Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 44 (1987)
Heft: 1

Artikel: Pathogenicity of "Crithidia fasciculata" in the haemocoele of "Glossina"
Autor: Ibrahim, E.A. / Molyneux, D.H.
DOI: https://doi.org/10.5169/seals-313814

Acta Tropica *44*, 13–22 (1987)

Department of Biological Sciences, University of Salford, Salford M5 4WT, UK

Pathogenicity of *Crithidia fasciculata* in the haemocoele of *Glossina*

E. A. Ibrahim, D. H. Molyneux

Summary

The mosquito flagellate *Crithidia fasciculata* produces intense haemocoelic infections following intrahaemocoelic inoculation into 5 species of *Glossina* – *G. austeni, G. fuscipes fuscipes, G. morsitans morsitans, G. palpalis gambiensis* and *G. tachinoides.* All *Glossina* inoculated with *C. fasciculata* died between days 4 and 9. Neither *Trypanosoma brucei* procyclics nor *Leishmania hertigi* promastigotes similarly inoculated into *Glossina* species, at the same dose, multiplied within the haemocoele and no deaths were recorded during the first 10 days post-injection. No mortalities amongst sham-injected controls occurred over the 10-day period.

Key words: *Crithidia fasciculata; Glossina;* tsetse flies; pathogenicity; *Trypanosoma brucei; Leishmania hertigi.*

Introduction

There has been much recent interest in the significance of haemocoelic infections of the subgenus *Trypanozoon* in *Glossina;* however, the role and significance of these infections remain to be fully determined. Recent reviews have summarized findings to date (see Molyneux, 1980, 1983; Evans and Ellis, 1983). Croft et al. (1982) and East et al. (1983) found that *Glossina* haemolymph possessed properties which inhibited motility in vitro of trypanosomes of all Salivaria subgenera whereas no similar effects were observed on epimastigotes of a bat trypanosome, *Trypanosoma dionisii,* promastigotes of *Leishmania hertigi* and choanomastigotes of the mosquito flagellate *Crithidia fasciculata.* Kaaya et al. (1986b) reported that *T. b. brucei* was found "to be pathogenic when

Correspondence: Dr. E. A. Ibrahim, Department of Biological Sciences, University of Salford, Salford M5 4WT, UK

inoculated intrahemocoelically". However in a later paper (Kaaya et al., 1986a) they state that following injection of live bloodstream forms of *T. b. brucei* into the haemocoele of *Glossina* a rapid and significant drop in trypanosome numbers occurred in the haemocoele; these results confirmed earlier observations (Croft et al., 1982; East et al., 1983). However, Poinar et al. (1979) and Kaaya et al. (1986b) reported that the bacteria *Serratia marcescens* and *Bacillus cereus* and *Escherichia coli* were pathogenic when inoculated intrahaemocoelically into *Glossina.*

Schmittner and McGhee (1970) observed a varied behaviour of 6 species of *Crithidia* when inoculated intrahaemocoelically into 3 different genera of insects (*Acheta, Drosophila* and *Tenebrio*); however, on some occasions *Crithidia* was highly pathogenic. Insect haemolymph is known to contain molecules capable of agglutinating trypanosomatid flagellates but the titres to different flagellates vary considerably (Ingram et al., 1983, 1984). Although no such trypanosome agglutinins have been detected in *Glossina* haemolymph (Ibrahim et al., 1984) inoculation of *Trypanosoma brucei* into the haemocoele of *Glossina* usually results in rapid clearance of flagellates and infections do not appear to establish readily in this site (Croft et al., 1982; Kaaya et al., 1986a) although there is evidence that certain stocks of *Trypanozoon* can produce infection (Otieno et al., 1976; Evans and Ellis, 1983; Kaaya et al., 1986b).

The non-pathogenic South American human trypanosome, *Trypanosoma rangeli,* however, is known to pass through the haemocoele into the salivary glands of *Rhodnius prolixus* and haemocoelic infections in this bug are known to be associated with pathogenicity (D'Alessandro, 1976; Ellis et al., 1980).

