Wildlife Disease Research and Economic Development

Proceedings of a workshop held in Kabete, Kenya, 8 and 9 September 1980

Editors: Lars Karstad, Barry Nestel, and Michael Graham

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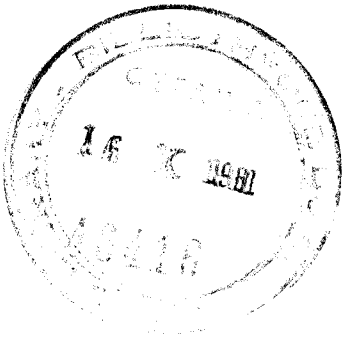

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Potential Application of Research on African Trypanosomiases in Wildlife and Preliminary Studies on Animals Exposed to Tsetse Infected with *Trypanosoma congolense*

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Wildlife has an essential role to play in the future socioeconomic plans for developing countries. However, consideration must be given to the fact that some species of wildlife can act as reservoir hosts of pathogens of man and his domestic animals. Such dangers must be recognized and clearly defined.

Of all diseases, trypanosomiasis provides one of the most complex and emotive interactions between wildlife, man, and domestic livestock because some wild animals are known to act as reservoir hosts for the human pathogens *Trypanosoma rhodesiense* (Heisch et al. 1958) and *T. gambiense* (P. De Raadt personal communication), as well as for the important pathogens of domestic livestock, namely, *T. congolense*, *T. vivax*, and *T. brucei* (Ashcroft 1959). As a result, several trypanosomiasis control campaigns have involved large-scale destruction of wildlife. However, such indiscriminate slaughter has on each occasion failed to control the disease and in some cases has exacerbated the problem because tsetse have readily adapted to other hosts (Buyst 1977). It is obvious that such strategies have to be reconsidered and that much more precise data are required on the role of different species of tsetse, different species of trypanosomes, and different species of wildlife in the transmission of infection between wild animals, man, and domestic livestock in order that effective control measures can be enacted.

In the past, it was generally assumed that wildlife was refractory to most infections, but as surveillance of disease intensified, more evidence became available to show that this was not the case. For example, certain species are known to be highly susceptible to trypanosomiasis (Carmichael 1934; Ashcroft et al. 1959; Desowitz 1960; Godfrey and Killick-Kendrick 1967). Over the last century, wild animal populations have been dwindling rapidly in the face of human population pressure and, whereas, in the past large-scale losses through disease probably went unnoticed, at the present time, certain species are in danger of extinction. As a result, there is now a definite need to be able to diagnose, treat, or control diseases such as trypanosomiasis in individual animals. At the same time, the establishment of game ranching projects enhances the risk of disease, a situation that will increase requirements for accurate diagnosis and understanding of disease processes. Thus, to quote Baker (1968) "more information on the pathogenicity of the salivarian trypanosomes to game animals, and on the pathology of the infections produced, is badly needed."

Currently, it is felt that the exploitation of trypanotolerant livestock, i.e. animals with reduced susceptibility to trypanosomiasis, offers one of the most promising approaches to the control of animal African trypanosomiasis (FAO 1979; Tsetse and Trypanosomiasis Control 1980). It is now established that certain breeds of cattle, sheep, and goats exhibit this trait to differing extents (reviewed by Murray et al. 1979). Wild animals have a reputation for being even more resistant to trypanosome infection and, in some cases, it is believed that they might be completely refractory to infection. Such conclusions are usually based on both the fact that wildlife survives in areas heavily infested with tsetse and on surveys involving the collection of single blood samples examined for the presence of trypanosomes using a range of light microscope parasitological techniques. This approach yields little useful information on the question of susceptibility of different species to trypanosomiasis.

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Ideally, what are required are sequential studies carried out on animals, not previously exposed to trypanosomiasis, following experimental infection with properly characterized trypanosomes where clinical, parasitological, immunological, and pathological parameters are assessed. In one of the few studies of this type, Ashcroft et al. (1959) evaluated the susceptibility of various species of wild animals, in most cases not previously exposed, to fly challenge and, in a few cases, to needle challenge with *T. rhodesiense* and *T. brucei*. They found a wide range of susceptibility between species and also some variation within species. Some species, including Thomson's gazelle, dik-dik, blue forest duiker, jackal, bat-eared fox, antbear, hyrax, serval cat, and monkey, became infected and usually died. Another category included less susceptible animals or even species refractory to infection. These could be divided into species that became infected and had parasitemias of considerable duration such as the common duiker, eland, Bohor reedbuck, spotted hyena, oribi, bushbuck, and impala, species usually infectible but with scanty parasitemias such as warthog, bushpig, and porcupine, and the baboon, which was refractory.

