3. Literature review

3.1 Significance of trypanosomosis

Trypanosomosis has for long enjoyed the dubious accolade of most important livestock disease in sub-Saharan Africa. Around nine million square kilometres are infested by tsetse flies (Budd, 1999), twice the area of the current European Union. An estimated 45 to 60 million cattle are at risk from trypanosomosis (Chadenga, 1994; Gilbert *et al.,* 2001) three to seven million die each year (Hadjuk *et al.,* 1994; FAO, 2000) and the productivity of the survivors in terms of draft power, milk production, growth and birth rate is lowered by 10–40% (Swallow, 2000). The direct and indirect costs have been estimated at 4.5 billion USD (ILRAD, 1994; Budd, 1999), which would make annual losses from trypanosomosis equal to one third the livestock GDP in sub-Saharan Africa. Moreover, trypanosomosis is a zoonosis; over 60 million people living in some 250 foci are at risk of contracting the disease (WHO, 1998); there are about 300 000 cases each year (WHO, 1998), most of which go untreated, and an estimated of 1.5 million Disability Adjusted Life Years (DALY) are lost annually from sleeping sickness (WHO, 2004b).

Unsurprisingly, trypanosomosis dominated the African medico-veterinary sphere for much of the last century, even being claimed as the single substantive cause of African under-development (Hoppe, 2004). Between 1905 and 1960 trypanosomosis commanded the attention of five imperial powers, dedicated government services and national, regional and international research institutions; 25% of colonial research spending went to trypanosomosis (Rogers and Randolph, 2002). Currently, there is probably more information on the molecular biology of trypanosomes than any other non-mammalian cell (WHO, 2001b), knowledge culminating in the recent decoding of the trypanosome genome (Berriman *et al.,* 2005). Yet, little of this knowledge is being applied directly to the management and control of the disease as it affects poor farmers in Africa.

As theoretical knowledge of the molecular biology of trypanosomes advanced, the last decades of the last century saw practical control of trypanosomosis on the African continent unravel (WHO, 2001b). With the onset of the Great African Depression in the 1980s (Leonard and Straus, 2003), tsetse programs, like other government services, became increasingly under-funded and dysfunctional and donors less willing to pay for large-scale, long-term control. At the same time the ideological basis for “rolling out the carpet”, military-style tsetse control operations was eroded by new ways of doing development (Chambers, 1983); the emergence of participation as the dominant development paradigm was hardly compatible with the traditional top-down campaigns for tsetse control, while the new pro-poor approaces widened the focus from cattle health to poultry and small ruminants (Perry *et al.,* 2002). The reliance of tsetse control on ground and aerial spraying of insecticides concerned the increasingly vocal and influential environmental lobby; minority voices even saw tsetse as the saviour of African wildlife and ecology, preserving the few remaining wilderness areas from unplanned development and man-made environmental destruction (Ormerod, 1979; Nelson, 2002). Evidence, too, was accumulating, that while tsetse

control was almost invariably followed by re-invasion, tsetse were being silently yet surely eliminated by changes in land use and climate, at no cost to the public purse (Bourn *et al.,* 2001). With the failure of socialism as a dominant political credo, and faced with massive debt and declining terms of trade, African governments under the pressure of Structural Adjustment Programmes shifted reluctantly from a welfarist to a market view; animal health service provision was re-conceptualised as a largely private good (Ahuja, 2004), and trypanosomosis control left to the farmers. In many respects it always had been; at their most extensive tsetse-control campaigns only covered a small proportion of the tsetse-infested areas.

The result is a decline in status of trypanosomosis from overarching problem to one of many factors in the complex of endogenous (i.e. both contributing to and caused by) constraints to African development, with its relative importance diminished by new preoccupations with AIDS, civil war and governance (Cross, 2001). Advocates for massive stand-alone trypanosomosis eradication campaigns remain, the recent call for elimination of tsetse from Africa using sterile flies being a remarkable case in point (PATTEC, 2001). But a consensus seems is emerging whereby tsetse eradication, even if feasible, is seen as not the best value for scarce development money (Bourn, 2002), and trypanosomosis control, if socially, economically and environmentally justified, a more useful, realistic and sustainable strategy for the next 100 years (Alsopp, 1994).

3.2 Epidemiology of trypanosomosis

African animal trypanosomosis (AAT) is a hyper-endemic, vector-transmitted disease of domestic livestock, showing a seasonal pattern where vector populations undergo seasonal fluctuations (Rogers, 1991). The *ab origo* sylvatic cycle of disease transmission is being replaced by a low-challenge, peri-domestic pattern dominated by livestock and man, and so fostering development of immune responses in hosts and selection of less virulent strains of trypanosomes (Bourn *et al.,* 2001). Where population pressure has forced settlement in fly belts, the disease seems to decrease in severity and importance with time (Bourn *et al.,* 1984), and while epidemics occur, they are generally the result of temporary biocenoses. For example, in Brukina Faso when farmers brought Zebu cattle south after the Sahelian droughts of the 70s and 80s they suffered devastating losses (Clausen *et al.,* 1992), but later studies in the same area showed a much lower prevalence and farmers living with disease (Woitag, 2003).

The epidemiology of AAT in tsetse-infested areas of Africa is determined by four biological factors operating within the physical environment, namely: trypanosomes, tsetse flies, reservoir hosts and cattle. Diverse farming systems; different cattle breeds varying in susceptibility; numerous other hosts, (wild animals and livestock) differing in their reservoir potential for trypanosomes and susceptibility to trypanosome stocks; and diverse tsetse species with varying ecological niches and host preferences and with differing vector competence for different strains of the three trypanosome species parasitic to cattle make this epidemiology a complex one.

3.2.1 Pathogen

AAT is caused by trypanosomes, unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae, and genus Trypanosoma (Levine *et al.,* 1980). The *Trypanosoma brucei* complex comprises three morphologically identical subspecies: *T. brucei brucei*, *T. b. rhodesiense*, and *T. b. gambiense*. Only the ancestral *T. b. brucei* is pathogenic to cattle, the other species causing acute sleeping sickness in east Africa and chronic sleeping sickness in west Africa respectively. *T. congolense* is divided into subtypes with different distributions and pathogenicity: savannah type, forest type, Tsavo type, and Kilifi type (Majiwa *et al.,* 1993). *T. congolense* is considered the most important cause of AAT in east Africa, and *T. vivax* in west Africa (Stephen, 1986). But some *T. vivax* stocks from east Africa can cause hyper-acute haemorrhagic disease with high mortality (Wellde *et al.,* 1983.) *T. b. brucei* is considered less pathogenic to cattle; however cases with central nervous system involvement and high mortality have been reported (Wellde *et al.,* 1989).

The 'vivax ratio' is the ratio of *T. vivax* to *T. congolense*; it is influenced by the species of tsetse, use of drugs, reservoir hosts and cattle immune responses (Ford, 1971). The vivax ratio is usually high where the overall prevalence is low; after successful vector control campaigns (perhaps reflecting greater importance of mechanical transmission); where diminazene is widely used; and where *G. palpalis*, (a poor vector of *T. congolense*), predominates (Harley and Wilson, 1968). In terms of pathology, a distinction can be made between the haematic (*vivax*, *congolense*) and humoral (*brucei*) trypanosome species; the former associated with anaemia and the latter also with tissue degeneration and inflammation (Losos and Ikede, 1972). Compared to *T. congolense*, the parasitaemia is higher but anaemia less profound in *T. vivax* infections. However, it is rarely possible to clinically distinguish disease caused by different trypanosome species and mixed infections are common.

Disease in cattle varies from hyperacute to chronic; the latter is more common in endemic areas. Signs are not pathognomonic, but a combination of the following are typically present: fever, anaemia, lymphadenopathy, dull and dirty coat, piloerection, change of hair colour, hair loss, weight loss, lacrimation, chancre, fatigue, anorexia, pica, abortion, salivation, nasal discharge, arched back, tucked-up abdomen, laboured respiration and jugular pulse (Stephen, 1986; Maré, 1988).

An important biologic feature of pathogenic trypanosomes is the Variable Surface Glycoprotein (VSG), a protein which forms a dense coat on the trypanosome surface. With time, the host mounts an effective immune response against trypanosomes with a specific VSG coat, removing these but not other trypanosomes that have switched to a new (temporarily unrecognisable) VSG coat. These variants form the next wave of infection. Antigenic variation of the surface coat is unique to trypanosomes and the basis of epidemiological features of intermittent parasitaemia; fluctuating fever; disease chronicity; long, largely asymptomatic, incubation period; and failure to develop effective post-infection immunity.

3.2.2 Vector

Tsetse flies (genus *Glossina*) are the primary vector of trypanosomosis and the only vector capable of transmitting trypanosomes cyclically. Thirty-one species and subspecies of tsetse have been identified. Species can be divided into three subgenera, based primarily on morphological features of the adult genitalia (Newstead *et al.,* 1924); a classification recently confirmed by comparative gene sequence analysis and by geometric wing morphometry (Patterson and Schofield, 2004).

