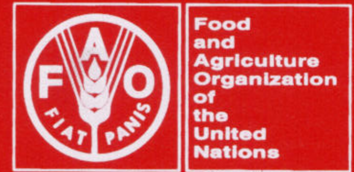
A field guide for THE DIAGNOSIS, TREATMENT AND PREVENTION OF AFRICAN ANIMAL TRYPANOSOMOSIS



A field guide
for
THE DIAGNOSIS, TREATMENT AND PREVENTION OF AFRICAN ANIMAL TRYPANOSOMOSIS

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(adapted from the original edition by W.P. Boyt)

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Preface

This new edition of the well-known field guide on African animal trypanosomosis adheres as much as possible to the original style and, particularly, to the intention of the author of the first edition in that it is essentially meant to be a guide for field control personnel. Its scope has been extended somewhat beyond that of the African continent, as trypanosomes of African origin have spread to the Americas as well as to Asia, and even to Europe, but the main emphasis remains on Africa. More attention is also given to methods of control of the disease other than those using chemotherapy and chemoprophylaxis, as it is being realized that drugs alone are not a sustainable answer, and have to be integrated into a multidisciplinary and flexible approach to control of the disease. For instance, the first edition did not touch upon the important subject of control of trypanosomosis through vector control.

Molecular methods have considerably changed virtually all aspects of our knowledge of African trypanosomosis, particularly in the fields of taxonomy, immunology and diagnosis. Nevertheless, this progress has not (yet) had much impact on the situation in the field, as will be evident from this second edition.

I am very grateful to Jan Slingenbergh and Brian Hursey of the Animal Health Service of FAO, Ron Dwinger of the Joint FAO/IAEA Division for Animal Health and Production, and Peter van den Bossche of the Regional Tsetse and Trypanosomosis Control Project in Harare, for all the time they spent in reading the manuscript and for their many valuable corrections, comments and suggestions. Thanks are due to Joachim Otte of the Animal Health Service of FAO for compiling the appendix on considerations of sample size and also to Giorgio Beccaloni of the same service for his skilful rendering of several of the original drawings and for creating new ones.

G. Uilenberg

Introduction

Livestock are of enormous importance in Africa, economically, for nutritional and agricultural purposes, and socially. The problem of African animal trypanosomosis[1] (AAT), also called "nagana", was recognized by African stockmen long before the cause of the disease was known, and many pastoralists associated the disease with the presence of tsetse flies. By experience and folk memory, methods of husbandry were evolved whereby domestic livestock could be maintained in strictly defined areas. By a process of trial and error it became known that certain zones were safe from the ravages of the disease, some were death traps and others were seasonally affected and fluctuated between good and bad years.

The impact of the tsetse-associated disease extends in sub-Saharan Africa over some 10 million km^2 (a third of the continent). Of these 10 million km^2 some 3 million are covered by equatorial rain forest; the remaining area contains some very good grazing areas, which perhaps fortunately have been protected so far by the tsetse fly against (over)grazing. The disease threatens enormous numbers of cattle and other livestock, while some trypanosomes affect humans also directly in most of this area by causing sleeping sickness. Over this vast area the process of development as seen elsewhere has been greatly hindered. Throughout the rest of the world progress has been attended by the increasing use of harnessed power, and the use of draught animals. For centuries trained oxen provided traction for cart and plough, while cattle also provided meat, milk and manure. Their absence over a great part of the African continent meant a constraint to the progress of development of huge proportions. The appearance of the internal combustion engine brought a change, but its use remains very limited in the small-scale farming practised in most African countries, and needs large amounts of scarce foreign exchange.

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[1] Formerly the disease was called trypanosomiasis, but international agreement has now been reached over the name trypanosomosis.

The successful treatment of human sleeping sickness, during the second decade of the twentieth century, was noticed by veterinary authorities, who attempted to adapt these methods to the control of animal trypanosomosis. The idea of preventing a constantly present disease by the use of drugs had been a reality in human medicine since quinine was first used in the control of malaria, and it was realized that the development of compounds with appreciable prophylactic effects against cattle trypanosomosis would be highly desirable. Sodium antimony tartrate was the only relatively successful remedy for African cattle trypanosomosis between the two world wars. It was however difficult to use, the tissue irritation attendant upon its injection required that it be administered intravenously and a complete cure could, at best, be assured only by repeated treatments.

During the late 1940s and early 1950s other drugs began to appear, which were immediately efficient in curing the disease and, additionally, produced appreciable periods of freedom from the disease after only one injection. Great optimism was expressed, the use of these compounds was extended and for the first time cattle could enter areas where previously this had not been possible.

It was not long however before the disease reappeared and drug-resistant strains of trypanosomes became a recognized problem. It has been said that "drug resistance attends chemotherapy like a faithful shadow" and so indeed it proved to be as further newly discovered compounds appeared, were hailed as being successful where others had failed, and in turn were overcome by the trypanosome. Pessimism then replaced recently expressed confidence. A period of concentrated logical research on drug resistance in the late 1950s and early 1960s reimposed a degree of order upon a scene of doubt and confusion and established the pattern of resistance, cross-resistance between alternative drugs and the existence of the so-called "avid" compounds which (at first) appeared to remain effective in the face of resistance to others.

Even now, several decades later, the position remains to a large extent unchanged, the same problems appear, virtually no new treatments have become available and the same mistakes are being made in their use.

Very few drugs are available for the control of animal trypanosomosis. Why this small range of effective treatments? Is there any likelihood of new and better therapeutic compounds in the near future? Regretfully, the answers are not encouraging. In order to establish the reasons, the problem must be examined on a wide scale.

African animal trypanosomosis is indisputably an enormous problem on the African scene, but the perspective alters when it is examined against a global background, even when taking into account mechanically and venereally transmitted African trypanosomes which have spread to other continents (*Trypanosoma evansi*, causing surra, mechanically transmitted *T. vivax*, and *T. equiperdum*, the causal agent of the venereal disease dourine). Placed in a global context, animal trypanosomosis is of less economic importance. The cost of the development of new compounds has increased enormously in keeping with inflation and increasingly exacting and expensive requirements of the licencing authorities on the absence of chronic toxicity, carcinogenicity and residue problems. At the same time, the market for recovering the enormous investments needed to develop new compounds to the commercial stage is limited and mostly poor. Logically, the economic stimulus required by the private industry for attracting their investment in research on new drug development is sadly lacking.

In parallel to research on trypanocidal drugs and their application, various ways of controlling the vectors of AAT, the tsetse flies, have been and are being explored. Tsetse flies are the natural vectors of AAT; in the fly the trypanosomes undergo a biological cycle which results in their multiplication, and the removal of these biological vectors is generally followed by the disappearance of the disease.

The subject of tsetse control is addressed in detail in the FAO publication series "Training manuals for tsetse control personnel" (Vols 4 and 5), and we limit ourselves to a general review.

Tsetse control became widespread with the arrival of synthetic insecticides in the 1940s. Selective spraying of the vegetational support of the flies and later the application of insecticides by

aircraft opened the way for large-scale tsetse eradication. However, although eradication of isolated tsetse populations was successful, this approach proved more difficult in areas not separated from the main tsetse belts. Complications arose with:

- The immensity of the tsetse-infested areas.
- Reinvasion of the treated areas from adjoining regions (tsetse flies do not recognize borders).
- The financial (and foreign exchange) cost. The requirement to maintain cleared areas permanently free from reinvasion makes the exercise very costly and has led to "donor fatigue".
- Even the best plans for the use of land cleared from tsetse flies are not always implemented, which may lead to uncontrolled settlement and land degradation. Environmental problems may also ensue from the side-effects of the insecticide itself, although the negative impact of a one-time application is limited and temporary.

Another approach towards tsetse eradication is the use of the so-called sterile male technique, or sterile insect technique (SIT). The principle of this method is based on sterilization of males, reared in the laboratory, through irradiation with gamma rays. Female tsetse flies, unlike males, only mate once during their life span, shortly after hatching, and thus will not produce any offspring after mating with a sterile male. Provided that sterile males are continuously released in numbers sufficient to compete with wild males, the population of tsetse will gradually decrease until it becomes extinct. Studies on this approach started in the 1960s. The method is attractive, as in itself it is not polluting,[2] and very selective. The sterile males will actively search for females belonging to the same species, also in sites where insecticide application is impossible, and this makes the method extremely alluring indeed. However, there are serious complications:

[2] There is indirect pollution in that the irradiating source creates nuclear waste after its useful life span has ended and it has to be replaced.

- Rearing tsetse flies is costly and labour-intensive and it is difficult to rear them in sufficient numbers for integrating SIT in large-scale eradication campaigns.
- Each species to be eradicated has to be reared separately.
- As the impact of SIT used by itself is only gradually felt, it is usually preceded by application of insecticides, to bring down the population to a low level and decrease the duration of the release of sterile males. The use of insecticides takes away the advantage of being selective and non-polluting. More recently, targets (see below) are also used prior to SIT.
- Further drawbacks are the same as with any tsetse eradication campaign (size of infested regions, reinvasion, donor fatigue, uncontrolled settlement).

A successful project using this technique, supported by the Joint FAO/IAEA Division for Animal Health and Production, has recently been completed against the isolated tsetse population of Unguja island (Zanzibar, United Republic of Tanzania), where some 1 600 km^2 were cleared of tsetse flies (*G. austeni*). The project was carried out from 1994 to 1997. Although an earlier successful eradication scheme in Burkina Faso, using traps followed by the release of large numbers of sterile males, succeeded in clearing over 3 000 km^2 of two species of riverine tsetse (*G. tachinoides* and *G. palpalis*) and one savannah species (*G. morsitans*), reinvasion occurred.

With the advent of artificial bait devices such as traps and insecticide impregnated screens, as well as the application of small quantities of persistent powerful (mostly synthetic pyrethroid) insecticides on the animals (which is far less polluting than their application on the vegetation), continued tsetse suppression has come within reach of local communities. However, such measures have to be sustainable and the village population has to remain motivated and understand that this type of control will go on and on. Eventually, the farming landscape may become unfriendly to tsetse survival (depending on the species) but this may take many years.

The present tendency is indeed towards control of tsetse flies, that is to say a continuous effort to keep the size of the populations down

to a level where the trypanosomosis problem is tolerable, instead of large-scale eradication schemes. Control has to be sustainable. This word is very much in fashion these days and needs perhaps an explanation. Sustainable control measures are those that are durable, that can be sustained over time. Sustainability may apply to financial aspects, for example, are funds for continuing the measures over the foreseeable future assured? (Think of donor fatigue, of foreign exchange and budget problems of national governments, of the cost-effectiveness of the measures.) Measures such as repeated large-scale insecticide application to the environment are not ecologically sustainable. The term sustainability can also apply to control measures against trypanosomosis itself. For instance, once resistance to a particular trypanocidal drug becomes generalized, then the use of this drug is no longer sustainable. If the cost of preventing the disease by the use of a drug exceeds the financial benefit that the owner derives from it, or if the foreign exchange situation of the country is such that continuous importation of the drug cannot be secured, then such drug use is no longer sustainable.

The control of the vectors of trypanosomes which are not transmitted by tsetse flies is even more difficult than that of tsetse flies. As we shall see, strains of two species of African trypanosomes have succeeded in adapting themselves to so-called mechanical transmission by various biting flies; there is no biological cycle in these flies, no multiplication, and the fly just acts as a needle: a fly feeding on an infected animal transfers a small quantity of infected blood to a second animal and directly infects it if the interval between feedings is sufficiently short for the trypanosomes to survive. This happens particularly when the first (infected) animal disrupts feeding of the fly by swishing its tail or some other movement, and it then continues feeding on the second host. The most important of these mechanical vectors are the tabanids (horse flies, etc.) and stable flies and related insects (the stomoxys group). Eradication of these flies, which are often occur in large numbers, is for the foreseeable future out of the question; control methods are generally not very effective. Trypanosomes of African origin which have dissociated themselves from tsetse flies will be discussed in a separate chapter in this publication.

What of the possibility of successful vaccination? The study of immunity has made enormous advances in recent years but, in the case of the protozoa in general, some of which "inhabit the very arsenals of the immune response", and trypanosomes in particular, many problems remains unsolved. This is in spite of many years of intensive research in various universities and research institutes throughout the world, including Africa. The main obstacles preventing vaccine development are the almost unlimited antigenic variation during infection by one single strain of trypanosome and the antigenic strain diversity within each of the several trypanosome species and types. Some scientists continue attempts at vaccine development using internal non-variable antigens (but a difficulty is that such antigens are out of reach of host antibodies as long as the trypanosome is alive and intact) or at immunizing against proteins causing pathogenic effects, instead of against the parasite itself. So far there is no breakthrough.

In some parts of Africa, rearing of ancient local breeds of trypanotolerant livestock offers another solution to the problem. Trypanotolerance in taurine (humpless) cattle is particularly well known and widespread in subhumid and humid West Africa, but the phenomenon had received little scientific attention, until studies on its nature began in the 1970s in the Centre de recherches sur la trypanosomose animale = Centre for Research on Animal Trypanosomosis (CRTA), now Centre international pour la recherche et le développement de l'elevage en zone subhumide = International Centre for Livestock Research and Development in the Subhumid zone (CIRDES), in Burkina Faso, and later in the International Trypanotolerance Centre (ITC) in the Gambia and in the International Laboratory for Research on Animal Diseases (ILRAD), now absorbed into the International Livestock Research Institute (ILRI) in Kenya. West African breeds of sheep and goats also possess a fair degree of trypanotolerance, although this has as yet not been studied extensively. It is also known at present that there are differences in susceptibility to trypanosomosis between various East African breeds of cattle and small ruminants. One of the limitations to the use of West African trypanotolerant cattle breeds is their small size; still, genetic studies might lead in the (probably rather distant) future to the transfer of the trypanotolerance trait to more productive breeds.

Chapter 1
African animal trypanosomes

LIFE CYCLES

Insects are usually involved in the natural transmission of the African pathogenic trypanosomes with which we are concerned in this field guide. When this is the case, the life cycle has two phases, one in the insect vector and one in the mammalian host. Transmission by insects may be cyclical by tsetse flies,[3] *Glossina* species, or mechanical by other biting flies (but apart from transmitting trypanosomes cyclically, tsetse flies can also act as mechanical vectors).

Cyclical transmission

When a tsetse fly hatches from its pupal case it is free from trypanosomes. Until its first bloodmeal, it is called a *teneral* fly. It acquires a trypanosomal infection when feeding on a parasitaemic (= having parasites in the circulating blood) mammalian host. The trypanosomes undergo a cycle of development and multiplication in the digestive tract of the fly until the infective metacyclic trypanosomes (metatrypanosomes) are produced. As indicated in Table 1 (p. 32), different trypanosome species develop in different regions of the digestive tract of the fly, and the metatrypanosomes occur either in the biting mouthparts or the salivary glands. The period from ingesting infected blood to the appearance of these infective forms varies from one to three weeks; once infective metatrypanosomes 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 tissues and the fly injects saliva to prevent

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[3] We assume that the reader has some knowledge of tsetse flies, as there simply is no scope in this manual for going into details. At present 23 different species and eight subspecies of the genus *Glossina* are recognized, belonging to three groups: *fusca* group or forest group, *palpalis* group or riverine group, and *morsitans* group or savannah group.

coagulation. Infection of the host takes place at this stage, with infective metacyclic trypanosomes in the saliva.

Although no classical sexual processes in the life cycle of trypanosomes have been described, it has been shown that exchange of genomic material (DNA) between trypanosomes sometimes occurs in the tsetse fly, although it is not clear how significant this is.

Life cycle in the mammalian host. The infective metatrypanosomes undergo development and multiplication at the site of infection where a swelling or chancre may be detected in the skin, and finally the mature blood trypanosomes (or trypomastigotes) are released via lymph vessels and lymph nodes into the blood circulation.

Reproduction in the mammalian host occurs through a process of binary division, details of which are described in Morphology, p. 14.

Trypanosomes feed by absorbing nutrients, through their outer membrane, from the body fluids of the host. The proteins, carbohydrates and fats are digested by enzyme systems within their protoplasm. Oxygen dissolved in the tissue fluids or blood plasma of their host is absorbed in a similar manner, to generate the energy necessary for the vital processes.

Waste products are disposed of by a reverse process, through the outer membrane, into the body fluids of the host. They include carbon dioxide formed during respiration, as well as more complex metabolic products.

Life cycle in the tsetse fly. The site of the different trypanosome species in the fly is indicated in Table 1. Blood stream forms (trypomastigotes) ingested by the fly undergo considerable changes, in morphology as well as in their metabolism. They change into long slender forms called epimastigotes, which multiply and finally give rise to the infective metatrypanosomes. For a detailed account of the different forms and the development in the tsetse fly, the reader is referred to standard text books (see Further reading on p. 157).

