SECTION B — ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

[See also 15: no. 7287.]


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Lack of resources, infrastructure and trained manpower are severe constraints on the application of relevant biotechnology in Africa. There is scope for achieving increased animal productivity with regard to improved disease diagnosis and control, nutrition and improvement of genetic composition. Trypanosomiasis is listed among the diseases limiting livestock production. The N'Dama and Muturu cattle (Bos taurus) of West Africa are trypanotolerant beef breeds and the possibility of transferring the genes responsible for this trait to other breeds, especially dairy breeds, should be explored. This would require collaborative research in breeding, recombinant DNA technology and genetics.


This paper briefly reviews previous economic studies carried out in Africa and then considers a four-year project which commenced in 1987 to examine social and economic aspects of tsetse and trypanosomiasis control in Zimbabwe. Benefit-cost analysis has included the consideration of possible environmental degradation associated with accelerated livestock production and improved understanding of the economics of draught animal production. Comparative cost studies have covered ground spraying with DDT and synthetic pyrethroids, aerial application of non-residual insecticides, use of odour-baited insecticide-treated screens and the direct application of synthetic pyrethroids to cattle. The project has provided
methodologies which need to be applied in the planning and appraisal of operations each year for future resource allocation to be most effective. Possible options are set out to improve resource management for tsetse and trypanosomiasis control in other parts of Africa.


African trypanosomiasis data are used to corroborate Bradford's Law, by applying the Bradford-Zipf bibliograph to the periodical literature, 1900-1983. The nucleus of periodicals was identified. The reference-scattering co-efficient was found to be 0.56. The ideal divisions of a collection of 670 journals with regard to the verbal formulation of the law are four zones with an initial zone of four most productive journals and bm of 5.13, a second moderately productive zone of 4 \( \equiv 5.13 \) or 21 journals followed by a third zone of 4 \( \equiv 5.13^2 \) or 105 journals and finally one with 4 \( \equiv 5.13^3 \) or 540 journals. Ranks of journals in what is regarded as current list (CL) covering 1978-1983 was compared to the ranking in the Far-Back-In-Time (FABIT) list (1900-1983) in order to examine the extent of bias in favour of the latter. The FABIT effect was significant. The total number of journals and articles that would be found in a 'complete' search of the documentation are 681 and 4440 as against 670 and 4100 found. Some implications of these findings for biomedical information services are discussed.

2. TSETSE BIOLOGY
(a) REARING OF TSETSE FLIES
(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

7219 Carlson, D.A. and Schlein, Y., 1991. Unusual polymethyl alkenes in tsetse flies acting as abstinon in *Glossina morsitans*. *Journal of Chemical Ecology*, 17 (2): 267-284. USDA, ARS, MAVERL, Gainesville, FL 32604, USA; Department of Parasitology, Hadassah Medical School, Hebrew University, Jerusalem, Israel. (Correspondence to Schlein.)

The major alkene of the male tsetse fly, *G. m. morsitans*, was isolated for characterisation by thin-layer and gas chromatography. The mass spectra of the alkene and the
alkene DMDS derivative indicated one isomer, 19,23-dimethyltritriacont-1-ene. The material is present at 1-2 µg/male fly and is partially transferred to the female preparatory to or during mating. A dose-dependent antiaphrodisiac effect was seen with exposed male flies using the isolated natural product, with 2 and 4 µg causing 80% loss of copulatory attempts, and 10 µg extinguishing the attempts. This effect was increased by addition of male-produced alkane. This compound and a 31-carbon homologue also appear in G. m. submorsitans. Similar quantities of alkenes that are species-specific appear in all tsetse males. Structures of male-produced trimethylalkenes that appear in two other species, G. palpalis palpalis and G. fuscipes fuscipes, were investigated.


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A study performed on three tsetse fly species (Glossina tachinoides, G. morsitans morsitans and G. fuscipes fuscipes) using scanning electron microscopy (SEM) brings new data on the morphology and location of some sensory organs of tsetse flies. Pictures illustrating morphology of proprioceptive hairs of the prothoracic organ are presented and for the first time the existence of hairs, probably mechanoreceptors, on the ptilinum, and chemoreceptors on the costal vein of the wings is indicated. This approach aims at better understanding of tsetse flies' perception of the environment in order to improve the trapping technology.


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Newly emerged G. m. morsitans tsetse flies lack a peritrophic membrane which develops to line the midgut.
fully after c. 80-90 h. Midgut lectins are mainly associated with the peritrophic membrane. Lectin levels in the blood-free gut of adult flies rise slowly up to 8 days and then rapidly to at least 14 days post-eclosion (when the last of our recordings was made). Despite starving flies for 4 days prior to the agglutination assay, gut lectin levels in older flies are 100-200 times more than those in newly eclosed flies. This is inconsistent with the idea that there is a simple relationship between lectins and the protection of tsetse flies against trypanosome infection. Various theories put forward to account for age-dependent variation in the ability of tsetse to become infected with trypanosomes are discussed in the light of these findings.


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The spontaneous intracellular activity of the thoracic ventral longitudinal eclosion muscle (VLEM) of *Glossina morsitans morsitans* is described for the period from eclosion up to a short time before the final breakdown of recorded fibres. The VLEM comprises a single motor unit with no inhibitory input. The firing frequency of the motor unit declines over 5 h after eclosion and leg release. Over a period of inactivity lasting 19-24 h in the sampled fibres, there is no loss of resting membrane potential and occasional miniature potentials. The inactivity is ended by the sudden onset of biphasic potentials very different in form to the motor potentials and having a greatly reduced amplitude. These potentials fired at 6 Hz, lasted 2-4 h and ended with a rise in frequency to 25 Hz. No further activity is recorded and the fibres are observed to lose their striations and rigor. Experiments to characterise the ionic basis of activity in the VLEM have been done on spontaneous and evoked activity. Like other insect muscles, the VLEM has a major Ca$^{2+}$ potential but, unlike insect skeletal muscles, it also appears to have a TTX-sensitive component. This Na$^+$ component is revealed by pre-treating the system in Na$^+$-free-choline saline, or by treatment with TEA in a Ca$^{2+}$-free saline. Neither EGTA nor cobalt abolishes this potential. Addition of EGTA does not inhibit nerve-evoked
activity, suggesting that the VLEM neuromuscular junction is in some way protected. The similarity of this muscle to insect visceral muscles is discussed.


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Lipophorin was isolated from the haemolymph of adult tsetse flies, *G. m. morsitans*, by ultracentrifugation in a potassium bromide density gradient. The tsetse fly lipophorin \((M \approx 600,000)\) has a density of \(\approx 1.11 \text{ g/ml}\) and consists of two apoproteins, apolipophorin-I \((\text{apoLp-I, } M \approx 250,000)\) and apolipophorin-II \((\text{apoLp-II, } M \approx 80,000)\), both of which are glycosylated as shown by staining with periodate-Schiff reagent. The protein complex is composed of 49% protein and 51% lipids. The finding of lipophorin in tsetse fly haemolymph suggests that, although these flies primarily utilise proline for their energy needs, there is an active transport mechanism for the supply of lipid requirements.


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The major protein in the milk gland secretions of the tsetse fly, *G. m. morsitans*, was isolated by a combination of gel permeation chromatography and crystallisation. It has a native \(M \approx 47,000\) and is composed of two identical poly-peptide chains \((M \approx 21,000)\) as determined by chemical cross-linking studies. The protein has no covalently-bound carbohydrates or lipids. Amino acid analysis of the protein revealed relatively high amounts of the aromatic amino acids, tyrosine \((9.1 \text{ mol.\%})\) and phenylalanine \((8.5 \text{ mol.\%})\). Immunoblotting experiments using antiserum against the protein revealed no cross-reactivity with any other milk proteins. Quantitation of the protein during the pregnancy cycle showed that synthesis of the protein by the milk glands of adult female flies starts as the larva molts into second instar and rapidly declines as it matures into third instar. It is proposed that the major milk gland protein could provide essential amino acids needed for puparium formation.
Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, Netherlands.
A comparison was made of the EAG responses of males and females of *Glossina morsitans morsitans*, *G. austeni* and *G. tachinoides* to various doses of compounds known to be components of ox and buffalo urine fractions which are attractive to tsetse in the field (phenol, 3- and 4-methylphenol, 3- and 4-ethylphenol, 4-n-propylphenol, dimethylsulfone). All three species did not respond to dimethylsulfone. The overall responses to the phenolic substances were higher in females than in males in *G. m. morsitans* and higher in males than in females in *G. austeni* and *G. tachinoides*. Response spectra of the species for the phenolic substances suggested that *G. m. morsitans* and *G. austeni* were most responsive to 3- and 4-methylphenol and 3-ethylphenol, whereas *G. tachinoides* was most sensitive to 3-ethylphenol and 3-methylphenol, and only moderately sensitive to 4-methylphenol. Cross-adaptation experiments, in which 1-octen-3-ol, acetone, 4-heptanone and 3-nonanone were also included, revealed that all phenolic compounds stimulated one and the same class of receptors, which differed from the class of receptors activated by 1-octen-3-ol. The ketones also had their own receptors. Hence, the flies can obtain information about the presence of attractants by at least three different receptor classes. It was concluded that phenol and any individual alkylphenol found in ox and buffalo urine should be attractive to tsetse flies, provided that stimulus intensity is above threshold and not beyond optimum. One class of receptors may respond more strongly in males than in females, whereas another class is more responsive in females than in males. This may result in a change in sex ratios in catches depending on the odour bait used.

