

Detection of infections of *Trypanosoma grayi* in *Glossina fuscipes fuscipes* in the Central African Republic

DNA probes have become frequently used tools for the identification of trypanosome infections in wild tsetse flies (McNamara *et al.*, 1989, 1995; Bourzat and Gouteux, 1990; Majiwa and Otieno, 1990). Such probes are particularly useful for the identification of infections that are indistinguishable by conventional dissection and microscopy (Lloyd and Johnson, 1924), such as immature infections of parasites in the *Trypanozoon* or *Nannomonas* subgenera and mature infections of parasites within the *Nannomonas* subgenus. Initially, radioactively labelled DNA probes were used for hybridization of dot- or touch-blots of tsetse midguts on nitrocellulose filters (Kukla *et al.*, 1987; Gibson *et al.*, 1988). More recently, PCR amplification of the target DNA sequences has allowed greater levels of sensitivity to be reached, enabling the low numbers of trypanosomes in the proboscis or salivary glands to be identified (Masiga *et al.*, 1992, 1996; Majiwa *et al.*, 1994).

Despite these technical improvements, field surveys of tsetse flies continue to produce many unidentifiable trypanosome infections in midgut samples. In particular, flies of the *palpalis* group take a high proportion of bloodmeals from reptiles and may therefore have infections of Stercorarian trypanosome species such as *Trypanosoma grayi*, which may be confused with immature infections of Salivarian trypanosomes (Dirie *et al.*, 1991; McNamara and Snow, 1991). The results of the present study are a case in point.

The study was carried out in the Commune d'Élevage d'Ouro-Djafon, Bambari, Central African Republic, on 25-28 September 1992 (Mission 1) and 9-12 January 1993 (Mission 2); 2 months prior to Mission 1, a trial of pour-on insecticide had been carried out (Gouteux *et al.*, 1996). A total of 355 *Glossina fuscipes fuscipes* was dissected and samples taken for bloodmeal and touch-blot analysis

(see Table). Infections of Stercorarian trypanosomes could be distinguished from those of Salivarian trypanosomes by the parasites' form (long and spindly) and their characteristic, rapid vibratory movements during microscopical examination of mid- and hind-gut sections. Such infections were by far the most common (85%); the combined prevalence of salivary gland infections (*T. brucei* ssp.) and infections of both proboscis and midgut (*Nannomonas*) was <1% and no proboscis-only infections (*T. vivax*) were found. Touch blots were subsequently hybridized with a DNA probe comprising sheared total DNA from *T. grayi* ANR4, a stock isolated from *G. palpalis gambiensis* in The Gambia, experimentally transmitted through crocodiles and characterized by isoenzyme and DNA probe analysis (Dirie *et al.*, 1991; McNamara and Snow, 1991). At high stringency (0.1 × standard saline-citrate at 65°C) this *T. grayi* probe hybridizes specifically with *T. grayi* and not with other trypanosome species (of the subgenera *Trypanozoon* or *Nannomonas*) (McNamara and Snow, 1991). Overall, 18 of the 355 touch-blots gave a positive hybridization with the *T. grayi* probe; only seven other touch-blots could be identified with any of the other available DNA probes (for *T. brucei*, savannah or forest *T. congolense*, *T. simiae* and *T. godfreyi*).

The results of the microscopical examinations showed low concordance with those obtained using the DNA probe for identification of *T. grayi*, particularly for Mission 2 (see Table). For Mission 1, of nine infections identified by microscopy as pure *T. grayi* or mixtures including this species, five were also found positive for this species using the DNA probe. For Mission 2, however, only six of the 40 infections thought to comprise or include *T. grayi* after the microscopy were positively identified by hybridization. The difference observed in the level of concordance between



TABLE

The numbers of infected flies amongst the 355 *Glossina fuscipes fuscipes* examined by microscopy and by use of a DNA probe specific for *Trypanosoma grayi*