We report here the pathogenicity of *C. fasciculata* to *Glossina* following intrahaemocoelic infection in comparison with *T. brucei* and *L. hertigi.*

Materials and Methods

Tsetse flies were obtained as puparia from the Tsetse Research Laboratory, Langford, Bristol (*G. austeni* and *G. morsitans*) or from l'Institut d'Elevage et Médecine Vétérinaire des Pays Tropicaux (IEMVT), Maisons-Alfort, Paris (*G. palpalis, G. fuscipes* and *G. tachinoides*). On emergence from puparia adult flies were maintained at 25 °C and fed 4 times a week on the ears of rabbits. The history, origin and isolation of the stocks of *T. brucei, L. hertigi* and *C. fasciculata* are as described in Ibrahim et al. (1984).

T. brucei, L. hertigi and *C. fasciculata* were maintained as previously described (Croft et al., 1982; Ibrahim et al., 1984). They were inoculated respectively as procyclics, promastigotes and choanomastigotes into male and female non-teneral *Glossina.*

Each fly was inoculated under aseptic conditions with 1 μl of parasite suspension containing $3–5\times10^3$ parasites, prepared from Cunningham's medium (Cunningham, 1977) (*T. brucei*) or Locke's solution (NaCl, 9.00 g; KCl, 0.42 g; $CaCl_2$ $2H_2O$, 0.24 g; $NaHCO_3$, 0.20 g; glucose, 1.0 g dissolved in 1 litre of distilled water) (*L. hertigi* and *C. fasciculata*). Insects were anaesthetized with CO_2 swabbed with 70% ethanol and injected with a fine hand drawn glass needle through the intersegmental membrane between thorax and abdomen into the haemocoele. After injection flies were examined to ensure no haemolymph leaked from the site of inoculation. The presence of living parasites was monitored by microscopic examination of the haemolymph obtained by severing a leg or by body

washing (for dissected flies) and homogenates of salivary glands, gut and larvae. Sham-controls consisted of flies which were either uninoculated or pricked by inserting a needle.

To determine the effect of intrahaemocoelic inoculation of trypanosomatid flagellates on the survival of the host, groups of 5 *Glossina* species were each injected with the above dose of *T. brucei, L. hertigi* or *C. fasciculata* parasites. A further group of *G. morsitans* was given a dose of 100–200 parasites of *C. fasciculata.* Both experimental and control flies were kept for 30 days post-injection. The experimental and control flies were monitored twice a day for moribund or dead flies, their sexes recorded and their thorax and abdomen dissected in Locke's solution and examined microscopically for living parasites.

The numbers of *C. fasciculata* within the haemocoele of *Glossina* at various times post-injection were determined following inoculation of *G. morsitans, G. tachinoides* and *G. palpalis* with 1 μl of parasite suspension in Locke's solution containing $3–5\times10^3$ parasite per fly. 1 μl of haemolymph was taken from *C. fasciculata*-injected flies and diluted with Locke's solution. A drop of the suspension was placed on a slide and examined microscopically for the presence of living parasites. Samples were counted on a haemocytometer and the concentration of parasites was calculated. The average of 2 counts was taken for each fly and the mean of 10 flies was taken for each sampling time.

