

## Trypanosomiasis and the conservation of black rhinoceros (*Diceros bicornis*) at the Ngulia Rhino Sanctuary, Tsavo West National Park, Kenya

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### Summary

Tsetse populations and trypanosome infections were monitored at the Ngulia Rhino Sanctuary to assess the impact of trypanosomiasis on rhinoceros. High densities of *Glossina pallidipes* were found near a permanent spring by the Ngulia escarpment; *G. longipennis* and *G. brevipalpis* were also present in lower numbers. Infection rates in *G. pallidipes* averaged 3·6%, with three times as many *T. vivax* as *T. congolense* infections. *T. simiae* and *T. brucei* were present at low frequency. DNA probes revealed that all mature *T. congolense* infections belonged to the Savanna subgroup. *G. pallidipes* fed on many hosts, with most meals taken from bovids and elephants. Rhino account for one of the blood meals in a small sample taken from *G. longipennis*. During a time of low tsetse densities (dry season), we estimated that the wild host population was acquiring seven infections per km<sup>2</sup> per day. At lower levels of challenge, an experimental rhino became infected with *T. congolense*. These results are discussed in terms of future plans for the repopulation of rhino in tsetse-infested areas in Kenya.

*Key words:* epidemiology, disease, *Glossina*, parasitology, *Trypanosoma*

### Résumé

On a surveillé les populations de tsé-tsé et les infections dues au trypanosome au Ngulia Rhino Sanctuary, pour évaluer l'impact de la trypanosomiase sur les rhinocéros. On a relevé de fortes densités de *Glossina pallidipes* près d'une source permanente, près de l'escarpement de Ngulia. Il y avait aussi des *G. longipennis* et des *G. brevipalpis* mais moins nombreuses. Le taux d'infection des *G. pallidipes* est de 3,6 en moyenne, dû trois fois plus à *T. vivax* qu'à *T. congolense*. *T. simiae* et *T. brucei* sont peu fréquents. Des tests d'ADN révèlent que toutes les infections déclarées par *T. congolense* appartiennent au sous-groupe Savanna. *G. pallidipes* se nourrit sur de nombreux hôtes, surtout sur les bovidés et les éléphants. Le rhino compte pour un seul repas dans un petit échantillon de *G. longipennis*. Lors d'une

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période où la densité de tsé-tsé était faible (saison sèche), nous avons estimé que la faune sauvage-hôte sacrifiait sept individus par jour et par km<sup>2</sup> à l'infection. A des niveaux plus bas d'expérimentation, un rhino "expérimental" a été infecté par *T. congolense*. On discute de ces résultats dans le cadre de projets de repeuplement par des rhinos, de régions du Kenya infectées par les tsé-tsé.

### Introduction

The conservation of black rhinoceros (*Diceros bicornis*) in Kenya is focused on the management of discrete breeding populations in eleven well-protected areas, including six national parks or reserves, and five private sanctuaries (Brett & Wanjohi, 1990). At the end of 1989, rhino in protected areas accounted for most of Kenya's population of 380–400 animals. Many of these areas are free of tsetse flies and hence are free of trypanosomiasis. Restocking of Kenya's free-ranging populations will require the movement of animals to tsetse-infested areas, exposing them to disease complications such as trypanosomiasis.

Historically, trypanosomiasis was thought to be of minor significance to rhino, as rhino thrived in tsetse-infested areas, and parasitological surveys failed to isolate parasites from rhino (Ashcroft, 1959; Dillmann & Townsend, 1979). In the 1960s, researchers became aware of the fact that rhino harbor latent trypanosome infections that become patent when animals are stressed (McCulloch & Achard, 1969; Clausen, 1981). These authors found that the disease could not be treated effectively with trypanocides, and hence, some animals died following translocation.

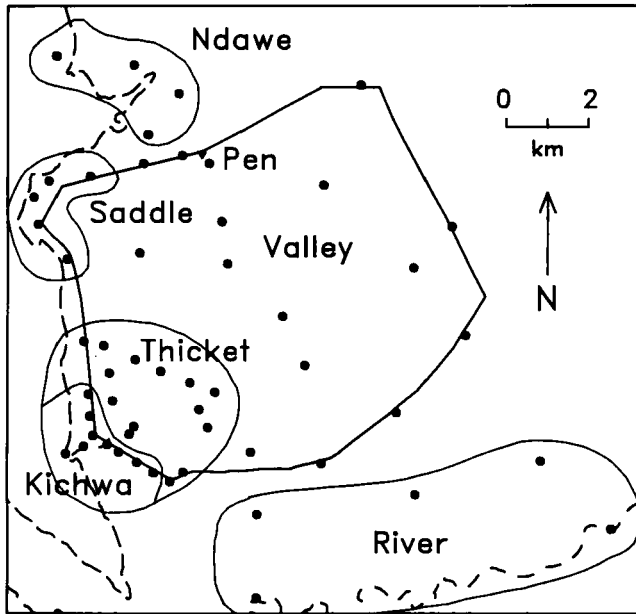
Results of these early studies have implied that health effects arising from trypanosome infection in rhino are found in unusual situations only, e.g. in young animals after loss of protective maternal antibodies (Clausen, 1981), or in old animals that are malnourished, parasitized, sick, or stressed (McCulloch & Achard, 1969). During translocations, the stress of capture and transport, the change in diet, the change in social status, etc. could convert a subclinical disease into a health problem.

