MEETING REPORT

Workshop: Harmonisation of the Activities of PAAT and PATTEC

A workshop was held at the FAO Headquarters, Rome, Italy, on 2-3 May 2002 in a further effort to harmonise the activities of the Programme Against African Trypanosomiasis (PAAT) and the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). Prof. P. Holmes, PAAT Chairman, chaired the meeting. Senior officers from PAAT, OAU-IBAR, PATTEC, SIT Forum, and the PAAT Secretariat were participants. The following is an edited version of the report of the meeting.

Background

Tsetse-transmitted trypanosomiasis is a disease unique to Africa affecting both humans and animals. This disease occurs in nearly 9 million km² in 37 sub-Saharan countries corresponding approximately to one-third of Africa’s total land area, and threatens an estimated 50 million people and 48 million cattle (in the tsetse affected countries more than 170 million cattle currently overcrowd the few tsetse free areas). The tsetse fly spreads sleeping sickness (Human African Trypanosomosis, HAT) among an estimated 500,000 people, the majority of whom will die for lack of treatment. Nagana (African Animal Trypanosomosis, AAT) has a severe impact on African agriculture; estimated annual losses in cattle production alone are in the range of US$ 1.0-1.2 billion. To this, we have to add the indirect negative effects engendered by trypanosomiasis on total crop production. The disease influences where people decide to live, how they manage their livestock and the intensity of crop agriculture. The combined effects result in changes in land use and the environment, and they affect human welfare and increase the vulnerability of agricultural activity.

Further, in tsetse infested areas of sub-Saharan Africa, half of the human population suffers from food insecurity. Approximately 85% of the poor are located in rural areas and more than 80% of the population depends on agricultural production for their livelihood.

Streamlining and harmonisation

In order to avoid duplication of work and to maximise the efficiency of various efforts it is essential that the four mandated organizations harmonise and concert their efforts in the fight against tsetse and trypanosomiasis (T&T) in people as well as in livestock. In response to this need, the PAAT was endorsed in November 1997 by the FAO conference. The Programme seeks to serve as a forum for FAO, WHO, IAEA and OAU-IBAR to harmonise their efforts and concert their forces and resources in order to:
• Ensure an harmonious, sustainable approach towards improved human health and sustainable socio-economic and agricultural development of tsetse-infested areas;
• Promote and coordinate international alliances and efforts assisting in harmonised interventions against T&T; and
• Achieve integrated tsetse/trypanosomiasis control/eradication in identified areas in Africa.

Through its activities, PAAT seeks to guide and assist in the development of the international policy framework, priorities, strategies and principles guiding the implementation of integrated intervention approaches involving:

(a) Socio-economic factors of tsetse/trypanosomiasis intervention
(b) Impact of human and animal trypanosomiasis on human well-being and African agriculture
(c) Research needs
(d) Community participation in integrated intervention campaigns
(e) Drug management
(f) Integration of control techniques
(g) Environmental considerations and sustainable land use options with or without different intervention approaches.

PAAT is essentially concerned with the development and application of science-based standards for assessing the economic, social and environmental benefits and costs of T&T management. Its studies and analyses balance human needs in terms of sustainable food security and livelihoods with the preservation of natural resources and prevention of environmental degradation.

In response to the African Heads of State and Government decisions, which were passed in Lomé, Togo and in Lusaka, Zambia in July 2000 and 2001 respectively, on the control and eventual eradication of tsetse flies, OAU launched African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) in October 2001.

In its Plan of Action, PATTEC highlights the ultimate desirable objective as eradication of tsetse and trypanosomosis from Africa through the progressive creation and subsequent expansion of tsetse-free zones. With a view to pursuing this objective, PATTEC undertakes to organise and coordinate the campaign, and to mobilise the necessary human, financial and material resources to do so.

PATTEC is a sharply focussed campaign committed to tsetse eradication. The approach is based on the area-wide concept to tsetse intervention, which is defined as the management and elimination of entire tsetse populations within circumscribed areas. Community-based initiatives will be an integral part of the tsetse control measures adopted during the campaign.

PATTEC demonstrates in a very clear manner African ownership and responsibility for elimination of an African problem.

PAAT is a broad based international inter-UN agency forum, which embraces all
those concerned with tsetse and African trypanosomosis research and intervention. PATTEC is a focused campaign within Africa primarily concerned with tsetse elimination.

PATTEC’s primary objectives are to catalyse, co-ordinate and support field projects that are directly concerned with tsetse suppression and its ultimate elimination.

Both PAAT and PATTEC have the common objective to deal with the tsetse and trypanosomiasis problem and, ultimately to remove this public health and agricultural development constraint from Africa. PATTEC has a clear time-bound objective to achieve this through tsetse eradication, initially by the creation of tsetse-free zones. PAAT works towards the same ultimate objective by harmonising the efforts of international organisations and groups. These involve not only generating support for PATTEC but also, through normative responsibilities of the mandated member organisations, taking forward standardisation issues and the provision of guidance, including that on policy development, sleeping sickness surveillance and treatment, the availability of trypanocidal drugs, drug resistance and the use of trypanotolerant livestock. All these issues are expected to remain important aspects in the short to medium term.

**Major Areas of Focus of the Workshop**

- Identification of expected workshop outputs;
- The concept of areawide integrated pest management for joint international action against T&T in the context of sustainable agriculture and rural development (SARD);
- Criteria / guidelines for joint international action against T&T in the context of SARD;
- Validation of two earlier identified projects, i.e. one related to the Ethiopian Southern Rift Valley system and the other to a transboundary area in Burkina Faso and Mali, with respect to these agreed criteria;
- Identification of sequential steps in project cycles, with particular reference to the respective and joint roles (collaboration and co-ordination) of PAAT and PATTEC in the development and implementation of projects, which require joint international action;
- Further areas of harmonised PAAT - PATTEC collaboration;
- The next steps;
- Recommendations.

**Expected Workshop Outputs**

At the beginning of the workshop the participants agreed to the following list of expected outputs:

- Define the nature of the relationship between PAAT and PATTEC.
- Identify areas of commonality and agree on a common approach.
- Establish guidelines / criteria for the development of robust programmes which are feasible and attractive.
- Identify issues to be addressed and by whom; e.g. project design and
implementation, funding, quality assurance.
- Draft next steps for concerted action.
- Agree on joint paper and communiqué.

**The Concept of Area-wide Integrated Pest Management**

Efforts against the tsetse and trypanosomiasis (T&T) problem should take full advantage of the benefits associated with an adherence to accepted principles of integrated pest management (IPM) or integrated disease and pest management (IDPM), such as:

a) IPM measures against T&T are implemented in the broader context of human well-being, poverty reduction and food security, with improved public health, enhanced livestock-agricultural development and sustainable and appropriate utilisation of available natural resources guiding the process of strategy development;

b) In any situation the most appropriate intervention measures are selected for application as part of the integrated campaign in a phased manner;

c) IPM measures take into account and capitalise from different favourable factors, such as agro-ecological production trends and climatic trends and variations.

Some aspects of the conventional application of IPM principles have certain disadvantages for implementation against trans-boundary key pests and diseases. For example, for many “normal”, non-trans-boundary agricultural pests, IPM measures usually are only initiated once the problem exceeds a predetermined economic threshold level. Also, a field-by-field approach, whereby some farmers may decide to apply control measures, whereas several of their neighbours opt not to initiate any intervention measures, usually requires a higher overall intervention intensity (i.e. overall amount of insecticides etc. used) than would be necessary if all farms and adjacent areas were treated in a well co-ordinated and synchronised manner.

The area-wide integrated pest management (AW-IPM) concept combines the obvious benefits of conventional IPM measures and, through a more preventive approach that targets entire insect pest populations, avoids the above disadvantages. As T&T is one of the trans-boundary pest insect and disease problems that constitute a key bottleneck for enhanced sustainable agriculture and rural development (SARD), the principles of AW-IPM should guide the planning and implementation of T&T intervention measures.

**Criteria/Guidelines for Joint International Action against T&T in the Context of SARD**

Joint international action against Human African Trypanosomiasis will be guided by WHO. Priority will be given to increase HAT control activities in areas where PAAT-PATTEC actions) are not being implemented.

