SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

7762 **Antwerp Trypanosomiasis Causal Model Group, 1992.** The causal model of sleeping sickness. (Meeting abstract no. 57.) *Annales de la Société belge de Médecine tropicale, 72* (Suppl. 1): 109. (See 16: no. 7766.) Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.

A group has been established to develop a causal model of human African trypanosomiasis, following the recommendations of the TDR/WHO sponsored workshop on modelling sleeping sickness epidemiology and control held at Antwerp in 1988 (see 12: no. 5938). Causal models link hypotheses about the causes of, and mechanisms leading to, particular phenomena and have proved useful in the selection and evaluation of interventions. A conceptual framework of the causal levels of sleeping sickness has been hypothesised.


This review considers the epidemiology of animal trypanosomiasis in the common fly belt of Malawi, Mozambique, Zambia and Zimbabwe and highlights the main constraints facing its control. The RTTCP was implemented in 1986 with the aim of eradicating tsetse-transmitted trypanosomiasis from the 322,000 km$^2$ fly belt. Its main objectives are to survey the distribution of tsetse flies and trypanosomiasis, to research and develop new survey and control techniques, to conduct emergency control operations, to monitor the environmental impact of insecticides, and training. Phase II of the Programme will prepare a comprehensive strategic plan for the progressive eradication of tsetse from the fly belt, in which control will be linked to sustainable land use. Although livestock production is secondary to arable farming, draught animals are severely affected by trypanosomiasis. Current control policies and strategies are discussed. Monitoring is carried out by examination of buffy coat preparations and blood smears but diagnostic methods need to be standardised and improved. A study of trypanosomiasis in goats has demonstrated the benefits of prophylaxis and good housing. The RTTCP has established a framework for a coordinated approach to tsetse control, but economic benefits must take into account the effects of other diseases.

7764 **Food and Agriculture Organization of the United Nations, 1992.**
Report of the consultation of the inter-secretariat coordinating group of the 
Programme for the Control of African Animal Trypanosomiasis and Related 
Rome; FAO. 44 pp. 
FAO, Via delle Terme di Caracalla, 00100 Rome, Italy. 
Past achievements and current activities of the 
Programme were reviewed. It was argued that 
achievements have been limited and that control 
strategies should aim at the enhancement of sustainable 
animal agriculture and proper land use. It was agreed 
that FAO should assume a monitoring role. Future 
policy and strategies were discussed. The influence of 
trypanosomiasis was reviewed in different regions of 
sub-Saharan Africa. Current environmentally acceptable 
control methods include prophylactic and curative drug 
use; vector control by SIT, selective ground spraying 
of residual insecticides, aerial spraying of non-
residual insecticides, traps and targets and 
insecticide-treated animals; and improvement of 
trypanotolerant breeds. Recommendations include: that 
the tsetse/trypanosomiasis situation be more accurately 
quantified; that increasing demand for arable land be 
evaluated according to changes in trypanosomiasis 
challenge; that control operations be incorporated into 
rural development schemes; that standardised diagnostic 
protocols be used; that planning and coordination of 
vector control activities be improved at national and 
regional level; that choice of control techniques be 
based on economic and environmental grounds; that 
trypanotolerant stock be given special attention; that 
routine economic feasibility studies be carried out; 
and that calculation of costs be standardised. 
7765 Lapeyssonnie, L., 1992. Géométrie et passion. La 
lutte contre la maladie du sommeil. [Geometry and 
passion. The control of sleeping sickness.] (Paper 
presented at the 26th Trypanosomiasis Seminar, Antwerp, 
Belgium, 11-13 December 1991.) Annales de la Société belge de 
Médecine tropicale, 72 (Suppl. 1): 7-12. (See 16: no. 
7766.)
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The words 'geometry and passion' are used to underline 
a conviction that nothing good or lasting can be 
obtained without the alliance of a rigorous choice of 
objectives and methods and a certain fervour in 
carrying out work. This conviction is illustrated by a 
brief history of human African trypanosomiasis control. 
Population displacement by colonial authorities earlier 
this century contributed to disastrous epidemics,
against which conventional control and diagnosis were ineffective. Against this background trypanosomiasis is seen as an 'administrative' disease, exacerbated by geographical and political factors and inadequate control. Rigorous control measures in the first half of this century were successful; by 1957 the incidence of new infection in Equatorial Africa, for example, had been reduced to 0.03%. Political and administrative problems have since resulted in a resurgence of trypanosomiasis which is now compounded by AIDS. Mobile control units could still be the key to successful control: they should redefine and update their objectives. Motivated and specialised auxiliary personnel who have been trained in field work and adequate funding are essential.


This supplement contains part of the proceedings of the 26th Trypanosomiasis Seminar which was held in Antwerp, Belgium, on 11-13 December 1991 and organised jointly by the Prince Leopold Institute of Tropical Medicine and the British Society for Parasitology. The papers covered three main areas of research: trypanosome genetics, trypanosome cellular and molecular biology, and tools and strategies for trypanosomiasis control. Eight of the 17 invited papers are included in this volume, together with abstracts of free communications and posters. The rest of the papers will be published elsewhere. (See also 16: nos. 7762, 7765, 7772, 7781, 7785, 7787-7789, 7795, 7797-7799, 7803, 7807, 7809, 7814, 7819-7821, 7830-7835, 7837, 7844, 7846, 7849, 7853-7855, 7858, 7859, 7863, 7865, 7866, 7869-7871, 7874-7876, 7878, 7880, 7882-7884, 7886-7889, 7891-7893, 7897.)


Institute of Commonwealth Studies, University of London, 27-28 Russell Square, London WC1B 5DS, UK. The effects of European colonialism on the exacerbation of human African trypanosomiasis in the former Belgian Congo are examined. Five main themes are explored: disease as a cause of historical change, epidemic disease as an insight into social relationships, medical imperialism as a facet of colonialism, the conflict between prevention and cure, and ecology. The epidemiology of sleeping sickness is considered in
relation to public health measures, population movements, immunology, geography and climate, and tsetse-trypanosome interactions. A history of control describes the work of scientists from the Liverpool School of Tropical Medicine between 1903 and 1905, who pioneered field survey methods including lumbar puncture. Control was initially based on the isolation of patients in camps or lazarets, coupled with severe restrictions on population movements. Effective medical control began to replace control by legislation after World War II. African perceptions of sleeping sickness, the effects of control on African societies and African reactions to medication, physical examination and isolation are discussed. Early authoritative control attempts are seen as a form of imperialism although beneficial public health measures developed as corollaries of this after the 1930s. By this time sleeping sickness was declining in northern Zaire but it remains unclear whether this was the result of control measures or the abandonment of the disruptive social engineering and economic exploitation of the early colonial period. Health legislation and instructions issued from 1888-1934 are listed in an appendix.

Murray, M., Stear, M.J., Trail, J.C.M., d'Ieteren, G.D.M., Agyemang, K. and Dwinger, R.H., 1991. Trypanosomiasis in cattle: prospects for control. In: Owen, J.B. and Axford, R.F.E. (eds), 1991 (see 16: no. 7769), pp. 203-223. Department of Veterinary Medicine, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK; ibid.; ILCA, P.O. Box 46847, Nairobi, Kenya; ibid.; ITC, P.M.B. 14, Banjul, Gambia; ibid. This review summarises attempts to control trypanosomiasis in cattle. Traps and targets and the direct treatment of cattle with insecticides are currently favoured for tsetse control. Trypanocidal drugs are still essential for disease prevention and treatment but no new commercial drug has been produced for 30 years. Successful drug control programmes have been carried out in villages and ranches but drug resistance may be a long-term problem. Antigenic variation has been a major constraint to vaccine development but this is not considered insurmountable. Trypanotolerance in cattle is associated with the ability to control parasitaemia and resist the development of anaemia. Both these criteria are highly heritable and are genetically correlated with production. They occur in indigenous breeds of cattle,
such as the N'Dama and the West African Shorthorn, which are being used in breeding programmes to maximise cattle production in tsetse-infested areas.


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The reviews presented at the symposium concern the genetic improvement of livestock for disease control as a necessary alternative to dependence on drugs. The text is divided into seven sections: principles and methodology of breeding for disease resistance (Sections 1 and 2), breeding for resistance to helminths, diseases involving flies and ticks, viral diseases, and bacterial/production diseases (Sections 3-6); major genes and animal disease (Section 7). Abstracts of ten posters presented at the symposium are also included. Four papers refer to animal trypanosomiasis (see 16: nos. 7768, 7813, 7824, 7826).