Mechanical transmission

By biting insects. The process is purely mechanical. A biting insect passes the blood forms from an infected animal to another in the

course of interrupted feeding. The time between the two feeds is crucial for effective transmission because the trypanosomes die when the blood dries. 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 flies themselves can of course also act as mechanical vectors.) This mode of transmission has proved to be sufficiently effective to maintain *Trypanosoma vivax* and *Trypanosoma evansi* in South and Central America, and the latter species in North Africa and Asia as well. No tsetse flies occur outside tropical Africa, apart from small tsetse pockets in the southwest of the Arabian peninsula.

By iatrogenic[4] means. This can occur when using the same needle or surgical instrument on more than one animal, at sufficiently short intervals that the blood on the needle or instrument does not dry. It is not an uncommon occurrence when animals are vaccinated or treated by injection, or when blood is collected from several animals in a row, without changing or disinfecting needles or pins. It may also occur when several animals are subjected at short intervals to a surgical intervention (dehorning, castration, etc.) without properly disinfecting the instruments.

Transmission by other means

- It is well known that carnivores may be infected with *T. evansi* and *T. brucei* by ingesting meat or organs from infected animals, as long as these are still sufficiently fresh to contain live trypanosomes. Infection occurs probably through the mucosa of the mouth (in which moreover bone splinters make wounds through which the parasites penetrate even more easily).
- Transmission of *T. evansi* in Latin America by the bites of vampire bats is common. These bats become infected by ingesting blood from infected horses or cattle, the

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[4] Iatrogenic transmission means that it is caused by the (veterinary) operator. Iatrogenic infections are induced (involuntarily) by the operator using unhygienic procedures, such as contaminated instruments.

trypanosomes multiply in the bats and these are thereafter able to transmit the disease to healthy animals. The trypanosomes apparently pass readily through the oral mucosa of the bat in both directions.

- All trypanosome species are occasionally transmitted congenitally, from the mother to the offspring, either through the placenta while the foetus is still in the uterus, or when bleeding occurs during birth. Congenital transmission of *T. vivax*, for example, has been observed in Latin America as well as in Africa, but its real importance is not well known.
- Venereal transmission is the normal means by which dourine of equines, caused by *Trypanosoma equiperdum*, is propagated. Because of its presence in the mucous exudate of penis and sheath of the stallion and the vaginal mucus of the mare, *T. equiperdum* is easily transmitted directly during copulation from an infected to a healthy animal and its geographical distribution is not restricted to specific climatic conditions. This species is essentially a tissue parasite and causes at most very low parasitaemias in the circulating blood of equines.

MORPHOLOGY

A sound knowledge of the basic features of the various trypanosomes enables the identification of each species and so the exact cause of the disease. Once the basic features possessed by all trypanosomes are appreciated, the diagnostic differences can be recognized and the species identified.

Basic morphology of trypanosomes.

Figure 1 is a diagrammatic illustration of the fundamental features of a trypanosome (trypomastigote) as seen in a stained preparation made from the blood of an infected animal.

Figure 1
Diagram of a trypanosome

The parasite consists of a single cell varying in size from 8 to over 50 µm.[5] All the activities associated with a living organism take place within this unicellular organism – nutrition, respiration, excretion, reproduction. The substance of which all living cells consist, the protoplasm, comprises three parts, an outer protective and retaining layer, the pellicle = cell envelope = cell membrane, within which the cytoplasm forms the bulk of the contents. Suspended in the cytoplasm are various structures, the most prominent being the nucleus, which may be regarded as the command centre of the cell and which also plays a major part in reproduction. It contains DNA (deoxyribonucleic acid), which is arranged in the form of genes and chromosomes; it represents the genetic information and is responsible for the manufacture of enzymes and other proteins of the cell.

Small granules (formerly called "volutin granules") can sometimes be seen in the cytoplasm; they may have various origins, they may be food or nuclear reserves, or result from a reaction between the trypanosome and the host's immune system.

Trypanosomes are thoroughly adapted to living and moving in the blood plasma or tissue fluid of the host. They are elongated and streamlined, and tapered at both ends. The pellicle, the outer layer of the cytoplasm, is flexible enough to permit a degree of body movement, while retaining a definite shape. As shown in Figure 1, a *flagellum* arises near to the posterior end from a parabasal body, and runs the length of the trypanosome; it may be continued beyond the anterior end of the body as a whip-like *free flagellum*. Along the length of the body the pellicle and cytoplasm are pinched up into a thin sheet of tissue called the *undulating membrane*, through the outer margin of which runs the flagellum, as shown in Figure 1.

Among other basic morphological features, a distinct well-defined body, the *kinetoplast*, is seen near to the posterior end of the trypanosome and differs in size and position according to the species. It is adjacent to the parabasal body (from which the

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One µm is a micron, a millionth of a metre or a thousandth of a millimetre.

flagellum arises), and so close to it that it cannot easily be seen separately with the light microscope. The kinetoplast has important functions in reproduction and metabolism and is probably essential for cyclical transmission by tsetse flies. (It is sometimes absent in a proportion of trypanosomes, especially of some strains of *T. evansi*, a species which has lost its ability of being cyclically transmitted.) The extent of the undulating membrane and the absence or presence of the free flagellum are also precious in specific identification of trypanosomes. Other morphological characters are the average length and the shape of the body.

Locomotion

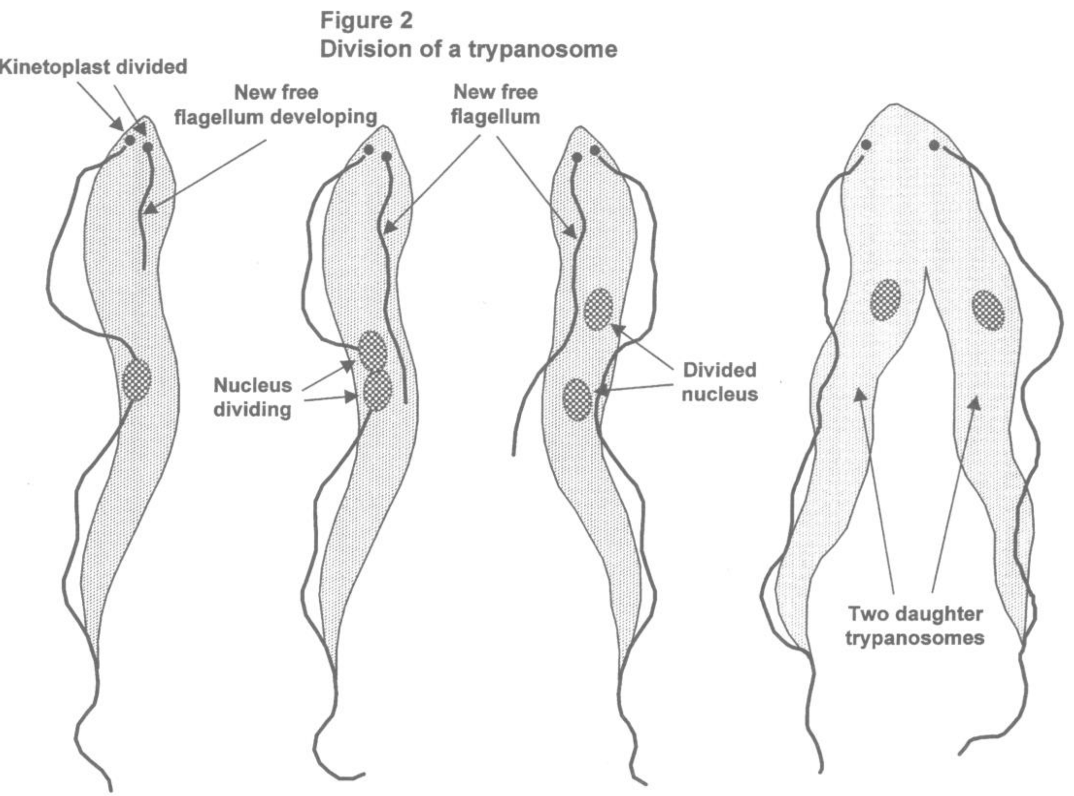
Trypanosomes move actively and progress by movement of the undulating membrane and the free flagellum (when present), which acts as a kind of propeller, thus drawing themselves through the blood plasma or tissue fluid. (The free flagellum, when present, arises from the anterior [front] end of the parasite.) The movement pattern as seen with the microscope in fresh blood preparation can be of some help in identifying the species involved, particular for *Trypanosoma vivax*, which moves rapidly forward between the blood cells, whereas other species often just wriggle around without showing much forward progress.

Reproduction

This is by a process of division to produce two daughter cells. However, as stated above, it has been shown that exchange and recombination of genetic material may take place in the tsetse fly between two trypanosomes, but it is unknown how frequently this occurs.

The division into two daughter cells (binary fission) follows the sequence of events illustrated in Figure 2. The kinetoplast divides first. A second parabasal body develops, from which a second flagellum develops. The nucleus divides next, followed by the rest of the trypanosome body duplicating all the structures present in the cytoplasm. The body then divides into two daughter cells, beginning at the anterior end. The process is rapid, and may result in a vast population in the host within a short period of time.

Figure 2
Division of a trypanosome



Differential morphology

There are distinct differences in appearance, shape and size between the various species of trypanosomes, allowing specific identification. It must be remembered, however, that in any biological material there is some variability. Also, trypanosomes are not rigid and continuously change their shape slightly; the individual parasite seen in the stained preparation presents the shape it had at the moment of dying. It has also been subjected to the unnatural stresses of drying out and being fixed and stained. Many variations in appearance are therefore seen, differing somewhat from the drawings in textbooks. It is thus necessary to observe carefully and systematically all the features in a sufficiently large number of individual trypanosomes; only after such an examination is it possible to arrive at a reasonably accurate diagnosis. There will be examples where trypanosomes are so few, or the staining so inadequate, that identification may not be possible or only after a prolonged search. It is also essential to examine several individual trypanosomes, because even if one specimen is sufficiently perfect to establish its identity beyond any doubt, further search may reveal another species and thus a mixed infection. Mixed infections occur more often in the field than was previously thought, as new more sensitive diagnostic techniques have shown (see Chapter 3 - Diagnosis).

For specific identification, a number of trypanosomes should be examined systematically for the presence or absence, size and position of a number of features:

(i) *Presence or absence of trypanosomes of different appearance.* If all individual trypanosomes are alike, the infection is called *monomorphic* (of one form); if there are distinctly different types it can be either a *polymorphic* (= pleiomorphic) species, or a mixed infection of different species.

(ii) *Presence or absence of a free flagellum.* In certain species there may be some trypanosomes with, and some without, a free flagellum.

(iii) *Size of the trypanosome* (expressed in µm).

(iv) *The size and position of the kinetoplast.* The position is related to proximity to the posterior extremity (rear end) of the organism.
(v) *The degree of development of the undulating membrane.* It may be conspicuous or inconspicuous.
(vi) *The shape of the parasite, particularly the shape of its posterior part.* The posterior extremity may vary from blunt to pointed.

It is important to remember that the successful use of this simple key depends on the presence in the preparation of a sufficient number of individual parasites. It is not always possible to make an accurate diagnosis if only one or two parasites can be found after a prolonged search. The task is rendered more difficult or even impossible if the stained preparation is of poor quality.

TAXONOMY AND NOMENCLATURE

Taxonomy is the classification and orderly arrangement of living organisms according to their structure and shape (morphology), their biological development (life cycles) and, more recently, their molecular structure, particularly that of their genome (molecular taxonomy). Nomenclature, the system of naming organisms, is based on their classification. Without going into great detail, let it be recalled that organisms are classified into large divisions called phyla (singular phylum), then into classes, orders and families. For example, the Diptera are an order of the class of insects, which belongs to the phylum of the arthropods, and the Glossinidae or tsetse flies are a family of the Diptera. The family Trypanosomatidae (which includes trypanosomes, but also for instance leishmanias) belongs to the order of the Kinetoplastida, the class Zoomastigophorea and the phylum Protozoa. Each species is furthermore given two names, the first, always spelled with a capital letter, places it in a relatively small group known as the genus (plural genera) and the last name, spelled with a small letter, indicates the species. The family Glossinidae comprises only one genus, *Glossina*, which includes over 20 species of tsetse flies. Sometimes a genus is subdivided into subgenera, and an additional name is sometimes added to denote a subspecies, when differences within one genus and/or species are so wide that a further breakdown is helpful (e.g.

Trypanosoma [*Trypanozoon*] *brucei gambiense*, which is the causal agent of classical human sleeping sickness; details of this name will be explained a little further on). The genus (or subgenus), species (or subspecies) may be further defined by adding the surname of the person who first described it and the year when the description was published (e.g. *Trypanosoma congolense* Broden, 1904 indicates that Broden in 1904 published the first description of this important pathogenic trypanosome of cattle in tropical Africa).

Another subdivision of the genus *Trypanosoma*, into two sections, is mainly based on the way in which the infective forms leave the intermediate insect host after their cyclical development.

In the section Stercoraria, development in the vector ends with the formation of infective metatrypanosomes in the posterior part of the digestive tract and transmission occurs through the faeces of the insect,[6] while in the Salivaria, with which we are mainly concerned in this field guide, the usual mode of transmission is inoculative, through the biting mouthparts of the vector (except for dourine).

In higher organisms a species is defined as a group of organisms that can interbreed with one another to produce fertile offspring. In the case of viruses, bacteria, and many of the protozoa, where no (classical) sexual processes are known to occur, the definition of species is more arbitrary and some scientists adhere to the principle that a valid species is one that is recognized by a good taxonomist. In addition to morphological and biological characteristics, molecular taxonomy is now increasingly used.

Once the identity of an organism has been stated in a scientific paper or book, it is customary to shorten the genus name to its initial capital letter and omit the name and year of the person who first described it (e.g. *Trypanosoma congolense* Broden, 1904 becomes *T. congolense*.)

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[6] The Stercoraria include for example the species *Trypanosoma cruzi*, which causes human trypanosomosis in Central and South America, also called Chagas disease; although it may be of even greater importance as a human disease than African sleeping sickness, it has fortunately no economic impact on livestock production.

Taxonomy and nomenclature of trypanosomes

Trypanosomes are unicellular organisms (Phylum Protozoa) belonging to the genus *Trypanosoma,* the family Trypanosomatidae and the order Kinetoplastida. Many species of trypanosomes occur as parasites in a wide variety of animals, and even plants. For the purpose of this field guide we are concerned with those causing disease (the pathogenic trypanosomes) in domestic animals in Africa (African animal trypanosomosis = AAT). Some of these parasites have been spread by humans from Africa to other continents. For example, *T. vivax* had been introduced to the Americas, by the importation of West African cattle in the eighteenth and nineteenth centuries, and it is likely that *T. evansi* had already "escaped" from Africa far earlier by animal movements (in particular camels) between Africa and Asia.

Trypanosomes are blood parasites (haemoparasites), from the word haem = blood, which in the vertebrate host occur in the blood and tissue fluid and within that group are known as haemoflagellates, as they progress actively by the movement of the thread-like filament called flagellum.

The trypanosomes causing AAT belong to three subgenera, as shown in Table 1.[7]

Specific morphology

The subgenus* Nannomonas *(the* congolense *group). *T. congolense* (see Figure 3). This is the smallest of the pathogenic trypanosomes, with a length of 9-22 μm.

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[7] A fourth subgenus, *Pycnomonas*, which comprises one species, *T. suis*, is a rare and poorly known parasite of pigs (and wild pigs such as wart hog), which we shall not mention any further.

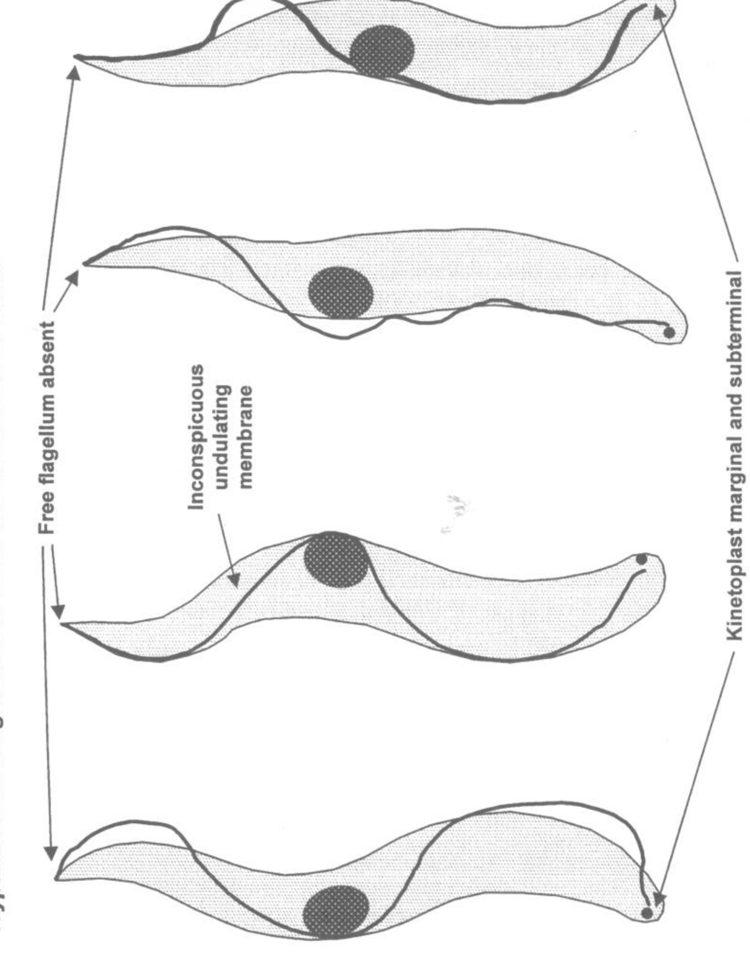

Figure 3
Trypanosoma congolense **as seen in a stained blood smear**

The blood forms are monomorphic, in that they lack a *free flagellum* (in the longer forms the shape of the anterior extremity may suggest the presence of a very short free flagellum). The use of the term *monomorphic* is somewhat misleading in this species in that there is a variation in size and shape between strains. Generally two variants are to be seen, a shorter form (9-18µ), the typical *congolense* type and a longer form (up to 25µ), with individuals intermediate in length between the two. The proportion of long and short forms varies in different cases and, it has been said, localities of origin. There is evidence which indicates that strains with the most long forms, the so-called "dimorphic" strains, cause a more severe form of trypanosomosis.

