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Miscellanea

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Trypanosomes and Experimental Trypanosomiasis in East African Bats

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Abstract

Using the haematocrit centrifuge technique, four hundred and twenty-seven bats from East Africa were examined for trypanosome infections. Approximately 21 % of the bats were found to be infected. The infection rate varied from zero to 73.3 %. No trypanosome was found in fruit-eating bats (Megachiroptera).

Three species of trypanosomes were found in insect-eating bats (Microchiroptera), none of the trypanosome was infective to mice or rats. The trypanosomes encountered in the survey were *Trypanosoma (Schizotrypanum) vespertilionis, T. (Megatrypanum) heybergi,* and *T. (M.) mpapuense*. New descriptions based on abundant materials are given for each of the species.

Trypanosoma rhodesiense and *T. brucei* produced a much more chronic infection in insect-eating bats *(Tadarida condylura)* than in mice. Since it is known that some species of *Glossina* feed on bats, we raised the possibility of insect-eating bats as potential reservoirs of these trypanosomes.

In experiments fruit-eating bats seem to be much more susceptible to *T. brucei* than the insect-eating bats. *T. vivax* is not infective to bats.

Introduction

The pathogenic trypanosomes of African mammals have been the subject of intensive studies since the beginning of the century. However, relatively little is known about the other trypanosomes of African mammals e.g. those of bats. Ed. and Et. Sergent (1905) described the first trypanosome from bats in Africa. A comprehensive review along with the original descriptions of the trypanosomes in bats has recently been published (Hoare, 1972). However, most of these descriptions were based on relatively few specimens. The incidence and distribution of these various trypanosomes of bats are not known since the infections in these animals are often low and hence are difficult and time consuming to detect by the examination of stained blood smears. Using the haematocrit centrifuge technique (Woo, 1969), we have examined the blood of bats from East Africa (Uganda, Kenya and Tanzania) to determine the species and incidence of trypanosome infections in bats in the different localities. In addition to reporting on the incidence and host range, new descriptions based on abundant materials are given for the species encountered in this survey.

The second part of this paper deals with the preliminary studies on experimental infections of bats with pathogenic trypanosomes.

Material and Methods

The bats were caught by either mist- or hand-nets. They were collected from various areas in East Africa. In most cases, cardiac blood was examined. About

0.1 ml of cardiac blood was withdrawn in a tuberculin syringe (using 24G x 1/2 needle) that had previously been rinsed with heparinized normal physiological saline. Two to three capillary tubes (each tube containing about 0.06 ml) of blood from each bat were sealed at one end with plasticine and centrifuged in an International (model MB) microcapillary centrifuge for four minutes at 12,000 r.p.m. After centrifugation, the tubes were examined under a microscope according to the technique described earlier (Woo, 1969). Thick and thin blood smears were also made from the blood and these were later stained in Giemsa's stain.

The image of the trypanosome was projected onto drawing paper using a camera lucida. A line was drawn down the middle of the trypanosome from one extremity to the other. The positions of the kinetoplast, the nucleus (at the center) and the anterior end and maximum width of the body were marked on this line. Measurements were made with a pair of fine dividers set so that the points were separated by a distance equal to one micron on the projected image.

Results

I. Trypanosomes in Bats

Four hundred and twenty-seven bats were examined from seven areas in Uganda, Kenya and Tanzania. Ninety bats (approximately 21.1 %) were found to be naturally infected with trypanosomes. The infection rates varied from zero in two species of bats to 73.3 % in one species (Table 1). No natural infection of trypanosomes was found in twenty-