A few attempts have been made to infect a small number of animals with *T. congolense* and *T. vivax*. With *T. congolense* some species such as oribi, bushpig, and porcupine were not infectible while others including the red-fronted gazelle, Bohor reedbuck, greater kudu, lesser kudu, waterbuck, Thomson's gazelle, steinbok, and elephant became infected and then recovered; a few animals such as jackals and certain species of monkeys were found to be highly susceptible and even died (Carmichael 1934; Ashcroft et al. 1959; Desowitz 1960; Roberts and Gray 1972). The number of species studied with *T. vivax* is even less. A single adult reedbuck did not become infected when challenged with *Glossina palpalis* infected with *T. vivax* (Desowitz 1960), while both duiker and red-fronted gazelle became infected but made a spontaneous recovery (Desowitz 1960; Roberts and Gray 1972).

Obviously much more data are required on the question of susceptibility of different species of wildlife to all the major African trypanosomes. Nevertheless, certain species show a remarkable degree of resistance to trypanosomiasis and offer an excellent model to study the mechanism(s) of host resistance with a view to possibly potentiating such traits in domestic livestock.

The availability of several species of wild animals reared in a tsetse-free environment by the Wildlife Diseases Section of the Veterinary Research Laboratories, Kabete, Kenya, has provided us with the unique opportunity to study many of the important questions raised in the preceding discussion. Accordingly, (1) Studies have been undertaken to evaluate the degree of susceptibility of different species of wild animals to *T. congolense*, *T. vivax*, *T. brucei*, and *T. rhodesiense*. This should provide knowledge on the impact of trypanosomiasis on wild animals per se as well as an evaluation of the possible role of different species as reservoir hosts for domestic livestock and for man. This study will include an investigation of infection rates and transmission characteristics of tsetse fed on trypanosome-infected wildlife. (2) Studies will be made of the mechanism(s) of susceptibility, including an evaluation of potential innate resistance factors as well as possible mechanisms of acquired resistance.

Reaction of Eland and Waterbuck to Bite of Tsetse Infected with *T. congolense*

In susceptible domestic animals and in man, a skin reaction develops at the site of challenge several days after the bite of a tsetse infected with trypanosomes (reviewed by Emery et al., in press). By analogy with the lesion considered pathognomonic of primary syphilis in man, which it resembles, this reaction has been termed the "chancre." From our studies in goats and cattle, it would appear that the skin acts as the site at which the parasites become established and proliferate prior to dissemination to the bloodstream. The intensity of the lesion is related to the number of trypanosomes inoculated into the skin and in our experience once a chancre has developed infection always follows. Thus, these early events are important in the establishment of infection and possibly affect the susceptibility of the host to the parasite. Consequently, a detailed examination of the lesion and associated changes has been undertaken in several wildlife species; as far as we are aware there is no published information on whether or not the chancre reaction develops in any species of wildlife apart from chimpanzees (Godfrey and Killick-Kendrick 1967).

In the present pilot study an eland and a waterbuck were bitten by *G. morsitans morsitans* infected with *T. congolense*. The eland and waterbuck were females aged 6 months and 14 months, respectively; both had been reared at Kabete and had no previous experience of trypanosomiasis.

Five infected flies were allowed to feed on the shaved flanks of each animal and subsequently these ten infected flies were fed on the flanks of two susceptible East African goats, five on each animal. Five uninfected flies were fed on the contralateral flank of each animal. The bite of the uninfected tsetse produced no detectable reaction in any animal,

whereas, with infected flies skin reactions developed at 9 of the 10 bite sites on goats, but only two of the five bite sites on the eland and three of the five bite sites on the waterbuck.

The kinetics of development of these early changes as they occurred in the eland and waterbuck (Fig. 1) were quite different from the pattern observed in the goat and in the bovine, the two domestic animal species we have studied most extensively (Emery et al., in press).