In the context of SARD and based on previous outcomes of PAAT meetings, the workshop developed the following criteria/guidelines for prioritising areas for joint
international action against T&T in the context of rural development and identified the factors contributing to increased feasibility and early success of project activities and sustainable outcomes as outlined below (Table 1).

Table 1. Criteria/guidelines for prioritising areas for joint international action against T&T in the context of sustainable agriculture and rural development (SARD).

<table>
<thead>
<tr>
<th>1. Severity of the impact of the tsetse/trypanosomiasis problem.</th>
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<tbody>
<tr>
<td>2. Desire/need for intervention by local communities and national governments.</td>
</tr>
<tr>
<td>3. Opportunity to support poverty reduction, increase food security and maximise socio-economic returns through enhanced SARD, such as:</td>
</tr>
<tr>
<td>(a) Expansion and intensification of mixed farming;</td>
</tr>
<tr>
<td>(b) Improved subsistence farming and/or production of cash crops;</td>
</tr>
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<td>(c) Land use and tenure as components of sustainability;</td>
</tr>
<tr>
<td>(d) Sustainable and environmentally appropriate utilisation of natural resources.</td>
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<tr>
<td>4. Factors contributing to increased feasibility and early success of project activities and sustainable outcomes, such as:</td>
</tr>
<tr>
<td>(a) Activities phased and initial objectives achievable within 5-7 years of a programme/project cycle;</td>
</tr>
<tr>
<td>(b) Natural barriers;</td>
</tr>
<tr>
<td>(c) Possibility for artificial confinement;</td>
</tr>
<tr>
<td>(d) Favourable agro-ecological production trends;</td>
</tr>
<tr>
<td>(e) Favourable climatic variations and trends;</td>
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<td>(f) Commitment and involvement of local authorities and communities;</td>
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<tr>
<td>(g) Existence of local technical and logistical support;</td>
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<td>(h) Existence of ongoing agricultural development project that identifies tsetse and trypanosomes control as a major constraint.</td>
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</table>

Validation of Identified Projects against Agreed Criteria

Two projects identified earlier, one within the Ethiopian Southern Rift Valley system and the other in a trans-boundary area in Burkina Faso and Mali, were assessed with respect to the agreed criteria described above. The meeting agreed that both projects met the criteria. With regards to the matter of isolation, both areas have natural barriers: in the case of the Ethiopian project (covering 10,500 km²) a short temporary artificial barrier of 8 km x 8 km will be required. In the West African project, natural barriers formed by the watersheds will be reinforced by extending farming practices coupled, where necessary, with temporary artificial barriers.

Sequential steps in Project Cycles and Roles of Partners

The workshop identified the sequential steps in project cycles and discussed the joint and respective roles of PAAT and PATTEC in the development and implementation
of projects, which require joint international action. The following was agreed (Table 2):

Table 2: Project Initiation, Implementation, Management and Supervision

<table>
<thead>
<tr>
<th>Activity</th>
<th>Partners involved</th>
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<tbody>
<tr>
<td>1. Project Initiation</td>
<td></td>
</tr>
<tr>
<td>a) Project identification</td>
<td>Affected countries, PATTEC, PAG-PAAT, International Organisations, donors and others</td>
</tr>
<tr>
<td>b) Consultation between/with government(s) of T&amp;T affected country(ies) and other partners</td>
<td>PATTEC, countries, beneficiaries, other partners and stakeholders</td>
</tr>
<tr>
<td>c) Establishment of Task Force</td>
<td></td>
</tr>
<tr>
<td>(i) Selection of Task Force members</td>
<td>PAAT and PATTEC, in consultation with national governments, donors and other stakeholders</td>
</tr>
<tr>
<td>(ii) Seek funding for Task Force</td>
<td>PAAT and PATTEC, in consultation with countries, donors, international organisations, the private sector and other sources</td>
</tr>
<tr>
<td>d) Preparation of Concept Note</td>
<td>Task Force</td>
</tr>
<tr>
<td>e) Concept evaluation</td>
<td></td>
</tr>
<tr>
<td>(i) Technical evaluation</td>
<td>PAAT-PAG</td>
</tr>
<tr>
<td>(ii) Prioritisation and recommendation to PMC</td>
<td>Technical Advisory Forum (TAF)</td>
</tr>
<tr>
<td>f) Preparation of Project Document</td>
<td>Task Force</td>
</tr>
<tr>
<td>g) Evaluation and approval of Project Document</td>
<td></td>
</tr>
<tr>
<td>(i) Technical evaluation</td>
<td>PAAT-PAG</td>
</tr>
<tr>
<td>(ii) Approval by government(s)</td>
<td>PATTEC</td>
</tr>
<tr>
<td>(iii) Final approval and submission to donor(s)</td>
<td>PATTEC-PMC</td>
</tr>
</tbody>
</table>
h) Mobilisation and Co-ordination of Resources

PATTEC-PMC (i) local and national governments and NGOs (ii) sub-regional, regional and international (iii) public/private investments; loans and grants

2. Project Implementation, Management and Supervision

| a) Selection and establishment of Management team | National governments, PATTEC, contributing partners |
| b) Independent project monitoring and evaluation; Establishment of procedures and team | PAAT and PATTEC in consultation with the national governments and donors |
| c) Field intervention | Project management team |
| d) Project progress evaluation and mid-term and final review | |
| (i) ongoing internal | project management |
| (ii) external | independent review team |

The criteria/guidelines for joint international action against T&T in the context of SARD and the sequential steps in project cycles (Table 2) and the possible roles of partners may need to be further improved and refined, as deemed necessary on the basis of experience that may be acquired.

Other Areas of PAAT-PATTEC Collaboration

PAAT and PATTEC agreed to continue collaborating in the following fields, which will be developed by the PAAT Secretariat and the PATTEC Co-ordination Office:

1. Information and communication, including PAAT-IS, PAAT-Link, newsletters, position papers as part of the PAAT Technical and Scientific Series, Tsetse and Trypanosomiasis Information Quarterly (TTIQ);
2. Publicity and awareness raising;
3. Training and capacity building;
4. Research priorities and operational research;
5. Normative issues, including quality assurance, good practices, standards, guidelines.

Next Steps

- Endorsement of the workshop’s recommendations on harmonisation of PAAT-
PATTEC by the four mandated organisations;
• Dissemination of the agencies’ endorsed workshop recommendations to the relevant parties;
• Initiation of PAAT-PATTEC co-operation on specific projects identified; refinement of guidelines / criteria and steps in project cycles, as required;
• Preparation and release of a joint communiqué on PAAT - PATTEC harmonisation;
• Development of proposals for continued collaboration in the areas identified in above.

Recommendations

PAAT-PATTEC harmonisation should be advanced as soon as possible by the respective partners along the lines identified in the workshop; and

To assist this harmonisation process and in recognition of the positive signals received from the higher management of IAEA and OAU the agreement on formal collaboration of IAEA and OAU in PAAT should be finalised.

NEW ELECTRONIC JOURNAL ANNOUNCED

Dr. Alberto Dávila and Dr. Kevin Tyler announce the launch of the new, free, electronic journal: Kinetoplastid Biology and Disease (http://www.kinetoplastids.com).

Kinetoplastid Biology and Disease offers a unique focus on species of the Order Kinetoplastida, the diseases they cause and the vectors which transmit them. It is an electronic publication which aims to strengthen ties between research and clinical/field applications, increasing dialogue between bench scientists, theoreticians and planners, and the professionals in the field. Kinetoplastid Biology and Disease accepts basic science, epidemiological, public health, clinical, veterinary and agricultural papers on trypanosomiasis, leishmaniasis and related diseases, meeting the criteria of peer review.

The advent of Kinetoplastid Biology and Disease enables the free dissemination of scientific information about kinetoplastid diseases and their control. This is a considerable advantage, since most scientific journals are not free and even the ones with cheaper subscription rates may not be accessible to researchers, clinicians, and field researchers in the poorest and affected developing countries. Moreover, the journal will serve as a focus in which the whole scientific community concerned with the kinetoplastids can participate: the aim is to educate, notify and debate about progress and direction. It is hoped that the new journal will serve as a vehicle to promote pragmatic research and as act a practical first step in tackling some of the communication difficulties that face those concerned with the eradication of these diseases.