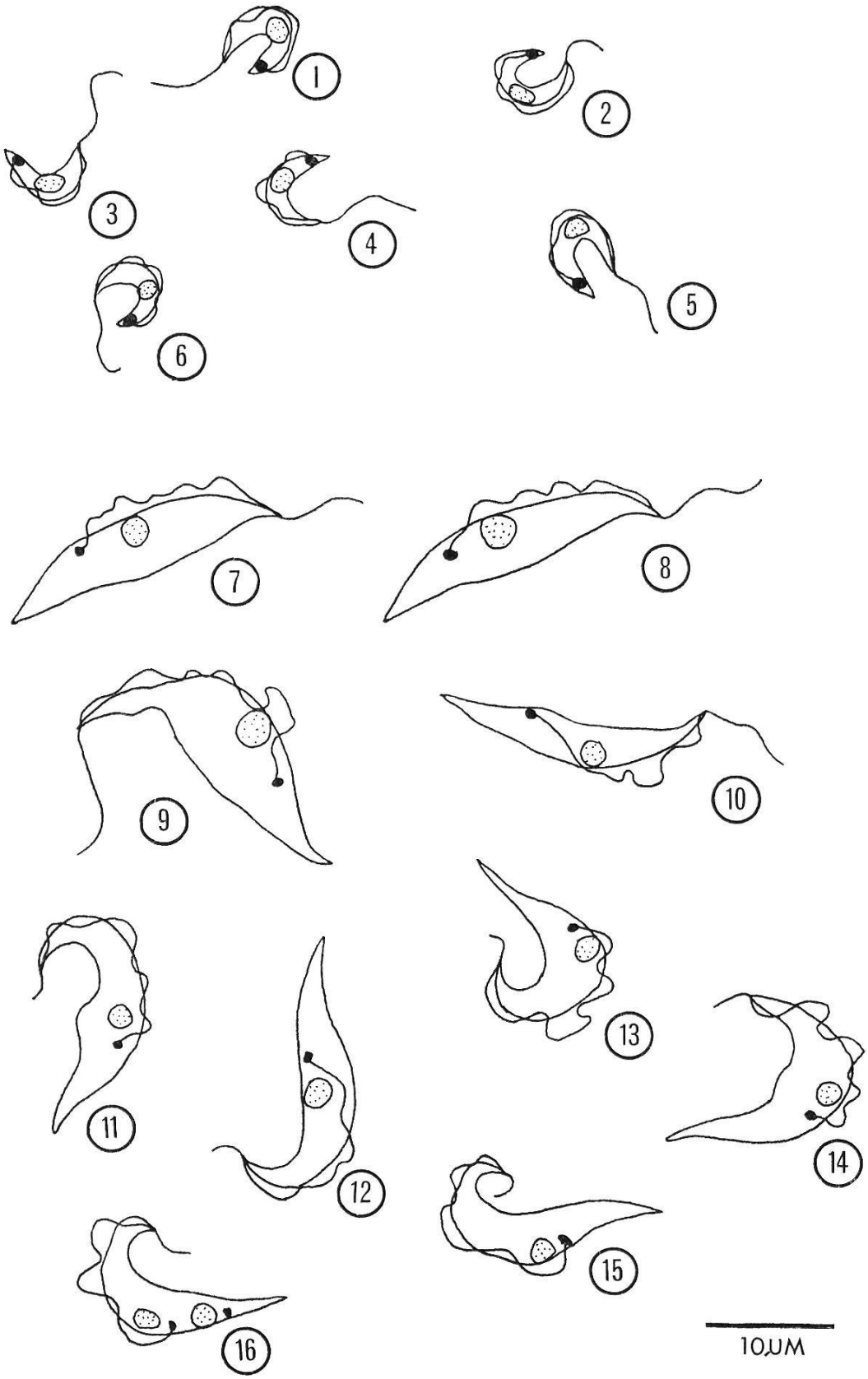
Table 1. The Incidence of Trypanosome Infections in Bats in East Africa

