (17.4 g/dL) and MCHC (39.6 g/dL).

A KWS veterinarian visited *Mweva* on week 60 for evaluation of a granulating wound and fever. It had nervous symptoms similar to those described for a black rhinoceros infected with trypanosomes in Zambia (Chomba et al. 2012). Specifically, *Mweva* had unilateral sensory loss (sight, hearing, touch). It was possible to inject into the neck the trypanocide melarsamine hydrochloride (Cymelarsan) manually, without immobilization. *Mweva* died 9 d later; gut stasis was noted at necropsy. No hematology records were retained postmortem. Histopathology was unremarkable except for a buildup of hemosiderin in the spleen. Histopathology did not detect severe meningoencephalitis, noted in black rhinoceros infected with *T. brucei* in Zambia (Anderson et al. 2011) and a key feature of the pathology of *T. brucei* (Figarella 2021).

Hematology

Statistical comparisons of hematological indices versus literature data were constrained by small sample sizes and by the opportunistic

nature of sampling. Qualitative comparisons based on 95% CIs for each data subset indicated that leukocyte profiles of (trypanosome-infected) Aitong animals differed from norms, with low neutrophils and eosinophils, and hence low total leukocytes, as well as low platelets (Table 1). Erythrocyte indices were also low (Hgb, PCV, MCH, MCHC, and MCV) except for RBC count. Circulating rubricytes, as well as immature cells, suggested a major disturbance of erythropoiesis.

Unknown trypanosome

The unusual *Nannomonas* found in three white rhinoceros remains unidentified due to the problem of finding a suitable host for characterization. The single photograph available (Fig. 1K) of this parasite resembled the morphology of *T. congolense*. Another unidentified *Nannomonas* found in black rhinoceros in contrast resembled *T. simiae* (Fig. 1L). That parasite infected four rhinoceros 4 wk after translocation to Ngulia but was not reported in Mihok et al. (1992a). It did not grow in laboratory animals including a pig, nor react with DNA probes. It differed from the unusual parasites at Aitong in that it readily infected *G. m. centralis* (gut and proboscis). It might have been *T. godfreyi*, for which we did not have a DNA probe, or a nonreactive form of *T. simiae* (Fig. 1M) or *T. simiae* Tsavo (Fig. 1N; Zweygarth et al. 1995). We attempted to type the *Nannomonas* from Ngulia with PCR in 2023 from a Giemsa-stained slide following Getahun et al. (2022) but were unsuccessful.

DISCUSSION

In 1994 the biological sampling protocols were standardized, when undertaken by the KWS and ICIPE, and these data were a reasonable representation of the hematological and diagnostic status of the animals. That said, the use of an immobilizing drug, and the method of delivery each may have profound adverse effects on white rhinoceros blood results, in addition to effects from transport or capture stress, and from anesthetic complications such as muscle tremors and respiratory depression (Kock and Burroughs 2021). To reduce blood count anomalies associated with these issues, immobilization was undertaken without animals being chased and recumbency times were kept to the minimum. Reversal of the neuroleptic requires use of morphine antagonists (diprenorphine at 2.5 mg per 1 mg etorphine administered intravenously), but in the white rhinoceros renarcotization may occur with this protocol (Rogers 1993). This does not affect results reported here, as it would be an effect postsampling and well before any further immobilization. Therefore, any effects should have been consistent to those affecting literature data for immobilized white rhinoceros. It is notable that in contrast to the sea-transported rhinoceros sampled in 1992, three trypanosome-free white rhinoceros sampled locally without major transport stress in 1994 had nearly normal erythrocyte profiles (Table 1). The two animals from the 1992 translocation to the Mara were infected with *T. brucei*, but it is not clear whether they were ever treated after this finding was disclosed. Importantly, no veterinary intervention was ever requested after tsetse suppression activities ceased. The animals were returned to Lewa Downs after 2 yr in good health with no further history of illness; hence, it can be assumed that management of the initial tsetse challenge was effective.