Kinetoplastid Biology and Disease is created as peer-review journal, freely available to anyone in the world with a networked computer. Kinetoplastid Biology and Disease is published by BiomedCentral (www.biomedcentral.com), and has a world-class editorial board (http://www.kinetoplastids.com/edboard/). It will be indexed by most of the major scientific indexing services such as PubMed. Articles will be available in html
and .pdf formats. *Kinetoplastid Biology and Disease* is supported by the “Vice-Presidencia de Desenvolvimento Institucional, Informacao e Comunicacao” and the “Instituto Oswaldo Cruz” of the Oswaldo Cruz Foundation. The journal is recipient of a Soros Foundation award for promoting open publishing.

In its first issue *Kinetoplastid Biology and Disease* presents:

Combating Kinetoplastid diseases.
A. Dávila and K. Tyler.
[http://www.kinetoplastids.com/content/pdf/1475-9292-1-6.pdf](http://www.kinetoplastids.com/content/pdf/1475-9292-1-6.pdf)

Salivaria or Stercoraria? The *Trypanosoma rangeli* dilemma.
E. Grisard.
[http://www.kinetoplastids.com/content/pdf/1475-9292-1-5.pdf](http://www.kinetoplastids.com/content/pdf/1475-9292-1-5.pdf)

What can we hope to gain for trypanosomiasis control from molecular studies on tsetse biology?
S. Aksoy, Z. Hao and P. M. Strickler.
[http://www.kinetoplastids.com/content/1/1/4](http://www.kinetoplastids.com/content/1/1/4)

From the cell biology to the development of new chemotherapeutic approaches against trypanosomatids: dreams and reality.
W. De Souza.
[http://www.kinetoplastids.com/content/pdf/1475-9292-1-3.pdf](http://www.kinetoplastids.com/content/pdf/1475-9292-1-3.pdf)

PCR identification of *Trypanosoma lewisi*, a common parasite of laboratory rats.
M. Desquesnes, S. Ravel and G. Cuny.
[http://www.kinetoplastids.com/content/1/1/2](http://www.kinetoplastids.com/content/1/1/2)

Molecular determinants and regulation of *Leishmania* virulence.
K.-P. Chang and B. S. McGwire.
[http://www.kinetoplastids.com/content/1/1/1](http://www.kinetoplastids.com/content/1/1/1)

The editors encourage colleagues to submit papers and send their feedback to "kbd@trypanosome.com"

**MEETING REPORT**

**IAEA/FAO Training Course on GIS, Ouagadougou, Burkina Faso, 6 - 24 May 2002**

A regional training course entitled "Establishing National GIS Capacity for Ongoing and Planned Tsetse / Trypanosomosis Intervention Campaigns" was held from 6th to 24th May 2002 in Ouagadougou, Burkina Faso. The training course was hosted by the Government of Burkina Faso through the National Radiation Authority for Atomic Energy, with the support of the International Atomic Energy Agency (IAEA) and the Animal Health Division of FAO, providing GIS software (ArcView and Spatial Analyst;
with CD-ROM, key and manuals) to the participant's countries, as well as the PAAT-IS CD-ROM and other relevant manuals.

The course focused on using Geographic Information System (GIS) based data collection and processing, as needed for integrated and area-wide tsetse and trypanosomiasis intervention campaigns and for post-intervention land-use monitoring. Nine participants from seven West African countries, i.e. Burkina Faso (three participants) and Cameroon, Côte d’Ivoire, Ghana, Mali, Niger and Senegal (one participant each), attended the course.

The students were introduced to the type of equipment and software needed for GIS based data collection and processing, and to modelling entomological, parasitological and other data/parameters into GIS-based data format. Particular attention was given to the screening and selection of the appropriate data sets, and to how these data have to be collected and processed. Attention was also given to translating data sheets into a spatial database format. The participants appreciated that digital mapping starts off with geo-referenced data gathering (e.g. number, sex, age and reproductive status of tsetse flies caught, trypanosomiasis prevalence/incidence, etc.).

Participants came to understand the principle of integrating the tsetse/trypanosomiasis data layers with gathered geo-referenced information on socio-economics, ecology or administrative data. The principles of linking information and data processing and analysis using ArcView were demonstrated and practised in special exercises.

Comments and recommendations were obtained from participants and lecturers. These will be used for further improving the course and eventually developing a manual for the use of GIS in support of tsetse/trypanosomiasis intervention campaigns. FAO, IAEA and PATTEC are currently considering the organisation of a similar GIS course later in 2002 or early in 2003 for Eastern and Southern Africa.

REQUEST FOR INFORMATION CONCERNING TSETSE DISTRIBUTION

Updating tsetse distribution maps

David Bourn, David Rogers and William Wint of Environmental Research Group Oxford Limited and Trypanosomosis and Land Use in Africa Group, Zoology Department, South Parks Road, PO Box 346, Oxford, OX1 3QE, UK [david.bourn@ntlworld.com], thank those who have already sent in details of tsetse distribution in eastern Africa, but report that they still need more records and wider coverage. Accordingly, they are making a further appeal for information. They ask the tsetse workers operating in this area for further collaboration.

They explain that they have been asked to update and refine predictions of potential tsetse distribution in eastern Africa, based on methods used to produce maps of potential tsetse distribution across the whole of sub-Saharan Africa for DFID’s Animal Health and Livestock Production Programmes and FAO, as well as the posters distributed at the October 2001 meeting of the ISTRC meeting in Ouagadougou.

To do this, they need the latest information on the distribution of individual tsetse species in eastern Africa to use as training data to determine the environmental correlates
of known distribution, from which other similar areas of potential distribution can be identified and mapped from multi-temporal satellite imagery. They ask, specifically, for any recent information, such as national tsetse distribution maps, local field survey maps, sketch maps, and geo-referenced digital data, relating to tsetse species distribution in Burundi, Ethiopia, Kenya, Rwanda, Somalia, Sudan, Tanzania and Uganda. They request names of whom to contact, or better still, the data to be faxed or e-mailed to them as soon as possible. Assistance would be very much appreciated and fully acknowledged. Phone and fax: (44) 01865 883281.
SECTION B - ABSTRACTS

GENERAL (INCLUDING LAND USE)


2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES


Robinson: Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria. [a.robinson@iaea.org]

The recent success of the sterile insect technique (SIT) in eradicating Glossina austeni from Zanzibar has stimulated interest in applying this technology to control Glossina pallidipes. However, little is known about the mating behaviour of this species in relation to the development and implementation of an effective SIT programme. The effect of age on male and female receptivity to mating was evaluated together with copulation duration, sperm transfer and the growth of the accessory gland and follicle A in males and females, respectively. Females and males reached their optimal sexual receptivity 9-13 days after emergence. Mean copulation duration was 20-30 min for mature males and females. The growth of follicle A and the accessory gland (apical body) was a function of age of females and males, respectively. Ovulation was not observed in virgin females up to 15 days of age whereas mated females ovulated by day 9. Males aged 7-15 days were equally effective in inseminating. Cages of males and females of different ages were set up to monitor puparial production in relation to optimization of mass rearing. The results are discussed in relation to the development of an efficient mass rearing protocol for this species and an optimal release strategy for sterile males.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY


Krasfur: Department of Entomology, 402 Science 2, Iowa State University
Genomic libraries enriched for simple sequence repeats were constructed for *Glossina morsitans morsitans*, *G. m. submorsitans*, and *G. m. centralis*. Sixteen microsatellite markers were isolated from the libraries and evaluated on flies from natural *G. m. morsitans* populations and other *Glossina* species in the morsitans and palpalis species groups. The primers amplified appropriate sized DNA fragments in the morsitans and palpalis groups. In *G. morsitans* s.l., eight of twelve dinucleotide repeats and four of twelve trinucleotide repeats were polymorphic. The polymorphic loci showed a mean 7.5 ± 4.8 alleles per locus and their mean heterozygosity was 55.8 ± 7.7%.