In the goat, the local skin reaction that developed from the bite of a tsetse infected with *T. congolense* was first detectable as a palpable discrete nodule 6–7 days after challenge. The lesion progressively enlarged and attained a more diffuse raised plaque with substantial subcutaneous oedema, heat, and pain, being most marked by about day 10. Thereafter, the reaction subsided rapidly and 5 days later

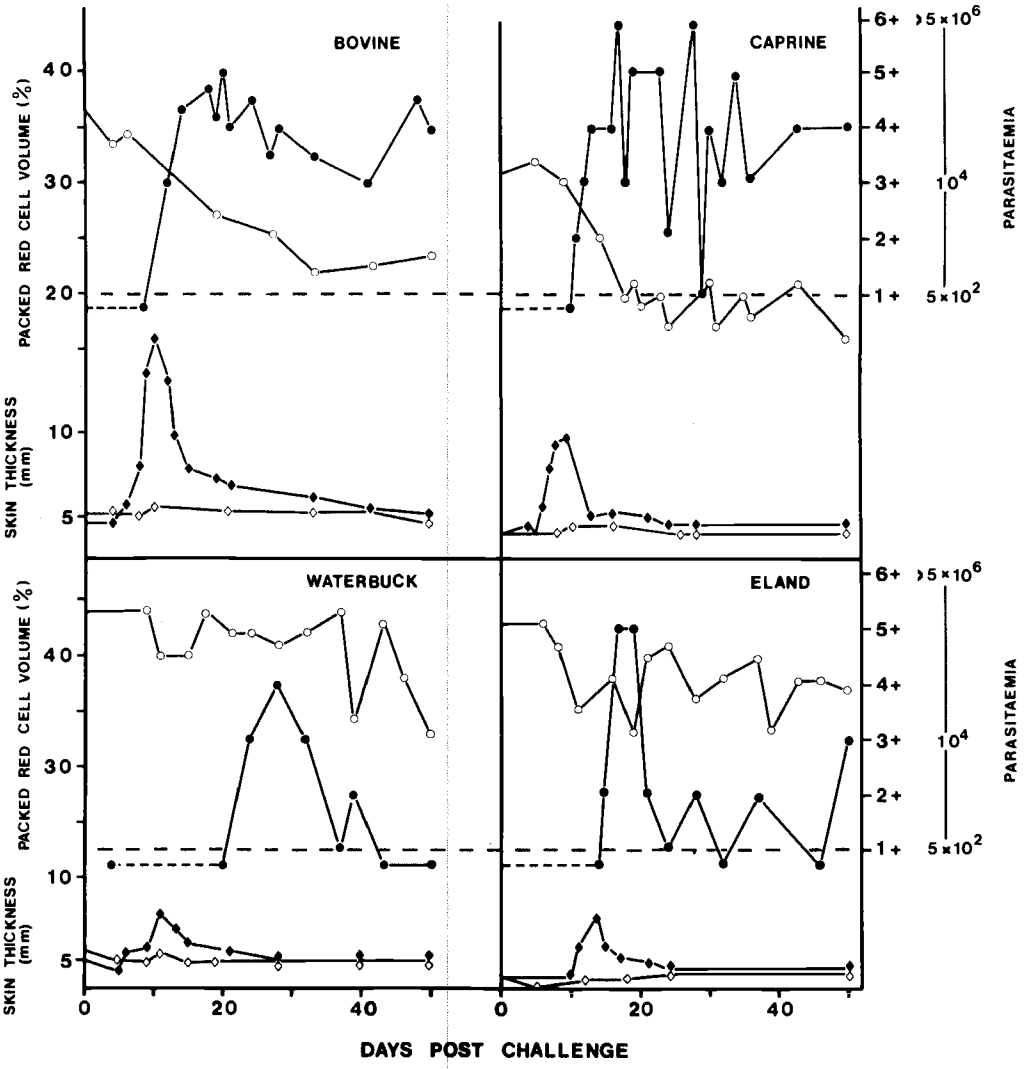

Fig. 1. Kinetics of development of the chancre in a bovine, a goat, a waterbuck, and an eland following challenge with G. m. morsitans infected with T. congolense: ◆ skin thickness at site of bite by infected tsetse; ◇ skin thickness at site of bite by uninfected tsetse on contralateral flank; ◯ packed red cell volume (%); ● parasitemia as detected by the method devised by Murray et al. (1977) using the scoring system described by Paris et al. (1980). The broken line indicates the limit of sensitivity for detection of trypanosomes.

the skin was almost back to normal. The skin had increased in thickness from 4.0 mm to reach 9.8 mm at the peak of response on day 10 (Fig. 1). The skin reaction that occurred in susceptible Boran, Friesian, and Charolais following the bite of a *T. congolense*-infected tsetse was similar to that described for goats. In some cases, the lesion reached as much as 100 mm in diameter at its maximum, by which time, skin thickness had more than doubled (Fig. 1).

