SECTION A – NEWS

PROGRAMME AGAINST AFRICAN TRYPANOSOMIASIS

There have been a number of staff changes recently within the PAAT Secretariat. Chris Jenner, who developed the PAAT Information System, has now left FAO, and Anne Jackson has taken over as co-ordinator of PAAT-L (address now PAAT-Link@fao.org). Jan Slingenbergh has been promoted to Senior Officer, and recruitment for a second FAO Rome-based Animal Health Officer to assist the PAAT Secretariat is now in progress.

The EU-funded Concerted Action programme ‘Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV)’, which underpins much of the work of the Research and Development Module of PAAT, recently held a very successful workshop at ITC (see p. 56). The outputs of the previous ICPTV Workshop on ‘Trypanocidal Drugs and Drug Resistance’ have been circulated through PAAT-L and it is hoped that the broad consensus achieved on tests for drug resistance will shortly be published.

A current issue of discussion within PAAT is how the established networks and activities of PAAT may be incorporated into a new global initiative on trypanosomiasis which is being prepared for presentation at a meeting of the Global Forum for Agricultural Research (GFAR) in Dresden, Germany, in May 2000. GFAR’s mission is to ‘mobilise the world scientific community in their efforts to alleviate poverty, increase food security and promote the sustainable use of natural resources’ (see http://www.egfar.org).

Another initiative, which is closely linked to PAAT, is the Concept Note being prepared by the OAU/IBAR Office in Nairobi for a potentially large EU-funded programme in West and Central Africa. The proposal builds on one of the areas of priority for control identified under the PAAT Plan of Action.

NEW APPOINTMENTS

ISCTRC Executive Committee: Francis Oloo

Francis Oloo was elected Chairman of the ISCTRC Executive Committee at its 25th meeting last October and will serve a two-year term. During his long career, which culminated in his appointment as the Head of the Kenya Tsetse Control Unit, he has participated in both national control and research activities, and now serves as the National Liaison Officer for the Kenya component of the EU-funded FITCA programme. As Chairman of the ISCTRC Executive Committee, he would welcome any views, requests and suggestions. He may be contacted through the IBAR Office, P.O. Box 66177, Nairobi, Kenya, or by e-mail at oloo@net2000ke.com.

CIRAD-EMVT: Emmanuel Camus

Dr Emmanuel Camus has recently been appointed Head of CIRAD-EMVT’s Animal Health Programme which is involved in trypanosomiasis research and development.

A new Joint Laboratory on Human and Animal Trypanosomiasis has recently been set up with IRD (previously ORSTOM), and has established close links with CIRDES (Burkina Faso), ILRI (Nairobi), IPR (Côte d’Ivoire) and OCEAC (Cameroon). Joint
activities will concentrate on four main topics: identification of vectors and pathogens; inter-relationships between hosts, vectors and parasites; management of risk areas; and identification of practically feasible strategies for disease prevention and control. The team comprises 12 full-time scientists and four full-time technicians from CIRAD and IRD. The laboratory is located in CIRAD-EMVT under the direction of Dr Gérard Cuny of IRD.

For further information contact: Dr Emmanuel Camus, Chef du Programme Santé Animale, CIRAD-EMVT, Campus International de Baillarguet, B.P. 5035, 34032 Montpellier Cedex 1, France (camus@cirad.fr).

**PROGRESS REPORT ON FITCA PROGRAMME**

Within the EU-funded Programme ‘Farming in Tsetse Control Areas of Eastern Africa’, Kenya has commenced work in several of the main areas of activity. The project is based in Western Kenya in order to link and co-ordinate with similar actions being undertaken in the corresponding border area of Uganda, the objective being to improve human welfare through the promotion of livestock production and the development of integrated crop/livestock farming systems. The main activities during the first year will be to concentrate on the collection of baseline data and to establish the required operational infrastructure.

Tsetse surveys are under way to establish the density and distribution of the main vector, *Glossina pallidipes*, and also to investigate the possible involvement of *G. fuscipes* in disease transmission. Supporting surveys for trypanosomiasis, in humans and livestock, have established *Trypanosoma vivax* as the main infection in animals and have also found an early stage case of sleeping sickness in the Busia District.

A range of strategies for implementing the programme and training the affected rural communities are under consideration for delivery through a partnership arrangement with other trainers and private health care providers active in this area.

A Participatory Rural Appraisal, carried out in five districts, has shown that poverty levels are greater than was anticipated and that livestock conferred greater wealth on owners. This will be followed by the collection of socio-economic and livestock production data to assess the economic status of the various components of the communities.

Extensive efforts have been put into creating a local awareness of the project’s objective, purpose and activities. At the same time, improved breeds of livestock are being introduced in order to train farmers on the husbandry required to maintain these animals under the challenge posed by trypanosomiasis.

More information on these activities is available from Francis Oloo at oloo@net2000ke.com.

**TRYPANOTOLERANCE WORKSHOP AND LIVESTOCK IN WEST AFRICA**

**ICPTV Workshop**

The workshop was attended by more than 30 scientists, research planners and policy makers from research institutes, international and regional organisations in 13 West and East African and European countries. Themes covered in formal presentations and discussion sessions included: Socio-economic and cultural aspects of the use of trypanotolerant livestock; characterisation of trypanotolerance using quantitative and molecular approaches to its exploitation and enhancement; breed characterisation for resistance in genotypes other than West African trypanotolerant livestock; and novel strategies for the immunological control of trypanosomiasis. The workshop also reviewed progress since a major international meeting on trypanotolerance held in 1993 in Nairobi, and formulated recommendations for future research in this area, based on scientific progress made in the interim, and changing donor priorities and policy. Avenues for seeking funding for the proposed research were also discussed.

The summary conclusions and recommendations of the workshop will be made available shortly over PAAT-L, the ICPTV website (PAAT/ICPTV common home page http://www.fao.org/paat/default.html), and in printed format. For further information on ICPTV, contact Dr Mark Eisler, ICPTV Co-ordinator, University of Glasgow, c/o ILRI, P.O. Box 30709, Nairobi, Kenya (tel./fax +254 2 631499; e-mail m.eisler@cgiar.org).

ITC and trypanotolerant livestock in West Africa

The International Trypanotolerance Centre recently hosted an ICPTV Workshop on the mechanisms of acquired and genetic resistance to trypanosomiasis (see report above). One of the reasons for holding the workshop in The Gambia was the fact that the national domestic ruminant stock is composed almost exclusively of N’Dama cattle, West African Dwarf goats and Djallonké sheep, known to be trypanotolerant, and ITC was selected because of its long experience in the field of research on trypanotolerance. It is scientifically and internationally recognised that this tolerance significantly contributes to their productivity of these breeds in tsetse-infested zones. The Gambian Authorities for Livestock Development address priorities and concerns for guaranteeing the conservation of this disease resistance trait as an integral part of a sustainable development tool for the livestock industry. However, trypanotolerance is not an absolute feature and its mechanism is not yet fully elucidated. Even N’Dama cattle suffer from trypanosomosis when submitted to high tsetse pressure.

The rapid growth of human population drives an increased demand in animal products, mainly meat and milk, and deserves increasing productivity of the local animal genetic resources. Therefore, breeding programmes aiming to improve the performance of the national stock are of high priority. This is a long-term effort and additional measures should be taken to satisfy the immediate needs of the expanding population. Cross-breeding programmes using upgraded exotic breeds could provide a short- to medium-term solution. However, it has to be kept in mind that interactions between natural and/or acquired resistance to the local pathological environment, nutrition and animal management exist. Therefore, both pure breeding and cross-breeding programmes should also aim at conserving a large degree of the disease resistance present in the indigenous breeds.

The understanding of the mechanisms of acquired and genetic resistance will help to better exploit the local breeds with a view to improving economically sustainable development of the local animal industry for the benefit of rural populations in West Africa.
KENYA TRYPANOSOMIASIS RESEARCH INSTITUTE

The KETRI/DFID-initiated Technology Transfer programmes are scheduled to begin in early 2000. Funds are being allocated to the Intermediate Technology Development Group (ITDG) to enhance community-based tsetse control in the Makueni and Kajiado Districts. The programmes are sponsored by DFID, through KETRI, for three years, and KETRI will monitor and evaluate their progress.

The December 1999 issue of the KETRI Newsletter covers, as well as the topic above, a commentary on the use of trypanocidal drugs; a survey of pastoralists’ knowledge of tsetse and trypanosomiasis control technologies; studies on a *Trypanosoma suis* isolate; trypanoresistance of the Orma Boran breed and the Orma/Zebu cross; and strengthening of camel research. Further details are available from: KETRI, P.O. Box 362, Kikuyu, Kenya (tel. +254 154 32960-4; fax +254 154 32397; e-mail ketri@net2000ke.com; http://www.net2000ke.com/ketri/default.htm).

DFID REVIEW OF THE UK’S TRYPANOSOMIASIS PROGRAMME

In 1998 the Livestock Production and Animal Health research programmes of the UK Department for International Development (DFID) commissioned a review of research related to tsetse and trypanosomiasis that had been funded by the UK government since 1980. The resulting analysis is published in three volumes with the overall title: *DFID-Funded Tsetse and Trypanosomiasis Research and Development since 1980*. Volumes 1 and 2 have already been published and volume 3 will be published by the end of May.

*Volume 1 – Scientific Review* presents five case studies on Zimbabwe, Kenya, The Gambia, Remote Sensing/Geographical Information Systems and The Trypanosome. Based on these studies, the effectiveness of the UK’s research is analysed by a panel of international scientists.

*Volume 2 – Economic Analysis* analyses the effectiveness of not just the UK but the whole international research effort and also predicts the costs and benefits of tsetse control on a continental scale. This analysis has already been presented to recent PAAT meetings.

*Volume 3 – Summary of Projects* contains summarised details of 112 projects that have been funded by the UK since 1980. The details include project objectives, outputs, conclusions and costs as well as a list of references.

Copies of each volume are available from: DFID Livestock Production Programme, NRInternational Ltd, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK (free to individual scientists and scientific institutions associated with tsetse and trypanosomiasis).

A 16-page colour booklet entitled *Tsetse, Trypanosomiasis and Africa – The Year 2000 Report*, targeted at non-specialist opinion formers and decision makers, is also due for publication later this year.

As a follow-up to *Volume 2 – Economic Analysis* it is hoped that a more detailed analysis of the costs and benefits of tsetse control, using specifically selected regions throughout Africa, will be forthcoming.
SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)


ITC, P.M.B. 14, Banjul, Gambia.

Detailed study of six areas of The Gambia, having a total of about 4000 N’Dama cattle, suggested ways to enhance their productivity, particularly by the provision of supplementary feed. Disease problems included Trypanosoma congolense infection, despite the relative resistance of the breed to trypanosomiasis, and Haemonchus contortus infestation. (Various aspects of the authors’ work have been published in 32 journal articles.)


Hendrickx: Projet Régional de Lutte contre la Trypanosomose, B.P. 2034. Bobo Dioulasso, Burkina Faso. [hendrickx.vangorp@fasonet.bf]

This memoir has sections entitled: ecoclimatic variables measured from the ground and by satellite; distribution and density of tsetse (subsections on Glossina tachinoides, G. palpalis palpalis, G. morsitans submorsitans, G. longipalpis, G. medicorum and G. fusca fusca); epidemiology of bovine trypanosomiasis; cattle rearing; priority areas for action.


