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Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **33 (1976)**

Heft 4

PDF erstellt am: **08.08.2024**

Persistenter Link: https://doi.org/10.5169/seals-312239

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Salivary Secretion in Wild *Glossina pallidipes* Austen. (Diptera, Glossinidae)*

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Abstract

The salivation behaviour of wild *G. pallidipes* obtained from Lambwe Valley and Kbwezi was studied. Salivation was measured by counting the number of salivary drops secreted per minute and measuring the sizes of the stained saliva after drawing them with a camera lucida.

The results confirmed observations obtained from laboratory bred flies. The quantity of saliva secreted by tsetse flies was significantly increased as the flies became hungrier. The proportion of flies salivating also increased with intensity of hunger.

Female *G. pallidipes* secreted significantly more saliva than the males.

There was no significant difference between the quantities of saliva secreted by infected and free tsetse flies; thus there is no evidence to support the suggestion that the presence of trypanosomes in the tsetse fly stimulated it to salivate copiously.

Flies having trypanosome infection in their salivary glands discharge large numbers of mature parasites in their saliva. Flies with trypanosomes in their proboscis discharged relatively few mature and immature parasites in their saliva.

Introduction

The diseases, trypanosomiasis and nagana have continued to challenge and constitute a serious threat to human life and livestock farming in tropical Africa. A recent survey by a research team from the Tsetse Fly Salivary Gland Project of the International Centre of Insect Physiology and Ecology, Nairobi, Kenya (ICIPE) recorded 10 cases of human sleeping sickness and several cases of the cattle disease in the Lambwe Valley area of Kenya. In spite of considerable research into the biology of the vectors, *Glossina* spp., there seems to be important gaps in our knowledge of the biology of these dipteran vectors of the diseases. For instance it was only in 1973 that the actual process of salivation by tsetse flies was studied in some detail at the ICIPE Research Centre, Nairobi, Kenya (YOUDEOWEI 1973, 1974, 1975). Salivation by the tsetse fly is important for the transmission of trypanosome parasites from an infected fly into the vertebrate host, but the actual mechanism of transmission of the infective trypanosomes has remained obscure. Work with laboratory bred *G. austeni, G. morsitans* and *G. pallidipes* showed that the quantities of saliva secreted by those flies increased with the intensity of hunger. An important finding was that the first salivary drop

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* ICIPE Publication No. 42.

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was 2.5 times the size of subsequent drops, indicating that even though an infected fly may not be fully engorged with blood when it pierces a host, the very first salivation would likely transfer a large quantity of trypanosomes into the host tissue. (For details see YOUDEOWEI 1974, 1975.) It is not certain whether the observations recorded with laboratory bred flies would apply to wild flies collected directly from the field. It was also not possible to study the salivation behaviour of infected flies due to the laborious process of obtaining enough laboratory bred flies infected with trypanosomes. The work reported in this paper therefore concentrated on a study of the salivation behaviour of wild flies and compared the results with those obtained for laboratory bred flies. Attention has been focussed mainly on *Glossina pallidipes* which is an important vector of trypanosomiasis in Kenya.

Materials and Methods

1. The Study Area

The tsetse flies used for this work were *Glossina pallidipes* collected from the Lambwe Valley near Homa Bay in the South Nyanza district of Kenya. This is an area on the North Western part of Kenya close to the shores of lake Victoria. The vegetation in the valley was mainly grassland with wooded thickets where the tsetse flies live. Observations were also made on specimens of the same species of tsetse at the Kibwezi forest about 200 kilometers South of Nairobi in the Machakos district of Kenya.

2. Trapping of Flies

In the Lambwe Valley where the fly population was high, two Langridge traps were set up close to a wooded thicket to collect the flies. The fly population at Kibwezi forest was not as high and so flies were caught by the "fly-round" hand net methods. When caught, the flies were sexed and kept individually in plastic tubes (4.2 cm x 3.2 cm) with nylon gauze at the two ends. They were then taken to the laboratory where observations were made.

3. Collection of Saliva

Saliva was collected on clean microscope slides from each fly by means of the batwing membrane technique (YOUDEOWEI, 1975a). The saliva collected on the clean microscope slides was fixed for 1 minute in methanol and stained for 40 minutes with Giemsa's stain and then air dried.