	Result of using probe during:			
	Mission 1		Mission 2	
	Positive	Negative	Positive	Negative
RESULT OF MICROSCOPICAL EXAMINATION				
Pure <i>T. grayi</i> infection	4	4	6	28
Mixed infection including <i>T. grayi</i>	1	0	0	6
Infection with unidentified trypanosome	1	4	0	3
Uninfected	2	102	4	190
Totals	8	110	10	227

the results of the two identification methods in Missions 1 and 2 may have been the result of a higher prevalence of infections with reptilian trypanosomes other than *T. grayi* during the latter period; there was a considerable increase in the prevalence of monitor lizard (*Varanus*) bloodmeals between Missions 1 and 2 (18% v. 83%; Gouteux *et al.*, 1996). These trypanosomes are unlikely to have been *T. varani*, which infects sandflies rather than tsetse (Dirie *et al.*, 1991), and presumably belong to other, as yet uncharacterized species. Several of the *T. grayi* infections detected by DNA-probe hybridization had not been identified by microscopy, emphasizing the utility of the DNA-probe methodology.

In conclusion, the present results show that infections with reptilian trypanosomes may reach high levels in the tsetse population and these may give a misleading impression of the actual numbers of immature, Salivarian infections present.

ACKNOWLEDGEMENT. We are grateful to T. Njoroge for assistance with the DNA-probe analysis during a training attachment from KETRI.

J. P. GOUTEUX
ORSTOM,
c/o Université de Pau et des Pays de
L'Adour,
Laboratoire de Mathématiques Appliquées,
IPRA, Avenue de l'Université,
F-64000 Pau, France

W. C. GIBSON*
Department of Genetics,
University of Leicester,
Leicester LE1 7RH, U.K.

Received 20 February 1996, Revised and accepted
4 March 1996

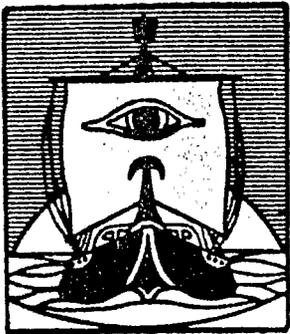
*Author to whom correspondence should be
addressed.

REFERENCES

- BOURZAT, D. & GOUTEUX, J. P. (1990). *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 43, 199–206.
- DIRIE, M. F., WALLBANKS, K. R., MOLYNEUX, D. H. & McNAMARA, J. (1991). *Annals of Tropical Medicine and Parasitology*, 85, 49–52.
- GIBSON, W. C., DUKES, P. & GASHUMBA, J. K. (1988). *Parasitology*, 97, 63–73.
- GOUTEUX, J. P., LE GALL, F., GUILLERME, J. M. & DEMBA, D. (1996). *Veterinary Research*, in press.
- KUKLA, B. A., MAJIWA, P. A. O., YOUNG, C. J., MOLOO, S. K. & OLE-MOIYOI, O. K. (1987). *Parasitology*, 95, 1–26.

- LLOYD, L. & JOHNSON, W. B. (1924). *Bulletin of Entomological Research*, 14, 265-288.
- MAJIWA, P. A. O. & OTIENO, L. H. (1990). *Molecular and Biochemical Parasitology*, 40, 245-254.
- MAJIWA, P. A. O., THATTHI, R., MOLOO, S. K., NYEKO, J. H. P., OTIENO, L. H. & MALOO, S. (1994). *Parasitology*, 108, 313-322.
- MASIGA, D. K., MCNAMARA, J. J., LAVEISSIERE, C., TRUC, P. & GIBSON, W. C. (1996). *Parasitology*, 112, 75-80.
- MASIGA, D. K., SMYTH, A. J., HAYES, P. J., BROMIDGE, T. J. & GIBSON, W. C. (1992). *International Journal for Parasitology*, 22, 909-918.
- MCNAMARA, J., DUKES, P., SNOW, W. F. & GIBSON, W. C. (1989). *Acta Tropica*, 46, 55-61.
- MCNAMARA, J. & SNOW, W. F. (1991). *Acta Tropica*, 48, 127-136.
- MCNAMARA, J. J., LAVEISSIERE, C. & MASIGA, D. K. (1995). *Acta Tropica*, 59, 85-92.

Vol. 90 No. 5
October 1996
ISSN 0003-4983



Published for the
**LIVERPOOL
SCHOOL OF
TROPICAL
MEDICINE**

Annals of **TROPICAL MEDICINE AND PARASITOLOGY**

By
**W.B. SAUNDERS
COMPANY LTD**

LONDON
PHILADELPHIA
SYDNEY
TOKYO
TORONT

PH 88
Saris