Joint FAO/IAEA Division, P.O. Box 100, A-1400 Vienna, Austria. [a.parker@iaea.org]

The second Seminar for Africa brought together 71 participants representing 24 nations, and several international agencies attended. The main focus was on tsetse SIT as part of an integrated area-wide approach, as well as aspects of tsetse biology relevant to SIT and mass-rearing. There were eight sessions devoted to: (i) Tsetse attractants (see 23:}
nos. 11349, 11353); (ii) Situation reports (nos. 11337-11339, 11354-11356, 11368, 11371); (iii) Tsetse-trypanosome interactions (nos. 11376, 11379); (iv) Tsetse genetics (nos. 11347, 11350, 11352); (v) Tsetse biology and biochemistry (nos. 11346, 11351, 11363, 11364); (vi) Prospects of tsetse SIT (nos. 11344, 11345, 11358, 11362); (vii) Tsetse SIT projects (entirely devoted to the eradication programme on Unguja Island, Zanzibar) (nos. 11361, 11367, 11370, 11373, 11374); and (viii) Disease diagnostics (nos. 11388, 11391). Each session of the seminar was followed by extended discussion of the presented papers. This was recorded by the session rapporteurs, and is given in their reports at the end of the Proceedings in conjunction with their own summary of each paper.


OAU/IBAR, P.O. Box 30786, Nairobi, Kenya.

Projects on farming in tsetse control areas in the Southern, Eastern and Western Africa Regions are at various stages of implementation with financial support from the European Union. Other donor agencies together with WHO are developing the Central African Regional Project, focusing special attention in those areas where sleeping sickness is still a major threat. Other activities of OAU/IBAR include regular meetings of the ISCTRC which are held every two years, training for medical, veterinary and entomological personnel in collaboration with other agencies, and dissemination of information. The OAU/IBAR office will be strengthened to enable it to play its role fully in co-ordination of the regional projects.

11339 **Keno Dassa, M., 1999.** Tsetse and trypanosomosis control in Ethiopia. *In: IAEA, 1999 (see 23: no. 11337), pp. 29-34.*

National Tsetse and Trypanosomiasis Investigation and Control Centre, P.O. Box 113, Bedelle, Illubador, Ethiopia.

Since the 1960s the magnitude of the problem has increased enormously and it is still increasing due to a number of factors which mainly include: (i) overpopulation and overstocking, (ii) the advance of tsetse flies into previously uninfested areas, and (iii) development of widespread drug resistance by trypanosomes. There are five economically important trypanosome species, *Trypanosoma brucei brucei, T. congolense, T. vivax, T. evansi* and *T. equiperdum.* Five tsetse species are distributed along the lowlands of western and south-western parts of the country; *Glossina morsitans submorsitans, G. pallidipes, G. fuscipes fuscipes* and *G. tachinoides* are the most important tsetse flies, whereas *G. longipennis* is of minor economic importance. The success achieved from small-scale tsetse control operations in the 1980s led to agreements with FAO, IAEA, UNDP, OAU/IBAR, the EU and the World Bank to conduct research and to extend sustainable tsetse control and agricultural development. Since 1994 the tsetse control operation has been totally dependent on government recurrent budget funding. Recent small-scale studies have demonstrated reductions in infection rates and higher animal yields following Spot-on deltamethrin applications with cattle. Work will be initiated with
IAEA/FAO assistance to establish Ag-ELISA capability in the NTTICC laboratory in Bedelle for monitoring the effectiveness of control operations in the upper Didessa valley and elsewhere, and to update the tsetse and trypanosomosis distribution maps and information on prevalence of trypanosomosis in Ethiopia.


Programme Conjoint OMS/UNICEF de Cartographie et de Gestion des Données en Santé Publique (HealthMap), WHO, 1211 Geneva 27, Switzerland.

An overview of the use of GIS in the control of tropical diseases is presented. Following an explanation of GIS, examples of their use are given using data from various Central and West African countries. In control programmes for human African trypanosomiasis, all villages visited by mobile teams in their survey areas are georeferenced using GPS and the information is integrated into a GIS and combined with historical and present epidemiological data. The epidemiological status of each village and the boundaries of foci can thus be clarified, and maps produced from these data make it easier to choose appropriate surveillance and detection strategies for each area.


Snow: 11 Newland Road, Banbury, Oxon OX16 8HQ, UK.

A technique for the rapid field assessment of African animal trypanosomosis (AAT) was developed during studies in The Gambia. This involved gathering indigenous information from rapid-appraisal questionnaires addressed to local informants, the results of single tsetse surveys and evaluations of the prevalence of trypanosome infections in village cattle. Local informants included livestock owners and herdsmen and trained personnel such as livestock assistants. The answers to the questionnaires were weighted in order to translate them into semi-quantitative ranked estimates (zero, low, medium, high or very severe) of the severity of AAT problems. A similar ranking was also defined for tsetse and prevalence data in The Gambia. The three assessment methods generally gave complementary results leading to similar conclusions about the severity of tsetse-trypanosomosis problems in a survey area; inconsistencies usually suggested that additional information was needed. The rankings of AAT intensity were used to develop management guidelines for minimising the impact of AAT at different levels through control interventions or improved livestock management. The methodology was designed to provide reliable, up-to-date and cost-effective assessments of AAT problems. Emphasis was placed on the importance of the involvement, priorities and perceptions of village livestock owners and herdsmen in making these assessments.
A study was carried out to quantify past outcomes of tropical pharmacology research and development (R & D) and to assess past benefits of the US Orphan Drug Act and potential benefits of the future European orphan drug regulation on tropical diseases. Of 1450 new chemical entities marketed between 1972 and 1997, 13 were specifically for tropical diseases and considered as essential drugs. Between 1983 and 1997, the US Orphan Drug Act approved 837 drugs and marketing of 152 new molecular entities (NMEs). Three NMEs have been designated for malaria and human African trypanosomiasis, and seven others, already commonly used in tropical diseases, received either orphan designation or an orphan approval for another indication. Pharmaceutical companies benefit from the US framework only when the US market exclusivity clause is applicable. Future European orphan drug regulation appears to be similar to the US Orphan Drug Act. It is concluded that the orphan drug programmes relating to rare diseases have met with some success. Considering tropical diseases as rare diseases seems inadequate to boost pharmaceutical R & D. However, some provisions of the European text may be relevant to tropical diseases, admitting the need for a more specific rule for evaluations of this kind of drug and recognising the existence of ‘diseases of exception’.


WHO, 20 avenue Appia, 1211 Geneva 27, Switzerland.

This report of the WHO Expert Committee on the Control and Surveillance of African Trypanosomiasis, which met in Geneva from 21 to 27 November 1995, reviews current epidemiological information on African trypanosomiasis and its vectors, and evaluates recent advances in drug treatment and the development of tools for the control and surveillance of the disease. Examples are provided of treatment schedules, vector control operations, indicators for monitoring control and surveillance activities and sample calculations for analysing the cost-effectiveness of different strategies. The report also gives methods for the cryopreservation of trypanosome-infected blood samples, and describes traps and screens for tsetse control. Although primarily addressed to health policy-makers in countries endemic for sleeping sickness, this report is also a useful reference source for health care staff at all levels, and for those engaged in research on the disease.
2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

[See also 23: nos. 11358, 11367.]


Djiteye: Laboratoire Central Vétérinaire, B.P. 2295, Bamako, Mali.

A technique for automatically marking adult G. austeni with fluorescent powder was assessed for the sterile insect project on Zanzibar. The technique using Day-Glo fluorescent powder was found to be a simple, cheap, efficient and discrete method with no deleterious effect on the sexual behaviour of flies or on the fertility of marked individuals. All G. austeni males marked with 1%, 0.5% and 0.25% Day-Glo (pink, orange) powder were identifiable at day 30 and day 37 post-emergence. Male flies marked with 0.5% Day-Glo appeared to be more competitive than mature controls of the same age (day 8 and day 15 post-emergence) and females marked with 0.5% pink Day-Glo fluorescent powder (copulated by marked males at the same dose) produced more pupae than non-treated females. Based on these observations a mixture of fine sand and Day-Glo powder in the proportion of 1 volume of mixture to cover 2 volumes of pupae is recommended for automatically marking adult flies during emergence. The proposed doses for the two tested colours (Rose Aurora and Orange Blaze) are 0.5% for identifications using stereo microscopy or UV lamp and 0.25% for the fluorescence microscope.


Opiyo: Entomology Unit, Agriculture and Biotechnology Laboratory, IAEA, Seibersdorf, Austria. [e.opiyo@iaea.org]

The progress made in tsetse fly rearing is now being used for eradication of G. austeni on the island of Zanzibar, Tanzania. In spite of these developments, the rearing system cannot be scaled up to an industrial level for the production of sterile tsetse flies. This is essential if area-wide application of the SIT is to be carried out, but the main constraints are labour-intensive activities and lack of standardisation through automation. At Seibersdorf an automated adult feeding and larval collection system with a capacity of 250,000 flies is being developed. Meanwhile, using the standard system, the holding capacity cages can be doubled from 100 to 200 flies per cage by use of inserts into the cages. Currently, adult sex separation is accomplished by chilling at emergence and again after mating. The requirement for sex separation after mating is being replaced by mating females at a ratio of 1 male to 4 females and not separating after mating. Using the differential developmental times of male and female pupae, it will be possible to have flies
of the required sex emerge directly into holding cages, thus eliminating the need for the first chilling. Further testing and evaluation of the system is continuing.


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Capacity methods are generally based on measuring of dielectric properties of matter (including living tissues) which are usually characterised by the dielectric constant. Since the cuticles of the tsetse fly pharate imago differ in morphological arrangement in the terminal abdominal segments (cuticle of males ‘folded’ four times due to the ventrally bent hypopygium; cuticle of females ‘folded’ only twice), we attempted to measure the dielectric constant (here expressed as capacity, measured in pF) of the posterior part of the puparium. The values obtained differ markedly in the male and female puparium (at least in *Glossina tachinoides*). However, it is very difficult to find a standard experimental procedure of measuring since the capacity methods used are extremely sensitive. Results can be strongly affected by the technical parameters of measuring (shape of electrodes, surrounding environment, RH, distance of the object from electrodes, etc.) and/or by the orientation of the individual male or female puparium (minimum differences in axes are necessary). Some preliminary experimental data on puparia of *G. palpalis palpalis*, *G. brevipalpis*, *G. fuscipes* and *G. tachinoides* are discussed and a measuring apparatus is suggested.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

[See also 23: no. 11376.]


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The application of recent advances in recombinant DNA/transgenic technologies provides approaches to improve the efficacy and reduce the cost of SIT for control of tsetse populations. The two potential applications of these studies for SIT are in the generation of trypanosome refractory lines and incorporation of natural mating incompatibility into the release strain. The symbiotic bacteria harboured in midgut tissue have been cultured, genetically transformed and reintroduced into tsetse. It is now possible to introduce and express anti-parasitic gene products in these endosymbionts to confer refractory phenotypes to engineered insects. As antiparasitic gene products, expression of single-chain antibody genes derived from transmission-blocking antibodies is being explored. Characterisation of symbiotic bacteria from tsetse ovaries has shown
that these are different from the gut organisms. Based on phylogenetic studies, these belong to the true Rickettsiacea, *Wohlbachia*. Presence of *Wohlbachia* in many insect systems provides a potent driving mechanism to spread desirable phenotypes in nature. Since all symbiotic bacteria in tsetse are maternally transmitted, gut organisms where refractory phenotypes are expressed can be introduced and spread into natural populations by use of cytoplasmic incompatibility induced by *Wohlbachia* symbionts (ovary). Experiments in progress are designed to determine the natural distribution of *Wohlbachia* infections in tsetse and the extent of the cytoplasmic incompatibility they confer. The availability of trypanosome refractory flies will increase the efficacy of sterile release males without the risk of trypanosomosis transmission to man and animals. The incorporation of natural incompatibilities into release strains would reduce cost and provide natural barriers for SIT operations.