Recent studies have now resulted in a subdivision of the species in several "types", which can be distinguished by isoenzymatic differences[8] and molecular techniques.[9] Only one type has received a separate species name, *T. godfreyi* (see below), as it is also different in its pathogenicity for various hosts, while the others are designated as *T. congolense* savannah type, *T. congolense* Tsavo type, *T. congolense* forest type, *T. congolense* Kilifi type. For the purpose of this practical field manual we shall use only the name of *T. congolense* for these four types, but it should be remembered that from a scientific point of view this name encompasses rather different parasites and possibly more than one species.

In stained specimens of *T. congolense* the cytoplasm stains a diffuse, even, pinkish colour and is seldom granular.

The nucleus is centrally placed. The *kinetoplast* is of medium size and is usually situated at the margin of the body, just in front of the posterior extremity (*marginal* and *subterminal*).

The *undulating membrane* is poorly developed and inconspicuous.

T. simiae (see Figure 4). Trypanosomes of this species are *polymorphic*, with a length of 12-24 µm. In typical cases some individuals are with and others without a free flagellum.

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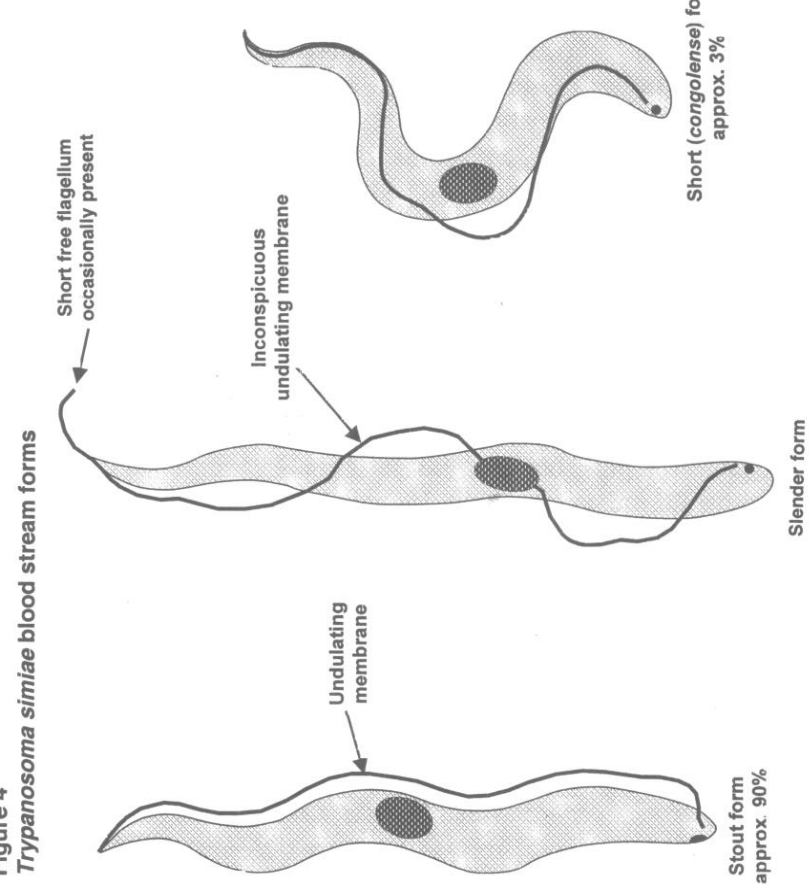
[8] Isoenzymes may be defined as enzymes which have identical functions but show molecular differences that can be detected with appropriate techniques.

[9] Such as those mentioned in Chapter 3 on diagnosis under molecular tests (DNA probes, PCR).

Figure 4
Trypanosoma simiae **blood stream forms**



The *kinetoplast* is of medium size, marginal and subterminal, as in *T. congolense*. Three morphological types can be recognized:

(i) Long stout forms, some of which may possess a *free flagellum*. Most parasites present in natural infections belong to this type. The *undulating membrane* is conspicuous and well marked.
(ii) Long slender forms, with sometimes a *free flagellum*, constitute a minority of the population. The *undulating membrane* is less prominent than in the preceding form.
(iii) A few short forms also occur, and are indistinguishable from typical *T. congolense*. (They are often called "congolense forms".)

T. godfreyi. This species has been separated recently from T. congolense in the Gambia, on the basis of isoenzymatic and DNA differences, but also because the disease it causes is different. It is pathogenic for pigs, but the disease is more chronic than the one caused by T. simiae. Morphologically it is similar to T. congolense, with a length of 9-22 µm (mean 13.7), but the undulating membrane is described as being usually conspicuous.

The wart hog appears to be its normal host and constitutes a reservoir of infection for domestic pigs.

***The subgenus* Duttonella *(the* vivax *group)*.** *T. vivax* (see Figure 5). This trypanosome as seen in the blood of mammals is also essentially *monomorphic*, with a *free flagellum*. Its length, including the *free flagellum*, varies from 18 to at least 26 µm. The following description concerns typical specimens.

The *kinetoplast* is large and terminal or almost so. It is much larger than in any of the other pathogenic species, and this is a distinguishing feature.

The nucleus is centrally placed, but the bulk of the cytoplasm is found in the posterior part of the body as this is somewhat swollen.

The *posterior extremity* is swollen and blunt.

Figure 5
***Trypanosoma vivax* blood stream forms**

Terminal large kinetoplast

Posterior end rounded

Inconspicuous
undulating membrane

Free flagellum always present

The undulating membrane is inconspicuous.

A more slender form is sometimes seen, which possesses a more pointed posterior extremity and has been thought to cause a more severe form of the disease. Such forms are commonly seen when *T. vivax* is dividing rapidly in the blood and it has also been reported that *T. vivax* in Latin America is more slender than the typical African parasite.

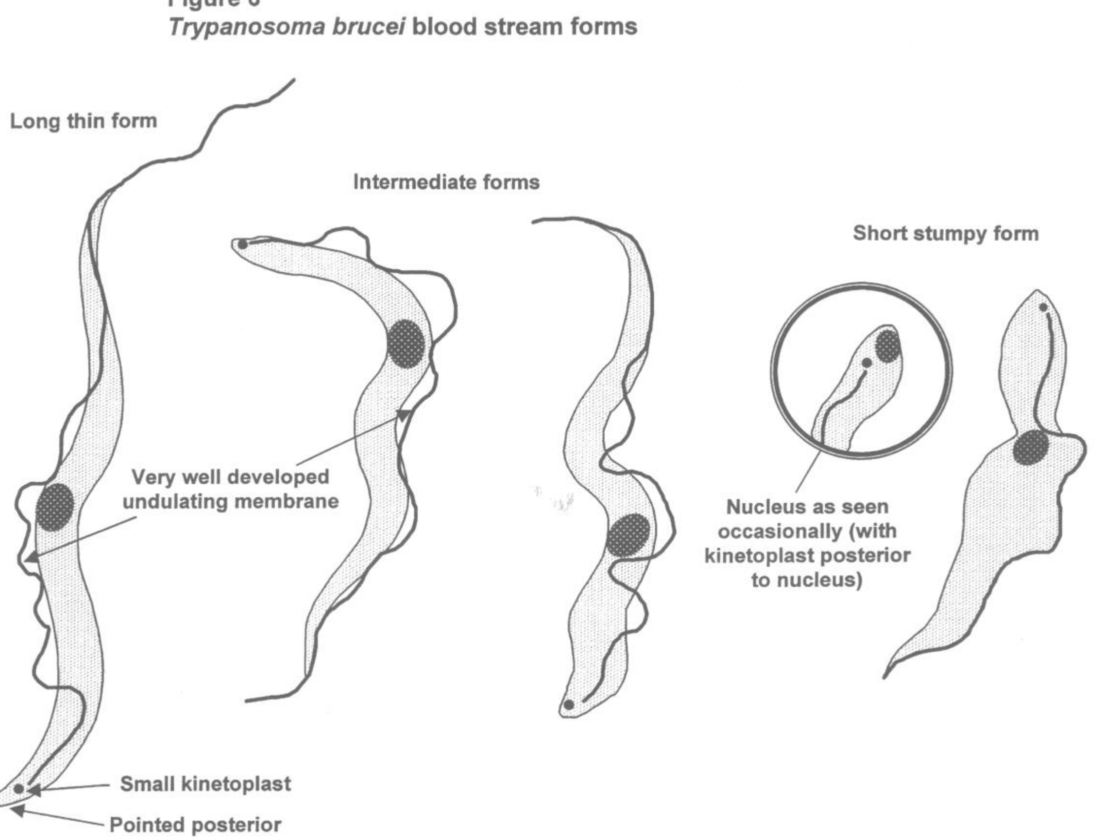
T. uniforme. Small trypanosomes (from 12 to 20 µm), otherwise similar to T. vivax, have been given the name T. uniforme. It is not recognized as a separate species by all specialists; some regard it as a subspecies (T. vivax uniforme).

The subgenus* Trypanozoon *(the* brucei *group). This group comprises five members: *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. evansi* and *T. equiperdum.* The three subspecies of *T. brucei* are normally transmitted by tsetse flies (in contrast to *T. evansi* and *T. equiperdum*) and are exactly similar in morphology, but only *T. brucei gambiense* and *T. brucei rhodesiense* are the cause of human sleeping sickness, the former mainly in West and Central Africa and the latter in eastern and southern Africa. *T. brucei brucei* is not infective to humans.

T. brucei (see Figure 6). *T. brucei* is *polymorphic*, with three main forms, all of which have a small *kinetoplast* and a conspicuous *undulating membrane*:

(i) *Long slender forms* (23-30 µm in length) with a *free flagellum*, which may be up to one half of the length of the organism. The posterior end is pointed and the nucleus is central. The *kinetoplast* is placed up to 4 µm in front of the posterior extremity.
(ii) *Short stumpy forms* (17-22 µm in length) normally without a *free flagellum*, but in which there may occasionally be individuals with a short free flagellum. The *kinetoplast* is usually subterminal. The position of the nucleus varies greatly and it is in some cases in

Figure 6
Trypanosoma brucei **blood stream forms**



the posterior part of the cell, sometimes so far posterior that the kinetoplast is anterior to it (so-called postero-nuclear forms). There is considerable variation in appearance between short stumpy forms, from broad, squat types (which include the postero-nuclear forms) to a form similar to *T. congolense*, although longer. In stained specimens blue volutin granules are often present in the cytoplasm, often arranged in a line along the margin of the cell.

(iii) *Intermediate forms*, varying in length between the two previously mentioned types. A *free flagellum*, of varying length, is always present. The nucleus is centrally placed. The posterior end is somewhat variable in shape, but usually bluntly pointed. The *kinetoplast* is close to the posterior extremity. Volutin granules are occasionally present but neither as common nor as plentiful as in the short, stumpy forms.

During the course of the infection, there is a change in the trypanosome population from the long thin forms, through the intermediate, to the short stumpy, and this altered appearance is accompanied by a change in the type of respiration, as the trypanosome prepares for its period within the tsetse fly. The short stumpy forms are adapted to living and developing in the tsetse, while long thin forms are the true mature blood forms which die in the gut of the insect. (Similar metabolic changes also occur in other trypanosome species, but there are no such obvious morphological changes associated with them as in *T. brucei*.)

As noted above, the species *T. brucei* is subdivided into three subspecies, *T. brucei brucei*, African trypanosome transmitted by tsetse flies, not infective to humans, *T. brucei gambiense*, the causal agent of classical or Gambian human sleeping sickness, and *T. brucei rhodesiense*, which causes the type of human sleeping sickness common in Zimbabwe.

T. evansi.[10] *T. evansi* cannot be distinguished on morphological grounds from the long slender forms of *T. brucei*, and it is almost

[10] Because this trypanosome is believed to be derived from *T. brucei brucei*, the name *T. brucei evansi* is sometimes used for it. Nevertheless, this particular trinomial name is invalid according to the rules of the international code for zoological nomenclature, as the name *evansi* was created before the name *brucei*. In order not to cause confusion, we will stick to using the commonly accepted species names of *T. brucei* and *T. evansi*.

certain that *T. evansi* has developed from *T. brucei* by continual mechanical passage (particularly through camels), the vectors being blood-sucking flies, especially those of the Tabanid family. The disease, often called *surra* in Asia and by a variety of names elsewhere, such as *el debab* (northern Africa), *mal de caderas* (Brazil), *murrina* (Central America), has spread over a wide area outside the tsetse belts in Africa, in the Near East, India, China and Southeast Asia, as well as in tropical America. Direct mechanical transmission has resulted in *T. evansi* losing its ability to undergo the developmental cycle in tsetse flies.

Its length is from 17 to at least 30 µm, and the description given for the long slender forms of *T. brucei* fits *T. evansi.* Nevertheless, short stumpy and intermediate forms may be seen rarely, irregularly and in very small numbers, including posteronuclear forms. A greatly varying proportion of individual trypanosomes may have no visible kinetoplast and there are even strains of *T. evansi* in which the kinetoplast is not apparent in any individual trypanosome, the so-called akinetoplastic strains (such strains have in the past been considered in Latin America as a separate species, *T. equinum,* but this name is no longer considered valid).

T. equiperdum. This is another trypanosome which is probably derived from *T. brucei.* Its morphology is identical to that of *T. evansi* and the long slender forms of *T. brucei*, but it is different in that it causes a natural disease only in animals of the horse family (horse, donkey, mule), among which it is transmitted by genital contact. The disease, dourine, is thus a venereal disease. As it is not dependent on insect vectors, it has spread as far north as Canada, Russia and other European countries, and as far south as Chile and South Africa. (It has since been eradicated in many countries.)

T. theileri *and other species of the subgenus* Megatrypanum (*Stercoraria*). *T. theileri* in cattle, domestic buffalo and various wild Bovidae (members of the family of bovines), and also related species such as *T. ingens* (antelopes and cattle), are normally non-pathogenic and only concern us because they can confuse the parasitological diagnosis of trypanosomosis. *T. theileri* is a

cosmopolitan species (occurring all over the world), transmitted by tabanid flies and probably also by ticks. It is a large species (from some 30 to over 60, even 100 µm), normally very scanty in the peripheral blood, but it is encountered relatively frequently by careful observers. Its large size and its morphology are distinctive features. In stained smears it cannot be confused with the pathogenic species, because of its large size, the position of the kinetoplast (far from the posterior extremity), and its finely pointed posterior extremity. Even in the buffy coat, its large size and sluggish movements often allow the diagnosis of *Megatrypanum* sp. Of the other species of *Megatrypanum* only *T. ingens* is also sometimes found in cattle; it is also an unmistakable huge trypanosome, deeply staining with Giemsa, and with a typical band-like transverse nucleus. (Non-pathogenic species of *Megatrypanum* also occur in sheep and goats, but they are so seldom encountered in blood preparations that we will not discuss them.)

TABLE 1
Classification of the pathogenic African trypanosomes[11]

Subgenus	Species/group	Development/transmission*
Duttonella	*Vivax* group: *T. vivax* *T. uniforme*	In tsetse: proboscis only Can also persist by mechanical transmission
Nannomonas	*Congolense* group: *T. congolense* *T. simiae* *T. godfreyi*	In tsetse: midgut and proboscis Not known to maintain itself exclusively by mechanical transmission
Trypanozoon	*Brucei* group: *T. brucei brucei* *T. brucei rhodesiense* ** *T. brucei gambiense* ** *T. evansi* *T. equiperdum*	In tsetse: midgut and salivary glands Oral transmission in carnivores Mechanical transmission[12] Venereal transmission

* Congenital transmission is not mentioned in the table but, in principle, any species may occasionally be transmitted in this way.

