Chapter 8

Control Options for Human Sleeping Sickness in Relation to the Animal Reservoir of Disease[1]

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Sleeping Sickness Epidemics

The Lake Shores region of East Africa has suffered from horrendous epidemics of human sleeping sickness throughout the past century. Human sleeping sickness remains endemic in several foci in eastern and northwestern Uganda, Tanzania, and elsewhere (Fig. 1). The disease exists in two forms and is caused by infection with either *Trypanosoma brucei rhodesiense* (acute sleeping sickness) or *T.b. gambiense* (chronic sleeping sickness). *T.b. rhodesiense* and *T.b. gambiense* coexist with a morphologically identical animal parasite *T.b. brucei* in geographically distinct foci across East Africa, *T.b. rhodesiense* to the east of the Rift Valley and *T.b. gambiense* to the west (Welburn, Fèvre *et al*. 2001). Although they are morphologically indistinguishable, transmitted by the same tsetse vector (genus *Glossina*), and share a wide range of vertebrate host species, the subspecies differ in one important aspect: their ability to infect people. *T.b. brucei* is sensitive to human serum and so confined to nonhuman hosts, while *T.b. rhodesiense* is resistant to human serum and infections in people cause sleeping sickness which, if untreated, leads to death. While viability of parasites in human serum forms the basis of differentiating the two subspecies, *T.b. brucei* and *T.b. rhodesiense* are essentially similar in all other respects (Ashcroft *et al*. 1959). Neither *T.b. rhodesiense* nor *T.b. brucei* causes clinical disease in cattle or other nonhuman hosts (Wilde and French 1945).

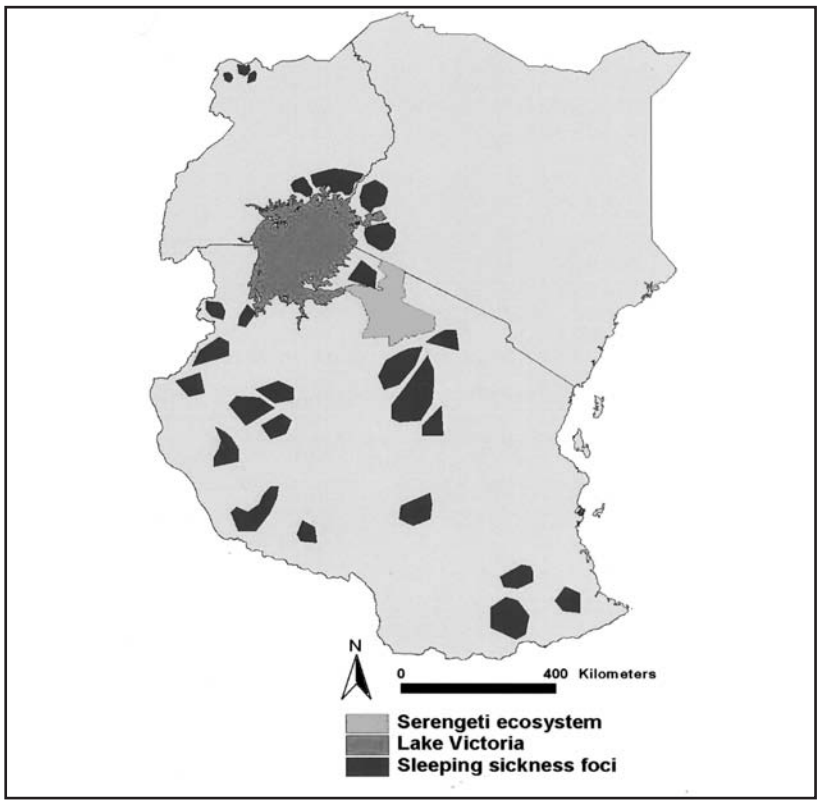

Fig. 1. Estimated locations of sleeping sickness foci in Kenya, Uganda, and Tanzania. Foci data reproduced with permission of the World Health Organization.

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A series of devastating sleeping sickness epidemics occurred in East Africa at the turn of the last century. The earliest reports of the disease in East Africa were when it was observed in Busoga, Uganda, in 1898. By 1908, a third of the population of the shore of Lake Victoria was dead, with the remainder evacuated in 1909. The vector of sleeping sickness in Busoga at this time was believed to be *Glossina palpalis,* and the disease believed to be caused by *T.b. gambiense*. However, close examination of the sleeping sickness reports (Köerner *et al*. 1995) and a retrospective study of patient records from southeast Uganda at this time (Fèvre *et al*. 2004) suggest that acute *T.b. rhodesiense* sleeping sickness was also present in the region.

