SURVEY FOR TRYPANOSOMES IN BLACK RHINOCEROS *(Diceros bicornis)*[1]

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Abstract: Blood samples were taken from 39 black rhinoceros *(Diceros bicornis)*, usually soon after they were captured. The blood was examined microscopically for trypanosomes, and most samples were tested for trypanosome serum antibodies and inoculated into small laboratory animals. Serum antibodies were found in most animals and trypanosomes identified as *Trypanosoma brucei* were found in 7 of 39 (18%) of the rhinoceros. Berenil (diminazene aceturate) did not effect complete elimination of trypanosomes. In spite of treatment, one rhinoceros died of trypanosomiasis.

INTRODUCTION

Trypanosomiasis has been reported in black rhinoceros *(Diceros bicornis)*[3,6] and wild animal trappers know that these animals, when exposed to stresses of capture and captivity, succumb to acute trypanosomiasis days or even weeks after they have been removed from tsetse areas. Harthoorn[3] recommended routine injection of Berenil[3] at capture to eliminate trypanosomes from black rhinoceros.

The purpose of the present survey was to obtain more information on the prevalence of natural trypanosomiasis in black rhinoceros, to compare diagnostic methods, and to determine the sensitivity of the rhinoceros trypanosomes to Berenil, as trappers complain that rhinoceros often achieve trypanosomiasis in captivity, even if they are injected with Berenil when captured, before transfer to holding grounds in tsetse-free areas.

MATERIALS AND METHODS

Blood samples were collected from 39 rhinoceros, 12 captured in Tanzania, between October, 1968 and February, 1969, 24 captured in Kenya between April, 1969 and November, 1970 and three held in captivity in Nairobi. Information on age, sex and location of capture are given in Table 1. The identification numbers are the same as used in bacteriological survey of black rhinoceros.[2]

Blood was collected from an ear vein, and heparinized blood was injected into rats, using 2 ml intraperitoneally (ip) and 2 ml subcutaneously (sc), and into mice, 1 ml ip and 1 ml sc. The numbers of rats and mice are given in Table 1.

The blood was also used to prepare thick and thin blood smears. All rhinoceros were injected intramuscularly with approximately 7 mg Berenil per kg body weight before they were released into their pens.

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[3] 4,4-diamidino-diazoamino-benzene-diacetamido acetate. Hoechst AG, Frankfurt (Main), Germany.

TABLE 1. Results from the blood tests from 39 black rhinoceros.

No.	Age	Sex	Collecting place	Blood smear	Serology CA.	Serology LAT.	Inoculation mice no. pos/no. inoc.	Inoculation rats no. pos/no. inoc.
1	Ad.	F	Arusha Chini	negative	1/640	1/1280		
2	Ad.	M	"	"	1/320	1/320		
3	Ad.	M	"	"	1/320	1/320		
4	Ad.	M	"	"	1/320	1/320		
5	Ad.	M	"	"	not tested			
6	Ad.	M	"	"	1/80	1/80		
7	1/2 gr.	M	"	"	1/640	1/640	0/6	
8	y. Ad.	M	"	"	1/640	1/640	0/6	
9	Ad.	F	"	"	1/320	1/640	0/6	
31	Ad.	M	"	"	not tested			
32	2/3 gr.	F	East of Lake	T. brucei	not tested			
33	2/3 gr.	M	Manyara	"	not tested			
10	1/2 gr.	F	Darajani	"	not tested		0/4	1/2
11	1/2 gr.	M	"	"	1/320	1/640	0/4	1/2
34	2/3 gr.	F	"	negative	1/160	1/160	0/4	0/2
12	Ad.	F	Kiboko	"	1/640	1/640	0/4	0/2
13	Baby	F	"	T. brucei	1/640	1/1280	0/2	1/1
14	y. Ad.	M	"	negative	1/20	1/20	0/4	0/2
15	1/2 gr.	M	"	"	1/640	1/640	0/4	0/2
16	Ad.	F	"	"	1/160	1/160		0/4
17	y. Ad.	F	"	"	1/160	1/160		0/2
18	Baby	F	"	T. brucei	1/640	1/640		2/2
19	1/2 gr.	F	"	negative	1/20	1/20		0/2
20	1/2 gr.	F	"	T. brucei	1/160	1/640		1/2
21	Ad.	M	"	"	1/20	1/20		0/2
22	Ad.	F	"	"	1/640	1/640		0/2
23	Ad.	M	"	"	1/320	1/320		0/2
30	Ad.	M	"	"	1/160	1/160		0/2
35	1/2 gr.	F	"	"	1/40	1/40		
36	Baby	M	"	"	not tested			0/2
37	Ad.	F	"	"	1/20	1/20		0/2

