Trypanosomosis, the disease and its control

– *An analysis of a new tsetse-repellent technology*

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ABSTRACT

Trypanosomosis, a disease caused by protozoan parasites of the genus, *Trypanosoma* and transmitted cyclically by the tsetse fly (*Glossina spp*) is arguably still the one of the main constraints to livestock production on the African continent, preventing full use of the land to feed the rapidly increasing human population.

Sleeping sickness, the disease caused in humans by other species specific of Trypanosoma, is an important yet neglected disease which poses a threat to millions of people in tsetse infested areas.

Current trypanosomosis control relies on trypanocidal drugs, use of trypanotolerant cattle breeds and control of the vector, namely the tsetse fly. None of these methods have the full potential to work in the long-term control of the disease. Most heavily relied on are the trypanocidal drugs and this has lead to an increasing problem with resistence in the target organisms.

A major project is underway in Kenya testing an alternative trypanosomosis control option. Research at the International Center of Insect Physiology and Ecology (ICIPE), has lead to the development of a tsetse fly repellent which is now being tested by ICIPE and the International Livestock Institute (ILRI) in a three year study. The goal is to take the repellent from the laboratory out to the farmers whose livestock are at risk of trypanosomosis.

The objectives of this report were to study Trypanosomosis and its control, and to join research staff from ILRI to take part in the ongoing project during field studies in Narok and Nguruman, Kenya.

The work was carried out as a Minor Field Study (MFS) at the Epidemiology center, ILRI, Nairobi, under the supervision of Dr. Thomas Randolph and PhD students Bernard Bett and Patrick Irungu.

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INTRODUCTION

Tsetse transmitted trypanosomosis in Africa is a serious constraint to both animal agriculture and human health. About 10 million square km of sub-Saharan Africa are infested by tsetse extending through 38 countries, and 30% of approximately 147 million cattle in the continent are said to be at risk from trypanosomosis (Murray and Gray 1984).

The incidence and severity of the disease in different regions are dependent upon local conditions. In some areas virtually no economic livestock development is achievable due to the disease. The impact of trypanosomes on African agriculture is most obviously felt at the herd level leading to reduced milk output, reduced live animal output and reduced efficiency of animals used for cultivation. In susceptible cattle breeds, the disease reduces calving by up to 20% and causes death of young stock. Meat and milk output is reduced by at least 50%(Swallow 2000).

Pastoral and agro-pastoral communities in Sub-Saharan Africa comprising predominately of small scale holders accounts for 20 and 240 million individuals respectively. Livestock constitutes the backbone of their livelihood, lifestyle and culture. Livestock production among these communities however are beset by technical, social, economical and disease constraints. Trypanosomosis is one of these constraints.

Trypanosomosis control has been based on the use of trypanocidal drugs, introduction of trypanotolerant cattle breeds and tsetse control using insecticides on traps/targets or livestock. All these methods have limitations.

The International Livestock Research Institute (ILRI), Kenya, is a research center within the Consultative Group of International Agricultural Research (CGIAR). CGIAR works with the common goals of “poverty alleviation, food security and environmental protection”. ILRI have several ongoing projects that focuses on Trypanosomosis and its control. The project described in this report concerns the testing of a new tsetse repellent control developed by researchers at the International Center for Insect Physiology and Ecology (ICIPE).

Objectives

The objectives of this report was to study African Animal Trypanosomosis in general and its control, and also to take part in the work done by ILRI in the field during the assessment of a new tsetse-repellent technology. The first part of the report is a literature review of trypanosomosis and its epidemiology and the second part is a description of the tsetse-repellent project.

TRYPANOSOMOSIS

African animal trypanosomosis (AAT) is a disease complex caused by tsetse fly transmitting *T.congolense*, *T.vivax* or *T.brucei* or simultaneous infection with one or more of these organisms. AAT is most important in cattle but can cause serious losses in pigs, camels, goats and sheep. Trypanosomes infects a wide range of hosts including wild and domestic animals which represents reservoirs for the parasite**.**

Infection results in subacute, acute or chronic disease characterized by intermittent fever, anemia, occasional diarrhea and rapid loss of condition and often terminates in death. In southern Africa the disease is widely known as" Nugana" which is derived from a zulu term meaning to be in low or depressed spirits.
Trypanosomes replicate in the tsetse fly and are transmitted through tsetse saliva when the fly feeds on an animal.

Human sleeping sickness

Human trypanosomosis is a major threat to human health in Africa. Approximately 35-55 million people in 36 African countries are at risk but only about 3 million of them are under surveillance (WHO 1979). Two species of salivarian trypanosomes causes infection in humans. Both of them causes "sleeping sickness" through invasion of the central nervous system (CNS). *T.rhodesiense* usually occurs with acute syndromes while *T.gambiense* infection may be initially asymptomatic, although at a later stage it affects the CNS (Urquhart *et al* 1996).

Humans are infected following a fly bite which occasionally causes a skin chancre at the site. The injected trypanosomes mature and divide in the blood and lymphatic system, causing symptoms of malaise, intermittent fever, rash and wasting. Eventually, the parasitic invasion reaches the CNS, causing behavioral and neurological changes. Death may occur (Kiminyo 2002).

Taxonomy

PHYLUM	Protozoa
SUBPHYLUM	Sarcomastigophora
CLASS	Zoomastigophorea
ORDER	Kinetoplastida
SUBORDER	Trypanostomatina
FAMILY	Trypanosomatidae
GENUS	Trypanosoma

Identification

The trypanosomes are elongated spindle-shaped protozoa ranging from 8.0 to 39 micrometers in length. All posses a flagellum which arises at the posterior end of the trypanosome from a basal body at the foot of a flagellar pocket. The flagellum runs to the anterior end of the body and is attached along its length to the pellicle to form an undulating membrane.The flagellum may continue forward as a free flagellum. Within a stained specimen a single centrally placed nucleus can be

seen, and adjacent to the flagellar pocket, a small structure, the kinetoplast, which contains the DNA of the single mitochondrion (Urquhart *et al* 1996).

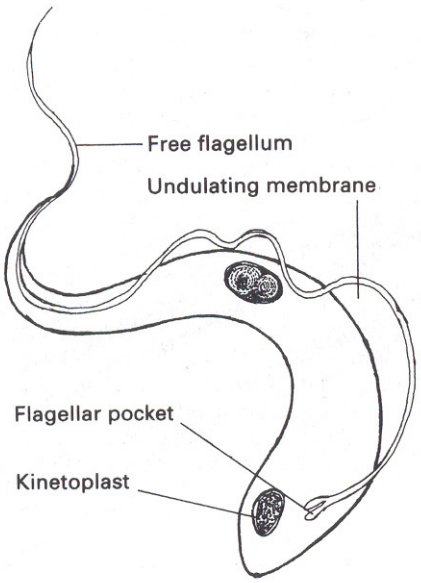

Fig 1 Trypanosome (Urquhart 1996).

T.congolense is monomorphic in form and 8.0 to 18 micrometers in length. It has no free flagellum and the undulating membrane is inconspicuous. The posterior end has a blunt shape.