Bats / Localities	Tororo	Nagongera	Lugala	Sipi	Aitong	Kisi	Serengeti	Percentage infection
Epomophorus wahlbergi	0/27 *	–	–	–	–	–	–	0
Tadarida condylura	9/178	1/38	–	–	–	0/7	–	4.9
Tadarida limbata	0/35	–	–	–	–	–	0/1	0
Pipistrellus nanus	–	–	1/3	–	–	–	–	33.3
Taphozous perforatus	5/11	0/2	–	–	–	–	–	38.5
Coleura afra	11/15	–	–	–	–	–	–	73.3
Hipposideros caffer	15/40	–	–	–	–	–	21/29	52.2
Rhinolophus spp.	–	–	–	2/10	–	18/22	4/4	66.6
Nycteris thebaicea	2/3	–	–	–	–	–	–	66.6
Scotophilus negritus	–	–	–	–	1/2	–	–	50.0

Figs. 7–10. Trypanosoma heybergi; note that the kinetoplast is about mid-way between the nucleus and the posterior tip, also the long free flagellum. (Specimens drawn from *Hipposideros caffer* No. 817, Serengeti, Tanzania.)

Figs. 11–16. Trypanosoma mpapuense; note that the kinetoplast is very close to the nucleus and the short free flagellum.

Fig. 16. Trypomastigote in cardiac blood, with 2 kinetoplasts and 2 nuclei. (Specimens drawn from *Nycteris thebaicea* No. 858, Tororo, Uganda.)



Figs. 1–16. Camera lucida drawings of trypanosomes found in bats during the present study.

Figs. 1–6. Trypanosoma vespertilionis; note the prominent kinetoplast and the long free flagellum, also the typical crescentric shape of the trypanosome. (Specimens drawn from *Coleura afra* No. 489, Tororo, Uganda.)

seven fruit-eating bats (Megachiroptera) examined while 22.5 % of the insect-eating bats (Microchiroptera) were infected.

Three species of trypanosomes were encountered in this survey. None of the 3 trypanosome was infective to mice or rats by intraperitoneal inoculation of infected blood from bats. The trypanosomes are identified as *Trypanosoma (Schizotrypanum) vespertilionis.* Battaglia, 1904; *T. (Megatrypanum) heybergi* Rodhain, 1923; and *T. (Megatrypanum) mpapuense* Reichenow, 1940.

T. (S.) vespertilionis is a *cruzi*-like trypanosome with the typical crescentric shape in stained specimens (Figs. 1–6). It is a very small organism with a large prominent kinetoplast at its pointed posterior tip (Table 2). The nucleus is in the posterior half of the body and its free flagellum is more than two-third its body length (Table 3). No dividing trypomastigote or other forms were seen in blood smears. In centrifuged tubes, the organism is very active and exhibits continous "tumbling" movements.

This trypanosome was recorded from *Coleura afra, Rhinolophus eloquens. Taphozous perforatus* (Tororo Area, Uganda) and *Pipistrellus nanus* (Lugala, Uganda).

T. (M.) heybergi is a relatively large trypanosome with its kinetoplast nearer to its nucleus than to its posterior tip (Table 2). The nucleus occupies a marginal position (Figs. 7–10) and is near the middle of the body (Table 3). The length of its free flagellum is quite variable and its undulating membrane well developed.

T. (M.) mpapuense like *T. (M.) heybergi* is a relatively large trypanosome with a pointed posterior tip (Figs. 11–16). However, its kinetoplast is closer to its nucleus than that of *T. (M.) heybergi* (Table 2). It has a short free flagellum, and the undulating membrane well developed. It probably multiplies in the blood as trypomastigote because we did see one trypomastigote with 2 kinetoplasts and 2 nuclei (Fig. 16).

It was not possible to differentiate between *T. heybergi* and *T. mpapuense* in unstained living preparations as seen in centrifuged capillary tubes; we therefore, have recorded the following bats as being infected with a large trypanosome subgenus *Megatrypanum; Hipposideros caffer, Nycteris thebaicea* (Tororo, Uganda), *Hipposideros caffer* (Serengeti, Tanzania); *Rhinolophus clivosus* (Sipi, Kenya) and *Scotophilus negritus* (Aitong, Kenya).

