International Centre of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya

A quantification of the risk of trypanosomiasis infection to cattle on the south Kenya coast

W. F. SNOW, S. A. TARIMO

Summary

The number of trypanosome-infected bites received by cattle grazed around an 8 ha area of forest harbouring a semi-isolated population of *Glossina pallidipes* Austen was estimated. The absolute size of the tsetse population was determined by mark-release-recapture techniques, the tsetse host range by the identification of blood-meals, and trypanosome infection rates by dissection of samples of tsetse. Feeding frequency was estimated and the number of cattle present was known. It was estimated that each cow received, from *G. pallidipes*, one infective inoculum of *T. congolense* every 5.8 days during the first experiment and 5.0 days in the second. For *T. vivax* results were 3.2 and 79.1 days, respectively.

Key words: tsetse; *Glossina pallidipes;* Kenya; population size; feeding patterns; trypanosome infection rate; epidemiology; "challenge"; mark-release-recapture techniques.

Introduction

The recent analysis by Rogers (1980) of the current state of knowledge concerning the risk of trypanosomiasis infection to livestock or "challenge", has provided both a lucid up-to-date summary and a stimulus to further consideration of a major aspect of the epidemiology of trypanosomiasis. Nevertheless, the definition by Smith and Rennison (1958) of "trypanosome challenge" as the number of infective bites from tsetse which a host receives in a unit of time remains the most useful summary of the concept. This topic is vital as a basis for the assessment of development potential in tsetse infected areas, the planning of

control strategies and the formulation of an epidemiological model of trypano-somiasis transmission.

The investigations reported below, involved the simultaneous application of several established techniques to estimate the number of infective bites received each day, from tsetse, by a small herd of cattle. It is suggested that this methodology can be used to define "challenge" in association with semi-isolated tsetse populations.

Materials and Methods

1. Description of the study site

The forest on Weni Maruma Farm, Diani 25 km south of Mombasa covered about 8 ha on the edge of the valley of a small river. To the north, west and south it was bounded by cashew and kapok plantations and to the east by an area of *Lantana camara* thicket, separating the forest from the main road south of Mombasa. Originally an area of lowland rain forest, few large trees remained but a high thicketed understorey was present and the forest edge was demarcated by dense *Lantana* thicket. Cattle, sheep and goats were grazed around the forest. Bushpig *(Potamochoerus porcus)* were common with occasional warthog *(Phacochoerus aethiopicus)*, bushbuck *(Tragelaphus scrip-tus)* and smaller antelope. A medium density population of *G. pallidipes* was present, with small numbers of *G. brevipalpis* Newstead and *G. austeni* Newstead. Although tsetse were widely distributed over the 400 ha farm the forest was considered to be the main focus of infestation.

The cattle herd maintained on the farm was intended for dairy production but has shown a steady decline which can be mainly attributed to intense challenge from trypanosomiasis. The herd declined from 110 animals in late 1977 to 46 in July 1980 and 37 in January 1981.

Two separate experiments were performed: one in July–August 1980 and the second during January–February 1981. Tsetse were sampled using pale blue biconical traps placed at roughly 50 m intervals along the north, west and south-west edges of the forest, where earlier surveys had caught most tsetse. During the two experiments 11 traps were used. Mean daily temperatures were 24° C in July–August 1980 and 28° C in January–February 1981.

From 1978 until 1981, a number of other surveys of *G. pallidipes* were made at Diani, which were not connected with the present study. Some references are made below to the data from these surveys.

2. Mark-release-recapture to estimate tsetse population size

Traps were set up on the morning of the first day. Tsetse were marked on the afternoon of day one, the morning and afternoon of the second day and the morning of the third day. Marking was with white cellulose paint mixed with coloured fluorescent powder, thinned with acetone and applied with a fine wire loop. Red, Orange, Yellow and Blue marks were used. After marking the tsetse were fed on a clean goat (1980) or rabbit (1981) and released inside the forest. The flies were then trapped continuously for 18 (1980) and 21 (1981) days and the traps emptied each morning. In effect two mark-release trials (afternoon day 1 + morning day 2 and afternoon day 2 + morning day 3) were running in parallel and pooled recaptures for the same days following each release were used as basis for the estimates of population size.