T.brucei is pleomorphic in form and can be seen in long slender, short stumpy or intermediate forms. Size wise it ranges from less than 18 to more than 39 micrometers in length. The slender form has a well developed free flagellum while in the stumpy form it is either short or absent. It has a pointed posterior end and an obvious undulating membrane.

T.vivax is monomorphic in form ranging from 20 to 27 micrometers. The undulating membrane is inconspicuous, the posterior end is broad and rounded and it has a short free flagellum (Urquhart *et al* 1996).

When studying the trypanosomes under the microscope *T.brucei* and *T.vivax* can be separated on the basis of their movement. In fresh unfixed blood films *T.brucei* moves rapidly within small areas of the microscope field whilst *T.vivax* moves rapidly across the whole field.

Life cycle of trypanosomes

Trypanosomes are parasites with a two-host life cycle: one mammalian and one arthropod. Tsetse flies ingest trypanosomes present in the blood or lymph while feeding on an infected host. Thereafter the trypanosomes lose their glycoprotein surface coat, and in the case of *T.brucei* and *T.congolense*, become elongated and multiply in the midgut before migrating forward to the salivary glands (*T.brucei*)

and the proboscis (*T.congolense*).There they undergo a transformation losing their typical trypanosome, or trypomastigote, form and acquire an epimastigote form characterized by the fact that the kinetoplast lies just in front of the nucleus. After a further multiplication of the epimastigotes they transform again into small, typically trypomastigote forms with a glycoprotein surface coat. These are the infective forms for the next host and are called metacyclic trypanosomes. The entire process takes at least two or three weeks and the metacyclic trypanosomes are inoculated into the new host when the tsetse fly feeds (Urquhart *et al* 1996).

With *T.vivax* a similar process of cyclic development takes place except that it occurs entirely within the proboscis.

At the site of inoculation the metacyclic forms multiply locally as the typical blood forms, producing within a few days a raised cutaneous inflammatory swelling called a chancre. Thereafter they enter the bloodstream, multiply and a parasitaemia, detectable in the peripheral blood, usually becomes apparent 1-3 weeks later. Subsequently, the parasitaemia may persist for many months although its levels may change due to the immune response of the host (Urquhart *et al* 1996).

Trypanosomes use cyclic transmission where the arthropod vector is a necessary intermediate host in which the trypanosomes multiply, undergoing a series of morphological transformations before forms infectious to the next mammalian host are produced. When multiplication occurs in the digestive tract and proboscis, so that the new infection is transmitted when feeding, the process is known as anterior station development and the various species of trypanosomes which use this process are often considered as a group, the Salivaria. All are trypanosomes transmitted by tsetse flies, the main species being *T.congolense, T.vivax* and *T.brucei*.

In other trypanosomes, multiplication and transformation occurs in the gut and the infective forms migrate to the rectum and are passed with the faeces, this is posterior station development and the trypanosome species using this process are the Stercoraria. In domestic animals these are all relatively non-pathogenic trypanosomes such as *T.theileria* and *T.melophagium* transmitted by tabanid flies and keds respectively.

Non-cyclical transmission is essentially mechanical transmission in which the trypanosomes are transferred from one mammalian host to another by the interrupted feeding of biting insects, notably tabanids and stomoxys. The trypanosomes in or on the contaminated proboscis do not multiply and die quickly so that cross contamination is only possible for a few hours (Urquhart *et al* 1996).

The salivarian trypanosomes, normally transmitted cyclically in tsetse flies may on occasion be transmitted mechanically (FAO 1998). The importance of this mode of transmission is variable from place to place, depending on the numbers of hosts and biting insects present, and also on the species of trypanosome. Large biting insects such as tabanids carry more blood and are more likely to act as mechanical vectors than for example mosquitoes. Tsetse themselves can also act as mechanical vectors.

Finally apart from classical cyclical and non-cyclical transmission, dogs, cats and wild carnivores may become infected by eating fresh carcasses or organs of animals which have died of trypanosomosis, the parasite penetrating oral abrasions (Urquhart *et al*1996).

The vector

Tsetse flies are classified into one genus - *Glossina,* of the family *Glossinidae*, order *Diptera* – the two winged flies.
The three main species of tsetse flies for transmission of trypanosomes are *G.morsitans* which favors the open woodlands of savannah*, G.palpalis* which prefers the shaded habitat immediately adjacent to rivers and lakes and *G.fusca* which favors the high dense forest areas (Leak 1998).

The most important subspecies from each group are:
G.Morsitans*: G.swynertoni, G.pallidipes and G.morsitans submorsitans*
G.Palpalis*: G.fuscipes, G.tachinoides and G.palpalis gambiense*
G.Fusca*: G.brevipalis and G.longipennis.*

The *morsitans* group is of great importance in the transmission of animal trypanosomosis and the *palpalis* group in the transmission of human sleeping sickness (Rogers1991).

Adult *Glossina* species are dull in appearance, varying in colour from a light yellowish brown to a dark blackish brown. In some species the abdomen may have alternate darker and lighter bands. The smallest species is 6-8mm long and the largest 10-14 mm (Jordan 1986).

The adult female produces a single egg, which hatches to a first stage larva in the uterus. After a period of development and moulting a third stage larva is deposited on the ground. Females produce one full grown larva every 9-10 days which then pupates in light or sandy soil. The adult fly will emerge after a puparial period that varies according to temperature but may be around 30 days at 24 degrees Celsius. Consequently tsetse flies have a very low rate of reproduction, closer to that of a small mammal than to most insects. The reproductive method of tsetse flies is known as adenotropic viviparity (FAO 1998).

When a tsetse fly hatches from its pupal case it is free from trypanosomes. Until its first blood meal, it is called a teneral fly and after its first meal it is called non-teneral. It acquires a trypanosomal infection when it feeds on a parasitaemic mammalian host. The trypanosomes undergo a cycle of development and multiplication in the digestive tract of the fly until the infective metacyclic trypanosomes are produced.

Different trypanosomes develop in different regions of the digestive tract of the fly and the infective metatrypanosomes occur either in the biting mouthparts or the salivatory gland of the fly. The period from ingesting infected blood to the appearance of the infective forms varies from one to three weeks and once infected metatryps are present the fly remains infective for the remainder of its life. During the act of feeding the fly penetrates the skin with its proboscis. By the rupture of small blood vessels a pool of blood is formed in the tissue and the fly

injects saliva to prevent coagulation. Infection of the host takes place at this stage, with infective metayclic trypanosomes in the saliva.

Tsetse flies once infected with trypanosomes are likely to transmit the parasite for the remainder of their lives.