The morsitans group is found mainly in savannah ecosystems and includes several important vectors of AAT including *Glossina morsitans spp.*, *G. pallidipes* and *G. austeni*. The palpalis group is found mainly in the riverine galleries of west and central Africa but can extend into savannah regions between river systems; less mobile than morsitans they rely on sight rather than smell to locate their hosts. Important AAT vectors in this group include *G. palpalis* and *G. tachinoides*. The fusca group are found mainly in forests and are therefore less important vectors; however the atypical *G. longipennis* and *G. brevipalpis* are found in drier areas of east Africa and are significant disease vectors.

Tsetse are unusual insects; females give birth to live offspring, both sexes feed obligatorily on blood, and mortality is low. Their longevity, mobility, and frequent feeding make tsetse highly efficient vectors, but the low rate of population growth means even small increases in mortality rate can result in population decline and even extinction (Hargrove, 2003a).

Vectorial capacity is the readiness to become infected while feeding on a vertebrate host and to subsequently develop an infection and transmit the trypanosome to another vertebrate host and varies from species to species (Challier, 1982). Infection rates in tsetse are determined by the parasite, the host, the vector, and the environment. They are generally low but vary greatly according to species of trypanosome, with *T. vivax* ranking the highest and *T. brucei spp*. ranking the lowest. Infection rates are influenced by endogenous factors (including tsetse species, strain, sex, age, nutritional status and interactions with micro-organisms within tsetse); by host factors (including tolerance of tsetse, immune state and attractiveness to tsetse); ecological factors (including climate, light, wind and biomass) and parasite factors (including species and infectivity) (Molyneux, 1980; Leak, 1999)

Biting insects may transmit tsetse mechanically (and this is how *T. vivax* is transmitted in South America and Mauritius). The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota*, *Liperosia*, *Stomoxys*, and *Chrysops* flies have also been implicated. According to Jordan (1986) and Leak (1999) who extensively reviewed the subject, there is little good evidence that mechanical transmission is of importance in Africa under natural conditions; reports of field occurrence may be due to insensitive sampling that does not detect low populations, and experimental studies that support mechanical transmission do not replicate natural conditions. Congenital transmission of trypanosomosis can take place (Melendez *et al.,* 1993) and carnivores can be infected (with *T. brucei*) by consuming infected meat; the importance of these transmission

routes is not known, but is not likely to be high. On the other hand, iatrogenic transmission is also possible and may be important when poor needle hygiene is practised.

3.2.3 Host

Cattle-infective trypanosomes circulate in a variety of wildlife hosts, which generally tolerate infections or have a state of pre-immunity. Small ruminants, equines, pigs, dogs and cats are also susceptible to some species of cattle-infective trypanosomes. The existence of reservoir and alternative hosts complicates the epidemiology of AAT, making it difficult to manage and perhaps infeasible to eliminate the disease.

Susceptibility of cattle to trypanosomosis depends on their breed, age, behaviour, previous exposure and health status; of these the most important factor is breed. Recent studies have shown that African cattle stem from the *in situ* domestication of a taurine (*Bos taurus*) wild ox that inhabited northern Africa around 9 000 years ago (Bradley *et al.,* 1996). In contrast, Zebus were mainly introduced from south Asia around 760 C.E. (Bradley *et al.,* 1998.) West African *B. taurus* breeds are trypanotolerant; that is, they can survive and be productive under trypanosomosis risk. This capacity is highly heritable and involves the ability to control parasitaemia, maintain weight and resist anaemia (Murray *et al.,* 1990). Some east African breeds are also, to a lesser degree, trypanotolerant (Dolan *et al.,* 1994; Magona *et al.,* 2004a). Although trypanotolerance is intrinsic, previous exposure to trypanosomosis is an important determinant of disease susceptibility (Clausen *et al.,* 1993); field studies have shown that N'Dama have a marked capacity to acquire resistance to both *T. vivax*, and to a lesser extent, *T. congolense* (Murray *et al.,* 2004). Moreover, there is evidence of a (lesser) ability to acquire resistance in *B. indicus* types in East Africa (Murray *et al.,* 1982).

There appears to be reverse age immunity to AAT (i.e. calves least susceptible). Different factors have been implicated: protection by maternal antibodies (Zapf, 1994); higher proportion of γδ T cells (Dwinger *et al.,* 1992); less exposure to infection; and less attractiveness to tsetse (Schofield and Torr, 2002). Other extensive studies have shown an inconsistent relation between age and prevalence (d' Ieteren *et al.,* 1988).

Tsetse flies perceive colour and are attracted to dark colours; one study showed brown and fawn coloured cattle were more likely to be infected than cattle of other colours (Carty, 2002). Conversely, in Senegal, no difference in parasitaemia or packed cell volume (PCV) was found between white, black and piebald cattle (Touré *et al.,* 1981).

Host behaviour affects susceptibility to tsetse, with more defensive behaviour associated with less successful tsetse feeding (Torr *et al.,* 2001). Recently it has been suggested, on the basis of experiments in mice, that females are more resistant to trypanosomosis (Turey *et al.,* 2005).

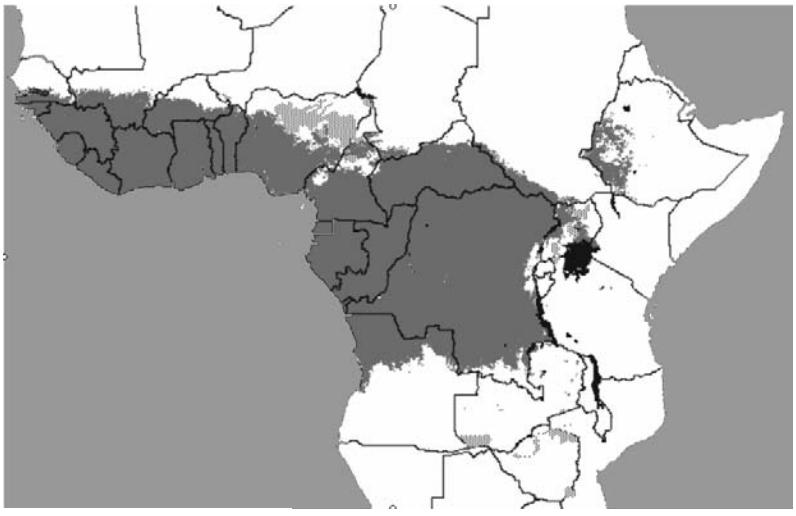
Stress, (due to another disease, malnutrition, water deprivation, heat or production such as pregnancy, lactation or work) is known to precipitate disease (Holmes *et al.,* 2000). Thus, good management may do much to mitigate the effects of trypanosomosis (Conner, 1992).

3.2.4 Environment

The environment provides conditions in which susceptible host and infectious vector can come together for long enough for transmission to occur; pathogens, vectors and hosts all occupy ecological niches determined largely, but not exclusively, by the environment. In west Africa, trypanosomosis is transmitted by savannah and riverine tsetse. The former are declining as the savannah habitat is changing due to human activities; in the moist savannah zone *G. morsitans* is at its northern ecological limit and so the removal of its habitat by human settlement and the introduction of agriculture is sufficient to cause its eradication (Budd, 2002). Riparian tsetse, on the other hand, can co-exist with human development. They are opportunistic feeders; where agricultural density is low they feed on wild reptiles and rarely carry pathogenic trypanosomes (de la Rocque *et al.,* 2001a). High transmission risk areas include watering places and adjacent to agricultural areas (de la Rocque *et al.,* 2001b).

Analysis with Geographical Information System (GIS) technology has deepened understanding of the spatial and temporal epidemiology of trypanosomosis, showing that distribution of tsetse flies is related to climatic conditions (Rogers and Randolph, 1993), and that satellite imagery can provide reliable surrogates for a range of climatic and geographic parameters (Hay *et al.,* 1996). This has allowed mapping of tsetse distributions with reasonable accuracy over a wide range of ecological conditions using Fourier processed time series of satellite imagery of various types, including vegetation cover, rainfall, temperature and elevation (Rogers and Robinson, 2004). These techniques use discriminant analytical and maximum likelihood methods and give accuracies of better than 85%. These models have been used to predict probabilities of fly distributions at 5 kilometre resolution for the whole sub-Saharan Africa and 1 kilometre resolution for a number of regions or countries (Fig 3.1). The relationships can then be applied to areas which have not been sampled, or where data is out-of-date, to provide a predicted probability of presence for areas outside the original training data set and to generate information which can be used for planning and *post hoc* evaluation of control.

