

EXPERIMENTAL INFECTION OF N'DAMA CATTLE WITH TRYPANOSOMES USING *GLOSSINA PALPALIS* GAMBIENSIS CAUGHT IN THE WILD

R. H. DWINGER, P. RAWLINGS, P. JEANNIN AND A. S. GRIEVE

International Trypanotolerance Centre (ITC), PMB 14, Banjul, The Gambia

SUMMARY

The transmissibility of trypanosome infection to N'Dama cattle by tsetse flies caught in the field was examined. Wild-caught Glossina palpalis gambiensis were transferred singly into small numbered cages and allowed to feed on 14 uninfected N'Dama cattle. Following a completed feed the tsetse were dissected and infection in the proboscis, the salivary glands and the gut was recorded. Each animal was bitten by a number of tsetse ranging from five up to 64 flies. Following dissection of the tsetse flies, seven of the cattle were found to have been bitten by a single infected tsetse, five by two, while the remaining two were each fed upon by three infected tsetse. The tsetse were harbouring either Trypanosoma vivax or a trypanosome species belonging to the Nannomonas subgenus or both species. The experimental animals were monitored daily over a period of three months for the appearance of trypanosomes in the blood and for antibodies in their sera. Other parameters such as body temperature, local skin reactions, packed red cell volume and weight changes were also measured. Trypanosomes were first detected eight days after the infective bite. Only five of the 14 cattle became infected, of which three had been exposed to a single infected tsetse fly. Trypanosoma vivax was detected in one animal, Trypanosoma congolense in two cattle and mixed infections of both species in the remaining two animals. These findings show that N'Dama cattle can become infected with trypanosomes through the bite of a single infected tsetse fly under field conditions. However, only five (possibly eight) of the 23 infected tsetse were able to transmit the parasites successfully.

INTRODUCTION

Animal trypanosomiasis is one of the major constraints to the production of adequate meat and milk supply to the increasing human population in large parts of Africa. In Central and West Africa a trypanotolerant cattle breed, the N'Dama, is able to survive and produce in areas where tsetse are prevalent. To investigate the trypanosomiasis risk to which these animals are subjected an epidemiological survey including an evaluation of the tsetse challenge is presently in progress in a few different geographical areas in The Gambia. As part of these investigations attempts are being made to determine the transmissibility of trypanosomes by tsetse to N'Dama. This paper deals with one aspect of parasite transmission, namely the ability of infected tsetse flies caught in the wild to transmit trypanosome infection to trypanotolerant cattle.

MATERIALS AND METHODS

Eighteen male N'Dama cattle, two to three years old, were selected on the basis of the absence of anti-trypanosomal antibodies using an indirect immunofluorescence test (Wilson, 1969). The sera of the animals were tested twice, one month and one week before the start of the experiment. The cattle were kept tethered in an area known to be free of tsetse flies and were each fed *ad libitum* on Gamba grass (*Andropogon gayanus*) supplemented with 6 kg, daily of a mixture of rice bran, groundnut cake, groundnut dust and salt in a ratio of 9:4:2:1.

Glossina palpalis gambiensis were captured in a forest near the village of Pirang by the Gambia river using F3 box traps (Flint, 1985). The tsetse were transferred singly into 6 × 3 cm plastic tubes having netting at both ends.

After shaving 20 small areas on the flanks of each of the 14 experimental animals tsetse were allowed to feed on the skin areas. All tsetse bite sites were marked with a felt-tipped pen. After tsetse had fed to completion they were dissected by the method described by Lloyd and Johnson (1924). The proboscis, salivary glands and gut of each tsetse were examined microscopically for the presence of trypanosomes. Tsetse with both gut and proboscis infection were considered to be infected with parasites of the subgenus *Nannomonas*, while those with infection in the proboscis only were considered to be infected with *Trypanosoma vivax* (Lloyd and Johnson, 1924). No salivary gland infections were