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Enzymes were investigated, by electrophoresis, in a population of *G. f. fuscipes* collected from Rusinga Island in Lake Victoria, Western Kenya. The following enzymes were tested: glucose phosphate isomerase, glucose-6-phosphate dehydrogenase (G6PDH), hexokinase, isocitrate dehydrogenase (IDH), malate-dehydrogenase (MDH),
phosphoglucomutase and xanthine dehydrogenase (XDH). Single monomorphic bands were stained by the following enzymes apparently under the control of single loci: G6PDH, MDH and XDH. The enzyme IDH showed two bands with very close mobilities and no variation among individuals in the population. Hence IDH was considered as representing a single locus. Glucose phosphate isomerase manifested three alleles and apparently six genotypes. Phosphoglucomutase manifested a double-banded pattern representing an autosomal locus.

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Trypsin is quantitatively the major proteolytic enzyme of G. p. palpalis. We found that 50–60% of total digestive proteolysis was carried out by trypsin. Trypsin was isolated from midgut homogenates by gel filtration on a Sephadex G-75 chromatograph followed by affinity chromatography on a soybean inhibitor CH-Sepharose 4B column. The procedure resulted in a 36-fold purification of trypsin with a high specific activity (238 U/mg) but a low yield of activity (35%). The purification of trypsin revealed that three fractions with trypsin activity were identified after polyacrylamide gel electrophoresis (PAGE). In comparison with the whole midgut homogenate, only minor protein impurities could be detected on the gel. Trypsin was further characterised using SDS-PAGE and isoelectric focusing. Isoelectric focusing of purified trypsin revealed at least three fractions with an isoelectric point of respectively pI = 5.1, pI = 5.3 and pI = 8.5. Only two fractions could be distinguished after SDA-PAGE. The molecular weight of these fractions was estimated to 24,000 and 26,500 Dalton. It is still unclear whether these fractions correspond to different trypsin-like enzymes. More thorough investigation is necessary to determine the possible differences between these fractions with trypsin activity.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES
[See also 15: nos. 7225, 7243.]
7228 Randolph, S.E., Rogers, D.J., Dransfield, R.D. and Brightwell, R.,

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Samples of *G. longipennis*, taken over 4 days in traps (baited with acetone, cow urine and octenol) at half-hourly intervals during their restricted activity periods at dusk and dawn, were analysed for their nutritional condition. For both sexes, trap catches were highest at the end of dusk, between 19.00 and 19.30 h, associated with a significant increase in numbers with the low haematin content characteristic of flies estimated to have fed more than 60 h previously. Also, the mean fat content of these flies was significantly lower than that of those trapped earlier. There was considerable day-to-day variation in the numbers of flies trapped, and in the numbers of low haematin and low fat flies. It is concluded that the nutritional condition of the fly probably influences the timing of the onset of activity, but possibly the fly's approach to the trap.


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Field studies in Zimbabwe elucidated how trees might be enhanced as baits for controlling *Glossina morsitans morsitans* and *G. pallidipes*. Catches from electrocuting devices at the bases of trees were near nil when sampling tsetse flies landing on the trunk but much greater when sampling them flying within 1 m of the trunk. Catches increased 5-8 times when 2 m of the trunk were blackened and given odour of acetone, 1-octen-3-ol, 3-n-propylphenol and 4-methylphenol, but were still only c. 30% of the catches from an odour-baited, free-standing, 1 m screen of black cloth. The upright trunk of real and model trees hindered their attractiveness but leaves and branches 5 m above ground had no clear effect. Real and artificial stumps of trees were as effective as the screen if they were 1 m, compact and sharply outlined. The practical and biological implications of the results are discussed, with particular reference to the use of insecticide-treated netting with modified tree stumps as baits for control.

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Following parturition, the third-instar larva of *Glossina morsitans morsitans* begins a wandering period in which it crawls to the site of pupariation. The duration of wandering can be drastically shortened by pinching or by denying the larva physical contact with the substrate. Contact with water increases the wandering period. Duration of subsequent activities appears to be rigidly fixed. At the end of the wandering period, the larva quickly progresses through a stereotypic sequence of behaviours that include immobilisation and excretion of a liquid from the anus, retraction of the anterior segments, cuticular shrinkage, and tanning. Muscular activity and mechanical changes in the cuticle are reflected in changes of haemocoelic pressure. Muscular contractions produce pressure pulses that gradually increase in frequency and intensity, reaching a peak during retraction of the anterior segments. Changes in the mechanical properties of the cuticle cause a more gradual elevation of baseline pressure as the cuticle shrinks and loses its plasticity. As tanning begins, muscular activity ceases and haemocoelic pressure gradually decreases. In spite of its unusual early development within the confines of the female's uterus, the free-living larva shows the full behavioural repertoire observed in other cyclorrhaphous Diptera at pupariation.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also *Tsetse and Trypanosomiasis Information Quarterly* 15: nos. 7229, 7255, 7288.]


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The movement of Mbororo stockbreeders from west to east is increasing due to various factors, including drought, and this is pushing them further and more permanently into the tsetse-infested humid savanna. In order to reduce trypanocidal and trypanopreventive drug use, a control trial of *G. f. fuscipes* was undertaken by local trapping, using two biconical traps (not impregnated with insecticide) at each watering place in 32 settlements. The stockbreeders collected the caught flies and maintained the traps. A reduction in apparent fly density was very obvious after 1 month and in general was above 90% after 2 months. The reduction was faster and more homogeneous in the dry season than in the rainy season. The stockbreeders managed their traps well and were motivated by this simple control technique. Problems arising from their semi-sedentary habit are discussed. The information and training already given to the stockbreeders will be intensified and their organisation into groups to facilitate the integration of this control method will be encouraged.


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Tsetse control methods are briefly surveyed with the conclusion that failure to substantially reduce animal trypanosomiasis over much of Africa is largely a consequence of the myth that total eradication is the only worthwhile goal. Control, rather than eradication, with low technology, local funding and community involvement, is more likely to be effective in the long run. To this end the NGU trap has been developed at Nguruman, Kenya. Blue cloth attracts the flies and black cloth elicits a landing response. Flies enter the trap from below and are attracted upwards towards light filtering through a muslin cone, where they are trapped in a plastic bag and die of heat stress. The traps are made by local people and baited with acetone and cow urine; they are very effective against *Glossina pallidipes*. Since 1987 fly density in the control zone has been maintained below 10% of the density outside the zone. New infections are few and drug use has dropped by 50%. This level of control is
achieved with two traps per km$^2$ which cost US $30 (excluding labour and transport costs) per year, compared with drug costs per km$^2$ of about US $150 per year.


This article reviews the methods and materials used for monitoring the impact of tsetse control on non-target insects and appraises the equipment used, ease of use and manufacture, and the value of the data retrieved. For monitoring terrestrial habitats, the use of Malaise, funnel, light, river, pitfall and sticky traps, groundsheets, sweep nets and odour-baited targets are described and discussed. Hives should be monitored to assess risks to bees. Aquatic habitats can be monitored by 'kick' sampling (where a net is held downstream of someone kicking up the substrate), Surber and cylinder samplers, drift traps, use of artificial substrates to measure invertebrate colonisation, and sampling of rooted vegetation. Bioindicators for demonstrating non-target effects or for signifying recovery and the interpretation of effects with emphasis on the contentious issue of acceptable damage are reviewed. Common problems are discussed, including experimental design, sampling error, identification, availability of bioindicators, methodology, hydrology and aquatic studies, soil biology and abiotic parameters. No lasting effects on non-target species from tsetse control are reported, except a predatory spider (Hersiliidae) from DDT spraying. Staff training and the development of appropriate methodology are future priorities.


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From 1978 to 1980, the basis of tsetse control in Côte d'Ivoire was an entomological survey which resulted in the establishment of tsetse distribution maps. From 1980 to 1982 different control techniques were tested. From 1982 to 1984 impregnated biconical traps and screens were tested in an area of 3600 km$^2$. After the tsetse population had been reduced by 95% using traps,
annual campaigns were carried out using this technique in an area of 16,000 km$^2$. Since 1989 the control area has been extended to 60,000 km$^2$ and the control techniques have been adapted to the ecological conditions, the different fly species and the different livestock management forms found in these areas. In addition to vector control, studies have been conducted on tsetse biology, epidemiology of trypanosomiasis and on the environmental impact of tsetse control techniques. From 1991 the indirect consequences of tsetse control, e.g. opening of new pasture areas, growth of livestock populations and their impact on the environment, will be studied by ecological monitoring. At the same time sociological aspects, e.g. possible conflicts between cattle breeders and other farmers in tsetse-free areas, and possibilities of an active participation of the population in tsetse control will be studied.