Unfortunately, data on trypanosomiasis health effects in wildlife are scanty (Olubayo *et al.*, 1991), and hence, it is difficult to assess the consequences of translocations of rhino to tsetse-infested areas. Our only recent experience prior to this study comes from the translocation of a male from Ol Jogi Ranch Game Reserve (a tsetse-free area) to the Ngulia Rhino Sanctuary in Tsavo West National Park in 1989 (a tsetse-infested area). The animal became visibly ill a few weeks later and had to be recaptured. It had acquired a *T. brucei* infection at Ngulia and was therefore treated with a trypanocide. Following release, it remained weak and died during a fight with another male. The death of this animal prompted us to initiate studies on trypanosomiasis during translocations. Here, we report the nature of the disease challenge at Ngulia and present recommendations for further research in order to support prudent health management of Kenya's existing protected populations.

### Methods

#### Study area

The Ngulia Rhino Sanctuary (3°2'S, 38°15'E) is located within Tsavo West National Park in a gently-sloping valley east of the Ngulia and Ndawe escarpments (Fig. 1). The full 69.3 km<sup>2</sup> sanctuary was completed in late October 1990 following a series of extensions of a low electrified fence that defines its boundary. During our study, water was available only at a natural spring and an artificial waterhole near the SW corner (Kichwa Tembo area).



**Fig. 1.** Map of the Ngulia Rhino Sanctuary in Tsavo West National Park, Kenya showing tsetse trapping sites (●), and location of the rhino pen (▼). 'Scarp' marks the 900 m contour line of the escarpments. — = Fence, - - - = scarp, - · - = river/spring.

### *Tsetse population surveys*

A tsetse trapping survey was carried out in a 125 km<sup>2</sup> area centred on the sanctuary during the 1990 dry season. Tsetse were caught in NG2G traps (Brightwell, Dransfield & Kyorku, 1991) baited with acetone (3000–5000 mg/h), cow urine (400–500 mg/h), and 1-octen-3-ol (0.3–0.5 mg/h). Most of the data were collected in early September when daily minima and maxima were about 18 and 36°C, respectively. Altogether, 53 sites were trapped for 2–6 days in five areas (Fig. 1):

**Kichwa.** A roughly 6 km<sup>2</sup> dense, moist thicket at the base of the Ngulia escarpment near Kichwa Tembo peak, opposite the original ranger's camp. Some common trees are *Melia volkensii*, *Delonix elata*, *Acacia tortilis*, *Platycelyphium voense*, *Boscia coriacea* and *Maerua* spp.

**Thicket.** A 12 km<sup>2</sup> dry thicket with vegetation similar to the Kichwa area, but with a lower density of trees and shrubs. The habitat breaks up gradually into bushland a few km from the escarpment.

**Ndawe.** A 7 km<sup>2</sup> dry thicket along the Ndawe escarpment north of the sanctuary. Tree and shrub cover mostly follow runoff associated with the escarpment. Vegetation grades rapidly into bushland about half a km from the base of the escarpment.

**Saddle.** A 5 km<sup>2</sup> area representing a narrow pass between the Ndawe and Ngulia escarpments. The area has sparse tree cover, but has areas of dense shrub cover. Although an unremarkable habitat, the area is used as a movement corridor by wildlife.

**River.** A large area of dry bushland with sparse tree cover extending from the Tsavo River to the southern edge of the sanctuary. Vegetation is dominated by *Acacia tortilis* and *Commiphora* spp. with very few large trees.

*Valley*. This area encompasses the majority of the sanctuary. It consists mostly of dry bushland with scattered trees; a few large areas of grassland are found near the rhino pen. Away from the escarpment, the *Melia-Delonix* association of the Kichwa and Thicket areas is replaced by *Adansonia digitata*, *Acacia tortilis*, *Commiphora* spp. and *Cassia abbreviata*.

