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Autor: Otieno, L.H. / Darji, N. / Onyango, P.

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The International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772, Nairobi, Kenya

Some observations on factors associated with the development of *Trypanosoma brucei brucei* infections in *Glossina morsitans morsitans*

L. H. Otieno, N. Darji, P. Onyango, E. Mpanga

Summary

The susceptibility of *Glossina morsitans morsitans* to *Trypanosoma brucei brucei* infection was shown to be age-dependent during the first 12 h: the youngest age group (1–8 h after emergence) being more susceptible than the older ones. The susceptibility was enhanced by cooling the young flies to a temperature of 0–5 ° C for 30 min. Male flies were found to be more susceptible than females. The number of trypanosomes ingested did not influence the subsequent salivary-gland infection rates observed in *G.m. morsitans;* however, there was a relationship between the number ingested and subsequent *T.b. brucei* midgut infections in the flies.

Key words: *Glossina morsitans morsitans; Trypanosoma brucei brucei;* infection rates; susceptibility; age-dependency.

Introduction

Mature infections due to *Trypanosoma (Trypanozoon) brucei brucei* trypanosomes are very rarely found in nature. For example, in an adequate sample of wild flies it is rare to find more than 0.1 or 0.2% carrying salivary-gland infections, although 20 or 30% of the wild game from which they derive their main food supply may be proved to be carrying trypanosomes (Duke, 1933a). Extensive investigations have therefore been carried out to determine factors responsible for the low infections in the tsetse (see reviews by Buxton, 1955; Jordan, 1974, 1976; Molyneux, 1977, 1980).

Correspondence: Dr. L. H. Otieno, The International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772, Nairobi, Kenya

In the present investigations, data is presented showing that tsetse environmental temperature at the time of infected bloodmeal, the number of trypanosomes ingested, and the sex of the fly are all important factors associated with infective development of *T.b. brucei* in the tsetse.

Materials and Methods

Strain of trypanosome

Trypanosoma brucei brucei, of a stock derived from EATRO 1969, was used in these experiments. This stock was originally isolated from *Crocuta crocuta* (hyaena) blood at Serengeti National Park, Tanzania in 1971 by Professor R. Geigy. It was first stabilated as EATRO 1857 after a short maintenance by serial passages in several mice and rats. This strain was tested for infectivity to man by inoculation of one human volunteer, who showed no signs of trypanosome infection. On the basis of this result, it has been classified as *T. brucei brucei.*

Target tsetse species

The tsetse species used for these experiments, *Glossina morsitans morsitans*, were obtained from the insectary of the International Centre of Insect Physiology and Ecology (ICIPE). This colony originated in 1968 with flies from the Tsetse Research Laboratory at the University of Bristol, Langford, England, which in turn originated from Zimbabwe (then known as Southern Rhodesia). Unless otherwise stated, male flies were used in these experiments.

Experimental animals

Random bred male rats of Wistar strain (160–200 g), and New Zealand white rabbits (2–2.5 kg) obtained from the ICIPE animal house, were used.

Temperature of maintenance after infective feed

Otieno (unpublished data, 1979) found that cooling newly-emerged *G.m. morsitans* to 0.5° C for 30 min delayed crop emptying for up to 6 h; control flies kept at 25° C emptied their crops within 2 h. It was therefore interesting to see if the short cold exposure influenced infection rates. Emerging *G.m. morsitans* were collected in polyvinyl (PVC) glue cages at one or two hourly intervals. The various groups of young flies collected were exposed to an infective blood meal 22–24, 20–22, 18–20, 16–18, 14–16, 12–14, 7–8, 6–7, 5–6, 4–5, 3–4, 2–3, and 1–2 h after emergence.

As soon as these flies had fed, one half of each age-group was transferred to a refrigerator at 0.5° C (for 30 min), and the other half kept at 25° C and 80% r.h. After the short cold exposure, all the flies were transferred to the insectary at a temperature of 25° C and maintained on two clean rabbits. The flies were dissected and examined for the presence of trypanosome infection 30 days after the infective bloodmeal. G test (Sokal and Rohlf, 1969) was used to analyse the figures.