The purpose of this study is to elucidate the production dynamics, growth characteristics and trends of African trypanosomiasis research literature between 1900 and 1985 through graphical methods. The data analysed comprised 5139 articles from Tropical Diseases Bulletin and Tsetse and Trypanosomiasis Information Quarterly. Counting technique was employed in sorting the articles according to yearly production and respective subject disciplines. The bibliometric ranks of the disciplines were determined based on (1) the total output and (2) the average decennial relative changes (Rc) in publications between 1936 and 1985 using 1936/45 as the base decade. The results show that the growth is neither linear nor logistic but exponential with an average 39.5-year doubling time. A marked feature is the occurrence of high 'epidemic' peaks between 1910–1914 and 1979–1985, a state in which publications were produced at relatively high level probably due to new discoveries or research orientation; and hence capable of quickly infecting a large number of scientists, enhancing productivity. There was also a low level of activity from 1914 which lasted for about 22 years. The foundation disciplines for African Trypanosomiasis
Research Programme (ATREP) are entomology and parasitology. Entomology had the highest bibliometric rank followed by parasitology. However, the Rc factors indicate that greater attention, as depicted by publication outburst, was given to physiology, immunology, biochemistry and epidemiology between 1976 and 1985 than had been before. In spite of its consistently higher output, entomology exhibited a greater fluctuating growth trend than all the other components. A possible explanation for this behaviour was ventured.

2. TSETSE BIOLOGY
(a) REARING OF TSETSE FLIES


The authors describe efforts at laboratory colonisation of *G. tachinoides* in April 1986 from wild puparia collected from the Yankari Game Reserve. The climatic conditions that hitherto prevented previous attempts at breeding the species at NITR were identified and corrected. A 7-day mating regime played a major role by ensuring optimal insemination of females. The colony exhibited adaptive trends towards the laboratory condition. However, antibiotics contained in the diet of the rabbit hosts probably affected the fertility of the female flies, resulting in the decline and collapse of the colony.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY


Elsen, Roelants, Lil, Bortel, Hees: Laboratory of Medical Ento-mology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium; Dujardin: Laboratoire Génétique des Vecteurs et Parasites, ORSTOM, B.P. 5045, 34032 Montpellier Cedex, France.
Two lines of *G. palpalis gambiensis* have been established in the laboratory from wild flies which originated near Bobo-Dioulasso, Burkina Faso. The first line was established in 1986-87 from colonies maintained by CRTA and ILRAD and a second line was established in 1989-90 from a colony maintained at Maisons-Alfort, Paris. These two lines, referred to as BO (Bobo-Dioulasso) and MA (Maisons-Alfort) respectively, differ in their rate of intestinal colonisation by the same isolate of *Trypanosoma brucei gambiense*. Significant genetic differences between the two lines (IH = 0.837) have been demonstrated, originating perhaps from the wild ancestors or from rapid genetic drift in the laboratory. Cellulose acetate electrophoresis of nine enzymes confirmed previous studies of chromosome C-banding. The BO line possesses a small supplementary apical band on L2 and shows variation at the level of L1, compared with the MA line. Genetic control of tsetse is theoretically possible if laboratory reared flies show significant differences from wild flies: this study supports the hypothesis.


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Electron microscopy shows that the peritrophic membrane of *G. m. morsitans* is trilaminate and suggests that the outer two layers contain negatively charged glycosaminoglycans. There are structural similarities between the vertebrate renal glomerular basement membrane and the tsetse peritrophic membrane. *In vitro* perfusion experiments show that the presence of calcium ions significantly increases the permeability of the peritrophic membrane to alkaline phosphatase. This effect appears to be saturated in 0.05 M solutions of calcium chloride. The presence of potassium ions at 0.05 M or reduction of the pH to 5.8 does not significantly alter permeability. The permeability of male peritrophic membranes is significantly higher than that of female membranes in some media which suggests a sexual dimorphism in peritrophic membrane composition. In the light of the electron microscopical results it is postulated that calcium ions exert their effect by neutralising anionic charges in the membrane.

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An odour-baited electric net placed in thick bush caught more *G. pallidipes* when sited at ground level (0.2-1.2 m) than when sited in the top of the bush (1.4-2.4 m) or just above it (2.6-3.6 m). However, a similar net running concurrently 4 m away in a game trail through the bush caught far more flies. When electric nets were placed in the centre of two adjacent game trails in this bush and then one of the trails was barricaded with branches from nearby bushes, the catch declined in that trail and increased proportionately in the other. It is inferred that tsetse flies navigate up host odour plumes by finding gaps in thick vegetation rather than by flying over the top or through it, and that game trails are important forms of such gaps. The implications for siting traps and targets are noted.


Recordings were made of the activation of hungry *Glossina morsitans morsitans*, *G. pallidipes* and *G. austeni* in response to odours from ox breath and ox urine, and a moving visual stimulus, in a wind tunnel. The spontaneous activity of *G. m. morsitans* was very low (less than 4% of males and 2% of females active per min during control periods). That of *G. austeni* and *G. pallidipes* was in the region of 20% except for *G. pallidipes* females when in excess of 40% were active during control periods. Addition of ox urine odours to the airstream had no effect on activity in any of the species investigated but addition of ox breath odours to the airstream significantly increased activity of *G. pallidipes* and of *G. m. morsitans*, although for the latter only approximately 12% of flies were active. For *G. austeni* the addition of ox breath odours resulted in a significant increase in activity of females but not of males. The moving visual stimulus resulted in a significant increase in the activity of both sexes of *G. austeni* and *G. m. morsitans* but no change in the activity...
of *G. pallidipes*. The low level of spontaneous activity and the low response to ox breath odours in a strain of *G. m. morsitans* maintained in the laboratory since 1969 was compared with a new colony of this species which originated from puparia collected in Zimbabwe in 1991. No difference in either spontaneous activity or the response to ox breath odour was recorded, but females from the new colony were significantly more responsive to a moving visual stimulus. In a further series of experiments the activity of *G. m. morsitans* and *G. pallidipes* was recorded at varying wind speeds. For both species, activity decreased as the wind speed increased. The results are discussed in terms of the likely host-location strategies used by these species.


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The tsetse adult extricates itself from the puparium and surrounding substrate by a series of muscular contractions that generate a stereotypic pattern of changes in haemolymph pressure. The digging action of the fly can be distinguished from a second pattern of haemoceolic pulsations that is used to remove obstacles from its path. When the fly is restrained extrication behaviour will persist for over 10 h. If the adult's legs are freed while the remainder of the body remains encased in the puparium, the fly fails to engage in extrication behaviour, a result which suggests that freedom of the legs switches off extrication behaviour and permits the onset of expansion of the body to its final adult size and shape. Expansion behaviour includes walking, grooming, pumping air into the gut, and contracting the abdominal muscles to generate rhythmic pulses of haemoceolic pressure. A barographic record of internal pressure changes reflects the dynamics of this morphogenetic process. Results from tsetse are compared with previous observations recorded in flesh flies. (The species studied were *Glossina brevipalpis*, *G. austeni* and *G. morsitans*.)
3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)


NRI, Central Avenue, Chatham Maritime, Chatham, Kent ME4 4TB, UK.

The research activities and results of NRI's Aerial Spraying Research and Development Project from 1986-90 are described. A prime objective was to assess the possibility of eradicating tsetse from hilly terrain using the sequential aerosol application technique (SAT). A Doppler-based navigation system was found to be highly satisfactory. Studies on optimal spray characteristics included aerosol and droplet behaviour, droplet size, drift, the effect of emission height and variable flow rate. Droplet v.m.d. should be between 20 and 30 µm and droplets can be expected to drift 10-15 km downwind. Operational swathe widths of 250-330 m for fixed wing spraying and 200 m for helicopters over rugged terrain are recommended. Larval development studies confirmed that sequential applications should continue to be 1-2 days shorter than the estimated first larval period. Residual populations of *Glossina pallidipes* in treated areas suggest a first application rate of 30 g endosulfan/ha is required in flat country, compared with 22 g endosulfan/ha or 0.25 g deltamethrin/ha for *G. morsitans*. In rugged terrain tsetse control was uneven like the insecticide distribution which was also affected by meteorology: use of helicopters is recommended. When properly carried out, SAT was found to give dramatic reductions in tsetse abundance. Aerial spraying operations in Zimbabwe and Zambia from 1982-86 and 1986-89 are summarised. Annual budgets and general recommendations are included. (See also 14: no. 6707.)

Mbororo herders and their cattle are increasing in the humid savannas of the Central African Republic, where trypanosomiasis control by chemotherapy is not realistic in the long term. A campaign to control the principal vector, *Glossina fuscipes fuscipes*, by trapping was initiated by the Agence Nationale pour le Développement de l'Elevage (ANDE). Traps were placed at cattle watering places and maintained by the herders themselves. Two to four biconical Challier-Laveissière traps, not impregnated with insecticide, were placed at each of 37 encampments. Reductions of 60-90% in apparent fly density were achieved after 1 month and reductions of 80-98% in subsequent months. Problems included lack of continual trap maintenance by nomadic herders, mobile herds coming into contact with tsetse in untrapped areas, the seasonal distribution of *G.f. fuscipes* and its role as vector, and practical problems of assessing the prevalence of trypanosomiasis in the cattle.