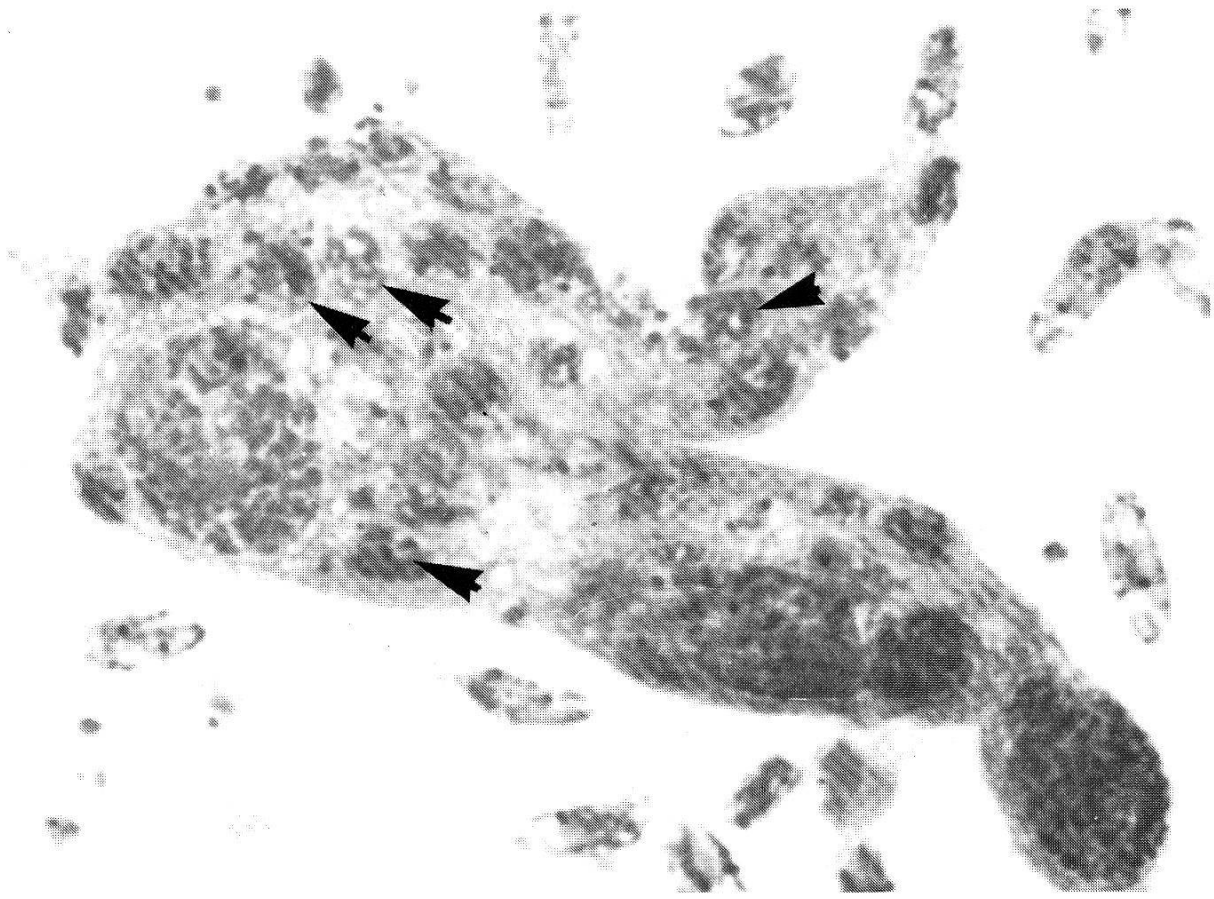
Results

Haemolymph and body washings were examined from 2880 *Glossina* previously inoculated with either *T. brucei, L. hertigi* or *C. fasciculata.* All *Glossina* species inoculated with *T. brucei* or *L. hertigi* cleared the inoculated parasites from the haemocoele between 24 and 72 h following injection. In contrast, none of the flies inoculated with *C. fasciculata* was able to do so. *C. fasciculata* survived and multiplied within the host haemocoele and from 6 to 8 days post-inoculation all flies showed intense infection of *C. fasciculata* (Figs. 1 and 2). All *Glossina* inoculated with $3–5\times10^3$ *C. fasciculata* died between days 4 and 9.