#### *Isolation of trypanosomes from tsetse*

The mouthparts and guts of tsetse were examined microscopically for the presence of trypanosomes. In addition to the dry season survey of 1990, we collected flies for dissection from the Thicket area in April, May 1990, and from the Kichwa area in January, March 1991.

From the location of infections, we scored the identity of the trypanosomes as follows: proboscis only (*Duttonella* subgenus, *T. vivax*), proboscis and gut (*Nannomonas* subgenus, *T. congolense* or *T. simiae*), proboscis and gut and salivary glands (*Trypanozoon* subgenus, *T. brucei*), gut only (immature *Nannomonas* or *Trypanozoon*, or *T. grayi* infection).

Probosces showing *Nannomonas* and *Trypanozoon* infections were injected into cyclophosphamide-immunosuppressed mice. Tail blood was checked three times a week for a month to detect infection. Isolates that grew were propagated in rats. At high parasitaemia, 100 *G. morsitans centralis* were fed once on the rat. The flies were dissected one month later to determine the infectivity of the isolate. In an attempt to isolate *T. vivax*, flies from the two 1991 surveys were kept alive by feeding them on rats. In Nairobi, infected probosces were placed into a culture system for propagation (Zweygarth, Gray & Kaminsky, 1991).

#### *Identification of trypanosomes with DNA probes*

We prepared dot blots of parasite DNA on Hybond-N nylon membranes (Amersham) from most gut infections. Blots were stored frozen between filter paper disks in polyethylene bags for 1–3 months following denaturation (Nyeko *et al.*, 1990). Blots were tested following standard methods with a panel of recombinant DNA probes for various trypanosome species groups and subgroups (Majiwa & Otieno, 1990).

#### *Identification of tsetse blood meals*

Recent tsetse blood meals were blotted onto filter paper, air-dried and stored for 7–9 months. Blots were eluted with PBS containing 0.1% sodium azide, and then diluted 1:10 in 0.05 M carbonate–bicarbonate buffer pH 9.6. For analysis, wells of a microtitre plate were coated with 100  $\mu$ l and then incubated overnight. After washing, the blood meal was identified in an enzyme immunoassay using rabbit anti-species immunoglobulin (IgG) horseradish peroxidase specific conjugate. The sample was scored as positive if the absorbance was three times greater than that of a control at 490 nm (Savigny & Voller, 1980).

#### *Monitoring of a translocated rhinoceros*

A male rhino was captured at Nairobi National Park on 30 August 1990 and moved to a pen in Ngulia, where it was held until release on 22 October (Fig. 1). At capture, and at two week intervals, blood was taken for routine haematology and

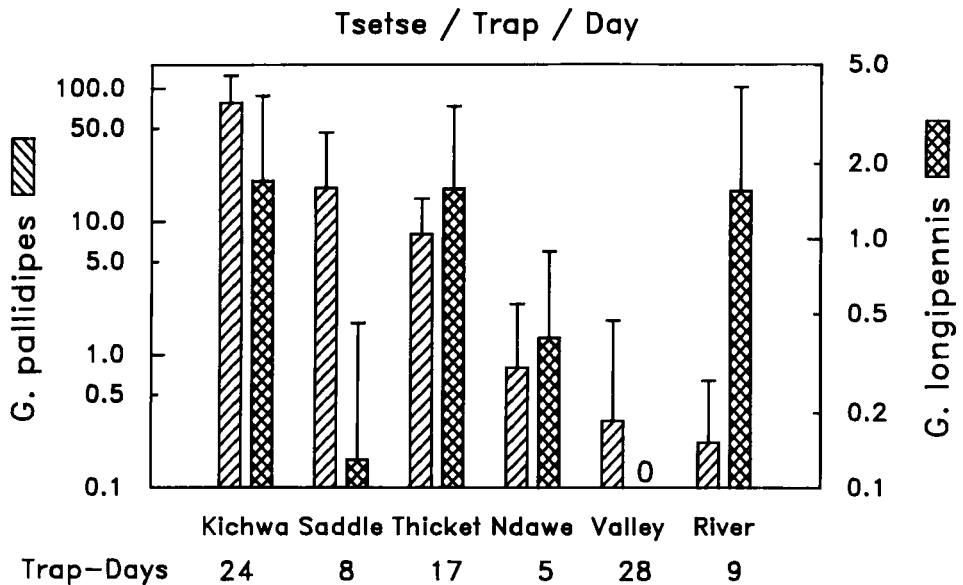


Fig. 2. Apparent densities of two species of tsetse in six areas of the Ngulia Rhino Sanctuary during the dry season (Aug.-Oct.) of 1990 calculated for the first two days of trapping. Error bars are one standard deviation.

parasitology (packed cell volume, erythrocyte, leukocyte and reticulocyte counts, thin and thick smears, haemoglobin concentration). We used the buffy coat concentration technique (Murray, Murray & McIntyre, 1977) for detection of trypanosomes. Blood samples were injected into immunosuppressed mice in an attempt to isolate trypanosomes. On the last two sampling occasions, 100 *G. m. centralis* were fed directly on the rhino and dissected one month later to detect infection (xenodiagnosis).