Bossche: RTTCP, Box A560, Avondale, Harare, Zimbabwe. [petervdb@rttcp.org.zw]

A total of 4420 male *G. m. morsitans* were captured on man fly-rounds in Katete District, Eastern Province, Zambia, between February 1991 and December 1993. Of these flies, 1680 were captured before June 1992, during which period 989 flies were also captured on odour-baited electric screens operated in the same area. Non-teneral flies were analysed for fat, haematin and residual dry weight (RDW) and their wing-vein length was measured. There were well marked annual cycles in wing length, fat and RDW. Flies were biggest at the end of the rainy season, and smallest at the end of the hot dry season. Fat levels were lowest before the onset of the rains and highest in the cool season. RDW was a function of haematin content and the degree of wing-fray; these factors were used to correct the RDW to zero haematin. Corrected RDW and wing-vein lengths were most highly correlated with relative humidity in the month prior to capture (r > 0.8 and 0.6, respectively). Correlations with saturation deficit were weaker; temperature accounted for < 20% of the variance. Fly-round flies had a consistently higher RDW than those from the electric screen, but their fat levels were lower. The distributions of log haematin levels differed little between the two sampling methods and were adequately described by a model where capture and feeding rates increased exponentially after each meal. The increase in the feeding rate after each meal differed little with season and was closely similar to that estimated for female *G. pallidipes* in Zimbabwe.

**Carlson, D.A. and Sutton, B.D., 1999.** Hydrocarbon profiles and sex pheromones in tsetse: who is related to whom and why, and do conspecifics always use the same sex pheromone components? *In: IAEA, 1999* (see 23: no. 11337), pp. 3-12.
Sex stimulant pheromones have been described in several species in the *morsitans* group that release sexual activity in males upon contact with conspecific females, and aid in species recognition. Isolation, identification and synthesis of non-volatile sex pheromones or aphrodisiacs have occurred for *Glossina morsitans morsitans* and *G. pallidipes*. The trimethyl-branched hydrocarbon Morsilure found active against *G. m. morsitans* in laboratory studies was also active in field studies. Bioactive compounds have been recently synthesised for *G. tachinoides*, and are implicated for *G. austeni*. Hydrocarbon profiles from some conspecific specimens nearly 100 years old from widely different locations were very similar, but others were not. Tsetse species considered for SIT projects should be investigated to ensure that they contain consistent hydrocarbon patterns and sex pheromones across the range of distribution. Also, analysis of hydrocarbons may indicate the presence of candidate sex pheromones in species for which pheromones are not yet known.


Department of Entomology, University of Alberta, Edmonton T6G 2E3, Canada. [rgooding@maildrop.srv.ualberta.ca]

Hybridisation experiments have been carried out using *G. m. morsitans*, *G. m. centralis*, *G. m. submorsitans* and *G. swynnertoni*. In most experiments, backcross males are sterile if they have an X chromosome from one taxon and a Y chromosome from another. Weak evidence for involvement of the autosomes in hybrid sterility was found in crosses between *G. m. morsitans* and *G. m. centralis*, and between *G. m. morsitans* and *G. m. submorsitans*. Statistically significant involvement of autosomes in hybrid male sterility was found in crosses between *G. m. centralis* and *G. m. submorsitans*. Intra-chromosomal recombination suggests that the X chromosome locus for compatibility of the X chromosome with the Y chromosome from another taxon lies closer to the locus *Est-X* than to the locus *G6pd*, and that the locus for inter-taxon compatibility of linkage group II lies closer to the locus *Odh* than to the locus *Est-1*. When *G. swynnertoni* are hybridised to *G. m. morsitans* or *G. m. centralis*, a higher than expected proportion of the backcross males are sterile. Asymmetries in the success of hybridisations of *G. morsitans* subspecies appear to be due to maternally inherited factors. In *G. m. morsitans × G. m. centralis* hybridisation, these factors may be slowly replaced or inactivated during recurrent backcross to *G. m. centralis*.

In *G. m. submorsitans × G. m. centralis* hybridisation, the maternally inherited sterility factors are rapidly replaced or inactivated during recurrent backcross to *G. m. centralis*. A significant proportion of backcross females in four hybridisation models (*G. m. submorsitans/G. m. morsitans*; *G. m. submorsitans/G. m. centralis*; *G. m. centralis/G. swynnertoni*; and *G. m. morsitans/G. swynnertoni*) do not produce offspring within 4 weeks of being inseminated by parental
taxon males. It is suggested that the effects of this phenomenon be considered when evaluating the potential of hybridisation as a genetic control method for tsetse.


Lambremont: Nuclear Science Center, Louisiana State University and A & M College, Baton Rouge, LA 70803-5820, USA.

Earlier studies revealed that female tsetse flies mated to radiation-sterilised males synthesised lipids from a common metabolic feedstock, acetate, and had a labelling pattern identical to normally-mated females with the exception of the diglycerides. More detailed analysis of neutral lipids and phospholipids by several chromatographic systems has substantiated the earlier findings, and revealed a very large accumulation of neutral lipids in the larva feeding on the uterine gland secretion in utero.


Malacrida: Department of Animal Biology, University of Pavia, I-27100 Pavia, Italy. [malacrid@ipv36.unipv.it]

Progress in the work on genome characterisation of tsetse flies has enabled us to extend information on the genetic characterisation of strains of G. austeni, G. fuscipes fuscipes and G. palpalis palpalis using RAPD (random amplified polymorphic DNA) and MLEE (multilocus enzyme electrophoresis) data. Biochemical and molecular keys are provided from investigation of the inter-taxon mating propensities in G. p. palpalis and G. f. fuscipes, using RAPD data, and sequence comparison of the mariner element in G. p. palpalis and other non-Glossinidae species.


Saini: ICIPE, P.O. Box 30772, Nairobi, Kenya. [rsaini@icipe.org]

Larvae of G. m. morsitans and G. m. centralis produce pheromones which attract respective gravid females and hence result in aggregation of pupae. Behavioural experiments indicated that females preferred to larviposit over moist sand conditioned by previously allowing larvae to pupate in it. Similar results were obtained with filter papers contaminated with the prepupation excretions of larvae and with volatiles collected from larvae prior to pupation. N-pentadecane and n-dodecane were identified as the dominant electrophysiologically active components of the larviposition pheromones of G. m.
morsitans and G. m. centralis, respectively, by GC-EAD and GC-MS analysis of the trapped larval volatiles. Both identified compounds were shown to significantly attract gravid females to larviposition sites in laboratory behavioural assays.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

[See also 23: no. 11339.]

11354 **Hargrove, J.W. and Packer, M.J., 1999.** Catches of tsetse flies (Glossina spp.) (Diptera: Glossinidae) from odour-baited traps and artificial refuges during the hot season in Zimbabwe. *In:* IAEA, 1999 (see 23: no. 11337), pp. 43-60.

Hargrove: IPMI, Tsetse and Trypanosomiasis Control Branch, P.O. Box CY, Causeway, Harare, Zimbabwe. [jhargrove@rttcp.org.zw]

Tsetse flies Glossina morsitans morsitans and G. pallidipes were sampled using odour-baited traps and artificial refuges at Rekomitjie Research Station, Zambezi Valley, Zimbabwe, between September 1988 and January 1989 when the weather was generally dry and hot. In September-November rain fell on four days only (nine days in December-January) and maximum temperatures were > 32\(^\circ\)C on 62 of 79 experimental days (22 of 34 in December-January). The sum of morning and evening trap catches declined during September and early October until the first rains fell. The following day, catches increased by an order of magnitude, then declined rapidly over the following 10 days. Thereafter catches increased steadily at a rate more than 1000 times greater than possible if it were due to birth alone. The reasons for these increases are not clear and further work is required to separate the effects of possible immigration and of possible changes in activity, and in ability to locate host odour sources. It was not possible to demonstrate any overall link between daily trap catches and any of the measured climatic variables, probably because of the crudeness of the measurements available. Refuge catches, on the other hand, were independent of the day of the experiment, but increased approximately exponentially with maximum temperature \((T_{\text{max}})\) for \(T_{\text{max}} > 32^\circ\)C. During October the mean ovarian age of G. pallidipes from traps increased by c. 45\% and from refuges by 100\%, and stayed at these high levels for the remainder of the experiment. Mean wing fray showed similar increases between September and November but decreased in December and January. Mean wing length decreased by c. 5\% during October and started to increase in December. Thoracic residual dry weight (TRDW) did not change between September and November, but showed consistent changes with age and pregnancy state. It increased rapidly during the first ovarian cycle and more slowly thereafter for the rest of life. TRDW increased during the first 80\% of pregnancy then declined by 5\% in flies with a late third instar larva *in utero*. Superimposed on these increases was an effect of fly size; moreover, flies from refuges had a significantly higher TRDW than those from traps.


Kitwika: TTRI, P.O. Box 1026, Tanga, Tanzania.
A research study to determine the relative abundance and economic importance of *Glossina* species was conducted in two sites of the Tanga region, Tanzania – the coastal area (Mivumoni) and open savanna (Gombero) – for 3 consecutive years, 1992-1994. The tsetse species were sampled using F3, epsilon, biconical, Ng and sticky panel retaining traps. A sample of the retained tsetse flies from each locality was dissected to determine infection rates. *Glossina pallidipes* was the most abundant in all sites and *G. brevipalpis* the least. *G. morsitans* was never caught in Mivumoni despite the fact that it is abundant at Mkwaja ranch, the two areas being separated only by a river. The tsetse flies caught at Gombero were actually caught in areas previously reclaimed from tsetse infestation according to the tsetse distribution map of 1973, indicating that the bush has regenerated and is again a suitable tsetse habitat. The infection rate in flies averaged 6.1% for Gombero and 7.4% for Mivumoni. Information obtained from farmers around these research sites shows that trypanosomosis is one of the most important livestock diseases in the area. Trypanosome species found were mostly *Trypanosoma congolense* and *T. vivax*. The results obtained indicate that tsetse distribution in the Tanga region has changed greatly over the past two decades. These findings suggest that the country-wide tsetse distribution and abundance is not clear and needs detailed study.


Nevill: Onderstepoort Veterinary Institute, Agricultural Research Council, Private Bag X5, Onderstepoort 0110, South Africa.

A long-term solution is being sought for the nagana problem in northeast Zululand. Trials are being conducted in the field to evaluate odours, colours, target sizes, etc. and suitable odour-attractants are being sought. So far, the 1:4:8 propyl phenol/octenol/methyl phenol mixture (effective for *G. pallidipes* and *G. morsitans* in Zimbabwe) has been found to be very attractive for *G. brevipalpis* but not for *G. austeni*. Phalogen blue flanked by panels of black is the most effective target arrangement for both species. The best trap to date is the XT sticky trap. Surveys suggest that *G. austeni* is the more widespread species. Tsetse density varies from rare in the extreme south (Umfolozi River) to abundant in areas with a high wild animal population.


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

Studies were made of the behaviour of *Glossina pallidipes* and *G. morsitans morsitans* during the hot season (September-November) in Zimbabwe. Attributes of samples of tsetse from refuges, odour-baited traps, targets and mobile baits were compared. Various arrangements of electric nets were used to study tsetse as they entered or left artificial refuges. The peak time of entry into a refuge varied between 08.00 h and
14.00 h and coincided with the time when the air temperature reached 32°C; the response was stronger if 32°C occurred earlier in the day. The peak time of exit varied between 15.00 h and 17.00 h, being significantly later on hotter days, but did not show a clear temperature threshold. Micro-meteorological measurements showed that refuges were significantly cooler than the surrounding riverine woodland during the day but warmer at night. There was no significant difference between the air temperatures in leafless mopane woodland and semi-evergreen riverine woodland during the day, but at night the riverine woodland was significantly cooler. Combining the micro-meteorological data with the estimated local movements of tsetse suggested that, during the hot season, tsetse experienced temperatures 2°C cooler than the daily mean in a Stevenson screen located in mopane woodland. Compared with the catches of tsetse from traps, refuges had higher proportions of G. m. morsitans, males, young flies and females in the later stages of reproduction, and it is suggested that, during the hot season, samples from refuges were less biased than traps with respect to species and sex composition, age and reproductive status. During the hot season, tsetse populations declined by c. 90% and although air temperatures exceeded lethal levels (c. 40°C), the refuge-entering responses meant that adult flies probably experienced a maximum of only c. 35°C. It is suggested that the decline in numbers is not due to direct mortality effects of temperature on adults but may be due, in part, to a doubling in the rates of reproductive abnormality during the hot season and an increase in adult mortality related to a temperature-dependent decrease in pupal period.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE-EFFECTS)

[See also 23: nos. 11339, 11344-11347, 11391.]


Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box 80, Legon C Accra, Ghana.

Animal trypanosomosis transmitted by some species of tsetse flies was identified in Ghana in the early 1920s. Since then several attempts have been made in the country to control both the disease and the vector, and these are discussed briefly. Research activities initiated in 1983 in establishing laboratory colonies of Glossina palpalis palpalis and G. tachinoides with the aim of applying the SIT as a component of programmes to eradicate tsetse and the disease in Ghana are described.


DFID Office for Southern Africa, 208 Infotech Building, 1090 Arcadia Street, Hatfield, 0083 Pretoria, South Africa.
Case studies, mainly in Zimbabwe but also in Zambia, investigated economic aspects of controlling savanna tsetse species which transmit bovine trypanosomiasis in southern Africa. Costs of the four major tsetse control techniques, each of which has been used on a large scale in the recent past, were analysed on a comparative basis. The costs of using odour-baited insecticide-treated targets compared well with traditional ground spraying using DDT, which is increasingly disfavoured on environmental grounds. The cheapest method of tsetse control is to treat cattle with appropriate insecticides. There are many situations where this is not feasible, for lack of cattle, but the approach is generally very promising and needs urgent technical development. Although aerial spraying is likely to be the preferred control method in some specific situations, it is the most expensive of the four techniques. Case studies showed that the policy of the Government of Zimbabwe was justified in relying on tsetse control rather than on the use of trypanocides. However, the comparative advantage is variable according to specific circumstances. A methodology for cost comparison was developed and demonstrated, based on simple economic models usable by planners without formal economics training. The emergence of bait techniques provides an opportunity for innovative strategies for tsetse and trypanosomiasis control in southern Africa, in which tsetse operations involve local communities and coordinate with rural development more closely than in the past. There is a key role for the economics profession in assisting to ensure that co-ordination is effective and appropriate.


AGCD, ASVEZA Project, Lusaka, Zambia.

Amongst the factors restricting livestock production in Zambia, trypanosomiasis plays an important part in the western province and in some areas of southern, central and eastern provinces as well as in the Lusaka province and 25% of the cattle population in the country is considered at risk. Trypanosomiasis control is organised by Zambia together with the bordering countries in a common programme (‘Common Fly Belt’) funded by different donors (EC, Belgium, Netherlands). Control is targeted at Glossina morsitans morsitans and G. pallidipes in the common fly belt and at G. m. centralis and G. fuscipes fuscipes in other areas. Trypanosoma congolense is the most important species in cattle. Control operations have resulted in the area infested with tsetse being reduced from 50% to 30% of the total country, and tsetse have disappeared from the southern province and from some areas in the other provinces. The average prevalence of trypanosomiasis in cattle is less than 10% in the controlled areas and even 0% in some locations. The different control methods used in Zambia are reviewed and the use of target screens considered. Community participation, private contractors and land use are also discussed.


Dyck: TTRI, P.O. Box 1026, Tanga, Tanzania. [adyck@compuserve.com]

Unguja island has provided a unique opportunity to eradicate the one tsetse species present, *G. austeni*, which is responsible for the cyclical transmission of trypanosomosis. Eradication of the vector will benefit the development of cattle production in Zanzibar, with positive economic and environmental impact, and also provide a model for future plans to eradicate tsetse flies from parts of mainland Africa. In the 1980s, a cooperative research project involving the Tsetse and Trypanosomiasis Research Institute (TTRI), the IAEA and the Department of Livestock Development, Zanzibar (DLDZ) was begun to improve fly rearing technology and to understand the ecology and behaviour of *G. austeni*. To evaluate the feasibility of using the SIT on Unguja island, ground releases of sterile male flies were initiated in 1990 using flies air-freighted from TTRI. Concurrently, FAO and the Government of Zanzibar started a programme of systematically treating cattle with insecticide in an attempt to eradicate the fly population with chemicals. In 1994 a formal project was started with the goal of eradicating the tsetse fly from Unguja island. The project has been executed by the governments of Zanzibar and Tanzania with the technical assistance of IAEA and FAO, and funding has been provided by the governments of Zanzibar and Tanzania, IAEA and various donor countries. Following the usual procedure of an SIT eradication programme, the fly population was first suppressed with insecticides (cattle pour-ons and insecticide-impregnated screens). Ground releases were initiated and then replaced with aerial releases when the number of sterile flies available was large enough. It is expected that eradication operations will be completed in 1997. To detect very low fly population densities, a systematic programme of taking blood samples from sentinel herd cattle and monitoring the transmission of trypanosomosis was initiated.


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The tsetse and trypanosomosis problem is characterised by many interdependencies involving agro-economical, social and environmental considerations. Any intervention will have a wide range of immediate and longer-term implications. Therefore, comprehensive planning is needed that retains a variety of options for problem intervention, including eventual tsetse eradication. The currently available and environmentally acceptable methods for tsetse and trypanosomosis management all have their specific limitations. Only a combination of several methods in an integrated, phased approach can effectively advance the establishment of viable agricultural systems. Since the trypanosomosis problem is not restricted to individual countries but affects entire sub-regions, an *area-wide* integrated pest management approach should be designed. The
potential of many available intervention methods and of new supportive technologies has not been sufficiently explored. This is particularly the case for the SIT which, contrary to other ‘conventional’ methods of tsetse control, has a unique efficiency pattern: increasing efficiency with decreasing target pest population density. A phased and complementary use of both conventional methods and the SIT will have maximum efficiency throughout the intervention campaign. The recent initiation of aerial releases of large numbers of sterile males over Zanzibar has received considerable attention. This effective and environmentally friendly intervention method can now be used even for inaccessible areas.

FAO/IAEA has launched an initiative to upgrade the SIT to an economically attractive alternative for integration into area-wide, sub-regional tsetse and trypanosomosis intervention campaigns. This will require methods to be developed for the release of at least 500,000 sterile males per week and to operate in areas as large as 10,000 to 20,000 km² at a time. The initiative consists of three components: (i) research and development on tsetse rearing automation, tsetse attractants and tsetse genetics; (ii) a shift from independent Technical Assistance support under the FAO/IAEA Agriculture programme to a concerted, impact-oriented effort of the ‘UN family’ and other key players; and (iii) concrete feasibility assessments of the SIT package as a component of area-wide, integrated tsetse and trypanosomosis management efforts at different selected sites.


ARS, USDA, Agricultural Research Center, Beltsville, MD 20705, USA.

The investigation involves a detailed analysis of the basic principles and suppression characteristics of various methods of tsetse control in use or under development. The methods include the application of insecticides, the use of attractants that simulate the attraction of host animals, the release of sterile males, autosterilisation, and the release of the parasite, Chrestomutilla glossinae (Hymenoptera; Mutillidae) into tsetse habitats. The suppressive actions of insect control procedures fall into two categories: those in which the effectiveness of the techniques is independent of the pest density, and those in which the pest density has a marked influence on the effectiveness of the control procedure. The former includes the application of insecticides and the use of tsetse fly attractants. The latter includes the release of sterile males and the release of parasites. The opposing suppressive actions of different methods of control depending on the pest densities gives the science of insect pest management the opportunity of integrate two or more control procedures and achieve more effective and more efficient control than can be achieved by the use of only one technique alone. Hypothetical tsetse population models are used to show why the integration of different techniques results in more efficient control. The release of sterile males alone for the suppression of normal density tsetse populations will be impractical because of the high cost of rearing the insects. This will also be the case for the technique involving the release of the parasite, C. glossinae. However, these techniques can be used to great advantage against naturally low populations or populations that have been greatly reduced by the use of insecticides, attractants or other means. In numerical terms the release of C. glossinae is considerably more effective than the release of sterile males. The outstanding advances that tsetse scientists have made on attractants
suggest that this method of control will become one of the primary means of suppressing tsetse populations. This technique is highly pest specific and would avoid the environmental hazards associated with the use of broad spectrum insecticides. However, the diminishing effectiveness as the pest population declines, and the contrasting increasing effectiveness when sterile males or parasites are released into the pest habitats, will result in a much more effective and efficient method of regulating tsetse populations when these two procedures are employed concurrently.


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Lure and kill techniques for insect pests are being developed in response to the need for more environmentally acceptable means of pest control. The development of insecticide-treated targets for tsetse control has led this technology and in many places has replaced the use of insecticide spraying operations for suppression of tsetse populations. Use of insect growth regulators (IGRs) on targets instead of conventional insecticides has been advocated for tsetse control and two successful demonstrations of this technique using the juvenile hormone mimic pyriproxyfen were undertaken in Zimbabwe. Similar demonstrations have been successful in Kenya and Côte d’Ivoire. Laboratory trials suggest that the chitin synthesis inhibitor triflumuron may more readily contaminate male tsetse and lead to transfer to females during mating. Triflumuron-treated, odour-baited targets have been used successfully to suppress tsetse populations in Zimbabwe. Since IGRs do not kill but sterilise insects, the advantages of their use in conjunction with SIT for pre-release suppression of a target population are discussed.


Mangwiro: Tsetse Control Branch, P.O. Box CY52, Causeway, Harare, Zimbabwe.

The efficacy of various pyrethroid insecticides for use on odour-baited targets to control tsetse was compared in Zimbabwe from 1986 to 1994. Formulations were applied to cotton cloth and polyester net and, at various intervals, the materials were bioassayed by exposing fed female *Glossina pallidipes* to cloth for 45 s or by inducing them to collide briefly with net. Trial formulations were compared with deltamethrin suspension concentrate (s.c.), the insecticide currently used in tsetse control operations in Zimbabwe. Applying 0.8% suspension of alphacypermethrin to cloth or net produced high mortalities for 9 months which was similar in performance to 0.4% suspension of deltamethrin s.c. Deltamethrin s.c. and β-cyfluthrin s.c. applied to cloth as 0.1% suspensions were equally effective, producing high mortalities for 2 months during the wet season, and 0.8% suspension of β-cyfluthrin was effective for 12 months. Suspensions of 0.1% lambda-
cyhalothrin capsule suspension or 0.1% lambdacyhalothrin wettable powder were significantly less effective than 0.1% deltamethrin s.c. Chemical analyses showed that increasing the concentration of insecticide applied to material increased the initial amount of insecticide on the material and decreased the subsequent rate of loss; 0.1% suspension of β-cyfluthrin s.c. applied to cloth produced an initial concentration of ≈ 280 mg/m² which declined by 94% in 12 months whereas 0.8% suspension showed no significant decrease in concentration (mean = 1304 mg/m²) over the same period. For controlling tsetse by means of pyrethroid-treated targets, it is suggested that β-cyfluthrin s.c. is as effective as delta-methrin s.c. but that alphacypermethrin s.c. should be used at twice the concentration of deltamethrin s.c. to obtain the same performance.


Maniania: ICIPE, P.O. Box 30772, Nairobi, Kenya.