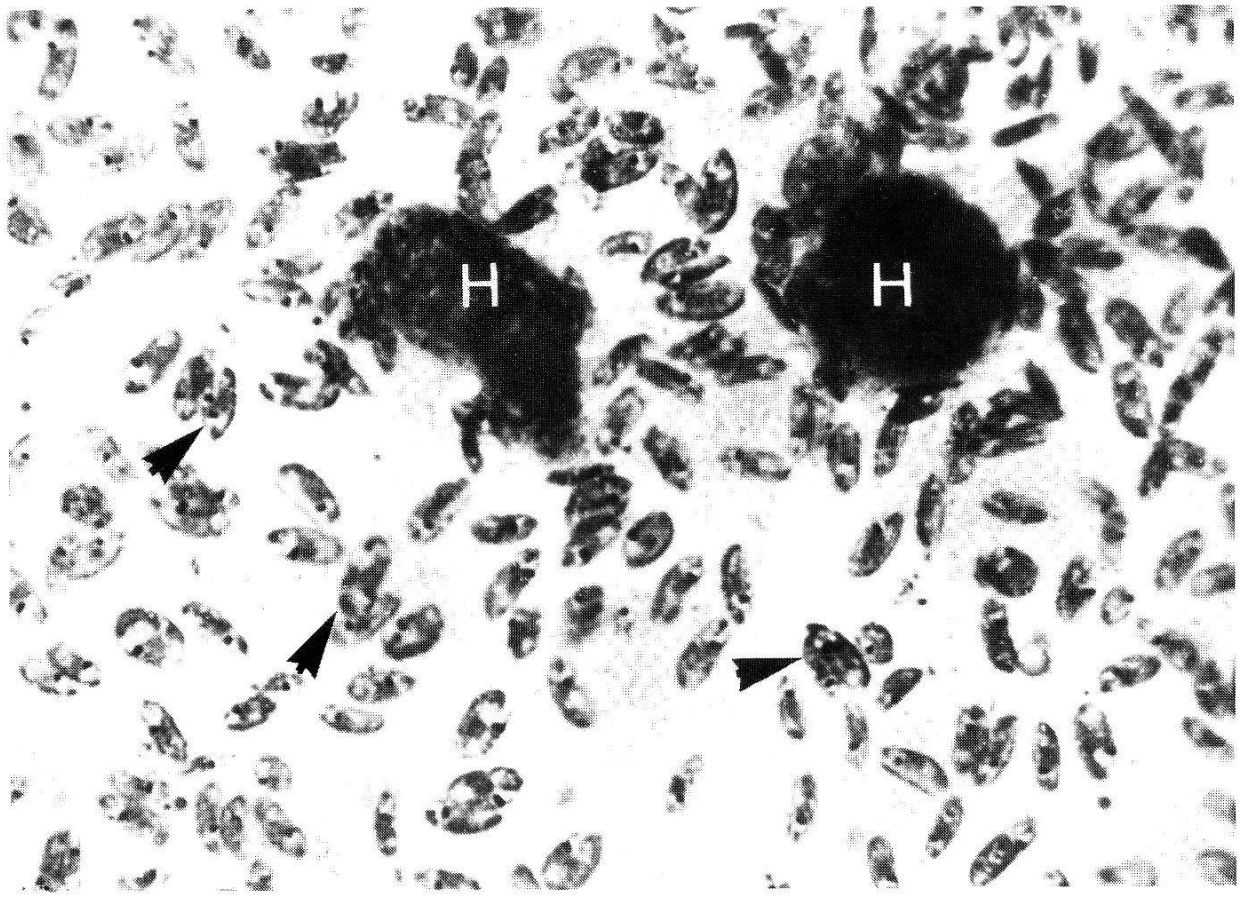
Highly motile *T. brucei* procyclics and *L. hertigi* promastigotes were seen in both haemolymph and body washings of *G. austeni, G. morsitans* and *G. tachinoides* up to 12 h post-injection, after which time it became increasingly difficult to find parasites. No *T. brucei* or *L. hertigi* were seen in either the haemolymph or body washings of *G. morsitans, G. austeni* and *G. tachinoides* on/or after day 4 post-inoculation.

Although *L. hertigi* and *C. fasciculata* were phagocytosed by haemocytes in vivo the phagocytic response of *Glossina* species against *C. fasciculata* was too weak to clear the parasites. Spindle cells and plasmatocytes (East et al., 1980; Kaaya and Ratcliffe, 1982) were observed to phagocytose *C. fasciculata* and *L. hertigi* but not *T. brucei* (Fig. 1).

