section b — abstracts

1. general (including land use)

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It is estimated that there are 173 million cattle, 191 million sheep and 158 million goats in Africa. Between 1981 and 1986, the numbers of cattle, sheep and goats increased by 7.3, 14.8 and 11.3% respectively. The tsetse-infested area of approximately 8.7 million km² has 43.6 million cattle, of which only 9.8 million are trypanotolerant. Approximately 8 and 9% of the total sheep and goat populations respectively are found in this area, the majority being trypanotolerant. The distributions of the land area used for livestock and of tropical ruminant livestock units in the different regions of Africa are as follows: East Africa, 25.2 and 52% respectively; West Africa, 21.2 and 20%; southern Africa, 12.71 and 12.7%; North Africa, 18.96 and 7.28%; Central Africa, 21.94 and 7.8%. Multidisciplinary research is needed to investigate ways of accomplishing improved ruminant productivity through development of appropriate low-cost technologies in line with the needs and aspirations of farmers.


This article describes the evolution of the extensive Fulani Mbororo livestock production in the Central African Republic. The main features are the degradation of pastures, the significant reduction in livestock production resulting from various diseases, mainly trypanosomiasis, and the increasing involvement of cattle owners in efforts to arrest the decreasing herd sizes. The causes and consequences of these different aspects are being studied, with particular regard for the impact that a livestock development programme could have in the coming years.

9532 Boid, R., Hunter, A.G., Jones, T.W., Ross, C.A., Sutherland, D. and


Thirty-six out of the 52 African countries are considered endemic for sleeping sickness. Disease focality makes global prevalence evaluation difficult. Some 55 million people are estimated to be at risk, of whom only 3.5 million are under surveillance. Available data indicate a prevalence of 0.6% in those surveyed but local prevalences can be much higher, e.g. 5% in several areas in northwestern Uganda and as high as 50-70% in some villages in Zaire and Angola. Extrapolation from these figures suggests over 300,000 individuals infected of whom only 20,000 are diagnosed and treated, the fate of the remaining 280,000 cases being unknown. Additional confirmation of the under-estimation of cases comes from serology. This suggests that, if the total population at risk were under serological surveillance followed by the use of proper parasitological diagnostic methods, the annual number of new cases could amount to over 0.5 million. The immunosuppressive nature of the infection also encourages secondary infections (especially in *Trypanosoma brucei gambiense* infection), thereby increasing morbidity and mortality. WHO has adopted a three-fold control strategy consisting of (i) active medical surveillance with mobile teams using refined diagnostic tools (serological and parasitological), (ii) fixed-post (passive) medical surveillance through dispensaries, health centres or hospitals (treatment being at specialised health centres), and (iii) vector control using impregnated screens and traps, targeted at high transmission rate areas, and with active community participation. The most effective strategy consists of implementing all three options together, although this will depend on financial constraints and on the local epidemiological and environmental
conditions.

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This letter comments on the article by Koerner et al. (see TTIQ, 18 (3): no. 8990) which put forward the view that the 1901 Busoga sleeping sickness epidemic was caused by Trypanosoma brucei rhodesiense rather than T. b. gambiense. Observers at the time considered 'Negro lethargy' in Uganda to be a different entity from the cases documented in West Africa (called 'Trypanosoma fever'), suggesting that the latter be renamed Gambia fever to avoid confusion. Other documentary evidence also seems to support Koerner et al.'s thesis that local social disturbances precipitated the outbreak. (See also 19: nos. 9536 and 9537.)

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It might be thought that by now human African trypanosomiasis would be well under control. Not only is this not the case but the disease is currently on the increase and remains a serious threat. However, because it is restricted to poor areas of intertropical Africa, pharmaceutical research ignores it for more lucrative and popular areas. Nevertheless, with the help of some dedicated researchers, and the creation of a mobilisation programme by the French government, some promising progress is being made, particularly in the use of community health workers, the use of GIS to locate areas for priority action, tsetse trapping, and the collection of blood samples on filter paper from all subjects at risk for serological examination at regional laboratories. Confirmation of infection in serological suspects is more difficult: methods for the detection of specific antigens are the most promising way forward. The suggested blanket treatment of all suspects with pentamidine, and CSF examination of all subjects with neurological problems, cannot be recommended: treatment should always have regard to the stage of the disease. Some new serological tests for the presence of antigalactocerebroside and antineurofilament antibodies (auto-antibodies) appear
promising in this respect. Perturbations of the circadian sleep-wake cycle and the related hormonal secretions are also linked to the stage of the disease. After the hopes engendered by DFMO, melarsoprol still remains the recommended drug for treatment of the nervous stage, with revised protocols being proposed to reduce the frequency of encephalopathies, relapses and length of hospitalisation. The development of promising new nitroimidazole drugs is not being pursued because of fears of possible, though unproved, carcinogenicity, in spite of a 3-5% incidence of fatal encephalopathies with the current arsenical drugs.

Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK.
This letter comments on the article by Koerner et al. (see TTIQ, 18 (3): no. 8990) which put forward the view that the 1901 Busoga sleeping sickness epidemic was caused by Trypanosoma brucei rhodesiense rather than T. b. gambiense and was brought about by social upheaval. The author considers the two propositions to be unrelated. Parasitological evidence presented in Reports of the Sleeping Sickness Commission of the Royal Society, 1903-1911, suggest that although many patients were in an advanced stage of the disease and died within months of admission, some survived 12 months or longer. Only scanty parasitaemia was found in patients and also in rats and dogs subinoculated with patients' blood or CSF. All this evidence points to T. b. gambiense. Bruce's 'easily achieved cyclical transmission' using monkeys and wild-caught flies is more likely to have been mechanical transmission or pre-existing infection in the wild flies. (See also 19: nos. 9534 and 9537.)

Medizinische Hochschule, D-30625 Hannover, Germany.
The author replies to Gibson's letter (see 19: no. 9536) concerning his original article on the 1901 Busoga sleeping sickness epidemic (TTIQ, 18 (3): 8990). The point of the original article was to suggest that sleeping sickness is not spread around Africa by itinerants (as for influenza) but rather expands and contracts from the 'true foci' in response to changes in fly density and/or distribution attributable to environmental pressures which are in turn influenced by socio-economic factors. Recent epidemics of sleeping sickness in Uganda (Trypanosoma brucei rhodesiense in the
south-east and *T. b. gambiense* in the north-west) fit this pattern. Despite greater mobility in modern Africa, no *T. b. rhodesiense* has been isolated from north-west Uganda, and no *T. b. gambiense* from the south-east, despite thousands of Sudanese refugees with *T. b. gambiense* settling in central Uganda. It is suggested that Busoga is and long has been a true *T. b. rhodesiense* focus and that Bruce only identified the 1901 trypanosomes as *T. b. gambiense* because they were similar to those recently described in West Africa. (See also 19: no. 9534.)


Mugangu: University College of Natural Resources, Agriculture and Veterinary Sciences at Mushweshwe, B.P. 270, Bukavu, Zaire.

Habitat selection by the African buffalo (*Syncerus caffer*) was studied in Virunga National Park, Zaire, between 1984 and 1989 in five different habitats (mudflat steppes, upland steppes, bushy steppes, woodland savannas, and forests) during dry and wet seasons. Buffalo generally selected habitats with high quality food and water, especially during the dry season, and with low risk of predation. One anomaly, selection of upland steppes during the wet season despite their high risk of predation, may be explained by the low abundance of tsetse flies there.


Epidémiologie et Santé animale, no. 28: 27-38.

Veterinary Epidemiology and Economics Research Unit, Department of Agriculture, University of Reading, Earley Gate, P.O. Box 236, Reading RG6 2AT, UK.

This paper outlines the various methods used for analysing the economics of disease control at the herd level. A partial budget provides the starting point, using mastitis control in the UK as an example, extending the analysis to several years and including the concept of discounting. The areas which pose particular problems in evaluating the impact of disease are discussed, focusing on the production parameters affected by the disease and the production systems involved. The difficulty of collecting data on the effects of disease on production and of establishing 'with' and 'without' disease control scenarios for evaluating project impact is highlighted. In this
context, the role of herd models is discussed and the different types of herd models, static and dynamic, deterministic and stochastic, are described. Finally, for the example of trypanosomiasis in Côte-d'Ivoire, a simple stochastic model is presented, simulating the variability of outcomes for individual livestock producers in the case of a transhumant herd with and without a tsetse control project using mono-pyramidal traps.