12207 **Boulanger, N., Brun, R., Ehret-Sabatier, L., Kunz, C. and Bulet, P., 2002.**


Boulanger: Réponse Immunitaire et Développement chez les Insectes, UPR 9022 du CNRS, Institut de Biologie Moléculaire et Cellulaire, 15 rue René Descartes, 67000 Strasbourg, France. [N.Boulanger@ibmc.u-strasbg.fr]

Several dipteran insects are vectors of parasites causing major human infectious diseases. Among these, the tsetse fly, *Glossina* spp., is responsible for the transmission of trypanosomes, the pathogens responsible for sleeping sickness in Africa. A better understanding of insect-parasite interactions will help establish new strategies to fight this important often fatal disease. Antimicrobial peptides (AMPs) are part of the humoral immune response in insects during bacterial, fungal and parasitic infections. Here, we studied the immune response of *Glossina morsitans* to bacteria and to *Trypanosoma brucei brucei* by analyzing the synthesis of AMPs as markers of the humoral immune response. By reversed-phase chromatography, mass spectrometry analysis, Edman degradation and *in vitro* antimicrobial assays of the hemolymph of immune-challenged adults of *G. morsitans*, we identified three AMPs: a cecropin, an attacin and a defensin. These three AMPs were found to be induced upon systemic bacterial infection and also after *per os* infections by bacteria and parasites.


Torr: NRI, University of Greenwich, Chatham Marine, ME4 4TB, Kent, UK.

In Zimbabwe, observations were made of the behaviour of individual stable flies (*Stomoxys* spp.) (Diptera: Muscidae) and tsetse (*Glossina* spp.) (Diptera: Glossinidae) feeding on cattle during the wet (*Stomoxys* and tsetse) and dry (tsetse only) seasons. For *Stomoxys* landing on adult cattle, only 27% took a full meal (mean feeding time = 147 s). Most *Stomoxys* left the host before completing their meal, largely due to disturbance by
the host's defensive behaviour (24%, mean time = 59 s) or other flies (44%, 71 s). The probability of a Stomoxys leaving the host progressively increased with time. Simultaneous observations of tsetse showed that, compared to Stomoxys, their feeding success was lower (15%), feeding was interrupted earlier (33 s) and the time taken to complete a meal was shorter (109 s). Further studies of tsetse across different seasons and hosts showed that feeding success varied according to host age (adult = 7%; calf = 3%) and was negatively correlated with the frequency of host defensive behaviour and the relative abundance of non-biting Diptera. Disturbances were more often caused by host behaviour (69%) than other flies (31%) and the probability of tsetse leaving decreased with time on the host. Overall, these results suggest that tsetse and Stomoxys have different feeding strategies. In particular, tsetse appear to be more responsive to host defensive behaviours, which reduces their feeding success relative to Stomoxys. These behavioural differences are consistent with the respective life-history characteristics of Stomoxys and tsetse.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES


Gikonyo: ICIPE, P.O.Box 30772, Nairobi, Kenya. [ngikonyo@isipe.org]

A previous study on the feeding responses of tsetse flies, Glossina morsitans morsitans, implicated the existence of allomonal barriers, both volatile and nonvolatile, on the nonpreferred host, waterbuck, Kobus defassa. In the present study, electroantennogram-active compounds in odours from waterbuck were compared with those of two preferred hosts of tsetse flies, buffalo, Syncerus caffer, and ox, Bos indicus. Odours from the three bovids were trapped on activated charcoal and/or reverse-phase (octadecyl bonded) silica and analyzed with a gas chromatography-linked electroantennographic detector (GC-EAD) and, where possible, identified by using gas chromatography-linked mass spectrometry (GC-MS) and chromatographic comparisons with authentic samples. The GC-EAD profiles (with G. m. morsitans antennae) of the odours of the two preferred hosts were comparable, comprising medium-chain, saturated or unsaturated aldehydes and phenols, with buffalo emitting a few more EAG-active aldehydes. Waterbuck odour gave a richer profile, consisting of fewer aldehydes but more phenolic components and a series of 2-ketones (C8-C13) and δ-octalactone. This bovid also emits moderate amounts of C5-C9 straight-chain fatty acids, some of which were detected in buffalo and ox only in trace amounts. However, these did not elicit significant GC-EAD responses. Waterbuck profiles from the antennae of G. pallidipes showed broad similarity to those from G. m. morsitans, although the composition of aldehydes and ketones was somewhat different, indicating species-specific difference in the detection of host odours. Certain waterbuck-specific EAG-active components, particularly the 2-ketones and lactone, constitute a candidate allomonal blend in
waterbuck odour.


Van den Bossche: Institute of Tropical Medicine, Veterinary Department, Nationalstraat 155, Antwerpen, Belgium. [pvdbossche@otg.be]

The seasonal changes in the distribution of Glossina morsitans morsitans Westwood (Diptera: Glossinidae) and its main host, cattle, were examined in a cultivated area of the plateau of eastern Zambia. During four consecutive years, the tsetse and cattle populations were monitored along a fly-round transect traversing the two main vegetation types in the study area. These were miombo, a one-storied open woodland with the genera Brachystegia and Julbernardia dominant, and munga, a one- or two-storied woodland where the principal tree genera were Acacia, Combretum and Terminalia. Concurrently, a capture/mark/release/recapture (CMRR) exercise was conducted along two other transects also traversing both vegetation types. The index of apparent abundance of tsetse (IAA) in miombo increased at the beginning of the rainy season (November), reached its peak at the end of the rainy season (April) and was low during the cold season (May to late August), but especially the hot dry season (September to late October). The IAA of tsetse in munga showed a pattern that was the reverse of that in miombo. The seasonal changes in the IAA of tsetse in both vegetation types were in accordance with changes in the movement patterns of tsetse between the two vegetation types as observed using CMRR. The distribution and abundance of cattle along the transect also showed a seasonal trend. This was especially so in munga, during the first three years of observations, where cattle abundance increased gradually from June onwards, reached a maximum at the end of the hot dry season (October-November) and declined steeply at the start of the rainy season (November-December). In both vegetation types, the monthly mean IAA of tsetse was positively correlated with the abundance of cattle in the previous month. It is concluded that the distribution of tsetse in cultivated area of the eastern plateau of Zambia undergoes substantial seasonal changes, which can partly be attributed to changes in the distribution of cattle. The implications of these observations for the control of tsetse are discussed.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)


Okiria: Livestock Health Research Institute, PO Box 96, Tororo, Uganda.
This study was conducted in Masaba and Masafu Sub-counties, Busia District, Uganda to assess the effect on the tsetse fly population of first treating all cattle with 1% w/v deltamethrin pour-on for a few months, followed by treating 10% of the cattle population. Treatment of all cattle for six months resulted in a significant reduction in the density of tsetse flies from 6.3 to 0.1 flies/trap/day (FTD), a 98.4% reduction. During the same period, the point prevalence of bovine trypanosomosis dropped from 37.7% to 2.9% (a 92.3% reduction). Treatment was resumed six months later, but this time only 10% of the cattle population received the pour-on treatment at three week intervals for a period of one year. This treatment maintained the tsetse fly density between 0 and 0.5 FTD and the prevalence of bovine trypanosomosis generally remained below 10%. In conclusion, under the local prevailing conditions, treatment of all communally grazed cattle with deltamethrin pour-on effectively suppressed the *Glossina fuscipes fuscipes* population. However, subsequent treatment of 10% of the cattle probably failed to control the tsetse fly population at a level sufficient to reduce trypanosomosis to acceptable levels.


Reichard: 3602 C. Las Colinas, Austin, TX 78731 USA.