TABLE I

Showing the relationship between trypanosome infections in tsetse flies and infections in N'Dama cattle on which they were fed

| Animal number | Total no. of tsetse | | | Dissection | | | Clinical signs | | | Trypanosome species in cattle |
|---------------|---------------------|------------|-----------------------|------------|-----------|-----------------|------------------------|------------------------------------|--------------|-------------------------------|
| | Complete feed | Probe only | Infected tsetse bites | Gut | Proboscis | Salivary glands | Appearance of chancres | Presence of fever and parasitaemia | | |
| 1 | 40 | — | 2 | + | + | — | + | + | <i>Tv/Tc</i> | |
| 2 | 33 | — | 3 | — | + | — | — | — | — | |
| 3 | 36 | 7 | 2 | — | + | — | — | — | — | |
| 4 | 45 | 6 | 2 | — | + | — | — | — | — | |
| 5 | 48 | 16 | 2 | — | + | — | + | + | <i>Tv/Tc</i> | |
| 6 | 35 | — | 2 | + | + | — | — | — | — | |
| 7 | 8 | — | 2 | + | + | — | — | — | — | |
| 8 | 9 | 4 | 1 | — | + | — | — | — | — | |
| 9 | 5 | — | 1 | — | + | — | — | — | — | |
| 10 | 16 | 2 | 1 | + | + | — | + | + | <i>Tc</i> | |
| 11 | 13 | — | 1 | + | + | — | + | + | <i>Tc</i> | |
| 12 | 50 | 1 | 1 | — | + | — | — | — | — | |
| 13 | 7 | 1 | 1 | + | + | — | — | + | <i>Tv</i> | |
| 14 | 22 | 2 | 1 | — | + | — | — | — | — | |
| 15(c) | — | — | — | — | — | — | — | — | — | |
| 16(c) | — | — | — | — | — | — | — | — | — | |
| 17(c) | — | — | — | — | — | — | — | — | — | |
| 18(c) | — | — | — | — | — | — | — | — | — | |

(c) = control animal. + = present. — = not present. *Tc* = *T. congolense*. *Tv* = *T. vivax*.

encountered indicating the absence of *T. brucei* in the captured tsetse. In order to ensure that animals were bitten by infected tsetse it was necessary to feed many tsetse flies on each animal. The number of complete feeds ranged from five to 50 per animal. On some animals an additional number of tsetse probed but did not feed (Table I). Four animals served as unchallenged sentinels in the experimental area.

The cattle were initially weighed and faeces were examined for the presence of helminth eggs. Blood samples were collected twice weekly for two weeks to determine the packed red cell volume (PCV). Following the bites of infected tsetse flies 10 ml blood samples were collected daily from the jugular vein into ethylenediaminetetra-acetic acid (EDTA)-coated vacutainer tubes. The PCV percentage was determined and the buffy coat was examined for trypanosomes by darkground (DG) microscopy (Murray, Murray and McIntyre, 1977). The number of trypanosomes was estimated by a previously described scoring method (Paris, Murray and McOdimba, 1982). Thin blood smears were prepared regularly according to standard procedures and examined to verify morphologically the infecting trypanosome species. Blood samples were collected for serum twice weekly and the separated serum stored at -20°C until required.

Antibody levels against *T. brucei*, *T. congolense* or *T. vivax* were measured using the immunofluorescent antibody test (Katende, Musoke, Nantulya and Goddeeris, 1987). The development of local skin reactions was assessed by measuring daily for a period of 14 days skin thickness at the bite sites using vernier callipers. The rectal temperature was determined daily early in the morning and the body weights of the animals were determined weekly using a mechanical weighing scale (Gascoigne Milking Equipment Ltd, UK). Sampling continued for a period of three months.

RESULTS

Two N'Dama were each bitten by three infected tsetse flies, five by two infected tsetse each and seven by a single infected tsetse each (Table I). *T. congolense* parasites were first detected between eight and 12 days while *T. vivax* was detected between 12 and 17 days after the infective tsetse bites (Table II). Two of the animals (numbers 1 and 5) showed mixed infections with *T.*

TABLE II

Time of appearance and disappearance of trypanosomes in N'Dama cattle bitten by infected tsetse flies (Glossina palpalis gambiensis) caught in the wild

| Animal number | First day p.i. of detecting trypanosomes | | Last day p.i. of detecting trypanosomes | | Day of termination of experiment |
|---------------|------------------------------------------|----------------------|-----------------------------------------|----------------------|----------------------------------|
| | <i>T. vivax</i> | <i>T. congolense</i> | <i>T. vivax</i> | <i>T. congolense</i> | |
| 1 | 15 | 8 | 48 | 78 | 84 |
| 5 | 12 | 41 | 56 | 103 | 103 |
| 10 | — | 12 | — | 89 | 103 |
| 11 | — | 11 | — | 70 | 84 |
| 13 | 17 | — | 28 | — | 84 |

— = not present. p.i. = post-infection.

congolense and *T. vivax*. Initially trypanosomes were seen daily in the blood samples but between 49 and 79 days the parasites were only detected intermittently; eventually these aparasitaemic periods occurred more frequently and became of longer duration. Animal number 13 which was infected with *T. vivax* showed a low and scanty parasitaemia which was undetectable after 28 days. Antibodies against trypanosomal antigens were found only in the five animals that became infected.