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

The application of systems analysis techniques identified a number of key factors which will determine future developments in the Gambian livestock industry, with especial reference to traditionally managed trypanotolerant, N'Dama cattle, and the importance of tsetse and trypanosomiasis. Different components of the analysis repeatedly identified the following key factors: human population growth; declining rainfall; loss of tree cover; trends in land-use including increased areas under cultivation, reduced fallowing and reduced access for grazing; livestock numbers; prices for groundnuts (the main cash crop) and cattle; and indicators of national wealth reflecting cash available to invest in livestock or to purchase livestock products. In many areas tsetse and trypanosomiasis appear to be in decline as a result of habitat destruction but they are likely to persist in others where trypanotolerant animals face a high risk of trypanosomiasis infection and active control may bring significant benefits. Several of the identified trends raise doubts concerning the long-term sustainability of traditional grazing on common land, although improved, intensive management strategies can do much to alleviate these problems.

2. tsetse biology
   (a) REARING OF TSETSE FLIES
   [See 19: nos. 9544, 9545.]

   (b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

A potent and specific inhibitor of the human coagulation protease thrombin was identified in salivary gland extracts of the tsetse fly, *G. m. morsitans*. This low molecular weight peptide (MW = 3,530 Da as determined by laser desorption mass spectrometry) was purified using a combination of size-exclusion chromatography and reverse-phase, high-performance liquid chromatography, respectively. Amino terminal sequencing of the purified protein reveals no homology to any previously identified serine protease inhibitor or naturally occurring anticoagulant. The tsetse thrombin inhibitor (TTI) is a stoichiometric inhibitor of thrombin, with an apparent equilibrium dissociation inhibitory constant \( K_{\text{app}} \) of \( 584 \times 10^{-15} \) M. In addition, it is also a potent inhibitor of thrombin-induced platelet aggregation. Like other haematophagous arthropods, tsetse flies appear to have evolved a novel protease inhibitor capable of antagonising host haemostasis and facilitating blood feeding.


The objective of this review is to provide an overview of the genetic variation in arthropods that transmit pathogens and to speculate on the significance of this variation. The review concentrates on publications appearing during the last decade and includes discussion of much of the recent information on tsetse genetics. Genetic mechanisms of susceptibility and refractoriness to pathogens have been established in several major vector-pathogen models, but a few of the most thoroughly studied models are artificial associations that have no epidemiological significance. Much remains to be done with models that will be of direct benefit to public health. The field is now opening to studies on the molecular aspects of vector-pathogen interactions and on the application of molecular genetic techniques for creation of strains of vectors that are incapable of transmitting pathogens. The usefulness of such information will depend upon a thorough understanding of the mechanisms that control
genetic variation (particularly variation in vector competence) in natural populations of vectors. Biochemical genetics, particularly studies of electrophoretic variation in enzymes, have been widely applied to vectors and have revealed that a significant amount of genetic variation exists in most vector populations. These techniques have demonstrated, or confirmed, the existence of sibling species and genetic structuring within several species of vectors. The application of biochemical and population genetics to colonies of vectors has raised questions of how well some colonies represent natural populations. These questions must be addressed (i) to reassure us of the validity of information obtained from experiments with these colonies, (ii) to determine for which goals the work with vectors is appropriate and useful, and (iii) to establish the usefulness of these colonies as sources of material for genetic control of vectors. There appears to be no end of suggestions for how vectors might be controlled, or replaced, in an effort to reduce the prevalence of diseases that are caused by vector-borne organisms. When championing the application of any new technique, it is advisable to bear in mind the significant amount of genetic variation that exists in vectors, the complex relationships that exist in all vertebrate-vector-pathogen systems, and the resiliency of such systems to perturbations.


McIntyre: Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada.

The pteridine content of the head capsule of teneral flies from 11 genetically selected lines (including eye-colour mutants) of *Glossina morsitans morsitans* and *G. palpalis palpalis* was examined using fluorescence spectroscopy. Wild-type *G. p. palpalis* had a greater pteridine content than did wild-type *G. m. morsitans*. Within *G. m. morsitans* there was a 25% variation in fluorescence values between genetic lines. Wild-type *G. p. palpalis* had the same pteridine content as brick mutants but more than tan mutants; in *G. m. morsitans* the salmon mutants had a higher pteridine content than did wild-type flies. Pteridine content did not account for the difference in eye colour between male and female
brick mutants. Accumulation of pteridines was not influenced by genotype in young flies, but in older flies salmon mutants accumulated pteridines more rapidly than did wild-type flies. Young flies, both wild type and salmon, accumulated pteridines more rapidly than did old flies. The results of the analysis of head capsule fluorescence in males from the parental lines and F1 and F2 generations of reciprocal crosses of the G. m. morsitans lines with the highest and lowest pteridine contents revealed that genetic control of pteridine content lies on the X chromosome and on one autosome.


Newly deposited pupae of G. pallidipes were incubated until emergence at constant temperature (20.5, 22.5 25.0, 27.5 and 29.5°C) and compared with pupae held at ambient conditions (19-31°C). Egg follicles and enclosed oocytes of newly emerged females were dissected and their lengths measured together with the lengths of the cutting blade of the hatchet cell of the right and left wings. Linear discriminant functions analysis showed that the mean length of egg follicle A accounted for 80% of the temperature-related variability. We therefore used a simple predictive equation for temperature experience based on mean follicle length in small batches of newly emerged females. The equation predicted with reasonable accuracy the temperature experience of 19 monthly samples of newly emerged G. pallidipes reared in an insectary under ambient conditions. Mean sizes of egg follicle A of the newly emerged tsetse may therefore be of utility in estimating temperature experience of pupae.


The egg follicle C of the left ovary developed first, indicating reversed ovarian sequences, in 5.8, 4.8, 4.2, 1.1 and 2.2% of newly emerged female G. pallidipes from pupae kept at 29.5°C 0.5, 27.5°C 1.0, 25.0°C 0.5,
22.5 $\pm$ 0.5 and 20.5 $\pm$ 1.0°C respectively, compared with 2.0% of flies from pupae maintained at ambient temperature (19-31°C) in the local colony. There was a highly significant positive correlation between the frequency of reversed ovarian sequences in flies and temperatures in the range 19.5-30.0°C. The incidence of the abnormality was very low (0.2%) in wild-caught females from the Lambwe Valley, probably reflecting the constancy of favourable temperatures in that location. Old females with altered ovarian sequence had apparently both normal pregnancy and alternate maturation of eggs between the ovaries. This appears to be the first report suggestive of a temperature-induced reversed sequential ovarian development in Glossina.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

[See also 19: nos. 9543, 9556.]


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In various vegetation types in Zimbabwe, the catches of *Glossina pallidipes* and *G. morsitans morsitans* at a target baited with odour (acetone, 1-octen-3-ol and two phenols) were positively correlated with catches of the same species at an unbaited net. No correlation existed between target catches and hand net catches of tsetse flies sitting on the vegetation. *G. pallidipes* females caught at a target and at an unbaited net were older than those caught from vegetation. Of the female *G. pallidipes* caught at the target, 46% were in the first 3 days of pregnancy. Of those caught at the unbaited net, significantly fewer, 21%, were in this stage. *G. pallidipes* males caught from vegetation contained more fat (3.07 ± 0.333 mg) than those caught at the unbaited net (2.06 ± 0.339 mg) or at the target (2.19 ± 0.218 mg).

It is inferred that target catches consisted predominantly of tsetse which were already in flight when they sensed the stimuli from the target, and that target catches were biased towards female *G. pallidipes* in the first 3 days of pregnancy.


Remotely sensed surrogates of meteorological data for

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This paper gives an overview of how certain meteorological data used in studies of the population dynamics of arthropod vectors of disease may be predicted using remotely sensed, satellite data. Details are given of the stages of processing necessary to convert digital data arising from satellite sensors into ecologically meaningful information. Potential sources of error in these processing steps are also highlighted. Relationships between ground-measured meteorological variables (saturation deficit, ground temperature and rainfall) and data from both the National Oceanic and Atmospheric Administration's polar-orbiting meteorological satellites and the geostationary Meteosat satellite are defined and examples detailed for Africa. Finally, the current status of existing satellite platforms and future satellite missions are reviewed and potential data availability discussed. How such satellite-based predictions have proved valuable in understanding the distribution of tsetse fly species in Côte d'Ivoire and Burkina Faso will be the subject of a future review (see 19: no. 9550).


Kitron: Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801, USA.