** Causal agents of human sleeping sickness.

[11] As noted in the text, recent studies, using modern molecular techniques, have shown that there are more types/species within each group than indicated in this table. So far, most of these types have not been given separate species names. In the absence of a clear vision of what constitutes a species in micro-organisms in which the rules for higher organisms do not easily apply, we will stick to the old nomenclature.

[12] Oral transmission in carnivores and vampire bats.

The pathogenic trypanosomes

Table 2 indicates the occurrence of the pathogenic African trypanosomes in common domestic animals. Also included are the two non-pathogenic species of *theileri* group mentioned above, which may give rise to confusion; they belong to the subgenus *Megatrypanum* and the section Stercoraria.

Within each species there is a great variety of strains which may be classified in a number of ways. One way of classification is according to the pathogenicity, virulence, or disease-producing potential of the strain, and this can be extremely variable. The course and outcome of trypanosomosis is in addition influenced by a whole range of coexisting factors and influences, which combine and react to exert profound effects. No attempt therefore has been made to include a column in Table 2 summarizing the severity of the disease produced in each animal species by each trypanosome species. Such an attempt would be meaningless and misleading. The only indication of pathogenicity in Table 2 is that in the second column the list of livestock species is tentatively ranked in descending order of importance. In the fourth column the susceptibility of the common laboratory animals is indicated, which can be of some importance in certain diagnostic procedures, as will be seen in Chapter 4.

TABLE 2
The occurrence of African trypanosomes in domestic animals

Trypanosome species	Domestic animals affected	Reservoir hosts	Laboratory animals
T. congolense	Cattle, camels*, horses, dogs, sheep, goats, pigs	Several groups of wild mammals	Rats, mice, guinea pigs, rabbits
T. simiae	Pigs	Wart hog, bush pig	Rabbits, monkeys
T. godfreyi	Pigs	Wart hog	None susceptible
T. vivax	Cattle, sheep, goats, domestic buffalo, horses	Several groups of wild mammals	Usually none susceptible
T. uniforme	Cattle, sheep, goats	Various wild ruminants	None susceptible
T. brucei brucei	Horses, camels*, dogs, sheep, goats, cattle, pigs	Several groups of wild mammals	Rats, mice, guinea pigs, rabbits
T. brucei gambiense, *T. brucei rhodesiense*	Human sleeping sickness; affect domestic animals as *T. brucei brucei***	Several groups of wild mammals (particularly *T. brucei rhodesiense*)	As for *T. brucei brucei* (after initial adaptation where *T. brucei gambiense* is concerned)
T. evansi	Camels, horses, dogs, domestic buffalo, cattle	Several wild mammals in Latin America	As for *T. brucei brucei*
T. equiperdum	Horses, donkeys, mules	None known	As for *T. brucei brucei* (after initial adaptation)
T. theileri and *T. ingens* (subgenus *Megatrypanum*)	Cattle, domestic buffalo*** (not pathogenic)	Various wild ruminants	None

* Camels are highly susceptible to *T. congolense* and to *T. brucei*, but do not usually penetrate into tsetse country.
** In particular, the behaviour of *T. brucei rhodesiense* in domestic animals is quite similar to that of *T. b. brucei*, whereas *T. brucei gambiense* is on the average more chronic (as it is in humans).
*** Of the two only *T. theileri* has been reported from domestic buffalo.

EPIDEMIOLOGY[13]

So many factors intervene in the epidemiology of African trypanosomosis that an entire book could be written on the subject. In the context of this guide we cannot possibly discuss all the possible scenarios and have to restrict ourselves to the main factors.

[13] The general term *epidemiology* tends to replace *epizootiology*, which was in common use in connection with diseases in animals.

For general principles of epidemiology, we refer to books such as those by Putt *et al.* (1986) and Martin, Meek and Willeburg (1987). It is essential to be familiar with the fundamental terms used in epidemiology, for example, the difference between *prevalence* and *incidence*. It is also essential to distinguish between *disease* as opposed to *infection*; particularly trypanotolerant animals may be infected without having clinical disease, in other words, they may be healthy carriers. *Prevalence* is the frequency of *existing* cases of disease, or of infection, at a certain time. *Incidence* indicates the frequence of *new* cases within a certain period of time. Where it is possible to determine prevalence and incidence by certain tests, it is important to use a correct sample size, and for those with some statistical background considerations on sample size have been included at the end of this manual.

One all-important factor is whether we are dealing with tsetse-transmitted trypanosomosis or not. If so, much depends on the *Glossina* species responsible for transmission. There is a large body of experimental evidence to show that host preferences and vector capacity differ greatly between groups and species of *Glossina*. For example, recent laboratory experiments with teneral tsetse flies in Burkina Faso have shown higher mature infection rates with the savannah type of *T. congolense* in *G. morsitans morsitans*[14] and *G. morsitans submorsitans* (both belonging to the savannah group of tsetse) than in *G. palpalis gambiensis* and *G. tachinoides* (*palpalis* or riverine group). *G. morsitans submorsitans* was the best vector of both the savannah and the riverine-forest types of *T. congolense*, while *G. m. morsitans* had the lowest vectorial capacity for the riverine-forest type; *G. palpalis gambiensis* was the least effective vector for the savannah type of *T. congolense*.

Savannah species are on the whole better vectors of the pathogenic trypanosomes of livestock. Also, where savannah tsetse (*morsitans* group) are the vectors, the risk of contracting the disease is widespread, although their distribution area in the dry season decreases. When riverine species are the culprits (in many parts of

[14] *G. m. morsitans* does not occur in Burkina Faso, nor in West Africa as a whole.

West and Central Africa), transmission occurs particularly along rivers with dense vegetation along the banks (the so-called gallery forests). Some of the forest species (*fusca* group) are confined to dense forest and are therefore not normally in contact with livestock, but some also occur on the forest edge and may locally play a significant role as vectors of AAT.

Populations of savannah species feed mainly on mammalian hosts, particularly bovids (antelopes, buffalo, cattle, sheep, goats) and suids (wart hog and bush pig), while riverine tsetse have a very wide range of preferred hosts, including reptiles and humans. Zebras, certain antelopes and also carnivores have little attraction for tsetse flies. The proportion of a tsetse population found infected with pathogenic trypanosomes therefore depends not only on its vector capability, but also on the hosts on which it mainly feeds. For instance, reptiles do not carry pathogenic trypanosomes,[15] and there are also major differences between suids and bovids, as the former will infect the flies particularly with *T. simiae* and *T. godfreyi*, while bovids are mainly the source of *T. vivax* and *T. congolense*).[16]

Herd management is also important. Daily activity patterns of the tsetse species involved and the grazing patterns of the herds are of great influence. If the herds graze on infested sites at the time of the day that the flies are most active, transmission will occur more frequently. In the Sahel zone, many of the cattle owners (e.g. the Baggara and the Fulani) are transhumant, because in the dry season the pastures and watering places in the Sahel are insufficient to maintain the large livestock populations. The zebu herds, accompanied by small ruminants, are then moved hundreds of kilometres to the south, where they may enter tsetse belts and contract AAT. Although the owners generally know the danger and recognize and associate tsetse flies with the disease, they are not

[15] But tsetse flies do get infected on reptiles with specific reptile trypanosome species, such as *T. grayi* of crocodiles, a species of the subgenus *Megatrypanum* (Stercoraria). Such infections may render the microscopical diagnosis of trypanosome infections in tsetse flies difficult (see Chapter 3 - Diagnosis).

[16] Host preferences of a tsetse population can be determined in specialized laboratories by serologically identifying the species from which the blood in fed flies originates (blood meal analysis).

always able to avoid infested areas. Particularly during dry years the southward migration is greater than usual, and the owners may deliberately choose between the risk of starvation of the herd and of tsetse-transmitted trypanosomosis.[17] At the beginning of the rainy season the transhumants start to move back to the Sahel pastures, in order to arrive when these are sufficiently lush. The animals infected in the tsetse belts are diseased by the time they reach the rainy season pastures, and may even die before, the physical effort of transhumance adversely affecting the outcome. Unless the animals are treated in time, great losses may occur and when there are large numbers of tabanids and other biting flies around during the rains, the infection may be further transmitted mechanically outside the tsetse belts.

Species and breed susceptibility are of course of great importance. Whereas in tsetse areas trypanosomosis is a very obvious problem in susceptible livestock, it may remain practically inapparent where trypanotolerant breeds are concerned (even if these breeds may not be very productive when challenge is high).

The risk to susceptible ruminants living in comparatively free areas surrounded by tsetse-infested regions, or at the edge of tsetse-belts, varies from year to year. Generally, tsetse fly populations during wet years will increase, spread, and persist during the dry season in areas from where they disappear in dry years.

Also, animals used for transporting persons or goods are sometimes particularly at risk. For example, although the classical breeding areas of camels in Africa are north of the tsetse belts, individual camels are used for the transport of merchandise to transhumant animal owners in their dry season grazing grounds in or near tsetse belts, and these camels risk contracting tsetse-transmitted trypanosomosis. The same applies to the riding horses of travellers and of the transhumant cattle owners. Interestingly, in recent years

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[17] Ironically, the few herds that remain behind in the Sahel during the dry season and live on the few remaining watering places and the pasture available around these, are usually in better shape at the end of the dry season than the herds returning from the coarse vegetation and the unhealthy areas further south.

there has been a tendency in Kenya to start keeping camels as far south as the Masai areas, because of the great losses in cattle caused by the severe droughts in the 1980s; this will of course increase contact between camels and tsetse fly and result in more disease.

The epidemiology of non tsetse-transmitted trypanosomosis (*T. evansi*, *T. vivax*[18]) is also influenced by many factors. There may be seasonal outbreaks, where the populations of biting flies (Tabanids, stable flies, etc.) are influenced by important seasonal climatic differences. The (chronic) disease sometimes becomes more clinically apparent during the dry season, when immunodepressive factors such the poor nutritional state of the animal diminish its defences, even when the initial infection occurred during the rains. The epidemiology is also greatly influenced by host preferences and diurnal (daily) behaviour patterns of the various local species of tabanids and other biting flies (e.g. whether the hours that they are active allow much contact with livestock or not).

The main reservoirs of *T. vivax* infection in Latin America are probably domestic ruminants themselves, but *T. evansi* has found new wild reservoirs such as blood-sucking vampire bats and the capybara, a giant rodent. The peculiar involvement of vampire bats in the transmission of *T. evansi* has been mentioned before in this field guide.

DISTRIBUTION

This section is to some extent a continuation of the previous one on Epidemiology, as many of the factors that determine the distribution of a particular trypanosome species are also involved in epidemiology; in fact, distribution dynamics form an integral part of the epidemiology.

As discussed in the previous section, trypanosomes that are normally cyclically transmitted by tsetse flies, can be transmitted mechanically (see Life cycles), and in the presence of large numbers

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[18] The epidemiology of dourine, as a venereal disease, is of course very different again, and will not be discussed here.

of biting flies trypanosomosis in domestic animals may extend beyond tsetse belts. Horseflies (tabanids) and stable flies (*Stomoxys* species) are particularly important as mechanical vectors. Nevertheless, the distribution of nagana in Africa largely coincides with that of its biological vectors, the tsetse flies, and the disease tends to die out in their absence. The presently accepted approximate distribution of the genus *Glossina* is given in Figure 7; animal trypanosomosis is certainly present in the whole of this area, and in some cases extends to a variable degree beyond it. Within this huge area, the situation is far from uniform. Individual tsetse species (and/or subspecies) are limited to certain regions and have a geographical distribution pattern which is determined by their different climatic and host requirements (just think of the savannah group, the riverine group and the forest group). Trypanosome subspecies, types and even species also have different geographical distribution patterns. For example, *T. godfreyi* is (so far) only known in the Gambia, *T. brucei gambiense* occurs in western and Central Africa, *T. brucei rhodesiense* in eastern and southern Africa, etc.

As far as the individual trypanosome species are concerned, seasonal outbreaks of *T. congolense* infection have been reported outside tsetse areas in the southern Sudan, for example, associated with large numbers of tabanids, but normally this trypanosome species is confined to tsetse belts and their near surroundings. It has not managed to escape from its biological vector.

The same holds for *T. simiae*; although it is thought that mechanical transmission by stable flies may be important once the infection has been introduced by tsetse flies into a piggery, the infection is not propagated outside tsetse areas. Knowledge of *T. godfreyi* is still insufficient.

The case of *T. vivax* is different. The infection can be seen in Africa at some distance from the edges of tsetse belts, and the author of this book diagnosed the parasite in the late 1950s in sedentary cattle herds all along the White Nile from Malakal in the southern Sudan up into the semi-desert of Khartoum Province, hundreds of kilometres from any tsetse belt. A similar situation has been reported

in Ethiopia, where *T. vivax* is commonly found in highlands too cold for tsetse survival. But the most remarkable fact is that *T. vivax* has been able to establish itself in the western hemisphere, in the absence of tsetse. These American strains of *T. vivax* are thoroughly adapted to mechanical transmission and all attempts to transmit them biologically through tsetse have failed. In the past, *T. vivax* has also been present on the Indian Ocean island of Mauritius, without tsetse, but has been eradicated there. There are also indications that *T. vivax* may sometimes persist at a low level, because of mechanical transmission, after tsetse flies have been eradicated from an area.

The distribution of *T. brucei* seems to be closely associated with that of its *Glossina* vectors (Figure 7), but it should be remembered that *T. evansi*, and probably also *T. equiperdum*, appear to have been derived from *T. brucei* and have adapted to mechanical and venereal transmission, respectively. *T. evansi* has been spread widely by biting insects outside tsetse-infested regions in Africa, and also outside Africa; it is present in tropical and subtropical areas of Africa north of the equator, in Asia, and in South and Central America from Panama to Argentina. *T. equiperdum* infection, as a venereal disease, is even less restricted by climate and in the past has spread as far as Canada and Russia in the northern hemisphere, and as far to the south as Chile and South Africa. Its present distribution is not very well known; *T. equiperdum* is sometimes difficult to distinguish from *T. evansi*. It has been eradicated from North America and most of Europe. It is certainly present in northern and southern Africa, and in tropical Africa at least in Ethiopia and probably the Sudan. It has made a comeback (or perhaps has been rediscovered) in Europe (Italy, Russia, possibly other countries), and is still present in parts of Asia, including Ouzbekistan and China. It is also believed to be still present in parts of South America, but there is little reliable information.

Figure 7
Tsetse distribution in Africa

Chapter 2
African animal trypanosomosis

In this chapter the course of the disease, the clinical symptoms, the post-mortem lesions, the pathogenesis (how the disease symptoms and lesions develop) and economical aspects are reviewed.

CLINICAL ASPECTS

Trypanosomosis is not one of those diseases that can easily and unequivocally be diagnosed on clinical grounds. In early trypanosomosis departures from the normal state are usually only slight and it is necessary to be keenly observant to appreciate them. People required to diagnose diseases have to be familiar with the various aspects of animals in good health in order to recognize changes from the norm, so that diseases can be diagnosed early and control measures be applied in time.

As far as cattle (taken as an example) are concerned, healthy bovines are bright, alert and aware of their surroundings. Grazing and browsing is followed by resting and ruminating. The eye is bright and clear and the coat smooth and shiny. The ears are held erect, the tail is continually swishing at irritating insects. As a herd animal, the individual bovine is normally rarely seen away from the group for any distance or length of time. The visible mucous membranes of the eye, nostril and vagina are a clear healthy pink colour. Pale mucous membranes indicate anaemia, the most important clinical sign of trypanosomosis, but anaemia occurs in other diseases too, particularly infections with other blood parasites and certain intestinal worms. While the faeces vary to a considerable degree in consistency according to the dryness of the grazing, they normally have a characteristic and inoffensive smell. Dark black or pale yellow or grey liquid faeces with a strong unpleasant odour and the splashing and staining of the hindquarters with liquid matter are always to be regarded with suspicion, although not necessarily related to trypanosomosis. On the other hand, faeces should not

consist of very hard balls of material covered with mucus or flecked with blood. The healthy animal keeps its nostrils clean with its tongue, and thick white or yellow discharge should never be apparent. The muzzle should be moist and clean. Breathing at rest should be quiet and regular, at about 10-15 times per minute, but the rate increases greatly when the animal is exposed to the sun in hot weather.

A tendency to sway and stagger indicates weakness or nervous incoordination. The bodily condition is an important indication, both of the individual animal and of the herd. All members of a herd are not usually stricken at the same time by disease, and poor condition of a whole herd, as well as of other herds in the vicinity, may reflect poor seasonal grazing or lack of water, while poor condition of a herd in contrast with others nearby may be a sign of poor management. When poor condition is noticed in some animals only, it is more likely to be caused by disease.