The effect of selected lipophilic materials on the loss of deltamethrin applied to cotton fabric and washed with water was studied. Corn oil, paraffin, linseed oil and silicone oil reduced the leaching of deltamethrin by water from the fabric. The cumulative sum of four washings of the treated cotton strips resulted in a total loss of 37.7% of deltamethrin from the cotton strip (without protectant) and 9.9% from the strip treated with deltamethrin and corn oil. The effect of the colour of fabric and a UV absorber compound on the photodegradation of deltamethrin was also studied. Photodegradation was much less on the blue or black fabric than on the white fabric, and 2,4-dihydroxybenzophenone reduced the photodegradation of deltamethrin applied to cotton fabric.


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Adult male tsetse, *G. m. morsitans*, exposed to spores of *B. bassiana* and *M. anisopliae* exhibited mortalities ranging from 98% to 100% by day 25 post-exposure. The
Metarhizium-infected tsetse developed circular abdominal lesions believed to correspond to the sites of penetration by the fungal germ tubes but similar lesions were not observed in tsetse exposed to spores of B. bassiana. Histological sections of tsetse fixed just prior to death revealed fungal hyphae in the fat body, muscles and gut walls of the infected insects, but the numbers of hyphae were scanty and no cellular immune reactions were observed in the infected tissues. After death, the fungi invaded the tissues, rapidly destroying most of the internal organs, and penetrated the cuticle, forming extensive surface mycelia. Scanning electron microscopy revealed an intricate network of hyphae on the cuticle surface. The potentials of B. bassiana and M. anisopliae for biological control of tsetse are briefly discussed.


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Biconical Challier traps and blue screens impregnated with deltamethrin were used to control riverine tsetse (Glossina palpalis and G. tachinoides) and trypanosomiasis in trypanotolerant cattle along the Sio river. In the experimental area 22 traps were deployed 100 m apart in 2.2 km of gallery forest and 32 screens (reimpregnated every 3 months) were placed in 32 ha of pasture. Infected cattle in both experimental and control areas were treated with Berenil monthly. Tsetse density per trap-day fell from 4.6 to 0.1 over the 12 month period December 1985 to December 1986, representing a decrease of 97.8%. At the same time the trypanosome infection rate decreased from 13.6% to 1.66%, a reduction of 88.1%. In contrast, the infection rate in the control herd fluctuated between 10.0% and 10.4% over the same period. Productivity increased in the experimental herd, with fewer abortions (none compared with up to 14.2% in the control herd), reduced calf mortality (by 91.2% compared to 76.8%) and increased calving rate (by 75.8% compared to 11.0%). The smaller increase in productivity observed in the control herd is attributed to the Berenil treatment. The cost of installing and maintaining the traps and screens was offset by the reduced amount and cost of Berenil required to treat the herd in the experimental area.
Simultaneous control of tsetse flies and ticks by the application of flumethrin-pour-on on cattle in Satiri, Burkina Faso. (Meeting abstract no. 73.) *Tropical Medicine and Parasitology*, 42 (4): 451.

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The effectiveness of the pyrethroid flumethrin against *Glossina palpalis gambiensis* was checked in field experiments during one year. Close to Satiri (southern Guinea savanna) 2000 cattle were treated monthly for simultaneous control of tsetse flies and ticks by pour-on application with flumethrin (1 mg active ingredient/kg bodyweight). Two hundred cattle were ear-tagged and their PCV and infection with trypanosomes monitored monthly. High challenge of tsetse flies (*G. p. gambiensis*, *G. tachinoides* and *G. morsitans submorsitans*), determined by biconical trapping, occurred before the experiment. After the fourth treatment with flumethrin no further tsetse flies were captured in the vicinity of the treated animals. Four weeks after treatment the tick reduction amounted to more than 80% per cow compared with the animals in the control area. The prevalence of trypanosomosis (*Trypanosoma vivax, T. congolense*) among the 200 cattle monitored decreased from 40% to zero.


A low-cost mono-screen trap for *G. f. fuscipes* suitable for use by a rural community in Uganda is described. The trap has a single blue/black screen and a cone made from mosquito netting. The supporting framework is made from indigenous plant materials. The differences in trap catches between the mono-screen, biconical, pyramidal and Vavoua traps were highly significant (*P* < 0.001). Taking the standard biconical trap as control, the mono-screen trap was 1.25 times as efficient and the pyramidal trap was 0.04 times as efficient. The cost of one mono-screen trap is estimated as 1800 Uganda shillings (= US $4.7), about half the cost of a pyramidal trap and one-quarter the cost of a biconical trap. The prospects for the use of the mono-screen trap by the community are discussed.

A new

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A process is described by which trapping technology is being taught to a rural community which has been affected continuously by an epidemic of sleeping sickness for over a decade. Through a systematic health education programme, people are actively involved in making and setting traps and in learning about the general characteristics of the tsetse fly and the disease. A mono-screen trap has been developed for community use and is being used to trap flies. This is the first time that this kind of community participation has been attempted in tsetse control and this approach is discussed in relation to other approaches.


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Targets of black cloth with or without flanking netting panels (c. 1 m tall × 1.7 m) baited with acetone (130 mg/h) and 1-octen-3-ol (0.5 mg/h), coated with deltamethrin suspension concentrate and deployed at 4/km², produced a decline of 3% per day in the apparent density of the tsetse fly *G. m. centralis* in 500 km² of the Western Province of Zambia. Flies were eradicated in a year as evidenced by the absence of catches from fly-rounds and traps and the elimination of the transmission of trypanosomiasis. The promise of the target technique is confirmed but the need for its further development is emphasised.

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

[See also 15: nos. 7221, 7225, 7261.]


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The RoTat 1/2 CATT test developed for *T. evansi* was used in comparison with other diagnostic tests for the detection of *T. b. rhodesiense* infection in the northern part of the Luangwa Valley. The human population, the domestic and a large number of game animals were positive with the RoTat 1/2 CATT, the Ag-ELISA, the IFAT and the radioimmunoprecipitation tests. Human sera from these areas precipitated the same $^{35}$S-methionine labelled trypanosome antigen components whereas few differences in band patterns were found between individual game animals. Surprisingly, however, *T. b. rhodesiense* could not be isolated from the Ag-ELISA and radioimmunoprecipitation positive patients from the Musenga and Kasyasya localities. The fact that the CATT positive humans were positive in antigen detection tests does indicate that in all probability they carry or had been carrying a subpatent infection. These results suggest that the reservoir for *T. b. rhodesiense* in that region is considerable, comprising the game animals and probably, to an even greater extent, the human population.


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Kingoyi forms part of the Niari focus in Congo and has been the scene of major epidemics. A previous study indicated that transmission took place within the village itself. Peridomestic populations of *Glossina palpalis palpalis* were recorded in 60 of 63 villages in the region and control was carried out in 55 of these villages from 1984-87. In November 1987 21 cases of sleeping sickness were detected at Kingoyi; all except two were in the first phase of the disease, indicating a recent resurgence. Tsetse density was evaluated at Kingoyi in the rainy season in February 1988. Only one female *G. p. palpalis* was taken using 21 pyramidal traps over 4 days and it was concluded that peridomestic tsetse were absent. The relative absence of livestock and game may have increased human-‘wild’ tsetse contact outside the village. This has important implications.
for control since the agricultural area surrounding the village covers some 80 km². The actual sites of transmission have not been determined but appear to be concentrated in two zones south of the village which will be given priority for control.


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Midgut infections in Glossina morsitans submorsitans and G. palpalis gambiensis in The Gambia were examined with DNA probes specific for Trypanosoma simiae, T. brucei and the three subspecific groups of T. congolense. The failure to diagnose some infections stimulated a second study in which two groups of unrecognised trypanosomes were isolated in vitro. One group had a Nannomonas cycle of development, identical to T. congolense and T. simiae. Like T. simiae this trypanosome was infective to pigs and did not infect rodents or ruminants; however, the infection in pigs was chronic, unlike the acute lethal parasitaemia characteristic of T. simiae. Comparison of isoenzymes and karyotype confirmed that the new trypanosome was biochemically distinct and represents a new species. It can be specifically identified with a DNA probe. The second group of stocks, all from G. p. gambiensis, had a stercorarian cycle of development, involving the midgut and hindgut of the fly. The trypanosome infected Nile crocodiles in the laboratory and was thus T. grayi. Using the extended range of DNA probes, T. congolense accounted for only the minority of Nannomonas infections in G. m. submorsitans; T. simiae, the new Nannomonas and savanna T. congolense occurred only in this fly. Riverine-forest T. congolense was present only in the G. p. gambiensis. This is the first description of an association between the different types of T. congolense and different fly species.