Satellite imagery, geographical information systems (GIS) and spatial statistics provide tools for studies of population dynamics of disease vectors in association with habitat features on multiple spatial scales. Tsetse flies (*Glossina pallidipes*) were collected during 1988-90 in biconical traps located along transects in Ruma National Park in the Lambwe Valley, western Kenya. Fine spatial resolution data collected by Landsat Thematic Mapper (TM) satellite and reference ground environmental data were integrated in a GIS to identify factors associated with local variations of
fly density. Statistical methods of spatial autocorrelation and spatial filtering were applied to determine spatial components of these associations. Strong positive spatial associations among traps occurred within transects and within the two ends of the park. From satellite data, TM band 7, which is associated with moisture content of soil and vegetation, emerged as being consistently highly correlated with fly density. Using several spectral bands in a multiple regression, as much as 87% of the variance in fly catch values could be explained. When spatial filtering was applied, a large component of the association between fly density and spectral data was shown to be the result of other determinants underlying the spatial distributions of both fly density and spectral values. Further field studies are needed to identify these determinants. The incorporation of remotely sensed data imagery into a GIS with ground data on fly density and environmental conditions can be used to predict favourable fly habitats in inaccessible sites, and to determine number and location of fly suppression traps in a local control programme.


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Three examples of the relevance of biodiversity to health are discussed: Glossina in Ankole, nutrition in Arnhemland, and child health in Yucatan. The spread of G. morsitans in the Ankole savanna grasslands, western Uganda, is mapped. With the social collapse that followed European invasion, the destruction and abandonment of settlements, which had previously been barriers to the fly's spread, allowed recolonisation by woodland and the animals which were the host species for G. morsitans. Invasion of the interlake area west of Lake Victoria began between 1890 and 1900 and the spread northwards continued until the rinderpest epidemic in 1919. After 1925 the northwards spread began again more slowly but between 1953 and 1958 the remainder of the Ankole savanna became overrun with tsetse. It appears that the intensive slaughter of elephants for big game hunting, cessation of their seasonal migration, and the subsequent absence of tree destruction resulted in a change of vegetation cover and faunal associations which encouraged the spread of G. morsitans.

9550 Rogers, D.J., Hay, S.I. and Packer, M.J., 1996. Predicting the

Trypanosomiasis and Land Use in Africa (TALA) Research Group, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

An example is given of the application of remotely-sensed, satellite data to the problems of predicting the distribution and abundance of tsetse flies in West Africa. The distributions of eight species of tsetse, *Glossina morsitans*, *G. longipalpis*, *G. palpalis*, *G. tachinoides*, *G. pallicera*, *G. fusca*, *G. nigrofusca* and *G. medicorum*, in Côte d'Ivoire and Burkina Faso were analysed using discriminant analysis applied to temporal Fourier-processed surrogates for vegetation, temperature and rainfall derived from meteorological satellites. The vegetation and temperature surrogates were the normalised difference vegetation index and channel-4-brightness temperature, respectively, from the advanced, very-high-resolution radiometers on board the National Oceanic and Atmospheric Administration's polar-orbiting, meteorological satellites. For rainfall the surrogate was the Cold-Cloud-Duration (CCD) index derived from the geostationary, Meteosat satellite series. The presence or absence of tsetse was predicted with accuracies ranging from 67 to 100% (mean = 82.3%). A further data-set, for the abundance of five tsetse species across the northern part of Côte d'Ivoire (an area of about 140,000 km²), was analysed in the same way, and fly-abundance categories predicted with accuracies of 30 to 100% (mean = 73.0%). The thermal data appeared to be the most useful of the predictor variables, followed by vegetation and rainfall indices. Refinements of the analytical technique and the problems of extending the predictions through space and time are discussed.


Monitoring of *G. austeni* populations in the forested areas of Unguja island of Zanzibar has since November 1990 routinely been carried out with the sticky panel trap because commonly used tsetse traps (biconical, Epsilon, F3) have proved to be unsuccessful in catching *G. austeni*.
Initial studies on the catching ability of various types of sticky panels for *G. austeni* indicated that the monopanel was as efficient in catching flies as the 3-dimensional version and the smaller legpanel. No significant differences in catch rate and sex ratio were observed with monopanels in various colours and colour combinations. Legpanels coloured white on one panel side and blue on the other side caught significantly more flies compared with other colour combinations, but female flies were under-sampled (32%). The type of sticky material applied on the panel influenced significantly the catch rate and female ratio. During long-term trapping with baby blue and white monopanels, females were under-sampled (38–46%) except when polyisobutyleneLMW was used as sticky material. Analysis of the age composition of the sampled *G. austeni* females revealed that teneral and nulliparous flies were well represented (11–24%). More than 20% of the trapped females were older flies, i.e. females with four or more ovulations, but this percentage dropped to 10% when Tanglefoot was used as sticky material.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE-EFFECTS)

[See also 19: nos. 9539, 9561, 9575.]


Blanc: NNRDF, P.O. Box 498, Oshakati, Namibia. The cost-benefit analysis of trapping for tsetse control performed by livestock farmers in the Central African Republic shows the value of this method for the farmers and the country. Advantages to the livestock farmers depend on the size of their herds and their management. Although only a small proportion of livestock farmers use the method, the Central African Republic benefits from this low financial risk programme, which involves limited investment. Most of the cost and handling are the farmers' responsibility following an information campaign.

contre *Glossina fuscipes fuscipes* en République centrafricaine. [Insecticide treatments (Pour on and Spot on) applied to cattle against *G. f. fuscipes* in the Central African Republic.] *Veterinary Research*, **27** (3): 273-284.

Gouteux: 32730 Montégut-de-Pardiac, France.

Four herds of Mbororo Zebu cattle (approximately 40 head each) in traditional Fulani rearing conditions were treated over a 12 month period. Flumethrin (Bayticol) Pour on was used every 3 weeks during the rainy season and then deltamethrin Spot on was used every 6 weeks during the dry season. The treatments had no impact on the apparent density of *G. f. fuscipes*, although they could have had a slight effect on the age structure and feeding patterns of the fly populations. They changed neither the trypanosome infection rates in cattle nor their PCVs. This trial shows that, under these experimental conditions, insecticide treatments were not effective for the control of trypanosomosis in cattle. Further trials should be carried out to assess the usefulness of this method when it is integrated with the trapping of tsetse flies.


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Attractants for tsetse flies are reviewed under the following headings: Techniques for studying tsetse behaviour (hand-catching versus automatic catching, electric nets, direct observation); Visual attractants (colour, movement, size and shape, patterning); Olfactory attraction (early studies, attraction of *Glossina pallidipes* and *G. morsitans morsitans* to host odours, attraction of other tsetse species to odours derived from hosts, contribution from laboratory studies); Other senses (tsetse pheromones, temperature and heat, sound and magnetic fields). Bait systems for tsetse control are discussed under: Artificial baits (early applications of bait systems, the Challier-Laveissière biconical trap and its derivatives, traps and targets designed using electric nets, maximising target/trap longevity) and Natural baits (treated cattle). Programmes of tsetse control using bait systems are described under the following headings: Patterns of bait deployment (control of *G. morsitans* group flies in savanna habitats, control of *G. palpalis* group flies in savanna regions, control of *G. palpalis* group flies in forest regions); Involvement of the local community;
Economic aspects of bait techniques. It is concluded that not only are bait methods capable of efficient and cost-effective control of tsetse flies; they are likely to be more sustainable in the long term than some other techniques.


Cuisance: CIRAD-EMVT, B.P. 5035, 34032 Montpellier Cedex, France.

Azatin EC\textsuperscript{b}, an extract from neem (Azadirachta indica) seeds, has been tested to examine its potential role in tsetse fly control by rural communities. Laboratory tests were performed on G. f. fuscipes by topical application or tarsal contact. The topical application of Azatin EC (30 g azadirachtin per litre) induced a low mortality rate at higher dosages only (2.61 \mu g of azadirachtin/fly) in young males (LD\textsubscript{50} = 0.747 \mu g) and old gravid females (LD\textsubscript{50} = 2.516 \mu g). Productivity (number of pupae/femalè) decreased 4.5-fold at higher dosages and pupal weight was significantly lower at the beginning of the pupal period. These parameters did not significantly differ from the controls thereafter. The emergence rate was significantly lower at dosages higher than 0.261 \mu g/fly. Flying and probing difficulties were observed at, and above, 0.261 \mu g/fly, indicating a probable muscular physiological imbalance.

However, tarsal contact with a piece of material treated with 3.9 g/m\textsuperscript{2} azadirachtin had no effect on the mortality, emergence rates or productivity for the three periods of time tested (1, 2 or 3 min). A strong repellent effect was nevertheless noticed after coating the ear of the host animal with a 0.3 g/l azadirachtin solution. At this concentration, 70% of the flies did not feed on the host, and, at 3 g/l concentration, this figure rose to 90%. The possible uses of this natural product in the field are discussed, in particular as a repellent for the protection of cattle in tsetse-infested areas.