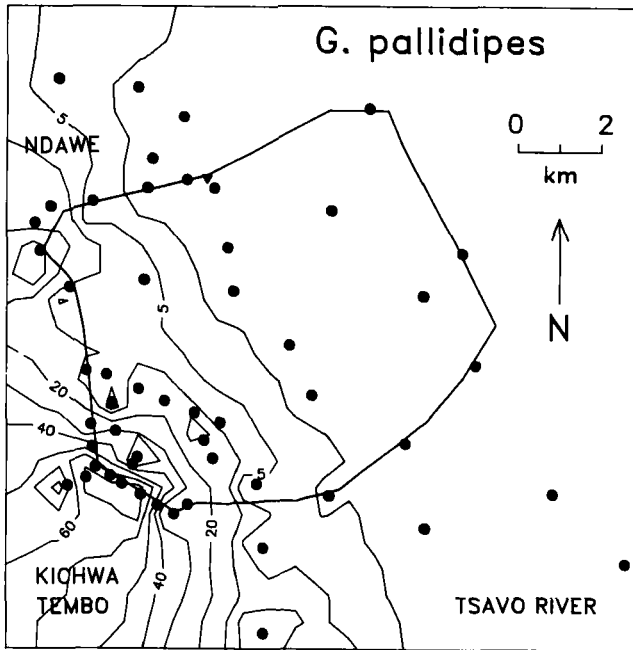
## Results

### *Tsetse population surveys*

*G. pallidipes* accounted for 96.3% of the 3204 tsetse caught in traps during the main dry season survey of 1990. *G. longipennis* and a very small number of *G. brevipalpis* accounted for the remainder. For consistency across areas, apparent densities of the two main tsetse species were summarized for the first two days of trapping (Fig. 2). Trapping indices reflect both activity and density, and hence, should be interpreted with caution (Williams, Dransfield & Brightwell, 1990).

Apparent densities of *G. pallidipes* varied over three orders of magnitude, with the highest densities ( $78 \pm 47$ , SD) found in the moist thicket at the Ngulia escarpment (Kichwa, Fig. 2). Modest densities of *G. pallidipes* were also found in the Thicket and Saddle areas. The highest dry-season catch recorded in a trap at the Kichwa area was 219 *G. pallidipes*. Densities of *G. longipennis* were mostly on the order of 1–2 flies, with a more even distribution among areas (Fig. 2).

From trapping indices, we calculated the spatial distribution of *G. pallidipes* (program SURFER, smoothing and interpolation of trapping indices on a spatial



**Fig. 3.** Estimated spatial distribution of *Glossina pallidipes* (— = fence, ▼ = pen, ● = trap site) at the Ngulia Rhino Sanctuary during the dry season (Aug.–Oct.) of 1990. Contour lines represent the estimated catch/trap/day in an odour-baited winged NG2G trap.

grid). Peak densities were found at the spring in the Kichwa area (Fig. 3). A smaller peak was discerned to the NW where the escarpments meet in a narrow pass. Together, these peaks produced a gradient of decreasing density away from the escarpment. Densities of less than one fly/trap/day were estimated for most of the sanctuary, including the area of the rhino pen.

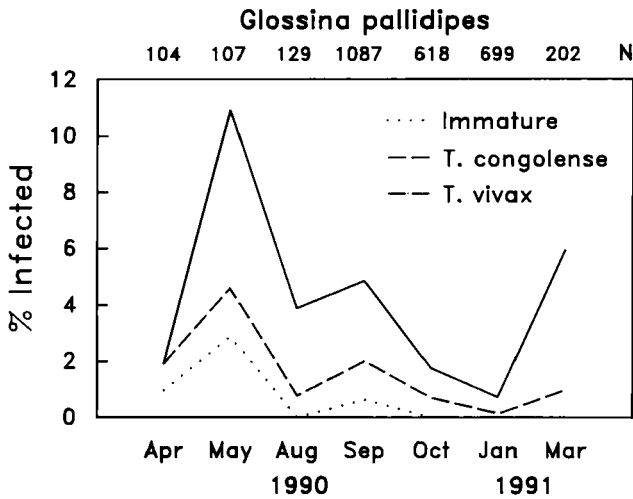
#### *Isolation of trypanosomes from tsetse*