Colony density and size, and numbers of white-browed sparrow-weavers were investigated in *Colophospermum mopane-Julbernardia globiflora* woodland treated from nought to five times with DDT for tsetse fly control. Colonies were absent from *Julbernardia* woodland. Estimated densities in sprayed and unsprayed mopane woodland were 74 and 71 colonies per km$^2$, respectively. Colony size varied with area and time but not with spray treatment. In 1987, colonies at the sprayed northern end of the study area were twice the size of those in the unsprayed, drought-affected south. Two years later, after good rains, colony size in sprayed and unsprayed areas was similar. Residues of DDT, DDD and DDE were measured in birds collected from sprayed and unsprayed areas 3, 5, 9, 12, 15 and 17 months after treatment. Residue levels in birds from the sprayed area fell from 3 months after treatment to reach background levels at 12 months. No adverse effects were noted. The most heavily contaminated bird contained 1083 µg DDT-
equivalents g\(^{-1}\) carcass lipid, less than half the maximum amount found in white-headed black chats *Thamnolaea arnoti* and red-billed wood-hoopoes *Phoeniculus purpureus*, whose populations collapsed after spraying. Dietary differences probably account for differing exposure and population responses to tsetse spraying operations.


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Thiodan, a u.l.v. formulation of endosulfan, was applied as an aerosol to field-caught *Glossina pallidipes* and *G. morsitans morsitans* held in a wind tunnel. Mortality was noted during a 48 h post-spray period and all female flies were dissected to assess their physiological age and stage of pregnancy. A second experiment using topical application of technical endosulfan to laboratory-bred flies was used to supplement data on *G. m. morsitans*. Male flies were found to be more susceptible than female flies. Physiological age was found to have little effect on tolerance to the insecticide, but the presence of a second- or third-stage larva in the uterus significantly increased tolerance compared with other pregnancy stages. Flies larvipositing during the topical application experiment were more tolerant than any other group of *G. m. morsitans*. It is common practice, whilst controlling tsetse using the sequential aerosol technique, to reduce application rates after the first cycle. This study indicates that application rates should be maintained or spraying should commence before newly emerged flies enter the later stages of pregnancy.

**7781 Laveissière, C. and Meda, H.H., 1992.** La lutte par piégeage contre la maladie du sommeil: pas aussi simple que l'on croit! [Sleeping sickness control by trapping: not as simple as one thinks!] (Paper presented at the 26th Trypanosomiasis Seminar, Antwerp, Belgium, 11-13 December 1991.) *Annales de la Société belge de Médecine tropicale*, 72 (Suppl. 1): 57-68. (See 16: no. 7766.)

IPR/OCCGE, B.P. 1500, Bouaké, Côte d'Ivoire.

A 2 year trial was carried out in Côte d'Ivoire to assess whether trapping can be used economically on a large scale with the help of rural communities. A
total of 38,660 insecticide-impregnated screens was distributed among 3671 farmers to cover the 1500 km² of the Vavoua focus and 400 traps were set up around villages. Testryp CATT was used to assess the incidence of trypanosomiasis before and after the trial. *Glossina palpalis palpalis* populations were reduced to zero in most villages and by more than 99.5% in the rest of the focus: disease transmission was arrested. Costs were around US $1/ha for the first year and US $0.15/ha for the second. The extent of community participation varied within and between different ethnic groups. People cannot be mobilised without understanding their habits and activities, their agricultural calendar and their movements. Successful control therefore depends not only on technology but on sociological studies. At present effective community participation seems possible only with imposed supervision.


Agrochemicals Unit, Joint FAO/IAEA Programme, IAEA Laboratories, A-2444 Seibersdorf, Austria.

Deltamethrin containing 96.3% DM1 epimer \((S)\)-\(\alpha\)-cyano-3-phenoxybenzyl \((1R,3R)\)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylate was allowed to stand in the dark at 21°C in 19 different solvents to study the effect of solvents on isomerisation of this insecticide. DM1 was converted to DM(2+2\(\|\)) epimer in methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 1-pentanol, acetone, and acetonitrile. Reaction was faster in the first four solvents than in the others and resulted in the formation of an equilibrium mixture of DM1 and DM(2+2\(\|\)) after 2-3 days. The reaction followed first-order kinetics and could be blocked by the addition of HCl or HBr. Bioassay of DM1 and DM(2+2\(\|\)) on 1-day-old female tsetse flies showed that DM1 was very toxic but DM(2+2\(\|\)) was relatively nontoxic to these insects. No isomerisation of DM1 took place in \(n\)-hexane, diethyl ether, ethyl acetate, \(p\)-dioxane, benzene, toluene, 2-methyl-2-propanol, 1-octanol, and 2-octanol in the dark.


Tsetse and Trypanosomiasis Control Branch, P.O. Box
Deposits of deltamethrin suspension concentrate (s.c.) on Terylene net and cotton cloth were bioassayed in Zimbabwe by exposing them to fed, female *G. pallidipes*, using a 45 s contact with cloth and a brief collision with net. On textured yarn net, the effective life of deposits produced from immersion in 0.1% deltamethrin was longer than on flat yarn net, apparently because the textured net held more insecticide and the insecticide was lost more slowly. The addition of an absorber for UV light (0.1% 2-hydroxy-4-methoxybenzophenone-5-sulphonic acid) did not significantly extend the effective life of deltamethrin deposits on cloth or net. Applying deltamethrin s.c. as a 0.6% suspension to cotton cloth produced mortalities of > 90% for 300 days. Applications of 0.8% deltamethrin on cotton cloth and textured net produced mortalities of > 70% for 12-16 months and 9 months respectively, compared to 4-10 months and 5-7 months respectively for applications of 0.1% deltamethrin. Chemical analyses indicated that the longer effective life of the 0.6-0.8% was due to a higher initial amount of insecticide and a lower rate of loss. It is suggested that for controlling tsetse in southern Africa, all-cloth targets sprayed with 0.6% deltamethrin s.c. will have an effective life of about one year.


As the tsetse fly, *G. m. morsitans*, extricates itself from the puparium and moves upward through the soil the ptilinum expands and contracts rhythmically and thus generates a stereotypic behavioural pattern that persists for up to 10 h if the tsetse fly remains confined. The response, which is easily recorded tensometrically from the movements of the ptilinum, can be exploited as a tool for evaluating the behavioural response of tsetse flies to various neurotoxic agents. The behavioural assay proves useful in providing precise information about the latency of the response.
and lethal time, and can suggest likely modes of action. For example, sublethal doses of pyrethroids reversibly suppressed the contraction cycles, a response consistent with the peripheral action of this insecticide. In contrast, chlorinated hydrocarbons greatly increased contraction frequency, a result consistent with the action of these agents on the CNS. Assays utilising eight commercial insecticide preparations (Pybuthrin, K-othrin, Vaztak, Reldan, Safrotin, Actellic, DDVP, Antrix) demonstrate the utility of this method for detecting subtle perturbations of the CNS and neuromuscular system.

4. epidemiology: vector-host and vector-parasite interactions
[See also 16: nos. 7775, 7819.]

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Maturation of Trypanozoon stocks in Glossina morsitans morsitans, which is controlled by a sex-linked gene, takes between 10 and 40 days. The maturation of Trypanosoma congolense is also controlled by a sex-linked gene. The expression of the gene for maturation in tsetse is dependent on interactions with the trypanosome genotype. Isolates of T. brucei brucei have a significantly greater transmission index (TI = number of salivary gland/midgut infections %) than T. b. rhodesiense isolates and similar differences have been found between stocks of T. congolense.

UTRO, P.O. Box 96, Tororo, Uganda; ibid.; TRL, Langford House, Bristol BS18 7DU, UK; Department of Biochemistry, Faculty of Science, Makerere University, Kampala, Uganda; ibid.
An epidemic of sleeping sickness, which started in 1976 in a focus within the county of Luuka in Central Busoga, has spread to cover the three districts of Busoga and large parts of the neighbouring districts of Tororo and Mukono. Forty-three isolates of the subgenus Trypanozoon from Busoga and Tororo (27 from man, nine from cows, two from pigs and five from tsetse
flies) were compared by thin-layer starch-gel electrophoresis for seven enzymes. Thirty zymodemes were identified; 17 of them were found circulating in the human population. The zymodemes seen previously in Busoga were still circulating, together with several new ones. Of the 16 isolates from cattle, pigs and tsetse flies, only two had the same profile, indicating a high degree of diversity. Two zymodemes from cows and a pig were identical to those found in man, implicating domestic stock in the transmission of human disease in south-east Uganda. A computer analysis of the results produced six main zymodeme groups. One comprised only isolates from man; two were composed of isolates from man, domestic animals and tsetse; and three consisted of stocks from domestic animals only. These groups quite probably indicate the different cycles of transmission involving man, tsetse fly and domestic stock.

Gibson, W. and Ferris, V., 1992. Sequential infection of tsetse flies with Trypanosoma congolense and T. brucei. (Meeting abstract no. 48.) Annales de la Société belge de Médecine tropicale, 72 (Suppl. 1): 104. (See 16: no. 7766.)