Epidemiology Research Unit, Box 4584, Johannesburg
Developments in the use of odour-baited traps offer exciting prospects for the effective control of tsetse flies and the trypanosome diseases of which they are the vectors. Work carried out at Nguruman in southwestern Kenya has led to the development of cheap and simple traps that can be made and serviced by local communities. Various models have been developed which enable us to understand the factors which determine the efficiency of traps which in turn will help us to determine the density of traps needed to achieve a set level of control and to design more effective traps. Four models are discussed here. The first is a model of the population dynamics of tsetse that relates the overall population loss rate to the mortality that we impose on adult flies. The second is a model of movement on a large scale that makes it possible to relate the adult mortality to the movement patterns and population dynamics of the flies, and to the properties of the trap. The third is a more speculative attempt to model the way in which individual flies locate traps once they are close enough to detect the odours. This should eventually make it possible to refine the parameters in the large scale movement models. The last is a model of invasions of flies into a cleared area. The development and testing of these models has relied extensively on the data collected in the field at Nguruman and in turn the models have helped us to interpret the data and to formulate new questions and experiments.

Zaranyika, M.F., Mambo, E. and Makhubalo, J.M., 1994. Organochlorine pesticide residues in the sediments of selected river bays in Lake Kariba, Zimbabwe. Science of the Total Environment, 142 (3): 221-226. Chemistry Department, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe. Sediment samples from seven of the major river bays (Charara, Nyaodza, Gachegache, Sanyati, Ume, Sengwa and Ruziruhuru) on the Zimbabwe side of Lake Kariba, an area subjected to spraying for tsetse control, were analysed for organochlorine pesticide residues by capillary gas chromatography and electron capture detection. The results obtained confirmed contamination of most of the bays by DDT and its metabolites, endosulfan, aldrin, dieldrin, endrin and heptachlor.

4. EPIDEMIOLOGY: VECTOR–HOST AND VECTOR–PARASITE

A new, selective approach to identifying protein ligand–receptor interactions between an arthropod vector and the parasite it transmits is described. Biotinylated vector proteins were incubated with living parasites in physiological conditions. After extensive washing, the parasites were subjected to SDS-PAGE and the polypeptides were electroblotted onto nitrocellulose membrane. Staining with avidin-horseradish peroxidase revealed only biotin-labelled proteins from the vector which were bound to the parasite. A multitude of tissue-specific proteins of G. p. gambiensis and G. m. morsitans, able to bind to cultured procyclic trypanosomes of Trypanosoma brucei brucei, T. b. gambiense and T. b. rhodesiense, has been demonstrated. The relevance of these interactions in relation to the developmental journey of the trypanosome in the tsetse fly is briefly discussed.


A rapid, visually read, dot-ELISA, developed for the detection and differentiation of trypanosome species in tsetse flies (Glossina spp.), was field tested alongside the standard fly dissection method on a ranch in southeastern Kenya. Of 104 G. pallidipes dissected, 2 were found to be infected with trypanosomes in their midguts. By the dissection method the infecting trypanosome species could not be identified, as both flies had no salivary gland infections. However, using the dot-ELISA, the 2 flies were shown to be infected with Trypanosoma congolense in their midguts. The midguts of an additional 6 (5.8%) of the 104 G. pallidipes tested positive for T. congolense in the dot-ELISA, even though no trypanosomes were seen on dissection. The infection rate for this fly species, as determined using the dot-
ELISA, therefore, was 7.7% for *T. congolense* in midgut infections compared to 1.9% identified by fly dissection. The salivary glands and mouthparts of the 6 additional tsetse flies identified by dot-ELISA were all negative as determined by the two techniques. None of 390 *G. longipennis* flies dissected and examined for trypanosomes in the midgut, salivary glands and mouthparts was shown, by this method, to be infected. Using the dot-ELISA, however, 17 (4.4%) of the flies tested positive for *T. congolense* in the midgut, whilst the salivary glands and mouthparts of the same flies were negative. Thus, the dot-ELISA appears to be more sensitive than the fly dissection method under field conditions. Moreover, the dot-ELISA can be performed in the field without electricity. It is simple to perform, and was not affected by high ambient temperatures (22-32°C), or by contamination of reactants with dust.


D'Amico: Maison du Parc, Gabas, 64440 Laruns, France.

The epidemiology of *T. vivax* infections was studied in gallery forest in the Ouro-Djafoun livestock area, Central African Republic, between July 1991 and July 1992. Previous studies have suggested that the usual cyclic transmission of *T. vivax* by the tsetse fly *Glossina fuscipes fuscipes* is probably not the only transmission route. This paper examines the possibility that stable flies are mechanical vectors of this trypanosome species. At the study site, at least five species or subspecies of stable flies were encountered: *Stomoxys nigra nigra* (approximately 60% of the sample), *S. taeniata*, *S. sitiens*, *S. omega omega* and *Haematobia* spp. The hypothesis that stable flies could be good vectors of *T. vivax* in this region is supported by three main observations: (i) stable flies were very abundant at the cattle resting site; (ii) an estimation of the 'contact index' between the cattle and stable flies demonstrated close interactions between cattle and stable flies at this site, particularly during the rainy season, and (iii) there was a good correlation (*P* < 0.05) between the apparent densities of stable flies at the resting site and the frequency of *T. vivax* infections in the cattle. The relevance of this phenomenon in terms of epidemiology and combating *T. vivax*-caused nagana is discussed.
Jusot, J.F., Vlas, S.J. de, Oortmarssen, G.J. van and Muynck, A. de, 1995. Apport d'un modèle mathématique dans le contrôle d'une parasitose: cas de la trypanosomiase humaine africaine à Trypanosoma brucei gambiense. [Contribution of a mathematical model to the control of a parasitic disease: the case of human African trypanosomiasis due to T. b. gambiense.] Annales de la Société belge de Médecine tropicale, 75 (4): 257-272. Muynck: Institut de Médecine Tropicale, Nationalestraat 155, B-2000 Antwerp 1, Belgium. T. b. gambiense sleeping sickness is lethal if not treated adequately. The endemicity was generally well under control in the 1960s. However, since the 1970s the disease has reappeared in most of its old foci, with alarming prevalence levels in several areas. Mathematical modelling provides a rational basis for finding the optimal strategies to control these resurgences. We present a deterministic model of the basic transmission of trypanosomiasis between human and vector hosts in natural situations. The parameters were quantified on the basis of available evidence from the literature. The model predicts a stable equilibrium state with very high prevalences, approximately 95% of humans and 27% of flies being infected. The model further shows that the build-up of an epidemic is initially very slow, and it takes several months before the equilibrium state is reached. Consequently communities have enough time to avoid catastrophic situations by migrating to safer areas. It is therefore unlikely that such high equilibrium situations will occur in practice. The expression of the basic reproductive rate $R_0$, the number of new infections during the lifetime of an infected subject, with high values of $R_0$, implies that efforts have to be substantial to diminish transmission to levels where the disease cannot maintain itself in the population. The smallest proportion of flies that it is necessary to eliminate by trapping or spraying has been calculated. In almost all situations a reduction of at least 90% is necessary, which is in accordance with the field experiences of vector control programmes. The present model can be considered as a starting point in the further development of a complete simulation model, which could be applied in supporting decision making in trypanosomiasis control.

Scharies, G. and Mehlitz, D., 1996. Sleeping sickness in Zaire: a nested polymerase chain reaction improves the identification of Trypanosoma (Trypanozoon) brucei gambiense by...

Scharres: Federal Research Centre for Virus Diseases of Animals/Institute for Epidemiological Diagnostics, Seestrasse 55, D-16868 Wusterhausen, Germany.

Blood samples collected in the sleeping sickness focus of Boma, Bas-Zaïre, from human patients and domestic animals were analysed by polymerase chain reaction (PCR) for the presence of trypanosome DNA. The comparison of PCR and miniature anion exchange centrifugation technique (m-AECT) results clearly showed that in domestic animals mixed infections (Trypanozoon/Trypanosoma (Nannomonas) congolense) were more frequently diagnosed by PCR than by m-AECT. *Trypanozoon*-positive blood samples were further analysed for *T. b. gambiense*. Amplified minicircle kinetoplast DNA (minicircle kDNA) was differentiated into *gambiense* and non-*gambiense* by hybridisation with DNA probes. To analyse blood samples, especially those with low parasite numbers, the amplification step needed to be improved by a nested PCR. Subsequent hybridisation was done with kDNA probes generated by PCR from blood samples which had been obtained from a human patient infected with *T. b. gambiense* and a pig infected with *Trypanozoon*. The hybridisation results clearly showed that at least two genotypes of *Trypanozoon* parasites occur in the sleeping sickness focus of Boma. One obviously corresponds to *T. b. gambiense* and was present in humans and two domestic animals (pig, dog). The other genotype seemed to be associated with *T. b. brucei* and could be detected only in the blood of domestic animals. This is the first time that field samples have been analysed by a technique which facilitates the molecular identification of *T. b. gambiense* without prior cloning, propagation and/or isolation of the parasites. This technique appears to be a promising tool for elucidating the significance of the animal reservoir in epidemiological studies of *gambiense* sleeping sickness.