ICIPE, P.O. Box 30772, Nairobi, Kenya; KARI, National Veterinary Research Centre, P.O. Box 14580, Nairobi, Kenya.
Teneral *G. m. morsitans* were fed on waterbuck (*Kobus defassa*) and Boran cattle (*Bos indicus*) infected experimentally with *T. congolense* clone IL2895. Infection rates in tsetse varied from 9 to 31% when fed on cattle, and from 2 to 59% when fed on waterbuck. In waterbuck, infections were often detected through the development of parasites in tsetse at times when parasitaemia could not be detected through microscopic examination of blood. Male and female, and 1- and 2-day-old flies were equally susceptible to infection on both hosts. Infection in tsetse was associated with a 14% absolute reduction in survival during the month following the infective feed.


Nekpeni, Dagnogo: CEMV, B.P. 2597, Bouaké 01, Côte d'Ivoire; Eouzan: IPR (OCCGE), Bouaké, Côte d'Ivoire. Trypanosome infection rates were calculated using 2153 *G. p. palpalis* caught in biconical traps in different biotopes related to human activity in the forest area of Côte d'Ivoire. The results showed that there was no preferential biotope for tsetse infected with trypanosomes. The most widespread species of trypanosome infecting *G. p. palpalis* was *T. congolense* (10.13%) followed by *T. vivax* (8.22%) and, more rarely, *T. brucei* (0.70%). Female tsetse were infected more (21.27%) than males (13.56%). In females the mean physiological age of first infection with *T. vivax*, *T. congolense* and *T. brucei* was 5, 15 and 35 days respectively. These different infection rates also varied according to season.


Maier: Institut für Medizinische Parasitologie, Sigmund-Freud-Strasse 25, 5300 Bonn 1, Germany. *Stomoxys calcitrans* has been found to be able to transmit erythrocytes and pathogens mechanically and by regurgitation *in vitro* (forced feeding capillary technique). The highest frequency and number of regurgitated erythrocytes was obtained when we offered agar-ATP-solution after blood feeding. The events of spontaneous regurgitation are rarely seen or missing.
when blood is fed in all the capillaries. In some preliminary experiments mechanical and regurgitative transmission of *Trypanosoma b. brucei* could be demonstrated *in vivo* too, but no transmission of *Plasmodium* and *Borrelia*. Mobility and degree of infectivity of the pathogens are obviously very important factors among others responsible for successful transmission of this kind.


Zoology Department, Howard University, 415 College Street, Washington, DC 20059, USA; ILCA, P.O. Box 5689, Addis Ababa, Ethiopia; ICIPE, P.O. Box 30772, Nairobi, Kenya.

The probability of a *Glossina pallidipes* picking up an infection from a single blood meal in Lambwe Valley was estimated to be 0.0092 for *Trypanosoma vivax*, 0.0028 for *T. congolense*, 0.00097 for *T. brucei* and 0.00024 for the human infective *T. brucei*. In cattle, the prevalence of blood protozoa was: *Trypanosoma* spp. 5.55%, *Babesia* spp. 2% and *Theileria* spp. 33.06%. Of the cattle with trypanosomes, 38.3% also had *Theileria* spp. and 2.12% also had *Babesia* spp.; 88.28% of the cattle with *Babesia* also had *Theileria* spp.


Tietjen, Tietjen, Mehlitz: Institut für Parasitologie und Tropenveterinärmedizin, Freie Universität Berlin, Königsbergstr 65, W-1000 Berlin 37, Germany; Welburn: TRL, Langford, Bristol BS18 7DU, UK; Kalunda: UTRO, Tororo, Uganda; Kakaire: Animal Health and Research Centre, Entebbe, Uganda.

Due to the high incidence of sleeping sickness in Uganda effective control strategies have to be established. The occurrence of animal reservoir hosts of East African sleeping sickness is well known. However, their epidemiological significance still needs clarification, particularly the frequency of the animal-tsetse-man cycle. A longitudinal study commenced in November 1990 in the south-east of Uganda (Mukono District), where the disease is endemic. The infection rates with *Trypanozoon* in man were 1.1% (n = 189), in pigs 61.7% (n = 120), in cattle 34.4% (n = 96), in dogs 10.0% (n = 20) and in goats 0% (n = 26).
In tsetse (Glossina fuscipes fuscipes) the infection rate with metacyclic Trypanozoon was 1.0% (n = 402). To assess human infectivity 113 parasite stabilates were produced for comparative behavioural, biological, biochemical and molecular biological examinations. The high infection rates with Trypanozoon in animals, especially in pigs and cattle, point to a significant epidemiological relevance of the animal reservoir in this region although the results of the characterisation of the isolates and the assessment of the proportion of the human infective Trypanozoon in animals is still awaited.


Laboratoire de Génétique des Parasites et des Vecteurs, ORSTOM, 2051 avenue du Val de Montferrand, B.P. 5045, 34032 Montpellier Cédex, France.

Six Congolese and three Zairian T. brucei stocks were studied by isozyme cellulose acetate electrophoresis. Twenty isozyme systems were used, of which only five showed variability. These five polymorphic systems made it possible to identify five different zymodemes. Zymodemes isolated from man were recorded also from pig and sheep, which confirms the results of previous authors. This supports the existence of an animal reservoir of human African trypanosomiasis in the Congo, which could play a role in the transmission of the disease, at least by the maintenance of residual foci.

5. HUMAN TRYPANOSOMIASIS
(a) SURVEILLANCE


The numbers of new cases detected in member countries of the OCEAC each year from 1982-88 are tabulated. In Cameroon almost one million people are at risk. The Mbam and Fontem foci together account for almost 95% of the cases. Both the Northern Province, with refugee populations from Chad, and the Eastern Province, where there has been a resurgence of the disease, require monitoring. Trypanosomiasis increased in the Central African Republic during the 1980s, especially in the Nola-Bilolo, Ouham and Upper M'Bomou regions. It is
estimated that some 120,000 people are at risk in these foci and all need regular surveillance. In Chad the foci are restricted to the south. Political and military unrest between 1978 and 1983 disrupted surveillance and resulted in large population movements; since then an increasing number of cases has been reported. In the Congo, historical foci persist and appear to be spreading. The largest focus is the Bouenza region, accounting for 71% of the total number of cases in 1986, followed by the Couloir along the River Congo. Some 340,000-420,000 people are believed to be at risk. The number of cases in Equatorial Guinea has declined due to effective surveillance and control. Relatively few cases occur in Gabon, where the main focus is the Komo estuary. The OCEAC and WHO have collaborated to increase effective control in these countries.


The Testryp CATT was performed on dried blood samples on filter-paper and on diluted blood using a microtechnique. This method was applied to both sample collection techniques and was evaluated in parallel with the classical Testryp CATT on whole blood, as described in the instructions provided with the reagents by the manufacturer. A total of 2087 people were tested; 453 samples were tested in the laboratory and 1634 during a field survey in five villages of a trypanosomiasis focus in Daloa, Côte d'Ivoire. This study demonstrated that the Testryp CATT micromethod on either type of sample collection gives results comparable to the Testryp CATT on whole blood. The collection of dried blood samples on filter-paper can be performed by non-specialist staff in trypanosomiasis control programmes of the national health services. In addition, a flask of CATT reagent will allow testing of six times more people by the micromethod than by the classical whole-blood method. The micromethod is suitable in the implementation of
programmes for the serological surveillance of populations at risk.


ORSTOM, B.P. 181, Brazzaville, Congo; ibid.; Division of Control of Tropical Diseases, WHO, 1211 Geneva 27, Switzerland. (Reprint requests to Cattand.)

Described is an evaluation of the card agglutination test (Tetryp CATT) applied to dried blood collected on filter-paper. The sensitivity of the test was compared for samples of diluted sera, whole blood and dried blood. Sera diluted 1:8 gave similar CATT results to those obtained with dried blood. The false negative rate was 5.8% and test specificity 100%. Use of CATT with samples of dried blood is recommended for screening populations at risk for trypanosomiasis in situations where specialised surveillance teams are not available to test sera or whole blood.


Laboratoire de Biologie des Populations, UFR de Sciences, Université Paris 12, 94010 Créteil Cedex, France; ORSTOM, B.P. 181, Brazzaville, Congo; ORSTOM, B.P. 5045, 34032 Montpellier Cedex 1, France.

Spontaneous auto-agglutination of red blood cells was assessed as an indicator for the diagnosis of human African trypanosomiasis. This test is easily carried out by health workers with minimum qualification. It presents a high sensitivity (0.91) and a high predictive value of a negative result (0.99). Although a positive result gives a low indication of infection, the health care workers should refer the patient to a screening centre.