The majority of *Glossina* inoculated with either *T. brucei* or *L. hertigi* or sham-injected, survived for upto 30 days and no deaths occurred during the first 10 days post-injection. In contrast, none of the *C. fasciculata*-injected flies survived after day 9. Statistical analysis (chi-squared and Student's t-test) of the bulk data showed no significant differences ($P > 0.05$) between the mortality rates amongst *T. brucei*- and *L. hertigi*-injected flies when compared to the mortality rates amongst the sham-injected controls. A highly significant ($P < 0.001$) mortality occurred amongst all *C. fasciculata*-injected flies on/or



1



2

Table 1. Concentrations of *C. fasciculata* in the haemolymph of *Glossina* species at various times following injection* of the parasite

Time post-injection (days)	Concentration of parasites in haemolymph (numbers/μl)**		
	G. morsitans	*G. palpalis*	*G. tachinoides*
1	445± 252***	318± 193	532± 214
2	942± 334	736± 248	1219± 316
3	4124± 716	3832± 978	5842±1293
4	7655±2792	8014±4145	10322±6384
5	11921±6144	13216±5393	16786±4621
6	27835±5136	24835±5236	30451±5621
7	38490±4581	33490±4534	44189±6474

* = initial dose injected per fly = 4×10^3 parasites
** = expressed as mean count ± standard deviation μl^{-1} of haemolymph
*** = each count represents a mean of 10 flies at each sampling time

after day 7 when compared separately to the deaths that occurred amongst the *T. brucei*- and *L. hertigi*-injected flies and the untreated and sham-injected control flies.

Examination of haemolymph of *G. morsitans, G. austeni, G. palpalis, G. tachinoides* and *G. fuscipes,* previously inoculated with *C. fasciculata,* demonstrated prolific multiplication of the flagellate (Figs. 1 and 2). When the flies were bled, the haemolymph was white or yellow in colour, indicative of an intense infection whereas clear haemolymph was obtained from parasite-free flies and flies with low levels of infection. The parasite concentration per μl of haemolymph at various times post-infection of 3 *Glossina* species with *C. fasciculata* is given in Table 1. No significant differences in parasite concentration were found between male and female *Glossina* species.

Data on the mortality of *Glossina* species following intrahaemocoelic inoculation of *C. fasciculata* ($3–5\times10^3$ parasites per fly) are presented in Table 2. The results demonstrated highly significant mortality ($P < 0.001$) amongst *C. fasciculata*-infected flies when compared to zero mortalities in the groups of control flies. None of the flies inoculated with $3–5\times10^3$ *C. fasciculata* survived after day 9 but 11 out of the 14 *G. morsitans* which survived until day 8 were found to be simultaneously infected with unidentified bacteria together with

Fig. 1. Smear of *G. palpalis* haemolymph infected with *C. fasciculata* showing flagellates within plasmatocyte. Note numerous intracellular parasites (some arrowed). Day 4 post-inoculation. ×3200.

Fig. 2. Giemsa-stained smear of massive pathogenic infection of *Crithidia fasciculata* in *Glossina morsitans* haemolymph. Such infections are typical of the levels of parasites seen in haemolymph from day 5 onwards until the fly succumbs. Dense bodies in centre are *Glossina* haemocytes (H). Dividing forms arrowed. ×1600.

Table 2. Mortality of *Glossina* species at various times post-injection of *C. fasciculata*

Species	Status/ treatment	Total no. of injected flies	No. dead flies									
			Days post-injection									
			1	2	3	4	5	6	7	8	9	10
G. morsitans . .	Untreated control	96	0	0	0	0	0	0	0	0	0	0
	Sham-injected	80	0	0	0	0	0	0	0	0	0	0
	Parasite injected	251	0	0	0	14	23	59	126	15	14	–
G. palpalis	Untreated control	55	0	0	0	0	0	0	0	0	0	0
	Sham-injected	57	0	0	0	0	0	0	0	0	0	0
	Parasite injected	192	0	0	0	84	57	27	11	13	–	–
G. tachinoides	Untreated control	45	0	0	0	0	0	0	0	0	0	0
	Sham-injected	45	0	0	0	0	0	0	0	0	0	0
	Parasite injected	183	0	0	0	27	35	25	46	50	–	–

0 = no deaths
– = no surviving flies

Table 3. Mortality of male and female *Glossina* species at various times post-injection of *C. fasciculata*

Species	No. and sex of inoculated flies		Days post-injection dead males									
	Male	Female	1	2	3	4	5	6	7	8	9	10
G. morsitans	124		0	0	0	16	26	46	36	–	–	–
G. palpalis	129		0	0	0	61	37	18	13	–	–	–
G. tachinoides	128		0	0	0	38	42	18	24	6	–	–
			Days post-injection dead females									
			1	2	3	4	5	6	7	8	9	10
G. morsitans		180	0	0	0	2	6	29	114	15	14	–
G. palpalis		123	0	0	0	28	31	27	22	15	–	–
G. tachinoides		91	0	0	0	2	8	24	51	6	–	–