In 1922, sleeping sickness was identified in Maswa District, Tanzania, and by 1946, 23,955 cases had been identified. Although cattle herds were inspected, especially animals that appeared sick, blood examination found not a single case and so efforts focused on the role of wildlife in maintaining disease (Davey 1924). In Ikoma, on the outskirts of what is now the Serengeti National Park, 2,119 cases were reported between 1925 and 1946; it was suggested that *T.b. rhodesiense* in people probably represented spillover infections from animal reservoirs (Fairbairn 1948).

In the 1940s, a second epidemic of sleeping sickness began in southeast Uganda, with 2,432 cases and 274 deaths confirmed in 1942. However, a new tsetse vector, *G. pallidipes,* was believed to be responsible for transmitting the zoonotic infection from game animals (MacKichan 1944).

Transmission from Game Animals

In 1947, Vanderplank (1947) fed tsetse flies on captive "wild" animals, including bush pigs and warthogs and showed that the level of transmission of *T.b. rhodesiense* by infected tsetse was very low and could be identified only through inoculation into rats. This was addressed by Jackson (1955), who rationalised that the life span of the fly was insufficient to maintain infections in areas of land cleared of human population. Therefore, because the disease did not disappear in the absence of man, it must be maintained by the abundance of game acting as a reservoir. Jackson suggested that "once disease has been introduced into an area, the game become infected with *T.b. rhodesiense* and they then act as reservoirs for maintaining the disease endemically."

Proof that game did harbor human-infective parasites and that those parasites were infectious to man was confirmed by the inoculation of human volunteers, which suggested that game was the primary reservoir of *T.b. rhodesiense* (Heisch *et al*. 1958). Studies on the feeding preferences of tsetse (Weitz 1963) supported this premise (Figs. 2a and 2b). The potential importance of domestic animals as a reservoir for *T.b. rhodesiense* was confirmed in the 1960s (Onyango *et al*. 1966), again using human volunteers.

A third epidemic in southeast Uganda began in the 1970s, peaked in 1980 with 9,000 cases, and fell to 7,000 cases in 1987. The vector was *Glossina fuscipes fuscipes,* and by this time cattle were found to be the major source of human-infective parasites. Very little wildlife remains in agricultural areas of Uganda, evidenced by the lack of tsetse blood meals taken on wildlife hosts (Fig. 2c). Today, the major focus of *T.b. rhodesiense* sleeping sickness in Uganda is in south-eastern Uganda, where *G.f. fuscipes* is the vector and cattle are the main animal reservoir (Welburn, Fèvre *et al*. 2001). Human sleeping sickness is endemic in 12 districts, and a recent extension in the geographic range has been linked to the movement of infected cattle (Fèvre *et al*. 2001). *T.b. gambiense* sleeping sickness remains active in the West Nile region in the northwest.

In Tanzania, sleeping sickness remains among the most serious threats to human health in those areas where it is transmitted. There are eight endemic foci of sleeping sickness in Tanzania and, although many have remained stable for many years, persistently active foci are found in Kigoma,

Fig. 2. Blood meal analyses of tsetse in Uganda. Figs 2a and 2b refer to the 1950s (Weitz 1963), during which two vector species were present, namely *G. pallidipes* and *G.f. fuscipes*.

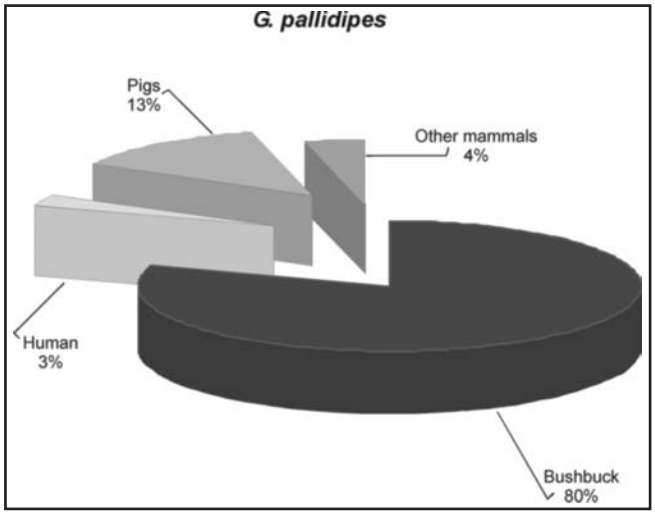


Fig. 2a.