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Flies were initially infected with bloodstream form T. congolense or T. brucei sspp., followed by sequential feeds containing T. brucei sspp. or T. congolense. Midgut trypanosome populations were then analysed by hybridising dot blots with species-specific DNA probes. Two different stocks of T. brucei were also fed in succession and the midgut trypanosomes analysed by molecular karyotype. It was found to be comparatively easy to infect flies with a second trypanosome species or stock, although the presence of an initial infection did not aid the establishment of a subsequent one. Flies therefore do not need to pick up different trypanosomes in a single feed to establish a mixed infection, which is essential for trypanosome mating.


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A guinea-pig chronically infected with *T. b. brucei* EATRO 1125 provided a single blood meal to 440 teneral *G. p. gambiensis*. Procyclic and metacyclic infections were subsequently found in 2.32% and 11.29% of the flies respectively. There was no significant difference in the vectorial capacity of male and female flies, of which 13.19% and 9.55% respectively carried metacyclic infections. Fly infection appears to be influenced by the level of parasitaemia, the density of stumpy forms at the time of the blood meal and fly maintenance.


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The polymerase chain reaction (PCR) has been used to identify developmental stage trypanosomes in tsetse by targeting repetitive DNA for amplification. This assay is sensitive and can detect one trypanosome; it shows no cross-reaction with non-target trypanosomes or with the excess of host DNA present in crude preparations. *Trypanosoma brucei, T. vivax, T. simiae* and the three subgroups of *T. congolense* have been identified by PCR. Mixed infections can be identified in multiplex reactions using combinations of primer sets.


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African trypanosome species were identified using the polymerase chain reaction (PCR) by targeting repetitive DNA for amplification. Using oligonucleotide primers
designed to anneal specifically to the satellite DNA monomer of each species/subgroup, we were able accurately to identify *Trypanosoma simiae*, three subgroups of *T. congolense*, *T. brucei* and *T. vivax*. The assay was sensitive and specific, detecting one trypanosome unequivocally and showing no reaction with non-target trypanosome DNA or a huge excess of host DNA. The assay was used to identify developmental stage trypanosomes in the tsetse fly, *Glossina morsitans morsitans*. The use of radioisotopes was not necessary and mixed infections could be detected easily by incorporating more than one set of primers in a single reaction. The use of crude preparations of template made the process very rapid. The methodology should be suitable for large-scale epidemiological studies.


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To determine whether the virulence of *T. simiae* is affected by the species of tsetse which transmits it, teneral flies were infected from inoculated pigs and then used to infect other pigs. The length of the patent period in these tsetse-infected pigs was recorded. Three stocks of *T. simiae* (CP 11 isolated from *G. austeni*, CP 813 from *G. pallidipes* and CP 1896 from a bushpig) and three species of tsetse (*G. brevipalpis*, *G. pallidipes* and *G. morsitans centralis*) were used. Stocks CP 11 and CP 813 cause hyperacute disease in pigs while CP 1896 causes chronic disease. The patent period was somewhat longer for each stock when transmitted by *G. pallidipes* than when transmitted by *G. brevipalpis*. The two pigs infected with stock CP 813 by *G. m. centralis* died within 2 days after patent infection. Both *G. brevipalpis* and *G. pallidipes* were resistant to infection with stock CP 1896, while *G. m. centralis* showed very low susceptibility to it; it was of low virulence to the pig infected by *G. m. centralis*.


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Earlier this century the postulate that *Trypanosoma brucei brucei* and *T. b. rhodesiense* had a common identity, and that human infectability was linked with resistance to
normal human serum (NHS) in vitro, were both finally refuted in the classic Tinde experiment. Interest in serum sensitivity was reawakened with the advent of the BIIT in 1970 and the studies that followed demonstrated the presence of both human-serum-resistant (HSR) and sensitive (HSS) variant antigen types within the surface antigen repertoire of a single *T. b. rhodesiense* organism. This confirmed the bimodal human-infectivity potential of some, if not all, *`brucei'* trypanosomes. Changes from sensitive (`S') to resistant (`R') forms in a *T. b. brucei* clone have been shown to occur in chickens and have also been reported in a `clean' bushbuck infected with a *T. b. rhodesiense* clone. The subsequent expression of `S' forms by *T. b. rhodesiense*, when isolated from man into clean rats, has also been demonstrated. Sera from some game animals in Zambia have been shown to be highly trypanolytic. *Trypanozoon* organisms are almost constantly in contact with mammalian blood elements, in the vertebrate and invertebrate hosts, and more recent studies have demonstrated changes in the serum sensitivity/resistance of *Trypanozoon* during metacyclic development in *Glossina*. In view of this, it is felt that the effects of physiological host factors on these parasites may well prove to be a scientifically lucrative field for further research. The bimodal potentiality for human infectivity is clearly a character of fundamental epidemiological and epizootiological importance in the transmission dynamics of this parasite complex.


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Teneral *Glossina morsitans morsitans* from outbred and susceptible stocks infected with *Trypanosoma (Nannomonas) congoense* developed, respectively, three and six times higher midgut infection rates than flies of the same stock which had previously taken a bloodmeal. Non-teneral *G. m. morsitans* remained relatively refractory to infection when infected at subsequent feeds. Differences in susceptibility to midgut infection between teneral flies from susceptible and outbred lines of *G. m. morsitans* disappeared in non-teneral flies, showing that maternally inherited susceptibility to midgut infection is a phenomenon restricted to the teneral state of the fly. Laboratory reared *G. m.*
morsitans were found to have become significantly more susceptible to trypanosome infection than wild flies from the population from which the colony was derived. The likely role of rickettsia-like organisms (RLO) in potentiating teneral susceptibility to midgut infection is discussed. The addition of the specific midgut lectin inhibitor D-glucosamine to the infective feed of non-teneral flies increased midgut infection rates to levels comparable with those achieved in teneral flies. It is concluded that the peritrophic membrane does not act as a barrier preventing non-teneral flies becoming infected. The relative refractoriness of non-teneral flies suggests that they do not play a significant part in the epidemiology of *Trypanozoon* or *T. congolense* infections.

5. human trypanosomiasis
(a) SURVEILLANCE

[See also 16: no. 7767.]


The quantitative buffy coat (QBC) technique uses a glass haematocrit tube pre-coated with acridine orange and anticoagulant. A cylindrical float inserted with the blood sample expands the buffy coat into bands after centrifugation. Motile, fluorescent trypanosomes occur just above the buffy coat and sometimes in the platelet layer within it. This method was successfully used to detect *Trypanosoma brucei rhodesiense* infection at Busoga, eastern Uganda, but did not show improved sensitivity compared with standard blood film examination or microHCT. Use of QBC at Moyo and Adjumani in northern Uganda showed the method to be a simple technique for the detection of both *T. b. gambiense* and *T. b. rhodesiense* in peripheral blood, which proved as sensitive as the entire standard diagnostic procedure being carried out concurrently by Médecins Sans Frontières including CATT, gland aspiration, microHCT and CSF examination. QBC has advantages over CATT, especially in the absence of false positivity, but further studies are needed to determine its sensitivity in the diagnosis of *T. b. gambiense* infection.

Tools developed for diagnosis of human African trypanosomiasis. (Meeting abstract no. 50.) *Annales de la Société belge de Médecine tropicale*, 72 (Suppl. 1): 105. (See 16: no. 7766.)

Laboratory of Serology, Institute of Tropical Medicine, Nationale-straat 155, B-2000 Antwerp, Belgium.

The Laboratory of Serology of the Institute of Tropical Medicine in Antwerp develops, evaluates and distributes serological tests for the diagnosis of human African trypanosomiasis. Work at the Laboratory includes the cloning and cryopreservation of VATs, immune trypanolysis with living trypanosomes of defined VAT, and direct and indirect agglutination tests. Direct agglutination using fixed, stained and freeze-dried trypanosomes involves the plate test with procyclic forms and CATT with defined VATs. Indirect agglutination using particles coated with variable antigens concerns the card latex agglutination test (CLAT) and the plate haemagglutination test. Other tests include immuno-fluorescence using fixed, freeze-dried VATs and ELISA using variable antigens. Ongoing research is aimed at the evaluation of recombinant or synthetic peptides and the development of antigen detection tests.


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The epidemiology and clinical features of trypanosomiasis are briefly reviewed. Laboratory diagnosis is considered in two stages: the establishment of suspicion through immunodiagnosis and subsequent parasitological confirmation. Reagents for IFAT and CATT are commercially available but not for indirect haemagglutination, ELISA, counter current immunoelectrophoresis and radial immunodiffusion. An antigen-trapping ELISA technique based on the production of a specific monoclonal antibody has been adapted for mass surveys as a card indirect-agglutination antigen test. Parasitological examination of lymph node aspirate, bone marrow and thick and thin blood films can confirm diagnosis. The most sensitive blood test is mAEC but the capillary tube centrifugation test is favoured because it is fast and inexpensive. A red blood cell lysis test has recently been developed and adapted for field work. The late stage disease can be confirmed in the CSF by
the presence of parasites after double centrifugation, a white blood cell count above 5/mm$^3$, the presence of Mott cells and an increase in total protein level. Immunofluorescence, CATT and IgM measurement can also be used with CSF. Some of the advantages and disadvantages of these tests are indicated.