Centro de Control de la Tripanosomiasis, B.P. 560, Bata, Equatorial Guinea; ibid.; ibid.; Hôpital de Luba, Luba, Equatorial Guinea; ibid.
The Luba focus was divided into three zones according to the prevalence of human trypanosomiasis: Epicentre A (27.5%), Epicentre B (8.3%) and Peripheral Zone C (3.0%). A different control approach was used in each zone. The entire population was serologically examined by IFAT, every 6 months in Epicentres A and B and annually in Zone C. Trypanosomiasis in seropositive cases was confirmed parasitologically, by CATT and by CSF examination. Cases with parasites and abnormal CSF were treated with melarsoprol and those with normal CSF with pentamidine. CATT- and parasite-negative cases were considered to be free of the disease. To assess the impact of vector control on disease transmission, 74 monopyramidal traps (18 traps/km²) were set up in Epicentre A. The most rapid decline in the number of cases was seen in this zone. In Epicentre B and Zone C, where there was no vector control, the reduction was more gradual and new cases were observed. However, by the end of the study the levels of infection were similar in all three zones, although the cost of vector control and 6 monthly surveillance in Epicentre A was twice that of 6 monthly surveillance only in Epicentre B and 5.5 times that of annual surveillance only in Zone C. Given the extent of the problem (63 new cases in 24,732 people examined), the rainforest ecosystem and limited funds and personnel, it is recommended that vector control be reserved for epidemiological emergencies only.

(b) PATHOLOGY AND IMMUNOLOGY
[See also 15: no. 7292.]
East and West African trypanosomiasis in man are reviewed under the headings: parasites and their transmission, pathogenesis and pathology, epidemiology, clinical cause, diagnosis, treatment and prevention.

(c) TREATMENT
Pentamidine concentrations in plasma, whole blood and CSF were determined in 11 patients with *T. b. gambiense* infection without CNS involvement in Côte d'Ivoire. Blood samples were drawn during a 48 h period after the first and last dose of pentamidine dimesylate given as 10 i.m. injections on alternate days. Maximum plasma concentrations were generally attained within 1 h after injection but varied extensively (420-13,420 nmol/litre). The median plasma concentration 48 h after the last dose was approximately 5 times higher than that after the first dose. The ratio between whole blood and plasma concentration was approximately 2. Small amounts of the drug were found in the CSF after the last dose. The findings showed inter-individual differences in the pharmacokinetics of pentamidine. The currently recommended daily dose regimen could be questioned, as drug accumulation was pronounced. All patients were cured and the concentrations attained should be considered as parasiticidal. Further studies on the kinetics and distribution after single and multiple doses of pentamidine as well as studies on the possible relationship between adverse effects and plasma concentrations are, however, needed.


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The history and current use of pentamidine against protozoal diseases is discussed with consideration of the treatment of African trypanosomiasis, leishmaniasis and *Pneumocystis carinii* pneumonia.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also 15: no. 7285.]

Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. A recent cross-sectional survey of bovine trypanosomiasis at the Obudu Cattle Ranch, located at an altitude of 1576 m on the Obudu Plateau in Nigeria, is presented. Blood samples from 68 adult cattle in three herds and 290 cattle (27 calves and 263 adults) in eight herds were screened for trypanosome infections in August 1989 and February 1990, respectively. Although the plateau is designated as tsetse-free, one (1.5%) (0.015, 95% confidence interval 0.029) and four (1.4%) (0.014, 95% confidence interval 0.013) of the ranch's cattle in August and February, respectively, had trypanosome infections. *Trypanosoma brucei* caused one of the infections while the others were caused by *T. vivax*.


Doka, Guedegbe, N'Diaye: Faculté des Sciences Agronomiques, Université Nationale du Bénin, B.P. 526, Cotonou, Benin; Baelmans, Demey, Pandey, Verhulst: Département de Production et Santé Animales, Institut de Médecine Tropicale, Nationalestraat 155, B-2000 Antwerp, Belgium. (Correspondence to Pandey.) Blood of different breeds of cattle, namely Lagune from the Atlantic province, Borgou and Borgou Zebu from the Borgou province, and Somba and Zebu from the Atacora province of Benin, were examined for trypanosome infection. Thick and thin blood smears for trypanosomes, CATT, IFAT and the trypanolytic test for antibodies to trypanosomes were used. Trypanosomes were detected in 19.3% (range 9.8-31.4%) of animals by examination of blood smears; antibodies to trypanosomes were found in 89.8% (range 88.4-100%) of samples by IFAT, 50.6% (range 34-87.5%) by CATT and 3.4% (range 1.1-7.1%) by trypanolytic test. *Trypanosoma vivax* and *T. congolense* were the main species in Benin with a low number of *T. brucei*. Zebu had lower infection rates than trypanotolerant breeds of Benin. The infection rates of various trypanotolerant breeds were not significantly different.

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A total of 33 domestic animals living in close contact with man in a human trypanosomiasis focus in South Congo were examined parasitologically and tested for serological markers of T. b. gambiense infection: 84.8% of the animals presented detectable T. congolense parasitaemia. The high rate of seropositivity observed with CATT (81.8%) contrasted with the low seroprevalence found with ELISA (< 13%). None of the 33 plasma samples showed lytic antibodies when analysed by immune lysis test against ten distinct T. b. gambiense predominant variable antigen types (LiTat 1.1 to 1.10). The results demonstrate the lack of specificity of CATT, and to a lesser extent ELISA, in detecting T. b. gambiense infection in animals. The seropositivity may be due to cross-reaction with certain T. congolense antigens. The absence of serological markers specific to T. b. gambiense confirms the parasitological data which estimate the prevalence rate of animals infected with Trypanozoon as less than 1% in the region.


CTVM, Easter Bush, Roslin, Midlothian EH25 9RG, UK.

A 12-month survey in three local government areas in Nsukka zone, Anambra State, Nigeria, revealed that out of 150 local and exotic breeds of pig examined, 46 (30.7%) were infected with trypanosomes. Both single and mixed infections of Trypanosoma brucei and T. congolense were observed. However, T. brucei was the predominant trypanosome encountered. The husbandry system in practice was the most significant factor influencing the prevalence of trypanosomes in the pigs. In addition significantly higher prevalences were recorded during the rainy seasons. Clinical trypanosomosis was encountered in only eight of the 46 positive cases seen, with anaemia, loss of weight and anoestrus being the most important effects associated with these
infections. The pathogenic and economic significance of these findings are discussed.


Institute of Tropical and Subtropical Agriculture, Agricultural University, 165 21 Prague 6, Suchdol, Czechoslovakia.
The author summarises the worldwide occurrence and socioeconomic aspects of animal trypanosomiasis for 1975-84 on the basis of the annual figures published in the FAO Animal Health Yearbook.


Central Veterinary Laboratory, MAFF, New Haw, Weybridge, Surrey KT15 3NB, UK.
The detection of antibodies against *Trypanosoma equiperdum* in 689 equid sera was compared by ELISA, the complement fixation test (CFT) and an indirect immunofluorescent test (IIF). CFT was the least sensitive technique, susceptible to anti-complementary factors and the most technically demanding. IIF was more sensitive, but was only suitable for testing limited numbers of samples. In this study, ELISA was the most sensitive test, the least labour-intensive and lends itself to a considerable degree of automation. It is suggested that ELISA would be relatively easy to standardise between laboratories and an ELISA protocol could be adopted as the internationally approved test for equine health certification.

(b) PATHOLOGY AND IMMUNOLOGY


Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. (Correspondence to Anika.)
The productivity of 76 newly imported pregnant Friesian cattle and two bulls under heavy trypanosome challenge in the rain forest belt of Nigeria is reported. At the first visit in August 1989 and within 7 months of arrival of the heifers, the herd population had been reduced by 26 (33.3%) as a result of deaths (six animals) and culls/salvages (20). The surviving 52 animals were generally in poor health with classical
symptoms of trypanosomiasis. Thirty-one (40.8%) of the pregnancies were unsuccessful because of abortions (13 animals), premature births (seven), embryonic deaths (five) and death of heifers (six). Of the 45 successful calvings, 16 perinatal deaths occurred. All serum samples were negative for brucellosis. Only 41 (63.1%) of the 65 productive heifers lactated, of which 24 (58.5%) yielded milk only for 6 months and less. The remaining 17 (41.5%) heifers were still at different stages of lactation ranging from 3 to 7 months within the period of analysis. Treatment with isometamidium (Samorin) at 0.5 mg/kg body weight cured the infection and prevented reinfections and/or relapses within 3 months of administration. A rise in the haematocrit and milk production after Samorin treatment was recorded. Careful analysis of the outbreak indicated that the reproductive wastages and poor lactational performance may have been induced by the severe trypanosomiasis diagnosed in the herd: in August 1989 *T. vivax* was diagnosed in 44% of the 52 surviving animals.


Impairment of T-cell function in Boran (*Bos indicus*) cattle during primary infection with *T. congolense* ILNat 3.1 was found to occur in peripheral blood, spleen and, in particular, the lymph nodes. Lymph node cells from infected cattle failed to proliferate in response to mitogenic stimulus and suppressed proliferation of both normal peripheral blood mononuclear cells and lymph node cells in co-culture assays. The addition of indomethacin, to inhibit prostaglandin synthesis, had no effect on the ability of lymph node cells from infected cattle to suppress the proliferative response of responder cells from uninfected cattle. The supplementation of the culture media with catalase, which degrades hydrogen peroxide, either alone or in combination with indomethacin, also did not result in restoration of proliferation. This suggested the presence of suppressor cells in lymph nodes of infected cattle which exert their effects via a prostaglandin-independent mechanism. By depleting lymph node cells from infected cattle of the monocyte-macrophage population using a cell sorter it was possible to abrogate the previously observed immunosuppression,
thus indicating a key role for these macrophages in the induction of trypanosome-associated immunosuppression.