In total, 3076 *G. pallidipes*, 189 *G. longipennis*, and 7 *G. brevipalpis* were dissected. Overall trypanosome infection rate in *G. pallidipes* was 3.58%, consisting of 2.21% *T. vivax*, 0.88% *Nannomonas* (mostly *T. congolense*), 0.03% *T. brucei*, and 0.46% immature infections (mostly unidentified). A similar infection rate was found in *G. longipennis* (3.17% overall, 1.58% *T. vivax*, 0.53% *T. congolense*, 1.05% immature). None of the 7 *G. brevipalpis* dissected had trypanosome infections.

In *G. pallidipes*, infection rates followed a possible annual cycle, peaking towards the end of the long rainy season in May, and declining therefore (Fig. 4). The high infection rate in early May 1990 was caused by a high prevalence rate of *T. vivax* (6.3%), possibly related to high densities of biting flies. The very low infection rate (0.7%) at the end of the short rainy season in late January 1991 coincided with very high tsetse densities, and the presence of many teneral (freshly-emerged) flies. Density of *G. pallidipes* estimated from six traps set in the Kichwa area for a single day was  $435 \pm 210$  tsetse/trap/day.

#### *Identification of trypanosomes*

Altogether, 47 isolates from *G. pallidipes*, and 1 from *G. longipennis* were injected into mice. As expected, 18 putative *T. vivax* isolates and 4 immature isolates failed



**Fig. 4.** Cumulative trypanosome infection rates detected by dissection in *G. pallidipes* captured at the Ngulia Rhino Sanctuary from April 1990 to March 1991.

to grow. Of 25 *Nannomonas* infections, only 6 were successfully isolated in mice. A single *T. brucei* infection from *G. pallidipes* was also isolated. Thirteen attempts to propagate *T. vivax*-infected probosces in culture were unsuccessful.

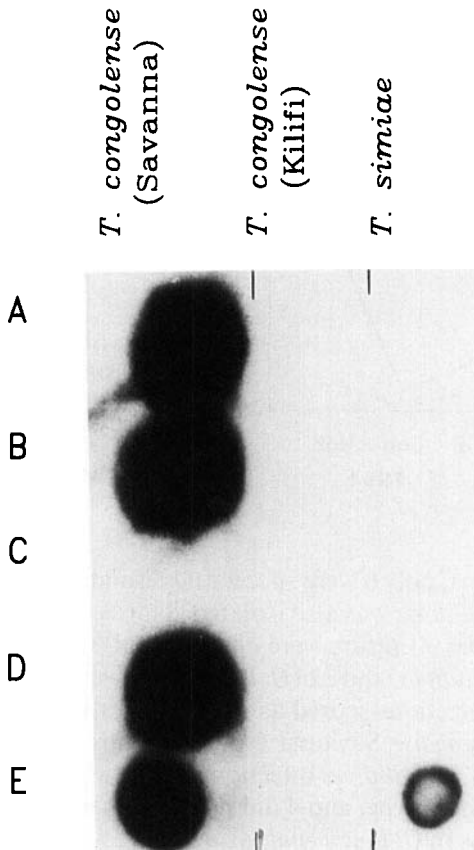
Overall, 29 infected guts of *G. pallidipes*, and 2 of *G. longipennis* were tested with DNA probes. In *G. pallidipes*, 21 infections scored as *Nannomonas* by dissection were found to represent 18 *T. congolense* Savanna type infections, 2 *T. simiae* infections, and 1 mixed infection. Of *G. pallidipes* infections scored as immature, 3 were found to be *T. congolense* Savanna type, and 4 did not hybridize with any of the probes. Two immature infections in *G. longipennis* also failed to hybridize. As shown in Fig. 5, negative and positive reactions were specific and unequivocal.

All of the *T. congolense* stocks isolated in mice and propagated in rats were transmitted easily to laboratory-reared *G. m. centralis*. The infection rates (almost all mature infections involving the hypopharynx) varied between 27% and 72% (mean 53%). The single *T. brucei* isolated that was tested produced a 32% immature and a 45% mature infection rate; these rates are unusually high for *T. brucei*.

#### *Identification of tsetse blood meals*

We analysed 70 blood meals from *G. pallidipes* and 8 from *G. longipennis* (Table 1); all were collected during the dry season of 1990, with most coming from the Kichwa area. *G. pallidipes* took the majority of its meals from bovids, but also took many meals from elephants. Some of the unspecified bovid meals may have been taken from lesser kudu, which were present in modest numbers (Table 1), but for which we had no antiserum. Surprisingly, only one meal was taken from a warthog, and no meals were taken from expected hosts such as bushbuck or giraffe. In the small sample from *G. longipennis*, one meal was taken from a rhino.