Laboratory studies were carried out to determine the effect of sex and age on the susceptibility of tsetse, *Glossina morsitans morsitans* and *G. m. centralis*, to the entomopathogenic fungus, *Metarhizium anisopliae*. Both species of host flies were susceptible to fungal infection. Female flies were generally more susceptible than male flies. Three host ages (40, 20 and < 1 day-old) were used; the youngest group was most resistant to fungal infection. Interactions between species, sex and age were significant on many occasions. Age usually accounted for the largest variability in mortality, followed by sex. All flies of age 40 days died between 7 and 8 days after infection whereas some of the younger flies, especially age < 1 day, lived longer than 10 days. Log10 day probit (LDP) mortality regressions fitted well to most of the data sets. LDP slopes were significant and high, ranging between 4.3 and 12.8, indicating a generally high mortality rate of increase over days. The slopes differed significantly between species, sexes and ages, but grouping by age was more intra-homogeneous than by species or sex. The 50% lethal time mortalities (LT₅₀) ranged between 4 and 7 days for age < 1, between 3 and 6 days for age 20, and was about 5 days for age 40, respectively. Corresponding LT₉₅ ranges were 8-20, 5-10 and 6-7 days for ages < 1, 20 and 40, respectively. The significance of these results in the fungal disease transmission by tsetse is discussed.


Msangi: TTRI, P.O. Box 1026, Tanga, Tanzania. [amsangi@costech.gn.apc.org]

After colonisation from pupae collected from Unguja island and many years of small-scale rearing, the *G. austeni* colony increased from 46,081 (31,986 producing and 14,095 pre-producing) in week 35, 1994, to 266,732 (197,572 producing and 69,160 pre-
producing) in week 39, 1995. The increase in colony size also led to an increase in puparia production from 10,090 to more than 75,000 per week. Release of sterilised flies increased from 1700 to more than 28,030 per week. Care in handling decreased mortality and percentage non-fliers and increased the percentage of flies successfully released from as low as 39.6% to 95.6%. Quality control assessment in the laboratory for released males showed 100% induced sterility in fertile females, an indication of complete sterilisation of flies released in the field. Following the refurbishment of the insectaries and implementation of state of the art methodology, the facility has the capacity to be a centre for mass-production of other species of tsetse for SIT application in Africa.


ICIPE, P.O. Box 30772, Nairobi, Kenya.

The project aims at contributing to improved livestock production in Africa for both food security and cash through the development and application of cost-effective, environmentally sustainable and culturally acceptable tsetse management strategies. Six specific objectives of the project include: development of improved trapping methodologies for Glossina pallidipes, G. fuscipes and G. morsitans spp. in different agro-ecological zones using kairomones, allomones and larviposition pheromones; development of cost-effective strategies for maintaining G. pallidipes at ultra low levels through barrier traps and allomones; clarification of some factors influencing the dynamics of trypanosomosis transmission at low levels of tsetse challenge; investigation of socio-economic aspects of NGU traps and determination of their cost-effectiveness and sustainability; evaluation of the potential of certain fungi as biological control agents for tsetse; and dissemination of information on achievements in tsetse research and on developed control strategies to interested governments, organisations and institutions throughout Africa.


Oloo: KETRI, P.O. Box 362, Kikuyu, Kenya. [ketri@net2000ke.com]

Kenya is infested with various species of the tsetse fly in approximately 25% of the country. These include areas where mixed farming is practised, hence making animal trypanosomosis a major constraint to rural production. Sustainable tsetse and trypanosomosis control requires the use of appropriate strategies which take into account environmental and socio-economic considerations to facilitate adequate community participation. The current methods of tsetse control include discriminate ground spraying with insecticide and odour technology (traps, targets, screens, pour-ons, dips). Ground spraying, mainly using synthetic pyrethroids, is confined to the sleeping sickness belt in western Kenya. Traps of various design, insecticide-impregnated targets and electric screens have shown tremendous success in reducing tsetse fly populations, often in excess of 90%. Pour-on technology has also demonstrated good results with fly reduction in
excess of 80%. The use of these methods is, however, limited by the cost of available formulations. These achievements have been reflected in lower animal trypanosomosis infections (by over 70%) and prospects of higher rural agricultural production. There is great potential for biological control which has not been exploited, probably due to the high costs involved. However, research institutions in Kenya could provide bases for the initiation of the use of SIT in integrated control programmes. In the control of animal trypanosomosis, the country has relied on chemotherapy and chemoprophylaxis using diminazene aceturate, and homidium bromide and chloride or isometamidium chloride as sanative pairs in cattle. Trypacide salts have been used for treatment of \textit{T. evansi} in camels, and melarsomine is also in current use. Even though information and necessary materials for tsetse and trypanosomosis control are available, there are resurgences of the flies and thus infections, especially during the rainy seasons. There is need to improve efficiency of surveillance and control programmes for animal trypanosomosis, and also for human trypanosomosis in the endemic areas of western Kenya.


Pan: 267 Woody Lane, Athens, GA 30605, USA.

The whole of Unguja island was divided into 38 blocks. In each block, 40-50 sentinel cattle covering the entire block were selected and bled at intervals of c. 2 months. Two months before the beginning of the first bleeding series all sentinel cattle were treated with a curative dose of Berenil (diminazene aceturate). A parasitological test (microhaemato-crit centrifugation technique, MHCT) and a serological test (antigen detection enzyme-linked immunosorbent assay, Ag-ELISA) were used to detect \textit{Trypanosoma brucei brucei}, \textit{T. congolense} and \textit{T. vivax} in the sentinels. Any sentinel animal which subsequently tested positive for trypanosomes was treated with Berenil 2-3 days later. Spot-on (deltamethrin) was applied to animals in blocks where positive cases were detected. Data are reported for blocks 1-29 where the disease prevalence was relatively low. Samples from the first eight bleedings have been examined. The MHCT results indicated that the three species of trypanosomes pathogenic to cattle were not present in blocks 1-29 after the fourth bleeding; the Ag-ELISA results, however, showed that antigens of the three species were present in the bovine samples.


Tsetse Control Division, P.O. Box 14, Maun, Botswana. [toppers@info.bw]

Tsetse control to protect the cattle industry was instigated 70 years ago. Early control efforts involved bush clearing, game destruction and the evacuation of people and cattle. Ground spraying with persistent insecticides such as dieldrin was used from the early 1960s but always proved difficult in the scattered islands of the Okavango. Eradication of \textit{Glossina morsitans centralis} from the isolated delta population was always
the objective but it was never to be achieved with ground spraying. Aerial spraying with fixed-wing aircraft applying sequential applications of low dosage insecticide aerosols began in 1972 and continued until 1991, each operation extending over 3000 to 9000 km$^2$. This greatly reduced the tsetse infestation and the incidence of trypanosomosis. There have been no cases of sleeping sickness reported in Botswana since 1983 and no positive diagnosis of trypanosomosis in cattle since 1987. In 1992 it was decided that aerial spraying should cease and tsetse control with odour-baited targets should be tried. A long period of drought, which had left much of the delta dry and more easily accessible, strengthened the argument for turning to this technology to eradicate the now recovering tsetse population and thus provide lasting protection for the flourishing tourist industry and the 300,000 cattle positioned around the periphery of the delta.


ICIPE, P.O. Box 30772, Nairobi, Kenya.

In a study to determine the costs of tsetse trapping in terms of construction materials, labour, transport and maintenance, 246 traps used to control *Glossina pallidipes* in an area of 100 km$^2$ at Nguruman, Kenya, were evaluated from November 1990 to July 1992. The total cost of materials, labour and servicing per trap per year was US$ 30.6 which, at that time, was equivalent to 1071 Kenya shillings.


Vreysen: Tsetse Eradication Project in the Southern Region, P.O. Box 19917, Addis Ababa, Ethiopia. [estc@telecom.net.et]

The progress of the release programme and the impact of the sterile male flies on the native fly population were evaluated during entomological monitoring with sticky panels. A total of 23 fixed monitoring sites (FMS), each consisting of a minimum of five blue/white sticky leg panels covering an area of not more than 1 km$^2$, were established over the release zone. The panels were checked and flies collected from once a day to once a week depending on the area. More than 90% and 95% of the total indigenous male and female flies, respectively, were trapped in the Jozani forest and surrounding forest habitats. In that area (FMS 1-6), the apparent density (no. flies/panel/day) of the indigenous female and male fly population decreased from an average 0.06 females/panel/day and 0.05 males/panel/day during the last quarter of 1994 to an average 0.02 females/panel/day and 0.04 males/panel/day as of week 20, 1995. No females were trapped in the area southeast of Jozani (FMS 7-22) and the apparent density of the wild male fly population was very low (average of 0.0007 after week 11, 1995). In both areas, the ratio of sterile to wild males increased with the increase in numbers of sterile males released and was consistently above 100:1 after week 35, 1995. The proportion of parous female flies showing evidence of mating with a sterile male increased significantly during
the one-year monitoring period, i.e. the rate of induced sterility increased from an average of 22% during the last quarter of 1994 to an average of 58% in weeks 41-46, 1995. Moreover, the percentage of fertile females (with a viable larva *in utero*) decreased significantly.


Vreysen: Tsetse Eradication Project in the Southern Region, P.O. Box 19917, Addis Ababa, Ethiopia. [estc@telecom.net.et]

The release of gamma-sterilised male and female *G. austeni* from light aircraft over the southern half of Unguja island of Zanzibar was initiated in August 1994. A twin engine aircraft (Piper Seneca, Piper Chieftain or Partenavia) transported the sterile flies twice a week from the mass-rearing facility at Tanga on the Tanzania mainland to the release zone on Unguja island. The sterile flies were packaged in carton release containers (11.5 × 9 × 5 cm) at densities of 50-200 flies per box. The boxes were dispersed through a specially designed fly chute in the aircraft’s cabin floor, along specific flight lines separated by 1-2 km swaths. All aircraft were equipped with the Global Positioning System which enabled accurate navigation. Sterile male flies were released in the more favourable forested habitats, whereas sterile female flies were dispersed in the drier areas of the eastern part of the island. The frequency of releasing the fly boxes (interval of 2-45 s) was calculated prior to each release based upon the release area, flight line, apparent density of the wild fly population, density of flies per release box and quantity of sterile flies available. During the 14 month release period (from week 34, 1994, to week 46, 1995) more than 1.7 million sterile male flies and 640,000 sterile female flies were dispersed over a total release area of approximately 600 km², representing one third of the entire surface of Unguja island. During routine quality control assessment of the transported flies, mortality and proportion of non-fliers of the female flies was in general lower than that of the male flies (4.8% and 3.0% mortality and 13.6% and 11.3% non-fliers for male and female flies, respectively). There was a significant correlation between male fly mortality during transport and the temperature in the aircraft during the evening releases.

4. **EPIDEMIOLOGY: VECTOR-HOST AND VECTOR-PARASITE INTERACTIONS**

[See also 23: nos. 11347, 11415, 11433.]

A DNA assay for bloodmeal identification in haematophagous insects was
developed. Specific host cytochrome B gene sequences were amplified by PCR and
classified on the basis of their mobility in a heteroduplex assay. In the blackfly *Simulium
damnosum* s.l. (Diptera: Simuliidae), human cytochrome B DNA sequences were
identifiable up to 3 days following ingestion of the bloodmeal. In *Glossina palpalis*
collected from tsetse traps in Côte d’Ivoire, bloodmeals were identified as taken from
domestic pigs on the basis of their heteroduplex pattern and DNA sequence. Evidently the
cytochrome B sequence shows sufficient interspecific variation to distinguish between
mammalian host samples, while exhibiting minimal intraspecific variation. The stability
of DNA in bloodmeals for several days post-ingestion by haematophagous insects allows
PCR-HDA assays to be used reliably for host identification.