Departments of Veterinary Pathology (Igbokwe) and Veterinary Surgery and Reproduction (Mohammed), University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria.

Four Sokoto Red goats were i.v. infected with T. brucei (NITR strain 8/18) and another four served as controls. Parasitaemia was detected in all infected goats from 4-21 days p.i., after which trypanosomes were only detected intermittently in two animals. The mean PCV and haemoglobin concentration of infected goats was lowered from day 7 p.i. and significantly so ($P < 0.05$) from day 11 p.i. The mean red blood cell count was significantly lower ($P < 0.05$) from 7-63 days p.i. Reticulocytes were not detected until 49-63 days p.i. and there was no significant change in mean corpuscular volume. The reticulocyte response was very low (0-2.4%), even when the PCV fell to 9-14%, and appears similar to that in cases of T. vivax and T. congolense infection in goats. The higher reticulocyte response reported for other host species infected with T. brucei is thought to be host-related.


Kamanga-Sollo, Musoke, Nantulya, Masake: ILRAD, P.O. Box 30709, Nairobi, Kenya; Rurangirwa: Small Ruminant Collaborative Research Support Programme, Nairobi, Kenya. (Correspondence to Musoke.)

Investigations were undertaken to evaluate the immune response of trypanotolerant N'Dama (Bos taurus) and susceptible Boran (Bos indicus) cattle to two Trypanosoma congolense VATs expressed in both breeds following tsetse-transmitted challenge. The VAT-specific antibodies of both IgM and IgG isotypes produced by both breeds had similar neutralising titres. The interaction between immune sera, trypanosomes and freshly isolated peripheral blood leucocytes (PBL) from uninfected N'Dama and Boran animals was studied. It was found that both N'Dama and Boran immune sera were able to induce adherence of trypanosomes to the N'Dama
The adherence-inducing activity was exhibited by both IgM and IgG antibodies, but IgG antibodies were more efficient in this respect. These results suggest that there are qualitative and/or quantitative differences in the immunoglobulin receptor function of PBL between the two breeds of cattle.


Department of Veterinary Medicine, Haryana Agricultural University, Hisar-125 004, India.

Some biochemical constituents were estimated during the course of *T. evansi* infection in pups. A group of five healthy pups 4-5 months of age were inoculated s.c. with $5 \times 10^5$ live *T. evansi*. Blood glucose, total serum proteins and alkaline phosphatase levels were estimated in blood/serum at various intervals. Blood glucose levels showed a progressive decline until death of the animals. A mean maximum decrease of 50.32% was observed after about 1 month p.i. The mean total serum protein values had a fluctuating trend and were significantly increased on 8, 22 and 28 days p.i. The changes in the levels of alkaline phosphatase were not significant.


Department of LPM (Poultry Science), College of Veterinary and Animal Science, Bikaner 334 001, India.

The propagation of *T. evansi* in bursectomised and non-bursectomised chicks revealed that bursectomised chicks developed earlier and more pronounced parasitaemia as compared to non-bursectomised chicks. It was observed that heavily infected chicks had body temperatures in the range of 90-102°F. The chicks which had parasitaemia and body temperatures of 104-105°F recovered from infection. The blood of some of the chicks with normal body temperature was found negative for the presence of *T. evansi* when examined microscopically but was found positive upon mice inoculation test. Such chicks recovered completely. These results suggest that probably higher body temperature is not conducive for the multiplication of *T. evansi* in chicks.

Luckins, A.G., McIntyre, N. and Rae, P.F., 1991. Multiplication of *Trypanosoma evansi* at the site of
Local skin reactions (chancres) developed at the sites of inoculation with *T. evansi* in rabbits and calves. Trypanosomes multiplied in the dermal collagen and, in the rabbit, were present in large numbers by 7 days p.i. Thereafter, however, numbers decreased and few parasites were observed by 11 days p.i. The presence of trypanosomes in the skin caused an extensive inflammatory reaction with disruption of collagen, oedema, necrosis of the skin and increases principally in neutrophils and lymphocytes. In calves, similar changes were observed although there were fewer trypanosomes present in the chancre and the cellular involvement was less extensive than that seen in the rabbit. This early extracellular proliferative phase of development of *T. evansi* may be of importance in naturally transmitted infections both in the initial establishment of the parasite in the mammalian host and in enabling the parasite to increase the numbers of antigenic variants expressed before the parasites invade the general circulation.


Department of Biological Sciences, Nigerian Defence Academy (NDA), P.M.B. 2109, Kaduna, Nigeria; Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; ibid.; ibid.

The course of experimental *T. vivax* infection in eight Uda rams was studied. All the infected animals became parasitaemic 2 days post-inoculation and remained so throughout the study period. A three-phase disease pattern was recognised, i.e. acute, subacute and chronic stages lasting 17-85 days. The disease was characterised by fever and a terminal decrease in rectal temperature despite an increase in parasitaemia with time for rams with acute and subacute infections. Mean weight loss was most marked in subacute followed by chronic cases. Gross pathological changes observed in some infected rams with subacute and chronic trypanosomosis were oedema of the face and lower jaw, hydropericardium and atrophy of the pericardial fat. Petechial haemorrhages were observed on the surfaces of
the heart and kidney of rams with acute infection. Anaemia was most severe in infected rams with acute disease, followed by those with subacute infections. Also, hypoproteinaemia was observed in all infected rams. Severe clinical findings associated with the death of all the infected rams during this study might indicate that the Uda breed of sheep is very susceptible to trypanosomosis. It is, therefore, recommended that this breed of sheep, which is strictly bred and reared in tsetse-free Sahel savanna, should not be introduced into endemic areas devoid of therapeutic cover and strict tsetse fly control.


Moskovskaya Veterinarnaya Akademiya, Moscow, Russia. Electron microscopy of tissue from three horses experimentally infected with Trypanosoma equiperdum, two field cases of dourine, and 50 rats inoculated with blood from the horses revealed destruction of the glycocalyx and organoid membranes, and accumulation of osmophilic granules. There was perivascular oedema in the internal organs (particularly liver) and changes in vascular endothelium.


Mwangi, Luckins: CTVM, Easter Bush, Roslin, Midlothian EH25 9RG, UK; Hopkins: Department of Pathology, Royal (Dick) School of Veterinary Sciences, University of Edinburgh, Summerhall, Edinburgh, UK. (Correspondence to Luckins.)

Marked enlargement of lymph nodes draining local skin reactions (chancres) occurred in sheep following intradermal inoculation of cultured metacyclic forms of T. congolesense. Histologically, these lymph nodes were characterised by follicular hypertrophy and hyperplasia, compression and relative reduction of the paracortical areas and expansion of the medullary regions. Immunohistochemical staining with monoclonal antibodies to ovine lymphocyte subsets and Fc receptor (FcR) bearing macrophages, revealed increased expression of B cells (CD45R+), major histocompatibility complex (MHC) Class II, FcR⁺ macrophages and CD1⁺ cells in the cortical and
paracortical areas. The paracortical areas were found to be sparsely populated by CD5+CD4+CD8+ cells, while the medullary areas contained numerous CD8+ cells and FcR+ macrophages. FcR+ macrophages were also present in cortical trabecular and subcapsular sinuses. As the chancre regressed, lymph node reactivity also subsided and fewer B cell follicles were observed and there was decreased expression of CD45R+ and MHC Class II+ cells.


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Plasma concentrations of the acute phase proteins (APP), C-reactive protein (CRP) and haptoglobin (Hp), increased markedly following experimental infection of dogs with T. brucei. The highest concentrations of CRP were observed immediately after peaks of parasitaemia. Treatment with curative doses of the trypanocidal drug suramin caused a rapid decrease in CRP. Relapse infections after subcurative treatment were followed by a reappearance of high plasma CRP concentrations. Haptoglobin remained elevated during the course of the disease. Curative treatment with suramin caused a gradual but slow decrease in Hp while subcurative treatment caused no significant changes. Thus, the estimation of CRP was useful in determining the presence of active infection and the success of chemotherapy. High Hp levels in severely anaemic dogs indicated that intravascular haemolysis does not contribute significantly to the anaemia associated with T. brucei infections in dogs. These conclusions need confirmation from a larger experiment.