The analysis of the results of the mark-release-recapture trials follows the method suggested by Begon (1979) for single releases and multiple recaptures based on Jackson's (1937) "positive" method. Data for the first 7 or 8 days was used, as this included the period of most recaptures. After this the small number of recaptures introduced a large sampling error.

3. Tsetse feeding patterns

Smears of the gut contents of recently blood-fed flies were made onto filter paper. These were submitted for identification by the Haemagglutination Inhibition (by Imperial College, U.K.) or the
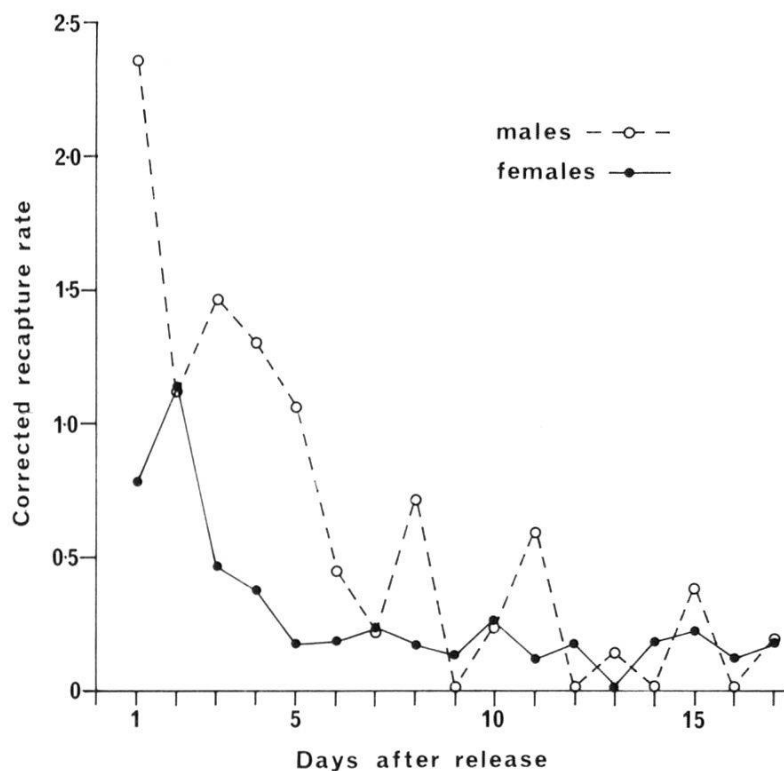
332

Fig. 1. Daily corrected recaptures for *G. pallidipes* marked and released at Weni Maruma Farm, Diani in July 1980.

Complement Fixation (by the Robert von Ostertag Institut, Berlin) tests to determine the host-range of *G. pallidipes* at Diani.

4. Trypanosome infection rates

Trypanosome infection rates were determined by dissection of samples of tsetse and their identity inferred from the location of trypanosomes in the fly. Thus, infections of the proboscis were assigned to *T. vivax;* gut and proboscis infections to *T. congolense* group; and proboscis, gut and salivary gland infections to *T. brucei*. Mixed *T. vivax / T. congolense* infections were identified only as *T. congolense* by this system.

Results

1. Mark-release-recapture

Recaptures of males declined rapidly after release, but although the numbers of females declined initially, a significant number of marked individuals were still taken towards the end of each experiment. This indicated that the tsetse population was indeed a semi-isolated one and that the area of dispersal was limited. The initial decline in numbers of marked flies may have represented the dispersal of tsetse from the point of release, with the subsequent stabilization of the number of recaptures reflecting an even mixing of marked flies within the population once the limit of distribution of the subpopulation had been reached. The corrected recapture rates (Rogers, 1977), those for 1980 are presented in Fig. 1, show a series of peaks at irregular intervals following