TABLE 1. (continued)

38	Ad.	M	"	"	1/640	1/640			0/2
39	2/3 gr.	M	"	negative	1/640	1/640			0/2
40	Ad.	M	"	"	1/640	1/640			0/1
41	Baby	F	"	"	1/640	1/640			0/2
42	2/3 gr.	F	"	"	1/160	1/640			0/2
24	y. Ad.	M	Darajani	"	1/5x	1/5	0/4		
25	y. Ad.	F	"	"	1/40x	1/40			
28	Baby	M	Amboseli	"	negative[x]		0/2		

y. = young
ad. = adult
gr. = grown
M = Male
F = Female
[x]blood taken 13 months after capture

Thin blood smears, fixed in absolute ethanol and the thick smears, unfixed, were stained in 10% Giemsa solution for 45 min, and each smear was examined microscopically for trypanosomes for at least 10 min.

Serum was examined for *T. brucei* antibodies. Serological methods included the capillary agglutination test (CA)[5] and the latex agglutination test (LAT).[1]

Inoculated rats and mice were examined for trypanosomes every second day by microscopy of wet blood smears. If no trypanosomes were found within the first 45 days after inoculation the animal was regarded as negative.

In an attempt to distinguish *T. brucei* from *T. rhodesiense*, a blood incubation infectivity test was employed.[7]

Three of the *T. brucei* strains isolated were subinoculated into rats and cattle, one cow and nine rats per isolate. When trypanosomes were demonstrated in blood of the cows and rats, approximately 5 days later, six of nine rats in each group were treated with Berenil, three at a dose of 3.5 mg/kg and three at 7 mg/kg. Three rats from each isolate were kept as infected controls. Two of the three cows were treated with 7 mg/kg Berenil when they became parasitaemic, approximately 5 days after *T. brucei* inoculation, the third was kept untreated for clinical observations for 23 days, then treated with 7 mg/kg Berenil.

RESULTS

Trypanosomes were found in blood smears from 7 of 39 (18%) rhinoceros in the survey (Table 1). Morphologically, all isolates were identified as members of the *T. brucei* group. Thirty-two of the 33 serum samples tested for serum antibodies were positive. Five of 26 blood samples injected into mice and rats yielded isolates of *T. brucei*. All the rhinoceros in which trypanosomes were found were subadults.

The rats that were injected with blood taken directly from the rhinoceros

developed parasitaemia between 15 and 32 days after inoculation. The trypanosomes did not kill the rats and the parasites could be reisolated from their blood for several months after inoculation.

Signs of trypanosomiasis in rhinoceros. Only one of the animals in the survey from which trypanosomes were isolated actually died of trypanosomiasis. Rhinoceros No. 10, weight approximately 350 kg, was given 3.15 g Berenil i.m. when caught and 8 days later a further 2.1 g Berenil i.m. Apart from a minor abscess, the animal appeared normal until 25 days after it was captured, when it was found with subcutaneous edema over the forepart of the body, especially around the eyes and lips. The animal was in good flesh, the conjunctivae were white, the respiration was 16/min and forced, and the body temperature was 39 C. The animal did not resist handling during injection with 600 mg Samorin[4] i.m. The swellings disappeared within a few days. The number of trypanosomes decreased and disappeared from the blood smears within 4 days after treatment, but the animal remained depressed and died 6 days after it first was observed sick. The most remarkable finding at post mortem examination was a 10 × 10 × 10 cm sharply demarcated area of dry necrosis in the muscle where the Samorin had been injected.

Additional blood samples were taken also from two of the other infected rhinoceros while they were in their holding pens. No. 11, weight about 250 kg, was given 2.1 g Berenil (8.4 mg/kg) just after capture and four days later the dose was repeated. Blood smears taken on day 12 and day 14 after captivity contained a few trypanosomes, but trypanosomes were not demonstrated in blood smears taken on the day 21, just before the animal died of septicemia.[2] In No. 18, no trypanosomes were seen in blood smears on day 21 and day 22 after captivity. The animal died on day 23 but showed no specific necropsy findings, and no trypanosomes were seen in tissue smears. The brain was not examined for trypanosomes.