Two examples of area-wide programmes, employing the sterile insect technique (SIT), which have eradicated a parasite and a disease vector common to domestic and wild animals are described. New World screwworm (NWS), *Cochliomyia hominivorax*, caused significant morbidity and mortality of livestock and wild mammals in tropical and subtropical areas of America before eradication was achieved in North America using the SIT and other components of an integrated pest management (IPM) programme. Movement of wild as well as domestic animals from an area which is infested with screwworm to a free area requires prophylactic treatment. Tsetse fly-borne trypanosomosis has an immense influence on the distribution of people and livestock in Africa. The immunotolerance of wildlife to the parasites is an important factor in maintaining some areas livestock free as wildlife refuges. Slaughter has ceased of wild hoofstock species considered to be disease reservoirs for control purposes. The SIT, combined with other IPM measures, has resulted in the eradication of the tsetse fly and trypanosomosis from Zanzibar. Other programmes in Africa are underway. Microbial ‘biopesticides’ have also been employed successfully against plant insect pests and some vectors of human disease. It seems likely that for the immediate future, wildlife may benefit from area-wide biological control programmes, intended mainly to protect humans and/or domestic animals.
4. EPIDEMIOLOGY: VECTOR-HOST AND VECTOR-PARASITE INTERACTIONS


Garcia: IRD, BP 1386, Dakar, Sénégal. [andre.garcia@ird.sn]

Human African Trypanosomiasis is related to behavioural risk factors but complex interactions exist between (i) environmental and behavioural risk factors, (ii) vector and (iii) human host. Our aim was to investigate the interrelationships between previously analysed risk factors and the roles of age and time of exposure according to ethnic group and migration status. However, this descriptive and retrospective study is based on cases only (no controls) and our results must therefore be regarded as hypothesis-generating. Individuals originating from areas where sleeping sickness is absent and who settle in an endemic area seem to develop the disease after a shorter time of exposure than native subjects from endemic areas. Our results emphasise the complexity of vector-transmitted disease epidemiology, involving behavioural and/or environmental risk factors on the one hand, and more individual ones such as ageing, immunity and genetic background on the other hand.


Lewis: The Scientist, 3535 Market St, Suite 200, Philadelphia, PA 19104, USA

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE


Solano: IRD, UR 035, Institut Pierre Richet, 01 BP 1500 Bouaké, Côte d’Ivoire. [solano@ird.ci]

Human African trypanosomiasis is a parasitic infection caused by protozoa belonging to Trypanosoma brucei subspecies. The clinical evolution of this disease is t
complex and might be because of the parasite itself, as genetic diversity has been observed in *T. brucei* ssp. We investigated the relationship between the genetic diversity of trypanosomes and the diversity of clinical patterns in Côte d’Ivoire. We studied clinical sleeping sickness cases, and genetically analysed the trypanosomes isolated from these patients. An important genetic monomorphism among stocks isolated in Côte d’Ivoire was observed by using various markers: isoenzymes electrophoresis, random amplified polymorphism DNA and PCR of microsatellite sequences. At the same time, the diversity of clinical patterns and evolutions was confirmed by clinical analysis. The existence of an individual susceptibility to disease (human trypanotolerance) should be taken into account even if our genetic conclusions might be distorted because the isolation success rates were particularly poor. In fact, we observed that the isolation success rate varied significantly depending both on the focus of origin ($P = 0.0002$) and on the ethnic group ($P = 0.0317$) of the patient. Further investigations are required in order to study a possible selective impact of the use of the kit for *in vitro* isolation of trypanosomes as an isolation technique.


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


Rickman: Orchard Cottage, Ford and Fairy Cross, Bideford EX39 5BU, N. Devon, UK. [ROYRICKMAN@aol.com]

It is argued that the quickest, and in the long term, the cheapest method of sleeping sickness control is to deplete the human reservoir of infection rapidly, while concurrently employing simple and practical measures to reduce human-tsetse contact in the key transmission areas. The main reservoirs for sleeping sickness are the early-stage haemo-lymphatic cases, as these are ambulant, have minor symptoms and the patients may have little interest in seeking medical aid especially at busier periods of the year. Rural health posts could be made more effective in diagnosing cases accurately if they were equipped with portable centrifuges and microscopes. Permanent medical surveillance teams equipped with new laboratory instruments as well as solid-tyred bicycles, could likewise help to deplete the parasite reservoir. Bush clearing and the use of tsetse traps at strategic points could reduce human-fly contact. Such measures should be highly cost-effective, in contrast to some of the more advanced techniques under discussion.

Solano: Institut Pierre Richet (IPR), IRD UR 035, BP 1500 Bouaké, Côte d’Ivoire. [solano@ird.ci]

During a medical survey of the sleeping sickness focus in Bonon, Ivory Coast, PCR with Trypanosoma brucei specific primers (TBR 1–2 from Parasitology 99 (1989) 57) was tested on DNA derived from blood samples. DNA purification using a chelating resin was performed either on whole blood or on the buffy coat prepared in two different ways. The preparation based on whole blood performed better than those using the buffy-coat. Using this first method, the sensitivity was 100% on parasitologically confirmed patients, and the specificity was 92%. However, problems of reproducibility of the technique were pointed out, particularly on samples from serologically positive but apparently apasitemic individuals. It is suggested that the PCR could help in the diagnosis of Human African Trypanosomosis, but the use of other primers should be investigated.

(b) PATHOLOGY AND IMMUNOLOGY


Bakheit: Centre for Infectious Medicine, Karolinska Institute, Huddinge University Hospital, (f-82), SE-141 86 Huddinge, Stockholm, Sweden.

Chemokine effects on leukocyte infiltration into the central nervous system (CNS) are key events in the inflammatory processes of neuroimmunologic and neuroinfectious diseases. Because chemokines may play important roles in proliferation and differentiation of brain cells and in the initiation and progression of CNS inflammatory disorders, we analyzed constitutive and inflammatory-induced expression of α and β chemokines in human first trimester forebrain cells. Dissociated cell cultures were studied for spontaneous chemokine induction and after stimulation with the trypanosome lymphocyte triggering factor (TLTF), which is a novel trypanokine secreted by African trypanosomes that triggers a complex of immune responses. LPS and variant surface glycoprotein (VSG) were used as controls. These results illustrate the ability of resident brain cells to constitutively express chemokine genes, which may suggest an important role for chemokines during brain development. Furthermore, TLTF-induced chemokine
expression in astrocytes and neurons indicate the capacity of TLTF to provoke neuroinflammation in the brain, which may have important therapeutic implications for the neurological manifestations of African trypanosomiasis.


Lejon: Institute of Tropical Medicine, Department of Parasitology, Nationalestraat 155, B-2000 Antwerpen, Belgium.

Concentrations of glial fibrillary acidic protein (GFAp) and light subunit neurofilament protein (NFL) in cerebrospinal fluid (CSF) were measured in patients with second-stage *Trypanosoma brucei gambiense* sleeping sickness. Correlations between GFAp and NFL in CSF as markers for astrogliosis and neurodegeneration, and clinical and biological data were investigated. Abnormal levels of GFAp and NFL were significantly associated with increasing CSF cell number and protein concentration, and with the absence of lymph nodes or the absence of trypanosomes in lymph node aspirate. A significant association was found between abnormal NFL and presence of trypanosomes in CSF, abnormal limb movements, difficulties in gait and coordination, and low Karnofsky index. By multivariate analysis, it was shown that increasing CSF cell number, increasing CSF protein concentration, and the absence of lymph nodes or the absence of trypanosomes in the lymph node aspirate were the best predictors for astrogliosis and neurodegeneration among the variables tested. These results demonstrate the importance of CSF cell count and protein determination in assessment of the severity of central nervous system involvement and reinforces the importance of laboratory diagnosis to assess the stage of the disease. The clinical symptoms studied were less useful in predicting astrogliosis or neurodegeneration.

(c) TREATMENT


Moore: Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.

An American tourist on a world tour was diagnosed as suffering from an infection of *Trypanosoma brucei rhodesiense* after arriving in Katmandu. As the patient had previously visited Brazil and Argentina, before proceeding to South Africa, Zimbabwe, Zambia, Tanzania and Kenya, it was necessary to make a differential diagnosis by, inter
alia, microscopical examination of the blood, in which trypanosomes (not of *T. cruzi*) were found. Clinical symptoms of the case are given in detail.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION


A fully automated image processing system for detection of motile microorganisms in biological samples is presented. The system is specifically calibrated for determining the concentration of *Trypanosoma cruzi* parasites in blood samples of mice infected with Chagas’ disease. The method can be adapted for use in other biological samples. A thin layer of blood infected by *T. cruzi* parasites is examined in a common microscope in which the images of the vision field are taken by a CCD camera and temporarily stored in the computer memory. In a typical field, a few motile parasites are observable surrounded by blood red cells. The parasites have low contrast. Thus, they are difficult to detect visually, but their great motility betrays their presence by the movement of the nearest neighbour red cells. Several consecutive images of the same field are taken, de-correlated with each other where parasites are present and digitally processed in order to measure the number of parasites present in the field. Several fields are sequentially processed in the same fashion, displacing the sample by means of step motors driven by the computer. A direct advantage of this system is that its results are more reliable and the process is less time-consuming than the current subjective evaluations made visually by technicians.