In four of the infected N'Dama cattle local skin reactions (chancres) first appeared between day 7 and day 10 after the infective bite. These lesions reached a maximum skin thickness one to two days later (Table I). At the time of maximum skin reaction a reddened, warm and painful swelling could be detected which remained discernible for another three to five days. The kinetics of the chancre formation were similar to those described previously for cattle (Dwinger, Grootenhuis, Murray, Moloo and Gettinby, 1986). All infected animals developed a transient but recurrent fever which was measured for the first time either on day 12 (animal numbers 1 and 5), day 15 (animal numbers 10 and 11) or day 16 (animal number 13) after the infective tsetse bite (Table I). In the infected cattle a slight decrease in PCV was noticeable; in the control animals this was not the case. During the period of infection the PCV level in the infected animals decreased steadily from an average of 26.8 to 23.6%, while the average PCV level in the controls increased from an initial 26.7% at the start of the experiment to 28.2% three months later. Only animal number 5 lost weight during the course of the experiment. It weighed 255 kg at the start of the experiment but lost 40 kg of body weight during the following two months. The other four infected animals gained an average of 9.5 kg in body weight while the four controls gained an average of 11.5 kg during the same period.

DISCUSSION

Although transmissibility of trypanosome infections by tsetse to cattle plays an important role in any attempt to model the risk of animal trypanosomiasis under field conditions (Snow and Tarimo, 1983; Rogers and Randolph, 1985) only one investigation has been reported to our knowledge dealing with this aspect of the disease. Wilson, Dar and Paris (1972) inoculated macerated proboscides of three different tsetse species infected with *T. vivax* or *T. congolense* into cattle. However, this technique does not take into account some of the factors which may play a role in the parasite transmission rate such as the interactions between tsetse flies, metacyclic trypanosomes and bovine skin. In our experiments only five of 14 N'Dama cattle bitten by infected tsetse flies became parasitaemic. Since the animals had been exposed to a total of 23 infected tsetse it can be deduced that only a maximum of eight tsetse were successful in transmitting the parasites to the cattle within the limits of the sensitivity of the dissection technique. At the same time our investigations did show that an animal belonging to a trypanotolerant breed can be infected with *T. vivax* or *T. congolense* using a single infected tsetse fly caught in the field (animals 10, 11 and 13).

A major drawback of using tsetse caught in the wild is that mixed infections of trypanosomes often occur (Otieno and Darji, 1979). It was therefore not possible to distinguish the transmission rate for parasites of the subgenus *Nannomonas* from that for *T. vivax*. If the experiment could be repeated using laboratory reared tsetse infected under controlled conditions it would be possible to examine the transmissibility for each subgenus or species separately.

Another problem was that during the dissection of tsetse flies the presence of *Nannomonas* subgenus infections can only be differentiated from *T. vivax* by finding trypanosomes in the midgut of the tsetse. As a consequence it is quite possible that, whenever both the gut and the proboscis were found to be infected, a mixed infection with parasites belonging to the *Nannomonas* subgenus and *T. vivax* could have been present. On the other hand, the two tsetse flies which had bitten animal number 5 were found to be infected in the proboscis only (Table I). Nevertheless both *T. vivax* and *T. congolense* parasites were detected in the blood of the animal. Apparently either no immature *T. congolense* were left in the insect gut at the time of dissection or the small number of immature *T. congolense* left was obscured by the full blood meal the tsetse had taken prior to dissection. Moreover, it is known that tsetse whether infected with *T. congolense* or *T. vivax* can rid themselves of parasites after feeding (Elce, 1971; Otieno and Darji, 1979). The presence of immature infections of *T. congolense* and/or *T. brucei* in the gut further complicates proper species determination morphologically in tsetse flies following dissection. Furthermore, it should be noted that of the 15 tsetse infected with *T. vivax* only three were successful in transmitting infection to the N'Dama (animal numbers 1, 5 and 13). Such a low transmission efficiency can be attributed either to the fact that an infected tsetse fly does not transmit infection at every feed (Wilson, Cunningham and Harley, 1966) or to the trypanotolerance of the cattle breed.