Identification of trypanosomosis in tsetse

The method most commonly used for detecting trypanosome infections in tsetse flies is that of dissection. The technique consists of dissecting out the organs of the tsetse fly in which trypanosomes develop, then examining them by microscopy. These organs are the midgut, salivary glands and mouthparts (specifically the labrum and hypopharynx). The type of trypanosomes present is usually determined solely according to the locations within the fly in which they are found. Thus an infection in the labrum or hypopharynx alone (the proboscis) is considered to be *vivax* type, infection in the proboscis and midgut the *congolense* type and infection of the proboscis, midgut and salivary glands a *brucei complex* infection.(Leak 1998)

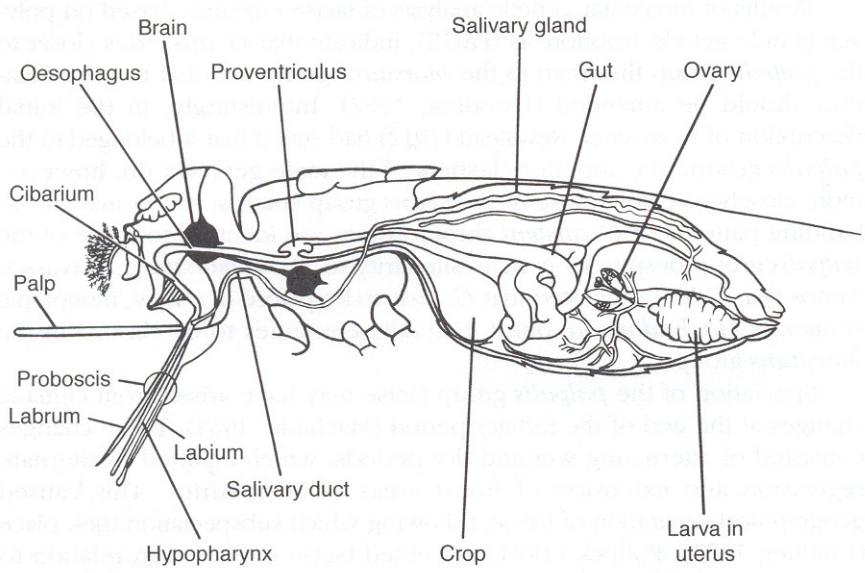

Fig 2 Basic anatomy of tsetse fly (female) (Leak 1998).

Pathogenesis

Initial replication of trypanosomes is at the site of inoculation in the skin, this causes a swelling and a sore (chancre). Trypanosomes then spread to the lymph nodes and blood and continue to replicate. *T.vivax, T.congolense* and *T.brucei* are characteristically present in the bloodstream. *T.brucei* is also found extravascularly in for example the myocardium, the central nervous system and the reproductive tract.

Lymphoid enlargement and splenomegaly develop associated with plasma cell hyperplasia and hypergammaglobulinaemia, which is primarily due to an increase in IgM. Concurrently there is a variable degree of suppression of immune responses to other antigens such as microbial pathogens or vaccines. Ultimately,

in infections of long duration, the lymphoid organs and spleen become shrunken due to exhaustion of their cellular elements.

Anemia is a cardinal feature of the disease, particularly in cattle, and initially it is proportional to the degree of parasitaemia. It is hemolytic in that the red blood cells are removed from the circulation by the expanding mononuclear phagocytic system. Cell degeneration and inflammatory infiltrates occur in many organs such as skeletal muscle and the CNS, but perhaps most significantly in the myocardium where there is separation and degeneration of the muscle fibers (Urquhart *et al* 1996).

The response of antibodies developed to the glycoprotein coat of the trypanosomes kills the parasites and results in the development of immunocomplexes. Antibodies however do not clear the infection since trypanosomes have genes that can code for a number of different surface-coat glycoproteins and therefore changes its surface antigenic makeup to evade the antibodies. Thus there is a persistent infection that results in a continuing cycle of trypanosome replication, antibody production, immunocomplex development and changing surface-coat glycoproteins.

Immunologic lesions are significant in trypanosomosis and it has been suggested that many of the lesions (eg anemia and glomerulonephritis) in this disease may be the result of deposition of immune complexes that interfere with, or prevent, normal organ function. Profound immunosuppression occurs following infection and this lowers the hosts resistance to other infections and thus results in secondary disease.

Clinical signs

Because simultaneous infection with more than one trypanosome species are very common and simultaneous infection with trypanosomes and other hemoparasites (*Babesia spp,Theileria spp, Anaplasma spp* and *Ehrlichia spp*) frequently occurs it is difficult to conclude which clinical signs are attributable to a given parasite.
Anaemia is for example seen in a whole series of diseases caused by blood parasites (in particular *Babesia* and *Anaplasma*) as well as in certain gastro-intestinal helminthes (*Haemonchus contortus*) and it is therefore not typical of trypanosomes by itself (Nyenko *et al* 1990).

T.vivax has a variable incubation period and although it is considered to be less virulent for cattle than *T.congolense*, mortality rates of over 50% can occur. There seems to be a marked variation in virulence of different strains of *T.vivax* but it remains the most important cause of trypanosomosis of cattle, sheep and goats in western Africa. It causes mild disease in horses and chronic disease in dogs.

T.brucei has a relatively short incubation period and causes severe to fatal disease in horses, camels, dogs and cats. It usually causes mild, chronic or subclinical disease in cattle, sheep, goats and pigs (Urquhart *et al* 1996).

The cardinal clinical sign observed in African animal trypanosomosis is anaemia. Also invariably present are intermittent fever, edema and loss of condition. Abortion is seen and infertility of males and females may be a sequel. The

severity of the clinical response is dependent on the species and the breed of the affected cattle and the dose and virulence of the infecting trypanosome. Stress such as poor nutrition, or concurrent disease, plays a prominent role in the disease process.

Within a week of infection with the hematic trypanosomes (*T.congolense* and *T.vivax*) there is usually a pronounced decrease in packed cell volume, hemoglobin, red blood cells and white blood cell levels and within 2 months these may drop to below 50% of their preinfective values (FAO 1998).

Anaemia

At an early stage there is phagocytosis of red cells by the white cells of the host. The red cells apparently become coated with material from lysed trypanosomes which tricks the phagocytes into mistaking them for foreign invaders and remove them (autoimmunity). It is possible that the anaemia caused by phagocytosis is increased by toxic substances emanating from the trypanosomes, which destroys cells directly by lysis (FAO 1998).

The hematopoietic system tries to compensate for loss of erythrocytes by increasing its activity but later in the chronic stages of trypanosomosis, other toxins from the parasite have a depressing effect on the hematopoietic system and the host is unable to produce as many red cells as are removed. The anaemia means a reduction in hemoglobin and therefore in the oxygen carrying capacity of the blood. Insufficient oxygen is available to the cells and the efficiency of their normal activities is reduced. A slow process of deterioration of health and condition sets in.

Immunodepression

Immunodepression can be explained in part by the depression of the hematopoietic system but as it occurs also in the acute stage, the ability of the immune system to react to invaders seems to be diminished before the hematopoietic system is depressed. Animals affected by trypanosomes often develop a lower antibody titre after vaccination against other diseases and secondary infections which the host would normally control may also arise during the disease (FAO 1998).