Sex of fly

24–36 h old male and female flies were fed on heavily parasitaemic rats and soon after engorgement they were separated into three groups: one was kept at 5° C, another at 25° C and the last group at 29° C (each for 90 min). After the short exposure to the respective temperature treatments, all the three groups were brought to the insectary maintained at 25° C and 80% relative humidity. The flies were examined for the presence of trypanosome infection 30 days after the infected blood meal.

Infective dose

The trypanosomes were separated from rat blood by means of DEAE cellulose (Whatman DE-52) anion-exchanger method according to Lanham and Godfrey (1970). The eluated trypano-

Table 1. *T. brucei* infection rates in young *G.m. morsitans*. Groups of newly emerged flies were fed on parasitaemic rat at hourly intervals for 8 h and thereafter fed at two-hourly intervals up to 24 h after emergence. After engorgement the flies were divided into two groups: one group was cooled at 5° C for 30 min. The other group was kept at room temperature as controls.

Time after emergence (h)	Cooled flies				Control flies			
	No.	Gut infections	Salivary gland infections	Salivary gland infections %	Exam.	Gut infec-	Salivary gland infections	Salivary gland infections %
22–24	15	3	1	6.6	15	1	1	6.6
20–22	13	4	2	15.4	15	1	1	6.6
18–20	15	4	2	13.3	14	4	1	7.1
16–18	19	7	5	26.3	14	3	1	7.1
14–16	29	4	3	10.3	23	5	3	13.0
12–14	2	–	–	–	3	–	–	–
Total	93	22	13	14.0	84	14	7	9.3
7– 8	11	7	5	45.4	10	3	2	20.0
6– 7	16	7	6	37.5	15	8	2	13.3
5– 6	10	5	1	10.0	11	3	2	18.2
4– 5	17	11	5	29.4	16	4	2	12.5
3– 4	20	5	5	25.0	16	7	3	18.8
2– 3	13	4	2	15.4	12	–	–	–
1– 2	16	3	2	12.5	19	3	2	10.5
Total	103	42	26	25.2	99	28	13	13.1

somes were washed twice by centrifugation in chilled PSG and suspended in 0.1 ml of the solution. The final concentration of trypanosomes was adjusted to 10^6 trypanosomes/ml. The suspension was then serially diluted to 10^6, 10^4, 10^3, 10^2 and 10 trypanosomes/ml. The dilutions were separately mixed with 3 ml of defibrinated rat blood and stirred well. The suspended trypanosomes were used to feed newly emerged (within 24 h of emergence) *G.m. morsitans* through membrane. The engorged flies were maintained on clean rabbits for 30 days, after which they were killed and examined for the presence of trypanosomes. Chi-square test was used to analyse these figures.

Results

Age of tsetse at infected bloodmeal

Table 1 summarizes the results obtained when young *G.m. morsitans* were cooled soon after ingesting blood infected with *T.b. brucei*. The Table shows that younger flies (1–8 h old) developed higher incidence of salivary-gland infections as compared to flies fed between 12–24 h after emergence. This was true both for control and flies cooled to 0–5° C for 30 min. The difference in infection

Table 2. The relationship between maintenance temperature after infective feed, sex of the fly and infection rates of *T.b. brucei* in *G.m. morsitans*. The flies were exposed to infected bloodmeal at 24–36 h after emergence and maintained at temperature shown below for 90 min.

Maintenance temperature	Sex of tsetse	No. of flies		
		exposed to infection	infected	%
5° C	♂	143	32	22.4
	♀	146	15	10.3
	Total	289	47	16.3
25° C	♂	134	36	26.9
	♀	128	7	5.5
	Total	262	43	16.4
29° C	♂	111	20	18.0
	♀	142	17	12.0
	Total	253	37	14.6

Table 3. *T.b. brucei* infections in *G.m. morsitans* after young (24-h old) flies were exposed to various concentrations of trypanosomes and maintained throughout at room (25° C) temperature. The table compares midgut and salivary gland infections observed.