An additional finding was the occurrence of chancre reactions in N'Dama cattle under field conditions following the bites of *G. palpalis*. Although the significance of the chancre reaction has previously been questioned since under field conditions local skin reactions have not been observed (Losos, 1979), our findings show that the chancre is not just an experimentally induced phenomenon and confirm similar observations by Roberts, Gray and Gray (1969). Of all the infected animals only animal number 13, infected with *T. vivax*, did not show any sign of local skin reactions. This agrees with observations on animals experimentally infected with different *T. vivax* stocks (Dwinger *et al.*, 1986).

In conclusion, we have demonstrated in preliminary investigations the transmission of trypanosomes from tsetse caught in the wild to a trypanotolerant cattle breed. Results indicate that a single infected *G.p. gambiensis* can transmit trypanosomes to a trypanotolerant cattle breed, but with low efficiency.

ACKNOWLEDGEMENTS

We wish to thank Dr D. Clifford for his support and Mr J. M. Katende for providing the IFAT reagents. The technical assistance of Mr J. Faye and Mr S. Kora is acknowledged. We are grateful to Drs P. R. Gardiner and S. K. Moloo for critical advice on the manuscript. This study was supported by funds from the European Development Fund and the Overseas Development Administration.

Accepted for publication February 1989

REFERENCES

- DWINGER, R. H., GROOTENHUIS, J. G., MURRAY, M., MOLOO, S. K. & GETTINBY, G. (1986). *Research in Veterinary Science*, **41**, 307-315.
- ELCE, B. J. (1971). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **65**, 239.
- FLINT, S. (1985). *Bulletin of Entomological Research*, **75**, 529-534.
- KATENDE, J. M., MUSOKE, A. J., NANTULYA, V. M. & GODDEERIS, B. M. (1987). *Tropical Medicine and Parasitology*, **38**, 41-44.

- LLOYD, L. & JOHNSON, W. B. (1924). *Bulletin of Entomological Research*, **14**, 265–288.
- LOSOS, G. J. (1979). **Pathogenicity of Trypanosomes.** (Eds G. LOSOS and A. CHOUINARD). IDRC-132e, Ottawa, pp 59–62.
- MURRAY, M., MURRAY, P. K. & MCINTYRE, W. I. M. (1977). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**, 325–326.
- OTIENO, L. H. & DARJI, N. (1979). *Annals of Tropical Medicine and Parasitology*, **73**, 583–588.
- PARIS, J., MURRAY, M. & MCODEMBA, F. (1982). *Acta Tropica*, **39**, 307–316.
- ROBERTS, C. J., GRAY, M. A. & GRAY, A. R. (1969). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **63**, 620–624.
- ROGERS, D. J. & RANDOLPH, S. E. (1985). Population dynamics and vectorial capacity of tsetse. WHO expert committee on the epidemiology and control of African trypanosomiasis, 8 pp.
- SNOW, W. F. & TARIMO, S. A. (1983). *Acta Tropica*, **40**, 331–340.
- WILSON, A. J. (1969). *Tropical Animal Health and Production*, **1**, 89–95.
- WILSON, A. J., CUNNINGHAM, M. P. & HARLEY, J. M. B. (1966). 11th meeting of the International Scientific Council for Trypanosomiasis Research. Publication no. 100. OAU, Lagos, pp 41–44.
- WILSON, A. J., DAR, F. K. & PARIS, J. (1972). *Tropical Animal Health and Production*, **4**, 14–22.

INFECTION EXPERIMENTALE DE BOVINS N'DAMA AVEC DES TRYPANOSOMES A L'AIDE DE *GLOSSINA PALPALIS GAMBIENSIS* CAPTUREES A L'ETAT SAUVAGE

Résumé—La transmissibilité de possibilité de transmission de la trypanosomose à des bovins N'Dama par des mouches tsé-tsé capturées sur le terrain a été examinée. Des *Glossina palpalis gambiensis* capturées à l'état sauvage ont été transférées individuellement dans de petites cages numérotées et placées pour se nourrir sur 14 bovins N'Dama indemnes.