Wasting

During the acute stage the appetite is variable, decreasing during the fever peaks. In the chronic stage when the fever reactions are less pronounced, the appetite is usually normal, almost until death even when extreme weakness prevents the animal from rising. The pronounced wasting is therefore not caused by starvation. There is consumption of the fat reserves during the recurring bouts of fever, but there is also severe degenerative changes of the muscle cells and other tissue cells and there is an increased breakdown of protein in muscles and elsewhere leading to atrophic degeneration. The decreasing supply of oxygen is also an important factor.

Pathology

No pathognomonic changes are seen. Anemia, edema and serous atrophy of fat are commonly observed. Subcutaneous edema is particularly prominent and is usually accompanied by ascites, hydropericardium and hydrothorax. The liver may be enlarged, and edema of lymph nodes is often seen in the acute disease but they may be reduced in size in the chronic stage. The spleen may be swollen, normal or atrophic. Necrosis of the kidneys and heart muscle and subserous petechial hemorrhages commonly occur. Gastroenteritis is also common, and focal polioencephalomalacia may be seen.

The hematic trypanosomes (*T.congolense* and *T.vivax*) cause injury to the host mainly by the production of severe anemia, which is accompanied in the early stages of the disease by leucopenia and thrombocytopenia. In the terminal stages of the disease caused by the hematic trypanosomes, focal polioencephalomalacia probably results from ischemia due to massive accumulation of the parasites in the terminal capillaries of the brain.

The lesions resulting from *T.brucei* (a tissue parasite) infection are remarkably different from those seen with the hematic trypanosomes. Anemia is an important lesion, but much more dramatic are the inflammatory degeneration and necrosis resulting from cellular invasion of various organs. Marked proliferative changes reflecting immune response are observed in most body tissues.

In cattle *T.vivax* occasionally causes a hyperacute hemorrhagic form of trypanosomosis. Prior to death animals are bleeding from many sites throughout the body, and at post mortem hemorrhages are widespread and extensive. The intestinal tract, from the abomasum to the rectum, contains large amounts of blood. Signs of bleeding are seen beneath the lining of various organs – the heart, pleural cavity, peritoneum, diaphragm – virtually every organ and tissue. The disease progresses so quickly to death that there is no loss of condition (FAO 1998).

Diagnosis

Trypanosomosis should be suspected when an animal in an endemic area is anemic and in poor condition. Confirmation depends on the demonstration of the organism in blood or lymph node smears.

In the early phases of infection especially with *T.vivax* and *T.congolense*, the parasite can readily be observed by microscopic examination of a wet-mount of blood slides. Thick blood films stained with Giemsa are also a good technique, but in thin fixed blood films which are favored for species identification, the parasite may be hard to demonstrate.

The haematocrit centrifugation technique plus dark ground/phase contrast buffy coat method (BCT method) concentrates parasites by centrifugation of blood, collected with heparin or EDTA, in microcapillary tubes. Trypanosomes are found in the buffy coat (the thin layer of white blood cells at the interface of the red cells and the plasma). When parasitaemia is low these techniques can be useful for demonstration of the parasite.

The sample may be examined either within the capillary tube (Woo technique) or by making a smear of the buffy coat after cutting the capillary at the interface (DG technique). For the DG technique, the smear is examined using dark ground or phase contrast microscopy under which live motile trypanosomes show up more clearly. Because *T.congolense* tends to associate with erythrocytes it is essential that buffy coat and adjacent erythrocytes be included in the smear. The packed red cell volume of the animal should also be measured before examination to aid in diagnosis (Leak 1998).

Stained lymph node smears are a method for diagnosis especially for *T.vivax* and *T.brucei*. In chronic *T.congolense* infections, the parasite localize in the microcirculation of the lymph nodes and in other capillary beds, allowing diagnosis by examination of lymphnode smears or smears made with blood collected from the ear. Early in infection, blood smears are optimal for demonstration of *T.congolense*.

These conventional techniques of microscopic examination for the presence of trypanosomes are still widely used but newer and far more sensitive methods are beginning to supplant them. Microscopic examination of blood samples is a direct method as it depends on actual detection of the parasite, whereas immunological methods detect parasites indirectly through antibodies or antigens.

Indirect serological methods:

- immunoflourescent antibody test (IFAT): The IFAT developed for trypanosomosis (Bailey *et al* 1967) uses known trypanosome antigens to coat glass slides or microtitre plate wells. Blood samples to be tested are added to the wells and incubated and then conjugate is added. After washing, the wells are examined with a fluorescence microscope. Apple green fluorescence occurs with positive blood samples.

- card agglutination test for trypanosomes (CATT): The CATT test for human trypanosomosis relies on the agglutination of formalin-fixed, stained and freeze dried trypanosomes by antibodies in the patients serum. The major antigen contributing to the test is the variant surface glycoprotein (VSG).

- antibody ELISA: antigens are adsorbed on to micro-ELISA plate wells ;in the presence of an enzyme-conjugated antiglobulin, the test serum reacts after incubation to give a chromogen-mediated colour change normally read with an ELISA reader. The test does not distinguish between current and previous infections as circulating antibodies remain in the host for some time after an infection is cured.

- antigen ELISA: In an attempt to identify current trypanosome infections more accurately, tests were developed to detect circulating antigens released by parasites in the blood of infected animals (Nantulya 1990) An ELISA plate is coated with monoclonal species-specific antibodies and then test serum is added. The coating antibody captured the antigen in the serum. A second enzyme-labeled antibody is then added which binds to

free combining sites of the captured antigen. Excess labeled antibody is then washed off and the reaction is revealed by addition of a substrate and chromogen.

- Polymerase Chain Reaction (PCR): The PCR technique for amplifying DNA samples has been developed as a diagnostic test for a number of parasites, trypanosomes amongst them. Trypanosomosis can be detected in both tsetse and cattle using PCR and DNA probes. PCR diagnostic tests are at present not sufficiently simple or reproducible for widespread use and are likely to be too expensive and technically demanding for general use in developing countries.

- DNA probes: The principle of DNA hybridization is that a single-stranded DNA fragment containing the specific DNA sequences is identified and preferably purified. It is then labeled, for example with a radioisotope, and used to probe whole parasite DNA or whole organisms. Prior to application of the probe, the test parasite DNA is denatured and split into single strands. When the probe is applied, the sequences in the probe will hybridize with complementary DNA sequences of the parasites. The results are then revealed by autoradiography. Species-specific DNA probes have been shown to detect simultaneous infection of cattle with *T.vivax, T.brucei* and *T.congolense* when conventional methods revealed only single infection

.

- latex agglutination antigen test: The principle of the test is that latex particles are sensitized with species-or subgenus-specific anti-trypanosome antibodies. If the relevant antigens are present in whole blood, plasma or serum, the antibodies capture the antigens. As there are several combining sites on the antigen molecules, aggregates form, which can be detected as an agglutination reaction. This test is simple to use under field conditions (Leak 1998).