II. Experimental Infection of Bats with the Pathogenic Trypanosomes

All bats used in the following experiments were examined for natural trypanosome infection by the haematocrit centrifuge technique and only negative bats were used.

(i) Experimental infection of insect eating bats *(Tadarida condylura).*

Table 2. Measurements * (in microns) of Trypanosomes from Bats

Trypanosome	Host	Locality	Sample size	PK	KN	PN	NA	PA	FF	TL	BW
Trypanosoma vespertilionis	*Coleura afra*	Tororo, Uganda	48	0.5–1.5 (1.1)	2.5–5.0 (3.5)	3.5–6.0 (4.6)	5.5–9.0 (7.0)	10.0–14.0 (11.3)	6.0–12.0 (8.7)	17.0–22.5 (20.0)	1.5–3.0 (2.3)
Trypanosoma heybergi	*Hipposideros caffer*	Tororo, Uganda	45	5.0–9.0 (7.0)	2.5–6.0 (3.6)	8.5–13.5 (10.6)	9.0–16.0 (13.0)	18.0–29.0 (23.5)	5.0–10.0 (6.8)	23.0–39.0 (30.3)	3.5–6.0 (4.3)
Trypanosoma mpapuense	*Nycteris thebaicea*	Tororo, Uganda	24	9.5–17.0 (11.4)	1.5–2.5 (1.9)	11.5–19.5 (13.4)	12.5–20.0 (16.4)	25.0–35.5 (29.8)	1.0–4.0 (2.2)	27.5–39.5 (32.0)	4.0–6.0 (4.9)

* Range followed by average (in brackets). Abbreviations in Tables 2–3, PK, the distance from posterior end to kinetoplast; KN, the distance of the kinetoplast to the centre of the nucleus; PN, the distance from the posterior end to the centre of the nucleus; NA, the distance from the centre of the nucleus to the anterior tip of the body (excluding the free flagellum); PA, the length of the body (excluding the free flagellum); FF, the length of the free flagellum; TL, the length of the body plus the length of the free flagellum; BW, the maximum width (excluding the undulating membrane).

Table 3. Body ratios * of Trypanosomes from Bats

Trypanosome	Sample size	PK/PA	PK/PN	PN/PA	FF/PA
Trypanosoma vespertilionis	48	0.07–0.16 (0.10)	0.12–0.60 (0.33)	0.32–0.52 (0.41)	0.57–1.09 (0.78)
Trypanosoma heybergi	45	0.25–0.35 (0.30)	0.52–0.73 (0.66)	0.41–0.50 (0.45)	0.25–0.44 (0.29)
Trypanosoma mpapuense	24	0.32–0.48 (0.38)	0.81–0.92 (0.85)	0.41–0.55 (0.45)	0.03–0.11 (0.07)

* Range followed by average (in brackets).

(a) Four *Tadarida condylura* and 4 mice of about the same size were each inoculated intraperitoneally with approximately 5×10^4 *Trypanosoma rhodesiense*. The trypanosome was the first mouse-passage of a strain originally isolated directly from a patient (ss 965) at the E.A.T.R.O. hospital. The blood of the bats and mice were examined by wet preparations (50 microscopic fields) and by the haematocrit centrifuge technique.

Five days after infection, all 4 mice were positive by wet preparation (1–3 trypanosomes per field) and by the 12th day all mice had massive numbers of trypanosomes in their blood. However, no trypanosomes were seen in the blood of the bats by wet preparations during the 12 day period. Since the bats could not be coaxed to eat in captivity all the bats were euthanised on the 12th day and trypanosomes were seen in their blood by the haematocrit centrifuge technique (1–3 trypanosome per 0.06 ml of blood). 0.1 ml of blood from each of the bats was inoculated into mice. All the mice subsequently became infected, thus confirming that the bats were infected and that the trypanosomes were still infective.

(b) The above experiment was repeated with the blood and metacyclic stages of *Trypanosoma brucei* Ulanga I.