Berenil sensitivity tests. Cattle subinoculated for the Berenil sensitivity test developed parasitaemia within 2-3 days. Only the cow injected with strain 13 was examined clinically. On the third day it had an elevated body temperature of 40 C. No other signs of disease were observed. Trypanosomes could be seen in wet blood smears from 3 to 6 days after inoculation. From 7 to 23 days, when it was treated with 1 mg/kg Berenil, trypanosomes could be demonstrated only by subinoculation into mice.

It was not possible to demonstrate any trypanosomes in the rats inoculated with blood from the cattle after the cattle had been treated with 7 mg/kg Berenil. All the rats treated with 3.5 mg/kg Berenil relapsed with parasitaemia within 23-25 days, and those treated with 7 mg/kg between 26 and 34 days, except the rats that were inoculated with strain 20 which remained negative for 45 days after they were given 7 mg/kg Berenil.

Blood incubation infectivity test. The results of this test indicated that the trypanosome strain isolated was *T. brucei* rather than *T. rhodesiense*.

DISCUSSION

There are few published reports about trypanosome infection in rhinoceros. Hoare[4] does not list the rhinoceros as a host for trypanosomes, and Weitz and Glasgow[9] place the rhinoceros in a group of favored hosts for the tsetse fly but also in a group with low or no prevalence of trypanosome infections. The present data would suggest that *T. brucei* infections are not uncommon in black rhinoceros.

[4] Isometa-midium chlorid. May & Baker, Dagenham, U.K.

The populations of rhinoceros in the survey areas probably act as reservoirs for trypanosomes, but it is not known what effect trypanosomes have on free-living rhinoceros. All the rhinoceros in the survey from which trypanosomes were isolated were apparently healthy when caught and, possibly, trypanosomes only become pathogenic when rhinoceros are stressed by other factors, e.g., drought, capture, etc.

Six of seven rhinoceros in which trypanosomes were demonstrated died of a variety of reasons, while in the entire survey only two others died. Only the death of No. 10, however, could be related directly to trypanosomiasis. All the deaths occurred within 3 months after capture.

While trypanosomes were found in all blood smears from rhinoceros blood samples which produced trypanosomiasis in laboratory animals, there does not seem to be any correlation between the biological and serological tests. If the serum antibody titers are compared with titers from cattle experimentally exposed to *T. brucei*, it is found that the titers are rather high in most of the rhinoceros.

Animals Nos. 24 and 25 kept in tsetse-free areas had low titers compared with most of the free-ranging animals, evidence that the titers may decline when rhinoceros are not regularly exposed to trypanosomes. The only serum samples without trypanosome antibodies was from No. 28 which was captured when only a few months old.

Although the tested strains do not show any particular resistence to Berenil, the repeated blood sampling from rhinoceros Nos. 10 and 11 indicate that it was not possible to eliminate the trypanosomes completely from the rhinoceros and, the observation that rhinoceros are extremely difficult to get back on their feet after they have been sick, makes it important to keep the animals under very close observation during the first four weeks of captivity. The losses from trypanosomiasis among rhinoceros are possibly due more to late treatment than to wrong treatment. On the other hand, depression and death of rhinoceros after drugs apparently have cleared the trypanosomes from the blood stream, may relate to invasion by *T. brucei* of the central nervous system. It may be worthwhile to consider treatment of such cases with drugs able to pass the blood-brain barrier.

The observed prevalence of *T. brucei* in rhinoceros, and the failure to demonstrate *T. congolense* and *T. vivax* in animals from areas like Kiboko where *T. congolense* and *T. vivax* are the predominant trypanosome species,[5] may be evidence for a particular susceptibility of the rhinoceros to infection with *T. brucei*.

Acknowledgement

The author would like to thank the Director of Veterinary Services for permission to publish this survey, to Mssrs. Binz and Rickman from the WHO/Trypanosomiasis Project, K41, Kenya, for having carried out the serological and the blood incubation infectivity tests, to Dr. G. Warui from the Veterinary Research Laboratory, Kabete, who kindly provided laboratory facilities and help with trypanosome identification; also to the personnel of the animal trapping company, Carr-Hartley & Sons, who were very cooperative and helpful during the whole survey and Dr. L. Karstad, who reviewed the manuscript.

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Received for publication 19 October 1979