In some slides the number of salivary drops secreted per minute was counted and recorded. The salivary drops were then drawn with a camera lucida to a magnification of x 100 and the area of each drop measured with a planimeter and recorded.

All slides were then examined under oil immersion magnification for the presence of trypanosome parasites. If present the number of parasites in the saliva was counted.

Results

1. Salivation in Relation to Hunger

The effect of hunger stage on the amount of saliva produced by female *G. pallidipes* was studied. The stage of hunger was determined

by a simple method after NASH (1969). Three hunger stages were recognised as follows:

Stage 1. Starved; with the abdomen empty and curved ventrally.
Stage 2. Intermediate; with abdomen not distended and not empty.
Stage 3. Gorged; with abdomen distended and full of ingested blood.

In all the samples of flies collected at Lambwe only hunger stages 1 and 2 were seen. For each hunger stage, 50 salivary drops were measured from flies and the data are presented in table 1. The figures presented in tables 1, 2 and 3 are relative and not absolute estimates of the quantities of saliva produced. The difficulty in obtaining absolute estimates of the quantities of saliva secreted by tsetse flies have been fully discussed elsewhere (YOUDEOWEI 1975b).

There was a highly significant difference between the relative quantities of saliva secreted by the flies at the two levels of hunger (table 1). The hungrier flies secreted the greater quantity of saliva. This confirms the results obtained with laboratory bred flies where YOUDEOWEI, 1975b showed that the quantity of saliva secreted by tsetse flies increased with the intensity of hunger.

Table 1. The effect of hunger stage on salivation in wild female *G. pallidipes*

Hunger stage	Mean ± S.E. area of salivary drop	Difference	t	No. of drops
1	25.79 ± 1.96 cm²			50
		6.67	2.83	
2	19.03 ± 1.33 cm²		$p > 0.01$	50

The proportions of males and females salivating at each hunger stage were also compared. The results show that whereas 22.9% (males) and 32.4% (females) of the hungrier flies did not salivate, 50% (females) and 66% (males) of the less hungry flies did not salivate. This is further evidence to demonstrate that the hungrier the fly the more readily it secretes saliva.

2. *Salivation in Male and Female* G. pallidipes

The number of salivary drops secreted per minute and the sizes of the salivary drops secreted by male and female flies at Lambwe Valley were compared. The results are presented in table 2 for flies at hunger stage 1.

There was no significant difference between the number of salivary drops secreted per minute by males and females (table 2) but there was a highly significant difference between the relative sizes of the

Table 2. The number of salivary drops per minute and the sizes of salivary drops secreted by wild male and female *G. pallidipes* at Lambwe

Sex	Mean ± S.E. No salivary drops/min.	Difference	t	No of flies tested
Males	7.9 ± 0.63	0.59	0.168 NS	87
Females	7.32 ± 0.78			71
Sex	**Mean ± S.E. area**	**Difference**	**t**	**No drops measured**
Males	19.4 ± 1.14 cm²	4.8	2.69	100
Females	24.2 ± 1.42 cm²		$p > 0.01$	100

salivary drops secreted by the sexes: the females, which are much larger in size, secreted larger salivary drops than the males (table 2).

3. Salivation in Infected Flies

The salivation behaviour of flies caught at Lambwe Valley and at Kibwezi forest was studied: particular attention being paid to those flies in which trypanosomes were detected. As it was impossible to identify an infected fly visually before it was dissected, detailed observations were made on each accurately labelled fly before it was dissected for its proboscis, salivary glands and entire gut to be examined for the presence of trypanosome parasites. The species of trypanosomes were identified on the basis of their morphology and location in the vector. Because of the natural occurrence of a very low infection rate the samples of infected flies examined was small. Out of a total of 212 flies examined at Lambwe, 9 (4.2%) were found infected with *Trypanosoma vivax, Trypanosoma congolense,* or *Trypanosoma brucei.* Only 1 specimen (0.5%) had salivary gland infection with *T. brucei.* At Kibwezi forest 229 flies were examined and 4 (1.75%) were found infected with trypanosomes; there was no salivary gland infection.