TRYPANOSOMOSIS CONTROL

Attempts to control trypanosomosis has been ongoing for the last hundred years. Early methods involved a combination of clearing vegetation, exterminating wildlife hosts and spraying of bushes. However these early control methods involved destruction of valuable resources and new and environmentally safer methods were developed. In Kenya two main control methods are in use:

- TSETSE FLY CONTROL for example odour baited insecticide treated targets and traps, insecticide treatment of animals either as a spray, dip or pour on, ground spraying, sterile insect technique and selective bush clearing.

- TRYPANOSOME CONTROL including trypanocidal drugs for curative and/or prevention, controlled grazing patterns, human sleeping sickness surveillance and use of trypanotolerant breeds of cattle.

Current control relies on three principal strategies: trypanocidal drugs, trypanotolerant cattle and tsetse control or eradication. Each has advantages and disadvantages, but generally none have proven to be fully satisfactory as viable, sustainable solutions.

Trypanocidal drugs

The control of trypanosomosis in domestic livestock depends mainly upon the use of drugs, either curatively or as a prophylactic. Resistance to the available drugs is on the increase and their continued use is expensive for livestock owners, it has been estimated that at least US$ 20 million (approximately 50 million doses) is spent annually to treat or protect animals exposed to trypanosomes in Africa. The actual amount of trypanocides used is difficult to estimate, particularly in recent years, since the distribution of trypanocides has become more decentralized, with a number of generic brands being sold, increasingly through traders and shopkeepers and less through official veterinary channels (Sones 1999).

Drugs currently recommended for chemotherapy of animal trypanosomosis come from only three closely related groups. These are the phenanthridines, isometamidium and homidium, and the aromatic diamidine, diaminazone. Only isometamidium and homidium are recommended for prophylaxis. The incidence of resistance to these drugs is apparently increasing and the main means of controlling the disease is therefore under threat (Peregrin1994).

Drug resistant trypanosomes develop through (i) under dosing, which may occur for a variety of reasons such as underestimation of animal body weight, over diluted solutions or incorrectly calculated dose volume.(ii) incorrect (and therefore ineffective) injection or (ii) an incorrect strategy of drug use (Leak 1998). Measures that may delay the development of drug resistance are to reduce the selection pressure on trypanosome populations by avoiding exclusive reliance on drugs for trypanosomosis control and avoiding mass treatment of livestock at short intervals.

Trypanotolerant livestock

At present**,** trypanotolerant livestock are only found in certain areas of West and Central Africa and although they retain a certain level of productivity under tsetse challenge conditions they are considered less productive in terms of meat and milk produced. Tolerant cattle have the ability to control parasitaemia and development of anaemia following a trypanosome infection. They are also known to be tolerant to streptothricosis, ticks and tick-borne diseases and to some extent, helminthiasis (D´Ieteren et al 1993).

In areas that are heavily infested with tsetse flies, or when a lower level of tsetse challenge is combined with other stress factors such as malnutrition, trypanotolerant breeds of livestock will need protection of trypanocidal drugs in order to be productive (FAO 2001).

Tsetse vector control

Tsetse vector control methods relying on large scale bush clearing and aerial spraying methods are no longer used due to environmental concerns.
Tsetse control currently relies on two bait systems: insecticide-treated traps and targets and insecticide treated livestock. Sterile Insect Technique (SIT) has also been used in efforts to eradicate tsetse flies in some areas.

Because of the stability of tsetse populations and their low reproductive rate, little sustained mortality pressure (additional to natural mortality) needs to be exerted on a population to cause its extinction (Weidhaas and Haile 1978).That makes them good candidates for traps and target control methods. Not to be forgotten though are the risk of reinvasion or immigration into an area already cleared of tsetse flies. The theory behind this control method is simple: the flies are visibly attracted to a trap or target, this attraction may be further helped by the use of olfactory attractants. When the tsetse lands on a trap or target they either receive a lethal dose of insecticide, or are caught in the trap and subsequently die (Leak 1998).

The effectiveness of traps and targets will depend on when the flies are active, how they move in their active state, whether they will move into the vicinity of a trap or target and finally, whether they are trapped or killed (Williams et al 1992).

Insecticide-treated livestock was developed as a method of tsetse control from the concept of baited traps and targets (Baylis and Stevenson 97). It is widely accepted by a majority of stockowners in Africa. The method has been used to control tsetse and trypanosomosis with varying results in Kenya (Stevenson et al 91). Most commonly used are the synthetic pyrethroids, and of these deltamethrin appears to be the most potent and it is also low in mammalian toxicity and has minimal environmental impact (Thompson et al 1991). Extensive use of insecticides on cattle for tsetse control appears to have the potential to interfere with endemic stability/immunity of cattle to several tick borne diseases. Thus the long term use of these products may jeopardize control of tick borne diseases (FAO 2001).

Sterile Insect Technique

Sterile Insect Technique (SIT) involves sustained and systemic release of sterile insects among the indigenous target population (FAO 2001). Males are sterilized by irradiation and then taken to a target area and released. Following mating with sterile males the females become infertile for the remainder of their life spans. By continually releasing sterile males in quantities over a time span that is sufficient to cover several generations of target populations, the fertile population is progressively reduced. Eventually, so few fertile insects remain that they cannot sustain the population. For maximum effectiveness, the sterile males released must outnumber the fertile native males by a considerable margin. One way this could be achieved is by suppressing the native population through other means before SIT (Bett 2003).

Vaccination

Attempts to vaccinate cattle against trypanosomosis started at an early stage in trypanosomosis research. Bevan (1928) for example tried to protect the animals by deliberately infecting them with known strains of trypanosomes and then treating them at various intervals following infection. These animals were said to be tolerant or to some extent "premunized" but the resistence broke down relatively easy. Attempts were also made to vaccinate cattle with killed trypanosomes by giving part-curative doses of trypanocidal drugs after infection, or by injection of small numbers of living trypanosomes.

These attempts failed and it is now recognized that the major obstacle to immunization lies in the phenomenon of antigenic variation in trypanosomes. Trypanosomes are covered by a dense coat of variant surface glycoproteins that stimulate antibody production in the host. The surface coat changes successively during the course of an infection, thus avoiding the immune response of the host. At present there are no drugs available that have the ability to interfere with antigen switching.

EPIDEMIOLOGY

The epidemiology of trypanosomosis depends on three factors, the distribution of the vectors, the virulence of the parasite and the response of the host.

The vector

The vectors: Of the group of *Glossina* flies, the savannah and riverine varieties are the most important since they inhabit areas suitable for grazing and watering. Although the infection rate of *Glossina* with trypanosomes is usually low, ranging from 1 to 20% of the flies, each is infected for life. Biting flies may act as mechanical vectors but their significance in Africa is still undefined (Leak 1998). Tsetse fly density is the most variable factor in the transmission of trypanosomosis. Climate affects tsetse abundance via one or more of four demographically important rates namely of birth, mortality, immigration and emigration (Rogers 1991).

Tsetse fly species differ in their susceptibility to trypanosomes and their subsequent ability, if infected, to transmit trypanosomes. For example, *G.fuscipes* appears to be a better vector of *T.vivax* to cattle than *G.pallidipes*, and *G.pallidipes* is a better transmitter of *T.congolense* than *G.swynnertoni* (Stephens 1986).