Figure 3.1 Example of a GIS- predicted map of tsetse distribution (palpalis group)



Source: Torr *et al.,* 2005

3.3 Management of trypanosomosis

Trypanosomosis is a major threat to animal and human health in sub-Saharan Africa; tsetse flies infest one third of the African continent, threaten the livelihoods of 250 million people, and cost farmers and consumers billions of dollars per year: estimates vary from 5 billion USD (FAO, 1962), to 1.9 billion USD (Jahnke *et al.,* 1988), to 1.3 billion USD (Kristjanson *et al.,* 1999). Unsurprisingly, trypanosomosis control (or more optimistically, eradication) has been a longstanding development preoccupation. There are three main strategies for actively managing animal trypanosomosis: vector avoidance/control/eradication, use of trypanotolerant breeds/species and treatment with prophylactic or curative medicines. Of these, as Table 3.2 shows, by far the most important is use of trypanocidal drugs.

Table 3.2. Relative importance of the three main trypanosomosis control strategies (Allsopp, 1998; Agyemang and Rege, 2004)

Strategy	Uptake
Vector control	Less than 1% of tsetse-infested area currently controlled
Trypanotolerant animals	Less than 20% of cattle at risk are trypanotolerant
Modern trypanocidal drugs	70% of cattle at risk are treated

3.3.1 Control of the pathogen

Although vector control and genetic resistance have potential for greater uptake, trypanocides remain the single most important strategy for managing trypanosomosis. Used in all cattle-keeping communities at significant risk of trypanosomosis, it is the only strategy sufficiently attractive to be adopted spontaneously. Both traditional and modern drugs are used to treat trypanosomosis. Traditional drugs are used across Africa; mostly derived from plants (Bizimana, 1994) but metaphysical or supernatural treatments are also important. Recent studies have revealed trypanocidal activity in many African plants (Adewunmi *et al.,* 2001; Hoet *et al.,* 2004; Bizimana *et al.,* 2005). But although anecdotal reports abound, there is as yet no conclusive evidence for the effectiveness of traditional medicines in the field treatment of bovine trypanosomosis.

Modern drugs, however, are very effective and reasonably safe. Historically, potassium arsenate was the first drug proven to affect trypanosomes, and the earliest report of its use was in 1887 in a sick horse probably suffering from AAT (Friedheim and Distefano, 1989). By the first decades of the last century antimony compounds were being widely used to treat bovine trypanosomosis. Relatively toxic, these compounds were succeeded by safer arsenical compounds, replaced by phenanthridinium compounds in the 1930s (Browning *et al.,* 1938). In 1949, quinapyramine was introduced by Imperial Chemical Industries and in the formulation known as Antrycide Pro-salt provided the first effective long-acting prophylactic drug for cattle trypanosomosis (Willet, 1963). In the next decade, more phenanthridinium compounds were introduced by Boots, namely prothidium, homidium bromide (in use since 1948 (Ford *et al.,* 1953) and the related drug homidium chloride, soluble in cold water). In 1955 a diminadine was introduced by Farbwerke Hoechst under the name of Berenil®; the active ingredient was diminazene aceturate, developed

eleven years earlier from congasin (Fussgänger, 1995). Diminazene is the only commonly used exclusively curative trypanocide. (Its chemical relation to pentamine, the human sleeping sickness drug, raises the possibility that drug resistance to diminazene may reduce effectiveness of treatments for sleeping sickness.) May and Baker introduced metamidium, to be replaced by its derivative isometamidium chloride; this has been marketed since 1961 as a prophylactic, and at half dose, as a curative (Rüchel, 1975). So at present there are essentially three drugs for the treatment of cattle trypanosomosis: isometamidium chloride (ISMM), diminazene aceturate (DIM) and homidium, the latter as chloride (HOM) and bromide (ETH). The different spectra of activity and some trade names are shown in Table 3.3.

Table 3.3 Trypanocides commonly used to treat cattle trypanosomosis

Drugs	Trade names	Active against
DIM	Azidine®, Azidin®, Babesin®, Babazene®, Berenil®, Beronal®, Crede-Bab-Minezene®, Diaceturate®, Diminaphen®, Diminasan®, Diminavet®, Diminaveto®, Diminazen®, Diminazene®, Dimisol®, Dizene®, Ganasag®, Ganaseg®, Lobazen®, Nozomil®, Pharzen®, Pirocide®, Sangavet®, Survidim®, Trycip®, Trypadim®, Veriben®	*T. congolense* and *T. vivax*. *T. brucei* at higher dose
ISMM	Trypamidium®, Samorin®, Securidium®	*T. congolense, T.vivax, T. brucei*
Homidium chloride	Novidium®, Babidium Chloride®, Ethydium Chloride®, Novidium Chloride®	*T. congolense, T. vivax*
Homidium bromide	Ethidium®	*T. congolense, T. vivax*

The main mode of action of ISMM is thought to be on the kinetoplast DNA (Newton, 1974). ISMM inhibits an enzyme needed for the decatenation and catenation of kinetoplastic DNA, which is structured as a dense network of interlocked minicircles (Shapiro, 1993). Catenation and relocking of the minicircle net-work is essential for DNA replication and, thus, for trypanosome division (Shapiro and Englund, 1990). The mode of action of diminadine drugs is not well understood; it is thought that molecules may bind to the DNA minor groove inhibiting transcription or DNA dependent enzymes (Wilson *et al.,* 2005). However, dyskinetoplasty alone could not account for the broad antiprotozoal and antimicrobial spectrum of the drug.

Several studies have shown that drugs make possible profitable cattle keeping even in areas of high trypanosomosis risk (Logan *et al.,* 1984; Trail *et al.,* 1985). Chemotherapy is more effective in areas of riverine tsetse than in areas of savannah, as riverine tsetse have lower infection rates and carry less pathogenic stocks (Ilemobade, 1987). Numerous studies have shown that use of trypanocides is the cheapest method of trypanosomosis control. Table 3.4 compares different control strategies; overhead costs (management, administration, training, surveys and research) are not included. As overheads are encountered for all control methods except spontaneous use of trypanocidal drugs by farmers, this underestimates the costs of other strategies.

Table 3.4 Direct and indirect costs of trypanosomosis control (modified from Shaw, 2003)

Technique	Costs per km^2 (US$)
Linear km of barrier using targets - barrier establishment - annual barrier maintenance	2 000 1 600
Ground spraying	265-315
Aerial spraying (Sequential Aerial Technique)	265-535
Sterile insect technique (SIT)	250-800
Low-density mono-pyramidal traps	26
Cattle insecticide treatment (pour-on, assuming 44 cattle per km^2)	50-120
Prophylactic trypanocides, assuming 15 cattle and 2 treatments/yr	30
Curative trypanocides, assuming 15 cattle, 10% prevalence and eight week duration of disease	4.5

Curative drugs are the cheapest strategy but losses are higher than with preventative strategies; it has also been suggested that the full costs of drugs (direct and indirect, including externalities) are underestimated by conventional cost effectiveness analysis (Barrett, 1997).

Emerging drug resistance is a major, perhaps the major, constraint to the long-term use of trypanocides. This is particularly serious as there is little likelihood of new drugs emerging in the near future, given the absence of any candidate drugs in the development pipeline, the long time needed for drug development and the lack of incentives for private sector product development.

While prospects for novel drugs in the short-term are not good, there are some promising new and old drug combinations and formulations to improve the pharmaceutical management of AAT.

- To extend the period of protection and decrease local reactions, alternative delivery systems such as dextran complexes, liposomal formulations, carrier erythrocytes, and polymers, have been developed, but have yet to be made commercially available. Although toxic reactions are less the costs are higher and in some cases the period of protection less (Peregrine, 1994).
- Suramin, used since 1925 to treat *T. simiae* and *T. evansi*, is not effective against the cattle-infective trypanosomes *T. vivax* and *T. congolense*, but compounds of suramin with other trypanocides (antrycide, prothidium, ETH and DIM) have broader action, less toxicity and prophylactic action. However, high costs have hindered development (Desowitz, 1957).
- Sustained Release Devices consisting of polymers loaded with ISMM and coated with dexamethasone to reduce tissue reactions at the implantation site extended the protection offered by ISMM three-fold (Diarra *et al.,* 1998).
- Combinations of drugs have had some success (Cawdery and Simmons, 1965).
- Sequential treatments (sanative pair) are widely recommended (Whiteside, 1960) on the basis that trypanosomes resistant to DIM were not resistant to ISMM/ETH and *vice versa*; hence using the two drugs sequentially (as a sanative pair) would eliminate resistant strains. Increasing reports of multiple drug resistance call this strategy into question (Clausen *et al.,* 1992).

Great efforts have been made to develop a vaccine to trypanosomosis. Trypanosomes' unique capacity for antigenic variation of the VSG coating the cell surface is the basis of their ability to

evade the host immune response (Barbet and McGuire, 1978), and because of this, prospects for the development of a vaccine are considered poor (Vickerman and Barry, 1982). Limited success is reported after immunizing with flagellar pocket antigen or tubulin (Balaban *et al.,* 1995, Lubega *et al.,* 2002). Other methods with some promise, but still far from field applicability, include immunological control of vectors, through immunizing hosts against tsetse rather than trypanosomes (Jacobs-Lorena and Lemos, 1995); immunizing against metacyclic forms of trypanosomes; making tsetse refractory to infection by trypanosomes (Hao *et al.,* 2001); and immunization of cattle against the mediators of cell damage during infection (Authie *et al.,* 2001).