In the eland, the skin reaction was mild and was detectable only by an increase in skin thickness without being indurated or circumscribed. The increase in skin thickness became apparent only on day 11 after the tsetse bite, reaching peak thickness on day 14 (from 4 mm to 7.8 mm) and then returning to normal by day 24 (Fig. 1).

In the waterbuck, a skin reaction was first detected on day 7 after tsetse challenge. The reaction was clearly observable on day 9 and by day 11 it attained its peak size of about 30 mm in diameter, with skin thickness increasing from 5 mm to 8.6 mm (Fig. 1). Thereafter, the lesion subsided and had disappeared by day 20.

The initial detection of the skin reaction always preceded the appearance of parasitemia. In the goat, the prepatent period ranged from 9 to 11 days, in cattle from 13 to 15 days, in the eland 13 days, whereas in the waterbuck parasites were not detected in the blood by microscopical examination until day 24.

In all four species, the appearance and development of the chancre was accompanied by significant enlargement of the draining prefemoral lymph node; the response, however, was more pronounced in goats and cattle.

In summary, the development of skin reactions following the bite of tsetse infected with *T. congolense* has been recognized for the first time in wildlife. A significant difference in the early events was found between domestic livestock and wild animals. There was some evidence that the transmission rate of the parasite was lower, i.e. the inoculation of trypanosomes from the tsetse into the skin of the host was not as successful in wild animals — only about half of the successful tsetse feeds in the eland and waterbuck resulted in the production of a skin reaction, a response considered indicative of the establishment of infection (Emery et al., in press). In domestic animals most tsetse feeds resulted in the production of chancres.

Furthermore, the size and severity of the skin reaction was significantly less in the wild animals. It is possible that this was related to the number of trypanosomes that became established in the skin as it has been shown, using intradermal needle inoculation of different numbers of trypanosomes, that the size and intensity of the reaction is dose dependent (Emery et al., in press). Alternatively, it might be that the rate of replication of the parasite was slower in the skin of these wild animals. In the waterbuck, despite the fact that trypanosomes were detected in the skin as early as day 11[3] they were not seen in the blood until day 24, further confirming the importance of the skin in the restriction of the trypanosome prior to bloodstream dissemination and indicating a possible role for skin reactivity in host susceptibility to trypanosomiasis.

The possible ways in which the skin might achieve this are not known but differences in skin structure do exist between different breeds of cattle and these may influence susceptibility. Carr et al. (1974) found an association between skin thickness and the prevalence of trypanosomes; within herds of East African Zebu, *T. congolense* infections were more common in thinner skinned animals. It is also known that skin vasculature can vary between certain indigenous African breeds of cattle and imported breeds (Amakiri 1976), the former usually being less susceptible to trypanosomiasis. We feel that the role of the skin in the transmission and establishment of infection is significant and that further studies on wildlife might elucidate the factors that influence these processes.

Another significant feature to emerge from this study was that the level and duration of parasitemias were less in the wild animals (Fig. 1). The level of the first peak of parasitemia in the eland was 10^5 trypanosomes per millilitre and in the waterbuck 5×10^4 trypanosomes per millilitre; whereas, in goats and cattle the level was as high as 10^6 trypanosomes per millilitre; the levels of the subsequent peaks of parasitemia were also less in the wild animals. The duration of sustained parasitemia in the eland and waterbuck was shorter as judged by the fact that they became negative for trypanosomes earlier than domestic animals and were negative on many more occasions over the period of 90 days up to the time this article went to press.

Associated with the appearance of parasitemia in goats and cattle, a significant drop in packed red cell volume (PCV) developed and this was progressive (Fig. 1). The majority of these animals had to be treated to prevent death. On the other hand, in both the eland and waterbuck, while a small drop in PCV occurred in association with parasitemia, no significant anemia developed and the PCV levels were soon back within normal range. An alternative explanation for the transient drop in PCV was that it was not related to parasitemia but was the result of repeated tranquilization and venipuncture.

[3]This was demonstrated by subinoculation into mice of a suspension prepared from a biopsy of a day 11 reaction.