Tsetse flies prefer to feed on particular hosts-the bushbuck for example is much preferred whilst the waterbuck is not. Cattle inhabit a medium position. There are also differences within one host species in that trypanosome infected animals attract tsetse more than uninfected hosts (Baylis and Nambiro 1993)

The parasite

The parasite: Since parasitaemic animals commonly survive for prolonged periods, there are ample opportunities for fly transmission. Perhaps the most important aspect of trypanosomosis which accounts for the persistent parasitaemia

is the way in which the parasite evades the immune response of the host through antigenic variation.

The repeated switching of the glycoprotein coat is now known to depend on a loosely ordered sequential expression of an undefined number of genes, each coding for a different glycoprotein coat. This together with finding that metacyclic trypanosomes may be a variation of antigenic types each expressing a different genetic repertoire, explains why domestic animals even if treated successfully, are often immediately susceptible to reinfection (Urquhart1996).

The host

The mechanisms underlying bovine trypanotolerance remains mostly unknown however, immune response to trypanosomes greatly differs between susceptible and resistant cattle. Whereas the immunity has a genetic basis, the intensity of the tsetse challenge has a strong influence on the degree of tolerance. Trypanotolerance has been defined as the relative capacity of an animal to control the development of the parasites and to limit their harmful effects, the most prominent of which is anemia (Murray *et al* 83).

Both acquired and innate resistance to African trypanosomosis can occur in cattle. The two most important trypanotolerant breeds are the *Bos taurus* subtypes N´dama and Baoule, whilst a degree of trypanotolerance has also been shown to occur in some *Bos indicus* zebu breeds example the Orma boran and the Maasai zebu (Mwangi *et al* 93).

The effects of trypanotolerant cattle on trypanosomosis transmission have not been investigated. It might be expected that both the probability of a fly becoming infected from an infected cow, and cows becoming infected from an infected fly would decrease (McDermott and Coleman,2001).

Herd management is also important. Daily activity patterns of the tsetse and the grazing patterns of the herds are of great influence. If the herds graze on infested sites at the time of day that the flies are most active, transmission will occur more frequently. The risk to susceptible livestock living in comparatively free areas surrounded by tsetse belts, varies from year to year. Generally during wet years tsetse populations will increase, spread and persist during the dry season, even in areas from where they disappear in the dry years.

NEW TSETSE REPELLENT TECHNOLOGY

Recent research at the International Centre of Insect Physiology and Ecology (ICIPE), Kenya, has led to the development of an alternative technology for tsetse control, potentially reducing dependency on trypanocides as well as environmentally hazardous acaracides. A prototype of the technology has been developed. Field testing is now underway to properly adapt it to the needs and circumstances of the targeted livestock keepers and to formulate appropriate strategies for its use in the integrated control of trypanosomosis.

Scientists at ICIPE and the International Livestock Research Institute (ILRI) work together on this major study, which goal is to move the repellent technology out from the laboratory and into a finished product ready to be used by livestock keepers in Africa. This requires further refinement of the repellent technology through laboratory studies. Focus will also be put on how the repellent could be integrated with other existing control procedures. Part of this process will involve evaluating its economic and technical acceptance into farmer livestock production systems. At the same time, it will be important to assess how the repellant technology will be produced and delivered to livestock keepers. The specific objectives include:

1. Further refining the technology, including evaluating other repellents and designing better deployment techniques for long-term use.

2. Evaluating the repellents as components of integrated control strategies, particularly together with trypanocidal drugs and with traps/targets.

3. Assessing the potential adoption of repellent –based technologies among pastoralists and agro-pastoralists in Kenya through on-farm trials and economic impact assessment.

The ultimate goal to is to reduce poverty amongst pastoral and agro-pastoral communities in sub-Saharan Africa whose livestock are at risk of trypanosomosis by the development of a more cost-effective and environmentally acceptable tsetse control technique (ILRI/ICIPE 2001).

Research program

The study is organized around five principal activities:

1 Optimizing the tsetse repellent prototype through laboratory studies.

2 Development of an epidemiological model prototype: Related research at ILRI and the Trypanosomosis research center (TRC) on trypanosomosis infection dynamics will be applied to evaluate the expected impact of repellent-based control. The prototype will be used to make the appropriate design of field trials, and as the model is refined based on the results of those trials it can be used to formulate recommendations for the implementation of the technology under different conditions.

3 Refining the tsetse repellent technology: researcher managed field trials. A series of field trials will be designed and implemented to validate critical parameters and results derived from the epidemiological modeling regarding optimal integrated control strategies. At the same time, the performance of the repellent and the repellent dispenser, their placement and deployment on the animal for long term use, and their toxicological assessment with respect to animal health will be evaluated.

4 Socio-economic evaluation of best-bet control strategies in farmer managed trials: Preliminary results from the modeling and researcher managed field trials will be used to identify the most appropriate repellent based control strategies. These best-bet strategies will then be evaluated on-farm using a participatory research approach in partnership with livestock keepers and local livestock health services. This will permit assessing the economic and technical incentives that will drive eventual uptake of the technology, as well as provide feedback for further adaptive research to ensure the technology is appropriate for pastoralist production systems.

5 Development of a business plan for production and delivery.

The specific part of the project that I had the opportunity to join during my Minor Field Study in Kenya was: a socio-economic evaluation of best bet control strategies in farmer managed trials. This part of the project is led at ILRI by Dr.Tom Randolph, and the doctoral students Bernard Bett and Patrick Irungu.
The objective of this study is to analyze the impact of tsetse- repellent technology on the epidemiology of trypanosomosis and asses ways of integrating it with other tsetse and trypanosomosis control technologies with a view of identifying a strategy or strategies that are effective and sustainable in tsetse and trypanosomosis control. Field trials were under way at the time of my visit, in the Narok district and the Nguruman region involving Maasai pastoral communities.

The repellent

The technology is based on a potent phenolic analogue of a mild natural repellent of the savannah tsetse species. The repellent has been derived by molecular optimization studies to act as an olfactory antagonist of a kairomone which flies use to locate the host for feeding. This repellent has been shown to substantially reduce tsetse challenge (>80%) and feeding efficiency on cattle (>90%).

Two types of potent tsetse repellents have been identified:

a) a single component synthetic compound which was discovered from a structure activity study of the phenolic constituents of body odours of the tsetse bovid hosts and their aged urine and

b) a natural multi component blend of odour constituents specific to tsetse refractory waterbuck (refractory to *G.morsitans* and *G.pallidipes*).

The repellent dispenser

The dispenser was developed so that a constant release rate of the repellent could be maintained for more than a month and secondly, the cattle could graze freely after the dispenser was placed on their bodies. The repellent technology will be especially suited for communities that depend on transhumance to maintain their livestock since the animals wears the repellent wherever they go.

