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A trypanosomiasis survey of wild animals in the Luangwa Valley, Zambia

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Summary

Between 1971 and 1974 546 wild animals of 34 species were examined for trypanosomes; 79 infections (9 mixed) were diagnosed and 29 stocks were cryopreserved. Of 14 stocks of the subgenus *Trypanozoon* tested by the blood incubation infectivity test three (two from *Kobus ellipsiprymnus* and one from *Phacochoerus aethiopicus*) retained their infectivity to rodents. There are indications that the number of animals harbouring trypanosomes is far in excess of those showing detectable parasitaemias even when using a combination of diagnostic methods. The standard microhaematocrit method involving microscopic examination of the buffy layer from a microhaematocrit tube between a slide and coverslip, and the inoculation of experimental animals produced the most satisfactory results.

Key words: Game animals; *Trypanozoon; Nannomonas; Duttonella;* isolation; characterization.

Introduction

Three trypanosomiasis surveys have been carried out on game animals in the Luangwa Valley:

1. Kinghorn and Yorke (1912 a and b): Location 12° 25′ S and 32° 02′ E, from June to April, including the rainy season. 119 ungulates of 13 species were examined.
2. Keymer (1969): Location 12° 50′ to 13° 21′ S and 31° 41′ to 32° 05′ E, during the dry season. 43 ungulates of 10 species were examined.
3. The authors (1971–1974): Location 13° to 13° 30′ S and 31° 30′ to 32° E. 546 wild animals of 34 species were examined during the dry seasons.

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The present survey was conducted to obtain further information on the incidence of trypanosome infections in wild animals and to compare the efficiency of various diagnostic methods.

Materials and methods

The survey area extended from 13° to 13°30′ S and from 31°30′ to 32° E on both banks of the Luangwa river at an altitude of 600–700 m. The average annual rainfall for the area is 750 mm falling mostly between December and February. All samples were taken during the dry season from June to October.

The vegetation is largely *Colophospermum mopane* woodland on brown Karoo sediments and *Brachystegia kirkia, Pterocarpus* and *Combretum* on sandier soils with typical riverine vegetation along the main drainage lines.

The area is a declared "Game Management Area" with all common game species represented. Three species of tsetse fly have been recorded: *Glossina morsitans orientalis, G. pallidipes* and *G. brevipalpis* (T. Sehof, Personal communication).

Scattered human habitations occur from Milyoti gate to Nyamaluma with concentrations around Kakumbi. Human sleeping sickness is endemic in this area.

Most animals were shot during the course of a pilot game utilization scheme. Blood samples were collected in EDTA-Vacutainers immediately after death. Large animals were bled from the jugular vein and small animals by cardiopuncture. Care was taken to prevent exposure of the samples to sunlight. The Vacutainers were immediately transferred to an insulated box which maintained a temperature of approximately 26° C. All samples were returned to the field laboratory within 3 h.

Diagnostic methods

1. Wet smears were prepared from blood containing EDTA and 100 fields were examined using 400× magnification.
2. Dry films stained with Giemsa were examined under high power oil immersion (200 fields).
3. Standard microhaematocrit method. After centrifugation at 15,000 r.p.m. for 5 min the microhaematocrit tube was cut about ½ cm on either side of the buffy-coat. Its contents was expelled on to a slide leaving the buffy-coat in a pin-head shape. A cover slip was lowered on to the buffy-coat which pressed it into a circular layer. The border between leucocytes and erythrocytes was examined for trypanosomes.
4. Microhaematocrit method (Woo, 1970). After microhaematocrit centrifugation the plasma bordering the buffy-coat was examined under a 10× ocular and a 40× oil immersion objective turning the microhaematocrit tube 4 times by 90 degrees during screening.
5. Inoculation of experimental animals. Three mice per animal were routinely inoculated intraperitoneally with 1 ml of blood. 8–10 mice were inoculated with blood from those animals with low parasitaemias of the *Nannomonas* subgenus. The limited stock of sheep, goats and pigs was reserved for the isolation of *T. vivax* and *T. simiae.*
6. *Blood incubation infectivity test (BIIT).* For the identification of all pleomorphic stocks of the subgenus *Trypanozoon* the BIIT (Rickman and Robson, 1970) was used in addition to the modification by (Awan and Dillmann, 1973). The human serum resistant stocks were retested at the London School of Hygiene and Tropical Medicine.