3.3.2 Control of the vector

Farmers have traditionally repelled the tsetse fly vector using wood smoke, rancid butter, camel fat, oil of *Khaya senegalensis* seeds, mud and other materials. These may have some benefit; a small wood fire has been shown to halve the number of tsetse attracted to cattle (Torr *et al.,* 2002). Farmers commonly avoid areas where tsetse are abundant either by concentrating cattle in highland areas (above 1500m) where tsetse populations do not persist, or in habitats where tsetse are less abundant, or by grazing cattle at night when most tsetse species are not active (Ford, 1971). That these methods are at best partially effective, is witnessed by the exclusion of trypanosusceptible cattle from the tsetse belt before the advent of trypanocides, despite flourishing indigenous veterino-medical systems (Mathias and McCorkle, 2004).

In contrast, modern strategies aimed at vector control are very effective (albeit in the short term). The relatively low population densities, specific ecological requirements, need for frequent blood meals and slow rate of reproduction means that tsetse populations can easily be reduced. Killing just 3-4% of the female population per day is enough to eradicate a population (Hargrove, 2003b). Many different strategies of vector control have been used:

- Control of tsetse started in the 20s and was initially through destruction of tsetse habitat or slaughter of wildlife hosts (Leak, 1999). Bush-clearing can lead to soil erosion and is difficult to maintain, while destruction of wild animals had become unacceptable by the early 60s on conservation and animal welfare grounds.
- Biological control using predators or pathogens has had little success, perhaps because most trials have used insects that occur in tsetse habitats (van der Vloedt, 1991).
- The sterile insect technique (SIT) temporarilyg eliminated tsetse from large areas of Burkina Faso, Tanzania and Nigeria. More recently SIT was used on Zanzibar, a small island with little risk of re-invasion; eradication was declared in 1997 and AAT has not recurred (Vreysen *et al.,* 2000). SIT is promoted as a means to eradicate tsetse from Africa, with large projects starting in west and east Africa (PATTEC, 2001a). Yet, fundamental questions on the feasibility, appropriateness and cost-benefit of this operation remain unanswered (Rogers and Randolph, 2002).
- Overall, the most important form of vector control has been the use of insecticides. Tsetse are exceptionally sensitive to insecticides because of their low genetic variation (due to low dispersal

rate, low reproductive rate, and selection for the most energetically-efficient individuals), and it is unlikely that resistance to insecticides can emerge (Krafsur, 2003). Ground-spraying of tsetse resting sites with residual insecticide was widely used following the introduction of cheap persistent insecticides such as DDT and dieldrin fifty years ago; more recent campaigns have used less toxic endosulfans or synthetic pyrethroid insecticides. Though effective, the method is labour intensive, logistically demanding, and potentially dangerous for the operators. Programs to control tsetse over wider areas developed aerial spraying techniques with air planes or helicopters. Widely used in the 60s and 70s, the high cost and resource requirement limited long-term use. More recently campaigns with sequential aerial technique (SAT) using modern global positioning systems (GPS) have been carried out (e.g. in Botswana). Low dosages of non-persistent insecticides are accurately applied along pre-planned flight lines (Allsopp, 1991). Hot fogging is a ground based method of applying insecticide similar to sequential aerial spraying (Wooff and Phillemon-Motsu, 1993). It is a non-residual technique and only affects the adult tsetse population. Despite widespread use and comprehensive investigation, there is little evidence of long-term negative environmental impact from the use of insecticides for tsetse control (SMEG, 1997; Douthwaite and Tingle, 1994), but concern persists. In 1988 the Regional Tsetse and Trypanosomiasis Control Programme for Southern Africa (RTTCP) rejected the idea of using environmentally-applied insecticides, due largely to concern about potential ecological impact (Grant, 2001), so that tsetse elimination was no longer feasible over such an area, and the programme was refocused on general rural development.

The cost and potential side-effects of ground and aerial spraying, stimulated interest in insecticide-treated traps/screens or baits. This was used as far back as 1910, when plantation workers with sticky paper on their backs were part of a successful tsetse control programme in the island of Principe (Buxton, 1955); traps were also reasonably successful in the 30s in south and east Africa (Leak, 1999). Recent years have seen extensive research into the design, fabric, colour, baiting, insecticide treatment and placement of traps and targets. Successful designs include the widely-used monoconical trap (Lancien, 1981), the pyramidal trap in the Congo (Gouteux and Lancien, 1986), the inexpensive Vavoua trap in Côte d'Ivoire (Laveissière and Grebaut, 1990), and the mono-screen trap for *G. f. fuscipes* in Uganda (Okoth, 1991). More complex cubical traps were developed for specific situations, such as the H-trap for *G. brevipalpis* in Kwazulu Natal (Kappmeier, 2000). Uni-dimensional targets, consisting of screens of cloth impregnated with insecticide are simpler and cheaper to construct. One example is the S-type target developed by Vale *et al.* (1985) for *G. pallidipes* and *G. m. morsitans* in Zimbabwe, consisting of a black rectangular sheet of cloth flanked by two sheets of black mosquito netting, fastened to a metal frame swivelling on a single pole sunk into the ground. In west Africa, Laveissière, Couret and Grébaut (1987) designed a similar screen (target) for use against *G. palpalis*; the final version consisted of a blue central cloth flanked by two strips of black mosquito netting. Low cost screens consisting of a sheet of polyester, with a framework of materials available *in situ* were developed in

west Africa. In Uganda very low cost targets consisting of sacking, patchwork, scare-crows or trees have been pioneered (Okoth, 1999). Carbon dioxide gas and natural (e.g. cattle urine) or synthetic (e.g. acetone) odours can dramatically increase effectiveness of screens/traps (Vale *et al.,* 1988) especially for *G. morsitans* and *G. pallidipes* species. Sterilising chemicals can be used instead of insecticides, and are a fall-back option if insecticides are not desired (Hargrove and Langley, 1990). Traps without insecticide have also been successfully used to suppress tsetse populations, but require more upkeep as minor damage may render them ineffective (Dransfield *et al.,* 1990).

A new option for control, applicable where zero grazing is practiced is insecticide-treated mosquito netting, surrounding but not covering the stable (Bauer *et al.,* 2005).

An extension of the stationary bait concept is the application of insecticides to livestock. Sprays (FAO, 1994), pour-ons (Bauer *et al.,* 1995), dips (Thomson *et al.,* 1992), ear tags (Kuepper and Harbers, 1997) aerosols and footbaths (Stachurski, *pers. com*.) have all been used. Studies on animal baits from six countries have been summarised by Torr *et al.* (2002). In general, trypanosomosis prevalence has been reduced to 5% or less. Where cattle distribution is patchy, the area involved is small, or re-invasion pressure is high the method is less effective (Hargrove *et al.,* 2003). There is also concern that insecticide treatment of cattle may disrupt enzootic stability to tick borne diseases and kill ecologically-important dung-beetles (Vale, 2002). If calves are not treated, and adults treated at low frequency this may not be a problem (CIRDES, 2000).

- The unwanted effects of insecticides are not present when repellents are used; recent work in Kenya suggests that these are very effective at protecting cattle (Saini and Hassanali, 2002). However, the cost-effectiveness and demand for this approach remains to be proven.

Tsetse control has been a front-line operation for more than 50 years with little long term success; vector control programs have cleared less than 2% of the tsetse habitat since the 70s (Budd, 1999). Tsetse fly far and fast, and re-invasion of cleared areas is a permanent threat; huge areas cleared in Nigeria, Cameroon, Botswana, Burkina Faso, Cote d'Ivoire and Zimbabwe have been or are in the process of being re-invaded. The failure of tsetse control is due partly to financial, but mainly to institutional factors, including: low responsiveness, poor quality, technical inefficiency, allocative inefficiency and inequity (Mills, 1995). There are little prospects for immediate improvement in these, given the current economic and institutional climate of sub-Saharan Africa.

3.3.3 Control through host resistance

Trypanotolerant taurine cattle are used as a strategy for AAT management mainly in west and central Africa where they comprise 20% of the bovine population. In Africa, as a whole there are 12 million trypanotolerant cattle, or 5% of the total population (Agyemang, 2000). Distinct humoral immune responses to trypanosome infection are the major feature of bovine trypanotolerance. Trypanotolerant cattle have other desirable characteristics including heat tolerance (Ferguson, 1987); resistance to helminths (Mattioli *et al.,* 1992), ticks (Mattioli *et al.,* 1993), and tick-borne diseases such as dermatophilosis (ILCA, 1979), anaplasmosis and babesiosis (Starkey, 1984);

and lower nutritional and husbandry requirements. Despite these advantages, wherever Zebu can be raised they displace the trypanotolerant breeds, implying farmers consider the advantages of trypanotolerant breeds outweighed by the disadvantages (Jabbar and Diedhiou, 2001). It was originally thought that trypanotolerant cattle were less productive than trypanosusceptible breeds, but later farm trials showed that they can be productive in terms of meat and milk (Feron *et al.,* 1988; Agyemang *et al.,* 1994). However, the slow increase in trypanotolerant cattle population compared to other breeds (Agyemang and Rege, 2004), and the lower price fetched in markets (Kamuanga *et al.,* 2001) indicate these cattle continue to be less preferred by farmers. More than three-quarters of the cattle in tsetse-infested areas are non-taurine, and taurines are likely to remain a minority choice for the foreseeable future.