333

Table 1. Estimates of the population size of *Glossina pallidipes* from mark-release-recapture trials at Weni Maruma Farm, Diani, south Kenya coast

	No. marked tsetse released	Percentage of marked tsetse recaptured	Estimated total population size (± 1 S.E.)
July–August 1980			
males	321	33.02	3386 (2442–4696)
females	761	26.15	7060 (4730–10547)
January–February 1981			
males	135	28.15	3003 (2407–3745)
females	409	29.10	6077 (4812–7681)

release. The mean interval between peaks was 3.75 days for both male and female *G. pallidipes* in 1980 and 3.2 days for males and 3.5 days for females in 1981.

The resulting estimates of the numbers of *G. pallidipes* at Diani are given in Table 1. It should be noted that the standard errors are calculated as logarithms (ln) and that this results in an asymmetrical range ± 1 standard error in Table 1 (Begon, 1979). Jackson's (1937) "positive" method allows for losses from a population, assuming that the mortality of marked is the same as for unmarked tsetse, and estimates gain rate.

2. Frequency of feeding

No direct measurements of the feeding frequency of *G. pallidipes* at Diani were made. However, the interval between peaks in the pattern of recaptures of marked flies (Fig. 1) suggested a feeding interval of perhaps four days during the first experiment and three during the second (cf. Rogers, 1977).

This estimate was supported by an indirect estimate of the feeding interval based on measurements of the length of the largest of the four ovarioles in the reproductive system of female *G. pallidipes* (W. F. Snow, unpublished data). These showed three frequency peaks of ovariole length in samples of trap-caught tsetse, suggesting three peaks of responsiveness to traps, and probably to hosts, during each interlarval period. From the formula of Glasgow (1963) an interlarval period of 11.6 days during July–August 1980 at a mean daily temperature of 24° C, and 8.8 days in January–February 1981 at 28° C was calculated. With three feeds per cycle, feeding frequency at Diani was estimated at once every 3.9 and 2.9 days during the two experiments. It has been assumed that the feeding intervals for males is the same as that of females at four and three days.

3. Feeding patterns

The results of the identification of blood-meals from *G. pallidipes* collected at Diani between October 1978 and January 1981 are summarized in Table 2.

Table 2. Identification of blood-meals from *Glossina pallidipes* at Weni Maruma Farm, Diani, south Kenya coast

Host	Percentage of total identified feeds by	
	males	females
Cattle	22.0	20.0
Unidentified bovid	29.3	20.0
Total bovid*	*61.4*	*49.0*
Bushpig	9.8	20.0
Warthog	2.4	6.7
Unidentified suid	14.6	15.6
Total suid*	*38.6*	*51.0*
Suid/Bovid mixed feeds	14.6	11.1
Suid/Sheep-goat mixed feed	–	2.2
Unidentified mammal	7.3	4.4
Total identified	41	45
% identified	89.1	83.3

* includes mixed feeds

The large number of unidentified bovid feeds was disappointing as they may have included both wild and domestic ruminants. However, since only cattle and a single sheep/goat meal were specifically identified, all bovid feeds (60% for male *G. pallidipes* and 50% for females) are considered as having come from cattle.

4. Trypanosome infection rates

The results of the infection-rate dissections are summarized in Table 3. A large proportion of blood-meals were taken from suids and the *"congolense"* type infections undoubtedly included an unknown proportion of *T. simiae*. *Trypanosoma brucei* was rare at Diani and was not encountered during the mark-release-recapture trials. No males were dissected during the first experiment and, for the estimation of "challenge", their infection rate was estimated on the assumption that they showed a similar ratio of infections to females as in the total of all dissections from this locality, i.e. for *congolense* $2.69/3.83 \times 3.16 = 2.22\%$ and for *vivax* $0.84/2.23 \times 6.65 = 2.50\%$ (see Table 3).