Results

From 1971 to 1974 546 animals of 34 species were examined. 79 trypanosome infections (9 of which were mixed) were diagnosed. These results and

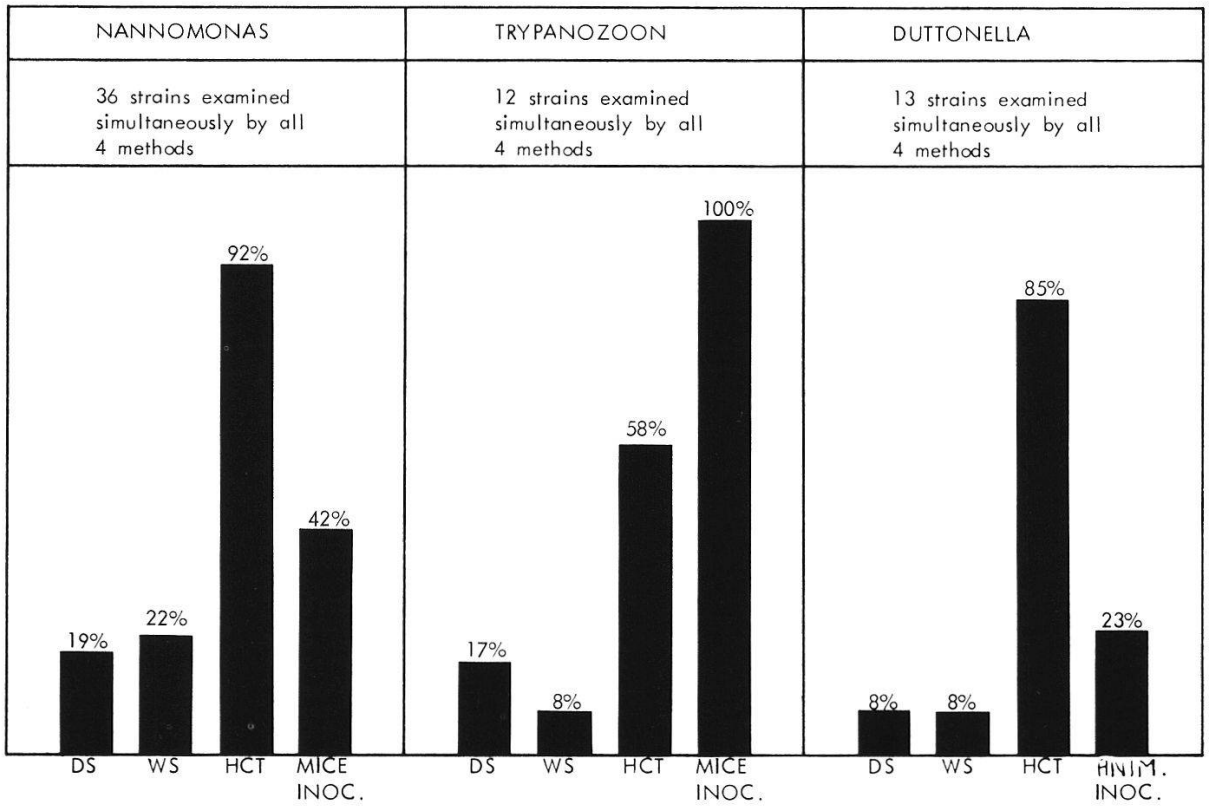

Fig. 1. Efficiency of various diagnostic methods. DS = stained dry smear; WS = wet smear; HTC = standard microhaematocrit method.

those of previous surveys are given in Table 1. The different trypanosome infections diagnosed in various species are given in Table 2. Also included in this table are the results obtained by different diagnostic methods; their efficiency is compared in Fig. 1.