0 = no deaths
– = no survivals

C. fasciculata. The bacterial infection apparently reduced the rate of multiplication of *C. fasciculata.* The longevity of *C. fasciculata*-infected *Glossina* species appears to be dose-related as no deaths occurred amongst infected *Glossina* during the first 3 days post-infection which coincided with low parasite counts

in the haemolymph (Table 1). Inoculation of *Glossina morsitans* with 100–200 *C. fasciculata* resulted in death 10 to 15 days post-injection.

Both male and female *Glossina* injected with *C. fasciculata* died between 4 and 9 days. Male flies tended to die earlier following intrahaemocoelic infection than females (Table 3). Statistical analysis of bulk data on mortalities of male and female *Glossina* species following *C. fasciculata* infection showed a highly significant ($p < 0.001$) death rate amongst male *G. morsitans, G. palpalis* and *G. tachinoides* between 4 and 5 days post-infection when compared with the mortality amongst female flies at the same sampling times. Male *G. morsitans* and *G. palpalis* did not survive after day 7; only 6 out of 128 (4.7%) *G. tachinoides* males survived until day 7 but the remainder were dead on day 8. *Glossina* remained active up to day 5 post-injection although the majority of the injected flies did not feed after days 6 and 7.

Discussion

The results show that intrahaemocoelic inoculation of *Crithidia fasciculata* into 5 *Glossina* species – *G. austeni, G. fuscipes, G. morsitans, G. palpalis* and *G. tachinoides* resulted in an intense multiplication of the parasite and always resulted in the deaths of the flies. In contrast, neither *T. brucei* and *L. hertigi* multiplied within the haemocoele. These flagellates disappeared completely from the haemolymph between 1 and 4 days.

Members of the genus *Crithidia* are known to easily adapt to new habitats and establish a flourishing population of parasites within a short period of time (Schmittner and McGhee, 1970; Wallace, 1943). Schmittner and McGhee (1970) reported the multiplication of 6 *Crithidia* species within the body cavities of *Drosophila virilis, Acheta domesticus* and *Tenebrio molitor.* In most cases deaths occurred amongst the hosts, especially those inoculated with *C. fasciculata.* They also reported that all moribund or recently dead insects were packed with flagellates. Similarly, fatal infections have been reported for *Galleria mellonella* larvae experimentally injected with *L. adleri* (Linder, 1960); for *Musca domestica* naturally infected with *Herpetomonas muscarum* (Kramer, 1961) and for *Rhodnius prolixus* infected with *T. rangeli* (D'Alessandro, 1976; Grewal, 1957; Tobie, 1970; Watkins, 1971). In the present work whilst all the *Glossina* species inoculated with $3–5 \times 10^3$ *C. fasciculata* died between 4 and 9 days post-inoculation, no deaths occurred amongst *T. brucei-, L. hertigi-* or sham-injected flies during the first 10 days. Such variations in the ability of insects to control intrahaemocoelic infections of trypanosomatid flagellates have been reported in other insect species by several authors. For example, Ivanoff (1925) found that trypanosomes survived for several days in *G. mellonella* haemolymph. Hoare (1938) observed a 100% survival of *T. cruzi* in all infected *G. mellonella* larvae, whilst Linder (1960) reported total failure of *T. cruzi* to develop within the same host, and Zeledón and De Monge (1966) observed a different behaviour to

T. rangeli in *Triatoma infestans* and *R. prolixus.* The parasite completely failed to develop when inoculated into *T. infestans* whereas a heavy infection occurred in the case of *R. prolixus.*

Inefficient phagocytic responses have been reported in many insect species which were unable to control intrahaemocoelically inoculated trypanosomatid flagellates. For example, by *T. molitor* inoculated with either *Leptomonas pyrrhocoris* (Zotta, 1921) or with *C. fasciculata* (Schmittner and McGhee, 1970) and by *G. mellonella* larvae inoculated with *H. muscidarum* and *L. culicidarum* (Linder, 1960). Furthermore, Zeledón and De Monge (1966) reported that blockage of phagocytosis with Indian ink particles in *T. infestans* infected with *T. rangeli* resulted in marked pathogenicity.