Catley: CAPE Unit, PACE Programme, OAU/IBAR, PO Box 30786, Nairobi, Kenya. [andy.catley@oau-ibar.org]

Participatory research on bovine trypanosomiasis was conducted with Orma pastoralists in Tana River District, Kenya. The use of participatory methods to understand local perceptions of disease signs, disease causes, disease incidence by cattle age group,
seasonal patterns of disease and preferences for indigenous and modern control methods is described. Results indicated that local characterization of diseases called gandi and buku by Orma pastoralists was similar to modern veterinary knowledge on chronic trypanosomiasis and haemorrhagic trypanosomiasis (due to *Trypanosoma vivax*), respectively. The mean incidence of gandi varied from 10.2% in calves to 28.6% in adult cattle. The mean incidence of buku varied from 3.1% in calves to 9.6% in adults. Pearson correlation coefficients for disease incidence by age group were 0.498 (P<0.01) and 0.396 (P<0.05) for gandi and buku, respectively. Informants observed cases of trypanosomiasis in 24.1% of cattle (all age groups); these cases accounted for 41.8% of all sick cattle during the preceding 12-month period. Eight indigenous and three modern trypanosomiasis control methods were identified. Results indicated that an integrated approach to trypanosomiasis control based on private, individual action was well established in the assessment area. When presented with four different trypanosomiasis control methods, community representatives selected ‘better use of trypanocides’ as the most preferred intervention and ‘community-based tsetse control’ as the least preferred intervention. This finding prompted researchers to modify the original project activities. Constraints facing the sustainability of community-based tsetse control are discussed.


Wildlife mortality involving bongos, *Tragelaphus eurycerus*, and other ungulates was investigated in the north of the Congo Republic in 1997. Four bongos, one forest buffalo, *Syncerus caffer nanus*, and one domestic sheep were examined and sampled. Although an outbreak of rinderpest had been suspected, it was found that the animals, which had been weakened by an *Elaeophora sagitta* infection and possibly also by adverse climatic conditions, had been exsanguinated and driven to exhaustion by an unusual plague of *Stomoxys omea*. Specimens of *Glossina brevipalpis* were caught at Bomassa camp.


Majiwa: ILRI, PO Box 51179, Nairobi, Kenya. [p.majiwa@cgiar.org]

Teneral tsetse flies infected with either *Trypanosoma brucei* or *T. vivax* were fed on healthy cattle. Blood samples collected daily from the cattle were examined by microscopy for the presence of trypanosomes, in thick smear, thin smear and in the buffy coat (BC). All the cattle fed upon by infected tsetse developed a fluctuating parasitaemia. DNA was extracted from the blood of these cattle and subjected to polymerase chain reaction (PCR) using oligonucleotide primers specific for *T. brucei* or *T. vivax*. The PCR products unique to either *T. brucei* or *T. vivax* were identified following amplification of DNA from the blood samples of infected cattle, whereas none was detectable in the DNA from the blood of the cattle exposed to non-infected teneral tsetse. In a concurrent set of experiments, one of the oligonucleotide primers in each pair was biotinylated for use in PCR-ELISA to examine all the blood samples, with this assay. Both the PCR and the PCR-ELISA revealed trypanosome DNA in 85% of blood samples serially collected from the cattle experimentally infected with *T. brucei*. In contrast, the parasitological assays showed trypanosomes in only 21% of the samples. In the blood samples from cattle experimentally infected, with *T. vivax*, PCR and PCR-ELISA revealed trypanosome DNA in 93 and 94%, respectively. Microscopy revealed parasites in only 63% of the BCs prepared from these cattle. Neither PCR nor PCR-ELISA detected any trypanosome DNA in blood samples collected from the animals in the trypanosomosis-free areas. However, both assays revealed the presence of trypanosome DNA in a number of blood samples from cattle in trypanosomosis-endemic areas.

(b) PATHOLOGY AND IMMUNOLOGY

Inbred Balb/c mice were infected with three clones of *Trypanosoma congolense* (Sam.28.1, Dind.3.1 and K60.1A) corresponding, respectively, to the three genetically distinct types (savannah, forest and kilifi) defined within this species, for the purpose of comparing their pathogenicity for a better understanding of the epidemiology of African trypanosomosis. Another clone of savannah type, IL 3000, was also tested simultaneously to study a probable strain variation. Both the clones of savannah type were found to be of extreme virulence with loss of appetite, rough hair, rapid respiration, lethargy, and all mice died within a week. Parasitaemias evolved rapidly to the first peak by day 3-5 post-inoculation without any remission and the course of disease was correlated positively with the prepatent period. The clones of the forest type and the kilifi type were of low virulence with chronic infection and symptoms progressively less patent throughout the infection; only one mouse died in each experimental group.


A study was carried out to assess the effects of concurrent *Trypanosoma congolense* and *Fasciola* infections in an endemic area in western Ethiopia. A total of 32 zebu cattle, confirmed positive for trypanosomosis and fasciolosis, were divided into 4 groups of equal number and received either isometamidium, or triclabendazole, or both, or none. Intensity of parasitaemia, fluke faecal egg output (epg), packed cell volume (PCV), differential eosinophil count and live weight gains were monitored weekly for a period of ten weeks. The results indicated that the initial parasitaemia due to natural infection with *T. congolense* ranged from 2+ to 4+ score in all treatment groups. Parasitaemia in Group I and II declined to zero level at week 3 post-treatment with isometamidium. Animals in Group III and IV (without isometamidium treatment) continued with fluctuating parasitaemia throughout the study period. Faecal examination showed a mean *Fasciola* epg ranging from 103 to 145 in all the groups prior to treatment with triclabendazole. One week after treatment, animals ceased excreting *Fasciola* eggs while non-treated subjects continued to do so with an overall mean epg count of 130 ± 11.68 and 148 ± 8.71 for Group II and IV, respectively. Assessment of mean PCV values of different treatment groups indicated 37.04 % and 26.81 % improvement in Group I and II, respectively. Animals in Group III and IV, however, showed a decline of 24.1 % and 42.63 %, respectively. Differential count results indicated a significantly higher eosinophil number in *Fasciola* positive animals (Group II and IV) than in Group I and III regardless of absence or presence of trypanosome infection. The results also indicated the presence of higher overall mean body weight gain in Group I and II (trypanosome
negative animals) than their Group III and IV counterparts, irrespective of the presence or absence of *Fasciola* infection. The present study strongly suggests that concurrent infections due to trypanosome and *Fasciola* infections are the most harmful form of parasitism in the study area, warranting due consideration for the control of these diseases.

(c) TRYPANOTOLERANCE


Naessens: ILRI, PO Box 30709, Nairobi, Kenya.

Natural resistance to African trypanosomiasis in certain *Bos taurus* cattle in West Africa, called trypanotolerance, may hold solutions for control of this economically crippling disease. Comparison of immune responses between trypanotolerant and trypanosusceptible cattle have shown some differences in antibody response, complement level and cytokine expression, but it is not known whether these differences are the cause of resistance. Two experiments were carried out to assess the contribution of the immune and haemopoietic systems to trypanotolerance. The production of haemopoietic chimaeras from trypanotolerant and susceptible twin calves and comparison of their responses after infection of singleton calves, allowed an assessment of the role of the haemopoietic system in trypanotolerance. An in vivo depletion of CD4 cells in the two breeds allowed an appraisal of the role of T and B lymphocytes in trypanotolerance. The results of the two experiments suggest that natural resistance comprises at least two mechanisms, an innate mechanism that controls parasite growth, and another, involving the haemopoietic system, that is able to limit anaemia. This supports the hypothesis that innate mechanisms in trypanotolerant cattle are more efficient in controlling disease, making them less reliant on antibody responses.