Trypanosomes of the subgenus *Duttonella* diagnosed and isolated via sheep and goats were considered to be *T. (Duttonella) vivax*. Trypanosomes of the subgenus *Nannomonas* diagnosed and isolated via mice were *T. (Nannomonas) congolense*. The 5 stocks from *Phacochoerus aethiopicus* which could only be isolated via domestic pigs were diagnosed as *T. (Nannomonas) simiae*. Three of the 14 stocks of the subgenus *Trypanozoon* isolated and tested by the BIIT retained their infectivity to rodents. Two were isolated from *Kobus ellipsiprymnus* and one from *Phacochoerus aethiopicus*, the latter being a new host record.

A total of 29 stocks was cryopreserved, 14 of the subgenus *Trypanozoon*, 3 *T. (Duttonella) vivax*, 9 *T. (Nannomonas) congolense* and 2 *T. (Nannomonas) simiae*.

Discussion

Relation of trypanosomiasis to age and sex, stress and localities

No direct relation between the incidence of trypanosomiasis and age or sex could be demonstrated. There are indications that certain age groups of various

Table 1. Animals examined during three surveys

Species (common name/scientific name)	No. examined			No. infected		
	A	B	total	A	B	total
1 Baboon (Papio cynocephalus)	1	20	20	–	0	0
2 Bat (Nycteris sp.)	–	2	2	–	2	2
3 Buffalo (Syncerus caffer)	4	19	23	0	2	2
4 Bushbuck (Tragelaphus scriptus)	15	23	38	9	14	23
5 Bushpig (Potamochoerus porcus)	4	–	4	0	–	0
6 Cane rat (Thryonomys sp.)	–	1	1	–	0	0
7 Civet (Viverra civetta)	–	6	6	–	1	1
8 Crocodile (Crocodylus niloticus)	–	1	1	–	0	0
9 Duiker (Sylvicapra grimmia)	–	7	7	–	3	3
10 Eland (Taurotragus oryx)	–	3	3	–	2	2
11 Elephant (Loxodonta africana)	–	20	20	–	2	2
12 Genet (Genetta genetta)	–	6	6	–	0	0
13 Giraffe (Giraffa camelopardalis)	–	1	1	–	1	1
14 Grysbok (Raphicerus sp.)	–	5	5	–	0	0
15 Hare (Lepus capensis)	–	10	10	–	0	0
16 Hartebeest (Alcelaphus lichtensteini)	7	–	7	1	–	1
17 Hippopotamus (Hippopotamus amphibius)	1	250	251	0	4	4
18 Hyaena (Crocuta crocuta)	–	7	7	–	4	4
19 Impala (Aepyceros melampus)	36	23	59	2	1	3
20 Jackal (Canis mesomelas)	–	1	1	–	0	0
21 Kudu (Tragelaphus strepsiceros)	8	13	21	5	11	16
22 Leopard (Panthera pardus)	–	2	2	–	0	0
23 Lion (Panthera leo)	–	6	6	–	6	6
24 Mongoose (Herpestes sanguineus)	–	2	2	–	0	0
25 Monkey vervet (Cercopithecus sp.)	–	18	18	–	0	0
26 Puku (Kobus vardoni)	15	24	39	3	1	4
27 Porcupine (Hystrix galeata)	–	1	1	–	0	0
28 Rhinoceros (Diceros bicornis)	1	5	6	0	0	0
29 Roan antelope (Hippotragus equinus)	8	11	19	1	2	3
30 Serval (Felis serval)	–	2	2	–	0	0
31 Warthog (Phacochoerus aethiopicus)	12	24	36	1	6	7
32 Waterbuck (Kobus ellipsiprymnus)	35	20	55	21	16	37
33 Wild cat (Felis lybiea)	–	1	1	–	0	0
34 Wild dog (Lycaon pictus)	–	2	2	–	0	0
35 Wildebeest (Connochaetes taurinus)	5	5	10	0	1	1
36 Zebra (Equus burchelli)	11	5	16	0	0	0
Total	162	546	708	43	79	122

A = Surveys by Kinghorn & Yorke and Keymer
B = Survey by the authors

Table 2. Comparison of the results of various diagnostic methods from animals found parasitologically positive