However, *T. brucei* procyclics failed to survive and reproduce within the haemocoele of the host and the parasites disappeared completely from the circulation between 1 and 4 days. The failure of *T. brucei* to survive and multiply within the haemocoele of *Glossina* species could be attributed to the anti-trypanosomal humoral activity of the haemolymph (Croft et al., 1982; Kaaya et al., 1986a). The presence of such a factor in a natural vector of trypanosomes indicates the existence of a potential natural control mechanism, which could explain why *T. brucei* infections of haemolymph are not frequently observed (Molyneux, 1983). Kaaya et al. (1986b) inoculated bloodstream forms of *T. b. brucei* intrahaemocoelically into *G. m. morsitans* and reported a dose of 2×10^2 parasites per fly as pathogenic and 10^3 parasites killed all flies within 72 h. However, Kaaya et al. (1986a), in experiments using the same *T. b. brucei* stock reported that 2% of flies inoculated intrahaemocoelically developed salivary gland and proboscis infections. They state "*T. b. brucei* injected into the haemocoele of *G. m. morsitans* disappeared rapidly from the haemolymph so that by 48 h post-inoculation only about 1% were present and became increasingly sluggish as they remained in the haemocoele. These observations strongly support the presence of an anti-trypanosomal factor in tsetse haemolymph".

No noticeable differences were observed in the rate of multiplication of *C. fasciculata* within the haemocoele of male and female *Glossina* species although a significantly higher percentage of mortality was observed amongst male flies on days 4 and 5 post-inoculation. However, the reasons for such differences are not known. Death which was associated with high numbers of *C. fasciculata* parasites might be attributed to competition for nutrients or toxicity of metabolic end products (Schmittner and McGhee, 1970). However, Tobie (1970) suggested that in *T. rangeli* infected *R. prolixus* the overwhelming of the haemocoele with parasites was responsible for the obstruction of haemolymph circulation with the resultant death of the insects.

The results reported here clearly demonstrate that whilst both *T. brucei* and *L. hertigi* were unable to thrive and multiply within the tsetse fly haemocoele, *C. fasciculata* was able to do so. Clearly, the haemolymph of *Glossina* is an excellent medium for the multiplication of *C. fasciculata* and the study empha-

sises that the control of trypanosomatid infections by insects is a variable characteristic which may be dependent on either the flagellate itself, the ability of the vector to evoke or evade a response, or to the physiological suitability of the haemolymph if that response is in some way circumvented.

Acknowledgments

This work received support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and the King Faisal Foundation to whom we are most grateful. We are also indebted to Dr. A. M. Jordan, Tsetse Research Laboratory, Bristol and Drs. J. Itard and M. Clair of IEMVT, Maison-Alforts, Paris, for the supply of *Glossina* puparia.

Croft S. L., East J. S., Molyneux D. H.: Anti-trypanosomal factor in the haemolymph of *Glossina*. Acta trop. (Basel) *39*, 293–302 (1982).

Cunningham I.: New culture medium for maintenance of tsetse tissues and growth of trypanosomatids. J. Protozool. *24*, 325–329 (1977).

D'Alessandro A.: Biology of *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920. In: Biology of the Kinetoplastida, ed. by W. H. R. Lumsden and D. A. Evans, Vol. 1, p. 327–403. Academic Press, London 1976.

East J., Molyneux D. H., Hillen N. A.: Haemocytes of *Glossina*. Ann. trop. Med. Parasit. *74*, 471–474 (1980).

East J., Molyneux D. H., Maudlin I., Dukes P.: Effect of *Glossina* haemolymph on salivarian trypanosomes in vitro. Ann. trop. Med. Parasit. *77*, 97–99 (1983).

Ellis D. S., Evans D. A., Stamford S.: Penetration of the salivary glands of *Rhodnius prolixus* by *Trypanosoma rangeli*. Z. Parasit. *62*, 63–74 (1980).

Evans D. A., Ellis D. S.: Recent observations on the behaviour of certain trypanosomes within their insect hosts. Adv. Parasit. *22*, 1–42 (1983).