5. Transmission of trypanosome infections to cattle

The proportion of bites from infected tsetse which can give rise to trypanosome infections in mammalian hosts is a critical factor in evaluating "challenge". Harley and Wilson (1968) estimated that only 20% of feeds from *G. pallidipes* infected with *T. congolense* contained sufficient trypanosomes to infect a

Table 3. Trypanosome infection rates in *Glossina pallidipes* at Weni Maruma Farm, Diani, south Kenya coast

	Proboscis only (*vivax* type)	Gut + proboscis (*congolense* type)	Gut + proboscis + salivary gland (*brucei* type)	Total number examined
July–August 1980				
males	not examined			
females	6.65%	3.16%	0	316
January–February 1981				
males	0	0.46%	0	217
females	0.23%	3.39%	0	443
All samples 1980–1981				
males	0.84%	2.69%	0	595
females	2.23%	3.83%	0.05%	2063

cow, although their observations involved only 25 feeds from 7 tsetse. Otieno and Darji (1979) showed that trypanosomes were present in 92% of salivations by *G. pallidipes* infected with *T. congolense* although only 18% contained more than the 10^2 minimum infective dose for cattle suggested by Harley and Wilson (1968). Otieno and Darji (1979) found that 69% of salivations by *G. pallidipes* infected with *T. vivax* contained trypanosomes but fewer were extruded than for *T. congolense*. This may suggest that fewer *T. vivax* are required to infect a mammal. If infection threshold numbers one order of magnitude less than for *T. congolense*, i.e. 10^1, are assumed, this value was exceeded in 20.3% of the probes reported by Otieno and Darji (1979). Wilson et al. (1972) showed that 29% of the macerated probosces of *G. pallidipes* infected with *T. vivax* produced an infection when inoculated into cattle. The product of this value and the frequency with which trypanosomes are present during salivation (Otieno and Darji, 1979) is 0.20 (see also Rogers, 1979), although this value does not take into account the number of trypanosomes extruded, and under natural conditions a proportion of salivations would contain sub-threshold numbers of trypanosomes. This limited data does suggest that the proportion of feeds from infected *G. pallidipes* containing an infective inoculum of *T. vivax* may be of the same order as for tsetse infected with *T. congolense*. This proportion is taken as 0.20 in the estimation of "challenge".

6. Cattle census

The dairy herd on Weni Maruma Farm comprised 46 cattle in July 1980 and 37 in January 1981.

Table 4. Estimation of the number of bites from *Glossina pallidipes* containing an infective inoculum of *Trypanosoma congolense* received by cattle at Weni Maruma Farm, Diani, south Kenya coast

Estimation	July–August 1980		January–February 1981	
	males	females	males	females
Estimated tsetse population (Table 1)	3400	7100	3000	6100
Feeding frequency (days)	4	4	3	3
Est. no. of tsetse feeding each day	850	1775	1000	2033
% of feeds on cattle (Table 2)	60	50	60	50
Est. no. of tsetse feeding each day on cattle	510	887.5	600	1016.7
% Infection rate (Table 3)	2.22*	3.16	0.46	3.39
Est. no. of infected tsetse feeding each day on cattle	11.32	28.05	2.76	34.46
Est. proportion of infective feeds (Harley and Wilson 1968)	0.2	0.2	0.2	0.2
Est. no. of infective feeds each day on cattle	2.26	5.61	0.55	6.89
No. of cattle present	46	46	37	37
Est. no. of infective feeds each day on each cow	0.05	0.12	0.01	0.19
Males + females = Total No. of infective feeds per cow per day	0.17		0.20	
Interval between infective bites (days)	5.84		4.97	

* Estimate from all dissections during 1980–1981 – no males dissected during this trial

Estimation of "challenge"

The steps in the calculation of estimated numbers of infective bites from *G. pallidipes* containing *T. congolense* received by each cow in the herd at Diani are set out in Table 4. A simple multiplicative model is used as a basis for these estimates (cf. Rogers, 1979).