Species	Diagnostic methods						Trypanosoma subgenus diagnosed						
	WS[1]	HCTW[1]	HCT[1]	DF[1]	inoc. exp. animals		D	N	T	M	DN	DM	NT
					mice[1]	others[1]							
Bat	1/2	0/1	2/2	1/2	0/2	–	–	–	–	2	–	–	–
Buffalo	1/2	0/1	2/2	1/2	0/2	–	1	1	–	–	–	–	–
Bushbuck	7/14	4/5	12/13	6/14	5/14	1/2	3	7	1	1	1	–	1
Civet	0/1	–	1/1	0/1	0/1	–	–	1	–	–	–	–	–
Duiker	0/3	3/3	2/3	0/3	1/3	–	1	2	–	–	–	–	–
Eland	0/2	–	2/2	0/2	1/2	–	–	1	–	–	1	–	–
Elephant	1/2	1/1	2/2	2/2	0/2	0/1	–	2	–	–	–	–	–
Giraffe	1/1	1/1	1/1	1/1	1/1	–	–	–	1	–	–	–	–
Hippopotamus	0/4	–	0/4	0/4	4/4	–	–	–	4	–	–	–	–
Hyaena	1/4	0/1	4/4	1/4	3/4	–	–	2	2	–	–	–	–
Impala	0/1	–	0/1	0/1	1/1	–	–	1	–	–	–	–	–
Kudu (greater)	1/11	4/6	10/11	1/11	3/11	3/7	3	3	–	1	4	–	–
Lion	2/6	0/2	4/4	2/6	4/5	–	–	3	3	–	–	–	–
Puku	1/1	–	–	1/1	–	–	1	–	–	–	–	–	–
Roan	0/2	1/2	2/2	0/2	0/2	–	–	2	–	–	–	–	–
Warthog	2/6	0/3	3/5	2/6	1/6	3/4	–	×5	1	–	–	–	–
Waterbuck	6/16	3/5	15/15	5/16	3/15	9/9	12	–	2	–	1	1	–
Wildebeest	0/1	1/1	0/1	0/1	0/1	–	1	–	–	–	–	–	–
Total	24/79	18/32	62/73	23/79	27/76	16/23	22	30	14	4	7	1	1

D = Duttonella
N = Nannomonas
T = Trypanozoon
M = Megatrypanum
DN = Duttonella-Nannomonas
DM = Duttonella-Megatrypanum
NT = Nannomonas-Trypanozoon
X = T. (Nannomonas) simiae
WS = wet smear
HCTW = microhaematocrit method (Woo)
HCT = standard microhaematocrit method
DF = stained dry film

[1] First number: no. of pos.; 2nd number: animals examined

species showed higher parasitaemias, which seem to be due to specific stress situations occurring at a certain age.

Young lions (*Panthera leo*) of 10–13 months appear to be exposed to social stresses, which can result in nutritional stress. This age group has to find new social status within the pride. Fighting for their share of food these lions frequently acquire serious wounds and are generally in poor condition. In these animals higher parasitaemias were usually found, but we could not determine whether their poor condition was the cause of the higher parasitaemias or vice versa. Similar observations were made with wounded buffaloes (*Syncerus caffer*) and those in poor condition. Bushbuck (*Tragelaphus scriptus*) rams showing wounds, apparently acquired in the course of territorial conflicts, were also found to have higher parasitaemias. These facts indicated that at least some higher parasitaemias were due to stress situations (Awan and Dillmann, 1973).

The infection rates of different species of mammals changed notably over the period of 2–3 years. Three reasons are considered to be responsible:

1. Changes in the population dynamics of a given species resulting in stress situations which in turn lowered the natural resistance to parasites and diseases.
2. Changes within the vector population in a given area altering the challenge situation.
3. Due to the limitations of the parasitological methods used for the diagnosis of trypanosomes only some of the existing low parasitaemias could be demonstrated.