Although rarely taken in the field, the body temperature can be a valuable indication of disease. Normal temperatures of various domestic animals are given in Table 3, but temperatures outside this range should not be regarded immediately as a sign of disease. The body temperature may vary during the course of the day, depending on the ambient temperature. In hot weather and when the animals are excited, the temperature may be well above what is considered to be normal, and be therefore meaningless in such conditions. In particular the camel, adapted to an existence during which there are great variations in the ambient temperature, shows large changes in body temperature, but even in cattle the differences may be surprisingly great. A lack of water, preventing the normal mechanism of heat loss through sweating, may also be responsible for a higher temperature in cattle.

In order to allow for the influence of various factors outlined above, *the best time to take the temperature of an animal is in the early morning in rested animals, before the ambient temperature has risen greatly.*

Despite these reservations, the temperature is an extremely useful guide to the presence of infectious disease, including trypanosomosis. The animal reacts to an infection by fever; it is a sign of the response of the bodily defences to the invasion by the infective agent. Successful treatment will result in the temperature returning to normal.

On the other hand, an abnormally low body temperature may be taken as a grave sign and can signal approaching death.

In the case of trypanosomosis the temperature fluctuates around an average which is higher than normal. This peaking is caused by antigenic variation (see below).

TABLE 3
Normal temperatures of domestic animals

Species	°C (upper limit under normal conditions ± 0.5°C)
Cattle	38.5
Sheep	39.0
Goats	39.5
Horses	38.0
Camels	37.5 (34.5-39.0)
Buffaloes (domestic)	38.3
Pigs	39.0
Dogs	39.0

In assessing a disease situation the opinion of the herdsmen should not be neglected, because they are continually in the presence of the animals and are best aware of the habits of the individual beasts, of local conditions, seasonal variations and past history. Patient enquiry will often result in the assembly of a great deal of indispensable information. Camel owners may be able to detect *T. evansi*-infected animals by the smell of their urine. Cattle owners often associate the loss of hair from the tail with chronic trypanosomosis; the author of this field guide does not know whether this association has ever been proved to exist.

Trypanosomosis is a collective term for a group of diseases brought about by one or more of the pathogenic trypanosome species. It should however be remembered that there are differences between infections caused by the various species and strains of

trypanosomes in different host species and host populations or breeds, and it must be stressed that there are great differences under the influence of various circumstances.

Typically, trypanosomosis is a wasting disease in which there is a slow progressive loss of condition accompanied by increasing anaemia and weakness to the point of extreme emaciation, collapse and death. However, there is a range of variation from the very acute disease in pigs caused by *T. simiae* to the usually mild condition caused by *T. brucei* or *T. evansi* in cattle. The individual and breed susceptibility are also of utmost importance. Infections that are on the average mild in trypanotolerant West African taurine cattle, such as the N'Dama and the Baoulé, may be severe in susceptible zebu and European taurine breeds.

Animals in good condition and not subjected to physical stress (such as having to transhume or walk far between pastures and waterpoints) usually suffer considerably less than animals on a poor nutritional level and having to expend much physical energy.

In horses extensive subcutaneous oedema is often seen in infections caused by trypanosomes of the subgenus *Trypanozoon* (*T. brucei*, *T. evansi*, *T. equiperdum*), but also by *T. congolense*. Infiltration with liquid of subcutaneous tissues may lead in acute *T. brucei* infections in sheep to swelling of the eyelids, the lips and the skin beneath the lower jaw.

Progressive symptoms of an affection of the central nervous system are not uncommon in horses infected with trypanosomes of the subgenus *Trypanozoon*; they may show staggering, paralysis, stupor, etc. similar to the symptoms of human sleeping sickness. Small ruminants infected with *T. brucei* may also show central nervous symptoms.

In the domestic pig, *T. simiae* produces a hyperacute, fulminating disease, which was described by Sir David Bruce (who first discovered it, and after whom the species *T. brucei* was named), as "the lightning destroyer of pigs". After a short incubation period

death occurs very rapidly and at post-mortem examination the picture is one of a complete capillary breakdown with haemorrhages and congestion in various organs throughout the carcass. The blood is found to be full of parasites, many sticking together in clumps (agglutination).

In cattle one sometimes encounters a hyperacute haemorrhagic form of trypanosomosis caused by *T. vivax*, normally a chronic disease. Prior to death animals are bleeding from many sites throughout the body, and at post mortem haemorrhages are very widespread and extensive. The intestinal tract, from the abomasum (fourth stomach) to the rectum, contains large amounts of blood. Large haemorrhages are seen beneath the linings of various organs, the heart, the pleural cavity, the peritoneum, the diaphragm – virtually in every organ and tissue. The disease progresses so quickly to death that there is no loss of condition. If treatment is given in the first few hours, recovery is remarkably rapid. There is a marked fall in the number of platelets in the blood, which play an important part in the process of blood clotting; their numbers are also deficient in some other diseases in which widespread haemorrhages are a feature.

In chronic trypanosomosis the chief pathological factor in the disease is a progressive anaemia with the profound effects this will have on various organs and systems and their basic functions throughout the body. However, anaemia is seen in a whole series of diseases caused by blood parasites (in particular babesiosis and anaplasmosis) as well as certain gastro-intestinal helminths (*Haemonchus*), and it is therefore not typical of trypanosomosis by itself. (The word *anaemia* means lack of blood, there is less haemoglobin, the iron-containing protein which transports oxygen to the various organs of the body and gives the blood its red colour.)

The following description would apply to an uncomplicated case of chronic trypanosomosis caused by *T. congolense* or *T. vivax* in a susceptible zebu.

The disease becomes apparent about seven to ten days after the infective tsetse bite, when the temperature will rise and the heart and respiratory rates increase. From this point onwards there will be a fluctuating but continuous slow deterioration in health with a steady loss of condition. A typical example of a temperature curve in trypanosomosis is shown in Figure 8.

The animal will become obviously sick with a variable appetite. It tends to leave the herd, seek shade and stand idly with hanging ears and tail, ignoring annoying insects. Its coat becomes dull and "staring" (lacking the sheen associated with good health). There may be some diarrhoea in the early stages.

Loss of condition will soon become obvious as first the fat beneath the skin and then the muscles themselves are greatly reduced and the underlying bones become apparent. The skin often loses its suppleness ("turgor") because of dehydration, the eyes are sunken and at this stage the classical signs of anaemia are obvious, the visible mucous membranes are pale and the blood is watery in appearance. The emaciation is associated with weakness and in the final stages results in inability to stand, and in pressure sores and ulceration of the skin over the bony prominences.

There is very often an increased secretion of tears (lachrymation). In early acute *T. vivax* infection in cattle trypanosomes may invade the anterior chamber of the eye, leading to the eye becoming bluish and opaque, and finally blindness may supervene. This condition can be confused with infectious keratitis (inflammation of the cornea) and conjunctivitis. When *T. vivax* is the cause, trypanocidal treatment leads to a rapid improvement of the eye condition. In dogs *T. brucei* also attacks the eyes and in this case in addition haemorrhages into the anterior chamber of the eyeball can result in blindness which will not respond to treatment.

POST-MORTEM FINDINGS

The usefulness of a post-mortem (PM) examination depends upon a basic knowledge of what is normal; this can only be obtained by practical experience, especially under the guidance of an experienced and skilled operator who can demonstrate PM techniques and pathological changes. Inspecting carcasses in an abattoir can be extremely useful, as long as one remembers that such carcasses have been bled, and the appearance of some organs therefore can be very different from those of animals which have died from other causes. For example, the lungs after slaughter and bleeding present an altogether different appearance from those of an animal which has died in a different way.

The PM findings in trypanosomosis can never by themselves lead to a certain diagnosis of the cause of death. There is not one single specific lesion.

In the acute stage there is loss of condition and anaemia, but not as severe as in chronic trypanosomosis. Miscroscopic examination of the blood will show that the haemopoietic system is actively trying to compensate for the loss of red cells (regenerative changes such as anisocytosis, normoblasts, Howel-Jolly bodies, basophilic punctations). The spleen is enlarged. The lymph nodes are enlarged and oedematous (containing more liquid than usual). The liver is enlarged and congested. The heart may be somewhat enlarged and may show a few haemorrhages on the muscle surface. There is also likely to be more fluid than normal in the chest, lungs, abdomen and pericardium (heart sac). The kidneys are pale and swollen. Subcutaneous oedemas may be present particularly in horses and sheep.

In chronic trypanosomosis the pathological changes seen at post mortem are more striking, without being typical. The carcass is emaciated and often dehydrated. The skin may show pressure sores and ulcers, when the animal has been unable to stand up for some time. The fat reserves under the skin have been used up and the skin is closely adherent to the underlying muscles and bone. The muscles have wasted to a remarkable degree and the underlying bones are prominent. The muscles are pale because of the anaemia and the blood is watery and pale, with an increased clotting time. The heart is

often enlarged and flabby because of muscle deterioration, and its weaker pumping action may have contributed to circulatory disturbances and increased fluid in the tissues (oedema). Unlike the picture seen in acute trypanosomosis, the lymph nodes are mostly normal or even hard, dry and reduced in size. The spleen is also normal in size or contracted with a drier pulp than normally seen.

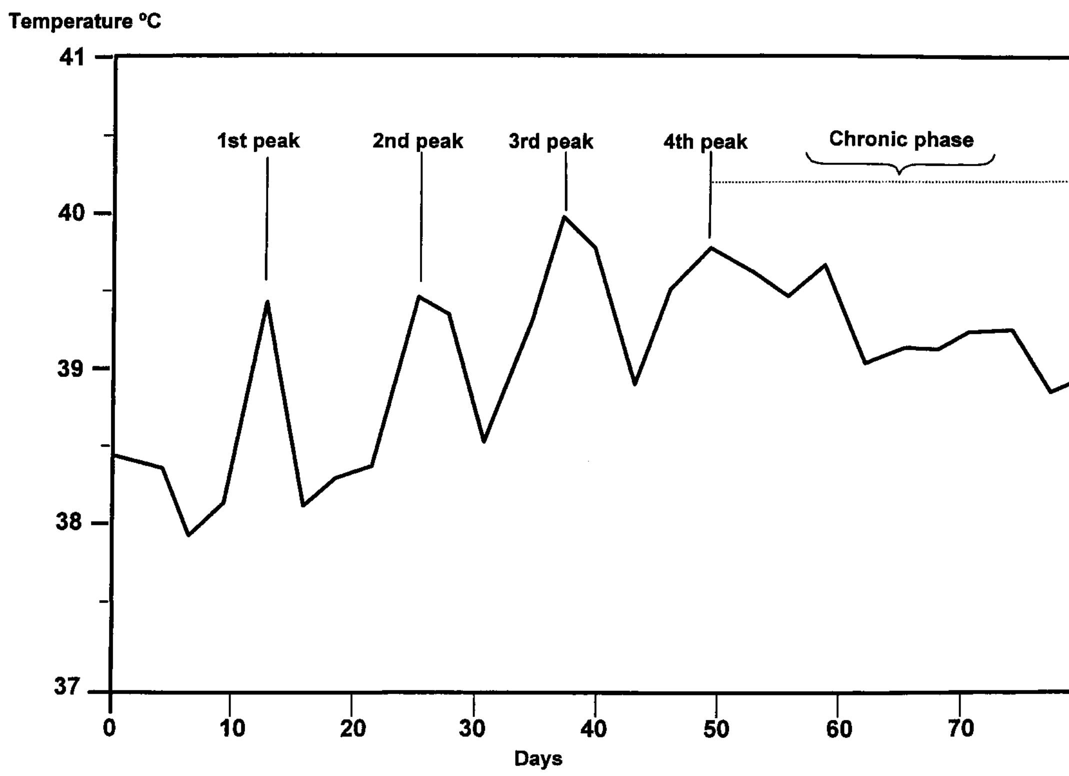
PATHOGENESIS

When the tsetse fly injects infective metacyclic trypanosomes into the skin of the host, there is a phase of local inflammation and a swelling, a so-called chancre, develops. The metatrypanosomes divide and multiply in the chancre and give rise to the typical blood forms which invade the lymphatics and lymph nodes, and then the blood stream.

Trypanosomosis, like other infectious diseases, starts with an increase of the body temperature, a hyperthermia. This is the result of the contact between the trypanosomes multiplying in the host and the defence system of the host. The surface proteins of the trypanosomes provoke the host in making specific antibodies against these proteins, and after a few days almost all of the trypanosomes in the blood are destroyed by these antibodies and the body temperature drops. However, a few parasites survive as they have been able to replace their surface proteins by different ones, against which the antibodies cannot act. These surviving trypanosomes are able to multiply, and cause a new peak of parasitaemia and hyperthermia, until the organism of the host makes specific antibodies against the new surface proteins. This seesawing process continues for a long time, as the trypanosome is able to make an almost unlimited number of antigenic variants, and the host responds to each of them, until either the antigenic repertoire of the trypanosome is finally exhausted, in which case self-cure of the host follows, or the ability of the host to react to all of the antigenic variants is overwhelmed, and the host dies.

A typical aspect of trypanosomosis therefore is the temperature curve: there are peaks every few days, particularly in the beginning. Antigenic variation becomes slower as the disease progresses and the intervals between temperature peaks become longer and the peaks are less high. Figure 8 illustrates this.

Figure 8
Temperature curve in a bovine suffering from *T. congolense* infection



One of the main symptoms of the disease is *anaemia* (decrease of haemoglobin in the blood). There are various theories on the pathogenesis of anaemia. In the early stages of the disease, it is believed to be caused in part by phagocytosis of red cells (their removal by a certain type of the host's white cells, the phagocytes). The red cells apparently become coated with material from lysed trypanosomes which tricks the phagocytes into mistaking them for foreign invaders and remove them. (This phenomenon is also called auto-immunity = immunity directed against cells of the host itself.) It is possible that the anaemia caused by phagocytosis is increased by toxic substances emanating from the trypanosomes which destroy red cells directly by lysis (haemolysis). The haemopoietic system (the system which produces red cells, mainly in the red bone marrow), tries to compensate for the loss of erythrocytes by increasing its activity but, later, in the chronic stages of trypanosomosis, othertoxins from the parasites have a depressing effect on the haemopoietic system, and the host is unable to produce as many red cells as are removed (even normally these cells have only a limited life span, and now they are removed even faster because of auto-immunity and haemolysis).

The anaemia means a reduction in haemoglobin and therefore in the oxygen-carrying capacity of the blood. Insufficient oxygen is available to the cells for their efficient functioning and the efficiency of their normal activities is reduced. A slow process of deterioration of health and condition sets in.

Trypanosomosis is also associated with *immunodepression*,[19] i.e. the host's immune system becomes less efficient to deal with infections. Although this can perhaps be explained in part by the depression of the haemopoietic system, which not only provides red cells but also white ones involved in the immune response, immunodepression occurs also in the acute stage; the ability of the immune system to react to invaders is already diminished before the haemopoietic system is depressed. Animals affected by trypanosomosis often develop a lower antibody

[19] Also called immunosuppression, but as there is never a total suppression of the immune system, the word immunodepression may be more appropriate.

titre after vaccination against other diseases, and secondary infections which the host would normally control may also crop up during the disease. For example it is common to find considerable numbers of *Babesia*, *Theileria* and/or *Anaplasma* in bloodsmears of animals suffering from AAT, in situations where normal animals are healthy carriers of these tickborne infections. Trypanosome infections disrupt the balance. Such concurrent diseases may also affect necropsy findings.

Various organs are affected by AAT, to some extent depending on the species involved. While (more or less hypothetical) toxins may be involved, as well as the anaemia (see above), the trypanosomes may also be more directly responsible. *T. congolense* is mainly confined to the blood, while *T. vivax* and *T. brucei* also invade the tissues. *T. vivax* is found in the lymph and even in the chamber of the eye and *T. brucei* is well known to invade the central nervous sytem in human sleeping sickness (*T. brucei gambiense* and *T. brucei rhodesiense*), but also in animals such as horses, goats and dogs. The nervous system is also affected in the later stages of dourine (*T. equiperdum*).

The *heart* is often affected by a myocarditis (inflammation of the heart muscle), and heart failure is often the direct cause of death. However, this depends to a large degree on the effort the heart muscle has to provide. Extensive myocarditis with the presence of trypanosomes in the heart muscle has been found in highly susceptible European cattle infected experimentally in Europe with pathogenic West African *T. vivax*, kept at rest in a stable and on a good level of nutrition, and which had shown no obvious signs of distress before they were slaughtered at the end of the experiment. Such infections could easily have caused fatal heart failure in African zebu cattle subjected to the stress of poor nutrition and walking long distances to watering points or during transhumance.

Oedemas (subcutaneous swellings caused by accumulation of tissue fluid) are often present in trypanosomosis, particularly in horses and dogs. There is evidence of increased permeability of blood capillaries, and therefore leakage of blood plasma leading to the swellings.

In chronic trypanosomosis the animal loses condition, there is *wasting*. During the acute stage, the appetite is variable, being decreased during the fever peaks. But 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 recurrent bouts of fever, but there are 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 cells are reduced in size and efficiency). The decreased supply of oxygen because of the anaemia is also an important factor (see above).