For this reason, research is ongoing into the transfer of the trypanotolerance trait to more desired breeds. Crossing trypanotolerant cattle with Zebus is widely practiced by farmers, and has received support from governments in terms of establishing breeding ranches, and in some countries, the importation of trypanotolerant nucleus herds. However, the polygenic-quantitative nature of trypanotolerance, its low to moderate heritability, and the technical problem of maintaining sufficient challenge to allow selection for adaptive traits without confounding of productivity traits, has limited controlled and scientific application of crossing (Soller, 1992). A more recent approach is the mapping of quantitative trait loci (QTL) (Hanotte *et al.,* 2003) allowing marker assisted selection (MAS) of target genes within breeds of tolerant animals, and marker-assisted introgression (MAI) of target genes from tolerant to susceptible breeds; this has been achieved recently in mice by Koudande *et al.* (2005). MAI and MAS coupled with artificial insemination and embryo transfer could produce rapid results; but experience with introducing superior animals to village herds in Africa has often been disappointing (Planchenault and Traore, 1993) and the uptake mechanisms for genetically improved animals, even if they could be produced on a significant level, are as yet unclear.

Although N'Dama can be economically productive even under conditions of high infection pressure (Itty, 1996), trypanotolerance is not an absolute phenomenon and in areas with high challenge or where cattle are stressed (by poor nutrition, ploughing or other disease), trypanocidal drugs are required for animals to be productive (Jordan, 1995). Field studies in the Democratic Republic of Congo (Trail *et al.,* 1992) and in Gabon (Trail *et al.,* 1993) showed that trypanosomosis was a pathological stress in N'Dama cattle as well as Zebu, with *T. congolense* infection having a more severe impact than *T. vivax* infection. Other experimental studies found 31% of trypanotolerant cattle died under heavy challenge (Roelants, 1986). This suggests that trypanotolerance may not be sufficient as a stand-alone strategy, but rather as part of an integrated control strategy (Murray *et al.,* 2004). In contrast to vector control or drug use there are few negative externalities associated with keeping trypanotolerant cattle, although it has been suggested that greater reliance on trypanotolerance would lead to higher levels of asymptomatic infection of cattle, thus increasing the risk of human sleeping sickness (Gibson and Bishop, 2005).

3.3.4 Practical aspects of control

Integrated control

Integrated control is an overall approach, in which different tactics are employed strategically based on a thorough knowledge of parasite life cycle and ecology. It avoids over-dependence on a single method, and recognises that no single method is likely to be completely effective, but in combination, satisfactory control may be achieved. Table 3.5 shows recommendations for integrating strategies of trypanosomosis control based on prevalence levels.

Table 3.5 Integrated control of trypanosomosis recommendations for different prevalence levels (adapted from Snow and Rawlings, 1999).

<table>
<tr><th>AAT Prevalence</th><th>Drugs</th><th>Vector control</th><th>Trypanotolerance</th></tr>
<tr><td>Zero</td><td>Do nothing.</td><td rowspan="2">Cost of vector control not justified at this level.</td><td>Introduce new breeds, e.g. European.</td></tr>
<tr><td>Low</td><td rowspan="2">Treat as and when required.</td><td>Introduce new breeds, e.g. pure Zebu.</td></tr>
<tr><td>Medium</td><td>Control not cost effective; unless major benefits from tick and nuisance fly control.</td><td rowspan="2">Keep trypanotolerant breeds.</td></tr>
<tr><td>High</td><td>Treat as and when required. Prophylaxis for valuable cattle.</td><td>Tsetse, tick and nuisance fly control using sprays/pour-ons; costs may be high and benefits small.</td></tr>
<tr><td>Very severe</td><td>Treatment on demand may not prevent significant production losses. Prophylactic treatment for all cattle.</td><td>Use of insecticide impregnated targets may be justified. Effective vector control likely to have important development benefits.</td><td>Keep trypanotolerant breeds, or shift from cattle to other species.</td></tr>
</table>

Trypanosomosis control can also be integrated at higher levels, for example with other animal disease control measures or with rural development objectives (Holmes, 1997). Combining tsetse with tick control is an option where animal baits are used to control tsetse. There are examples from Burkina Faso (Bauer *et al.,* 1992), Kenya (Baylis and Stevenson, 1998), Tanzania (Fox *et al.,* 1993), Zimbabwe (Van den Bossche and Mudenge, 1999) and Ethiopia (Leak *et al.,*1995) where the treatment of cattle with pyrethroids to control tsetse has had an impact on ticks and tick borne diseases (TBDs). Most studies showed benefits, with reductions in mortality from TBD and fewer ticks. However, reduction of ticks could disrupt enzootic stability (the epidemiological phenomenon seen in some TBDs whereby infection is high but clinical disease low; it is notable with babesiosis and heartwater, occasional with anaplasmosis, and rare for theileriosis). Enzootic stability arises when animals are infected at a young age, reverse age immunity exists, and initial infections protect from later infection. If tick numbers are reduced animals will be infected at a later age when disease is more serious and losses will be higher. Disruption of enzootic stability can be disastrous, for example when tick control stopped because of unrest in Zimbabwe, more than a million cattle died from TBD (Peter *et al.,* 2005). One study in Zambia found that cattle protected with pour-ons had less immunity to *babesia* than controls, suggesting enzootic stability was

disrupted by tsetse control; in this case, there was no report of resultant increased mortality or morbidity. Infrequent treatments, restricted to adult bovines may effectively control tsetse without disrupting enzootic stability (CIRDES, 2000). Other concerns over the use of insecticides in integrated tick/tsetse control are environmental harm (especially to beneficial insects, such as dung beetles) and fostering of resistance in ticks (Eisler *et al.,* 2003).

It has long been known that helminth infections increase the pathogenicity of trypanosomosis and *vice versa*. A recent study has shown synergetic (multiplicative) effects of combining trypanosomosis prophylaxis with worm treatment in calves (Karanja, 2005). Conversely, a study in goats found effects of anthelminthic and trypanosomosis control were additive and independent (Hendy, 1989). Worm treatments which result in health and production benefits may not be cost-effective in village cattle (Itty, 1997; CIRDES, 2000); it is possible that integrated approaches addressing multiple diseases may be more economically attractive.

As both are spread by the same vector, tsetse control can address simultaneously both animal and human trypanosomosis (Magona and Walubengo, 2005). Another integrated approach is treating cattle in order to decrease the transmission of sleeping sickness. This has been successfully used in Uganda where cattle were the main reservoir host for *T. brucei* and *T. rhodesiense* (Hide *et al.,* 1998). A promising innovation is integration with malaria control; in some tsetse-infested areas, the main malaria-carrying mosquito is *Anopheles arabiensis*, which obtains half its blood meals from humans and half from cattle, and malaria has been successfully controlled by insecticide treatment of cattle (Gibson, 2002).

Community control

The proactive involvement of farmers in tsetse control is recent and not without critics. Participation means different things to different people and proponents recommended that the word should not be used without qualification and definition (Table 3.6). There are interesting differences between Anglophone Africa, where participation is often used in the sense of empowering communities (Dransfield *et al.,* 1991), and Francophone Africa where it is more commonly used in the non-transformative sense of informing, educating or involving communities (Laveissière and Penchenier, 2000). Bait methods (traps/screens or treated cattle) for tsetse control are particularly suited to community use. Some of the first community projects had the primary objective of controlling human sleeping sickness epidemics. The 80s and 90s saw projects in Cote d'Ivoire (Laveissière *et al.,* 1994), Uganda (Lancien, 1991; Okoth 1998), Angola (Abel *et al.,* 2004) and the Sudan (Joya and Okoli, 2001). These were generally effective in reducing fly numbers and disease incidence, and the community was actively involved in placing and maintaining traps. However, community contributions were low, interest declined with time, and effective control of the project remained with project staff (WHO, 2004a), suggesting participation was at a low level (Table 3.6).

