Trypanosoma simiae in the white rhinoceros (Ceratotherium simum) and the dromedary camel (Camelus dromedarius)

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Abstract

Trypanosoma simiae was identified as the cause of a disease outbreak in dromedary camels (*Camelus dromedarius*) introduced to Tsavo East National Park, confirming the susceptibility of camels to this pathogen. *T. simiae* was also isolated from a new host, the white rhinoceros (*Ceratotherium simum*) through xenodiagnosis with a susceptible tsetse species (*Glossina morsitans centralis*). A white rhinoceros showed some evidence of anaemia and lymphopaenia when harbouring *T. simiae*, but did not suffer any long-term health effects.

1. Introduction

Trypanosoma (*Nannomonas*) *simiae* is a well-characterised parasite of suids because of its importance as an acute pathogen of domestic pigs. The wild reservoir in savannah appears to be the wart-hog (Claxton et al., 1992), with the bush pig probably acting as a reservoir in forested areas. *T. simiae* can be identified on morphological criteria, but it is easily confused with other pathogenic trypanosomes by inexperienced workers. Hence, unequivocal identification requires

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demonstration of rapid, acute effects on pigs, combined with a lack of infectivity for rodents. Some *T. simiae* isolates can infect sheep, goats, rabbits and certain primates; there are also occasional reports of infections developing in other animals (Stephen, 1986). Unfortunately, routine identification of the parasite has been problematic due to the need for subinoculation of pigs.

Tsetse flies in East and West Africa harbour *Nannomonas* infections that infect pigs, but are not *T. simiae* or the common Savannah type of *Trypanosoma congolense* (Dukes et al., 1991; Majiwa et al., 1993). These parasites appear to be new genotypes of *T. congolense* rather than new forms of *T. simiae*, leaving the identity of field isolates not killing pigs open to interpretation (Claxton et al., 1992).

Fortunately, a simple alternative to pig inoculation exists for the unequivocal identification of *T. simiae* based on hybridisation of fixed parasites with DNA probes (Majiwa and Webster, 1987). DNA probes together with xenodiagnosis with tsetse flies (Mihok et al., 1991) are now being used to isolate and characterise cryptic infections in wildlife (Mihok et al., 1992a,b). This paper reports a new host record for *T. simiae* in the white rhinoceros (*Ceratotherium simum*), and confirms historical reports of the pathogenicity of *T. simiae* in the dromedary camel (*Camelus dromedarius*).

2. Materials and methods

2.1. Animals

Twelve camels were moved to Ithumba in Tsavo East National Park, Kenya in March 1992. Two fell sick in May and were treated with quinapyramine sulphate (Trypacide, May and Baker, 2 mg kg^{-1}). A month later, six were observed to have clinical symptoms of trypanosomosis, but could not be treated with Trypacide due to unavailability of the drug. Instead they were treated with diminazene citrate (Veriben, Sanofi, 2 mg kg^{-1}) as an emergency measure, along with tetracycline and ivermectin. A week later, a further two animals fell sick and one died. At this time, all animals except 'Rapsu' were treated with Trypacide. Blood samples were obtained from seven of the camels a few days later for parasitology and haematology. Veriben-treated animals improved without apparent toxic effects and no further deaths occurred before the animals were moved. Ithumba is infested with tsetse, with *Glossina pallidipes* as the main vector.

Seven healthy white rhinoceroses were sampled in August at Lewa Downs Ranch near Isiolo. Five animals had just arrived in Kenya from the Umfolozi/Hluhluwe Game Reserves in South Africa. The other two were born in Kenya on Lewa Downs and Solio Ranches; they were sampled prior to translocation to Aitong in the Maasai Mara. Tsetse are present at low density near Isiolo, but trypanosomosis has never been diagnosed in cattle at Lewa Downs. The ranch harbours many wild animals, including wart-hogs.

2.2. Sampling

Rhinoceroses were immobilised with etorphine, with or without acepromazine and hyaluronidase; camels were restrained manually. Heparinised blood was collected for a complete blood count on a Coulter Model ZM equipped with a 256 Channelyzer. About 1 ml blood was injected into three cyclophosphamide-immunosuppressed Swiss mice (200 mg kg^{-1}). Mouse blood was checked three times a week for 1 month for the presence of trypanosomes. Sheep were also injected with blood from the South African animals and checked daily for changes in body temperature.

About 100–200 teneral, laboratory-reared *Glossina morsitans centralis* were fed on each animal's blood through a silicone membrane after a few hours delay. Flies were maintained on uninfected rabbits changed weekly; they were dissected at 1 month to check for trypanosome development in the proboscides, salivary glands and guts. Infected flies were used to test cyclical passage of isolates by feeding them on mice, rabbits and pigs; stabilates of cryopreserved blood were used for syringe challenge in a few cases.

Parasitaemia was assessed by examining the buffy coat for trypanosomes with dark ground microscopy using the index of Paris et al. (1982). Blood from the original animals, and parasites developing in pigs or the guts of tsetse were dot blotted onto Hybond N^+ (Amersham) membranes for screening with a panel of trypanosome-specific DNA probes including *T. simiae*, Trypanozoon (*Trypanosoma brucei* and *Trypanosoma evansi*), *Trypanosoma vivax* East African, and *T. congolense* Savannah, Kilifi, Tsavo, and West African Riverine/Forest types (Majiwa et al., 1993).