(d) TREATMENT


A survey was carried out in Kaduna State of Nigeria to establish the indigenous knowledge system for treating trypanosomiasis in domestic animals. Questionnaire and interviews were, respectively, administered to, or conducted with about 200 livestock farmers and traders spread around the state. Data obtained revealed the use of several plants either alone or in combination, for the treatment and management of trypanosomiasis. The most common plants encountered were *Adansonia digitata*, *Terminalia avicennioides*, *Khaya senegalensis*, *Cissus populnea*, *Tamarindus indica*, *53*
Lawsonia inermis, Boswellia dalziellii, Pseudocedrela kotschyi, Syzygium guineense, Sterculia setigera, Afzelia africana, Prosopis africana and Lannea kerstingii. The method of preparation and mode of administration of some of these plants in the treatment of trypanosomiasis are reviewed and discussed.


Jenkins: Immunology and Disease Resistance Laboratory, Agriculture Research Service, US Department of Agriculture (USDA), Beltsville MA 20705, USA. [mjenkins@anri.barc.usda.gov]

Protozoa are responsible for considerable morbidity and mortality in domestic and companion animals. Preventing infection may involve deliberate exposure to virulent or attenuated parasites so that immunity to natural infection is established early in life. This is the basis for vaccines against theilerosis and avian coccidiosis. Vaccination may not be effective or practical with diseases, such as cryptosporidiosis, that primarily afflict the immune-compromised or individuals with an incompletely developed immune system. Strategies for combating these diseases often rely on passive immunotherapy using serum or colostrums containing antibodies to parasite surface proteins. Subunit vaccines offer an attractive alternative to virulent or attenuated parasites for several reasons. These include the use of bacteria or lower eukaryotes to produce recombinant proteins in batch culture, the relative stability of recombinant proteins compared to live parasites, and the flexibility to incorporate only those antigens that elicit “protective” immune responses. Although subunit vaccines offer many theoretical advantages, our lack of understanding of immune mechanisms to primary and secondary infection and the capacity of many protozoa to evade host immunity remain obstacles to developing effective vaccines. This review examines the progress made on developing recombinant proteins of Eimeria, Giardia, Cryptosporidium, Toxoplasma, Neospora, Trypanosoma, Babesia, and Theileria and attempts to use these antigens for vaccinating animals against the associated diseases.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS


Boulangé: ILRI, POBox 30709, Nairobi, Kenya.

A 69-kDa immunodominant protein of Trypanosoma congolense was identified as a member of the hsp70 family that is homologous to mammalian BiP. We report here the
expression of the gene encoding the *T. congolense* BiP in a bacterial system. Dot blotting of the truncated recombinant proteins confirmed that BiP antigenicity is mainly located in the C-terminal third of the molecule. A recombinant fragment corresponding to this region was used as an antigen in an indirect ELISA and an initial evaluation of its diagnostic potential for bovine trypanosomosis was performed. The test showed limited sensitivity for detection of primary-infected cattle but was capable of accurately detecting secondary infections. As BiP and its derivatives may be produced at low cost under stable forms allowing standardization of the tests, they warrant further evaluation as antigens for serodiagnosis of bovine trypanosomosis.


Bubis: Departamento de Biologia Celular, Universidad Simón Bolívar, Apartado 89.000. Valle de Sartenejas, Baruta, Caracas 1081-A, Venezuela. [jbubis@usb.ve]

*Trypanosoma evansi* and *Trypanosoma vivax* are the most extensively distributed trypanosomes responsible for diseases in livestock. Western blot and indirect immunofluorescence assays revealed a high immunological cross-reaction between these two parasites. An antigen with an apparent molecular mass of 64 kDa (p64), which exhibited cross-reactivity with *T. vivax*, was purified to homogeneity from a Venezuelan isolate of *T. evansi*. This antigen is glycosylated, contains a glycosyl-phosphatidylinositol anchor and appeared to be localized through the cell except in the nucleus, indicating that it could primarily be confined to the parasite surface. These results, together with its relative abundance and apparent molecular weight, suggest that p64 probably corresponds to the soluble form of a variable surface glycoprotein from *T. evansi*. Anti-p64 polyclonal antibodies, raised on mice, recognized a 53 kDa polypeptide band from a Venezuelan isolate of *T. vivax* on Western blots. Additionally, sera obtained from naturally infected animals also recognized p64, suggesting its potential use as a diagnostic reagent. Mild acid treatment only slightly decreased the immunorecognition of p64, suggesting its potential use as a diagnostic reagent. To date, p64 represents the first antigen isolated and partially characterized from *T. evansi*.

(b) PATHOLOGY AND IMMUNOLOGY

(c) CHEMOTHERAPEUTICS


Laurens: Laboratoire de Pharmacognosie, UPRES-A 8076 CNRS, Faculté de Pharmacie, Université de Paris XI, 92296 Châtenay-Malabry, France.
Bioguided-fractionation of a CH$_2$Cl$_2$ extract of the stems of *Uvaria klaineana* (Annonaceae) led to isolation of klaivanolide, a novel bisunsaturated 7-membered lactone (5-acetoxy-7-benzoyloxymethyl-7H-oxepin-2-one), together with benzyl benzoate. Klaivanolide showed potent in vitro antileishmanial activity against both sensitive and amphotericin B-resistant promastigote forms of *Leishmania donovani* with $IC_{50}$ values of 1.75 and 3.12 µM, respectively. The compound also showed in vitro trypanocidal activity against trypomastigote forms of *Trypanosoma brucei brucei* GVR 35. Its structure was established by 1D and 2D NMR and other spectroscopic techniques.


Lalmanach: Laboratoire d’Enzymologie et Chimie des Protéines INSERM EMI-U 00.10, Université François Rabelais, Faculté de Médecine, F-37032 Tours, France.

Trypanosomes are the etiological agents of human sleeping sickness and livestock trypanosomosis (nagana), which are major diseases in Africa. Their cysteine proteases (CPs), which are members of the papain family, are expressed during the infective stages of the parasites’ life cycle. They are suspected to act as pathogenic factors in the mammalian host, where they also trigger prominent immune responses. *Trypanosoma congolense*, a major pathogenic species in livestock, possesses at least two families of closely related CPs, named CP1 and CP2. Congopain, a CP2-type of enzyme, shares structural and functional resemblances with cruzipain from *T. cruzi* and with mammalian cathepsin L. Like CPs from other trypanosomatids, congopain might be an attractive target for trypanocidal drugs. Here we summarise the current knowledge in the two main areas of research on congopain: first, the biochemical properties of congopain were characterised and its substrate specificity was determined, as a first step towards drug design; second, the possibility that inhibition of congopain by hostspecific antibodies may mitigate the pathology associated with trypanosome infection, was explored.


Kita: Department of Biochemical Chemistry, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. [kitak@m.u-tokyo.ac.jp]

Parasites have developed a variety of physiological functions necessary for their survival within the specialized environment of the host. Using metabolic systems that are
very different from those of the host, they can adapt to low oxygen tension present within the host animals. Most parasites do not use the oxygen available within the host to generate ATP, but rather employ systems anaerobic metabolic pathways. The enzymes in these parasite-specific pathways are potential targets for chemotherapy. Cyanide-insensitive trypanosome alternative oxidase (TAO) is the terminal oxidase of the respiratory chain of long slender bloodstream forms of the African trypanosome, which causes sleeping sickness in human and nagana in cattle. TAO has been targeted for the development of anti-trypanosomal drugs because it does not exist in the host. Recently, we found the most potent inhibitor of TAO to date, ascofuranone, a compound isolated from the phytopathogenic fungus, Ascochyta visiae.


Nyasse: Department of Organic Chemistry, Faculty of Science, University of Yaoundé 1, Cameroon. [bnyasse@uycdc.uninet.cm]

Phytochemical study of the stem bark of *Enantia chlorantha* resulted in the isolation of two protoberberines 1 and 2. These alkaloids as well as commercially available berberine were modified chemically and tested *in vitro* against *Trypanosoma brucei* proliferation as well as on three targeted glycolytic enzymes. The inhibitory activities observed were in the range of 20 µM (ED₅₀ values).