ECONOMIC ASPECTS

Tsetse-transmitted trypanosomosis

In high challenge areas, and in the absence of trypanotolerant breeds, tsetse flies and AAT prevent the keeping of livestock, at least of those species that are affected by AAT. In that case, the economic impact on livestock production is most pronounced. In several African countries livestock (draught oxen) and their products (manure) play an important role in crop production, and the integration of livestock in crop agriculture is therefore severely affected.

Keeping non-susceptible livestock, in particular poultry, may be the best answer to solving the problem of providing sufficient animal proteins in the human diet. Or game farming may be a feasible option. Sometimes the area is more suitable for the production of valuable agricultural crops which can be sold and the income used to buy animal products from elsewhere.

All kinds of situations exist between this extreme case and the absence of trypanosomosis; the economic impact varies accordingly. Many factors are involved when economic aspects are considered, such as:

- Species, type, productivity, value and susceptibility of livestock. For instance: exotic dairy cattle with a high production are both very valuable and very susceptible; exotic

breeds in general are of high monetary value and more susceptible than indigenous ones having been exposed to the disease since many generations. Nevertheless, indigenous draught oxen are valuable and, when worked hard, have an increased susceptibility to the effects of the infection. Horses are particularly susceptible to trypanosomes of the subgenus *Trypanozoon*, cattle much less so. Trypanotolerant breeds can survive and even produce where other breeds can only be maintained under intensive and expensive chemoprophylactic or chemotherapeutic regimens.

- Challenge, which depends on species of fly present (vectorial ability, host preferences), density of the fly, their daily activity patterns and the grazing patterns of the livestock, and wild reservoir hosts).
- Presence of drug resistance. (As we shall see further on, very few drugs against trypanosomosis remain commercially available.)
- Type of production (commercial, subsistence, transhumant).
- Economic situation and management of the country (part of national budget and foreign exchange allotted to livestock resources).
- Commercial factors, such as "dumping" of surplus meat by the European Union (which decreases the price of locally produced animals), or an unrealistic exchange rate, may bias cost-benefit aspects of trypanosomosis control.

The economic impact is made up of direct losses (consisting of loss of production, mortality, abortion), as well as the cost of control (which includes the cost of drugs, their transport to the field site, the salaries of the operators, etc.).

The loss of *potential* production (i.e. the production that could be achieved if trypanosomosis did not occur) are indirect losses. At present unused grazing areas in many of the tsetse-infested areas of Africa could support a large ruminant livestock population. However, the control of the fly should only be envisaged when really needed, and then only when proper and sustainable land-use plans have been elaborated, and when the political will and

legislative means to carry such plans through are assured. If not, overgrazing is bound to occur, followed by erosion and, depending on the climatic zone, by desertification, leading to permanent loss of the land. In the meantime, the presence of tsetse fly preserves these areas.

Because livestock keepers avoid certain tsetse-infested areas, cattle distribution is often imbalanced or even distorted. From the continental cattle distribution we know that this indirect effect of AAT is very important; only 10 million out of 165 million head of cattle in the tsetse-infested countries of sub-Saharan Africa are distributed within the limits of the continental fly belt while most of the remainder is distributed at the perimeter of the fly distribution. At the local level, it is extremely difficult to clarify this point because nobody is sure about the magnitude of these indirect losses. Still, the collective, indirect AAT losses are estimated by FAO to be in excess of the 0.6 to 1.2 hundred thousand US dollar direct losses incurred by trypanosomosis-affected cattle.

Increasingly, tsetse and trypanosomosis control schemes become concentrated in selected areas of high priority. These are areas where control is technically feasible, where the economic returns are considerable and where the transformation of the landscape, from bush to farmland, already occurs because of demographic pressure. It is in such dynamic environments, which become progressively less suited for tsetse survival, that it is economically attractive to intervene.

It is important to monitor the changes during such interventions. Data have to be collected to check on what happens to tsetse-transmitted trypanosomosis, how farming practices and the landscape change. Close monitoring makes it possible to adjust control programmes for technical reasons, or make the programme more efficient in agricultural (economical) terms, or adapt to environmental degradation risks.

Simulation models are useful to examine the economic impact and to decide upon suitable control strategies to achieve a positive cost-benefit result, taking into account the range of various parameters in any particular situation (see, for example, Brandl, 1988). Because of

the land-use aspects, there is a growing tendency towards the collection of georeferenced data, which may be plotted on maps; computerized versions may be examined in the so-called geographical information systems (GIS).

Mechanically transmitted trypanosomosis

Studies on *T. vivax* and *T. evansi* in Latin America show that their economic impact can be quite severe. Trypanosomosis of domestic animals has been ranked as third in importance in Colombia, after ticks/tickborne diseases and liver fluke. Even the inapparent losses of subclinical infections by *T. vivax* may be considerable and the same certainly applies to mechanically transmitted trypanosomosis in Africa; futher economic studies are necessary in order to obtain reliable figures.

Chapter 3
Diagnosis

Diagnostic procedures vary not only according to the tools available, but often even more to what one wishes to know.

If owners report disease problems in their cattle herd or flock of small ruminants, in a region where tsetse flies are known to occur, one is not really interested in knowing which species of trypanosome is or are causing the disease. It is enough to confirm that the disease is indeed trypanosomosis, as the control measures are generally similar for all species. Direct parasitological diagnosis in wet blood films or buffy coat preparations may be all that is needed for this purpose. Even though such methods are not the most sensitive and a proportion of the infections will be missed, as long as a sufficient number of animals is examined, the diagnosis of trypanosomosis will be reached. Once the disease has thus been confirmed, clinical diagnosis of individual animals is indicated, unless treatment of the whole herd appears to be preferable. Control measures should be instituted as fast as possible, and no time should be lost by sending specimens to a distant laboratory. So-called "pen-side testing" is needed in this case.

For mechanically transmitted trypanosomosis in herds in tsetse-free regions, it is usually enough to confirm that the disease is caused by trypanosomes. A more precise diagnosis is usually even less important here; for instance there is only one species of trypanosome causing surra in camel herds. For animals such as horses, which are kept individually, or at least not usually in large numbers, a more sensitive method is preferred, or, if none is available, direct parasitological methods should be carried out repeatedly over a length of time, to increase the chances of finding the trypanosomes (or the reliability of a negative result).

On the other hand, in research, such as epidemiological surveys, requirements may be far more precise. Assessment of the seasonal occurrence of different trypanosome species demands the most sensitive and specific method applicable in the circumstances in which the researcher has to work. The accuracy of the work will be increased if two or more methods are used that can complement each other. Thus, if the investigator can fall back on a fairly sophisticated laboratory, it may be possible to use the potentially very sensitive polymerase chain reaction (however, at present, available reagents are so specific that they will not detect all different types of a trypanosome species and reagents are not [yet] available for all the known types, see further on). Sanitary surveillance associated with eradication schemes (for example in the case of dourine) or the prevention of the introduction of trypanosomosis (dourine, surra) across national borders, also presents particular requirements (the sensitivity of the test used is essential in this case).

In regions where the disease is known to occur, clinical symptoms and post-mortem lesions are important indications, especially in combination with the history of the disease and the region in which it occurs. However, symptoms and lesions of trypanosomosis are never pathognomonic (which means specific for the disease), and suspicion has to be confirmed by other means. Clinical signs and PM lesions are reviewed in Chapter 2.

LABORATORY METHODS

Parasitological diagnosis[20]

Microscopical diagnosis. *Direct methods: wet blood films (= fresh blood preparation).* A simple technique is to examine fresh blood between a coverslip and a slide with the microscope, using medium magnification (usually a dry objective of 40x or even less, and eye-pieces of 5-10x). Trypanosomes are seen either directly, moving between the blood cells, or indirectly, as they cause the blood cells to move. The lower the magnification, the larger the field (and the quantity of blood) observed, and the faster the examination; the preferred approach depends on the experience of the operator.

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[20] The parasitological diagnosis of dourine is discussed in Chapter 5, p. 138, as this venereal disease is a special case.

Advantages

- Simple and inexpensive.
- If trypanosomes are found, the disease is diagnosed on the spot.

Disadvantages

- Unless the animals are brought to the veterinary centre, or the blood (with an anticoagulant) can be taken quickly to the centre, a field microscope has to be taken to the herd, as the parasites lose their mobility after a limited time.
- Limited sensitivity, the detection limit is usually around 10^4 trypanosomes per ml of blood.[21]
- The species of trypanosome cannot be identified. (*T. vivax* can often be strongly suspected if the parasites move quickly forward through the microscopical field.)

Fresh preparation of lymph. Trypanosomes, particularly T. vivax, are sometimes found in lymph collected from a lymph node, when they are not found in blood. The opposite may also occur however, and the procedure is not commonly carried out.

The lymph is usually collected from a swollen prescapular lymph node, and examined between slide and coverslip, like blood. The presence of trypanosomes is usually only seen indirectly, by the movements of the lymphocytes, because the great density of the lymphocytes will obscure the trypanosomes.

Thick blood film. A drop of blood is applied on a clean slide and spread out with the corner of another slide, or with a match or a needle, to produce a circular area to a thickness such that, when dry, the hands of a watch or small print can be seen through the film. One may also let the drop run over the inclined slide until the appropriate thickness is attained. Next, the film is thoroughly dried, and in the laboratory stained without fixation, usually with Giemsa stain. Giemsa stain is aqueous and removes the haemoglobin by lysing the red blood cells. Fixation with methanol is not carried out, as it would prevent lysis, which is necessary in order to see through the several layers of blood cells, which otherwise would obscure the parasites.

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[21] The sensitivity depends to some extent on the species concerned, *T. brucei* and *T. vivax*, being larger and the latter also more mobile, are more easily noticed than *T. congolense*.

If lysing and staining are done before the blood has thoroughly dried, the film may be washed off the slide. On the other hand, if many days elapse between the preparation of the film and lysis and staining, lysis may be incomplete, especially if the film has been exposed to heat or sun. It may help to lyse such films in distilled (or at least clean) water before staining, but it may difficult to obtain satisfactory results. Examination is carried out under the microscope, preferably using an oil immersion objective of 40-50x. Specific diagnosis of the trypanosomes is sometimes possible using the 100x oil immersion, but is usually difficult or impossible because the process of lysis distorts the parasites.

Advantages

- Simple and inexpensive.
- A field microscope is not needed, as the blood films are taken back to the centre for processing and examination at ease.
- It is sometimes (but mostly not) possible to identify the trypanosome species seen.

Disadvantages

- An immediate diagnosis of trypanosomosis on the spot is not possible.
- The sensitivity of the method remains limited.

Thin blood smears. These are made as in the case of blood smears to detect other blood parasites. They are fixed by methanol and stained with Giemsa stain, or with one of the more recent fast stains such as Diff-Quik, RAL 555, Field's stain, which have the advantage of acting much faster than Giemsa. They are read using an oil immersion objective (40-50x for scanning, 100x for identification of trypanosomes). The morphology of the different species has been indicated in Chapter 1 (p. 22).

Advantages

- Specific diagnosis of trypanosomes is possible.

Disadvantages

- The sensitivity is extremely low, and the main use of thin smears is in fact the specific identification of trypanosomes

found in wet or thick smears. But when only a few parasites have been seen in a fresh preparation or a thick film, the thin smear may well be negative.

Thin smears of lymph. Lymph aspired from a prescapular lymph node, instead of being examined as a fresh preparation (or after a fresh preparation has been found positive), can also be made into a thin smear, fixed and stained, which will make specific identification possible. The smears should be very thin, as the many lymphocytes, which are also stained, complicate the visualization of the parasites. For this reason thick lymph smears are not suitable for diagnosis, lymphocytes cannot be lysed as can red cells.

Concentration methods: buffy coat examination (also called the Woo method). It may be useful to discuss here first the constituents of blood. Blood consists of a liquid, the plasma, in which blood cells are suspended. There are three main kinds of blood cells, the red cells (erythrocytes), white cells (leukocytes, of which there are several types) and thrombocytes (or platelets, responsible for starting the process of blood clotting). If a blood sample is taken into an ordinary glass tube, the blood will coagulate, clot, as one of the blood proteins, fibrinogen, changes under the influence of the platelets into insoluble fibrin, which forms a mesh of strands in which the blood cells are trapped. This mesh contracts and the serum (= plasma without fibrinogen) separates.

Clotting of blood can be prevented by adding an anticoagulant, such as sodium citrate, heparin or EDTA. If such blood is left alone in a tube, the cells which are slightly heavier than the plasma, sediment into a column beneath the plasma. This process can be much accelerated and the separation improved by spinning the tube in a centrifuge at high speed. It will then be seen that most of the white cells settle at a slower rate than the red cells, as their specific gravity is a little less than that of the red cells, and form the so-called buffy coat between the much thicker red cell layer and the column of plasma (Figures 9 and 10). The specific gravity of trypanosomes, which are free in the plasma, is also slightly less than that of the red cells and so they tend to concentrate at the limit of

plasma and the buffy coat, as well as in the buffy coat. (This also depends on the species, the specific gravity of *T. congolense* is almost the same as that of the red cells, and they are thus less concentrated.)

This concentration technique has been standardized by the use of microhaematocrit tubes and a specially designed centrifuge (Figure 11). The tubes are capillary tubes, which can be obtained treated inside with an anticoagulant or, if untreated, an anticoagulant must be added to the blood before it is taken up in the tubes. The rotor plate, which can take many tubes of different animals at the same time, is spun at high speed (12 000 rpm) for five minutes.