5. human trypanosomiasis

(a) SURVEILLANCE

[See also 19: nos. 9533, 9535, 9562.]

9563 Rogalle, T., Doua, F., Brault-Noble, G., Fidier, N. and Buguet, A., 1995. La technique QBC dans la détection des trypanosomes. [The quantitative buffy coat technique
The quantitative buffy coat (QBC) technique is described in detail. Eight patients, already known to be positive for trypanosomiasis by CATT, by direct microscopical examination and by mAEC, were tested by the QBC technique. Confirmation of diagnosis was obtained in all patients in less than 5 min, confirming the method's sensitivity and ease of use. Part of the interest of this technique is in the possibility of the additional rapid diagnosis of malaria and filariasis using the same blood sample.


A trial of a new simplified procedure and a smaller kit for the **in vitro** isolation of trypanosomes (miniKIVI) is described. The study was conducted in Sinfra, a village 80 km southwest of Yamoussoukro, Côte d'Ivoire, on 14 sleeping sickness patients previously diagnosed by CATT and mAEC. Each patient was bled twice: 5.0 ml of blood was inoculated into the standard KIVI and 0.5 ml into the miniKIVI. All blood samples gave rise to positive cultures by one or both methods, whatever the level of parasitaemia in the initial sample, and no bacterial or fungal contamination was observed. The blood from 11 patients yielded positive cultures in both systems; two KIVIs and one miniKIVI failed to grow organisms. The incubation period varied between 9 and 22 days, was usually the same in the KIVI and the miniKIVI, and appeared to be independent of the number of trypanosomes inoculated. The efficiency of the miniKIVI is thus at least comparable to that of the standard KIVI, is simple to use, lighter in weight and less costly. Although the long prepatent period reduces its value for routine diagnosis, it may be useful as a standard for evaluating new diagnostic techniques in the field.

(b) **PATHOLOGY AND IMMUNOLOGY**

[See also 19: nos. 9599, 9600, 9601.]

**9565 Brabin, L. and Brabin, B.J., 1992.** Parasitic infections in...
women and their consequences. *Advances in Parasitology*, **31**: 1-81.
Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.
The clinical course and results of parasitic infections are often different in men and women because exposure to infection may be related to behaviour and work patterns of males and females, which are frequently distinct. Immunity to infection and response to treatment may also differ between the sexes. Pregnancy may alter susceptibility to infection, infections during pregnancy can influence the outcome of pregnancy, and maternal immune status is related to the development of infant immunity. The effect of the major tropical diseases (onchocerciasis, filariasis, malaria, schistosomiasis, African trypanosomiasis and leishmaniasis) on women is examined. The epidemiological evidence for sex differences in parasite prevalence, density and clinical disease manifestations, evidence for sex differences attributed to exposure, to hormonal and genetic factors, and to immune stimulation during pregnancy, and parasitic infection and pregnancy outcome, are reviewed.

Two patients with *Trypanosoma brucei rhodesiense* infection were followed clinically, biologically and by nuclear magnetic resonance imaging (MRI) for a year. One patient was at the haemolymphatic stage and had a normal MRI throughout. The other presented with CNS involvement. Cerebral tomo-densitometry was normal but MRI showed signs of meningitis. After two courses of treatment with melarsoprol, a severe encephalitis occurred preceded by trembling and associated with drowsiness, a tetrapyrimal syndrome, right-sided hemiplegia, a static and dynamic bilateral cerebellar syndrome and swallowing difficulties. Tomodensitometry without injection was normal; MRI revealed abnormalities in areas suggested by the neurological picture. The good correlation shown by MRI with the clinical and biological picture suggests that it could be a useful method for understanding the nature of an encephalitis and evaluating the prognosis.

9567 Goichot, B., Buguet, A., Brandenberger, G., Tapie, P., Bert, J.,

Thyrotropin (TSH) levels were compared during a 24 h period in six untreated patients with *Trypanosoma brucei gambiense* sleeping sickness, selected during a medical investigation in Congo, and in five healthy African subjects. Blood was withdrawn continuously via a catheter and sampled into 10 min aliquots in an adjoining room. TSH was measured by a commercial IRMA kit. Sleep was recorded by continuous polysomnography and scored visually. The integrity of the sleep-wake cycle varied greatly among patients, ranging from major disruption with insomnia to almost undisturbed nocturnal sleep. Mean TSH levels were slightly higher in the patients than in the controls, although the difference was not significant. The nocturnal surge was preserved in all but one patient and its amplitude was not different between patients and controls. There were more TSH pulses in the patients, maybe due to fragmented sleep with many awakenings. The relationships between sleep structure and TSH variations were preserved, with decreasing TSH levels during slow-wave sleep and increasing levels after awakenings. We conclude that, contrary to other biological rhythms, the nycthemeral pattern of TSH is preserved in the sleeping sickness patients. The TSH nocturnal surge persisted, unlike in other nonthyroidal illnesses. The relationships between TSH variations and sleep structure are also preserved, demonstrating the robustness of this association.


Several explanations for the major histocompatibility complex (MHC) polymorphism have been proposed. We argue that the only one consistent with the entire body of knowledge about the MHC is an explanation based on the immune response to parasites. Furthermore, we propose that parasites coevolving with their hosts have had a major influence on MHC polymorphism, whereas
parasites that switched hosts recently and became very virulent have had little effect. The latter category includes micro- and macroparasites responsible for the major human infectious diseases (including *Trypanosoma brucei gambiense*). The hypothesis explains why no convincing association between human leucocyte antigen (HLA) alleles and resistance to infectious disease can thus far be documented and indicates the direction in which the search for such associations should be taken.


Buguet: Unité de Physiologie de la Vigilance, Département des Facteurs Humains, Centre de Recherches du Service de Santé des Armées Emile Pardé, B.P. 87, F-38702 La Tronche Cedex, France.

Human African trypanosomiasis (sleeping sickness) is a unique disease model of disrupted circadian rhythms in the sleep-wake cycle and cortisol and prolactin secretion. This study examined the temporal relationship between growth hormone (GH) secretion and the sleep-wake cycle in eight infected African patients and six healthy indigenous African subjects. Twenty-four-hour sleep patterns were recorded by polysomnography and hourly blood samples analysed for plasma GH. No relationships between the mean normalised plasma GH levels (Z scores) and the sleep stages (wakfulness, sleep stages 1 and 2 ('light' sleep), slow-wave sleep (stages 3 and 4), and rapid eye movement (REM) sleep) were found in the patients or healthy subjects. However, when the time of sampling of the plasma GH concentrations was lagged by 16 min with respect to the occurrence of the various sleep stages, significant correlations were found between plasma GH concentrations and slow-wave sleep in both healthy subjects and patients. Thus, the association between slow-wave sleep and GH secretion persisted even in the presence of disrupted circadian rhythms, further supporting the concept that sleep and the stimulation of GH secretion are outputs of a common mechanism.


Vincendeau: Laboratoire de Parasitologie, Université de Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux Cedex,
France.

Human African trypanosomiasis (HAT) is characterised by a major deregulation of the immune system. Hypergammaglobulinaemia, auto-antibodies and immunodepression are cardinal features. Parasitaemia occurs in waves due to the successive appearance of parasites with different variable surface glycoprotein (VSG) antigens. Antigenic variation enables parasites to elude the host's immune defences. Although high levels of immune complexes have been detected during HAT, it seems unlikely that they play a significant pathophysiological role. Numerous auto-antibodies have been detected. B lymphocyte activation is uncommon. In vitro T lymphocytes do not proliferate normally, but synthesise cytokines, such as interferon-γ, which enhance parasite development. Macrophages bind and destroy parasites in the presence of antibodies. They also synthesise large quantities of TNF-α which promote parasite destruction but also increase the severity of clinical symptoms. Nitric oxide synthesised by activated macrophages has an antiparasitic effect but induces immunosuppression. In the meningoencephalitic stage of HAT, a severe inflammatory reaction is observed. This event is preceded by astroglia which could be induced by astrocytes secreting TNF-α and IL-1. Auto-antibodies against the central nervous system (e.g. anti-galactocerebrosides, anti-tryptophan-like auto-antibodies) may also be involved in the development of encephalitis. VSG antigens play a key role in the immunopathology of HAT (antigenic variation, induction of cytokines and auto-antibody production). Successive relapses occur with the appearance of new antigenic variants and production of antibodies. The resulting continuous stimulation of the immune system leads to deregulation of immunoglobulin production and the cytokine network.

(c) TREATMENT

[See also 19: no. 9535.]