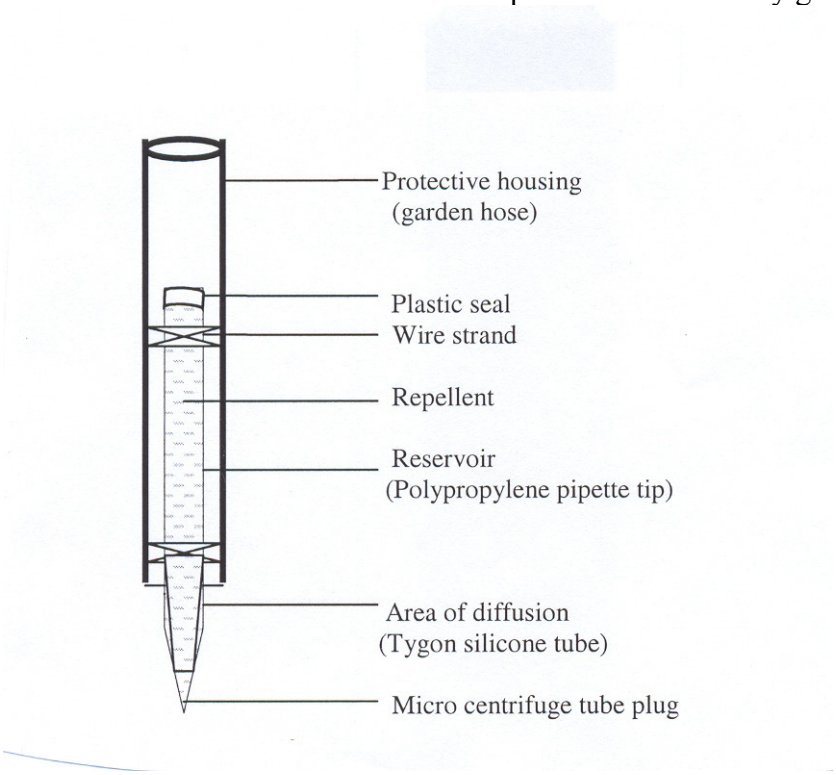

Fig 3 Schematic drawing of the repellent dispenser

The dispenser was prepared from 10ml polypropylene pipette tips, tygon silicone tubing, clear garden hosepipe and 1ml micro centrifuge tubes (cut in halves).

The pipette tip was cut on the lower (narrow) side to produce a uniform cylindrical plastic barrel (the reservoir) of about 9cm in length and one of the ends sealed with a plastic stopper. At the lower end of the reservoir, a tygone silicone tubing (4cm in length) was inserted and this acts as the diffusion area of the repellent. The open end of the silicone tubing was plugged with a 1ml micro centrifuge tube plug after the repellent was poured into the reservoir. The reservoir was then pushed inside a clear garden hose (of about 12cm in length) so that only the tygon silicone was exposed. The garden hose helps protect the dispenser when the cattle are moving.

This dispenser is capable of releasing an average of 4.5mg of the repellent per hour. This rate was arrived at after several laboratory experiments to determine the rate of release and the length of the tygone tubing selected. Since previous field experiments indicated that 9mg/hr of the repellent provide maximum protection to cattle, two such dispensers were used. The dispensers are tied around

the neck of a cow with a belt so that the tygon silicone tubing faced downwards and the dispensers were near the forelegs of the animal. Several field experiments were undertaken to determine the optimum position to place the repellent dispenser on the body of the animals.

The main technical problems that were addressed or solved during the process included the selection of the appropriate polymer for optimal diffusion of the repellent, and minimizing breakage of the twines used to tie the dispensers, damage by vegetation, sun, heat, and leakage of the dispensers caused by the cracking of the dispenser tubing (ILRI/ICIPE 2001).

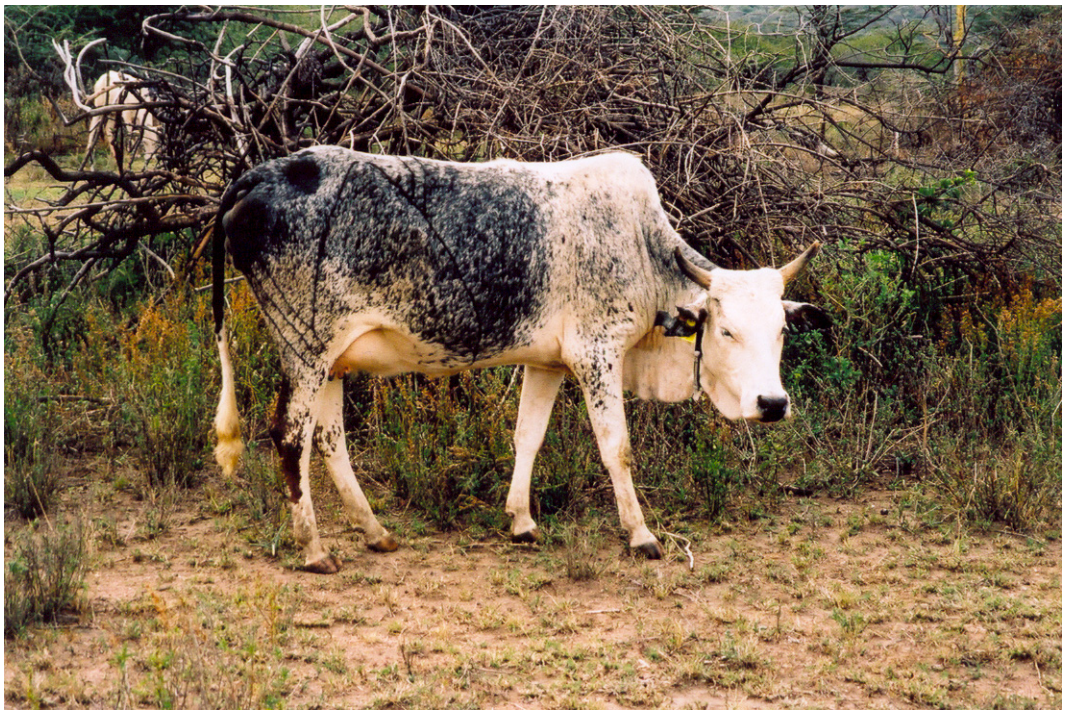

Fig 4 Maasai livestock with tsetse-repellent collar

Modeling

Modeling is a growing field in epidemiology and in this study it was used as a basis for the development of appropriate field studies. Modeling is a mathematical way of understanding the complex epidemiology of a disease such as trypanosomosis.

A hypothesis is made regarding factors influencing the transmission of trypanosomes and the probable impact of trypanosome control measures. The crucial starting point is to develop a sound framework that incorporates the essential biological features of trypanosomosis transmission. Employing this framework models can be developed that use available data to investigate assumptions and hypotheses and predict changes in trypanosomosis incidence. Field studies and experiments can be planned that provide information to support or define theories, to revise models, to confirm predictions and to provide further insight into trypanosome biology and control (McDermott and Coleman 1999). In

this study, plenty of data was already available from earlier research and this was used to calibrate the model. More data is being collected from the field during the project.