Taking the first column of Table 4 as an example for estimating "challenge" by this method, the mark-release-recapture trial had indicated that 3400 male *G. pallidipes* were present at Diani in July–August 1980 (Table 1). With a feeding interval of four days, 850 (3400/4) male *G. pallidipes* were feeding daily. Of these 60% were feeding on cattle (Table 2). Thus 510 (850 × 0.6) tsetse were feeding on cattle each day. An infection rate for *T. congolense* in male *G. pallidipes* was estimated at 2.22% (Table 3) giving a value of 11.32 (510 × 0.0222) infected tsetse feeding on cattle each day. If only 20% of bites from infected flies are infective, 2.26 (11.32 × 0.2) infective bites were received by cattle each day. During these observations, 46 cattle were present and it was estimated that each cow received 0.05 (2.26/46) infective bites per day. A complementary figure for the number of bites received from female *G. pallidipes* was also calculated and the sum of values for males and females gave the estimated total number of infective bites received per cow per day as 0.17.

The results of the estimation of infective feeds per cow are presented both as infective bites per day and its reciprocal which represents the interval, in days, between infective feeds. This latter expression of the estimate is perhaps an easier way of visualising a field situation. The interval between infective bites containing *T. congolense* was 5.8 days (1980) and 5.0 (1981) per cow for the two experiments. Using the same analysis, but appropriate values for infection rates, corresponding values of 3.2 and 79.1 days were obtained for *T. vivax*.

Discussion

Individually none of the methods employed in this study are original, but their simultaneous application to estimate risk of trypanosomiasis infection is novel. However, many aspects of the data input used in this method to estimate "challenge" could be improved.

Mark-release-recapture techniques, whilst offering the most reliable method of estimating the number of tsetse present, still present many problems in their application and interpretation (cf. Roff, 1973). A very small proportion of the total population was marked during the present trials and more accurate estimates could be obtained by more extensive marking and sampling, and the use of multiple mark methods. The feeding of the flies prior to release probably decreased the initial mortality of marked relative to unmarked flies which could have led to an underestimate of population size. However, a far more serious limitation of single mark-release methods with tsetse relates to the fluctuating availability of the flies in relation to the hunger/feeding cycle (Randolph and

338

Rogers, 1978; Rogers and Randolph, 1978). Tsetse are maximally responsive to traps when they are hungry, but become unresponsive and consequently rarely occur in the traps after feeding. This variation in availability of marked flies to the traps may have led to a major overestimate of population size, possibly by a factor as large as $\times 2$. However, it was not possible to make a valid correction for this source of error in the Diani study, particularly since tsetse were fed before release.

The estimates of challenge for *T. congolense* during the two experiments were very similar at 5.8 and 5.0 days. In contrast, the estimates for *T. vivax* of 3.2 and 79.1 days were very disparate, due to the difference in infection rate in tsetse. A series of observations over two years (S. A. Tarimo, unpublished data) indicated that infection rates in *G. pallidipes* at Diani varied between 2.3 and 8.8% for *T. congolense* and 0 and 6.7% for *T. vivax*. It was a matter of chance that similar *T. congolense* but low and high *T. vivax* infection rates were observed during the two experiments.

Previously, the closest approach to an absolute estimate of "challenge" in terms of the number of infective bites received by a host in unit time was briefly reported by Cawdery (1958, 1959). The number of *G. swynnertoni* and *G. pallidipes* feeding on one bullock, as representative of the animals in a herd, were counted and their trypanosome infection rates determined (Cawdery, 1958). From this data, "challenge" was estimated as 0.071 infected feeds per animal per day (comprising 0.038 by *G. swynnertoni* and 0.033 by *G. pallidipes*). Direct observation of the number of tsetse attacking livestock is rarely practical under field conditions where "challenge" estimates must be considered in relation to normal livestock management practices. All catches from livestock by humans are confused by the apparent repellency of man for some tsetse (Hargrove, 1976). This factor probably resulted in an under-estimate of attack rates by Cawdery (1958). In the study at Diani, indirect estimates of tsetse biting rates were derived from determination of tsetse population size and feeding frequency. In a later report (Cawdery, 1959) the estimate of challenge was used to predict the appearance of patent trypanosomiasis in a herd of untreated, sentinel cattle. Predicted and observed patterns were very similar although Cawdery (1959) assumed that all infected tsetse transmitted infective inocula of trypanosomes at each feed. From the discussion above this is definitely not the case and would have caused an overestimate of "challenge" in Cawdery's (1959) calculations.