Overall, 6/10 rhinoceros sent to the OWC in 1994 died from trypanosomiasis (KWS 2022). It is worth noting that the use of the serum antigen-ELISA trypanosome test kit for bovines proved to be unreliable in white rhinoceros, as eventually proved to be the case for bovines (Eisler et al. 1998). Five animals sampled with *T. brucei* infections and one without, were negative for microfilaria, which concurs with the rarity of evidence for *Stephanofilaria* spp. in white versus black rhinoceros in Kenya (King'ori et al. 2024). *Ehrlichia* sp. is present in elephants in Kenya (King'ori et al. 2019), but its presence in white rhinoceros has not been studied. Serological evidence suggests that it infects black rhinoceros (Kock et al. 1992b). The presence of unusual

cytoplasmic inclusions suggestive of *Ehrlichia* sp. in two animals that died may be of significance. Idiopathic hemosiderosis occurs in captive black rhinoceros but is otherwise rare (Kock et al. 1992a; Dennis et al. 2007; Radeke-Auer et al. 2023); hemosiderosis was recorded in the one white rhinoceros case examined histologically amongst these mortalities in OWC. There was no evidence for any other cause of, or potentially contributory factor to, the death of the animals.

In Kenya, PCR has suggested the presence of several known *Nannomonas* types in highland black rhinoceros introduced to Meru National Park. This area had high numbers of *G. palllidipes* ($\sim$ 50 per trap) when a few animals were assessed to investigate significant mortalities after translocation (Obanda et al. 2011; Mutinda et al. 2012). Although no trypanosomes were detected in smears, PCR detected mixed trypanosome infections in two subadult black rhinoceros that were chosen for diagnosis. Parasites were typed as *T. congolense* forest and savanna types, *T. godfreyi*, *T. simiae*, and *T. simiae* Tsavo. One of the black rhinoceros had a PCV of 18% and died despite treatment (Obanda et al. 2011). Our failure to identify the *Nannomonas* from black rhinoceros from Ngulia after the fact may have reflected the use of a stored, stained slide for this attempt, or may simply have been related to the fact that there are still several wildlife trypanosomes that cannot be identified (Auty et al. 2012a). Although no trypanosomes were detected on blood collected postmortem from three white rhinoceros, this may be unreliable depending on the degree of autolysis. Blood cell histograms are still available for all animals and might provide further insights into how rhinoceros react to trypanosome infections.

Following our study, a further six privately owned white rhinoceros from highland sanctuaries were translocated to OWC (1996, 2003, 2009), with two removals. Three animals were registered in OWC in 2007 (KWS 2007). An animal was born at OWC in 2008 and currently resides at the boma with an adult female. The fate of the four individuals surviving from the 1994 introduction and subsequent introductions is unclear, but they are presumed dead. As a calf, the animal born in 2008 developed a condition that sometimes led to convulsions (epileptic like seizures) but it has survived for over a decade after several treatments with the trypanocide quinapyramine (sulphate and chloride in a 3:2 ratio; Triquin, Vetoquinol, Paris, France). This drug is typically used for *T. evansi*, which is not transmitted by tsetse (Desquesnes et al. 2013; Harit et al. 2019).

We summarize our findings as follows:

- The lessons learned from this experience in Kenya are important to future conservation management of white rhinoceros, particularly in the context of movement into areas infested with tsetse fly.
- White rhinoceros are particularly susceptible to a normally rare trypanosome in wildlife (*T. brucei*). Subpatent infections are common and include the new finding of an unknown species or DNA type of *Nannomonas*. The pathogenic effect is acute to chronic, moderate to severe, and can lead to death.
- *Trypanosoma brucei* is the usual human pathogen causing sleeping sickness and there is potential for zoonotic transmission. Where white rhinoceros are infected in proximity to humans, this risk is not insignificant given their large body size, attraction to biting flies and tolerance of human presence.
- We recommend PCR combined with proof of parasite identity through inoculation of blood into a variety of animals (not just rodents), as well as xenodiagnosis (e.g., with cryopreserved blood) for future characterization of the parasites infecting rhinoceros.
- Management of infections by prophylactic treatment with trypanocides and clinical treatment is useful but may only provide a temporary benefit. Chronic ill health is common and may manifest as neurological symptoms because parasites cross the blood brain barrier.
- Routine hematology is only partially informative because of similarities to other stress