Grewal M. S.: Pathogenicity of *Trypanosoma rangeli* (Tejera, 1920) in the invertebrate host. Exp. Parasit. *6*, 123–130 (1957).

Hoare C. A.: Development of mammalian trypanosomes in the body-cavity of caterpillars. Trans. roy. Soc. trop. Med. Hyg. *32*, 8–9 (1938).

Ibrahim E. A. R., Ingram G. A., Molyneux D. H.: Haemagglutinins and parasite agglutinins in haemolymph and gut of *Glossina*. Tropenmed. Parasit. *35*, 151–154 (1984).

Ingram G. A., East J., Molyneux D. H.: Agglutinins of *Trypanosoma, Leishmania* and *Crithidia* in insect haemolymph. Dev. comp. Immunol. *7*, 649–652 (1983).

Ingram G. A., East J., Molyneux D. H.: Naturally occurring agglutinins against trypanosomatid flagellates in the haemolymph of insects. Parasitology *89*, 435–451 (1984).

Ivanoff M. E.: Un nouveau mode de conservation et d'envoi des *Trypanosoma* et des spirochètes dans le larve de *Galleria mellonella*. C.R. Soc. Biol. *181*, 230–232 (1925).

Kaaya G. P., Ratcliffe N. A.: Comparative study of haemocytes and associated cells of some medically important dipterans. J. Morphol. *173*, 351–365 (1982).

Kaaya G. P., Otieno L. H., Darji N., Alemu P.: Defence reactions of *Glossina morsitans morsitans* against different species of bacteria and *Trypanosoma brucei brucei*. Acta trop. (Basel) *43*, 31–42 (1986a).

Kaaya G. P., Ratcliffe N. A., Alemu P.: Cellular and humoral defenses of *Glossina* (Diptera: Glossinidae): reactions against bacteria, trypanosomes, experimental implants. J. med. Entomol. *23*, 30–43 (1986b).

Kramer J. P.: *Herpetomonas muscarum* (Leidy) in the haemocoele of larval *Musca domestica* L. Entomolog. News *72*, 165–166 (1961).

Linder J.: Behaviour of various Trypanosomatidae in larvae of the bee-moth *Galleria mellonella*. Bull. Res. Counc. Israel *8E*, 128–134 (1960).

Molyneux D. H.: Host-trypanosome interactions in *Glossina*. Ins. Sci. Appl. *1*, 39–46 (1980).
Molyneux D. H.: Host-parasite relationships of Trypanosomatidae in vectors. In: Current topics in vector research, ed. by K. F. Harris, p. 325. Praeger Publishers, New York 1983.
Otieno L. H., Darji N., Onyango P.: Development of *Trypanosoma (Trypanozoon) brucei* in *Glossina morsitans* inoculated into the tsetse haemocoele. Acta trop. (Basel) *33*, 145–150 (1976).
Poinar G. O. jr., Wassink H. J. M., Leegwater-van der Linden M. E., van der Geest L. P. S.: *Serratia marcescens* as a pathogen of tsetse flies. Acta trop. (Basel) *36*, 223–227 (1979).
Schmittner S. M., McGhee R. B.: Host specificity of various species of *Crithidia* Leger. J. Parasit. *56*, 684–693 (1970).
Tobie E. J.: Observations on the development of *Trypanosoma rangeli* in the haemocoele of *Rhodnius prolixus*. J. Invert. Pathol. *15*, 118–125 (1970).
Wallace F. G.: Flagellate parasites of mosquitoes with special reference to *Crithidia fasciculata* Leger, 1902. J. Parasit. *29*, 196–205 (1943).
Watkins R.: *Trypanosoma rangeli:* effect on excretion in *Rhodnius prolixus*. J. Invert. Pathol. *17*. 67–71 (1971).
Zeledón R., De Monge E.: Natural immunity of the bug *Triatoma infestans* to the protozoan *Trypanosoma rangeli*. J. Invert. Pathol. *8*, 420–424 (1966).
Zotta G.: Sur la transmission expérimentale du *Leptomonas pyrrhocoris* Z. chez des insectes divers. C.R. Soc. Biol. *85*, 135–137 (1921).