There is evidence from work in cattle and in mice that the capacity to control parasitemia might be related to a superior immune response to the trypanosome. Studies in N'Dama (a trypanotolerant breed of cattle), have indicated that these cattle have the ability to acquire and mount a greater immune response than Zebu (Desowitz 1959). At the same time, work on mice indicates that reduced susceptibility and the greater ability to control trypanosome parasitemia is associated with superior immune responsiveness and the ability to produce IgM antibodies (Murray et al., in press). Furthermore, studies at ILRAD on sera from N'Dama and Zebu suggest that the recognition of a small number of common trypanosome antigens by the animal's humoral immune system is correlated with N'Dama's greater capacity to control and abort trypanosome infections (Shapiro and Murray, in preparation).

In the current project, we intend to evaluate the immune response to the trypanosome of wildlife hosts of different species and also to investigate if wild animals that recover from trypanosome infections recognize the same common antigens as trypanotolerant cattle. Such antigens might be of major importance in developing new immunodiagnostic tests or even vaccines.

It should also be considered that many species of wild animals could be poor hosts for trypanosomes for non-immunological reasons such as the absence of essential nutrients or the presence of deleterious factors in their bloodstreams. Some of these factors have been recognized already. It has been shown that cotton rats are completely refractory to *T. vivax* because of a trypanocidal factor identified as a serum macroglobulin (Hudson and Terry 1970). The only way in which *T. brucei* can be distinguished from *T. rhodesiense* is by its inability to infect man; the lytic host factor responsible has been identified as a high density lipoprotein (Rifkin 1978). Other factors that could be important in host susceptibility to trypanosomiasis might include complement reactivity and the activity of the mononuclear phagocytic system. The fact that certain species of wildlife are resistant or even refractory to trypanosome infection offers an opportunity to investigate the existence of such factors. Their identification and isolation might be important for future use in the management of the disease in man or in domestic livestock.

It is likely that there are several other factors that influence host survival in endemic tsetse areas and thus contribute to the overall picture of trypanotolerance. It is well established that certain species of tsetse exhibit definite host feeding preferences (Weitz 1963), although these traits are by no means stable and can be influenced by environmental factors (Moloo 1973; Moloo et al. 1980). The significance of such tsetse preferences was shown recently by Roberts et al. (1980) when they compared the attractiveness of cattle and oryx to tsetse under critical experimental conditions. They found that five times as many flies were attracted to cattle and they were able to count full engorgement on cattle of 279 tsetse during the period of observation; only 4 tsetse were seen to obtain partial blood meals from the oryx during the same time.

Other factors important for survival are likely to include the capacity to forage and utilize food and the ability to regulate body temperature and conserve water, traits well developed in wild animals (EAVRO 1967). Recognition and understanding of all these traits must yield essential information that will aid future control of trypanosomiasis.

In conclusion, it appears to us that nature has presented to man a vast animal kingdom whose excitement and beauty can be viewed by all. She has achieved this by a rigorous process of selection for important characteristics that allow survival under the most severe ecological pressures. The recognition and delineation of the mechanisms underlying these characteristics will not only help us in our battle to conserve the wild animal kingdom but also aid us in developing new tools to treat, control, and even prevent some of the most important diseases that afflict man and his domestic animals.

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Amakiri, S. F. 1976. Arteriovenous anastomoses in the skin of tropical cattle. Acta Anat. 96, 285–300.

Ashcroft, M. T. 1959. The importance of African wild animals as reservoirs of trypanosomiasis. E. Afr. Med. J. 36, 289–297.

Ashcroft, M. T., Burtt, E., and Fairbairn, H. 1959. The experimental infection of some African wild animals with *Trypanosoma rhodesiense*, *T. brucei* and *T. congolense*. Ann. Trop. Med. Parasit. 53, 147–161.

Baker, J. R. 1968. Trypanosomes of wild mammals in the neighbourhood of the Serengeti National Park. Symp. Zool. Soc. Lond. 24, 147–158.

Buyst, H. 1977. The epidemiology of sleeping sickness in the historical Luangwa Valley. Ann. Soc. Belge. Med. Trop. 57, 349–359.

Carmichael, J. 1934. Trypanosomes pathogenic to domestic stock and their effect in certain species of wild fauna in Uganda. Ann. Trop. Med. Parasit. 28, 41–45.

Carr, W. R., MacLeod, J., Woolf, B., and Spooner, R. L. 1974. A survey of the relationship of genetic markers, tick-infestation level and parasitic diseases in Zebu cattle in Zambia. Trop. Anim. Hlth. Prod. 6, 203–214.