All these infected flies did not seem to secrete more saliva than the uninfected flies; thus it was not possible to identify an infected fly by the relative quantities of saliva it produced. A comparison of the sizes of the salivary drops secreted by infected and uninfected flies is given in table 3.

It is clear from this table that there was no significant difference between the sizes of the salivary drops secreted by uninfected and infected flies. There is therefore no experimental evidence to support the

Table 3. Comparison of the sizes of salivary drops secreted by uninfected and infected wild *G. pallidipes.* Data from Lambwe and Kibwezi pooled

State of flies	Mean ± S.E. area of salivary drop	Difference	t	No of drops measured
Infected	20.18 ± 2.9 cm^2			25
		1.49	0.38 NS	
Uninfected	21.67 ± 2.7 cm^2			25

NS = Not significant.

suggestion that the presence of trypanosome parasites in the tsetse stimulates it to salivate.

Counts of the numbers and form of trypanosomes in the saliva showed that in a salivary gland infection the saliva was teeming with mature infective forms of *T. brucei* (table 4). In the case of proboscis infections however, the saliva contained relatively few trypanosomes, most of them being immature, epimastigote forms. Although the pro-

Table 4. Distribution of Trypanosome parasites in the saliva of wild *G. pallidipes.* Data from Lambwe Valley and Kibwezi forest, Kenya

Slide	Site of infection			No of	Species of
	Proboscis	Gut	Salivary gland	parasites	trypanosomes
Lambwe					
493	+	–	–	12	*T. congolense*
577	+	+	+	241	*T. brucei*
528	+	–	–	17	*T. congolense*
581	+	–	–	2	*T. congolense*
785	+	+	–	20	*T. congolense*
862	+	–	–	0	*T. vivax*
867	+	–	–	9	*T. vivax*
892	+	–	–	6	*T. congolense*
917	+	–	–	43	*T. congolense*
Kibwezi					
98	+	–	–	15	*T. vivax*
100	+	–	–	8	*T. vivax*
128	+	–	–	27	*T. congolense*
488	+	–	–	0	*T. vivax*

\+ Denotes parasites present.
– Denotes parasites absent.

boscis was heavily packed with trypanosomes very few of them seem to have been discharged in the saliva.

Discussion

The results obtained with the wild flies have confirmed the significance of the physiological state of hunger in the salivary secretion of tsetse flies. In both laboratory bred and wild flies salivation was increased as the flies became hungrier; females tending to secrete more saliva than the males probably due to the larger size of their salivary glands. The proportion of flies salivating as well as the quantity of saliva produced increased with the intensity of hunger. It can thus be concluded that hunger is an important physiological state in the tsetse fly which favours the deposition of trypanosome parasites from an infected fly into the vertebrate host.

The distribution of trypanosomes in the saliva of infected flies raises the important question of the actual mechanism of transmission of these parasites from the infected fly into the host tissues. In the case of *T. brucei* infecting the salivary glands, numerous mature infective parasites are ejected with the saliva and this is likely to be the major mode of transmission. In the case of *T. congolense* where infection is only in the proboscis, very few parasites are ejected with the saliva; some of these being immature and therefore not infective. It is likely that only the trypanosomes which find their way into the hypopharynx are ejected with the saliva and there must be an alternative route through which trypanosomes which mature in the proboscis are transmitted into the host during feeding. This whole issue will be fully discussed in another paper (OTIENO & YOUDEOWEI, in preparation).

It is reasonable to suggest that the presence of trypanosomes in the fly, particularly in the salivary glands, would stimulate the infected fly to salivate copiously. This would be a natural adaptive mechanism to ensure the successful completion of the life cycle of the parasite in its vertebrate host. There was, however, no experimental evidence to support this suggestion; there was no significant difference between the sizes of salivary drops secreted by infected and uninfected flies.

Acknowledgements

I am grateful to Professor Thomas R. Odhiambo, Director, ICIPE Research Centre for his comments on the manuscript and for permission to publish this paper. I also thank the authorities of the University of Ibadan for granting me permission to travel to Kenya for research. This work was financially supported by the UNDP, the Rockefeller Foundation, New York and the International Atomic Energy Agency (IAEA) Vienna to whom I am greatly indebted.

The technical assistance of Miss Nafisa Darji and Philip Onyango is gratefully acknowledged.

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