Early diagnosis and treatment of sleeping sickness are essential, both for effective cure and to break the parasite's transmission cycle. Six categories of diagnostic criteria and tools are distinguished: clinical signs and symptoms, bioclinical parameters, and parasite, antibody, antigen and trypanosomal DNA detection tests. Parasite detection tests include the quantitative buffy coat technique, which is a promising new tool for blood examination, and bone marrow puncture. The reliability of antibody detection tests (CATT, ELISA and others) depends on the distinction between variable and invariable antigens. An innovative assay detecting antibodies to parasite enzymes has recently been introduced and a breakthrough is expected for the introduction of perfectly defined recombinant or synthetic antigens. Antigen detection tests are mainly ELISA systems using polyclonal or monoclonal antibodies to one or more invariable antigens. A sensitive test for specific trypanosomal DNA will probably be developed, although its applicability to field survey remains questionable. Reliable parameters for the meningoencephalitic stage of the disease are the increase of white blood cells, total protein and albumin and the presence of IgM and anti-trypanosome antibodies. At present no method can distinguish between relapse and reinfection.

patients and trypanosome cryostabilates, antigen preparations and experimental antisera are collected and distributed. Serodiagnostic test systems including immune trypanolysis, immunofluorescence, ELISA, CATT and card latex agglutination (CLAT) are also distributed. New test systems are developed and evaluated, serological tests are carried out on request, and advice, demonstrations and training are provided.

7799 Muynck, A. de, Henry, M.C., Mentens, H. and Nzaba, P., 1992. Développement d'un outil de dépistage des trypanosomés à *T. gambiense*, à l'aide d'un système de score basé sur des critères cliniques et épidémiologiques. [Development of a tool for detecting trypanosomiasis due to *T. gambiense* with the aid of a score system based on clinical and epidemiological criteria.] (Meeting abstract no. 58.) *Annales de la Société belge de Médecine tropicale*, 72 (Suppl. 1): 109-110. (See 16: no. 7766.)

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Trypanosomiasis detection programmes using mobile equipment are restricted for economic and logistic reasons and lack of support. An alternative method of effective diagnosis is essential. A score system using epidemiological and clinical criteria has been elaborated, based on data collected at Kwamouth, Zaire, from 2357 people of whom 118 were trypanosomiasis patients. Each criterion was analysed statistically and the following were shown to be significant: age, general condition of the patient, foot pains, pruritis, character change, apathy, Winterbottom's sign and sleep disturbance. These significant criteria were weighted according to their sensitivity, specificity and positive predictive value. Use of a score card based on these criteria by primary health care centres would provide an efficient approach to diagnosis.


In north-west Uganda the prevalence of African human trypanosomiasis reaches 30% in some areas and repeated screening of whole populations is carried out. A survey of former trypanosomiasis patients showed that about half the individuals who were seen 24-36 months after treatment remained CATT-positive, excluding cases...
of relapse and reinfection. This suggests that CATT screening in areas where a proportion of the population has already been treated might increase the number of false-positive cases and lead to an overestimate of the prevalence rate.


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A recently described simple kit for isolating African trypanosomes in vitro (KIVI) was tested further with blood samples from man and other animals in Côte d'Ivoire and République du Congo. A high rate of success was achieved, with positive cultures being found 5-36 days after inoculation. The method was also of value in diagnosis. Parasitaemia was initially detected by the haematocrit method; in addition, the mini-anion exchange column was used for human blood, and lymph fluid from patients with swollen glands was examined. The card agglutination test (CATT) was applied to the human blood samples. In Côte d'Ivoire, all five parasitaemic patients, who were also positive by CATT, yielded positive KIVI cultures. Of 15 animals, two parasitaemic and ten apparently aparasitaemic individuals gave positive cultures. In the Congo, none of the 22 animals was parasitaemic and none gave a positive culture. Of 647 human subjects initially screened, 61, mostly with a positive CATT, were examined by KIVI; 20 gave positive cultures. Seven of these cultures originated from patients in whom no trypanosome had been seen in blood or lymph fluid, although blood from two parasitaemic patients failed to yield positive KIVI cultures. Some patients with CATT-negative whole blood and/or serum were positive by KIVI.

(b) PATHOLOGY AND IMMUNOLOGY


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Improvements were made in the immunodetection of anti-galacto-cerebroside (anti-GalC) antibody in sera of patients with human African trypanosomiasis by thin-layer chromatography, enzyme-linked immunosorbent assay and immunoadsorption. Rabbit anti-GalC antibodies were used to standardise these techniques and demonstrate their specificity. Anti-GalC antibodies were found in the sera of 42.8% of 63 patients with human African trypanosomiasis. Thirty-four control subjects living in the same endemic area were also tested. Anti-GalC levels were higher in human African trypanosomiasis patients with neurologic disturbances compared with patients without such disturbances. These antibodies were distributed mainly between the IgG and IgM classes, but 28% of the patients with human African trypanosomiasis had increased IgA levels without anti-GalC antibody activity.


The trypanocidal activity of human plasma and its relationship to high density lipoproteins (HDL) have been investigated. The trypanolytic activity of normal plasma was greater than plasma from cirrhotic patients with low levels of HDL. Depletion of all or part of plasma apoA-I (the major protein of HDL) abolished or decreased the trypanolytic effect. Trypanocidal activity was associated with the denser and smaller particles of subfractionated HDL and with the water soluble protein (apolipoprotein) fraction. Purified apoA-I was trypanolytic but other apolipoproteins isolated from human HDL were non-trypanolytic. Baboon (a non-permissive host of T. b. brucei) apoA-I was trypanolytic though less potent than human apoA-1 but apoA-1 from permissive hosts (cattle and sheep) was inactive. Sera from transgenic mice expressing human apoA-1 showed trypanolytic activity in vitro but this was only moderately greater than control sera and the mice were fully susceptible to T. b. brucei infection, perhaps
because of an anti-trypanolytic effect of mouse apolipo-proteins. Although human apoA-1 exhibits trypanolytic activity, its identification as the main trypanolytic factor of human plasma remains equivocal.


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The clinical symptoms of a man who became ill 2 days after returning to the USA from a short visit to Tanzania are described. The illness commenced with the appearance of a painless red nodule on the site of an insect bite. Various possible diagnoses are discussed but none correctly identified the patient's illness except trypanosomiasis, which was confirmed by blood film examination. Methods for the laboratory diagnosis of trypanosomiasis are discussed, such as the examination of thick and thin blood films and buffy coats and centrifuged CSF. Serological tests are used in endemic areas but are not considered reliable for diagnostic purposes. The patient was successfully treated with five successive doses of suramin. The clinical symptoms of the disease and side effects of the drug throughout the course of treatment are described. White blood cell and platelet counts were made at intervals. Of particular interest was the extent of apparent immune complex disease manifested by thrombocytopenia, leucopenia, generalised oedema and perhaps headache. The vectors, transmission, epidemiology, pathology and treatment of human African trypanosomiasis are reviewed.

(c) TREATMENT
[See also 16: no. 7856.]


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A biological assay was developed for measuring melarsoprol in serum and CSF of patients with human trypanosomiasis.
African trypanosomiasis. Trypanosomes were cultivated in microtitre plates for 72 h with melarsoprol (Mel B) in concentrations of 1.25 µg/ml to 2.2 ng/ml. The minimum inhibitory concentration of Mel B for a reference *Trypanosoma brucei rhodesiense* clone was determined by micro-scopical examination. Samples of serum or CSF were incubated under the same conditions and the highest dilution determined which caused death of all trypanosomes. The melarsoprol concentration of the sample was then calculated using the sample dilution and the determined minimal inhibitory concentration of the trypanosome population used for the assay. The test was validated using a number of reference samples and it was used for melarsoprol determination in serum and CSF samples taken from two treated patients. A sample size of 100 µl was sufficient to perform the assay. The lower detection limit was 9 ng/ml (22.6 nmol/ml). The assay has potential for measuring other trypanocidal drugs in body fluids.


The suggestion that subcurative chemotherapy may be a cause of post-treatment reactive encephalopathy (PTRE) in mice infected with *Trypanosoma brucei brucei* and that patients require more aggressive treatment (see 15: no. 7571) is challenged. Subcurative drugs such as pentamidine and suramin sodium, which cross the blood-brain barrier poorly, and diminazene do not cause PTRE in humans and eflornithine (DFMO) rarely induces this condition. The murine model is also challenged: eflornithine monotherapy is not very effective in mice but is curative in over 90% of human patients with *T. b. gambiense* infection. The minimum effective dose of the highly curative drug melarsoprol, which frequently induces PTRE, is unknown and may be overcurative at current rates of administration; more research is necessary.