Table 3.6 Different types of participation (adapted from Pretty, 1994)

Participation	Involvement of communities	Power of communities
Passive	People are told that a project is happening in their area.	No power over project.
Extractive	People answer questions in an extractive one-way process. Results are not shared with communities.	Power to opt out.
Enquiring	People are consulted by external agents. Discourse is two-way. External agents define problems and solutions but may modify them as a result of community views.	Power to opt out. Power to disagree.
Paid for	People provide resources or labour in return for incentives e.g. contribute labour for subsidised screens, bring cattle for subsidised treatments.	Power to withhold involvement, bargain for more incentives.
Functional	People participate by forming groups to meet pre-determined project objectives.	Power to make minor decisions and 'right' decisions i.e. approved by the project.
Interactive	People participate in joint analysis and in forming new or transforming existing institutions. They have real control over decision-making.	Power to make all decisions, project may pressurise to make 'right' decisions.
Self-mobilisation	People take the initiative to form and transform institutions independently of the external agent. External agent provides advice.	Power to make 'wrong' decisions; project advises and does not control.

Projects with an animal health focus have been described in Kenya, Ethiopia, Uganda, Zambia and Zimbabwe, Botswana and Burkina Faso (Barrett and Okalii, 1998; Kamuanga, 2003). Again, technical results were good but levels of participation low, and farmer contributions insufficient to maintain activities without the presence of external support (Brightwell and Dransfield, 2001; Kamuanga, 2003). While some researchers (Barrett and Okali, 1998; Catley and Leyland, 2001) argue this is due to the generally low level of participation and continued domination by administrators and experts, others believe there are intrinsic economic incentives for the failure of community control without external support. These include: the free-rider problem, as vector control is a non-excludable public good so there are incentives to enjoy the benefits without paying the costs (McCarthy *et al.,* 2003); time consistency issues because farmers are less willing to pay when it appears the problem is gone; and equity problems as some benefit more from control. A recent expert committee was unanimous that when methods of vector control are included in public health intervention packages, they should be made available at no cost (WHO, 2005).

Autonomous control

The single most effective method of trypanosomosis control has been environmental change through human settlement, agricultural expansion, deforestation and hunting: so called autonomous control. An exhaustive case study of five African countries (Ethiopia, Kenya, Nigeria, Zimbabwe and the Ghana) found that land cultivation was increasing by 2-5% per year and in all

countries tsetse (especially savannah species) declining as the result of autonomous, anthropogenic control (Bourne *et al.,* 2001). This is unsurprising, considering that the population of Africa has increased from 133 to 900 million people in the last 100 years. Mc Dermott *et al.* (2000) modelled the effects of autonomous control and climate change on tsetse populations predicting that tsetse will disappear from 30% of their current area by 2050, persist at reduced levels over 20% and remain in high numbers over 50% of their current distribution, with greatest decline in west Africa, and smaller areas of decline in eastern, central and southern Africa. To put this expected reduction of 30% of tsetse infested area in perspective, large-scale tsetse eradication campaigns have only cleared 2% of tsetse-infested land since the 70s (Budd, 1999).

3.4 Trypanocidal drug resistance

Modern medicines make dramatic differences to human and animal health; at the start of the third millennium the world population of both is without precedent (six billion humans and 20 billion domestic animals). Used properly, veterinary drugs permit higher levels of production, improve animal welfare and safeguard the livelihood assets on which 700 million poor farmers in developing countries rely. Used improperly, veterinary drugs waste scarce resources, occasion avoidable sickness and death, mask poor production and promote drug resistance leading to exacerbated disease in animals and humans.

3.4.1 Definition and aetiology

Drug resistance is the heritable loss of sensitivity of a micro-organism to an antimicrobial to which it was before sensitive. Modern cattle trypanocides were introduced in the 50s and the first cases of resistance were reported in the next decade (although trypanocidal failures have been reported since the 20s). Currently resistance to trypanocides has been reported from 15 countries (Geerts and Holmes, 1998; Diall *et al.,* 2003; Mamoudou, *pers. com*.). In west Africa, resistance was first identified in 1984 (Authié) and not long after, multiple drug resistance was confirmed (Clausen *et al.,* 1992). Drug resistance in trypanosomes is likely to be promoted by the same factors implicated in development of resistance to other microbes, that is: large-scale drug use; sub-curative doses; using drugs in immuno-compromised patients; and using drugs that are slowly eliminated from the body (Holmes *et al.,* 2004). In addition, some drugs can induce cross-resistance to other trypanocides. Use of quinpyramine rapidly leads to multiple resistance to DIM, HOM, ETH and ISMM (Ndoutamia *et al.,* 1993). The exact mechanisms of drug resistance are imperfectly known, but reduced drug uptake has emerged as a common characteristic of drug-resistant trypanosomes. In the case of *T. brucei* cellular uptake of major drugs against occurs via a specific nucleoside transporter (P2), the loss of which results in drug resistance (Carter *et al.,* 1995).

3.4.2 Extent and impact of resistance

Although resistance has been reported for many years, only recently have methodologies existed for estimating its regional prevalence. Studies applying these methodologies typically report wide

variation in levels of resistance or its risk factors from village to village in a given geographical area (Sinyangwe *et al.,* 2004; Tewelde *et al.,* 2004).

The impact of resistance can be indirectly assessed from farmers' current reliance on trypanocides, the single most important strategy for trypanosomosis control. If resistance renders drugs ineffectual, then the 40-60 million trypanosusceptible cattle in the tsetse belt would not survive long. To date, few studies have been carried out on the impact of resistance. One study in a part of Ethiopia with a high prevalence of drug resistant *T. congolense*, found that despite high calf mortality and abortion, cattle production was profitable (Itty *et al.,* 1995). Experiments have shown that high levels of treatment in mice infected with resistant trypanosomes can prolong lifespan even if there is no complete cure (Mdache *et al.,* 1995), and this may also be true for cattle. While some studies suggest that resistant trypanosomes are less pathogenic, more recent studies have contradicted this (Rowlands *et al.,* 2001). Resistant trypanosomes isolated by the project discussed in this thesis proved highly pathogenic to calves (Bocoum, 2003). An additional adverse effect of trypanocide resistance in domestic animals has recently been suggested: selection of cross-resistance to drugs used to treat human sleeping sickness (Fevre *et al.,* 2001).

3.4.3 Management of resistance

Resistance management has three components: detection and surveillance of resistance; prevention of resistance; and containment or eradication of resistance.

Surveillance and monitoring of resistance

Detection and monitoring is the first, indispensable step to the control and management of resistance (WHO, 1997). There are four methods for detection of trypanocide resistance: field tests, laboratory tests in animals, *in vitro* tests and combined *in vivo/in vitro* tests; none is without problems.

1. Longitudinal parasitological data collected in the field may allow recurrent infections to be distinguished from new infections, indicating resistance (Rowlands *et al.,* 1993; Schuckken *et al.,* 2004). However, uncertainty and variation in incubation period, and low sensitivity of field diagnosis makes this an imprecise method. More reliable are controlled clinical trials in which cattle are randomly allocated to two groups, one of which is treated with ISMM, and both groups are checked every two weeks for the presence of infections (Eisler *et al.,* 2000). A third field test combines ELISA tests for drug detection with parasitological tests for the presence of trypanosomes; the simultaneous presence of trypanosomes and a high drug concentration is suggestive of resistance (Eisler *et al.,* 1997a).
2. Laboratory tests have fewer confounding factors than field tests. They can be carried out in ruminants or mice, while the former is more informative on resistance as it affects cattle, the costs can be prohibitive. The procedure is to infect a small group of animals with trypanosomes, and treat with trypanocides. Animals are then continuously monitored and the effective dose that gives temporary clearance or cure of the parasites in 50 or 95% of the mice

can be calculated usually using probit techniques. Unfortunately, most *T. vivax*, and many *T. congolense* isolates, do not grow in mice. Moreover, the curative dose in cattle cannot be directly extrapolated from mice tests (Sones *et al.,* 1988).

3. *In vitro* tests have been developed in which trypanosomes are grown in cell culture and then exposed to trypanocides; these tests allow screening of large numbers of isolates and are less objectionable on animal welfare grounds, but difficulty of *in vitro* cultivation of *T. congolense,* expense, and the high level of expertise and laboratory resources required are constraints. In the Growth Inhibition Assay (Kaminsky and Zweygarth, 1989) culture-adapted trypanosome blood-forms are incubated for 24 or 48 hours with different concentrations of trypanocides. For the Long-term In Vitro Viability Assay (Kaminsky *et al.,* 1989), the observation period is ten days. Another modification is the Low Inoculation Long Incubation Test (Brun and Lun, 1994), in which micro-plates are used and observation is for four days. Other tests are based on the reduced uptake of trypanocidal drugs associated with resistance. Uptake can be assessed using radioactive 14C-ISMM or fluorescence tests (Brun and Kunz, 1989).
4. Combined *in vitro/in vivo* tests include the Drug Incubation Infectivity Test (Kaminsky *et al.,* 1990) in which trypanosomes are incubated with trypanocides and then inoculated into rodents, who are observed over 20-30 days. In the Drug Incubation Glossina Infectivity Test, trypanosomes are exposed to the drug *in vitro* and then fed to tsetse flies to check whether they develop into metacyclic forms (Clausen *et al.,* 1999).