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The effects on the fertility of small East African male goats of intravenous infection with T. (b.) evansi were...
studied. Six infected bucks developed erratic, low but persistent parasitaemia, the PCV dropped gradually but significantly \((P < 0.001)\) and they became emaciated. Half of these bucks developed clinical orchitis. Two bucks died of the disease during the experiment. Semen from all the infected bucks deteriorated in quality and quantity and those with clinical orchitis became totally aspermic. Spermatozoal abnormalities and the number of dead spermatozoa rose significantly. Later in the disease, the testicles of the infected bucks atrophied. Histologically, the testicles from the infected animals became devoid of spermatozoa, the testicular blood vessels contained microthrombi and there was infiltration of inflammatory cells. Subsequently, diffuse calcification set in, with calcium deposits obliterating most of the seminiferous vesicles and ducts and also the epididymal ducts.


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Five waterbuck and four Boran cattle were infected with *T. congolense* IL2895 using *Glossina morsitans morsitans*. At the same time, two waterbuck and two cattle were inoculated intravenously with bloodstream forms. With both methods of challenge, cattle had short prepatent periods followed by a continuous high parasitaemia. All cattle became severely anaemic and had to be treated with trypanocidal drugs to prevent death. In contrast, tsetse and intravenous challenge of waterbuck resulted in a long prepatent period, followed by brief, intermittent levels of low parasitaemia, and eventual selfcure. Waterbuck did not become anaemic, even during short bouts of parasitaemia which in general were very low. Both cattle and waterbuck developed parasite-specific antibodies, but some waterbuck failed to develop neutralising antibodies. These results suggest that the ability of the waterbuck to resist trypanosome infection may not be mediated entirely by antibody-dependent immune processes.

The examination of 122 Alsatians presented for treatment during 1978-85 showed 19 (15.6%) were infected with trypanosomes. This incidence is higher than that in local dogs, where 158 (8.7%) were infected out of 1822 examined over the same period. The mortality rate was 63.2% and 45% for Alsatians and local dogs respectively. *Trypanosoma brucei* was identified in five and *T. congolense* in two of seven Alsatian cases. Symptoms included anorexia, lethargy, pyrexia, ocular disturbances, regional oedema and cerebral derangement typified by initial excitement and opisthotonus followed by coma; *T. brucei* was associated with one case of abortion. Eight (67%) out of 12 cases treated with Berenil suffered from relapse or reinfection and died 1-6 weeks after treatment; some dogs developed CNS derangement due either to trypanosomal encephalitis or Berenil toxicity and died within 7 days. Six (85.7%) out of seven dogs responded to treatment with Samorin but four of these subsequently relapsed; no toxicity was associated with this drug.


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Young boars were placed on diets with either low or high dietary energy and subsequently infected with a virulent stock of *T. brucei*. The effects of dietary energy level and infection on some serum biochemical parameters were evaluated up to 7 weeks p.i. There were no significant changes in serum electrolyte (Na+, K+) concentrations resulting from dietary energy level and/or the infection. Serum total protein and albumin levels significantly decreased in both groups of infected boars, the decline being greater in those on the low-energy diet. Infection was accompanied by a rise in serum transaminase (serum aspartate and alanine aminotransferase) levels which were higher in infected boars on the low-energy diet. The serum testosterone concentration declined in both groups of infected boars with the fall being more pronounced in the group on the low-energy diet. The results indicated that the reproductive efficiency of boars may be modulated by...
nutrition and that adequate feeding may assist in ameliorating the deleterious effects of trypanosomiasis on production in endemic areas.


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The susceptibility of N'Dama cattle (Bos taurus) to four consecutive infections with different tsetse-transmitted clones of T. congolense was compared with that of Borans (Bos indicus). All animals were aged 13 months at the start of the study and had been born and raised free from trypanosomiasis under the same management and nutritional conditions, thereby limiting environmental factors that could have influenced susceptibility. While cattle of both breeds were equally susceptible to the establishment of trypanosome infections, the N'Damas exhibited superior resistance. Despite infection with virulent parasites, the N'Damas gained weight at the same rate as uninfected control animals, they did not develop anaemia to the extent that trypanocidal drug treatment was required, and all made a spontaneous recovery to normal haematological values within 2-4 months. In contrast, all the Borans needed treatment during the course of the four infections because of severe anaemia and showed markedly reduced liveweight gains. These clinical differences in the N'Damas were associated with two repeatable characteristics, namely, the ability to control parasitaemia and to 'resist' anaemia, processes that did not appear to be linked. Also in contrast to the Borans, the N'Damas were able to mount accelerated haemopoietic responses, resulting in the reduced severity of anaemia following a primary infection. These findings pose the question as to whether the ability to control parasitaemia and to 'resist' anaemia could be used as criteria for identifying resistant or trypanotolerant cattle.

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Eight trypanotolerant N'Dama cattle controlled an infection of *T. congolense* ILNat 3.1, transmitted by *Glossina morsitans centralis*, more efficiently than a group of similarly infected trypanosusceptible Boran cattle. All eight N'Damas maintained their PCV above 15% throughout the primary infection whereas the PCV of six of the eight Borans dropped below 15%; these latter animals were treated with diminazene aceturate to prevent possible death. Lymphocyte, neutrophil and platelet counts also decreased in the Boran during the primary infection. In contrast, a lymphocytosis was observed in the N'Dama; and although the neutrophil and platelet counts decreased, the drop was less severe than in the Boran. Two years after the primary infection and immediately prior to a homologous rechallenge infection, all eight N'Damas had neutralising anti-metacyclic trypanosome variant-specific antibodies present in their sera compared to five of the eight Borans. Following the homologous rechallenge infection the eight N'Damas became parasitaemic but there were no alterations in their erythrocyte or leucocyte counts. The Borans became highly parasitaemic and developed severe, chronic anaemia and leucopenia. Thus, the trypanotolerant N'Damas controlled a primary infection of *T. congolense* more efficiently than trypanosusceptible Boran cattle and eliminated a homologous rechallenge infection without the pathology associated with the disease.


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A study was conducted using 32 mature 22 kg West African dwarf goats to measure the effect of *T. vivax* infection on energy and nitrogen metabolism. Sixteen goats were infected i.v. with $14 \times 10^6$ *T. vivax*. Sixteen control goats were sham-injected. Digestibility and metabolisability of energy and N balance were measured for each goat. Heat production and energy balances were measured per treatment group from 1 week before infection to 6 weeks after infection. Goats were fed alfalfa pellets (10% above maintenance). Treated goats had a reduced ($P < 0.05$) PCV (38-40% before infection v. 20-25% 6 weeks after infection) and an increased ($P < 0.05$) rectal temperature. Log parasitaemia/ml was about 6.0 to 6.2. Parasitised goats showed increased urine creatinine excretion at week 2 p.i. After infection, feed intake was reduced (about 15%; $P < 0.05$) and greater variability in intake was noted. Treated and control goats had similar N output and energy output in urine. Metabolisability of energy intake was similar at 42.7 v. 42.1% in treated v. control goats, respectively. Heat production in infected goats was increased by about 15%. Treated goats lost more weight and had a lower N balance than control goats ($P < 0.05$). The calculated maintenance energy requirement for infected goats ($464$ kJ ME/kg$^{75}$) was 25% greater than for control goats ($375$ kJ ME/kg$^{75}$).


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The influence of *Trypanosoma* infection on the production of TNF, IFN- and IL-2 in trypanotolerant cattle and dogs was studied. Peripheral blood lymphocytes (PBL) of *Trypanosoma*-infected and uninfected Zebu and Baoulé cattle were stimulated with the T cell mitogen Concanavalin A (Con A) and their supernatants were tested for IFN- and IL-2 activities. Con A-blasts served as indicator cells for the IL-2 test. PBL of both cattle races were able to produce IL-2 since their supernatants could induce the proliferation of IL-2-dependent Con A-blasts. As checked by their
antiviral activity, the above mentioned supernatants contained IFN because they could protect Madin Darby Bovine Kidney (MDBK) cells from an infection with the vesicular stomatitis virus (VSV). Similarly, PBL from infected and uninfected pariah dogs were stimulated with Con A. Regardless of the infection, Con A-stimulation induced the cells to produce IL-2, indicating that the Trypanosoma infection did not suppress the production of this growth factor. Serum samples of infected and uninfected dogs were tested for TNF and IFN. The TNF test was performed using L929 cells as indicator cells and human recombinant TNF (hrTNF) as reference. HrTNF was able to kill most of the L929 cells. However, sera of the infected dogs had no influence on the growth of L929 cells, indicating that the serum samples did not contain TNF. Serum samples of the same infected dogs protected dog fibroblasts from an infection with VSV suggesting that IFN was produced during the course of the Trypanosoma infection in pariah dogs.