effects resulting from transport or confinement or sampling (where neuroleptics are used for immobilization). Disturbed erythropoiesis is the best indicator of infection, with low Hgb, PCV, MCH, MCHC, and MCV, accompanied by low neutrophils and platelets. An inadequate response to anemia is also often noted, with metarubricytes present on blood smears.

- Given the endangered status of the white rhinoceros, requiring metapopulation management and use of ex situ sanctuaries, extreme caution is advised when considering locations where tsetse and trypanosomes exist and animals are free range.
- Ongoing tsetse suppression methods within fenced systems are probably the only ethical, safe, and reliable control option to justify such animal introductions. Efficacy of tsetse suppression should also be validated with appropriate monitoring.
- Altogether, vulnerability to trypanosome infection for naïve black and white rhinoceros remains a complex issue with many knowledge gaps (du Toit et al. 2005).

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LITERATURE CITED

Alvernita G, Khairani KO, Dariyan, Lukitaningsih D, Supriyono, Pahlawan DS, Kalbuadi ZG, Hadi UK. 2016. The tabanid fly biodiversity and its potency as transmission vector of trypanosomiasis to the Javana rhino population with the Uung Kulon National Park. In: *Proceedings of the 3rd International Wildlife Symposium*, University of Lampung, Bandar Lampung, Indonesia, 18–20 October, pp. 109–113.

Anderson NE, Mubanga J, Fevre EM, Picozzi K, Eisler MC, Thomas R, Welburn SC. 2011. Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS Negl Trop Dis* 5:e1211.

Andriansyah DC, Candra D, Agil M, Handayani SU, Tiuria R, Koesumawati U, Radcliffe RW. 2008. Disease surveillance around Way Kambas National Park to support Sumatran rhino conservation and health. In: *Proceedings of the 3rd International Meeting on Asian Zoo/Wildlife Medicine and Conservation*, Bogor, Indonesia, 19–22 August, pp. 128–129.

Auty H, Anderson NE, Picozzi K, Lembo T, Mubanga J, Hoare R, Fyumagwa RD, Mable B, Hamill L, et al. 2012a. Trypanosome diversity in wildlife species from the Serengeti and Luangwa Valley ecosystems. *PLoS Negl Trop Dis* 6:e1828.

Auty H, Morrison LJ, Torr SJ, Lord J. 2016. Transmission dynamics of Rhodesian sleeping sickness at the interface of wildlife and livestock areas. *Trends Parasitol* 32:608–621.

Auty HK, Picozzi K, Malele I, Torr SJ, Cleaveland S, Welburn S. 2012b. Using molecular data for epidemiological inference: Assessing the prevalence of *Trypanosoma brucei rhodesiense* in tsetse in Serengeti, Tanzania. *PLoS Negl Trop Dis* 6:e1501.

Brooks MB, Harr KE, Seelig DM, Wardrop KJ, Weiss DJ. 2022. *Schalm's veterinary hematology*. John Wiley & Sons, Hoboken, New Jersey.

Chomba C, Squarre D, Kamboyi H. 2012. Notes on black rhino mortalities in North Luangwa National Park, Zambia. *Pachyderm* 52:88–90.

Clausen B. 1981. Survey for trypanosomes in black rhinoceros (*Diceros bicornis*). *J Wildl Dis* 17:581–586.