Tapia: Department of Physical Chemistry, Uppsala University, P.O. Box 532, S-75121 Uppsala, Sweden. [orlando.tapia@fki.uu.se]

8. TRYpanosOME RESEARCH

(a) CULTIVATION OF TRYpanosomes

(b) TAXONOMY, CHARACTERISATION OF ISOLATES


Agbo: Division of Animal Sciences, Section for Animal Genomics, Institute
Genetic analysis of *Trypanosoma* spp. depends on the detection of variation between strains. We have used the amplified fragment length polymorphism (AFLP) technique to develop a convenient and reliable method for genetic characterization of *Trypanosoma* (sub)species. AFLP accesses multiple independent sites within the genome and would allow a better definition of the relatedness of different *Trypanosoma* (sub)species. Nine isolates (three from each *T. brucei* subspecies) were tested with 40 AFLP primer combinations to identify the most appropriate pairs of restriction endonucleases and selective primers. Primers based on the recognition sequences of EcoRI and BglII were chosen and used to analyse 31 *T. brucei* isolates. Similarity levels calculated with the Pearson correlation coefficient ranged from 15 to 98%, and clusters were determined using the unweighted pair-group method using arithmetic averages (UPGMA). At the intraspecific level, AFLP fingerprints were grouped by numerical analysis into two main clusters, allowing a clear separation of *T. b. gambiense* (cluster I) from *T. b. brucei* and *T. b. rhodesiense* isolates (cluster II). Interspecies evaluation of this customized approach produced heterogeneous AFLP patterns, with unique genetic markers, except for *T. evansi* and *T. equiperdum*, which showed identical patterns and clustered together.


Noga: Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillborough Street, Raleigh, North Carolina 27606, USA.


Dacks: Program in Evolutionary Biology, Canadian Institute for Advanced Research, Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, N.S., B3H 4H7, Canada. [jdacks@is2.dal.ca]

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

Steverding: School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK. [dsteverding@hotmail.com]


Steverding: School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK. [dsteverding@hotmail.com]


Clayton: ZMBH, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany [cclayton@zmbh.uni-heidelberg.de]


Barry: Wellcome Centre for Molecular Parasitology, University of Glasgow, Anderson College, 56 Dumbarton Road, Glasgow G11 6NU, UK. [j.d.barry@bio.gla.ac.uk]


Vellieux: Laboratoire de Biophysique moléculaire, Institut de Biologie Structurale J.-P. Ebel CEA CNRS UJF, 41 rue Jules Horowitz, 38027 Grenoble Cedex 01, France. [vellieux@ibs.fr]


Sollner-Webb: Department of Biological Chemistry, The Johns Hopkins University of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA. [bsw@jhmi.edu]

12249 Drozdz, M., Palazzo, S.S., Salavati, R., O’Rear, J., Clayton, C. and Stuart,

Stuart: Seattle Biomedical Research Institute, University of Washington, Seattle, WA, USA. [kstuart@u.washington.edu]


Clayton: ZMBH, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany [cclayton@zmbh.uni-heidelberg.de]


Gilbert: Welsh School of Pharmacy, Cardiff University Redwood Building, King Edward VII Avenue, Cardiff CF10 3XF, UK.


Hajduk: Department of Biochemistry and Molecular Genetics, Schools of Medicine and Dentistry, University of Alabama, Birmingham, Alabama 35294 USA [shajduk@uab.edu]


Pérez-Montfort: Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apartado Postal 70242, 04510 Mexico DF, Mexico.


Cross: Laboratory of Molecular Parasitology, The Rockefeller University,

Sollner-Webb: Department of Biological Chemistry, John Hopkins University School of Medicine, Baltimore, Maryland 21205, USA [bsw@jhmi.edu]


Hill: Department of Microbiology, Immunology, and Molecular Genetics, University of California at Los Angeles, 10833 Le Conte Avenue, Los Angeles, CA 90095, USA [kenthill@mednet.ucla.edu]


Horn: Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK. [david.horn@lshtm.ac.uk]


Donelson: Department of Biochemistry, University of Iowa, 300 Eckstein Medical Research Building, Iowa City, IA 52242 USA [john-donelson@uiowa.edu]


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


Klingbeil: Department of Biological Chemistry, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205, USA. [mklingb@mail.jhmi.edu]


Donelson: Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242, USA. [john-donelson@uiowa.edu]


Stuart: Seattle Biomedical Research Institute, 4 Nickerson Street, Seattle, Washington 98109-1651 USA.


Koslowsky: Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan 48824, USA.


Wang: Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-0446, USA.


Mottram: Wellcome Centre for Molecular Parasitology, University of Glasgow, The Anderson College, Glasgow G11 6NU UK.

Oldfield: Department of Chemistry, University of Illinois at Urbana - Champaign, 600 South Matthews Avenue, Urbana, Illinois 61801, USA. [eo@chad.scs.uiuc.edu]


Frasch: Instituto de Investigaciones Biotecnologicas, Universidad Nacional de General San Martin, INTI, Avenue, General Paz s/n, Edificio 24, Casilla de Correo 30, 1650 San Martin, Pcia de Buenos Aires, Argentina. [cfrasch@iib.unsam.edu.ar]


Kristensson: Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.


Ouellette: Centre de recherche en Infectiologie du CHUL, 2705, boul. Laurier, Sainte-Foy, QC, Canada G1V 4G2. [marc.Ouellette@crchul.ulaval.ca]


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


Bindereif: Institut für Biochemie, Justus-Liebig-Universitat Giessen, Heinrich-Buff-King 58, D-35392, Giessen, Germany. [albrecht.bindereif@chemie.bio.uni-giessen.de]


Pellé: ILRI. P.O. Box 30709, Nairobi, Kenya. [r.pelle@cgiar.org]


Hassanzadeh: Cellular Immunology Unit, Vlaams Interuniversitair Instituut voor Biotechnologie, Vrije Universiteit, Pardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium. [reza@bigben.vub.ac.be]


Rascón: Instituto de Biología Experimental, Universidad Central de

Krauth-Siegel: Biochemie-Zentrum Heidelberg, Ruprecht-Karls-Universität, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany. [krauth-siegel@urz-heidelberg.de]


Ferguson: Division of Biological Chemistry and Molecular Microbiology, The Wellcome Trust Biocentre, School of Life Sciences, University of Dundee, DD1 5EH Dundee, UK. [m.a.j.ferguson@dundee.ac.uk]


Sanchez: Department of Molecular Microbiology and Immunology, Oregon Health and Science University, 3181 S.W. Sam Jackson Park Road, L220, Portland, OR 97201-3098, USA. [sanchezm@ohsu.edu]


Ullu: Department of Internal Medicine, Yale Medical School, 333 Cedar Street, New Haven, CT 06529-8022, USA. [elisabetta.ullu@yale.edu]


Duszenko: Verfügungsgebäude der Universität Tübingen, Auf der Morgenstelle 15, 72076 Tübingen, Germany. [michael.duszenko@uni-tuebingen.de]

Schneider: Department of Biology/Zoology, University of Fribourg, CH-1700 Fribourg, Switzerland.


Tyler: Northwestern University Medical School, Department of Pathology, 303 E. Chicago Avenue, Chicago, IL 60611, USA. [k-tyler@northwestern.edu]


Borst: Department of Molecular Biology and Centre of Biomedical Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. [pdorst@nki.nl]


Steyaert: Department of Ultrastructure, Vlaams Interuniversitair Instituut voor Biotechnologie, Vrije Universiteit Brussel, Pardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium. [jsteyaer@vub.ac.be]


Stuart: Department of Pathobiology, University of Washington, Seattle, Washington 98195, USA. [kstuart@u.washington.edu]


Seebeck: Institute of Cell biology, University of Bern, Baltzerstrasse 4, CH-

Kubrzycki: Department of Biochemistry and Microbiology, Rhodes University, Grahamstown 6140, South Africa. [i.zubrzycki@ru.ac.za]