	No. trypanosomes/ml of suspension				
	10	10^2	10^3	10^4	10^6
Flies examined	69	68	63	66	41
Gut infections	23.2%	25.0%	15.9%	19.7%	41.5%
Salivary gland infections	8.7%	5.9%	9.5%	10.6%	12.2%

rates between the two groups of flies are statistically significant ($P < 0.05$). It was particularly interesting to note that flies fed between 6–8 h after emergence showed the highest infection rates (up to 45.4%).

The cooling effect on infection rates was more noticeable on younger flies (25.2%) than older flies (14.0%). This difference is also statistically significant ($P < 0.05$). It is clear therefore that the effect of cooling did not influence infection rates in flies fed 12 or more hours after emergence.

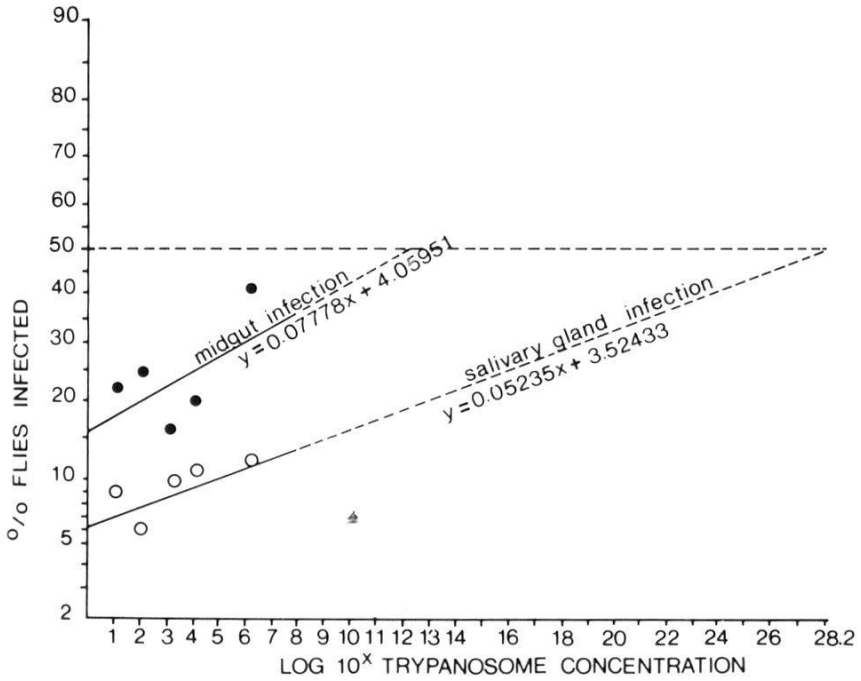

Fig. 1. Titration of the minimum infective dose of *T.b. brucei* to *G.m. morsitans*. The percentage number of flies responding to infection is plotted against $\log_{10}$ trypanosome concentration used.

Sex, age and temperature at infected bloodmeal (Table 2)

When flies subjected to 5° C, 25° C and 29° C temperature treatments were examined for the presence of trypanosomes in their salivary glands, the following results were obtained: 16.3%, 16.4% and 14.6%, respectively. These results compare well with 14.0% observed (Table 1) for flies infected at 12–14 h after emergence. The results confirm that the temperature of maintenance of *G.m. morsitans* infected after 24 h of emergence is not crucial as far as the ensuing infection is concerned. However, the experiments showed clearly that male *G.m. morsitans* were more susceptible to *T.b. brucei* infection than female flies. The differences were statistically highly significant ($P < 0.001$).