Da Silva TR, Bomfim SRM, Cavalcanti FBP, Lopes WDZ, Utsonomiya YT, Cadioli FA. 2023. "Lysis and concentration technique" improves the parasitological diagnosis of *Trypanosoma vivax*. *Vet Parasitol* 323:110043.

Dennis PM, Funk JA, Rajala-Schultz PJ, Blumer ES, Miller RE, Wittum TE, Saville WJA. 2007. A review of some of the health issues of captive black rhinoceroses (*Diceros bicornis*). *J Zoo Wildl Med* 38:509–517.

Desquesnes D, Holzmuller P, Lai DH, Dargantes A, Lun ZR, Jittaplapong S. 2013. *Trypanosoma evansi* and surra: A review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomed Res Int* 2013:194176.

Desquesnes M, Gonzatti M, Sazmand A, Thévenon S, Bossard G, Boulangé A, Gimonneau G, Truc P, Herder S, et al. 2022. A review on the diagnosis of animal trypanosomoses. *Parasit Vectors* 15:64.

du Toit R, Lovemore M, Dunham KM. 2005. *The reintroduction of rhinos to Gonarezhou National Park, Zimbabwe: A feasibility study*. SADC Regional Programme for Rhino Conservation, Harare, Zimbabwe, 102 pp.

Eisler MC, Lessard P, Masake RA, Moloo SK, Peregrine AS. 1998. Sensitivity and specificity of antigen-capture ELISAs for diagnosis of *Trypanosoma congolense* and *Trypanosoma vivax* infections in cattle. *Vet Parasitol* 79:187–201.

Figarella K. 2021. Neuropathogenesis caused by *Trypanosoma brucei*, still an enigma to be unveiled. *Microb Cell* 8:73–76.

Gakuya F, Ombui J, Maingi N, Muchemi G, Ogara W, Soriguer RC, Alasaad S. 2012. Sarcoptic mange and cheetah conservation in Masai Mara (Kenya):

Epidemiological study in a wildlife/livestock system. *Parasitology* 139:1587–1595.

Getahun MN, Villinger J, Bargul JL, Muema JM, Orone A, Ngiela J, Ahuya PO, Saini RK, Torto B, Masiga DK. 2022. Molecular characterization of pathogenic African trypanosomes in biting flies and camels in surra-endemic areas outside the tsetse fly belt in Kenya. *Int J Trop Insect Sci* 42:3729–3745.

Gibson W, Backhouse T, Griffiths A. 2002. The human serum resistance associated gene is ubiquitous and conserved in *Trypanosoma brucei rhodesiense* throughout East Africa. *Infect Genet Evol* 1:207–214.

Gibson WC, Stevens JR, Mwendia CMT, Makumi JN, Ngotho JM, Ndung'u JM. 2001. Unravelling the phylogenetic relationships of African trypanosomes of suids. *Parasitology* 122:625–631.

Gillingwater K, Mamabolo MV, Majiwa PAO. 2010. Prevalence of mixed *Trypanosoma congolense* infections in livestock and tsetse in KwaZulu-Natal, South Africa. *J S Afr Vet Assoc* 81:219–223.

Govender D, Oosthuizen MC, Penzhorn BL. 2011. Piroplasm parasites of white rhinoceroses (*Ceratotherium simum*) in the Kruger National Park, and their relation to anaemia. *J S Afr Vet Assoc* 82:36–40.

Granier N. 1995. White rhinos to Kenya. *Swara* 18:10–12.

Harit A, Kumar RR, Vatsya S. 2019. Cerebral trypanosomosis in buffalo—a case study. *J Entomol Zool Stud* 7:1037–1039.

Hutchinson R, Gibson W. 2015. Rediscovery of *Trypanosoma* (*Pycnomonas*) *suis*, a tsetse-transmitted trypanosome closely related to *T. brucei*. *Infect Genet Evol* 36:381–388.

IUCN/SSC. (2013). *Guidelines for reintroductions and other conservation translocations*. IUCN Species Survival Commission, Gland, Switzerland, viiii + 57 pp.