Dumas: Institut de Neurologie Tropicale, Faculté de Médecine, 2 rue du Dr Marcland, 87025 Limoges Cedex, France.
The development of DFMO raised hopes of a more effective and safer treatment for late-stage trypanosomiasis but the complicated therapeutic regimen, some treatment failures, especially in children, and its cost have dashed these hopes. This has led to renewed interest in the better use of existing drugs and in combination therapy. With recent upsurges in the disease, new lines of research are being pursued, with trials of promising new compounds such as the nitroimidazoles. More research on the pathogenesis of the disease and host-parasite interactions is leading to a better understanding particularly of the immunology of trypanosomiasis, with a vaccine perhaps becoming more than just intellectual speculation.


Pépin: Infectious Diseases Section, Centre Hospitalier Universitaire, 3001 12ème Avenue Nord, Sherbrooke, Quebec, J1H 5N4, Canada.

Six hundred and sixteen patients with early T. b. gambiense trypanosomiasis (no trypanosomes in the CSF and a CSF white cell count [WCC] of 1-5/mm$^3$) were treated with a combination of pentamidine (6 i.m. injections of 4 mg/kg) and suramin (2 i.v. injections of 20 mg/kg) in Nioki hospital, Zaire, between 1983 and 1992; 46 (7.5%) of them subsequently relapsed. There was no increase in the frequency of treatment failure during this 10 years' period. Relapses were more frequent in children aged 0-17 years (19 of 163, 11.7%) than in adults (26 of 420, 6.2%) (relative risk [RR] = 1.88, 95% confidence interval [CI] 1.07-3.31, $P = 0.04$). Even within this small range of CSF WCC, the risk of treatment failure increased in parallel with the WCC count and reached 10 of 36 (27.8%) in patients with a CSF WCC of 5/mm$^3$. Treatment failures were more frequent (5 of 30, 16.7%) in a small group of patients treated with a combination of diminazene (3 i.m. injections of 7 mg/kg) and suramin (one i.v. injection of 20 mg/kg) than in the pentamidine/suramin group (RR = 2.23, 95% CI 0.96-5.21, $P = 0.08$). Our data support the view that CNS involvement occurs early in Gambian trypanosomiasis, which in turn raises doubts about the usefulness of adding suramin to pentamidine, as the former drug, which is more expensive than
pentamidine and has to be administered i.v., penetrates poorly into the CSF and may potentially decrease free pentamidine levels in blood and CSF.


Simarro: Fundació CIDOB Universidad Autónoma de Barcelona, Elisabets 12, 08001 Barcelona, Spain.

A 26-year-old man from the Luba focus in Equatorial Guinea was diagnosed with *Trypanosoma brucei gambiense* infection in 1986. He was treated with a single dose of pentamidine followed 48 h later by melarsoprol given in 3 series of 3 injections, the dose being progressively increased. Because follow-up lumbar punctures showed an elevated CSF WBC count (on one occasion with trypanosomes seen in the CSF), he received two additional courses of melarsoprol in 1987 (plus suramin) and 1988 (plus pentamidine). In 1989 he again had trypanosomes in the CSF and an elevated CSF WBC count and was treated with oral eflornithine (30 g daily, every 6 h for 4 weeks). In 1990 he was again found to have relapsed and was then treated with a combination of oral eflornithine and i.v. melarsoprol. Follow-up for 2 years indicated that he had been cured by this combination therapy. Previously synergism between these two drugs has been suggested and experience with this patient reinforces this idea.

6. animal trypanosomiasis
(a) SURVEY AND DISTRIBUTION
[See also 19: no. 9553.]


Bengaly: CIRDES, 01 B.P. 454, Bobo-Dioulasso 01, Burkina Faso.

The sensitivity and specificity of the antigen detection ELISA for the diagnosis of African animal trypanosomiasis was assessed in naturally-occurring infections in Burkina Faso. A total of 1633 cattle was sampled in a trypanosomiasis endemic area and examined for trypanosomes by the dark ground/phase contrast buffy-coat technique (BCT) and for circulating antigen by ELISA. Fifty sera from Markoye, a tsetse-free area in northern Burkina Faso, and 49 sera from Germany were also tested. In the trypanosomiasis endemic area, BCT
detected 144 (8.8%) positive animals (93 Trypanosoma vivax, 48 T. congolense, 3 mixed infections). The Ag-ELISA detected 108 (75%) of the BCT-positive cattle (39 of the 51 T. congolense-BCT-positive cattle, but only 17 of the 96 T. vivax-BCT-positive cattle). It also showed that mixed infections were frequent, especially those involving T. brucei and T. congolense. BCT indicated that the predominant species was T. vivax followed by T. congolense, whereas Ag-ELISA showed T. congolense to be most frequent followed by T. brucei and T. vivax. In the tsetse-free area, Ag-ELISA detected one positive animal carrying T. brucei and T. congolense and showed an apparent specificity of 98%. No serum from Germany was detected positive. This study suggests the joint use of Ag-ELISA and BCT for the diagnosis of animal trypanosomiasis, particularly for epidemiological studies in endemic areas.


As part of a study on livestock productivity under trypanosomosis risk in the region of Boundiali, northern Côte d'Ivoire, 21 herds of cattle (N'Dama, Baoulé and Zebu crosses) and 20 flocks of Djallonké and Djallonké ∃ Sahel sheep were monitored monthly for body weight, PCV and trypanosomal parasitaemia over various periods between January 1984 and December 1992. A tsetse control campaign using biconical traps impregnated with alpha-cypermethrin started in December 1987. Tsetse control reduced the relative tsetse density by over 95% between 1988 and 1992, and this was associated with reductions in the prevalence of Trypanosoma congolense over the same period of over 90% in both sheep and cattle. Average reductions in the prevalence of T. vivax were lower, on average 68% in adults and 85% in young animals. Attempts were made in the design of the study to allow comparisons between controlled and uncontrolled areas; however, there were too many confounding and uncontrollable factors to allow such comparisons to be made. It was necessary, therefore, to compare data collected from all herds and flocks before and after the intervention, with the consequential difficulties in accounting for uncontrollable year-to-year variations in factors
affecting trypanosome prevalence in livestock.

(b) PATHOLOGY AND IMMUNOLOGY
[See also 19: nos. 9539, 9583, 9585.]

Trypanosoma congolense: high erythropoietic potential in infected yearling cattle during the acute phase of the anemia. *Experimental Parasitology, 82* (2): 104-111.
Andrianarivo: School of Veterinary Medicine, Pathology, Micro-biology and Immunology, University of California, Davis, CA 95616, USA.
The erythropoietic response was evaluated in four naive yearling N'Dama (*Bos taurus*) calves and four age-matched Boran (*B. indicus*) calves which developed anaemia over a 140-day primary infection with *T. congolense* clone IL13E3. Similar levels of parasites were detected in the two breeds until 42 days p.i. During this period, there were no breed differences in mean PCV, possibly explained by a rapid and greater colony-forming units-erythroid response in the bone marrow of the Boran calves. However, this early erythropoietic response was transient and, despite the persistent severe anaemia, subsided from 70 days p.i. onward. In contrast, in the N'Dama calves, the mean PCV was gradually compensated from 56 days p.i. onward and reached 30% by 126 days p.i., with a return of the erythroid progenitor levels to near pre-infection values. These results suggest that the age of the Boran cattle has an important impact on the early bone marrow response in primary *T. congolense* infection and confirmed the high erythropoietic potential of young calves.

College of Veterinary and Animal Sciences, Mannuthy, Trichur 680 651, India.
Three case reports are presented of *Trypanosoma evansi* trypanosomiasis in dogs in Trichur, India. The main clinical signs were corneal opacity, anorexia, oedema of limbs, anaemia and general weakness. Diagnosis was by wet film examination. The dogs were treated with a single i.m. injection of diminazene aceturate at 8 mg/kg. Two were cured and one died.

Nine West African Dwarf goats were each infected experimentally with $3 \times 10^7$ *Trypanosoma vivax* parasites. The changes in the plasma concentration of nonesterified fatty acids (NEFA) were monitored during the infection and the level of hepatic triacylglycerols and glycogen was measured postmortem. During the infection the goats had higher plasma NEFA concentrations than nine uninfected control goats and at postmortem their total liver triacylglycerol and glycogen contents were found to be increased. These observations suggest that the mobilisation of the goats' defence mechanisms against the *T. vivax* infection induced a more intensive fat mobilisation resulting in changes in fat metabolism of the liver.