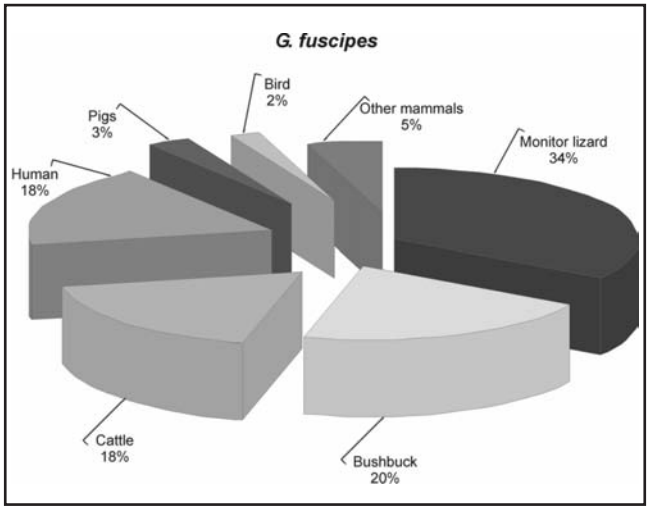
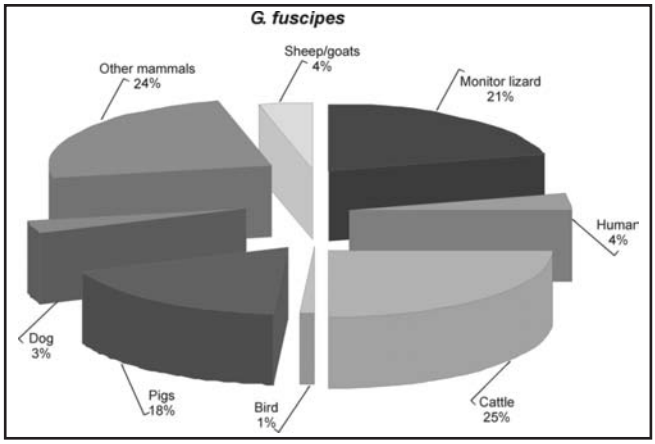


Fig. 2b.

Fig. 2c. Refers to the present (Waiswa *et al*. 2003) and shows that bushbuck have been lost as a source of bloodmeals and that domestic livestock have become a major source of feeds for tsetse. These animals are also reservoir hosts of *T.b. rhodesiense*.



Arusha, Tabora, Kasulu, and Kidindo (Mwambembe 1998). At least 500 new cases of sleeping sickness are recorded annually in Tanzania.

Characterisation of *T. brucei* resistant to human serum

T. brucei s.l. (s.l. = *sensu lato*; i.e., includes all subspecies of *T. brucei*) can be identified in the blood of wild animals and domestic livestock. However, the fact that *T.b. brucei* and *T.b. rhodesiense* are morphologically identical creates difficulties in assessing the risk posed to man from wildlife and domestic livestock when using traditional microscopy-based screening methods. *T.b. brucei* has been reported in cattle (Onyango *et al*. 1966), bushbuck, duiker (Heisch *et al*. 1958), hartebeest (Ashcroft *et al*. 1959), zebra (McCulloch 1967), wildebeest, topi, waterbuck, impala, warthogs (Baker *et al*. 1967), and lions (Sachs *et al*. 1967, Baker 1968). However, it was not known how many of these *T. brucei* s.l. were human infective. Early work in this area relied on the use of human volunteers to determine whether parasites observed in animals were infective to man. Heisch *et al*. (1958) took blood from a bushbuck and infected a rat, and subsequent inoculation to a person resulted in infection with trypanosomes in that person; similarly, Onyango *et al*. (1966) showed that some *T. brucei* s.l. isolated from infected cattle could infect people.

A novel test, the blood infectivity incubation test (BIIT), was developed in the 1970s. It tested the parasites' ability to survive challenge with human serum in a mouse model (Rickman and Robson 1970), which eliminated the need to experiment on human subjects. Geigy *et al*. (1973) validated this method on wildlife material and tested blood from Coke's hartebeest, lion, spotted hyaena, and waterbuck. They showed that blood from a *T. brucei* s.l.-infected Coke's hartebeest was infectious to human volunteers *and* was positive as human-infective using the BIIT. BIIT has since been used to show that reedbuck, waterbuck, spotted hyaena, lion (Gibson and Wellde 1985), and domestic pigs all harbour human-infective parasites (Waiswa *et al*. 2003). This technique is still in use today and can give an indication of the prevalence of human-infective parasites.