Jain NC. 1993. *Essentials of veterinary hematology*. Lea & Febiger, Philadelphia, Pennsylvania.

Jalovecka M, Hajdusek O, Sojka D, Kopacek P, Malandrin L. 2018. The complexity of piroplasms life cycles. *Front Cell Infect Microbiol* 8:248.

Khayale C, Kariuki L, Chege G, Sibanda M, Mulama M, Okita-Ouma B, Amin R. 2020. Progress on the Kenya black rhino Action Plan (2017–2021). *Pachyderm* 61:109–119.

King'ori E, Obanda V, Chiyo PI, Soriguer RC, Morrondo P, Angelone S. 2019. Molecular identification of *Ehrlichia*, *Anaplasma*, *Babesia* and *Theileria* in African elephants and their ticks. *PLoS One* 14:e0226083.

King'ori E, Waiguchu G, Ruoro M, Muriithi K, Mumbi C, Omondi M, Aminga D, Angwenyi S, Mijele D, Chiyo PI. 2024. Identifying risk factors for *Stephanofilaria*-caused ulcerative dermal lesions, in black and white rhinoceros' meta-population in Kenya. *Transbound Emerg Dis* 2024:2655970.

Kock MD, Burroughs R. 2021. *Chemical and physical restraint of African wild animals*. 3rd Ed. Michael D Kock, IWVS (Africa), Greyton, South Africa, 476 pp.

Kock N, Foggin C, Kock MD, Kock R. 1992a. Hemosiderosis in the black rhinoceros (*Diceros bicornis*): A comparison of free-ranging and recently captured with translocated and captive animals. *J Zoo Wildl Med* 23:230–234.

Kock N, Kock M, Pawandiwa A, Matambo T. 1989. Postmortem findings in translocated black rhinoceroses (*Diceros bicornis*) in Zimbabwe. In: *Erkrangungen der Zootiere: Verhandlungsbericht des Internationalen Symposiums über die Erkrankungen der Zoo-und Wildtiere* 31:275–279.

Kock ND, Jongejan F, Kock MD, Kock RA, Morkel P. 1992b. Serological evidence for *Cowdria ruminantum* infection in free-ranging black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses in Zimbabwe. *J Zoo Wildl Med* 23:409–413.

Kock R, Wambua J, Mwanzia J, Saigwa K. 1995. Effects of translocation on the black rhinoceros (*Diceros bicornis*)—red and white cell parameters. In: *Erkrankungen der Zootiere: Verhandlungsbericht des Internationalen Symposiums über die Erkrankungen der Zoo-und Wildtiere* 37:33–39.

Kock RA, Mihok S, Wambua J, Mwanzia J, Saigawa K. 1999. Effects of translocation on hematologic parameters of free-ranging black rhinoceros (*Diceros bicornis michaeli*) in Kenya. *J Zoo Wildl Med* 30:389–396.

Kenya Wildlife Service. 2007. *Conservation and management strategy for the black rhino (*Diceros bicornis michaeli*) and management guidelines for the white rhino (*Ceratotherium simum simum*) in Kenya (2007–2011)*. 3rd Ed. Kenya Wildlife Service, Nairobi, Kenya.

Kenya Wildlife Service. 2022. *White rhino (*Ceratotherium simum*) conservation & management action plan (2021–2025) in Kenya*. Kenya Wildlife Service, Nairobi, 88 pp.

Ledoka MV. 2008. *Molecular characterization of trypanosomes commonly found in cattle, wild animals and tsetse flies in KwaZulu-Natal, South Africa, 2005–2007*. MSc Thesis. Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa, 100 pp.

Lord JS, Lea RS, Allan FK, Byamungu M, Hall DR, Lingley J, Mramba F, Paxton E, Vale GA, et al. 2020. Assessing the effect of insecticide-treated cattle on tsetse abundance and trypanosome transmission at the wildlife-livestock interface in Serengeti, Tanzania. *PLoS Negl Trop Dis* 14:e0008288.