11376 **Elsen, P., Abbeele, J. van den, Roelants, P. and Claes, Y., 1999.** Vectorial
capacity and isoenzymatic analysis of *Glossina austeni* bred at IAEA-Vienna.
*In*: IAEA, 1999 (see 23: no. 11337), pp. 77-90.

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Cyclical transmission studies with *G. austeni* from IAEA show very low vectorial
capacity (1.5%) for *Trypanosoma brucei brucei* and much higher (16.3%) for *T.
congolense*. The proportion of midgut infections that provide mesocyclic forms is lower
than the proportion of mesocycles providing metacyclic infections. This seems to
indicate the existence of a more important barrier at the beginning of the cycle of the
parasite, which could explain the much later appearance of the metacyclic forms in this
species. Indeed, a comparison of the dynamics of the metacyclogenesis between *G.
austeni* and *G. morsitans morsitans* indicates that the colonisation of the salivary glands
occurs much later in *G. austeni*, taking place after day 17 when it is already finished in *G.
m. morsitans*. The isoenzymatical characterisation of *G. austeni* has been established for
14 enzymes (20 loci) of which 7 are monomorphic (12 loci). Two loci,
phosphoglucomutase and one of superoxide dismutase, are sex-linked with the Y
chromosome. The isoenzymatical comparison for the 8 polymorphic loci between flies
remaining completely negative and those developing metacyclogenesis indicates a
significant difference in the global allelic frequency at the level of the females in the case
of *T. congolense* and at the level of the males in the case of *T. b. brucei*. Taken separately,
the enzymes aconitase and alkaline phosphatase are the ones playing a significant role in
these differences.

Influence du nombre de repas infectieux sur la compétence vectorielle des
mouches ténérales de *Glossina morsitans morsitans* (Mall) infectées par
*Trypanosoma (Nannomonas) congolense* IL 1180. [Influence of number of
infectious meals on the vectorial competence of teneral tsetse flies *G. m.*}

Kazadi: Department of Animal Health, Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.

Teneral tsetse flies, *G. m. morsitans* (Mall), aged < 32 h were divided into two groups and infected initially with *T. congolense* IL 1180. Flies of group A were maintained on healthy rats and those of group B fed exclusively on parasitaemic rats to ensure regular re-infection. In group A flies, values of 0.65 ± 0.09, 0.73 ± 0.10 and 0.47 ± 0.11 were recorded for mesoprocyclic and metacyclic indices and vectorial competence (VC), respectively. The corresponding values for group B flies were 0.76 ± 0.08, 0.79 ± 0.08 and 0.60 ± 0.10. In group A, the mesoprocyclic index and the VC of the females were significantly higher than those of the males. In group B, no such significant difference was seen between the sexes. For both sexes taken together, the mesoprocyclic index and the VC of the flies of group B were higher than those of flies of group A. Males of group B revealed a higher VC than those of group A, while no significant difference in VC was observed in the females of the two groups.


Ethiopian Health and Nutrition Research Institute, P.O. Box 1242, Addis Ababa, Ethiopia.

Laboratory-reared *Glossina morsitans morsitans*, fed on immobilised Swiss albino mice, were used in a study to compare the desirability of preserving the bloodmeal on filter paper smears or in whole intact dried specimens. The two techniques showed similar absorbance values, and the inclusion of 25% ethanol appeared to improve the detection of the bloodmeal in both cases. Since bloodmeals preserved on filter paper deteriorated rapidly with time, preservation of insects as dried whole specimens is recommended if bloodmeal identification is not performed immediately.


ILRI, P.O. Box 30709, Nairobi, Kenya.

The susceptibility of colonies of *Glossina morsitans centralis, G. austeni, G. palpalis palpalis, G. palpalis gambiensis, G. fuscipes fuscipes, G. tachinoides* and *G. brevipalpis* were compared. Mature infection rates ranged as follows: 0-97.1% for *Trypanosoma vivax*, 0.3-49.2% for *T. congolense*, and 0-40.4% for *T. brucei brucei*. Thus there were differences in the susceptibility of different tsetse species and subspecies to these pathogenic *Trypanosoma* species. Further study revealed that even the same species of tsetse from different populations may show significantly different susceptibility in
trypanosome infections, e.g. *G. pallidipes* from allopatric populations in Kenya. Two colonies of *G. pallidipes* which originated from allopatric populations in Kenya were established. One was from Ngoruman, Rift Valley Province, the other from Shimba Hills, Coast Province. Infection rates by stocks of *T. vivax* in the two allopatric populations of *G. pallidipes* were very high, and ranged from 71.3-80.0%. Therefore, the vector aspects of *vivax*-trypanosomiasis probably do not differ significantly between these two areas of Kenya. However, the colony of *G. pallidipes* which originated from Ngoruman was significantly more susceptible to infections with stocks of *T. congolense*, *T. simiae* or *T. b. brucei* than the colony which originated from Shimba Hills. If the observed differences in the susceptibility of the two *G. pallidipes* colonies to infections with the above pathogenic *Trypanosoma* species reflect transmission of trypanosomes by the two allopatric populations of tsetse in the field, then the epidemiology of trypanosomiasis must differ between these two areas of Kenya.


Sasaki: Laboratory of Entomology, Faculty of Dairy Science, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan.

A survey of flies associated with wild chimpanzees was carried out at the Mahale Mountains National Park, Kigoma District, Tanzania, during the rainy seasons (November-December) of 1995 and 1996. A total of 16 genera and 35 species belonging to five families were collected. These included two tsetse fly species, *Glossina morsitans morsitans* and *G. longipennis*. Although *G. longipennis* rarely feeds on primates, many bloodmeals from this species were experimentally confirmed to be of primate origin.

### 5. HUMAN TRYPANOSOMIASIS

**(a) SURVEILLANCE**


Steverding: Abteilung Parasitologie, Hygiene-Institut der Ruprecht-Karls-Universität, Im Neuenheimer Feld 324, D-69120 Heidelberg, Germany.

A sensitive and specific method was developed to identify *T. brucei* ssp. using PCR to amplify conserved expression-site-associated gene 6 and 7 DNA target sequences. Amplification of 10% of the DNA in a single trypanosome produced sufficient PCR product to be visible as a band in an agarose gel stained with ethidium bromide. Fifty-nine blood samples from serologically positive cases of sleeping sickness from Equatorial Guinea and Angola were analysed by PCR, and tissue fluids were examined for trypanosomes. The PCR test detected 20 (87%) of the 23 parasitologically positive cases,
with a specificity of 97%. In five cases, the parasite was demonstrated by PCR 4-6 months prior to parasitological detection, showing the potential of this assay in early diagnosis of *T. b. gambiense* infections in apparently aparasitaemic sleeping sickness patients.


Truc: IRD, B.P. 5045, F-34032 Montpellier, France.

Stage determination of human African trypanosomiasis is based on the detection of parasites and measurements of biological changes in the cerebrospinal fluid (CSF) (concentration of white blood cells > 5 cells per mm$^3$ and increased total protein levels). The patient is treated accordingly. Demonstration of the absence or presence of trypanosomes by the double centrifugation technique is still the only test available to clinicians for assessing treatment success. In this study, the polymerase chain reaction (PCR) was evaluated as a tool for assessing the disease stage of 20 patients from Côte d’Ivoire and for determining whether treatment had been successful. Fifteen patients considered to be in the advanced stage of the disease were all PCR positive although trypanosomes were demonstrated by double centrifugation in only 11 patients. The five remaining patients, who were considered to be in the early stage, were negative by both methods. Following treatment, 13 of the 15 second-stage patients were found to be negative for the disease in at least two samples by PCR and double centrifugation. Two others were still positive by PCR immediately and 1 month after the treatment and, although trypanosomes were not found by the double centrifugation technique, CSF cell counts and protein levels remained abnormal. Further evaluation of the PCR method is required, in particular to determine whether PCR assays could be used in studies on patients who fail to respond to melarsoprol, as observed in several foci.

(b) PATHOLOGY AND IMMUNOLOGY


Buguet: Unité de Physiologie de la Vigilance, CRSSA, B.P. 87, F-38701 La Tronche, France.

To determine whether the disruption of the circadian sleep/wake cycle observed in human African trypanosomiasis (HAT) can be reversed after trypanocidal treatment, 10 Congolese patients infected by *Trypanosoma brucei gambiense* underwent 24-h polysomnographic recordings before treatment with melarsoprol and after each of three weekly treatment sessions. Polysomnography consisted of a continuous recording of the electroencephalogram, electromyogram and electro-oculogram on a Minidix Alvar polygraph. Sleep traces were analysed in 20-s epochs for wakefulness, REM sleep and
NREM sleep (stages 1, 2, 3 and 4; stages 3 and 4 representing slow-wave sleep (SWS)). As previously described, the 24-h distribution of the sleep/wake cycle was disturbed in proportion to the severity of the illness. The overall amounts of each sleep/wake stage did not change after treatment. However, the patterns of occurrence of sleep episodes, REM sleep and SWS phases were determinant in the evaluation of treatment efficacy. The trypanocidal action of melarsoprol led to a reduction in the number of sleep episodes, except in one patient whose health condition worsened during the third treatment session: sleep onset REM sleep phases (SOREMPs) decreased and the number of SWS episodes during a sleep episode increased. It is concluded that in HAT the reversibility of the sleep/wake cycle alteration and that of sleep structure constitute the basis for an evaluation of the healing process.


Malesker: Alegent Health Immanuel Medical Center and Department of Pharmacy Practice, Creighton University, Omaha, NE 68178, USA.

We report the first apparent case of a splenectomised individual who developed severe trypanosomiasis with central nervous system involvement. The patient was a 41-year-old man who participated in an East African safari. Upon his return to the United States, the patient presented with an infection with \textit{Trypanosoma brucei rhodesiense} that was treated successfully with suramin and melarsoprol. The onset of symptoms, laboratory studies and disease progression did not differ from cases previously reported in the literature. The role of the spleen in trypanosomiasis is not well understood and the few reports available describe only animal models. In this case, asplenia had no apparent effect on the onset of symptoms and overall severity of the illness. Further studies are necessary to ultimately define the role of the spleen in trypanosomiasis.

(c) TREATMENT

[See also 23: nos. 11342, 11382, 11383.]


Pécoul: Access to Essential Drugs Project, Médecins Sans Frontières, C.P. 6090, CH-1211 Geneva 6, Switzerland.

The authors comment on the letter by Sjoerdema and Schechter (see 23: no. 11386) and describe the part being taken by MSF and WHO to try to find an industrial partner who is willing to produce eflornithine at a reasonable price. MSF is ready to ensure a market for the drug by guaranteeing purchase for 2-3 years and organisation of distribution to sleeping sickness programmes in affected countries.

In the 1980s the curative effects of eflornithine for Trypanosoma brucei gambiense sleeping sickness were discovered, especially for cases which were refractory to melarsoprol. In spite of its approval by the US Food and Drug Administration in 1990 for marketing for sleeping sickness and its designation as an orphan drug (i.e. useful to fewer than 200,000 patients in the USA), it is not currently available. The authors plead that eflornithine be manufactured again at low cost to combat the current resurgence of sleeping sickness.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also 23: nos. 11341, 11360, 11370.]


Department of Clinical Veterinary Medicine, Madingley Road, Cambridge CB3 0ES, UK.

An intermediate-technology pattern matching model and decision support system for veterinary diagnosis is described. Six diseases of cattle occurring in the tropics, including trypanosomiasis, are used to illustrate the model. The pattern matching model is composed of a series of transparent overlays and a template. Each transparent overlay represents a sign state and contains sign frequency information for the diseases on the template. By superimposing multiple transparent overlays upon the disease template, a ranked list of differential diagnoses can be obtained. Ranking is by summation of disease sign frequencies. Modifications to accommodate observational uncertainty are presented. Disease prevalences can be represented in the model.