A la suite d'un repas complet, les mouches ont été disséquées et on a enregistré l'infection dans le proboscis, les glandes salivaires et l'intestin. Chaque animal a été piqué par un nombre de mouches allant de cinq à soixante quatre. A la suite de la dissection des glossines, on a trouvé que sept bovins avaient été piqués par une seule mouche infectée, cinq par deux mouches, alors que les deux derniers avaient servi à nourrir chacun trois glossines infectées. Les mouches tsé-tsé hébergeaient soit *Trypanosoma vivax* ou une espèce de trypanosomes appartenant au sous genre *Nannomonas* ou par les deux espèces.

Les animaux d'expérience ont été suivis quotidiennement pendant trois mois pour observer l'apparition des trypanosomes dans le sang et des anticorps dans leur sérum. D'autres paramètres tels que la température corporelle, les réactions cutanées locales, le volume érythrocytaire et les variations pondérales ont également été mesurés. Les trypanosomes ont été détectés pour la première fois huit jours après la morsure infectante. Seuls cinq des 14 bovins se sont infectés, parmi lesquels trois avaient été exposés à une seule mouche infectée. On a détecté *Trypanosoma vivax* chez un animal, *T. congolense* chez deux bovins et des infections mixtes des deux espèces chez les deux derniers animaux infectés. Ces observations montrent que les bovins N'Dama peuvent devenir infectés par des trypanosomes par la pique d'une unique mouche tsé-tsé infectée dans les conditions du terrain. Cependant, seulement cinq (peut-être huit) des 23 glossines infectées ont été capables de transmettre les parasites avec succès.

INFECCION EXPERIMENTAL DE GANADO N'DAMA CON TRIpanosomas USANDO *GLOSSINA PALPALIS GAMBIENSIS* ATRAPADAS EN EL CAMPO

Resumen—Se estudió la transmisibilidad de infecciones con tripanosomas a ganado N'Dama, a través de moscas tsetse atrapadas en el campo. Las *Glossina palpalis gambiensis* atrapadas, fueron transferidas individualmente a jaulas numeradas, donde se alimentaron sobre 14 animales N'Dama sin infección alguna.

Una vez completada una comida, las moscas se disecaron buscándose infecciones en la proboscis, glándulas salivares e intestino. Cada animal fue picado por un buen número de moscas, en un rango de cinco a 64. Seguida a la disección de las moscas tsetse, siete bovinos fueron picados por una sola mosca infectada; cinco por dos, mientras que los otros dos fueron cada uno picado por tres tsetse infectadas. Las moscas estaban infectadas con *Trypanosoma vivax* o con especies de tripanosomas pertenecientes al subgénero *Nannomonas*, o por ambas especies.

Los animales fueron seguidos clínicamente día a día por un período de tres meses por la presencia de tripanosomas en la sangre y anticuerpos en el suero. Se tomaron en cuenta otros parámetros como la temperatura corporal, reacciones cutáneas locales, volumen corpuscular celular y peso corporal. Los primeros tripanosomas se detectaron ocho días después de la picadura de moscas infectadas.

Solamente cinco de los 14 bovinos se infectaron, de los cuales tres, habían sido expuestos a una única tsetse infectada. Se detectó *Trypanosoma vivax* en un animal, *T. congolense* en dos bovinos más e infecciones mixtas en los otros dos.

Estos hallazgos demuestran, que el ganado N'Dama puede infectarse con tripanosomiasis, seguidamente de la picadura de una sola tsetse infectada bajo condiciones de campo. Sin embargo, solamente cinco (posiblemente ocho) de las 23 tsetse infectadas, fueron capaces de transmitir el parásito exitosamente.

FIRST INTERNATIONAL CAMEL SYMPOSIUM
Dubai – United Arab Emirates
March 1991

The first International Camel Symposium will be held at the Dubai Trade and Exhibition Centre in March 1991. The 4-day programme will include research and clinical communications, plenary papers and a poster session and will cover all aspects of camel (dromedary) physiology, nutrition, medicine and surgery. The following themes will be emphasised:

- Clinical diseases
- Reproduction and obstetrics
- Surgery, anaesthesia and injury treatment
- Physiology, nutrition and exercise

Presentations are also encouraged in other areas of applied research.

Commercial exhibitions will be welcomed.

Those wishing to offer papers/posters for consideration or requiring additional information regarding the scientific programme or commercial exhibition should contact the following:

Dr A. M. Billah,
PO Box 11808,
Dubai, United Arab Emirates.
Tel: (International code 9714) 379375. Fax: 379030.

The final date for submission of titles is 15th June, 1990 and of selected abstracts is 15th October, 1990.