Minimum infective dose

Attempts to determine the least number of trypanosome organism infective to *G.m. morsitans* revealed that the flies fed on dilutions containing as low as 3.3 trypanosome/ml were as readily infected (8.7%) as those (12.2) exposed to higher concentrations of trypanosomes (Table 3). In general, no statistically significant differences were observed in infection rates among the flies fed on the various trypanosome concentrations. It was, however, important to note that the flies which were exposed to the highest trypanosome concentrations showed the highest incidence of immature (midgut) infections. Percentage of gut and salivary gland infections were plotted against trypanosome concentration on a probit/logarithmic scale (Fig. 1). There is no heterogenous deviation from expectation for the curves ($\chi^2_{df=3} = 6.69$) for gut infection; ($\chi^2_{df=3} =$

0.847) for the salivary gland infection. The curves show clearly that, it is virtually impossible to get 50% salivary gland infection (10^{28} trypanosomes/ml). It may be interesting to consider these curves as models to predict *T.b. brucei* infection rates in *G.m. morsitans.*

Discussion

In the experiments reported in the present study, it was the intention to see if the susceptibility to infection in the case of *G.m. morsitans* was related to any particular age group before the infective bloodmeal. It may be noteworthy to mention that Gingrich et al. (1982) have recently shown that mature male *G.m. morsitans* fasted for four days before infected bloodmeal developed infection rates comparable to tenerally infected flies. Nevertheless, the data presented have confirmed earlier observations (Van Hoof et al. 1937; Wijers, 1958) regarding the general role of the fly age at the time of taking an infecting feed. The critical factor was not just a first bloodmeal but how young they were before the first bloodmeal was offered. The most susceptible age appears to be somewhere around 6–8 h after emergence, when up to 45.4% infection rate was achieved. The difficulty in handling flies of this age made it difficult to carry out more observations with flies of the very young age group. Besides, flies less than 8 h old ingest very little blood, as such great care is needed to ensure that such flies have actually fed. It is noteworthy that Jenni (1977), by modifying the standard procedures for infecting and maintaining infected flies, similarly obtained very high (44%) salivary-gland infections among 91 *G.m. morsitans* infected with a population derived from a cloned-metacyclic *T.b. brucei.* He attributed the high salivary-gland infections partly to the slow rate of crop emptying of engorged flies in response to a drop in temperature.

Duke (1933b) examined his earlier records of *T.b. gambiense* and *T.b. rhodesiense* transmission by *G.p. palpalis* and found that there was no significant difference between the two sexes. However, when the data were based on infection rates on all flies showing developmental forms in the midgut and the proventricular region, he found significantly higher infection rates in the female flies. Burtt (1946), on the other hand, found a higher proportion of male *G.m. morsitans* became infected with *T.b. rhodesiense* than female flies. He found that, in every case, the number of females which survived to be examined was significantly less than that of male flies. He was therefore led to believe that the high mortality rate in the female flies was primarily responsible for the disparity found in the infection rate in the two sexes. Van Hoof (1947), carrying out transmission experiments with *T.b. gambiense* using *G.p. palpalis*, found greater frequency of salivary-gland infections in male than in female flies. On the other hand, Fairbairn and Culwick (1950) found that the effect of temperature differed with sex of the fly; therefore each sex must be considered separately. In male flies, there was a very significant positive regression of infection rate

on pupal temperature; but the infection rate was not influenced by the temperature to which adult flies were exposed. In female flies, they found that the infection rate was governed by a combination of pupal temperature and the temperature of fly maintenance. Baker and Robertson (1957) found no significant differences between infection rates in male and female *G.m. morsitans* maintained under identical laboratory conditions. In the experiments reported here, it has been shown very clearly that male *G.m. morsitans* were far more susceptible to the stock of *T.b. brucei* used than females. This observation confirms Burtt's findings, but contrasts with those of Fairbairn and Culwick (1950) and Ford and Leggate (1961) who laid a lot of emphasis on temperature. Our findings did not show any relationship between temperature and the sex of fly as far as infection rates were concerned.

The reasons for higher incidence of *T.b. brucei* infections in males are not clear. It is possible that female flies digest their bloodmeal faster than males, thereby subjecting the ingested trypanosomes to the unfavourable environment created by the release of proteolytic enzymes (Vundla and Otieno, in preparation). It may be important to refer to Maudlin's (1982) recent findings that susceptibility to *T. congolense* infection in *G.m. morsitans* was clearly dependent on maternal phenotype. This is the first instance, to our knowledge, when it has been shown experimentally that susceptibility of tsetse to trypanosome infection is genetically controlled.

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