Although a gamut of tests have been developed, common disadvantages include high cost, high resource requirement, limited applicability to trypanosome species/strains, imprecise extrapolation to cattle, and animal welfare issues. Surveillance of resistance would be greatly facilitated by the development of cheaper, faster, more sensitive tests, with one possibility being tests for genetic markers. Resistance markers for *T. brucei* (Mäser *et al.,* 1999) and more recently *T. congolense* (Delespaux *et al.,* 2005) have been developed and seem promising, but have yet to be comprehensively assessed as a tool for detecting resistance in trypanosomes.

Preventing the development and deterioration of resistance

Rational Drug Use (RDU) is the cornerstone of efforts to combat resistance (WHO, 2001c), given that trypanocidal drugs will be used long into the future and that no strategy in existence or development, short of an unforeseen scientific breakthrough, will eliminate AAT in the current economic and social conditions in Africa. Inappropriate drug use in the community is a major factor in the development of resistance (Gonzeles *et al.,* 1999); studies from human medicine have linked resistance to inappropriate treatments resulting from lack of knowledge (Kunin, 1993; Bruneton *et al.,* 1997), inadequate diagnosis (Hogerzeil, 1993; Bosu, 1997), incorrect drug selection (Hossain, 1982; Hui, 1997) and incorrect drug regimen (Gumodoka *et al.,* 1996; Chalker, 1997). RDU occurs when medicines appropriate for the disease are administered correctly for adequate time periods and at the lowest cost to the client and their community (WHO, 1987). The successful application of RDU in human medicine is well documented (Radyowijati and Haak, 2003). Ross-Dengen *et al.*

(1997) reviewing 59 studies on RDU, mainly in developing countries, found more than 40% had high impact (>25% improvement over controls) and almost 40% moderate impact (10-25% improvement over controls). However, RDU has rarely been applied to livestock systems in developing countries. A complication is that most animal health treatments in Africa are given at community level, and RDU strategies may be need to be applied at both farmer and service provider level. RDU interventions have been divided into four categories summarised in Table 3.7 and discussed in the next section.

Table 3.7 Specific strategies for improving RDU (WHO, 2001)

Training and information	**Regulatory**
• Mass media (radio, television, newspaper) • Provision of printed material, • Continued professional development • Counselling, training of groups or individuals • Academic detailing • Decision-support to change prescription behaviour	• Restriction of antimicrobials to prescription only • Licensing manufacture, importation, distribution and sale of drugs • Registration and inspection of drug sellers • Standards-based marketing authorisation and registration of drugs • Quality control of products and services • Professional bodies to regulate conduct and quality of health service providers and education • Restrictions on drug sales promotion and advertising
Managerial	**Economic/policy**
• Lists of essential drugs and formularies • Evidence-based standard treatment guidelines (non-statutory standards) • Drugs/therapeutics/ethics committees • Peer review and learning structures • Audit and feedback of prescribing practice • Performance targets • Price and quality information (score cards, ranking, quality marks) • Course of therapy packaging • Dispensing and prescribing controls	• Subsidies/taxes on pharmaceutical products to influence price and hence purchasing behaviour • Competition in the provision of health services and products to decrease price and drive up quality • Pharmacy cross-subsidies to encourage service provision in under-served areas • Tax breaks for compliance with regulations, research, relocation to rural areas • Orphan drug provisions to incentivise new products for neglected diseases • Increasing patent length, height and breadth to encourage drugs with new modes of action rather than 'copy-cat' products • Removing/placing tariff and non-tariff barriers on pharmaceutical trade • Stimulating research and development by surrogate markets or tournaments/prizes • Tradable permits for resistance

The objectives of strategies are typically to: avoid use of drugs by disease prevention, reduce use of drugs by replacing with alternatives, ensure drugs are only given when clinically needed, give the appropriate drug at the appropriate dose, and ensure correct administration of the drug. The strategy of RDU can be forwarded by four types of interventions as described below:

- Informational/educational interventions provide information or training to health providers or users. They are often politically unthreatening and easy to implement, and are the most widely used intervention. Many reviews and evaluations have shown that success is greater (often >20%

improvement) when: multiple channels of information provision are used and multiple sessions take place; a problem-oriented approach is followed; there is focus on a single issue; participatory and interactive approaches (including peer review) are adopted; both users and providers are targeted; training occurs in the work-place and local opinion leaders and role models are involved (Fresl and Wolfheim, 1997; MSH, 2001; Smith *et al.,* 2001). Strategies which have little impact include: mass media approaches (changes in behaviour are commonly 10% or less, but this may be cost effective when populations are large (Atkin, 2001)); dissemination of printed information and guidelines (Davis *et al.,* 1995); and passive educational methods (Avorn *et al.,* 2001).

- Managerial interventions shift the way services are delivered into more preferred paths and are potentially powerful ways of encouraging RDU. An important caveat is that there should be effective management in place, often not the case for public services in developing countries. However, managerial initiatives may be effective in the private sector of developing countires, assuming this is functional and that businesses have incentives to comply with initiatives, and initiatives do not counter their economic interests. Managerial interventions have even been successful when used in the informal (illegal) private sector (Ross-Dengan, 1996).
- Regulatory initiatives are widely promoted (with the failure of regulation usually seen as justifying more regulation). Regulation can have impacts given an enabling environment, for ecample Spain (with poorly implemented legislation and antibiotics freely available from pharmacies) has higher resistance than comparable European Union countries with better regulation (Baquero *et al.,* 1996). The problem with regulation is that most drug rules are against the economic interests of those regulated, and therefore difficult to implement and sustain. Less than one in six WHO country members have well-developed and implemented drug regulation; predictably, these are mainly industrialised and rich countries (WHO, 1999). Very few developing countries have operational licensing, inspection or quality control systems for human drugs (RMD, 1993) or health delivery (Bennett *et al.,* 1996); in the absence of effective regulation of human health, it may be unrealistic to assume that effective regulation of veterinary drugs is attainable. The problem is often implementation failure rather than policy or regulation failure, and has multiple causes, largely unresolvable in the near term: poor governance; financial constraints; inadequate human resources; lack of information; weakness of consumer and professional groups; and low prioritisation of drug regulation are often most cited (WHO, 1997).
- Economic incentives or market-based instruments change behaviour by providing financial rewards or imposing financial costs. Although often considered as an alternative to regulation, they require some legislation and regulation for their creation and function. Theoretically more effective and less costly than command and control regulation, they have been little used in the pharmaceutical sector in developing countries. Price is the single most important determinant of quantity of drug use, although it appears to have little effect on the quality of drug use. When Iceland stopped subsidizing antimicrobials, drug use and resistance fell, although both remained high in similar countries which continued to subsidize (Stephenson, 1996). Other macro policy

interventions to contain and prevent microbial resistance (such as legislation change, global control of drug availability, patent laws, and competition policy) are considered to have potential to contain and prevent resistance, but there is relatively little information on their application and impact. A promising recent trend is initiatives to create surrogate markets through purchase commitments or tax-breaks for drugs that treat neglected diseases. Human trypanosomosis is considered a candidate for these, with possible spill-over benefits to animal trypanosomosis if new treatments can be found (PIU, 2001). A major constraint to the more widespread use of policy and economic interventions in the management of trypanocide resistance is the lack of compelling evidence on the costs of resistance or its impacts on farmers and national economies, and the absence of powerful lobbying and advocacy groups to represent those most affected.

In practice, a combination of strategies are often recommended and used. A recent review of 177 antimicrobial resistance related studies, came to the main conclusion that that there is no single resistance-combating strategy that is likely to be completely effective. Rather, multidisciplinary antimicrobial programmes (that include a combination of the above interventions) may be the most effective approach to countering antimicrobial resistance (Smith *et al.,* 2001).

These are general strategies for antimicrobial resistance, In the specific case of trypanocide resistance, guidelines for action at different levels of resistance have been suggested (Table 3.8). Repeating treatments was found to be effective but impractical because of the need for monitoring relapses (Ilemobade and Na Isa, 1981); this strategy may be worth revisiting in light of alternative service delivery mechanisms. Increasing dosage was not found useful in the field (Ilemobade, 1979), and there was concern that high doses of DIM would produce *sterilisatia magna*, or increased sensitivity to infection resulting from treatment (Rüchel, 1975). However, in mice experimentally infected with DIM-resistant trypanosomes, the use of higher doses of DIM resulted in higher curative rates and when there was no complete cure, life span was prolonged (Mdache *et al.,* 1995), suggesting regimes with increased dosage may be worth revisiting. A recent report found that repeating treatment with HOM cured mice infected with resistant *T. congolense*, when a single-dose of HOM, even at high levels (60 mg/kg) was not effective (Silayo *et al.,* 2005). In the case of ISMM the narrow therapeutic index limits potential to increase dose and frequency; hepatotoxicity and fatalities have been reported after repeated treatments (Eisler *et al.,* 1997b). The use of dextran complexes allows a high dose to be given without attendant toxic reactions, but the period of protection is short (Aliu and Chineme, 1980). Changing the trypanocide may be useful when cross-resistance does not exist; this principle underlies “sanative treatments” that is, giving sequential treatments with two trypanocides that do not show cross-resistance (Whiteside, 1962). This was successfully used in Nigeria until undermined by the emergence of multiple resistance. Packaging trypanocides as single doses has been recommended and also not using prophylactic trypanocides as curatives (Touratier, 1985). Ready-to-use formulations have been developed which improve dosing and injection hygiene, reducing the risk of resistance. Unfortunately the higher price of these preparations has meant that demand is extremely low

(Sones, 2005). Actions to counter resistance must not only be effective but also acceptable and affordable; factors largely ignored in the drawing up of guidelines for trypanocide resistance.