The modest number of meals remaining unidentified was probably due to our use of a stringent criterion for identification. All gut samples were examined at the time of collection for the presence of avian or reptilian erythrocytes (nucleated cells), but none were found.



**Fig. 5.** Autoradiograph of dot blots (five rows) of crude homogenates of infected guts of *G. pallidipes* tested with three recombinant DNA probes (three columns) for different *Nannomonas* group trypanosomes. Flies A, B, D hybridized only with the probe for *T. congolense* Savanna type; fly E had a mixed infection and therefore also hybridized with the probe for *T. simiae*; fly C had an immature infection that did not hybridize with any probe. None of the flies reacted with the probe for *T. congolense* Kilifi type.

#### *Monitoring of a translocated rhinoceros*

The rhino survived confinement in the pen at Ngulia, was released, and is still alive. Erythrocyte indices at Ngulia were similar to baseline values obtained at capture in Nairobi, except on one occasion when there was evidence of dehydration (increase of packed cell volume from 42 to 57%). Overall, we never observed qualitative or quantitative signs of anaemia, an indication of trypanosomiasis in susceptible animals. Leukocyte counts were, however, consistently lower than those at capture, possibly indicating parasitism or a general stress reaction. When xenodiagnosis was performed on two occasions, tsetse revealed the presence of *T. congolense* Savanna group trypanosomes in the rhino (32, 39%). Buffy coat examination and inoculation of blood into mice failed to detect this infection.

#### *Trypanosomiasis challenge to rhino*

To estimate trypanosomiasis challenge to rhino, we assembled various parameters from our study and from the literature. As we were unable to obtain good quantitative information for *G. longipennis* (a potentially important vector; see Table 1 and Weitz, 1963), we were forced to limit this analysis to the common tsetse at Ngulia, *G. pallidipes*.



**Table 1.** Blood meals taken by *Glossina* spp. captured in traps at the Ngulia Rhino Sanctuary, Kenya during the dry season of 1990 (August to October), and numbers of large mammals found at or near the 69 km<sup>2</sup> sanctuary

Species fed upon	Common Name	<i>G. pallidipes</i>	<i>G. longipennis</i>	Number
<b>Suidae</b>				
<i>Phacochoerus aethiopicus</i>	Wart Hog	1	0	40 ± 5
<b>Giraffidae</b>				
<i>Giraffa camelopardalis</i>	Giraffe	0	0	100 ± 10
<b>Bovidae</b>				
<i>Cephalophus grimmia</i>	Grey Duiker	NT	NT	10 ± 5
<i>Madoqua kirki</i>	Kirk's Dik-Dik	5	1	abundant
<i>Oreotragus oreotragus</i>	Klippspringer	NT	NT	6
<i>Tragelaphus scriptus</i>	Bushbuck	0	0	present <sup>1</sup>
<i>Tragelaphus imberbis</i>	Lesser Kudu	NT	NT	70 ± 10
<i>Tragelaphus oryx</i>	Eland	NT	NT	6
<i>Oryx gazella</i>	Oryx	0	0	4
<i>Kobus defassa</i>	Waterbuck	NT	NT	present <sup>1</sup>
<i>Alcelaphus buselaphus</i>	Hartebeeste	NT	NT	20 ± 5
<i>Aepyceros melampus</i>	Impala	NT	NT	200 ± 20
<i>Gazella granti</i>	Grant's Gazelle	NT	NT	10
<i>Syncerus caffer</i>	Buffalo	5	0	12 <sup>2</sup>
Unspecified Bovid		9	2	
<b>Equidae</b>				
<i>Hippotrigis tragga</i>	Zebra	NT	NT	50 ± 10
<b>Rhinocerotidae</b>				
<i>Diceros bicornis</i>	Black Rhinoceros	0	1	10
<b>Elephantidae</b>				
<i>Loxodonta africana</i>	Elephant	15	1	60 ± 5
<b>Carnivora</b>				
<i>Crocuta crocuta</i>	Spotted Hyaena	NT	NT	5
<i>Panthera leo</i>	Lion	NT	NT	6
<i>Panthera pardus</i>	Leopard	NT	NT	2
<i>Acinonyx jubatus</i>	Cheetah	NT	NT	2
Unidentified		35	3	

Notes: NT = Not tested; estimates of animal numbers are based on Kenya Wildlife Service ranger patrols.

<sup>1</sup>Bushbuck and waterbuck are present in the area, but are rarely seen at the sanctuary.