The advent of biochemical methods of parasite characterisation offered new ways to examine strains of *T. brucei* s.l. resistant to human serum. Hyaena and oribi were added to the growing list of wild animals believed, on isoenzyme analysis, to act as reservoirs of human disease along with dogs, goats (Gibson and Wellde 1985), and pigs (Enyaru *et al*. 1993) from the domestic pool. These biochemical techniques were followed by molecular methods of strain typing, and a battery of techniques is now available for examining the population structure and strain composition of *T.b. brucei* and *T.b. rhodesiense,* including analysis of restriction fragment length polymorphisms (RFLP) (Hide *et al*. 1994), analysis of variability in mobile genetic elements by PCR (MGE-PCR) (Tilley *et al*. 2003), and minisatellite marker analysis (MacLeod *et al*. 2000). These studies have all confirmed that the domestic reservoir of *T.b. rhodesiense* in southeast Uganda lies principally with cattle and that, in other foci, where there are still significant proportions of wildlife hosts, interactions between the wildlife and domestic animals in the tsetse habitat determine the degree of importance of different hosts.

Up to now, none of the methods described above has enabled us to accurately determine the prevalence of *T.b. rhodesiense* in domestic livestock, wild animals, or tsetse, because all of the methods require significant quantities of parasite material. This means that the parasite material from the host animal must first be amplified in mice prior to application of the technique. However, not all *T. brucei* s.l. observed in the field amplify in mice; up to 50% of *T. brucei* s.l. from cattle in southeast Uganda are lost during mouse passage (Welburn unpublished data). Therefore, measurements of the prevalence of *T.b. brucei* and *T.b. rhodesiense* in livestock using BIIT, RFLP, MGE-PCR, and minisatellite analysis are underestimates.

Recently, a major breakthrough led to a solution to this problem: the discovery that a single gene can be used as a marker to differentiate *T.b. brucei* from *T.b. rhodesiense*. The *SRA* (serum-resistance-associated) gene (Xong *et al*. 1998) has been used to confirm the human-infective status of parasites in cattle in southeast Uganda (Welburn, Picozzi *et al*. 2001). In that study, 46% of the local Zebu cattle were infected with *T. brucei* s.l., and up to 18% of the cattle in Soroti district were infected with *T.b. rhodesiense*. It is likely that, in the absence of tsetse control measures, the majority of the local cattle in this region are infected with *T. brucei* s.l. and that the true infection rate in these animals can be determined only by longitudinal screening; PCR-based screening indicates that infection rates are far higher than previously thought. Because the *SRA* gene is present in all *T.b. rhodesiense* isolates, and is essentially conserved across East Africa (Gibson *et al*. 2002), we are now in a position to

Fig. 3. Measuring the risk of different hosts acting as reservoirs for human sleeping sickness parasites. $p1$ = Probability of tsetse feeding on cattle, warthogs, or people; *Inf* = *T.b. rhodesiense* infection rate in cattle, warthogs, or people; $p2$ = probability of a tsetse picking up a *T.b. rhodesiense* infection from cattle, warthogs, or people. Values in the graphics are shown as proportions rather than percentages. See text for references.