During acute *T. vivax* infection of calves, produced by i.v. inoculation, the mean PCV and red blood cell counts of the infected animals decreased significantly ($P < 0.05$) between days 6 and 13 p.i. The moderately severe normocytic anaemia started to develop during the first wave of parasitaemia which occurred from day 2 p.i. and peaked between days 4 and 5 p.i. The mean erythrocyte glutathione (GSH) concentration of the infected calves decreased significantly ($P < 0.05$) from 58.4 ± 11.4 mg/100 ml red blood cells (RBC) on day 0 p.i. to 44.5 ± 12.8 mg/100 ml RBC on day 5 p.i. As the GSH values recovered on day 6 p.i. and increased thereafter, another slight decrease ($P > 0.05$) in GSH concentration occurred on day 12 p.i. at the second peak of parasitaemia followed by a significant ($P < 0.05$) increase to 79.1 ± 14.6 mg/100 ml RBC on day 13 p.i. In the uninfected calves, the mean GSH values ranged from 47.7 ± 7.0 to 60.8 ± 6.8 mg/100 ml RBC. When washed, erythrocytes of the infected and uninfected calves were separately challenged with hydrogen peroxide. They produced comparable amounts of thiobarbituric acid reactive substances as a measure of
by-products of lipid peroxidation. This suggested that the ability of the erythrocytes to prevent peroxidative injury was not reduced, because GSH regeneration was probably enhanced and the antioxidant capacity of the erythrocytes was maintained.

Animal Health Research Centre, P.O. Box 24, Entebbe, Uganda.

The effect of animal trypanosomiasis on antibody response to rinderpest vaccination under field conditions was investigated. Three groups of cattle were randomly selected from animals volunteered by stock owners and were kept in their usual habitat throughout the trial. The pre-treatment surveys showed that none of these groups had a rinderpest antibody prevalence of more than 20% and that each of the groups included some cattle that had trypanosomiasis as detected by microscopic examination of Giemsa stained blood smears. The first group was vaccinated with a standard dose of tissue culture rinderpest vaccine (1 ml subcutaneously) and given a prophylactic dose of Samorin (isometamidium chloride). Group II was vaccinated 30 days following Samorin treatment. Group III was vaccinated but was not given any Samorin until day 63 post-vaccination. Antibody response to the vaccine, measured by competitive ELISA technique on days 7, 14, 21, 63 and 400 post-vaccination, showed that group II animals had an antibody prevalence of 90% by day 14 and group I animals attained a 90% prevalence on day 63. The antibody prevalence of group III animals never attained 90% and rose above 80% only after Samorin treatment on day 63. It was concluded that pathogenic trypanosomes to some degree cause a delay in antibody response to the rinderpest vaccine.

(c) TRYPANOTOLERANCE
[See also 19: no. 9576.]

ILRI, P.M.B. 2248, Kaduna, Nigeria.

A total of 505 lactation records from 340 N’Dama cows were matched with health records recorded on the same animals over a period of 4 years with the aim of
establishing linkages between three criteria of trypanotolerance (frequency of infection, control of parasitaemia, control of anaemia) and performance traits (milk offtake, calf weaning weight, calving interval, cow productivity index). The longer term objective is to include one or more of these criteria in a selection index for genetic improvement programmes, if found to be associated with performance traits. Least-squares analyses showed that the control of anaemia measured by PCV was more frequently linked with performance traits than the other criteria. Infected cows which maintained above-average PCV values had an 84% higher cow productivity index than those with below-average PCV. Uninfected cows achieved a 60% higher cow productivity index than infected cows. These results suggest that selection of animals based on some of the criteria will give correlated response in performance traits, provided the criteria have genetic basis.


Research at the International Livestock Research Institute, formed recently by the amalgamation of ILRAD and ILCA, includes molecular genetic studies on resistance to trypanosomiasis in cattle, and on genetic characterisation of African breeds of cattle, including microsatellite genotyping of samples from more than 100 sub-Saharan cattle breeds. The preservation of genetic resources will be essential to protect trypanotolerant breeds. Marker assisted selection will be used to identify individuals carrying the most advantageous combinations of genes.


B-cell activation and the quantity and isotype of antibody produced at the cellular level were measured in six trypanotolerant N'Dama and five trypanosusceptible Boran cattle. The frequencies of spleen cells secreting total and parasite-specific IgM and IgG were measured prior to and 16, 28 and 35 days after a primary challenge with *T. congolense*. Boran
cattle had higher frequencies of splenic cells secreting IgM specific for trypanosome-derived variable surface glycoprotein (VSG), cysteine protease (congopain, CP), and heat shock protein (hsp70/BiP) and the nonparasite antigen, ovalbumin, than did N'Dama cattle. In contrast, the number of VSG-specific IgG-secreting cells was significantly greater in N'Dama than in Boran cattle. During infection, low titres of anti-VSG IgM were detected transiently in the serum of all animals. However, N'Dama had significantly more VSG-specific IgG in blood than Boran during infection. The peripheral blood mononuclear cell population of N'Dama cattle contained a higher percentage of surface IgM-positive B-cells prior to and throughout infection than were found in the blood of Boran. In addition, during infection N'Dama cattle had more circulating lymphocytes that could be activated in vitro to undergo differentiation into IgM- and IgG-secreting cells. These findings demonstrate differences in the frequency of trypanosome-specific antibody-secreting cells in the spleen and in the activation state of B-cells in the blood between N'Dama and Boran cattle during a primary infection with *T. congolense*.


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

A small number of West African *B. taurus* cattle breeds, including the N'Dama, constitute a valuable genetic resource by virtue of their ability to remain productive under trypanosomiasis challenge. However, introgression of *B. indicus* genes into the trypanotolerant breeds, particularly by introduction of Zebu bulls, is a threat to this resource. This work describes the characterisation and cloning of a bovine randomly amplified polymorphic DNA (RAPD) that is generated in polymerase chain reaction (PCR) with the 10 base primer ILO1065 from *B. indicus* male templates, but not from *B. taurus* male templates or female templates of either type. Male-specific sequences with homology to the RAPD also occur in *B. taurus* breeds. This suggests that the polymorphism may be due to base substitution(s) in an ILO1065 priming site, or insertion/deletion events either affecting priming sites or occurring between sites on the cattle Y chromosome. We have shown that cattle, whether *B. indicus* or *B. taurus* phenotype, which possess a typically *B. indicus* metaphase Y chromosome on
the basis of QFQ banding, have a *B. indicus* ILO1065-generated genotype. The ILO1065-primed RAPD can be used in a simple dot blot assay as a probe of RAPD-PCR products, to provide a convenient, reliable and effective means of detecting introgression of Zebu genes in *B. taurus* cattle populations.


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It has been reported that some breeds of cattle such as the N'Dama mount a more effective antibody response to the variable surface glycoprotein coat of trypanosomes and that this may contribute to their ability to control the infection. Thus we have investigated antibody responses to surface exposed epitopes of the variable surface glycoprotein in *Trypanosoma congolense*-infected N'Dama (trypanotolerant) and Boran (susceptible) cattle. Similar titres and isotypes were found in both N'Damas and Borans indicating that trypanotolerance is not associated with superior antibody-mediated destruction of trypanosomes. However, significant differences in antibody responses to cryptic VSG epitopes and non-trypanosome antigens were identified. Trypanosusceptible Boran cattle had low IgG responses to cryptic epitopes but high IgM responses to non-trypanosome antigens such as β-galactosidase. In contrast the N'Dama cattle had significantly higher IgG responses to cryptic VSG epitopes and negligible responses to β-galactosidase. These results indicate differences in the induction of anti-trypanosome immune responses between trypanotolerant and susceptible cattle infected with *T. congolense*.

(d) TREATMENT


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

The pharmacokinetics of diminazene in plasma and pseudo-afferent lymph were investigated in East Africa Galla goats. The efferent prescapular lymphatic duct of three goats was cannulated 8 weeks after surgical
removal of the lymph node. Thereafter, 3.5 mg of diminazene base/kg of body weight was administered to these goats and to three noncannulated goats. Using high-performance liquid chromatography, concentration of diminazene was determined in plasma and lymph collected up to 96 h after treatment. Maximal concentrations of diminazene in plasma of noncannulated goats (median (range), 4.30 (4.28-5.01) µg/ml), plasma of cannulated goats (3.94 (2.94-4.06) µg/ml), and lymph (1.06 (0.73-1.86) µg/ml) were significantly different ($P < 0.05$): values in lymph were considerably lower than those in plasma from noncannulated and cannulated animals. Time to reach maximal concentration did not differ significantly between lymph and plasma of noncannulated and cannulated goats. Over the first 24 h after drug administration, concentration of diminazene in plasma of noncannulated goats was generally higher than that in lymph, but thereafter was similar. Apparent volume of distribution of diminazene in the plasma of noncannulated (2.57 (1.93-2.60) L/kg) and cannulated (2.30 (1.04-2.40) L/kg) goats did not differ significantly. Penetration ratio of diminazene into lymph, compared with plasma, of cannulated goats was 1.69:1. Disposition of diminazene in goats is thus characterised by higher concentration in plasma than in lymph, although the drug persists longer in lymph than in plasma. The latter observation may account for the enhanced therapeutic efficacy of diminazene in the early stage, compared with later stages, of a tsetse fly-transmitted trypanosome infection.