<sup>2</sup>Modest numbers of buffalo at the Tsavo River and in the Ngulia Valley also visit the area (the number refers to the individuals living within the fenced area).

When rhino are present in sufficient numbers, *G. pallidipes* will take about 10% of its meals from rhino (Wilson *et al.*, 1972; Phelps & Vale, 1978). Based on mark-recapture studies of tsetse at Nguruman (fig. 4 in Dransfield *et al.*, 1990), and at the Lambwe Valley (M. M. Mohammed-Ahmed, unpubl.), we estimated density in the Kichwa area to be about 2200 ± 1000 flies per km<sup>2</sup> during the dry season of 1990 (mean and range). These flies had mature infections consisting of 1.05% *T. congolense*, 2.27% *T. vivax*, 0.16% *T. simiae* and 0.05% *T. brucei*.

From our data on mouse inoculations of *T. congolense* Savanna type isolates, perhaps 26% of infections in tsetse would give rise to infections in a host (Wilson, Dar & Paris, 1972). Given a feeding interval of roughly 3 days (Randolph, Rogers & Kiilu, 1991), two infective bites per km<sup>2</sup> per day would be present for each 1% increment in infection rate ( $2200 \times 0.01 \times 0.26/3$ ). This gives a total of about seven new infections per km<sup>2</sup> per day at a total infection rate of 3.6%. These infections would be distributed in a roughly 10+ km<sup>2</sup> area near the waterhole and spring by Kichwa Tembo among hosts according to their densities, and the feeding preferences of tsetse.

From observations of wildlife (Table 1), and from similar data obtained in Tsavo East (Leuthold & Leuthold, 1976), we estimate that a minimum of about 100 preferred hosts used the waterhole and spring in the Kichwa area on a daily basis. For the ten rhino present at the time of our study, each rhino could have been challenged by one potentially infective *T. congolense* bite every 5 days (inverse of: two bites per km<sup>2</sup>  $\times$  10 km<sup>2</sup>  $\times$  10% of the preferred hosts divided by ten rhino at a 1% infection rate with *T. congolense*). Although based on many rough estimates, this final number is a reasonable indication of the maximum level of challenge; actual levels of challenge may be lower, but are unlikely to be higher.

## Discussion

### *Tsetse and trypanosomiasis at Ngulia*

This survey has confirmed the existence of tsetse and trypanosomiasis at Ngulia at levels of significance to the health of rhino. Challenge to animals arises mostly from high numbers of infected *G. pallidipes*, with a much smaller potential for infection through *G. longipennis* and *G. brevipalpis*. Nevertheless, the presence of low numbers of *G. longipennis* is significant, as this species feeds primarily on rhino (Weitz, 1963). Unfortunately, very little is known about *G. longipennis*, or the parasites it harbours, as it is not sampled as easily as other tsetse (Kyorku *et al.*, 1990).

As in other trypanosome infection surveys (Ryan *et al.*, 1986), *T. vivax* and *T. congolense* accounted for most of the infections detected in tsetse. The substantially higher infection rate with *T. vivax* was presumably related to the few blood meals taken from warthogs, and the many meals taken from bovids (Snow *et al.*, 1988). The trypanosome infection rate in *G. pallidipes* (3.6%) varied seasonally (Fig. 4), with high rates occurring after rainy periods. Given differences in sampling methods, our results are similar to those recorded for other wildlife areas in Kenya (e.g. Mara Reserve: Wilson *et al.*, 1972; Shimba Hills Reserve: Tarimo, Snow & Butler, 1984; Ruma National Park: Majiwa & Otieno, 1990). In areas where cattle are important hosts, infection rates are often higher, and are dominated by *T. congolense* (Kiboko: Ogawa, 1981; Kenya Coast: Tarimo *et al.*, 1984).

Although Ngulia is close to the locality where Kilifi-type *T. congolense* was first isolated, we did not detect this parasite subgroup in our DNA blots, nor did we detect the presence of West African Riverine/Forest *T. congolense*. The potential presence of uncharacterized forms of *Nannomonas* group parasites or *T. grayi* (a parasite of reptiles: Minter-Goedbloed *et al.*, 1983; McNamara & Snow, 1991; Dirie *et al.*, 1991) was, however, suggested by our inability to identify some immature infections. In contrast, we identified all mature infections, with most

representing *T. congolense* Savanna type. This apparent lack of parasite diversity at Ngulia contrasts with the heterogeneity observed in other studies that have used DNA probes: *G. pallidipes* from Ruma National Park in the Lambwe Valley (Majiwa & Otieno, 1990), cattle from Uganda (Nyeko *et al.*, 1990), and tsetse from the Gambia (McNamara & Snow, 1991).