Majiwa PAO, Maina M, Waitumbi JN, Mihok S, Zweygarth E. 1993. *Trypanosoma (Nannomonas) congolense*: Molecular characterization of a new genotype from Tsavo, Kenya. *Parasitology* 106:151–162.

Makhulu EE, Villinger J, Adunga VO, Jeneby MM, Kimathi EM, Mararo E, Oundo JW, Musa AA, Wambua L. 2021. Tsetse blood-meal sources, endosymbionts and trypanosome-associations in the Maasai Mara National Reserve, a wildlife–human–livestock interface. *PLoS Negl Trop Dis* 15:e0008267.

McOdimba FA. 1990. The effect of temperature and storage on the infectivity and motility of African animal trypanosomes in the blood of different hosts. *Acta Trop* 47:53–60.

Mihok S, Kock R, Masake R. 1996. Health implications of translocations of endangered species in Africa: Trypanosomiasis in rhinoceros. In: *Proceedings Joint Conference*, Junge RE, editor, American Association of Zoo Veterinarians, Wildlife Disease Association, American Association of Wildlife Veterinarians, East Lansing, Michigan, 12–17 August, pp. 423–424.

Mihok S, Munyoki E, Brett RA, Jonyo JF, Röttcher D, Majiwa PAO, Kang'ethe EK, Kaburia HFA, Zweygarth E. 1992a. Trypanosomiasis and the conservation of black rhinoceros (*Diceros bicornis*) at the Ngulia Rhino Sanctuary, Tsavo West National Park, Kenya. *Afr J Ecol* 30:103–115.

Mihok S, Munyoki EN, Masaninga F, Ndegwa PN, Olubayo RO. 1994a. Isolation of *Trypanosoma* spp. from wild tsetse flies through procyclic expansion in *Glossina morsitans centralis*. *Acta Trop* 56:25–37.

Mihok S, Olubayo RO, Darji N, Zweygarth E. 1993. The influence of host blood on infection rates in *Glossina morsitans* sspp. infected with *Trypanosoma congolense*, *T. brucei* and *T. simiae*. *Parasitology* 107:41–48.

Mihok S, Olubayo RO, Moloo SK. 1992b. Trypanosomiasis in the black rhinoceros (*Diceros bicornis* Linnaeus, 1758). *Rev Sci Tech* 11:1169–1173.

Mihok S, Otieno LH, Darji N. 1989. *Isoenzyme polymorphism and the blood incubation infectivity test (BIIT): Contradictory predictions of human infectivity for* Trypanosoma brucei. *Publication No. 115*. International Scientific Council for Trypanosomiasis Research and Control, Nairobi, Kenya, pp. 137–141.

Mihok S, Otieno LH, Darji N, Munyinyi D. 1992c. Influence of D(+)-glucosamine on infection rates and parasite loads in tsetse flies (*Glossina* spp.) infected with *Trypanosoma brucei*. *Acta Trop* 51:217–228.

Mihok S, Zweygarth E, Munyoki EN, Wambua J, Kock R. 1994b. *Trypanosoma simiae* in the white rhinoceros (*Ceratotherium simum*) and the dromedary camel (*Camelus dromedarius*). *Vet Parasitol* 53:191–196.

Miller M, Buss P, Wanty R, Parsons S, Van Helden P, Olea-Popelka F. 2015. Baseline hematologic results for free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa. *J Wildl Dis* 51:916–922.

Miller MA, Buss PE. 2015. Chapter 55. Rhinoceridae (Rhinoceroses). In: *Fowler's zoo and wild animal medicine*. Vol. 8. Miller RE, Fowler ME, editors. Saunders, St. Louis, Missouri, pp. 539–546.

Miller MA, Olea-Popelka F, Buss PE. 2022a. Effect of boma confinement on hematologic and biochemical values in free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa. *J Wildl Dis* 58:735–745.