Desowitz, R. S. 1959. Studies on immunity and host–parasite relationship. I. The immunological response of re-

sistant and susceptible breeds of cattle to trypanosomal challenge. Ann. Trop. Med. Parasit. 53, 293–313.

1960. Studies on immunity and host–parasite relationship. II. The immune response of antelope to trypanosomal challenge. Ann. Trop. Med. Parasit. 54, 281–292.

EAVRO. 1967. Annual report. Physiology Section. Animal Production Division, East African Veterinary Research Organization, 56–65.

Emery, D. L., Akol, G. W. O., Murray, Max, Morrison, W. I., and Moloo, S. K. The chancre—early events in the pathogenesis of African trypanosomiasis in domestic livestock. In Third International Symposium on Biochemistry of Parasites and Host-Parasite Relationships. Topic: The Host-Invader Interplay. Beerse, Belgium. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands (*in press*).

Food and Agriculture Organization of the United Nations. 1979. Report of the Second Consultation on the Programme for the Control of African Animal Trypanosomiasis. 5–7 December 1978. Lusaka, Zambia. AGA-803 (1978).

Godfrey, D. G. and Killick-Kendrick, R. 1967. Cyclically transmitted infections of *Trypanosoma brucei*, *T. rhodesiense* and *T. gambiense* in chimpanzees. Trans. R. Soc. Trop. Med. Hyg. 61, 781–791.

Heisch, R. B., Macmahon, J. P., and Manson-Bahr, P. E. C. 1958. The isolation of *Trypanosoma rhodesiense* from a bushbuck. Brit. Med. J. II, 1203–1204.

Hudson, K. M. and Terry, R. J. 1970. Natural immunity of the cotton rat to *T. vivax*. Trans. R. Soc. Trop. Med. Hyg. 64, 170–171.

Moloo, S. K. 1973. Relationship between hosts and trypanosome infection rates of *Glossina swynnertoni* Aust. in the Serengeti National Park, Tanzania. Ann. Trop. Med. Parasit. 67, 205–211.

Moloo, S. K., Kutuza, S. B., and Boreham, P. F. L. 1980. Studies on *Glossina pallidipes*, *G. fuscipes fuscipes* and *G. brevipalpis* in terms of the epidemiology and epizootiology of trypanosomiases in south-eastern Uganda. Ann. Trop. Med. Parasit. 74, 219–237.

Murray, Max, Murray, P. K., and McIntyre, W. I. M. 1977. An improved parasitological technique for the diagnosis of African trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg. 71, 325–326.

Murray, Max, Morrison, W. I., Murray, P. K., Clifford, D. J., and Trail, J. C. M. 1979. Trypanotolerance — a review. Wld. Anim. Rev. 31, 2–12.

Murray, Max, Morrison, W. I., Clifford, D. J., Murray, P. K., and McIntyre, W. I. M. Susceptibility to African trypanosomiasis. Studies in cattle and in mice. ISCTRC. 16th Meeting, Yaounde, Cameroon, 1979. OAU/STRC (*in press*).

Paris, J., Murray, Max, and Agure, R. 1980. An evaluation of the sensitivity of current trypanosome parasitological diagnostic techniques. In Report of the Expert Consultation on Research on Trypanosomiases. 1–5 October, 1979. FAO, Rome. (AGA-801, 1979). Appendix IV, 40–50.

Rifkin, M. R. 1978. Identification of the trypanocidal factor in normal human serum: high density lipoprotein. Proc. Natl. Acad. Sci. USA, 75, 3450–3454.

Roberts, C. J. and Gray, A. R. 1972. Trypanosome infections in captive antelope. Trans. R. Soc. Trop. Med. Hyg. 66, 335.

Roberts, L. W., Bhogal, M. S., and Karstad, L. 1980. 1. Trypanosomiasis. A. Resistance of oryx to tsetse and trypanosomes. Wildlife Disease Research. Semi-Annual Progress Report. January–June 1980. Wildlife Diseases Section, Veterinary Research Laboratories, Kenya, 2–3.

Tsetse and Trypanosomiasis Control: A Strategy for the Future in Africa. OAU/STRC/IBAR. 1980.

Weitz, B. 1963. The feeding of *Glossina*. Bull. Wld. Hlth. Org. 28, 711–729.