Two unique features of tsetse from Ngulia are the low frequency of mixed infections, and the low frequency of *T. simiae* (a parasite of suids). The absence of bushbuck as a host for *G. pallidipes*, the few meals taken from warthogs, and the large number of meals taken from elephants (Table 1), may account for these observations. Bushbuck have high trypanosome prevalence rates (Ashcroft, 1959) and often account for the majority of meals in *G. pallidipes* (Turner, 1987; and others).

#### *Potential impacts of trypanosomiasis on rhino*

*T. brucei*, a trypanosome of particular significance to the health of rhino (Clausen 1981), is present at modest frequencies in wild mammals (Ashcroft, 1959), but for unknown reasons, is rare in tsetse (Rogers & Boreham, 1973). At Ngulia, *T. brucei* was extremely rare in *G. pallidipes*, but was readily transmitted to *G. m. centralis*. These results are puzzling and are at odds with the catalyst for our study: the infection of the Ol Jogi male with *T. brucei* after translocation. Nevertheless, our estimates of disease challenge in the Kichwa area provide a probable explanation.

In the absence of any information on tsetse ecology, the Ol Jogi male was inadvertently confined in an older pen near Kichwa Tembo during March–April 1989, a time of expected high infection rates (Fig. 4), and particularly high tsetse densities (the long rainy season). This animal was presumably exposed to daily or at least weekly trypanosome infection. Hence, it succumbed to complications resulting from trypanosomiasis. Under natural conditions, animals often feed and drink near Kichwa Tembo after sunset, limiting their contact with tsetse flies, and hence reducing their overall disease risk. This was clearly not possible in confinement for this animal.

The discovery of *T. congolense* in the experimental rhino is the first record of this parasite in a rhino. Although rhino clearly suffer health effects from *T. brucei* and *T. vivax* (McCulloch & Achard, 1969; Clausen, 1981), *T. congolense* had no detectable effect on the animal's health. These results are encouraging, but must be interpreted in terms of the low level of parasitaemia reached for this single case. The diversity of parasite types found at Ngulia suggests that results will not be the same for all cases. For example, a second experimental rhino moved in 1990 acquired a *T. vivax* infection that resulted in high parasitaemia accompanied by anaemia, thrombocytopenia, and lymphopaenia (unpubl. data).

Except for this recent observation, and a few cases diagnosed by McCulloch & Achard (1969), researchers have failed to detect *T. vivax* in rhino (Ashcroft, 1959; Dillmann & Townsend, 1979; Clausen, 1981). *T. vivax* is common in tsetse in areas where rhino live, and hence should be part of the parasite fauna of rhino. Its rarity, however, may be related to the insensitivity of parasitological techniques (Dukes *et al.*, 1991), rather than the rarity of infections. Supportive evidence comes from Clausen's (1981) efforts to isolate *T. brucei* from rhino. He was successful in isolating this parasite primarily through inoculation of blood into rats; mice never

became infected. We similarly failed to detect *T. congolense* through mouse inoculation, but were successful through xenodiagnosis. Given these problems, a variety of techniques and animals (e.g. donkeys?) should be used to detect infections in rhino. We particularly recommend that tsetse xenodiagnosis be incorporated whenever practical.

### Conclusions

The acquisition of *T. congolense*, *T. brucei* and *T. vivax* infections by the three animals moved from highland areas to Ngulia has confirmed the susceptibility of rhino to trypanosomiasis when moved from a tsetse-free to a tsetse-infested area. Although deaths can result from disease problems, they are unlikely to occur if infections are managed with prudent monitoring during confinement. Disease risks cannot be eliminated, but can be reduced to a minimum by moving animals to areas with low risk based on tsetse ecological and epidemiological surveys.

The odd nature of trypanosome parasites found in rhino (a perissodactyl) requires further study. Epizootiological studies of potential vectors are required to define the kinds of parasites that infect rhino as opposed to the more commonly-studied artiodactyls. These studies should focus on *G. longipennis*, a tsetse species that lives at low density in the typical dry bushland habitat of lowland rhinos, and is known to feed on rhino (Weitz, 1963). The biology of this species is poorly understood (Kyorku *et al.*, 1990). We recommend that an effort be made to produce a library of trypanosome isolates from rhino and from *G. longipennis* to supplement our current stock of cryopreserved isolates from *G. pallidipes*. It would then be possible to compare trypanosomes from different sources with biochemical and molecular techniques to elucidate transmission cycles. Stocks from existing sanctuaries such as Ngulia also need to be screened for patterns of drug resistance, so that preliminary insights can be gained into how to treat sick rhino with conventional trypanocides.

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