The efficiency of the recently developed drugs
nifurtimox and DFMO is reviewed, with reference to their adverse effects and potential for large scale use. A comparison is made with the arsenical drug melarsoprol which is still widely used and may have lethal side effects. The results of treatment regimes using nifurtimox and DFMO, with particular reference to trials in Zaire and Sudan, are described. It is concluded that both drugs are effective control agents for early and late stage gambiense sleeping sickness; neither can yet be recommended for the treatment of rhodesiense patients. The toxic effects of DFMO are within acceptable limits but its high cost and preferred i.v. administration with a recommended dose of 100 mg/kg every 6 h for 14 days means that it is unlikely to replace melarsoprol. Nifurtimox has practical advantages over DFMO, requiring an oral dose of 5 mg/kg three times a day for 14 days. There is evidence to suggest that melarsoprol, nifurtimox and DFMO may act synergistically when at least two are administered concurrently, with the potential for increased effectiveness, shorter treatment regimes and reduced toxicity.

6. animal trypanosomiasis
(a) SURVEY AND DISTRIBUTION


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Two enzyme-linked immunosorbent assays (ELISA), one based on a mouse anti-T. brucei group-specific monoclonal antibody and the other on rabbit anti-T. evansi polyclonal antibodies, have been evaluated for their ability to detect circulating trypanosome antigens in camel sera as a means for the diagnosis of T. evansi infections. All 91 sera from a negative control camel herd from Kenya gave negative antigen-ELISA results in the monoclonal antibody-based ELISA and only two of them (2.2%) gave false positive results in the polyclonal antibody-based ELISA. In subsequent analyses of sera from infected camels (as determined by mouse inoculation), the monoclonal antibody-based ELISA
detected antigens in 90 (83.3%) out of the 108 sera tested. This percentage was lower for the polyclonal antibody-based ELISA which was able to detect antigens in 67 (60.9%) out of the 110 sera tested. The two tests detected probably different antigens and when the results were combined, 99 out of 107 (92.5%) sera were shown to be ELISA positive. In a survey involving 316 camels from the Gao and Nara areas, in Mali, a high proportion of animals tested were antigen positive (43.5 and 42.9%, respectively for the mono- and polyclonal antibody-based ELISA) compared to only 22 (7.0%) diagnosed by the parasite detection techniques. Thus, these immunoassays were at least six times more sensitive than the haematocrit centrifugation technique. As a large proportion of cases may be antigen positive but parasite negative, these two surra immunoassays should be used in routine diagnosis in addition to the parasite detection techniques in the dromedary camel.


Nitcheman, Lemesre, Grebaut, Cavaleyra, Frézil: ORSTOM, Centre de Montpellier, B.P. 5045, 34032 Montpellier Cedex, France; N'Dokoue, Le Gall, Blanc, Gouteux, D’Amico: ANDE, B.P. 1509, Bangui, Central African Republic; Nantulya: ILRAD, P.O. Box 30709, Nairobi, Kenya; Cuisance: CIRAD-EMVT s/c ORSTOM, Centre de Montpellier, B.P. 5045, 34032 Montpellier, Cedex, France.

Animal trypanosomiasis causes serious losses in production, accounting for some 3.9 thousand million francs CFA each year in the Central African Republic. Specific monoclonal antibodies against non-variable antigens of Trypanosoma congolense, T. vivax and T. brucei have been developed at ILRAD and tested at ORSTOM using the ELISA technique on bovine sera from the Central African Republic. A preliminary study of 606 sera from Zebu
cattle demonstrated the superior sensitivity and specificity of this technique compared with classic parasitological methods. The latter showed 12% of the animals to be infected and only 4% to carry two species of trypanosome, compared with serological results of 67% and 66% respectively. The rate of multiple infection by all three trypanosome species was shown to be 31% by the serological method, as opposed to 0% by parasitological methods. The preliminary results are encouraging and large scale trials will be carried out.


A total of 183 camels from Kenya were examined for circulating trypanosomal antigens by four methods: (1) a monoclonal antigen-detection enzyme-linked immunoassay (Ag-ELISA) and circulating anti-trypanosomal antibodies; (2) antibody-detection enzyme-linked immunoassay (Ab-ELISA); (3) buffy coat examination (BCE); (4) mouse subinoculation (MI).

Thirty-seven camels (20%) were parasite-positive by BCE and 60 camels (33%) were parasite-positive by MI. Sixty-three camels (34%) tested positive on Ag-ELISA. Of the 24 camels which could not be detected by BCE, Ag-ELISA detected 18 (75%). Ab-ELISA detected 90 (49%) positive camels. Of all the parasite-positive camels (61), Ag-ELISA detected 93% and Ab-ELISA 95%. Based on the results of 55 camels, there was a significant statistical difference ($P < 0.0001$) in Ag-ELISA optical density (OD) values (of either serum or plasma antigen analysis) between parasite-positive and parasite-negative camels. No significant difference was observed in Ab-ELISA OD values between parasite-positive and parasite-negative camels. Diagnosis of *T. evansi* infection in camels by the use of Ag-ELISA alone or in combination with BCE could therefore be a more preferred approach in assessing patent infection than the use of Ab-ELISA.

A survey was carried out to determine the incidence of trypanosomes in Fulani Zebu cattle in Anambra State that had been established in the area for at least 3 years. It is recommended that the microHCT method be used routinely for screening cattle. This method gave an average 16.7% incidence from four sites which can be compared with incidences of 1.3% and 7.6% from northern Nigeria. The incidence of bovine trypanosomiasis is therefore significantly higher in the south, where tsetse flies are more frequent, but the cattle appear healthier and more productive than their counterparts in the north. This apparent resistance to infection is probably enhanced by good nutrition. The main constraint on cattle productivity in the south appears to be land shortage rather than the presence of tsetse flies.

(b) PATHOLOGY AND IMMUNOLOGY
[See also 16: nos. 7825, 7827.]


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Five trypanotolerant N'Dama (*Bos taurus*) and five susceptible Boran (*Bos indicus*) cattle were challenged by tsetse flies infected with *T. congolense* IL 13-E3. These animals had experienced five previous infections with *T. congolense*, each terminated by drug therapy. Immunoblotting and ELISA were used to determine isotype and specificity of antibody responses to trypanosome invariant antigens. Both IgM and IgG1 were elicited, but the IgG1 responses were directed against a greater diversity of antigens. A 69 kD antigen was the major invariant antigen which elicited IgM antibodies in both breeds, but the N'Damas also responded with high levels of specific IgG1. Analysis of isotypic responses to whole trypanosome extract also revealed lower levels of IgG1 and higher levels of IgM in the Borans than in the N'Damas, suggesting that a dysfunction in the switch from IgM to IgG might occur in infected Boran cattle. A 33 kD antigen appeared to elicit only IgG1. Sera from all five N'Damas and the two Borans which were most resistant to the disease reacted with this antigen.
prior to and following reinfection. Furthermore, during the primary *T. congolense* infection in the same animals, anti-33 kD antibodies were detectable in all five trypano-tolerant N'Damas, but in none of the five susceptible Borans. Thus, the presence of antibodies to the 33 kD antigen to *T. congolense* appeared to be associated with a capacity to control the disease.


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The various facets of resistance to infection that are mediated by innate and acquired mechanisms of immunity are reviewed. Three sets of genes govern host response to infection: those which control innate immunity, those which determine the specificity of acquired immune responses and those which affect the quality of acquired immunity. The relative virulence of *Trypanosoma brucei* in mice is discussed as an example of innate immunity. Studies on trypanosome subclones selected for different degrees of virulence suggest a complex interplay between host and parasite, with dominant mouse genes controlling antibody-mediated clearance of parasitaemia segregating independently of recessive genes controlling resistance to infection lethality. In this case parasite-specific antibody responses were not directly associated with host resistance.

7814 **Haeren, C. van, Baderha, B., Büscher, P. and Verhulst, A. 1992.** Total haemolytic complement activity and immunoconglutinin levels in sera of goats infected with *Trypanosoma brucei brucei*. (Meeting abstract no. 40.) *Annales de la Société belge de Médecine tropicale,* 72 (Suppl. 1): 99. (See 16: no. 7766.)

Laboratory of Serology (Haeren, Baderha, Büscher) and Laboratory of Zootechnics (Verhulst), Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.

Six Saanen goats were subcutaneously infected with one VAT and six others with a mixture of five VATs of *T. brucei*. Sera were collected from 7 days pre-infection to 42 days p.i. Parasitaemia remained low in all cases but the infection showed morbid development. The
haemolytic complement activity (expressed as CH50 units/ml) via the classic (CPW) and alternative (APW) pathways was studied. There was a definite decrease of APW haemolytic activity from 10 days p.i. in both groups of goats but this did not fall to zero. The CPW haemolytic activity also decreased from 10 days p.i. and fell to zero before 40 days p.i. in all cases. A steady increase in the conglutination reaction began in most cases from 10 days p.i. and continued during the period of observation.