Miller MA, Olea-Popelka F, Buss PE. 2022b. Effect of capture and immobilization on boma adaptation in free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa. *J Wildl Dis* 58:816–824.

Mohamad A, Vellayan S, Radcliffe RW, Lowenstine LJ, Epstein J, Reid SA, Paglia DE, Radcliffe RM, Roth TL, et al. 2004. Trypanosomiasis (surra) in the captive Sumatran rhinoceros (*Dicerorhinus sumatrensis sumatrensis*) in peninsular Malaysia. In: *Proceedings of the 11th International Conference of the Association of Institutions for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress*, Petaling Jaya, Malaysia, 23–27 August, Universiti Putra Malaysia Press, Serdang, Malaysia, pp. 187–189.

Moodley Y, Westbury MV, Russo IRM, Gopalakrishnan S, Rakotoarivelo A, Olsen RA, Prost S, Tunstall T, Ryder OA, et al. 2020. Interspecific gene flow and the evolution of specialization in black and white rhinoceros. *Mol Biol Evol* 37:3105–3117.

Mutinda M, Otiende M, Gakuya F, Kariuki L, Obanda V, Ndeere D, Ndambiri E, Kariuki E, Lekolool I, et al. 2012. Putative filariosis outbreak in white and black rhinoceros at Meru National Park in Kenya. *Parasit Vectors* 5:206.

Mwanzia JM, Kock R, Wambua J, Kock N, Jarret O. 1995. An outbreak of sarcoptic mange in the free-living cheetah (*Acinonyx jubatus*) in the Mara region of Kenya. In: *Proceedings of American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians Joint Conference*, pp. 105–112.

Ndegwa PN. 1997. *Studies on ecology and epidemiological importance of* Glossina swynnertoni *Austen, near Aitong, Maasai Mara, south western Kenya*. PhD Dissertation, University of Nairobi, Nairobi, Kenya. 145 pp.

Ndegwa PN, Mihok S, Oyieke FA. 2001. Habitat preferences and activity patterns of *Glossina swynnertoni* Austen (Diptera: Glossinidae) in Aitong, Masai Mara, Kenya. *Insect Sci Appl* 21:113–122.

Nijhof AM, Penzhorn BL, Lynen G, Mollel JO, Morkel P, Bekker CPJ, Jongejan F. 2003. *Babesia bicornis* sp. nov. and *Theileria bicornis* sp. nov.: tick-borne parasites associated with mortality in the black rhinoceros (*Diceros bicornis*). *J Clin Microbiol* 41:2249–2254.

Njagu ZW. 1998. *Role of the monitor lizard* Varanus niloticus laurenti *in the epidemiology of tryposomiais along the shores of Lake Victoria, Kenya*. PhD Dissertation. Kenyatta University, Nairobi, Kenya, 172 pp.

Obanda V, Kagira JM, Chege S, Okita-Ouma B, Gakuya F. 2011. Trypanosomosis and other co-infections in translocated black (*Diceros bicornis michaeli*) and white (*Ceratotherium simum simum*) rhinoceroses in Kenya. *Sci Parasitol* 12:103–107.

Okello I, Nzalawahe J, Mafie E, Eastwood G. 2023. Seasonal variation in tsetse fly apparent density and *Trypanosoma* spp. infection rate and occurrence of drug-resistant trypanosomes in Lambwe, Kenya. *Parasitol Res* 123:46.

Okita-Ouma B. 2004. *Population performance of black rhinoceros (*Diceros bicornis michaeli*) in six Kenyan rhino sanctuaries*. MSc Thesis, Conservation Biology, Durrell Institute of Conservation and Ecology, University of Kent, Canterbury, UK. 112 pp.

Olubayo RO, Mihok S, Munyoki E, Otieno LH. 1994. Dynamics of host blood effects in *Glossina morsitans* sspp. infected with *Trypanosoma congolense* and *T. brucei*. *Parasitol Res* 80:177–181.