The estimates of the frequency of infective bites to cattle on the farm at Diani seem realistic for a high "challenge" situation. It would be relatively simple to test these estimates of frequency of infective bites, and the methods used, by the introduction of clean, susceptible cattle and monitoring them for infection. Parallel veterinary input and knowledge of trypanosome infection rates in wild animals in the vicinity would give the basis on which to construct an epidemiological model of the situation at Diani.

Begon M.: Investigating animal abundance, 97 p. Edward Arnold, London 1979.

Cawdery M. J. H.: Estimation of trypanosome challenge, p. 18. EATRO Ann. Rep. No. 8, 1956–1957 (1958).

Cawdery M. J. H.: Estimation of challenge, p. 61–62. EATRO Ann. Rep. No. 9, 1957–1958 (1959).

Glasgow J. P.: The distribution and abundance of tsetse, 252 p. Pergamon Press, London 1963.

Hargrove J. W.: The effect of human presence on the behaviour of tsetse (*Glossina* spp.) (Diptera; Glossinidae) near a stationary ox. Bull. ent. Res. *66*, 173–178 (1976).

Harley J. M. B., Wilson A. J.: Comparison between *Glossina morsitans, G. pallidipes* and *G. fuscipes* as vectors of trypanosomes of the *Trypanosoma congolense* group: the proportions infected experimentally and the numbers of infective organisms extruded during feeding. Ann. trop. Med. Parasit. *62*, 178–187 (1968).

Jackson C. H. N.: Some new methods in the study of *Glossina morsitans*. Proc. zool. Soc. Lond. *1936*, 811–896 (1937).

Otieno L. H., Darji N.: The abundance of pathogenic African trypanosomes in the salivary secretions of wild *Glossina pallidipes*. Ann. trop. Med. Parasit. *73*, 583–588 (1979).

Randolph S. E., Rogers D. J.: Feeding cycles and flight activity in field populations of tsetse (Diptera: Glossinidae). Bull. ent. Res. *68*, 655–671 (1978).

Roff D. A.: On the accuracy of some mark-release-recapture estimators. Oecologia *12*, 15–34 (1973).

Rogers D. J.: Study of a natural population of *Glossina fuscipes fuscipes* Newstead and a model of fly movement. J. anim. Ecol. *46*, 281–307 (1977).

Rogers D. J.: Epizootiology: the tsetse-cattle interface. Report of the expert consultation on research on trypanosomiases. Appendix Vl p. 61–67. 1–5 October 1979. FAO, Rome, AGA-801 (1979).

Rogers D. J.: A manuscript incorporating a review of the present state of knowledge of trypanosome risk to cattle in tsetse infested areas and research approaches for more accurate estimation of risk. Unpublished report, non-staff traveller for FAO (1980).

Rogers D. J., Randolph S. E.: Metabolic strategies of male and female tsetse (Diptera: Glossinidae) in the field. Bull. ent. Res. *68*, 639–654 (1978).

Smith I. M., Rennison B. D.: Some factors concerned in trypanosome challenge, p. 63–66. Proc. 7th Meet. Int. Sci. Comm. Trypanosom. Res. (Brussels) (1958).

Snow W. F.: Tsetse ecology and epidemiology of trypanosomiasis in The Gambia and on the south Kenya coast: a comparison, p. 369–381. Proc. 16th Meet. Int. Sci. Comm. Trypanosom. Res. Cont. (Yaounde) (1979).

Wilson A. J., Dar F. K., Paris J.: A study of the transmission of salivarian trypanosomes isolated from wild tsetse flies. Trop. anim. Hlth Prod. *4*, 14–22 (1972).