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The course of *Trypanosoma congolense* infection in Scottish Blackface sheep was followed for 96 days. Infected animals developed fluctuating parasitaemia, macrocytic normochromic anaemia and leucocytosis which was principally a lymphocytosis. Following treatment with the trypanocidal drug, diminazene aceturate at 84 days after infection, the haematological values returned to normal within 12 days. Infected sheep developed hypocholesterolaemia and hypophospholipidaemia leading to a reduction in total serum lipids. This study has shown that sheep infected with *T. congolense* develop anaemia, the onset of which follows the first wave of parasitaemia. The changes in blood lipids observed in infected sheep appeared to be related to the intensity and duration of parasitaemia.


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An epidemiological survey was conducted in The Gambia from November 1987 until October 1989 on trypanosomosis in N'Dama cattle raised in areas of low and high tsetse infestation, with the purpose of determining some production parameters of this breed under the traditional management system and the factors influencing them. The monthly births, mortalities, weight changes and strongyle egg excretion are
presented, together with the haematocrit and monthly and yearly prevalence of trypanosomosis. The authors conclude that profitable animal husbandry is possible with N'Dama cattle in such a trypanosome-infested region but the returns depend on feed availability rather than on trypanosomosis prevalence. Better results are obtained with enforced control of bushfires and more comprehensive herd management, including selective culling, deworming and feed supplementation in so far as economic conditions allow.


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The effects of trypanosomiasis on sheep and goat production in East and West Africa are briefly reviewed. Small ruminants may not show clinical symptoms but experiments and field surveys have shown them to be fully susceptible to infection and the economic impact of trypanosomiasis on these animals has been shown to be substantial. Pathogenic effects include anaemia, immunosuppression, retarded growth, weight loss and poor reproductive performance. Sheep infected with Trypanosoma vivax and T. brucei showed significant decreases in semen volume, sperm counts and sperm viability which may be associated with severe testicular degeneration, itself resulting from changes in levels of plasma testosterone. In female goats infected with T. congolense luteal dysfunction resulted in persistent corpora lutea, reduced plasma progesterone concentrations and alterations in oestrous cyclicity, with degenerative changes in the ovaries, pituitary and thyroid glands. Some West African Dwarf breeds, such as Djallonke goats, show a degree of trypanotolerance comparable to that of N'Dama cattle and some East African sheep also show trypanotolerance. However, the development of trypanotolerant breeds may be limited by ecological conditions.


KETRI, P.O. Box 362, Kikuyu, Kenya; University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK; ibid.; ibid.

When beagle dogs were infected with T. brucei, a marked reduction in the plasma concentration of atrial
natriuretic factor (ANF) occurred in the terminal stage of the disease during weeks 3 and 4. At the same time there was an increase in plasma renin activity (PRA) after infection. Ultrastructural studies of the atria of these dogs demonstrated a reduction in ANF granules. The changes in ANF and PRA occurred in association with severe pancarditis and the development of heart failure. By impairing the ability of the heart and kidneys to regulate blood volume, the alterations in ANF and PRA could be involved in the pathogenesis of heart failure in *T. brucei*-infected dogs.


KARI, P.O. Box 274, Uthiru, Nairobi, Kenya.

Four male East African goats were i.v. infected with *Trypanosoma evansi* and observed for 60 days p.i. All four animals showed a drop in PCV, fluctuations in parasitaemia and loss of weight. Direct microscopy showed the presence of trypanosomes in synovial and peritoneal fluid and CSF, and also in lymph node fluid after mice subinoculation. Two of the goats died and pathological changes included necrotic foci in the liver, kidneys and lymph nodes, and bronchopneumonia. It is concluded that goats may act as reservoir hosts for *T. evansi* infecting camels.

7820 Olubayo, R.O., 1992. Changes of peripheral blood leukocytes subpopulation in African buffalo and N'Dama cattle following tsetse transmitted *Trypanosoma congolense* infection. (Meeting abstract no. 39.) *Annales de la Société belge de Médecine tropicale*, 72 (Suppl. 1): 98. (See 16: no. 7766.)

KARI, P.O. Box 274, Uthiru, Nairobi, Kenya.

The prepatent period in three African buffaloes infected with tsetse-transmitted *T. congolense* was 15–25 days, compared with 10–11 days in three N'Dama cattle. Both species were able to control parasitaemia. The buffaloes showed mean parasite counts of $10^7 - 10^8$/ml of blood whereas the N'Damas had counts of $5 \times 10^7 - 5 \times 10^8$/ml. A moderate drop in PCV was observed in both species during the first 40 days p.i., after which the buffaloes maintained their PCV at pre-infection levels while in the N'Damas PCV was maintained at 28% below the pre-infection level. Analysis of lymphocyte subpopulations using a panel of monoclonal antibody markers and fluorescence activated cell sorter showed a significant increase of myeloid (CD11b) cells in the
buffaloes. These cells did not occur in the N'Dama cattle and may be responsible for the control of parasitaemia in buffaloes.


Marked changes in lymphocyte populations were found to occur in sheep infected with *T. evansi*: the number of B cells increased from 22 days p.i. and the numbers of lymphocytes expressing CD4 and CD8 antigens decreased. The proportion of B cells expressing CD5 antigen increased from 5% to over 90% later in infection. Analysis of lymphocyte populations from sheep immunised with *P. haemolytica* showed differences in response between *T. evansi*-infected and uninfected animals. Uninfected animals showed an increase in CD5⁺ T cells, CD4⁺ cells and B cells, CD8⁺ lymphocytes showed little change and the CD4/CD8 ratio remained high. In infected sheep, the number of CD5⁺ cells decreased and there was no increase in B cells, although there was an increase in immature T cells expressing CD4 and CD8 simultaneously. These changes may be responsible for the impaired immune responses seen in *T. evansi*-infected animals.


National Animal Production Research Institute, Ahmadu Bello University, P.M.B. 1096, Shika-Zaria, Nigeria.

Twelve Yankasa rams aged between 2.5 and 3 years with good semen characteristics were used in this 15-week study. Six rams were infected with *T. vivax*, while six served as controls. The infected rams developed chronic trypanosomosis accompanied by fluctuating pyrexia, lethargy, anaemia, scrotal oedema and cachexia. There was a drastic and progressive deterioration in semen quality in all infected rams manifested by a decrease in volume or cessation of semen production, oligozoospermia, a sharp decrease in progressively motile sperm, elevated numbers of dead (eosinophilic) sperm and 100% morphological abnormalities of sperm in most animals. The rams were all deemed unfit for breeding by 3 weeks p.i.
Uninfected rams were healthy and had good semen characteristics throughout the investigation. The results show that rams infected with *T. vivax* may become infertile within a short interval due to rapid deterioration of semen characteristics and this trypanosome species may be an important causative agent of infertility in endemic areas.

(c) TRYPANOTOLERANCE

[See also 16: nos. 7768, 7816, 7827.]


CIRAD-EMVT, CRAAG, B.P. 1232, 97184 Pointe-à-Pitre Cedex, Guadeloupe; CRTA, 01 B.P. 454, Bobo-Dioulasso 01, Burkina Faso; ibid.; ibid.

This study was made on 1016 cattle of the Baoulé taurine breed (*Bos taurus*) renowned for their trypanotolerance. Entomological, parasitological and immunological surveys were carried out to verify the levels of tsetse and trypanosome challenge and their relative impact in the different ecosystems encountered. Socio-economic, epidemiological, therapeutic and zootechnical data confirmed that this Baoulé population was highly trypanoresistant. This characteristic is genetically determined but proportionately maintained by the intensity of natural field pressure. Immunogenetic research was carried out within several systems, firstly to determine the genetic situation of this population (stabilised or not) and secondly to identify breed markers and/or markers that correlate with pathological and zootechnical data. Results from these studies will be published later.


ILCA, P.O. Box 46847, Nairobi, Kenya; ibid.; Department of Veterinary Medicine, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK.

Trypanotolerance in N’Dama cattle is reviewed with reference to livestock performance and the genetics of trypanotolerance traits. Some N’Dama cattle are more
resistant to trypanosomiasis than others and the degree of resistance can be assessed by measuring the PCV of infected animals. This simple procedure indicates the extent of anaemia, the control of which is a factor of trypanotolerance. Heritability estimates for this factor are high and there are positive correlations with production traits. The method enables young cattle with increased resistance to trypanosomiasis to be identified and used in breeding programmes to improve both productivity and disease resistance.