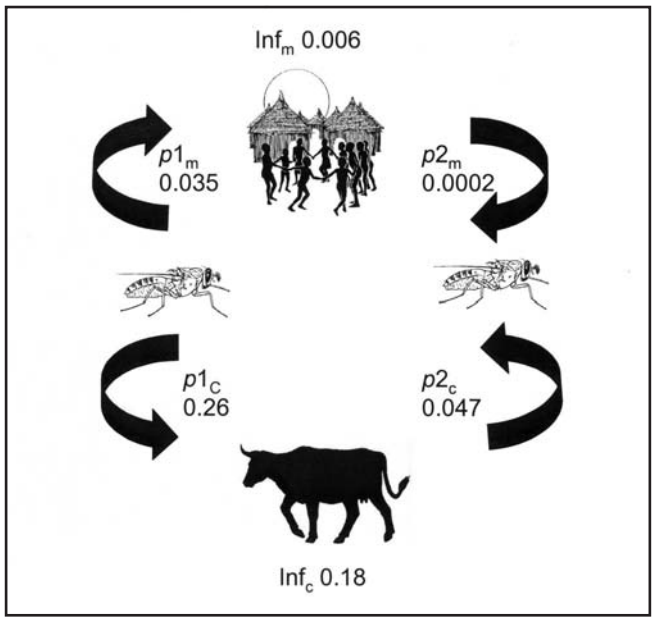


Fig. 3a. *SRA* screening of domestic cattle in southern Uganda has shown that 18% of cattle are carrying human-infective *T.b. rhodesiense* (Inf_c), while the infection rate in people is 0.6% (Inf_m). Tsetse bloodmeal analysis shows that 26% of tsetse bloodmeals are taken on cattle ($p1_c$), while only 3.5% are taken on human hosts ($p1_m$). Thus, the probability of a tsetse picking up *T.b. rhodesiense* from cattle is 4.7% ($p2_c$) and from people 0.02% ($p2_m$). Cattle are 223 times more likely to be a source of infection for tsetse than people are.

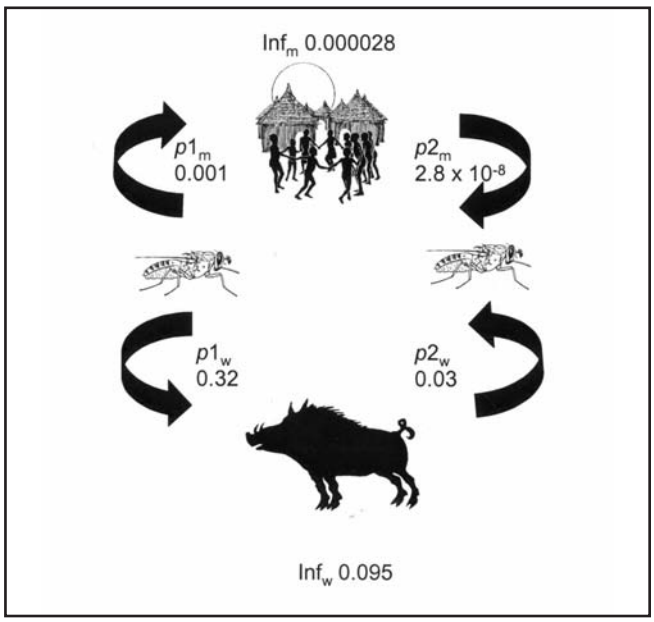


Fig. 3b. *SRA* screening of wildlife samples in the Serengeti shows that 9.5% of warthogs are carrying human-infective *T.b. rhodesiense* (Inf_w), while the infection rate in people is 0.0028% (Inf_m). Tsetse bloodmeal analyses show that 32% of tsetse bloodmeals in the Serengeti are taken from warthogs ($p1_w$), and 0.1% are taken from people ($p1_m$). Thus, tsetse have a 3% probability of becoming infected from warthogs ($p2_w$) and a 0.0000028% probability of becoming infected from people ($p2_m$). Warthogs are 1.07 million times more likely to be the source of a human-infective parasite to the fly than a person is.

accurately assess the risk posed by wildlife and domestic animals to man in this region. Blood-spot samples can be collected in the field onto filter cards that fix the DNA in situ, and these cards can be processed for *T. brucei* s.l. and *SRA* screening without needing amplification in laboratory animals (Welburn, Picozzi *et al*. 2001; Picozzi *et al*. 2002). For the first time, we are able to accurately assess the relative risks posed to man from wildlife and domestic livestock in East Africa and to design control strategies accordingly.

Case study – southeast Uganda

With the advent of molecular tools for epidemiology, modern control strategies can be designed to determine the source of infection as wildlife, domestic livestock, or both. Thus, the limited resources for disease control can be effectively apportioned.

Southeast Uganda has experienced epidemics of sleeping sickness on three occasions during the last century: the great epidemic of 1901–1920 and further epidemics in the 1950s and 1970s. For the first epidemic, when knowledge of the epidemiology of the disease was poor, the colonial solution was to remove the entire human population from the affected area. For subsequent epidemics, the first line of response has been to implement tsetse control – but is this really necessary and does it offer a sustainable solution?