Each of the 10 bats were inoculated intraperitoneally with approximately 1×10^5 trypanosomes. Bats were sacrificed at 24 hours intervals and their blood examined by wet preparation and inoculated into mice.

Trypanosomes were detected in cardiac blood 4 hours after infection by the haematocrit centrifuge technique. Twenty-six hours post infection, trypanosomes were detected by wet preparations; small number of trypanosomes were present in cardiac blood of all sacrificed

bats during the seven days. After the 7th day, the experiment was again terminated because the bats could not be coaxed to eat in captivity. All mice inoculated with blood from all the infected bats became infected.

The salivary glands of a *Glossina morsitans*[1] experimentally infected with *T. brucei* were ground up and injected intraperitoneally into 3 bats. The infections in all 3 bats were detected within 5 days of infection and the trypanosomes were infective to mice.

(ii) Experimental infection of fruit eating bats *(Epomophorus anurus).*

Three fruit eating bats were infected with approximately 1×10^4 *T. brucei.* All 3 bats died in approximately 3 days. At the time of death, one bat had about 2×10^5 trypanosomes in one mm^3 of blood.

Seven bats were each inoculated with approximately 2×10^4 blood forms (directly from an infected cow) of *Trypanosoma vivax.* The trypanosome is a virulent West African strain. The trypanosomes were detected for 3 days post infection by the haematocrit centrifuge technique. Subsequent daily examinations were negative.

Discussion

According to the "Host-parasite check-list" in Hoare (1972) there is no record of trypanosome described from fruit-eating bats (Megachiroptera) in Africa. In our own survey, we did not find the fruit-eating bats in Tororo, Uganda to be infected.

In the insect-eating bats (Microchiroptera) we found that about 21 % of them were infected. Three species of trypanosomes were encountered, namely, *T. (S.) vespertilionis, T. (M.) heybergi,* and *T. (M.) mpapuense.* Our description of *T. vespertilionis* conforms closely to that found in the literature; however, there is one difference and this is in the position of the nucleus. In the trypanosome that we studied, the nucleus is in the posterior region of the body while in most descriptions of the trypanosome, the nucleus is in the anterior region of the body. HOARE (1972), in his review concluded that undue emphasis had been placed on the position of the nucleus in this trypanosome and felt that the variation is too great for it to have taxonomic value. After studying our own specimens, we tend to agree with him.

Our description of *T. heybergi* is very similar to the original description of the trypanosome from *Nycteris hispida* in the Congo (RODHAIN 1923). Similarly, our measurements of *T. mpapuense* closely resemble that given by REICHENOW (1940) when he described the trypanosome

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[1] The infected *G. morsitans* was kindly supplied to us by Dr. R. STEIGER of the Swiss Tropical Institute.

from *Nycteris aethiopica* in Tanzania. One small difference is in the length of the free flagellum. Our strain seem to have a slightly longer free flagellum, but since the original description was based on fewer specimens, we feel that this difference is not very significant.

Since *Glossina morsitans* and *G. tachinoides* feed readily on bats (NASH 1941) and since our experimental work shows that one of the common insect-eating bats *(Tadarida condylura)* can be infected, we feel that these bats may be potential reservoir hosts of pathogenic trypanosomes (subgenus *Trypanozoon*) in endemic areas of Africa where insect-eating bats and tsetse flies are abundant. The course of infection in the insect-eating bats infected with blood and metacyclic stages of *Trypanosoma rhodesience* and *T. brucei* was much more chronic than that in mice. Although insect-eating bats had not been in the past considered as potential reservoir hosts in Africa, the vampire bat of South and Central America has been recognized to be an important vector and reservoir host for *T. (Trypanozoon) evansi* (HOARE 1965).

We did examine a very small number of insect-eating bats from endemic trypanosomiasis areas (3 from Lugala, Uganda; 2 from Aitong, Kenya, and 34 from Serengeti, Tanzania); however, none of them were infected with the pathogenic trypanosomes. Perhaps a more extensive survey in these or other regions may prove to be more rewarding.

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