Figure 9
The components of blood

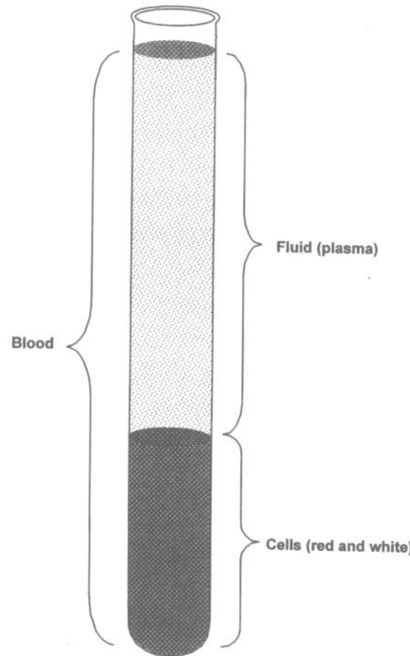


Figure 10
Blood components after centrifugation

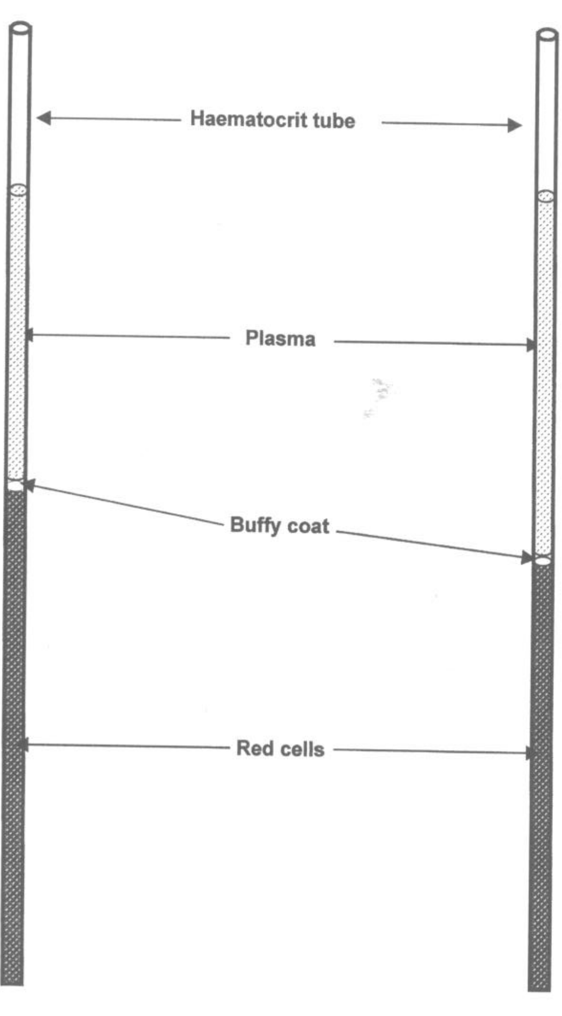
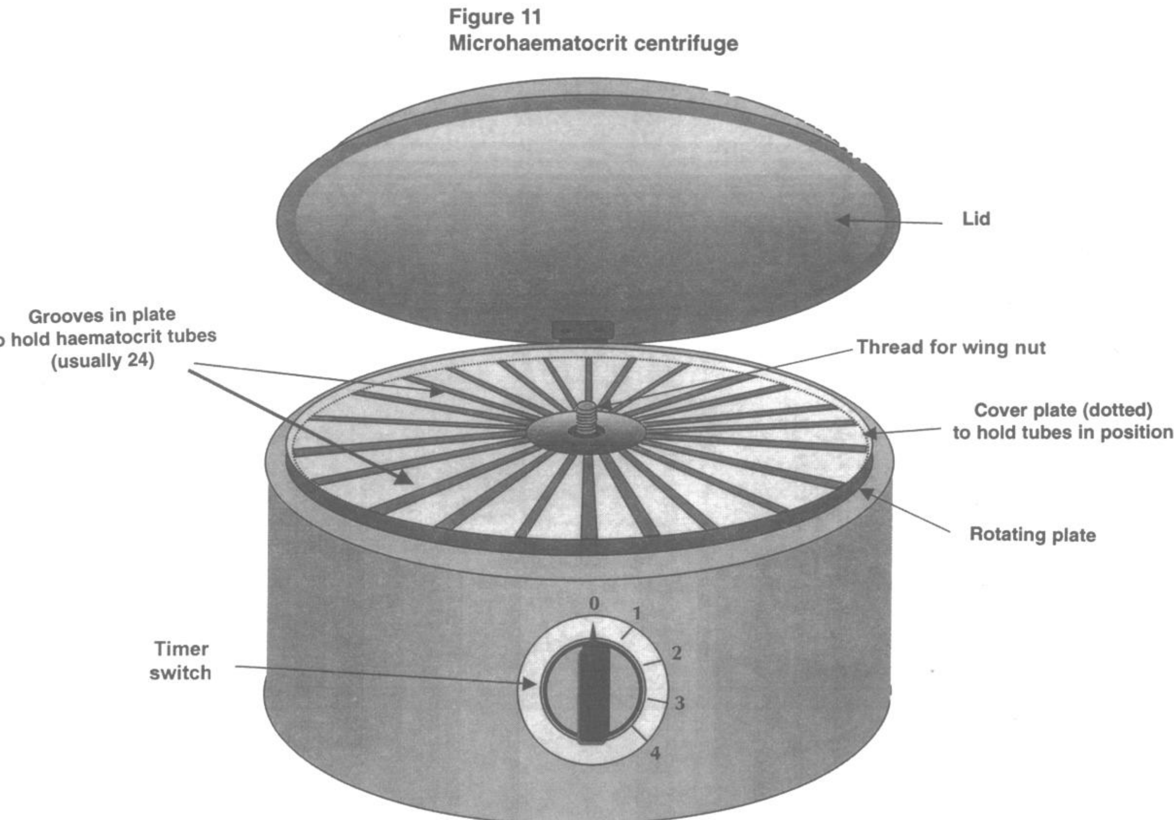
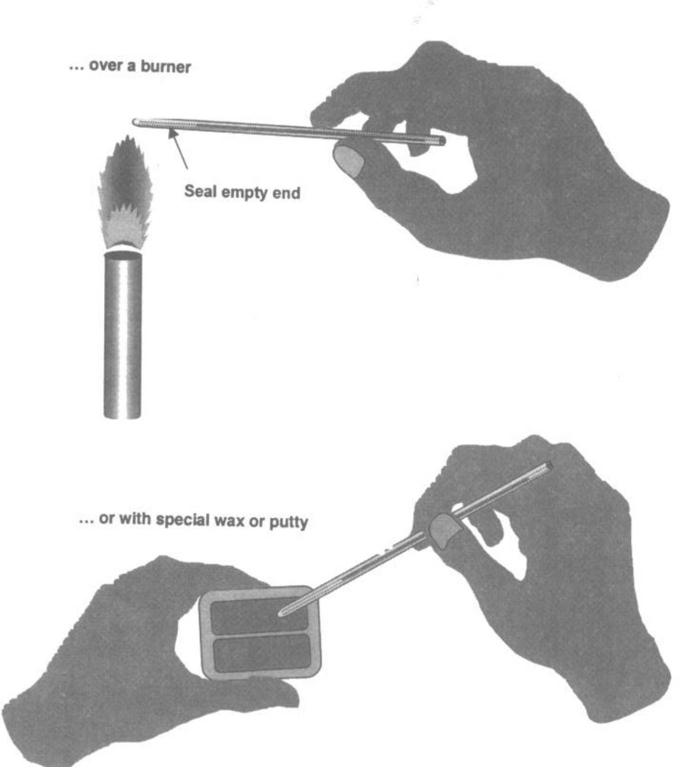


Figure 11
Microhaematocrit centrifuge



The capillary tubes are open at both ends. Blood is taken up at one end, until about three-quarters of the length of the tube is filled. Filling occurs by capillary attraction and practice will show how to apply and incline the tube to achieve this. It is important not to have the blood column interrupted by air. The other end of the tube is then sealed over a burner (taking care not to char the blood) or by special wax or plasticine (Figure 12). The tubes are then placed in the grooves (individually identified by a number corresponding to the number of the blood sample) of the rotor plate, with the sealed end outwards (to prevent the blood from being thrown out during centrifugation); the cover is closed and screwed down, and the timing is set for five minutes. After centrifugation, the tubes are removed, care being taken that it remains known to which animal each of the tubes corresponds.

Figure 12
Sealing haematocrit tubes

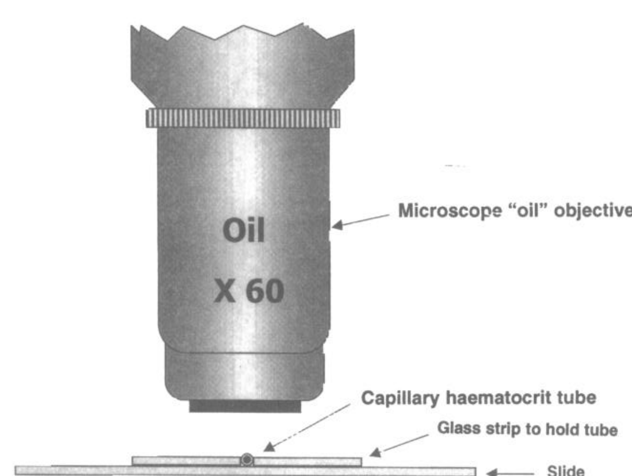


The packed cell volume (PCV) value (= the length of the column of concentrated cells, expressed as a percentage of the total length of the blood column) can be read directly in a special reader, which can be individually adjusted for the length of the blood column in each tube; the PCV gives a valuable indication on the presence and degree of anaemia.

The tube is then examined for the presence of trypanosomes, either by direct examination of the buffy coat/plasma junction, or after making a smear of this area.

Direct examination. The tube is placed on a microscope slide in a slot formed by sticking two pieces of glass to the slide 1.5 mm apart (Figure 13). Immersion oil is placed on the capillary tube, to fill the space between the glass bars and the tube, over the region of the tube where the parasites, if present, are concentrated and will be visible (buffy coat, and buffy coat/plasma junction). The best results are obtained with a special oil immersion objective lens, magnifying 40-60 times, which has an extended working length so that one can focus deeply into the tube as it is rotated during examination, and with a special substage condenser providing phase contrast illumination. This is the classical Woo method.

Figure 13
Section through a slide adapted to hold haematocrit tubes



Advantages

- The sensitivity is higher than that of the direct methods, of the order of 10^3 trypanosomes per ml of blood, but this also depends on the species of trypanosome. For *T. vivax*, a large and very mobile species, the method has been reported to detect 80 percent of cases where the numbers of trypanosomes per ml of blood are between 300 and 700, and 100 percent for numbers of more than 700 per ml. It has been reported that a shorter time of centrifugation, three minutes only, may result in a higher sensitivity for *T. vivax*.
- The special centrifuge can be run on a portable generator, so that diagnosis in the field is possible, if one has also a field microscope.
- The PCV value is obtained at the same time.

Disadvantages

- Special equipment is needed.
- No specific identification is possible, although the type of motility may give some indication.

Darkground/phase contrast buffy coat technique. The haematocrit tube is snapped 1 mm below the buffy coat/red cell junction, to include the upper layer of red cells. Clean and precise snapping can be obtained by marking the spot with a small glass saw or diamond pen. The contents, including the first centimetre above the buffy coat, is then gently expressed on to a clean slide, so that some plasma is included with the buffy coat. (The tube may also be snapped in this second site, and the contents of the intermediate section thus obtained is expressed on the slide.) The expressed contents are carefully mixed and then examined under a coverslip, using a special condenser providing dark ground phase contrast background illumination, which renders the trypanosomes much more visible. The material can also be treated as a smear, to be fixed and stained for specific identification of the parasites.

Advantages and disadvantages (compared to the classical Woo method)

- It is largely a matter of personal preference and experience, but the sensitivity may be somewhat greater. Visualization of the trypanosomes may be easier. There is no need for a special objective with an extended working length. Stained smears may moreover enable a specific diagnosis.

A comparison of these various parasitological techniques has shown that their sensitivity differs to some extent according to the trypanosome species concerned, but that in general the descending order of sensitivity is dark ground/buffy coat - classical Woo method - thick film - wet film - thin film.

Lysis and centrifugation. By hypotonic shock or even better the use of appropriate concentrations of certain detergents it is possible to obtain lysis of the red cells, without affecting the mobility of the trypanosomes, which can then be concentrated by centrifugation. Sodium dodecyl sulphate (SDS) has been reported as particularly suitable, in a concentration of 0.12 percent. Larger volumes of blood can be examined by this method than by the haematocrit method. Equal volumes of blood (e.g. 5 ml), to which an anticoagulant has been added, and PSG buffer containing 0.24 percent of SDS are mixed and left at ambient temperature for at least ten minutes. The mixture is then centrifuged for ten minutes at 2.250 g. The sediment is examined between slide and coverslip, if necessary after adding a minute quantity of PSG.

Advantages

- The sensitivity is higher than that of the preceding methods, of the order of 10^2 trypanosomes per ml.

Disadvantages

- More steps are necessary and the method takes more time because of the time needed for lysis and longer centrifugation, for a gain in sensitivity which is after all limited.

Use of experimental animals. Low or subpatent (= not patent, not observable by microscopical examination of blood) parasitaemias of certain species and strains of the pathogenic trypanosomes can be revealed by injecting laboratory animals with the blood to be examined. The sensitivity of this method varies according to the species or even strain present and the susceptibility of the experimental animals used. The last column of Table 2 (Chapter 1) indicates experimental animals that are susceptible to each trypanosome species. It should however be realized that within each trypanosome species there are important strain differences, in this respect too. Also, there may be important differences

in the susceptibility to a given trypanosome species or strain between inbred laboratory strains of an experimental animal species.

The two species of experimental animals most commonly used are laboratory rats and mice. They can be bred and maintained by diagnostic laboratories, provided that strict attention is paid to housing, hygiene, correct feeding, and breeding..

A blood sample, with anticoagulant, from the suspected domestic animal is injected in one or several rats or mice. This is mostly done by the intraperitoneal route (in the abdominal cavity), using 1 or 2 ml for a mouse and up to 5 ml in rats. The technique of inoculation is easily mastered after a demonstration and a little practice. The blood of the rodent is then examined frequently by snipping off a minute piece of the tip of the tail and transferring the resulting drop of blood to a slide for microscopical examination under a coverslip. Regular examination (preferably daily, at least twice a week) should continue for at least two months after inoculation.

Some more details are given below for the different trypanosome species.

T. congolense. Rats and mice are commonly used, although rabbits and guinea pigs may also be considered.

Some strains of *T. congolense* are readily established in rodents, whereas others do not easily infect them. In some cases the less virulent strains have long incubation periods, up to two weeks or more and some may not establish in rodents at all or the parasitaemia may be fleeting and short lived.

Generally speaking, rats are more easily infected than mice and should be used for isolating strains of known low pathogenicity.

T. simiae. Only rabbits may be susceptible to the species and not all strains establish themselves in this host. Some strains are virulent for monkeys and it was in this animal that the species was first discovered, hence its name.

After passage through rabbits or monkeys, *T. simiae* often becomes less virulent for domestic pigs and loses its polymorphic characteristics, existing as the long stout form only.

T. godfreyi. This trypanosome apparently does not infect laboratory animals.

T. vivax. With rare exceptions, this species does not infect any of the laboratory animals. There have been (very few) reports of adaptation of *T. vivax* to rodents by serial passages and other methods, and rare strains will grow well in laboratory rodents straight away after isolation. In general the use of laboratory animals is not suitable for the diagnosis of *T. vivax*.

T. brucei. Rats and mice are very susceptible to most strains of *T. brucei brucei* (and *T. brucei rhodesiense*, much less *T. brucei gambiense*) and the blood of apparently healthy cattle in tsetse country may prove to be infected at a subpatent level with this trypanosome when blood is injected into rodents. But even with this species there is a range of sensitivity; some strains appear in the blood after 48 hours or less, rapidly reaching enormous numbers and killing the rodent in a few days, others take longer to appear and may cause a disease lasting many weeks.

Rapid serial passage through rodents of the same species, i.e. transferring infected blood to the next animal as soon as the trypanosomes appear, increases the virulence for the rodent species to a maximum which kills rapidly. At the same time the parasite loses its polymorphic characteristics so that it shows virtually only the long slender type. As the polymorphism is lost, so is the ability to infect tsetse flies. (It is in this way that *T. evansi* is believed to have originated from *T. b. brucei*, following rapid serial mechanical passages by biting flies in camels.)

Laboratory rodents are used to reveal the almost true prevalence of *T. brucei* in domestic or wild animals; one single trypanosome is often enough to establish infection in a rat or mouse. Only *T. brucei gambiense* will often not readily establish in rats or mice at the first

isolation, although it may adapt to rodents after passage through other hosts, such as monkeys.

T. evansi. Strains of this species are infective for laboratory rodents in a similar manner as *T. b. brucei* and *T. brucei rhodesiense*, so that rat and mouse inoculation is used to reveal subpatent infections with this parasite.

T. equiperdum. Even in the normal equine host, the parasites are exceedingly scanty in the circulating blood, and isolation in laboratory rodents is rarely successful and not normally used for diagnosis. However, once adapted to rodents following rapid serial passages, it becomes indistinguishable from rodent-adapted strains of *T. brucei* and *T. evansi.*

General. As mentioned above, strains of trypanosomes that give fleeting low parasitaemias in rodents can usually be adapted to them by making serial passages in rodents as soon as the parasites are seen. Gradually the parasitaemias become higher and the incubation period shorter.

Advantages

- When rodents are susceptible to the particular trypanosome species and strain, the method is highly sensitive, and definitive as the parasites are actually seen. It can thus be of great value as a research tool, but not for all species.

Disadvantages

- Apart from the question of cost and the logistics of transport to and from the field, it is also not a suitable method for routine diagnosis because suitable rodents have to be maintained and monitored over long periods.

A word should be said here about the possibility of parasitological diagnosis of trypanosomes in the biological vectors,* Glossina *spp. It is used only in epidemiological studies, not in routine diagnosis. An operator skilled in dissecting tsetse flies can tentatively determine the infection rates of tsetse flies with

different trypanosome species by microscopical examination of the various parts of the digestive tract (see Table 1).

- *Infection in the proboscis alone is caused by* **Duttonella** *(***T. vivax** *or* **T. uniforme***);*
- *Infection in the salivary glands is caused by* **Trypanozoon** *(***T. brucei** *subspp.);*

Uncertainties remain, however:

- *Infection in the midgut alone may be caused by immature infection by* **Nannomononas** *(***T. congolense, T. godfreyi, T. simiae***), by* **Trypanozoon***, or even by the crocodile parasite* **T. grayi** *(of which the mature metatrypanosomes occur in the hindgut);*
- *It is not possible to determine definitely by microscopical examination whether infection in the midgut and other parts of the digestive tract is caused by a single or a mixed infection.*[22]

Indirect methods

Serological tests. The aim of serological tests is to detect specific antibodies (which are blood proteins belonging to the immunoglobulins), developed by the host against the infection or, inversely, to demonstrate the occurrence of circulating parasitic antigens in the blood by the use of characterized specific antibodies. The detection of antibodies indicates that there has been infection, but as antibodies persist for some time (weeks, sometimes months) after all trypanosomes have disappeared from the organism (either by drug treatment or self-cure) a positive result is no proof of active infection. On the other hand, circulating trypanosomal antigens are eliminated quickly after the disappearance of the trypanosomes, and their presence therefore shows almost always that live trypanosomes are present in the animal.

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[22] Using appropriate molecular techniques on the parasites in the various parts of the digestive tract (PCR, nuclear probes), the investigator can acquire more certainty but, as stated before, specific molecular tools are not (yet) available for all types of every trypanosome species.

Validation and standardization of procedures is important if one hopes to compare results obtained in various countries and by various teams. Validation and standardization of some tests are coordinated by the Joint FAO/IAEA Animal Health and Production Division in Austria. The World Organization for Animal Health (OIE) in Paris issues the *Manual of standards for diagnostic tests and vaccines*, which should be consulted for further details. Serological tests are mainly used as tools for research, for monitoring trypanosomosis control programmes and for surveys, not so much for the routine diagnosis of the disease in the field, and we will indicate only the principles here.