Dwinger: Animal Production and Health Section, IAEA, P.O. Box 100, A-1400 Vienna, Austria. [r.dwinger@iaea.org]

Monoclonal antibodies directed at epitopes of Trypanosoma brucei brucei, T. congoense and T. vivax have been used to capture and detect trypanosomal antigens in bovine blood samples using an enzyme-linked immunosorbent assay (ELISA) developed elsewhere. The test has been transformed into a ready-to-use kit format for distribution.
among a network of 15 African research institutes. The specificity of the test was assessed under experimental and field conditions and found to be 96 ± 2% for T. b. brucei, 99.5 ± 1% for T. congolense and 99 ± 1% for T. vivax. Following a validation period under field conditions, adjustments were made to the protocol to increase the sensitivity of the ELISA and to improve the suitability of the test for laboratory use under African conditions. The Ag-ELISA is now being applied in conjunction with conventional parasitological techniques such as the dark ground/buffy coat technique (DG/BCT) to monitor progress in various tsetse and trypanosomosis control programmes and in a tsetse eradication effort on the island of Zanzibar. The two tests complement each other, since infections not detected by one test may be detected by the other. In general, the serological test tends to produce more false negatives during subacute infections, while the parasitological techniques tend to produce more false negatives during chronic infections. Since the sensitivity of the ELISA is not optimal, research efforts at the FAO/IAEA Agriculture and Biotechnology Laboratory will be focused on improving this aspect. However, these efforts are severely hampered by the lack of a diagnostic test that can be used as a 'gold standard'. The use of the polymerase chain reaction (PCR) for verifying doubtful test results and as a possible candidate for a 'gold standard' to diagnose trypanosomosis is discussed. Finally, future plans are outlined to initiate the use of geographical information systems to assess the impact of tsetse control and eradication programmes on land use and disease distribution.


Department of Veterinary Medicine, Infectious Diseases and Fish Diseases, Cairo University, Egypt.

The prevalence of T. evansi in 104 imported Sudanese dromedary camels was examined at Cairo and Giza abattoirs using a latex agglutination test for the detection of circulating trypanosomal antigens. Blood smear examination and mHCT detected latent parasitaemia in five camels (4.8%). Thirty camels serologically positive for antibodies (28.84%) had circulating trypanosomal antigens, while eight (7.69%) were negative for antigens. Twelve camels with detectable levels of circulating antigens (11.5%) tested negative for antibodies. Forty-two camels (40.30%) were positive for circulating antigens. It is concluded that the presence of circulating trypanosomal antigens does not correlate with the presence of specific antibodies. Detection of circulating T. evansi antigens using the latex agglutination test was found to be more sensitive and reliable for diagnosing carrier camels.


Magona: Livestock Health Research Institute, P.O. Box 96, Tororo, Uganda.
An animal trypanosomiasis survey was carried out at five major landing sites (Kitamiro, Lingira, Lukale, Buwanzi and Kirongo) on the Buvuma islands in March 1997. Owners brought a total of 118 cattle, 32 pigs, 259 goats and 60 dogs for examination at the five sites. All animals were bled and examined for trypanosomiasis using the buffy coat technique. Trypanosomes were found only at Kitamiro, Buwanzi and Kirongo, with overall prevalences of 30%, 26.3% and 11.2%, respectively. The highest prevalence was in pigs (50%), followed by cattle (18.6%), dogs (3.3%) and goats (3.1%). The predominant species in all animals was *Trypanosoma brucei*; *T. vivax* was found only in cattle and goats, and *T. congolense* was detected only in cattle and pigs. Two mixed infections were found: *T. vivax/T. brucei* in cattle and *T. congolense/T. brucei* in pigs. The distribution of animal trypanosomiasis appeared to coincide with the distribution of cattle and pigs, with pigs constituting the most important reservoir hosts for human and animal trypanosomiasis on the islands.


Blood and serum samples were collected from the tsetse-free zone in the central highlands of Ethiopia to determine the specificity and establish percentage positivity cut-off points of the Ag-ELISA. Blood samples collected from these areas were negative for trypanosomosis using standard trypanosome detection methods (STDMs, i.e. thick and thin blood smears and haematocrit capillary technique). Ag-ELISA, in contrast, detected circulating trypanosome antigens in 8.2% of the serum samples collected. In the same way, samples were gathered from a tsetse-infested zone in the upper Didessa valley in western Ethiopia to assess the sensitivity of the Ag-ELISA. In this case, STDMs detected trypanosome infections in 15.8-16.7% of the blood samples, while the Ag-ELISA indicated the presence of circulating trypanosome antigens in 38.6% of the serum samples tested. Ultimately, Ag-ELISA was used to monitor the effectiveness of the tsetse control campaign in the upper Didessa valley. There were enormous differences in the prevalence rates of trypanosomosis, as revealed by the STDMs and Ag-ELISA, between the tsetse-controlled and tsetse-infested zones of the upper Didessa valley. Generally in this work, Ag-ELISA revealed the presence of circulating trypanosome antigens in only 43.7% of patent infections. Nevertheless, the test detected 318 more cases which were not diagnosed by any one of the STDMs used. More interestingly, Ag-ELISA indicated the widespread presence of *T. brucei* in cattle in all the sampling zones.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 23: no. 11335.]

Ajuwape: Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Haematological changes produced by trypanosome infection in aparasitaemic but antigenaemic naturally infected Zebu cattle were studied. Of 125 cattle examined, only 6 (4.8%) were parasitologically positive, while 52 of the remainder (43.7%) were ELISA-positive. *Trypanosoma vivax* accounted for most infections, with smaller numbers of *T. congolense* and *T. brucei* and some mixed infections. Compared to the non-antigenaemic group, the antigenaemic cattle showed significant decreases in erythrocytic parameters (PCV, haemoglobin concentration, red blood cell count, mean corpuscular haemoglobin concentration and mean corpuscular volume). However, no significant difference was observed between the total serum protein and the total and differential absolute leucocyte counts of antigenaemic and non-antigenaemic cattle.


Audu: Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

The course of experimental infection and pathogenicity of an isolate of *T. evansi* from a camel in northern Nigeria were investigated using eight infected and six uninfected (control) Yankasa sheep. Each sheep was infected i.v. via the jugular vein with c. $2.0 \times 10^6$ *T. evansi*. The effects of the parasite on body temperature, PCV, haemoglobin, erythro-cytes and total protein were monitored three times a week for c. 9 weeks. Body weights were determined once a week for the duration of the experiment. All the infected sheep were positive, with a prepatent period varying between 3 and 6 days. *T. evansi* produced parasitaemic waves at an average interval of 8.3 days. Two distinct forms of the disease were produced: acute (4-14 days p.i.), from which four sheep died, and chronic (43-59 days p.i.), from which two died. The other two sheep survived. Mean rectal temperatures were significantly elevated in infected sheep ($P < 0.05$). Anaemia was a distinct feature of the disease, and the mean values of the haematological parameters dropped significantly ($P < 0.05$). Observed clinical signs included pale mucous membrane, epiphora, loss of appetite, emaciation, dullness and rough hair coat together with fluctuating pyrexia which in most cases coincided with the rise in parasitaemia. It is concluded that this isolate of *T. evansi* is pathogenic for Yankasa sheep.

This experiment studied the effects of body condition, long- and short-term levels of nutrition and trypanosomosis infection on the productivity of N'Dama cows using a $2 \times 2 \times 2$ factorial cross-over design. Pre-partum, 23 cows received supplements for 6 months (H), the other group of 20 for 2 months (L). Both groups grazed native pastures. Two days post partum, half the cows from each group were placed on a basal (B) or supplemented (S) plane of nutrition. The diet of concentrate, groundnut hay and andropogon hay was the same, only the quantities differed. Four weeks post partum half the animals in each group were inoculated with *T. congolense* organisms (I), the others acting as controls (C). The trial continued for a further 6 weeks. Pre-partum nutrition (H, L) had no effect on dry-matter intake (DMI) but pre-partum feeding (H) improved post-partum productivity, evident by higher dam liveweights ($P < 0.05$), body condition ($P < 0.001$), calf birth weight ($P < 0.05$) and calf liveweight gain ($P < 0.01$). Post-partum nutrition had no effect on productivity. Trypanosome infection caused a reduction ($P < 0.05$) in total DMI. The decline in groundnut hay and concentrate intake was proportionally ($P < 0.001$) greater in the S-I group than in the B-I group. A low plane of nutrition pre-partum depressed milk yield but increased fat concentration ($P < 0.05$). Infection significantly reduced milk offtake ($P < 0.05$). The reduction in milk offtake ($P < 0.01$) and calf liveweight ($P < 0.05$) were proportionally larger in the B-I than in the S-I group. Infection caused a decline in milk protein concentration ($P < 0.05$) and protein yield ($P < 0.01$) which was independent of dietary effects. Infection reduced ($P < 0.01$) the PCV but there were no interactions with diet. None of the cows was pregnant 150 days post partum but seven were cycling, 3 of 5 in the H-S-I group, 2 of 7 in the H-B-I group, 1 of 5 in the L-B-I group and 1 of 5 in the L-S-C group. These results suggest that S-I cows attempted to maintain milk yield at the expense of liveweight whereas the B-I cows had insufficient liveweight reserves that could be mobilised. This suggests that the nutritional balance and changes in weight at the time of infection might be more important than historical planes of nutrition.


Kock: Kenya Wildlife Service Veterinary Section, P.O. Box 40241, Nairobi, Kenya.

Haematological data obtained during routine translocation of 74 black rhinoceros in Kenya between 1991 and 1995 were examined, and subsets of data from 43 rhinoceros that were translocated to different regions of Kenya were compared. The findings suggest that transport and confinement stress may lead to gastric ulceration with haemorrhage, and that, in many animals, exposure to trypanosomes contributes to anaemia.

Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

The pathogenicity of T. brucei of cattle origin isolated from Kaura Local Government Area of Kaduna State was observed in eight (five infected and three control) 8-12 months old domestic boar pigs over a period of 12 weeks. All the five infected pigs became parasitaemic within a period of 2-7 days. Fluctuating levels of parasitaemia and pyrexia occurred in the infected pigs while mortality was observed in two. Other symptoms included anaemia, anorexia, rough hair coat, mucopurulent ocular discharge, emaciation, hyperaemia of the skin and incoordination leading to wobbling of the hind limbs. The infected pigs had significantly ($P < 0.001$) lower mean PCV and total plasma protein. Grossly, the infected pigs showed haemorrhages and congestion of organs. Significant ($P < 0.001$) differences were noticed in the mean body weight gain between infected and control pigs. The mean organ weights of the infected pigs were lower than those of controls, while their corresponding organ/body weight indices were higher. Histopathological changes such as mononuclear cellular infiltration by lymphocytes, plasma cells and macrophages, including lesions indicative of glomerulonephritis and broncho-pneumonia, were observed. The clinical, haematological and histopathological aberrations were more severe in the pigs that died. The results showed that this isolate of T. brucei is pathogenic to pigs and may adversely affect pig production. The possible involvement of cattle as reservoir hosts for infections causing outbreaks in domestic pigs is suggested.

(c) TRYPANOTOLERANCE

[See also 23: no. 11407.]


Goldammer: Department of Veterinary Pathobiology, Texas A & M University, MS Building 1197 RM 312, College Station, TX 77843, USA.

About 5% of the total African cattle population have a natural resistance to trypanosomiasis. In a preliminary study, markers linked to trypanotolerance were identified on different chromosomes. The use of this information in breeding programmes needs a close linkage of DNA markers to the trait. Research on domestic cattle in Africa therefore aims to generate a high resolution marker map for quantitative trait loci (QTL) regions significantly affecting trypanotolerance. Microdissection was used for the direct analysis of chromosome regions correlated to economically important traits. The chromosome fragment BTA7q14-q22, which corresponds to the QTL linked to parasitaemia, was isolated several times and used for the generation of chromosomal libraries. Primers designed from chromosome fragment-specific sequences were used for the isolation of bovine-specific bacterial artificial chromosomes (BACs) which can now
serve as starting material for the identification of informative markers within the QTL region.