Despite huge resource allocation to control tsetse flies, they still persist across southeast Uganda and sleeping sickness remains endemic. There are, however, some striking differences in the tsetse ecology of southeast Uganda today compared with that during the 1950s epidemic. In 1950, there were two main vector species: *G. pallidipes* and *G.f. fuscipes*; today, there are almost no *G. pallidipes* present and the predominant vector species is *G.f. fuscipes*. Moreover, the host-feeding preference of these flies was very different in 1950 than it is today (Figs. 2a–c). Bloodmeals from *G.f. fuscipes* now show that the flies feed on reptiles and cattle with almost no feeding on wild game in this once game-rich region. Infection rates in *G.f. fuscipes* remain low, 1:300 *T. brucei* s.l. and less than 1:1,000 *T.b. rhodesiense* (Hide *et al*. 1996), while *T. brucei* s.l. infection rates in cattle (Welburn, Picozzi *et al*. 2001) and pigs (Waiswa *et al*. 2003) are very high (*T. brucei* s.l. infection rates in 200 cattle screened for *T. brucei* was 44%). For the implications of these results on disease transmission, see Fig. 3a.

Such detailed information can be used to design control activities. In a region where such a high proportion of cattle

are infected with *T. brucei* s.l. and the vector shows low infection rates, it would be appropriate to target limited resources at removing the domestic reservoir of disease (i.e., treating cattle) and to use insecticides (livestock "pour-ons" or restricted environmental applications) to control transmission. The data show that reliance on treating human cases, while essential, will not greatly affect the transmission of the sleeping sickness, whereas interventions aimed at controlling the parasite in cattle will have profound public health implications in terms of preventing outbreaks of sleeping sickness (Welburn, Fèvre *et al*. 2001).

Case study – Musoma, Serengeti, Tanzania

Musoma district, Tanzania, had been free of human sleeping sickness since 1954, when the last three cases were reported. Disappearance of sleeping sickness was associated with the closing of the gold mines in the district, with the resultant evacuation of the mining settlements reducing man-fly contact. The surrounding areas to the east and south of Ikoma (designated the Serengeti National Park and Maswa, Ikorogono, and Grumeti Game Reserves) were sparsely populated but contained large numbers of game animals (Fig. 1). The decade that followed saw the development of the region as a tourist attraction and an increase in the human population. In the mid to late 1960s, the region experienced a resurgence of the disease: 1965 (1), 1966 (4), 1967 (6), 1968 (14), 1969 (six cases, of which two were tourists [Onyango and Woo 1971]). It was estimated that 40,000 tourists visited the region in 1971 (Onyango and Woo 1971).

In 1971, a tsetse survey was conducted in Musoma District: 6,348 *G. swynnertoni* and 623 *G. pallidipes* were caught, but no mature *T. brucei* s.l. salivary gland infections were detected in any of these flies. Of 862 bloodmeals analysed, only two were from primates, with warthog and buffalo being the most favoured hosts for *G. swynnertoni.* Furthermore, those animals with *T. brucei* infections were not, with the single exception of the warthog, hosts favoured by the tsetse fly. Warthog, with only a 7.7% (1/13) *T. brucei* s.l. infection rate, provided 25.6% of the bloodmeals of *G. swynnertoni*. It was concluded that the warthog was five times more likely to be the source of *T. brucei* s.l. infections in *G. swynnertoni* than all the other *T. brucei* s.l.-infected host animals together, simply because of the feeding preference of the fly (Rogers and Boreham 1973). Rogers and Boreham (1973) also did not find a mature *T. brucei* s.l. infection in 3,500 *G. swynnertoni*. In 1971, 3,000 people in Ikoma-Serengeti area were screened for *T.b. rhodesiense* and no evidence was found of infection, despite the fact that, four months prior to this study, four employees of the National Park had been diagnosed with sleeping sickness (Onyango and Woo 1971).