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

The absorption, distribution and elimination characteristics of $^{14}$C homidium have been studied in non-infected and *Trypanosoma congolense*-infected cattle treated with $^{14}$C homidium chloride by either i.m. or i.v. injection at a dose level of 1 mg/kg body weight. Results show that the mean (± SD) elimination of the drug from plasma followed a bi-exponential process, with half-lives of 0.084 ± 0.006 h and 97.66 ± 16.28 h for the distribution and elimination phases after i.v. injection, respectively. Bioavailability of the i.m. dose was 62.5% and 57.8% in non-infected and
trypanosome-infected cattle, respectively. Absorption was rapid, with a $t_{\text{max}}$ of 15 min and a mean $C_{\text{max}}$ (± SD) of 268.4 ± 4.09 ng/ml following the i.m. dose in non-infected cattle. The major route of excretion was via faeces. Approximately 90% of the total dose given to non-infected i.m.-treated cattle was excreted within 14 days. Following i.m. administration of the drug, residues remained high in the major excretory organs, with the liver having concentrations of 1411 and 1199 ng/g after 14 and 28 days, respectively. Over the same period, the values in the kidneys were 649 and 448 ng/g. Concentrations in the liver 14 and 21 days following i.v. treatment were 2195 and 2454 ng/g, respectively. These results show that there was no significant difference in liver drug residues between 14 and 21 days, or 28 days depending on the treatment given, suggesting that once the drug is in this organ, it is released back into the circulation at an extremely slow rate.

7. experimental trypanosomiasis

(a) DIAGNOSTICS

[See also 19: no. 9627.]


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Gujarat Agricultural University, c/o Patani Surgical Hospital, Mansarovar Road, Palanpur, Gujarat 385 001, India.


Department of Biochemistry, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 19: no. 9631.]


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Department of Biochemistry and Molecular Genetics, School of Medicine, University of Alabama, Birmingham, AL 35294, USA.

The authors comment on a paper by Tomlinson et al. (see *TTIQ*, **18** (3): no. 9071) which re-examines the involvement of high-density lipoproteins (HDL) in trypanosome killing and concludes that human serum-mediated lysis of *T. b. brucei* is not an exclusive property of human serum HDLs. The fact that HDL-deficient sera from Tangier patients had high levels of trypanolytic activity supports this view. When the lytic component of both normal and Tangier sera were examined, most of the trypanolytic activity in normal serum was found in the very high density subclass of HDL, while the trypanolytic activity of the Tangier sera fractionated with the lipoprotein-deficient components. However, when fractionated by gel filtration chromatography, both normal and Tangier sera displayed two peaks of trypanosome lytic activity, one at 150,000-600,000 MW and the other at > 1,000,000 MW.


Raper: Department of Medical and Biochemical Parasitology, New York University Medical School, New York, NY 10016, USA.

Natural immunity of humans to the cattle pathogen *T. b. brucei* has been attributed to the presence in normal human serum (NHS) of lytic factors for the parasites. We and others have shown that NHS contains two
trypanolytic factors (herein termed TLF1 and TLF2) that can be separated by gel filtration. TLF1 copurifies with a subclass of high density lipoprotein (HDL), whereas TLF2 has a much higher molecular weight and does not appear to be a lipoprotein. We find that the trypanolytic activity of purified TLF1 is totally inhibited by exogenous haptoglobin (Hp) at concentrations (0.1 mg/ml) lower than those present in NHS (0.2-2 mg/ml). In contrast, exogenous Hp (up to 2.5 mg/ml) has no effect on the lytic activity of either NHS or isolated TLF2. Hp-depleted sera from patients with intravascular haemolysis is severalfold more trypanolytic than NHS. These sera contain only TLF1, and their lytic activity is totally abolished upon the addition of Hp (0.1 mg/ml). When NHS containing different Hp allotypes is fractionated by gel filtration, TLF1 activity is either revealed or remains masked, depending on whether it coelutes with Hp. Masked TLF1 activity in the column fractions is revealed if Hp is removed by density gradient ultracentrifugation. We conclude that endogenous Hp inhibits TLF1 activity, and that TLF2 is the main trypanolytic factor in NHS.


Tomlinson: Department of Pathology, New York University Medical Center, 550 First Avenue, New York, NY 10016, USA.

Analysis of 20 samples of normal human serum (NHS) showed no correlation between the trypanolytic activity of unfractionated NHS and its haptoglobin (Hp) concentration. The Hp allotype (Hp1-1, Hp2-1 or Hp2-2) of each serum sample was determined. No relationship between serum trypanolytic activity and its Hp type was discernible. These data support the authors' previous conclusion that TLF2 and not TLF1 is the main trypanolytic factor in NHS.


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It is hypothesised that rapidly dividing parasites producing high parasitaemias within an individual host
are in different environmental settings. It is further suggested that these infrapopulations experience the drastic environmental changes of free-living forms in an island environment and that, in chronically infected animals, the environmental conditions will over time select the parasites best suited to grow in their changing habitat. Evidence is presented to demonstrate that the host environment (mice) does change during an infection with African trypanosomes (Trypanosoma brucei rhodesiense) and that, with time, each host becomes environmentally unique. Data are also provided to show that parasites cloned from different hosts are phenotypically different and are assumed to be genetically different as well. The evidence provided is consistent with the hypothesis that each individual host provides a unique habitat in which selection occurs, and that the rapidly dividing protozoans, such as the African trypanosomes and plasmodia, are continuously evolving in the individual host.


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Ajaiyeoba: Department of Pharmacognosy, 
University of Ibadan, Ibadan, Nigeria.

In vivo trypanocidal activities of new S- 
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brucei, T. b. gambiense, T. b. rhodesiense, T. congolense; mice.]

Bacchi: Haskins Laboratories, Pace University, 
41 Park Row, New York, NY 10038-1502, USA.

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Bouteille: Institut de Neurologie Tropicale, 
Faculté de Médecine, 2 rue du Dr Marcland, 
87025 Limoges Cedex, France.

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Brun: Swiss Tropical Institute, P.O. Box, CH- 
4002 Basel, Switzerland.

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(methylsulfonio)-2-cyclopentene (AdoMao): an 
irreversible inhibitor of S-adenosyl-methionine 
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Topical chemotherapy for experimental African 
trypanosomiasis with cerebral involvement: the use of 
mealsorprol combined with the 5-nitroimidazole,

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8. trypanosome research
(a) CULTIVATION OF TRYpanOSomes
(b) TAXONOMY, CHARACTERISATION OF ISOLATES


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Within the subgenus Nannomonas, Trypanosoma congolense and \(T. simiae\) are the only recognised species. Here, the isolation and partial characterisation of a new Nannomonas-type trypanosome from a Glossina pallidipes caught at the Ngulia Rhino Sanctuary, Tsavo West National Park in Kenya is described. A trypanosome culture was
initiated with metacyclics derived from a single tsetse. Organisms were propagated axenically as trypomastigote forms at 35°C initially in the presence of bovine aortic endothelial cells. Culture-derived bloodstream trypomastigotes were transformed into procyclics at 26°C which further on transformed into epimastigotes and finally into pig-infective metacyclics. On Giemsa-stained slides the organisms had a marginal kinetoplast close to the posterior end. The undulating membrane was well developed in some organisms but inconspicuous in others. The undulating membrane extended to the tip of the flagellum. A free flagellum seemed to be present only in some trypanosomes. The parasites caused a very mild infection in domestic pigs accompanied by a low parasitaemia. Infections were either self-limited or could be cured by treatment with the trypanocidal drug, diminazene aceturate. The trypanosomes did not infect mice, goats or steers. DNA from the trypanosome isolate hybridised with DNA probes for the Nannomonas subgenus; it did not hybridise with DNA probes for Kilifi-type and savanna-type T. congolense, T. simiae or T. brucei.

(c) LIFE CYCLE, MORPHOLOGY, BIOCHEMICAL AND MOLECULAR STUDIES
[See also 19: nos. 9558, 9602.]

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Courtoy: Cell Biology Unit, Catholic University of Louvain and International Institute of Cellular and Molecular Pathology, avenue Hippocrate 75, B-1200 Brussels, Belgium.

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exogenous DNA into *Trypanosoma brucei* requires a high degree of homology between donor and target DNA. *Molecular and Biochemical Parasitology*, 76 (1-2): 215-229.

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Boothroyd: Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA.

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Feizi: Glycobiology Group, Glycosciences Laboratory, Northwick Park Hospital, Watford Road, Harrow, Middx HA1 3UJ, UK.

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