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Thirty-two mature dwarf goats weighing between 16 and 30 kg (22.7 ± 3.7, SD) were used to study the effect of T. vivax infection on rectal temperature (RT), feed intake (DMI), and metabolic rate. Sixteen of the goats were infected i.v. with 14 ± 10^6 T. vivax each; the 16 others served as controls. Animals were fed at about 1.1 times maintenance. Heat production was measured from 1 week pre-infection to 6 weeks p.i. From data on successive 9 min periods, heat production was calculated per 24 h period and separately for 0700 to 2000 (day period) and for 2000 to 0700 (night period). Rectal temperature was measured twice weekly. Compared with controls, animals infected with T. vivax developed and maintained a 1°C higher RT and a higher metabolic rate. After the prepatent period of 5 to 7 days, during which RT remained normal, all infected goats had a period of about 7 days with constant high temperatures. After
that initial episode, RT fluctuated. Heat production of infected animals was increased by 15.6 kcal day$^{-1}$ kg$^{-0.75}$, or about 16%. This increase in heat production was greater during the night (22 kcal day$^{-1}$ kg$^{-0.75}$) than during the day (14 kcal day$^{-1}$ kg$^{-0.75}$). After *T. vivax* infection, large differences in DMI among animals were apparent. In four animals, a clear relation between DMI and RT was noted, but in 12 animals no such relationship was apparent.

(c) TRYPANOTOOLERANCE

[See also 15: nos. 7268, 7277, 7280, 7281.]


ILCA, P.O. Box 46847, Nairobi, Kenya; ibid.; OGAPROV, Moanda, Gabon; ibid.; ILRAD, P.O. Box 30709, Nairobi, Kenya. (Correspondence to d'Ieteren.)

Antigen-detection enzyme immunoassays (ELISA) were used for the diagnosis of *Trypanosoma vivax*, *T. congolense* and *T. brucei* in N'Dama cattle in Gabon, Central Africa. The assays are based on monoclonal antibodies which recognise trypanosome antigens specific for each of the three species and animals were termed 'antigenaemic' when found positive by this technique but not found parasitaemic by the buffy coat technique. One hundred and forty-eight 1-year-old animals were exposed to a medium natural tsetse challenge and an average of six assays per animal were carried out over a 92-day period. Blood samples were routinely examined 11 times over this period and 28% of animals were detected as parasitaemic by the buffy coat technique; 90% of these were antigen-ELISA positive. More importantly, 40% of the animals with negative parasitological findings were also found to be antigenaemic. Parasitaemic animals with above-average PCV values had 32% higher daily weight gains than those with below-average, while antigenaemic animals showed no significant linkage between PCV values and weight gain. Thus only the 28% of animals with detectable parasitaemias could have been used for selection decisions based on PCV values. Antigenaemic animals grew at the same rate as negative animals and had 22% superior growth rates to parasitaemic animals. When antigenaemic animals were classified as having more ability to control parasite growth than parasitaemic animals, a significant sire effect suggested some possibility of a degree of
genetic control being involved. Thus the ELISA could offer a practical possibility for selection of trypanotolerant animals based on infection criteria.

(d) TREATMENT
[See also 15: no. 7275.]

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Four populations of Trypanosoma congolense and T. b. brucei were isolated from cattle under different management practices and environments in Zambia. All four isolates had varied responses to both diminazene aceturate (Berenil) and isometamidium chloride (Samorin) as curative drugs in infected mice. Trypanosomes from a traditionally managed herd in a high-tsetse-challenge area had the strains most resistant to Berenil, with maximum curative dose of 45 mg kg\(^{-1}\) bodyweight. Another isolate from a high-tsetse-challenge area was evidently resistant both to Berenil at 40 mg kg\(^{-1}\) and to Samorin at 4 mg kg\(^{-1}\). The strains most susceptible to both Berenil and Samorin were from a commercially managed herd of cattle under medium tsetse challenge. They responded to recommended cattle standard doses of 3.5 mg kg\(^{-1}\) or 7 mg kg\(^{-1}\) Berenil and to as little as 0.25 mg kg\(^{-1}\) Samorin. It is evident that trypanosome strains resistant to Berenil and/or partially resistant to Samorin exist, and that both T. congolense and T. b. brucei are implicated.

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The ten compounds considered include two trypanocides, diminazene aceturate (Azidin, Ganasag, Berenil) and isometamidium chloride (Samorin, Trypamidium). Summary monographs of each drug are presented, covering chemistry, identity, use, metabolic studies, residue studies and methods of analysis, and an appraisal of the residue data in terms of their significance for the
safety assessment of the drug. Recent publications relevant to the safety assessment of each compound are listed. The recommended therapeutic dose of diminazene is 3.5 mg/kg i.m. or 2.0 mg/kg i.v.; i.m. use produces significant residues in the liver and kidneys which are lower than those reported for isometamidium. The recommended withdrawal period prior to slaughter of 20 days for cattle and sheep is thought to be adequate. Isometamidium is administered at rates of 0.5 or 1.0 mg/kg, usually by i.m. suspension. Significant and persistent residues, which are increased with i.v. administration, occur at the injection site, liver and kidneys. Depending on the toxicological evaluation, it is recommended that these tissues should be discarded prior to human consumption.

Centre de Recherches Zootechniques Wakwa, P.O. Box 65, Ngaoundere, Cameroon.
Cattle in one of two adjacent pastures in Cameroon which were equally infested with *Glossina morsitans submorsitans* and *G. longipalpis* were dipped once a week in 25 p.p.m. Butox (deltamethrin) after both groups of cattle had been cleared of trypanosomiasis (mainly *Trypanosoma congolense* and *T. vivax*) with Berenil (3.5 mg/kg). Only 3.9% of the Butox-treated herd were infected at the end of the 7-month test period, as opposed to 37% of the undipped herd. However, levels of trypanosome infection in field populations of *G. m. submorsitans* and *G. longipalpis* were higher in individuals from the treated pasture. Numbers of *Amblyomma variegatum* and *Musca domestica* were also reduced by the dipping.

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Isometamidium chloride (Samorin) is a widely used and highly effective trypanocide for the treatment of bovine trypanosomiases. However, the appearance of isometamidium-resistant populations of *T. congolense* in Africa makes it necessary to develop methods for the rapid and reliable detection of drug resistance in the laboratory. Currently available tests are time-consuming and/or expensive. In the present study, the short-term *in vitro* incubation of trypanosomes in a range of isometamidium concentrations and the infectivity of
the parasites in mice has been assessed. A series of *T. congolense* isolates were used which were known to differ in their *in vivo* sensitivity to the drug. The results showed a close correlation between the known level of resistance and the capability of trypanosomes to remain infective after incubation in isometamidium. Thus isolates displaying a high level of resistance *in vivo* remained infective following incubation in higher concentrations of drug. This assay may provide a simple and reliable method for detecting drug resistance in *T. congolense*.

7. EXPERIMENTAL TRYPANOSOMIASIS
(a) DIAGNOSTICS
[See also 15: no. 7264.]
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(b) PATHOLOGY AND IMMUNOLOGY
[See also 15: nos. 7312, 7348.]
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Although high density lipoprotein (HDL) particles purified from human serum by ultracentrifugation are known to lyse *T. b. brucei*, it is unclear whether individual differences in the trypanocidal activity of human serum reflect changes in the concentration of HDL.
In the present study, trypanolytic activity, whether assessed *in vitro* or *in vivo*, was greater with plasma from normal healthy individuals than with plasma from patients with various hepatic diseases and associated low levels of HDL. For all subjects taken as a single group there were highly significant positive correlations between the plasma concentration of apolipoprotein (apo) A-I, the major protein constituent of HDL and trypanolysis *in vivo* ($r = 0.93, n = 10, P < 0.001$) or *in vitro* ($r = 0.77, n = 36, P < 0.001$).

Removal of plasma apoB-containing (i.e. non-HDL) lipoproteins by precipitation revealed that the trypanocidal activity was also significantly correlated with HDL-cholesterol and HDL-apoA-II, as well as with HDL-apoA-I, but not with HDL-apoE. Depletion of all or part of plasma apoA-I by non-ultracentrifugal methods abolished or decreased the trypanolytic effect of the plasma. The findings from these experiments, which were designed to avoid alteration in the composition of HDL by ultracentrifugal forces, provide additional support for the proposal that the trypanocidal action of human plasma resides with native HDL particles.


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Shapiro, S.Z., 1989. The potential for \textit{Trypanosoma} vaccine development. \textit{In}: Wright, I.G. (ed.), \textit{Veterinary protozoan and hemoparasite vaccines} (Boca Raton, Florida, USA; CRC Press Inc.), pp. 131-163. Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA.

This chapter, divided into five sections, focuses on vaccine development against members of the salivarian trypanosomes. The first section introduces the genus \textit{Trypanosoma} with special reference to the African trypanosomes. Section 2 considers attempts at immunisation against African trypanosomiasis, discussing early experiments, antigenic variation and vaccination with nonvariable antigens. Section 3 discusses the parasite and disease, including trypanotolerance. The fourth section considers possible approaches to immunological control of trypanosomiasis, considering empirical approaches and then theoretical targets. Section 5 is the author's conclusions.


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Some recent successful examples of antiparasitic drug development, based on biochemical knowledge, are discussed under the headings: combination of salicylhydroxamic acid and glycerol (against *Trypanosoma brucei* and *T. b. rhodesiense*); DL-alpha-difluoromethylornithine (against *T. b. gambiense*); and allopurinol riboside (against *Leishmania*).


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