At the same time, 115 mammals from 13 species were screened, and 12 (10%) *T. brucei* s.l. infections were found: five from lions, one from warthog, three from hartebeest, two from hyaena, and one from a waterbuck. Parasites resistant to human serum were identified in five of the 12 *T. brucei* s.l. infections (a hyaena, two lions, the waterbuck, and the hartebeest). From the absence of tsetse infected with *T. brucei*, it was concluded that the "fly" and "game" areas did not generally overlap (Geigy *et al*. 1971). A follow-up survey involving 798 head of cattle in Ikoma area showed that 28 (3.5%) were infected with *T. brucei* s.l. determined by microscopy and mouse inoculation of 260 samples; ten were tested by BIIT, of which four gave positive results. This suggested that 1.4% of cattle were harbouring *T.b. rhodesiense* (Mwambu and Mayende 1971). A survey of 95 wild game animals from four species (lion, hartebeest, waterbuck, and spotted hyaena) inoculated into rats found forty *T. brucei* s.l. infections (42%), from all except the waterbuck. Spotted hyaena and hartebeest showed the highest ratio of *T.b. rhodesiense* to *T.b. brucei* (4/13 and 1/4 respectively), while only one of 24 lion-derived *T. brucei* s.l. were human infective (Geigy *et al*. 1971). The combined results of three surveys in 1966–1967, 1970, and 1971 suggest that approximately 50% of lions, 40% of hyaena, and 17% of hartebeest carry *T. brucei* s.l. infections. In 1972, a follow-up tsetse survey in Serengeti found nine strains of *T. brucei* s.l. (all BIIT negative) isolated from 11,060 *G. swynnertoni*, an infection rate of 0.08%, or less than one mature *T. brucei* infection per 1,000 tsetse flies (Moloo and Kutuza 1974). Fig. 3b shows the implications of the wildlife infection rates on transmission of *T.b. rhodesiense* in this setting where wildlife is plentiful.

Recently, the Serengeti has again been affected by sleeping sickness; nine cases in tourists associated with Tanzanian National Parks were reported through TropNetEurope (a sentinel surveillance network of clinical sites throughout Europe) (Sinha *et al*. 1999, Moore *et al*. 2002, Ripamonti *et al*. 2002, Jelinek *et al*. 2002). In 1998, the annual incidence of trypanosomiasis in tourists was 13/450,000. The response of the National Park was to implement a tsetse-suppression programme. Although information about this project is scarce, a dramatic drop in tsetse fly populations has been reported. A recent survey of 518 cattle from 11 villages bordering the Serengeti National Park using DNA probes found 23 *T. brucei* s.l. infections, giving a *T. brucei* s.l. point prevalence of 4.4%. Of these, 6/518 (1.16%) were *SRA* positive, i.e., human-infective *T.b. rhodesiense* (Picozzi, unpublished data). These came from 4 villages. Of 232 wildlife samples that were also screened, 8 (3.4%) were positive for *T. brucei* s.l. Nine lions were sampled, one was confirmed positive for *T. brucei* s.l.; 6/21 (29%) warthogs, 1/46 (2.2%) topi, and 1/68 (1.5%) wildebeest were also positive for *T. brucei* s.l. (Kaare 2003). The *SRA* gene was found in 2/21 (9.5%) warthogs (Picozzi, unpublished data).

The livestock population in Tanzania stands at 15.64 million head of cattle, 10.68 million goats, and 3.49 million sheep (Government of Tanzania 1998); 98% of the cattle population are from the traditional sector, while a small percentage are improved breeds (the main use of which is for crossing with indigenous stock to improve productivity). The pastoralist system is the major means of livelihood in semi-arid areas using extensive rangeland resources. Stock keep-

ing is based on highly mobile grazing and watering patterns, and there is potential for extensive interaction between tsetse, domestic animals, and wildlife. Extensive grazing systems and commercial farms may encroach on national parks, forest reserves, and other previously marginal land.

Summary

It is clear that sleeping sickness parasites are successful in both domestic livestock reservoirs and wildlife reservoirs, particularly warthog. We suggest that effective management of sleeping sickness in nonwildlife areas such as southeast Uganda depends on targeted treatments of the domestic animal reservoir either through use of chemotherapeutic drugs and/or "pour-on" insecticides. Such activities would also impact on trypanosomes that are pathogenic for cattle but not human infective, which cause substantial losses to the agricultural sector (Welburn, Fèvre *et al*. 2001).

In and around the Serengeti National Park and other such extensive areas with abundant wildlife, the transmission cycle appears to involve domestic livestock in villages on the park boundary interacting with wildlife and tsetse. Wildlife and domestic transmission cycles are no longer separate in such a situation, in an era of increasing contact between the two landscape systems. In this situation, control may depend on limiting the degree of interaction between livestock and wildlife, the use of chemotherapeutic drugs in cattle, and controlling tsetse through "pour-on" insecticides on cattle. In wildlife areas, there may be a case for the use of stationary tsetse targets and traps. There is also a need for a policy of non-encroachment of pastoralists into the national parks.

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