Infection rates

Overall infection rates obtained from any survey are irrelevant if certain factors are not taken into consideration: variety of species examined, number of samples taken and diagnostic methods applied. Simple manipulations of these factors will provide infection rates varying from 0–100%. Simply by excluding primates from the 1972 sample a drastic change in the overall infection rate from 8–19% could be achieved. Furthermore it was found that daily and even hourly variations in individual parasitaemias of trypanotolerant animals are common. Recent daily examinations by one of the authors on individual parasitaemias in trypanotolerant cattle over a period of 153 days showed positive results on only 56 days.

Blood collection

The method described above was preferred, as the larger volumes collected and the precautions taken increased the survival time of trypanosomes far in excess of 3 h, sometimes up to 3 days after collection. This was necessary as local conditions prevented immediate examination of the samples.

Diagnostic methods

Results obtained from wet smears can vary, depending from where and how long after death the blood sample is collected. With one bull elephant (*Loxodonta africana*) 10 wet smears from the jugular vein showed 8 negative and 2 positive smears, while of 10 wet smears from the ear vein 2 were negative and 8 positive.

The same comments apply to dry smears. In 1968–1969, 1800 dry smears from 360 elephants were taken from the jugular vein (immediately after death), the heart, the lungs, the liver and lymph glands (2–8 h after death) and stained by Giemsa. Only two smears were positive, each containing 2–3 trypanosomes of the *Nannomonas* subgenus. Results obtained by this method alone appeared to be very unreliable.

The standard microhaematocrit method proved to be the most reliable, even the sluggish organisms of the *Nannomonas* subgenus could be diagnosed provided the parasitaemia was not too low, as shown in hippopotamus (*Hippopotamus amphibius*) (Dillmann and Awan, 1972).

The microhaematocrit method as used by Woo (1970) was very successful for all stocks of the subgenus *Trypanozoon* and *Duttonella* which were still agile and lively. However it was observed that sluggish organisms of *Nannomonas* and the short forms of pleomorphic stocks of *Trypanozoon* were centrifuged into the buffy-coat layer, thus escaping detection. It is therefore advisable to use both microhaematocrit methods.

Mice were used successfully for the isolation of pleomorphic stocks of *Trypanozoon* from low parasitaemias, not detectable by other methods. Less satisfactory results were obtained when attempting to isolate organisms of the subgenus *Nannomonas.* Some of these stocks originating from lion (*Panthera leo*), kudu (*Tragelaphus strepsiceros*), eland (*Taurotragus oryx*), bushbuck (*Tragelaphus scriptus*), elephant (*Loxodonta africana*) and other species failed to produce detectable parasitaemias in mice. This may be due to the fact that sometimes only 3 mice per animal were inoculated (Geigy et al., 1971).

For the isolation of *T. congolense* from lions (*Panthera leo*) 10 mice per animal were inoculated. In one case all 10 mice remained negative up to day 27. On day 30 one trypanosome was found in one mouse. It was therefore decided to continue the examination of this mouse and two others beyond the usual period of 30 days. On day 31 no trypanosomes were detected even when using the microhaematocrit method. On day 82 the mouse again showed a very low parasitaemia which disappeared completely the following day, all other mice remaining negative. Again on day 90 a few trypanosomes were observed and three more mice were subinoculated. After 6 days this stock was isolated from a very slowly rising parasitaemia in one of the 3 mice.

Only a very limited stock of goats, sheep and pigs was available for experiments. They proved to be of minor importance except for isolating *T. vivax* and *T. simiae.*

Efficiency of various diagnostic methods

Fig. 1 clearly indicates that the inoculation of laboratory rodents produced a high rate of diagnostic success when dealing with organisms of the subgenus *Trypanozoon.* However for the diagnosis of stocks belonging to *Nannomonas* or *Duttonella* the standard microhaematocrit method produced results which could not be improved by the inoculation of experimental animals.

Isolation of human serum resistant stocks

The isolation of human serum resistant stocks from waterbuck (*Kobus ellipsiprymnus*) and warthog (*Phacochoerus aethiopicus*) seems to be epidemiologically important in this area of Zambia. Both species were frequently found close to human habitations, even in areas not now teeming with game. The role of these animals as a natural reservoir for human sleeping sickness requires further investigation.

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