3. Results

3.1. Camels

All seven camels had normal erythrocyte profiles (packed cell volume (PCV) 26–30%), with elevated leucocyte counts ($17-26 \times 10^9 \text{ l}^{-1}$) consisting mostly of lymphocytes ($8-19 \times 10^9 \text{ l}^{-1}$). Parasites with the morphology of *T. simiae* were detected at about $10^4-10^5 \text{ ml}^{-1}$ in the untreated camel, Rapsu, and one of the treated camels, 'Boji'. None of the mice injected with camel blood developed infections. In three of four camels, xenodiagnosis revealed the presence of *Nannomonas* infections. Infection rates in tsetse fed on the camels varied from 30 to 53%, with maturation rates (proboscis infections of all positive flies) varying from 29 to 43%.

A pig injected with stabilated blood of the untreated camel Rapsu died of overwhelming parasitaemia on Day 10, with parasites first detected on Day 8. The prepatent period was much longer in a pig injected with blood of the treated camel Boji (20 days). This pig was treated with 20 mg kg^{-1} diminazene aceturate plus 50 mg kg^{-1} Ro 15-0216 (Zweygarth and Röttcher, 1987) on Day 21; it relapsed, was treated again, relapsed again and then died. Parasites developing in both pigs

failed to infect mice. DNA blots of all camel-origin parasites reacted with the probe for *T. simiae* only.

3.2. White rhinoceros

Based on data for captive animals (Zoological Society of London, C. Hawkey Lynx database), haematological indices for the seven white rhinoceroses were within normal ranges (PCV: 35–45%; leucocytes: $5–13 \times 10^9 \, 1^{-1}$; lymphocytes: $1–4 \times 10^9 \, 1^{-1}$). The South African animals had been travelling by sea for a few weeks and had lost weight, but appeared healthy. The resident Lewa Downs animals appeared robust, despite the fact they were sampled during a severe drought. Other wildlife on the ranch, particularly zebra, were dying of unknown causes at this time.

Although parasites were not detected through microscopy or mouse inoculation in any of the seven animals, tsetse fed on resident 'Chuma' developed a *Nannomonas* infection (21% infected, 10% maturation rate). Chuma had the lowest leucocyte count $(5 \times 10^9 \, 1^{-1})$, the second lowest PCV (36%), and the lowest platelet count $(172 \times 10^9 \, 1^{-1})$ of the seven rhinoceroses. Inoculation of blood from the South African animals into sheep resulted in no detectable health effects.

One tsetse with a mature infection from the flies fed on Chuma was fed on a pig. Parasites were detected on Day 7 and the pig died of overwhelming parasitaemia on Day 8. DNA blots of tsetse guts and the blood of the pig reacted with the probe for *T. simiae* only. Mice injected with the pig's blood failed to become infected. A rabbit challenged by tsetse fed on Chuma harboured *T. simiae* at about $10^4 \, \text{ml}^{-1}$ on Day 10. It was anaemic (PCV 19%), and died a few days later. Blood from the rabbit failed to infect mice.

The white rhinoceros 'Chuma' is still healthy 20 months after translocation to the Maasai Mara.

4. Discussion

Through xenodiagnosis, careful parasitological follow-up, and the use of DNA probes, it has been shown for the first time that the white rhinoceros can act as a cryptic reservoir for *T. simiae*. Given the insensitive techniques used in most wildlife trypanosome surveys, the presence of *T. simiae* in non-suid wildlife should be reassessed. For example, the cryptic presence of *T. simiae*, or other unusual parasites (Majiwa et al., 1993), could account for the many trypanosomes detected in wildlife that fail to grow in rodents. Unfortunately, except for the historical study of Bruce et al. (1913), and the recent study of wart-hogs by Claxton et al. (1992), no concerted effort has been made to assess the importance of wildlife reservoirs for *T. simiae*. Recent experiments with various host bloods suggest that bovine blood is not toxic to *T. simiae* (Mihok et al., 1993), suggesting the possibility of diverse reservoirs.

Despite low parasitaemia and growth in a non-suid host, *T. simiae* in white

rhinoceros was transmissible to tsetse, and was pathogenic for pigs. The cryptic presence of this infection in a white rhinoceros resembled previous findings for a black rhinoceros acquiring a *T. congolense* infection after translocation (Mihok et al., 1992a). In both cases, infection at very low parasitaemia had no severe health effect, outside of marginal anaemia and lymphopaenia. Minor health effects for *Nannomonas* parasites contrast with the detrimental effects observed for *T. brucei* and *T. vivax*, which reach higher parasitaemia (Mihok et al., 1992a,b).

Although *T. simiae* was once documented as a pathogen of camels in Somalia (Pellegrini, 1948), the isolation of *T. simiae* during a disease outbreak in Kenya was unexpected (Röttcher et al., 1987; Dirie et al., 1989). In laboratory experiments, Zweygarth et al. (1987) failed to infect camels with two stocks of *T. simiae*, placing historical reports in doubt. This failure may have been related to attenuation of *T. simiae* when transmitted by syringe challenge (Stephen, 1986). The current results have now confirmed without doubt that *T. simiae* is a pathogen of the dromedary camel.

The 3 week prepatent period for the parasite isolated from camel Boji was unusual for 'classical' *T. simiae*, which kills pigs rapidly (Stephen, 1986). In future, characterisation of field isolates typed objectively with DNA probes should better reveal the degree of natural variation in *T. simiae*. DNA typing, for example, would have been useful in identifying the chronic parasite isolated from a warthog by Claxton et al. (1992), especially given the existence of new *Nannomonas* parasites infective for suids (Dukes et al., 1991; Majiwa et al., 1993).

The isolation of *T. simiae* from two camels after presumed curative treatment is another unusual feature of this study. Ithumba, located within Tsavo East National Park, has no history of trypanocide use, but is close to Galana Ranch, an area with a history of chemotherapeutic treatment of cattle (Dolan et al., 1992). Incipient trypanocide resistance developing in different areas of Kenya will clearly pose serious restrictions on the introduction of camels for tourism in tsetse-infested areas.

Acknowledgements

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