Hanotte: ILRI, P.O. Box 30709, Nairobi, Kenya.

A preliminary search for quantitative trait loci (QTLs) in a cattle F₂ resource population segregating for trypanotolerance has revealed three large candidate chromosomal regions (Bta02, Bta05, Bta07). In order to narrow down the position of the trypanotolerant QTLs, comparative chromosomal maps between cattle, humans and mice are being built. Using a cattle radiation hybrid panel, framework maps are first constructed with type II markers (microsatellites). Type I markers (genes) are then positioned within these maps. Their relative chromosomal positions, compared to their positions in humans and mice, allow definition of conserved syntenic regions between the three species. The large number of genes being identified in humans and mice may assist selection of candidate genes.


Bradley: Department of Genetics, Trinity College, Dublin 2, Ireland.

The possibility of detecting regions of the genome under selection through effects on variation at linked neutral genetic markers was investigated. In particular, we studied three genomic regions which had been identified by mapping in a cross-bred pedigree as harbouring quantitative trait loci (QTLs) for trypanosomiasis tolerance. Population genetic analysis was used with mapped microsatellites to try to infer selection for trypanotolerance in West African cattle populations.


Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK.

This chapter reviews the genetic basis of immunity in cattle, and genetic resistance to leucosis, brucellosis, dermatophilosis, mastitis, trypanosomiasis, helminthiasis and ticks.

11401 Teale, A., Agaba, M., Clapcott, S., Gelhaus, A., Haley, C., Hanotte, O., Horstmann, R., Iraqi, F., Kemp, S., Nilsson, P., Schwerin, M., Sekikawa,

Teale: Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK.

A two species comparative genome scanning approach has been taken to mapping, and eventually identifying, genes at quantitative trait loci (QTLs) influencing resistance to trypanosomosis due to infection with *Trypanosoma congolense*. Using two large murine F2 and two F6 advanced intercross populations, QTLs influencing survival time in mice following challenge with *T. congolense* were mapped to small confidence intervals on MMU1, 5 and 17. The QTL on MMU17, *Tir1*, has a sufficiently small confidence interval (≤ 1 cM) to justify an immediate search for positional candidate genes at the DNA level. Mapping of trypanotolerance loci in an F2 cattle resource population is now beginning to reveal bovine resistance QTLs. A key strength of the comparative strategy derives from overlaying the genetic maps of murine and bovine QTL-containing regions. If, and where, the same genes are used by the two species, this may facilitate localisation of one or more of the genes contributing to variation in resistance to *T. congolense* trypanosomosis.

(d) TREATMENT

[See also 23: no. 11369.]


Murilla: Radioisotope Laboratory, KETRI, P.O. Box 362, Kikuyu, Kenya.

Two ELISAs for the determination of homidium [bromide] in serum of treated cattle have been developed and evaluated. One is a direct competition assay and the other an indirect competition assay. Both are highly sensitive with a limit of detection of 0.1 ng/ml of serum. Homidium levels were measurable in serum of cattle for over 2 months following administration of a single i.m. dose of 1 mg/kg body weight. The level of sensitivity makes these assays potentially useful tools in the pharmacokinetic evaluation of homidium and for investigating drug resistance or causes of drug failure. The indirect competition assay was chosen as being most suitable for further studies.

Pharmacokinetic studies on the trypanocidal drug homidium bromide using a competitive ELISA (detection limit 0.1 ng/ml) are reported for non-infected Friesian and Boran steers following treatment with homidium bromide at a dose of 1.0 mg/kg body weight. Following i.v. treatment of five Friesian steers, the mean serum drug concentrations were 31.9 ± 2.1 and 3.9 ± 0.4 ng/ml at 1 and 24 h, respectively. The decline in serum drug concentration was tri-exponential with half-lives of 0.064 ± 0.037 h for $t_{1/2\alpha}$, 7.17 ± 1.87 h for $t_{1/2\beta}$ and 106.3 ± 6.6 h for $t_{1/2\gamma}$ for distribution and elimination phases 1 and 2, respectively. The drug was detectable in serum for 17 days following treatment. The mean residence time (MRT) was 63.4 ± 7.5 h. Following i.m. treatment of five Friesian steers, the drug concentration at 1 h after treatment was 72.5 ± 2.2 ng/ml. This declined to 9.8 ± 1.8 ng/ml at 24 h. Low concentrations of between 0.1 and 0.3 ng/ml remained in circulation for up to 90 days post-treatment. Following i.m. treatment of five Boran steers, the mean serum drug concentration at 1 h after treatment was 112.1 ± 40.3 ng/ml. By 24 h after treatment, the concentration had fallen to 13.0 ± 3.3 ng/ml. Thereafter, the serum drug concentration-versus-time profile and the pharmacokinetic parameters obtained following non-compartmental analysis were similar to those obtained following i.m. treatment of Friesian steers.


A known nomadic cattle herd in the Bahr El Arab tsetse belt was divided into three equal groups of 100 each and given treatments against trypanosomiasis during the dry season (January to May) 1987. Group 1 was given Samorin (isometamidium chloride) (1 mg/kg), examined and re-treated with the same drug once every 2 months. Group 2 was given ethidium (homidium) bromide (1 mg/kg), examined and re-treated with the same drug each month. Group 3 (control) was treated symptomatically with Berenil (diminazene aceturate). Infection rates, mean PCV values and mortality rates were monitored for each group. Group 1 cattle had lower trypanosome infection rates, higher mean PCV values and no mortalities at the end of the experiment. Group 2 had the lowest mean PCV values and significantly higher trypanosome infection rates, with a 2% mortality rate at the end of the experiment. Controls showed intermediate values for trypanosome infection rates and PCV values, with no mortality. These results are discussed in relation to practical control of bovine trypanosomiasis in the area.

A procedure for the evaluation of chemical equivalence of proprietary formulations of isometamidium is described. The method combines the analysis of the principal component (isometamidium), HPLC profiling of related substances and determination of the inorganic impurity, ammonium chloride, using a modification of the Berthelot (Indophenol) reaction. Application of these procedures to analyses of commercially available sachets from four different batches of Samorin and four different batches of Veridium has demonstrated that there are marked qualitative and quantitative differences between batches from these two sources. Whilst Samorin samples showed inter-batch consistency of composition, there was considerable inter-batch variation between the samples of Veridium.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

(b) PATHOLOGY AND IMMUNOLOGY

[See also 23: nos. 11410, 11439, 11447.]


N. Inoue: Research Center for Protozoan Molecular Immunology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan.


Iraqi: ILRI, P.O. Box 30709, Nairobi, Kenya.


Bakhiet: Division of Infectious Diseases (F-82), Karolinska Institute, Huddinge University Hospital, S-14186 Huddinge, Stockholm, Sweden.

Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Jabalpur 482 001, M.P., India.

(c) CHEMOTHERAPEUTICS

[See also 23: nos. 11416, 11431, 11435.]


Grassi-Zucconi: Department of Cell Biology, University of Perugia, I-06100 Perugia, Italy.


Department of Biology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia.

The ethanol extract of root wood and the acetone extract of dried leaves of *M. stenopetala* were found to be active *in vitro* against trypomastigotes of *Trypanosoma brucei*, with ED$_{50}$ values of 9.2 and 10 µg/ml respectively.


Houghton: Pharmacognosy Research Laboratories, King’s College London, Manresa Road, London SW3 6LX, UK.

Dichloromethane extracts of the root bark and stem bark of *Kigelia pinnata* collected from Zimbabwe exhibited antitrypanosomal activity against *T. b. brucei* *in vitro*. Four naphthoquinones were isolated from both root and stem bark and assessed for
activity against *T. b. brucei* and *T. b. rhodesiense* bloodstream form trypomastigotes *in vitro*. One compound with a furanonaphthoquinone structure was found to possess pronounced activity against both parasites with IC₅₀ values of 0.12 and 0.045 µM, respectively, although it was less active than pentamidine. The other compounds exhibited lesser activity.


Grant: Bioengineering Unit, Wolfson Centre, University of Strathclyde, Glasgow G4 0NW, UK.


Zhou: Shanghai Institute of Pharmaceutical Industry, 1111 Zhong Shan Bei Yi Lu, 200437 Shanghai, China.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERISATION OF ISOLATES


MacLeod: Wellcome Centre of Molecular Parasitology, Anderson College, University of Glasgow, 56 Dumbarton Road, Glasgow G11 6NU, UK.

The issue of whether genetic exchange occurs at a significant frequency in natural populations of *T. brucei* is controversial and one of the arguments against a high frequency has been the apparent lack of host infections with mixtures of trypanosome genotypes. Three minisatellite markers (MS42, CRAM, 292) within the coding regions of three genes have been identified and PCR-based methods developed for detecting variation at these loci using crude lysates of infected blood as templates. Initial PCR analysis, using primers flanking the repeats, of DNA from two cloned stocks of the parasite (STIB 386 and TREU 927) has shown that two DNA fragments of different size were amplified from each stock. Analysis of the inheritance of these fragments into the F₁ progeny of crosses demonstrated that the different size fragments were alleles that segregated in a Mendelian manner. The alleles at each of the three loci segregated independently, consistent with their localisation on three different chromosomes.
Analysis of a series of cloned isolates from tsetse flies showed that these loci were highly variable, giving heterozygosities of 94% and the identification of 12 distinct alleles in a sample of 17 cloned isolates. In order to determine whether isolates are heterogeneous in terms of trypanosome genotype, the allelic variation at these three loci was examined in uncloned samples from tsetse flies isolated in Kiboko, Kenya, and Lugala, Uganda. A significant proportion of the isolates (36% in Lugala and 47% in Kiboko) contained more than two alleles at one or more of the loci, thus demonstrating that a high proportion of tsetse flies were infected with more than one genotype of trypanosomes. This was established unequivocally for two isolates (845 from Lugala and 927 from Kiboko) by generating a series of cloned trypanosome lines from each and determining the genotype of each clone: one isolate (927) contained seven different genotypes with a high proportion of the possible combinations of alleles at each locus. These results indicate the possibility of frequent genetic exchange in the field and imply that a significant proportion of mammalian hosts must contain mixtures of different trypanosome genotypes. These findings demonstrate the advantages of using minisatellite markers for the analysis of the population structure of *T. brucei*.

(c) LIFE CYCLE, MORPHOLOGY, BIOCHEMICAL AND MOLECULAR STUDIES


Field: Wellcome Trust Laboratories for Molecular Parasitology, Department of Biochemistry, Imperial College of Science, Technology and Medicine, Exhibition Road, London SW7 2AY, UK.

11417 **Bastin, P. and Gull, K., 1999.** Assembly and function of complex flagellar structures illustrated by the paraflagellar rod of trypanosomes. [*T. brucei.*] (Review.) *Protist, 150* (2): 113-123.

Bastin: School of Biological Sciences, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, UK.


Bastin: School of Biological Sciences, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, UK.

11419 **Bastin, P., Pullen, T.J., Sherwin, T. and Gull, K., 1999.** Protein transport and flagellum assembly dynamics revealed by analysis of the paralysed

Bastin: School of Biological Sciences, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, UK.


Blackwell: Cambridge Institute of Medical Research, Wellcome Trust/MRC Building, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 2XY, UK.

The common strategies employed by the three trypanosomatid genome projects (Trypanosoma brucei, T. cruzi and Leishmania major) are outlined and highlights of the projects to date are presented. All data obtained from these projects are made available through the public domain (for T. brucei see http://parsun1.path.cam.ac.uk).


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