The Ross-Macdonald model of malaria transmission has been adapted for trypanosomosis transmission by Rogers (1988) and Milligan (1990). It was modified to take into account the following features of African Trypanosomosis:

1) More than one vertebrate host species is usually involved, man and domestic animals (in human sleeping sickness) or domestic and wild animals (in cattle trypanosomosis).

2) The vertebrate must first incubate the infection for a period of time before transmission can take place.

3) Once the vertebrates recover or are cured of the disease, they are immune to reinfection for a further period. This immunity is lost at a constant rate and the hosts then re-enter the susceptible category.

4) Not all infected blood meals eventually produce mature infections in the vectors. The average proportion of such meals that give rise to mature infections is smaller for *T.congolense* than it is for *T.vivax*.

5) After taking an infected blood meal vectors must survive for a further period before they can transmit the disease. Losses occur through natural mortality during this period, reducing the prevalence in the vector.

The model assumes that hosts cannot be infected if they are already incubating, infected or immune to trypanosomosis. Little is currently known about the effects of super-infection on the durations of incubation, infection or immune periods so that this cannot yet be allowed for in the model. Mixed infections in vertebrates other than humans are quite common (Rogers 1988).

TESTING OF THE SYNTHETIC REPELLENT AT NAROK AND NGURUMAN

The synthetic repellent developed by ICIPE is being evaluated over a period of one year in two trial sites, Nikinje in Megwara sub-location, Narok district and Nguruman in Nguruman sub-location, Kajiado district. The evaluation is being carried out by the TRC / ILRI in collaboration with ICIPE. Entomological, veterinary and socioeconomic studies are being conducted simultaneously in each of these sites. During my Minor Field Study I followed PhD students Bernard Bett and Patrick Irungu in their work with this project, specifically participating in the veterinary and entomological parts described below.

Fig 5 Map of Kenya

Veterinary

There are 24 herds to be sampled monthly, 12 in each of the trial sites. Out of the 24 herds 12 are treatment herds and 12 are control herds. The herds were chosen so that each treatment herd has a control herd that lives under the same conditions and the same tsetse challenge. Herd sizes range from 9 to 110 animals.

All the animals in these herds have been ear-tagged at the start of the trial. Descriptive information was obtained from each animal on age, sex, breed and color. Herd level attributes like herd size and grazing patterns were recorded.

All the animals were blanket treated at the start of the trial with diminazene aceturate at a dose of 7 mg/kg bw. Any new animals brought into the herd are ear tagged and treated with diminazene aceturate at the earliest opportunity.

During sampling we weighed the animals using a weighing band. The animals were also scored on body condition using scoring system ranging from 1-9 with body condition classified from L- (marked emaciation) to F+ (heavy deposit of fat clearly visible).

From all the animals in these herds, we obtained venous blood from the ear veins using a pair of capillary tubes and trypanosomosis diagnosis were carried out using buffy coat technique (BCT).

Centrifugation of the collected blood were carried out for 5 minutes at 1200 rev/min. Packed cell volume (PCV) was then determined using a haematocrit reader.

Thin blood smears were prepared and stained with Giemsa for all samples positive for trypanosomosis on BCT and those that had a PCV of 20 or less.

All positive cases for trypanosomosis and tick borne diseases were treated appropriately if by the time of sampling the farmer had not already treated them.

All the animals in the treatment herds - 6 at Narok and 6 at Nguruman - were fitted with repellent collars at the start of the trial. The status of the collars and the repellent dispensers are checked every month and recorded. Replacement of any lost collars and monthly replenishment of repellent is done by ICIPE.

Fig 6 Field work at Narok site

Entomology

Tsetse densities are monitored using baited biconical traps in Narok and NGU traps in Nguruman. The baits used are: phenol sachets and acetone. (The biconical trap is one of the most widely used traps for sampling, especially of *palpalis* group species. The NGU traps were developed primarily to provide an effective, cheap and easily made trap for community based control of *G.pallidipes*.)

Traps were deployed for a period of 72 hours in each site and harvested every twenty-four hours. We recorded the fly catches for each site and day specifying

the sex, age and species of captured flies. We also recorded other biting flies caught.

The flies were then dissected to determine trypanosomosis prevalence.

Feeding preferences of tsetse is determined by identifying the residual undigested blood meals in their midguts. Blood is expressed on Whatman filter papers and analyzed using ELISA technique.

Farmer reports

Farmers included in the field trials are asked to record: when they treat an animal, when an animal dies, when an animal gives birth, when an animal is sold or bought and also when an animal is given out for other purposes e.g. cultural. They are also requested to keep the empty trypanocide packages so that the amount used can be checked every month. The grazing patterns of the animals are also recorded.

Potential problems

Tsetse flies would adapt to the repellent within a certain time of its introduction. Therefore there is a strong indication for finding ways of integrating the repellent with other existing control technologies in order to reduce the selection pressure put upon the fly population. It has been observed that resistance to other compounds (antimicrobials, trypanocides, antihelmintics and insecticides) systematically occurs in target organisms approximately 10 years following their introduction to the market (Waller 1994).

Using the repellent alone will not lead to a reduction in tsetse fly numbers as it does not kill the flies. The tsetse would simply shift their source of blood meals to either wildlife or untreated livestock. This is an especially important problem because of the way animals in Maasai neighboring communities are not strictly kept apart and use the same grazing areas and watering holes. This would also put neighboring untreated animals under a higher tsetse challenge.

The farmers are used to diagnosing and treating their animals for trypanosomosis and even though they are asked to record all treatments during the study this may be hard to control. The power of the test results will be reduced if unknown amounts of trypanocides are indeed used during the trial.

Potential for impact

A successfully adapted and commercially produced repellent product is expected to have significant impact. The current widespread use of trypanocides, which often represent the single largest livestock health input in pastoralist communities, demonstrate pastoralists recognition of trypanosomosis as a critical livestock health constraint, and more importantly, their willingness to act and invest in protecting their livestock. Assuming that the proposed research confirms the preliminary results suggesting demonstrable reduction in disease incidence when the repellent is used, then uptake should follow as pastoralists realize savings from lower trypanocide use, depending upon the longevity of the eventual commercial repellent product. It is expected to be attractive for transhumant

pastoralist communities for whom alternative tsetse control with traps and targets is often impractical (ILRI/ICIPE 2001).

RESULTS

At the time for my Minor Field Study the field study was still in its first months and therefore no final results are presented in my report. At the Narok site all animals in the 12 herds were blanket treated with diminazene aceturate (7mg/kg bw) and all animals in the 6 treatment herds were fitted with repellent dispenser collars as this was the first month of the trial at this site.

At the Nguruman site the test was into its second month and no sick animals were expected or found since all cattle had received blanket treatment with diminazene aceturate (7mg/kg bw) the previous month. In the 6 treatment herds the repellent collars were controlled on all animals and refilled. A few collars were missing and some were broken in the silicone part but most importantly all repellent dispensers were empty at the time of control. Since the repellent dispensers are designed to last more than a month, continually releasing the repellent, this is a major concern.

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