One thousand and twenty-eight cow-year records were available from 260 N'Dama cows each having at least 2 years of monthly matching health and performance data over a 5-year period under a medium natural tsetse challenge in Gabon. Four hundred and fifty-eight calf/dam pairs were also available where the calf had been reared to weaning, both had monthly matching records and each cow had weaned at least two calves. Evaluations were carried out on effects of, and linkages between, environmental and stress factors, number and species of trypanosome infections, curative drug treatments given, anaemia measured by PCV, and performance measured by calf weaning weight, cow calving rate and cow weight change over the lactation period. Major findings were that over the period from calf birth to weaning, while calves and their dams grazing together had similar numbers of trypanosome infections detected, the *Trypanosoma vivax*: *T. congolense* ratios were very different: 1:0.7 in calves; 1:2.8 in cows. This indicated that some ability to control the development of parasitaemia following *T. vivax* infection might be being acquired, from weaning onwards. In cows, relationships between lowest PCV recorded and curative drug treatments given suggested that between 20 and 32% of trypanosome-infected cows were not being identified by the buffy coat parasitological diagnostic technique. The high level of curative treatment given (to 13.7% of cows over the calendar year, and to 40% of...
calves from birth to weaning) will have tended to reduce the variance and linkages between aspects of infection and PCV values, especially in calves. In calves, the influence of trypanosome infections, in both calf and dam, on their respective PCV values and hence on calf weaning weight was apparent. There was a 0.91 ± 0.40 kg increase in calf weaning weight for each 1% increase in calf average PCV, and a 0.95 ± 0.39 kg increase for each 1% increase in cow average PCV. In cows, there was a similar pathway of influence of *T. congolense* infection through the PCV values to calving rate which was not significant with *T. vivax* infection. There was a 3.3 ± 0.65% increase in calving rate for each 1% increase in average PCV. Repeatabilities of performance traits were in the normal range. Repeatabilities of numbers of trypanosome infections detected by the buffy coat technique were too low to have any practical significance. Repeatability of average PCV at 0.40 ± 0.03 could allow PCV when infected to be used as one criterion of trypanotolerance.


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The genetic improvement of livestock resistance to disease depends upon studies of laboratory model systems. A range of such host–parasite systems, selected to cover the main groups of parasites responsible for disease in farm animals, is reviewed. Murine systems have contributed greatly to the understanding of trypanotolerance and genetic variation in response to infection. Studies with *Trypanosoma congolense* have shown that resistance in mice is controlled by a single gene. Resistant strains produce variant antigen-specific IgM antibodies earlier in infection than susceptible ones, which may show a preferential switch to IgG isotypes. However, work with *T. brucei rhodesiense* indicates that whereas isotype-specific responses may correlate with the ability to control parasitaemia, they do not correlate with ultimate survival. Differences in cytokine release or, with *T. b. brucei*, parasite-induced immune suppression operating against B cell function may be involved.

(d) TREATMENT

Trypanosomose et traitements trypanocides chez le veau Ndama en milieu fortement infesté de glossines (ranch de Madina-Diassa au Mali). [Trypanosomosis and trypanocidal treatment in N'Dama calves in an area of high tsetse infestation (Madina-Diassa ranch, Mali).] Revue d'Élevage et de Médecine vétérinaire des Pays tropicaux, 45 (2): 155-161.
Laboratoire Central Vétérinaire du Mali, B.P. 2295, Bamako, Mali.
This work aims at contributing to the knowledge of trypanosomosis epidemiology in calves of trypanotolerant breeds and at defining an appropriate treatment to improve the survival of such calves in a tsetse infested area. The first study was a parasitological and clinical survey of 100 calves from the day of birth to the age of 1 year. According to the results of this survey, the period from birth to 3 months is a 'critical' period in the life of the calves, due to a high infection rate and mortality related to trypanosomosis. The purpose of the second study was to investigate the possible interference of early trypanocidal treatments with the later expression of trypanotolerance. For this purpose three groups of animals over 1 year old were established. The groups had different trypanosomosis history due to the different treatments they had undergone during their first year of life. All the animals were exposed to trypanosomosis without treatment and followed up parasitologically and clinically during the second year. The results showed no interference of early trypanocidal treatments (including preventive ones) with the expression of resistance in potentially trypanotolerant animals.

Departments of Veterinary Medicine (Otesile), Veterinary Micro-biology and Parasitology (Fagbemi, Akinboade) and Veterinary Physiology and Pharmacology (Makinde), University of Ibadan, Ibadan, Nigeria.
Growing pigs were placed on feeds with high (group A), medium (B) and low (C) dietary energy and were infected with a virulent stock of T. brucei. Eight weeks later, the infected pigs were treated with isometamidium chloride at 1 mg/kg live weight and all pigs were subsequently placed on a high energy diet to
investigate their response to therapy. Clearance of *T. brucei* from blood was completed 72 h after treatment. There was no evidence of relapsed infection up to 8 weeks after treatment. Red blood cell parameters returned to normal 4-6 weeks after treatment with responses being fastest in group A. Eight weeks after treatment, pigs in groups A, B and C had gained about two-thirds of the live weight gains of their non-infected pair-fed controls. It appears that the retarded weight gain as a result of the infection persisted after therapy since drug-treated pigs did not gain as much weight as their non-infected controls.


The trypanocidal activity of an arsenical compound (RM 110; mel Cy; Cymelarsan) was evaluated against *T. b. brucei* and *T. b. evansi* in cultures, in goats and in pigs. The trypanosome stocks used differed in their levels of susceptibility to Cymelarsan in an *in vitro* test, their IC$_{50}$ values (drug concentration which inhibits growth by 50%) ranging from 4.8-5.1 nM for susceptible, and 26.9 nM for a resistant stock. Goats infected with a susceptible *T. b. evansi* stock were cured after a single injection of 0.3 mg/kg Cymelarsan. In three out of four goats chronically infected with the same stock a single injection of 0.625 mg/kg Cymelarsan effected a cure, whereas the goat in which the infection relapsed was finally cured after injection of 0.625 mg/kg on each of three consecutive days. A single dose of 2.5 mg/kg did not cure goats infected with an arsenical-resistant *T. b. brucei* stock. One of two pigs chronically infected with arsenical-susceptible stocks of *T. b. brucei* was cured after a single injection of 0.625 mg/kg Cymelarsan, whereas the other one relapsed and died. In conclusion, the results may indicate that the dose of 0.25 mg/kg recommended by the manufacturer is too low and that a single injection may not cure animals with CNS involvement with certainty. The recommended dose might therefore have been applied strictly for the treatment of camels only.
7. experimental trypanosomiasis

(a) DIAGNOSTICS

Identification of *Trypanosoma brucei gambiense* by PCR.
(Meeting abstract no. 51.) *Annales de la Société belge de Médecine tropicale*, **72** (Suppl. 1): 105-106. (See **16**: no. 7766.)

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Antibody detection ELISA/*T. b. gambiense* using variable antigens. (Meeting abstract no. 36.) *Annales de la Société belge de Médecine tropicale*, **72** (Suppl. 1): 96-97. (See **16**: no. 7766.)

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Increased knowledge of VAT diversity in *Trypanosoma brucei gambiense* suggests that two or three antigens should be combined for a full range ELISA test. Semi-purified antigens of LiTat 1.3 and 1.6, skimmed milk powder for blocking aspecific protein binding, peroxidase conjugated anti-human IgG, ureumperoxide as substrate and ABTS as chromogen were used for an updated test version in microtitre plates. Sera from 172 *gambiense*-infected patients and 234 control sera were tested separately with either antigen. At OD cut-off values of 0.3 and 0.4 specificity was 99.5% and 100% respectively. Corresponding overall sensitivity was 98.2/95.3% for LiTat 1.3 and 91.8/83.1% for LiTat 1.6. The combined results at the same cut-off values were 99.1/100% specificity and 99.4/97.6% sensitivity. A reliable assay using mixed antigen preparations is being developed.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **16**: nos. 7813, 7826.]

Endotoxins and the pathogenesis of *T. b. brucei* infections in mice. (Meeting abstract no. 33.) *Annales de la Société belge de Médecine tropicale*, **72** (Suppl. 1): 93-94. (See **16**: no 7766.)

Alafiatayo: Department of Biological Sciences, University of Salford, Salford M5 4WT, UK.

Prostaglandin D production by cultured mouse fibroblasts induced by *T.*
b. brucei products and endotoxin. (Meeting abstract no. 32.) Annales de la Société belge de Médecine tropicale, 72 (Suppl. 1): 92–93. (See 16: no. 7766.)

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Kennedy: University Department of Neurology, Southern General Hospital, Glasgow G51 4TF, UK.

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(c) CHEMOTHERAPEUTICS

[See also 16: nos. 7872, 7878.]
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CTVM, Easter Bush, Roslin, Midlothian EH25 9RG, UK.

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Department of Veterinary Physiology, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK.


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8. trypanosome research

(a) CULTIVATION OF TRYPANOSOMES
[See also 16: no. 7801.]
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(b) TAXONOMY, CHARACTERISATION OF ISOLATES
[See also 16: no. 7786.]
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(c) LIFE CYCLE, MORPHOLOGY, BIOCHEMICAL AND MOLECULAR STUDIES
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Laboratoire d’Immunologie et Parasitologie Moléculaire, Université de Bordeaux II, 146 rue Léo Saignat, F-33086 Bordeaux Cedex, France.

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Swiss Tropical Institute, Socinstrasse 57, Postfach, CH-4002 Basel, Switzerland.

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Department of Biochemistry, University of Bath, Bath BA2 7AY, UK.

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