Otiende MY, Kivata MW, Makumi JN, Mutinda MN, Okun D, Kariuki L, Obanda V, Gakuya F, Mijele D, et al. 2015. Epidemiology of *Theileria bicornis* among black and white rhinoceros metapopulation in Kenya. *BMC Vet Res* 11:4.

Pohlin F, Hooijberg EH, Buss P, Huber N, Viljoen FP, Blackhurst D, Meyer LCR. 2020. A comparison of hematological, immunological, and stress responses to capture and transport in wild white rhinoceros bulls (*Ceratotherium simum simum*) supplemented with azaperone or midazolam. *Front Vet Sci* 7:569–576.

Radeke-Auer K, Clauss M, Stagegaard J, Bruins-Van Sonsbeek LGR, Lopez J. 2023. Retrospective pathology review of captive black rhinoceros *Diceros bicornis* in

the EAZA Ex-situ Programme (1995–2022). *J Zoo Aquarium Res* 11:298–310.

Rodrigues CMF, Garcia HA, Rodrigues AC, Pereira DL, Pereira CL, Viola LB, Neves L, Camargo EP, Gibson W, Teixeira MMG. 2020. Expanding our knowledge on African trypanosomes of the subgenus *Pycnomonas*: A novel *Trypanosoma suis*–like in tsetse flies, livestock and wild ruminants sympatric with *Trypanosoma suis* in Mozambique. *Infect Genet Evol* 78:104–143.

Rogers PS. 1993. Chemical capture of the white rhinoceros (*Ceratotherium simum*). In: *The capture and care manual*, McKenzie AA, editor. Wildlife Decisions Support Services, Lynnwood Ridge, South Africa, pp. 512–533.

Rookmaaker K, Antoine PO. 2012. New maps representing the historical and recent distribution of the African species of rhinoceros: *Diceros bicornis*, *Ceratotherium simum* and *Ceratotherium cottoni*. *Pachyderm* 52:91–96.

Silva I, Thananjayan K, Dissanayake DRA, Fernando WCR, Murugananthan M. 2017. Relationships of mean corpuscular volume with diameter and surface area of canine erythrocytes. *Sri Lanka Vet J* 64:13–16.

Tajeri S, Haidar M, Sakura T, Langsley G. 2021. Interaction between transforming *Theileria* parasites and their host bovine leukocytes. *Mol Microbiol* 115:860–869.

Taylor RD. 1986. The unsuccessful introduction of white rhinoceros to Matusadona National Park, Kariba. *Pachyderm* 6:14–16.

Trivedi S, Burnham CM, Capobianco CM, Boshoff C, Zheng Y, Pettiglio JW, Ange-van Heugten K, Bissell HD, Minter LJ. 2021. Analysis of blood biochemistry of free ranging and human-managed southern white rhinoceros (*Ceratotherium simum simum*) using the I-STAT alinity v®. *Vet Med Int* 2021:2665956.

Truc P, Formenty P, Duvallet G, Komoin-Oka C, Diallo PB, Lauginie F. 1997. Identification of trypanosomes isolated by KIVI from wild mammals in Côte d'Ivoire: diagnostic, taxonomic and epidemiological considerations. *Acta Trop* 67:187–196.

Welburn S, Picozzi K, Coleman PG, Packer C. 2008. Patterns in age-seroprevalence consistent with acquired immunity against *Trypanosoma brucei* in Serengeti lions. *PLoS Negl Trop Dis* 2:e347.

Zweygarth E, Mihok S, Majiwa PaO, Kaminsky R. 1995. A new *Nannomonas*-type trypanosome; isolation, *in vitro* cultivation and partial characterization. In: *Proceedings of the first international congress of parasitology and tropical medicine on trend in the management and control of tropical diseases*, Sinniah B, editor. Malaysian Society of Parasitology and Tropical Medicine, Kuala Lumpur, Malaysia, 24–27 August, pp. 188–190.

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