Table 3.8 Guidelines for drug resistance (modified from ICPTV, 1999)

<table>
<tr><th colspan="2">Resistance to a single trypanocide</th><th>Multiple resistance</th><th>Use of ISMM</th></tr>
<tr><td>0 % resistance prevalence</td><td>Sanative pairs
Vector control
Avoid high risk areas
Avoid block treatments</td><td rowspan="4">Stop use of quinapyramine in cattle

Only treat clinical cases

Intensify vector control

Consider zero-grazing or fly-proof stabling

Increase the proportion of trypanotolerant cattle</td><td rowspan="4">Avoid the use of continuous ISMM prophylaxis

Consider the use of prophylaxis only in cattle exposed to heavy challenge for a defined period, e.g. transhumance or high seasonal challenge

Never administer ISMM more frequently than every 3 months</td></tr>
<tr><td>1 – 30%</td><td>Sanative pairs
Treat only clinical cases
Investigate trypanocide use
Vector control
Temporal and spatial surveillance</td></tr>
<tr><td>31 – 60%</td><td>Use sanative pair
Monitor treatment outcomes
Treat inter-current infections
Vector control
Improve husbandry and nutrition
Trypanotolerant animals</td></tr>
<tr><td>>60%</td><td>Treat only clinical cases
Zero-grazing
Vector control
Trypanotolerant breeds/species</td></tr>
</table>

Containment of resistance

While an armentarium of RDU interventions offers hope for prevention of resistance, there is less information on containing or removing resistance when established. However, containment of resistance in hospitalt facilities is relatively well documented (Goldman *et al.,* 1996). A recent paper reviewing 230 studies concluded that with appropriate actions, resistant organisms could be effectively controlled and even eliminated. At community level, there is reasonable evidence that when irrational drug use is controlled, resistance levels decline (Binyon and Cooke, 2000); several studies show evidence of a ‘critical threshold’ of use below which resistance declines even without total removal of the antimicrobial (Seppela *et al.,* 1997). But in general once resistance is established, it is difficult to remove and prevention is considered preferable. While much is known about the management of antibiotic resistance, to date no studies have been carried out on containing trypanocide resistance and there are important unanswered questions on the mechanisms of resistance (single or multiple), genesis of resistance (uni- or multi–focal), spread of resistance to new areas (role of vectors and cattle movement) and persistence of resistance. But it is known that AAT cannot persist in the absence of tsetse and so vector eradication should be an effective means of eliminating resistance. Methods for eradication of localised tsetse populations are highly effective and community-based bait methods, using insecticide-treated cattle and traps, are particularly attractive being low-cost, environmentally-friendly and empowering to local communities; these could be used to eliminate ‘pockets’ of resistance, although the low sustainability of vector control throws doubt on its appropriateness as a long-term, large-scale

trypanosomosis control operation (Brightwell *et al.,* 2001; Randolph *et al.,* 2003). Uncertainty surrounding the effectiveness and permanence of resistance containment can be reduced by complementing epidemiological field studies with mathematical models (Austen and Anderson, 1999). Models have been successfully developed for modelling relationships between antibiotic use and resistance and have proved useful for designing more effective resistance control programs (Bonten *et al.,* 2001).

3.5 Modelling trypanosomosis control and drug resistance

Quantitative, population-based approaches to trypanosomosis epidemiology and control are under-researched (Anderson and May, 1991), and greater and more sophisticated combination of theory, models, diagnostics and field studies will provide researchers with a framework for better understanding the epidemiology of trypanosomosis and for making predictions of the likely effects of implementing different control options (McDermott and Coleman, 2001). Mathematical models are formal frameworks expressing relations between factors involved in disease.

3.5.1 SEIR models

Compartmental prevalence models of diseases start from the basic premise that the population can be subdivided into a set of distinct classes (compartments), dependent upon their experience with respect to the disease. The basic model classifies individuals as Susceptible, or Infectious or Recovered (and not susceptible, i.e. immune); this is the SIR model (Anderson and May, 1991). Where disease incubation is a feature, a category for Exposed (incubating, not infectious) can be added (the SEIR model). The rate at which individuals move from one compartment to another can be modelled by differential equations; the transition rate from susceptible to infected is the force of infection, and from infected to recovered is the rate of recovery. Many permutations in compartments and transitions are possible.

To model a specific disease such as AAT it is first necessary to have a complete picture of the biology of the disease (e.g., the duration of the period of infectivity, incubation period, immune status after infection). Next data on the demographic, epidemiologic, and biologic characteristics of the infection (transition rates) and the population (birth, death, in-migration, out-migration rates) are added. Finally, a parsimonious model is constructed. Modelling can give insight into why and under what conditions a disease will persist, and the anticipated levels of disease under different scenarios. Models for trypanosomosis have been adapted from the Ross-Macdonald model of malaria transmission (Ross, 1911; MacDonald, 1957), first by Rogers (1988) and subsequently by Milligan and Baker (1988), Milligan (1990) and McDermott and Coleman (2001). Different compartments and transition paths are used in these models: Tables 3.9 and 3.10 present a summary of the state variables and dynamic equations used for modelling AAT.

An important parameter is the basic reproductive ratio, R_0, defined as the average number of secondary cases caused by an infectious individual in a totally susceptible population. As such, R_0 predicts the initial rate of increase of the disease over a generation. When R_0 is greater than 1, the

disease can enter a totally susceptible population and the number of cases will increase, whereas when R_0 is less than 1, the disease will always fail to spread. Therefore, in its simplest form R_0 tells us whether a population is at risk from a given disease. The derivation R_0 for AAT is shown in Table 3.10; the importance of bite rate (w) is evident.

Table 3.9 Description of state variables (all duration in days)

n	Incubation period cattle	w	Bite rate of tsetse on cattle per day
a	Detection of illness	t	Proportion cattle treated ISMM
i	Duration of illness in cattle	c	Proportion detected cattle treated DIM
f	Proportion of animals who self-cure	v	Bites on host causing infection in vector
j	Time to self-cure	b	Vector bites causing infection in host
m	Case fatality	G	Vector population
h	Proportion of tsetse meals from cattle	o	Incubation period in tsetse
u^{-1}	Life expectancy of tsetse	p	Duration immunity/protection in cattle

Table 3.10 Dynamic disease equations and basic reproductive ratio for AAT

$\delta S/\ \delta t = R.p^{-1} – S.h.w.b.I.V.H^{-1} - S.t + I.f.j^{-1}$ $\delta E/\ \delta t = S.h.b.w.I.V.H^{-1} – E.t - E.n^{-1}$ $\delta I\ /\ \delta t = E.n^{-1} - I.a.c – I.t - I.m – I.f.j^{-1}$ $\delta R/\ \delta t = I.t + I.a.c + E.t + S.t - R.p^{-1}$	$R_o = \frac{b.\exp(-u.o)\ .\ G.w^2.v.i}{u.\ (S+E+I+R)}$

Modelling can give important insights into disease control. MacDonald's mathematical model for malaria predicted that adulticides are more effective than larvicides in malaria control. Because DDT had just become available, this conclusion was of enormous practical significance, arguably, "*the single most important insight into public health from modelling*" (Bradley, 1982). Rogers' model showed sleeping sickness could not be maintained in humans alone and an animal reservoir was required, and predicted that sudden removal of domestic animals from a village could result in a mini-epidemic of sleeping sickness; both findings with important implications. McDermott and Coleman (2001) were able to predict the necessary cover (80%-90%) of the cattle population by a hypothetical vaccine in order to provide herd immunity and effectively control trypanosomosis. Coleman *et al.* (1999) also assessed the relative impact of mass treatment of cattle versus the treatment of human cases on the prevalence of sleeping sickness in South-Eastern Uganda and concluded mass treatment of cattle with coverage of 80% would break the transmission of Rhodesiense sleeping sickness to humans.

Although these results are interesting, mathematical models for trypanosomosis have yet to be convincingly validated in the field or make a significant contribution to the policy or practice of trypanosomosis control. The substantial input requirements of models in terms of detailed disease epidemiology are only partially met in the case of AAT and models for vector transmitted disease that rely heavily on assumptions may diverge too far from reality to give accurate results (Dye, 1992). Despite these reservations, models are useful tools for understanding, if not always predicting, epidemiology, and have at least the potential to improve management of trypanosomosis.