Antibody-detecting tests. Many different types of serological tests have been in use for many decades, and there is little point in enumerating them all. We will mention and describe the general principles of some of the most commonly used, the indirect immunofluorescent test, the indirect ELISA and, particularly for the detection of antibodies to *T. evansi*, a card agglutination test. Variations of these and other tests are used for specific scientific purposes. It is quite possible that other tests will become routine in the near future, such as agglutination assays using latex beads coated with either antigen or antibody; time will tell.

The indirect fluorescent antibody (IFA) test

- A smear of blood containing fixed trypanosomes constitutes the antigen (which is actually of course a mixture of a great number of antigenic proteins). Let us assume that we wish to carry out the test on sera of cattle. The bovine serum to be examined is put into contact with the smear, and immunoglobulins (antibodies) against the trypanosomal antigens attach themselves to the trypanosomes in the smear, and remain stuck to the smear even when the serum is washed off.
- In order to show that antibodies have reacted with the antigen, a commercial preparation containing antibodies (also immunoglobulins) raised in laboratory animals, usually rabbits, against bovine immunoglobulins, is applied to the smear, and washed off after being allowed to react. If these rabbit antibodies remain stuck to the slide, the test is positive, as they

indicate the presence of anti-trypanosome bovine immunoglobulins which have reacted with the antigen slide.

- In order to show the presence or absence of the rabbit immunoglobulins, and thus indirectly the presence or absence of specific antibodies against trypanosomes in the bovine serum, the rabbit anti-bovine immunoglobulins in the commercial preparation are conjugated with (fixed to, "labelled" with) a fluorescent dye, usually fluoresceine, which can be detected by looking at the smear with ultraviolet light. The combination of immunoglobulins and fluoresceine is called a conjugate, and in this case it is a rabbit anti-bovine conjugate. If the microscopic examination shows the trypanosomes in the antigen smear to be fluorescent, the test result is positive.
- One can "titrate" the bovine serum (determine the level of specific anti-trypanosome immunoglobulins) by using serial dilutions of the serum and determining the end point, the highest dilution still giving a positive test result.

Figure 14 shows the principle of the test.

There are quite a number of possible pitfalls associated with this test, and it is carried out only in the laboratory by qualified laboratory personnel.

<u>Advantages</u>

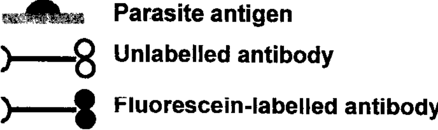
- The antigen can be produced comparatively easily and in large quantities by making smears of laboratory animals with a high parasitaemia of the trypanosome species against which antibodies are to be detected.

<u>Disadvantages</u>

- The test can only be carried out in the laboratory and the procedure is rather long and complicated, as well as to some extent subjective (i.e. titration by different operators may give somewhat different results).

Figure 14
Immunofluorescent antibody test

Indirect test



- Special equipment is needed (a special fluorescence microscope or at least a special fluorescence attachment), as well as a source of UV light.
- The commercial conjugate is expensive, and each batch has to be tested as to which dilution is optimal. Species-specific conjugates are needed; these are commercially available for most domestic animal species (e.g. rabbit anti-sheep conjugate, rabbit anti-horse conjugate, etc.), but not for instance for camels, nor for wild animals.[23]
- The test is not sufficiently specific to distinguish between the different species of pathogenic trypanosomes, although in the field this is not always an important consideration. It is known that even antibodies to *T. cruzi* (in Latin America) may cross-react with *T. evansi* antigen.
- Only limited numbers of sera can be examined in a given time period.

The indirect enzyme-linked immunosorbent assay (ELISA). The principle of this test is in fact very similar to that of the IFA test. The binding of anti-trypanosomal antibodies to the antigen is shown by a conjugate of antibovine (if the test serum is bovine) immunoglobulins labelled with an enzyme, which can be visualized by adding an appropriate chromogenic substrate (i.e. the interaction between enzyme and substrate will create a colour). The use of substrate can be compared to the use of UV rays to visualize the fluorescent conjugate in the IFA test.

Usually solubilized antigens obtained from disrupted trypanosomes are used (instead of smears containing whole trypanosomes), and the soluble antigens are coated in the wells of microtrays ("stuck" on the surface of the well, as it were). Each microtray contains many (usually 96) wells. This makes it possible to process many sera at the same time, using multichannel pipettes. Only small quantities of sera and conjugate are used. The test results

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[23] It is in principle possible in well-equipped and well-staffed laboratories to make conjugates which are not commercially available, directed against immunoglobulins of other animal species, but it is not an easy procedure.

can be read visually, but this introduces an element of subjectivity, and a special ELISA reading instrument will quickly give the optical density (OD) of each well (showing quantitatively the intensity of the interaction between the enzyme and the substrate), thus helping to speed up the processing of large numbers of sera. The ELISA lends itself to standardization and automation.

Nevertheless, the use of antigens derived from whole trypanosomes means that the test is not necessarily more species-specific than the IFA test. More specific results can be obtained by the use of characterized species-specific antigens, which are produced by molecular engineering. But, as stated before, such a level of specificity may not be required in the field. Even using such specific antigens, it may not be possible to distinguish between very closely related trypanosomes, such as *T. evansi* and *T. equiperdum*, and even *T. brucei*, in the horse family, or *T. brucei* and *T. evansi* in other animals.

Advantages

- The test lends itself to automation and standardization, and to processing large numbers of sera. However, as there are many pitfalls associated with this test as well, only well-equipped specialized laboratories can be expected to provide standard procedures and reagents, and individual operators should not attempt to modify the test procedure prescribed by organizations such as OIE and FAO/IAEA.

Disadvantages

- It is also not a test that can be conducted in the field; an adequately equipped laboratory is needed (the ELISA reader being the major equipment).[24]
- The reagents are comparatively expensive.

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[24] It should, however, be noted that it may not be impossible to develop eventually so-called "pen-side" (or "crush-side") ELISAs, which should give a positive or negative visible result on a specially prepared and impregnated stick or paper ("dip-stick tests"). Such tests, perhaps not always very satisfactory, exist for certain other diseases; for some, especially diseases of poultry, the market is so extensive and rewarding that they have been commercialized.

- The antigens are not as easy to produce as the antigen smears for the IFA test.
- If antigens of whole trypanosomes are used, the test is not species-specific.

Card agglutination test. This has been developed from a commercial test for the diagnosis of human sleeping sickness (the Testryp® CATT), into a commercial kit for *T. evansi*, CATT test *T. evansi*®. For the detection of antibodies to surra (*T. evansi* infection) serum samples are mixed on a plastic card with fixed and stained trypanosomes as antigen and the test is positive when the antigen agglutinates. A titre can be determined by serial dilutions of the serum.

The great advantage of this test is that in principle it is easy to carry out even in the field. Its specificity and sensitivity appear to need further evaluation, and in the experience of the author reading the test results is not always easy.

Antigen-detecting tests (Ag-ELISA). These tests have been developed for the detection of circulating trypanosomal antigens. The surface antigens of trypanosomes are variable; only one or two of the many different variants are present in the blood at any one time and, unless one would possess mixtures of antibodies to all the possible variants, their detection is not reliable. Therefore, the tests that have been developed are based on so-called monoclonal antibodies against invariable (internal) antigens. This needs some explanation.

Among the constituents of the immune system are various types of lymphocytes. Each of the lymphocytes that manufacture antibodies in response to an infection only makes one type of antibody, directed against only one antigen of the infectious organism. In fact, antibodies are so specific that they only fit on a particular site of an antigen (a so-called *epitope*), like a key in a lock. By genetic engineering it is possible to fuse single lymphocytes with cancer cells of a mouse; single-fused (hybridized) cells will grow (because of their cancerous component) in cell culture or in mice, and produce a single type of antibody, a so-called monoclonal antibody.

Such monoclonal antibodies in principle react very specifically with one antigen only, or rather fit in one particular shape of epitope only.

The principle of detecting circulating antigens using *monoclonal antibodies* is again quite simple (but there are many problems).

The wells of a microplate are coated with a monoclonal antibody, which is specific for an invariable (non-surface) antigen of a trypanosome species. Serum of the animal to be tested is allowed to react in one of the wells, and if there is the corresponding circulating antigen, its presence in the well after washing off the test serum can be shown by finishing off the test with a conjugate of the same monoclonal antibody marked with a suitable enzyme, and the presence of the enzyme is shown by use of the suitable chromogenic substrate, as in the normal ELISA.

Tests based on this principle, using monoclonal antibodies supposedly specific for the various subgenera, species or types of pathogenic trypanosomes, have been widely tested and distributed to National Agricultural Research Systems (NARS) in Africa for AAT diagnosis and elsewhere for the diagnosis of surra and *T. evansi* infections. It has however become apparent recently that the sensitivity of this type of test is not as high as was claimed, and even that positive results are not reliable, at least with the monoclonal antibodies that were in common use. On the one hand false positive results may occur, on the other hand there are cross-reactions between species.

The fact that the sensitivity of this test is often low and irregular can easily be understood, as the antigens detected are only released in the blood stream when the trypanosomes die and disintegrate. Thus, in the early stage of the disease, before the first peak of antibodies causes massive lysis of the parasites, the trypanosomes are healthy, and the test is negative. Thereafter it is likely that the result is negative during each peak of healthy parasites covered by a new type of surface antigen, and only becomes positive when the immune system has caught up and produced the appropriate antibody, causing the death of this particular trypanosome variant,

and thus the release of internal antigens. The sensitivity for *T. vivax* is particularly low, considerably lower than the buffy coat technique for the detection of parasites.

The fact that even positive results are not reliable and not specific is more difficult to explain. On the one hand it should not be forgotten that the reaction between an antibody and an epitope is like that of a key in the three-dimensionally appropriate lock. The same three-dimensional configuration sometimes exists on a very different protein of a very different organism. But on the other hand it appears also likely that the monoclonal antibodies that were selected for the test, although they were promising in initial laboratory tests, were released at too early a stage for evaluation and distribution in the field. Fortunately field evaluation has successfully detected their shortcomings. False positive results and cross-reactions appear to be particularly frequent with the Ag-ELISA for *T. congolense* and that for the *T. brucei* group.

Until monoclonal antibodies are developed which give more reliable results, it appears best not to use these tests but, as they had already been widely advertised and distributed, some mention of these problems was required here.

Molecular tests. The principle of molecular tests is the demonstration of the occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, species or even type or strain. Nucleotides are the constituents of DNA (deoxyribonucleic acid), the molecules which constitute the genes on the chromosomes in the cell nucleus. A positive result indicates active infection with the trypanosome for which the sequences are specific, as parasite DNA will not persist for long in the host after all live parasites have been eliminated. These tests are not only suitable for detecting parasites in the mammalian host, but also in the insect vector. The general principle of these tests will be shortly explained here, but as they can only be carried out reliably in well-equipped laboratories by specifically trained staff, and are still mainly research tools, no technical details will be given.

It seems appropriate to recall first the general structure of DNA, which contains the basic genetic information for all living organisms (except for some viruses which possess only RNA, another form of nucleic acid). DNA occurs as a double helix (= screw-shaped coil, or a spiral staircase), made up of two strands of nucleotides or bases, which are linked together by hydrogen bonds. There are only four different nucleotides, adenine (A), thymine (T), cytosine (C) and guanine (G). All genetic information (the genetic code) depends on the linear sequence of these four bases. The bonds between the two strands either connect A to T or C to G, i.e. the only possible base pairs are A-T and C-G.

The hydrogen bonds between base pairs can be broken by heating DNA, resulting in separation of the strands. The bonds will be restored when the sample is cooled down.

This basic knowledge will make it easy to understand the first of the two main molecular methods for the diagnosis of disease.

DNA-probes (nucleic acid probes).[25] The sample to be examined is heated to separate the two strands of DNA (this is also called denaturing of DNA), and these are fixed to a membrane, so that they cannot recombine again on cooling. A probe is then added. A probe consists of a linear sequence of nucleotides of a certain length, which has been prepared to correspond with a similar sequence of nucleotides in one of the strands of the parasite which the test is meant to detect. The probe will link (hybridize) with that part of the parasite DNA strand which is the mirror image of the base sequence of the probe. Depending on the sequence of DNA that has been selected for the probe, the test can be more or less specific, certain sequences are common to all species of a subgenus (and thus will for example not allow to distinguish between *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. evansi* and *T. equiperdum*, but indicate the presence of trypanosomes of the subgenus *Trypanozoon*), while other sequences are so specific that they only

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[25] Some nucleic acid probes use RNA instead of DNA, but we will discuss here only DNA probes.

occur in each species, or subspecies, or even type. Whether hybridization has occurred or not is demonstrated by showing that the probe remains fixed to the sample after washing. For this it is of course necessary to "label" the probe, and this can be done by incorporating radioactive isotopes in the probe molecule, and showing that the radioactivity persists. The method is suitable for simultaneously processing large numbers of samples.

Unfortunately, radio-isotopes are not suitable for use in field laboratories. The procedure is long and involves quite a number of steps, but the main reasons are that special training is essential for working with radioactive materials and a special infrastructure for the safe disposal of radioactive waste is necessary; another reason is that the radio-isotope normally used (^{32}P) decays quickly (has a short half-life) and frequent delays in transport and storage (such as customs) mean that the probe is often out of date by the time it arrives at the site where it is to be used. Because of this, radioactive DNA probes are not used for routine diagnosis, and remain essentially research tools.

For these reasons DNA-probes have been developed without a radioactive label, but labelled for instance with an enzyme, which can be demonstrated by an ELISA. However, such probes mostly have a considerably lower sensitivity than the radioactive ones, and are as or even more complex, involving many steps.

It should be realized that DNA-probes for trypanosomosis are not commercially available, and a laboratory that intends to use this technique either has to prepare its own probes or obtain them from other laboratories such as ILRI.

The polymerase chain reaction (PCR). This is another molecular method of detecting parasite DNA. It is based on the use of an enzyme, DNA polymerase, which amplifies (multiplies, copies) sequences of DNA bases, until sufficient material is produced to be detected. It does so by polymerization ("sticking together") of nucleic acids. Parasite DNA is denatured (separated by heat into the two single strands). Two "primers" are used, which are short

sequences of nucleotides (one for each DNA strand), each constructed so as to be complementary to a specific site on one of the two single parasite DNA strands. The primers attach to the sites for which they are complementary and DNA polymerase then starts to reproduce the rest of each complementary sequence which follows from that primer. This occurs in opposite directions until the entire sequence of double-stranded DNA between the primers has been doubled (as a complementary strand is produced from each primer). The polymerase can of course only do its work when nucleic acids are added to the test material. The cycle is then repeated, the two double-stranded DNA sequences are again denatured, the primers attach again, the polymerase amplifies, etc. In the end, the PCR product is submitted to electrophoresis and the bands are detected by special staining.

This procedure is extremely sensitive, as even minute quantities of parasite DNA can be amplified into a detectable quantity if the number of cycles is sufficiently high. It can also be highly specific, or less so, depending on the primers available for the reaction. Some primers will amplify a piece of DNA that is specific for a subspecies, type or even strain.

Important advantages of PCR over DNA probes are the greater sensitivity and the fact that no radioactive isotopes are needed. But there are also many pitfalls and disadvantages. Just to mention a few: false negative results may for instance be obtained if the specificity of the primers is too high, e.g. many infections by *T. vivax* are not recognized by existing primers, which do not recognize all types of the species. (The situation is better for *T.* [*Nannomonas*] *congolense*, as there are at present specific primers for the savannah, riverine-forest, Kilifi and Tsavo types of *T. congolense*, and also for *T.* [*N*]*) simiae.*) Whole blood contains factors which inhibit the PCR, another reason for false negative results.[26] Strict spatial separation of

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[26] It has been reported that the PCR for the detection of active *T. vivax* infections in blood only detects parasitaemias of over 1 000 trypanosomes per ml. This is rather similar to the sensitivity of parasitological techniques. PCR carried out on the pellet resulting from plasma centrifugation is very sensitive (parasitaemias of some ten trypanosomes/ml are detected). DNA purification is even more sensitive, but requires a commercial kit and is more expensive and time-consuming.

the various steps of the PCR procedure is required, and a number of controls have to be used, as otherwise there is a considerable danger of obtaining false positive results by contamination of samples with other, non-relevant DNA. These and several other possible causes of false positive and false negative results prevent the use of PCR as a routine in the field, or even in laboratories which have not been adequately set up and equipped for this purpose. The technical staff has to be adequately trained and has to be aware of the possible pitfalls. Also, the test is relatively expensive, mainly because the special polymerase used for the test, which is thermostable (= can withstand the repeated high temperatures needed for the cycles of denaturing DNA), has been patented and is expensive. On the other hand, as for DNA probes, there are no PCR kits or primers commercially available for trypanosomes. Each laboratory has to make its own primers, adapted to its intentions, or rely on those that have been made by other laboratories (and by no means cover all possible requirements).

In order to detect and avoid false positive results, it is possible to combine PCR with the use of DNA probe technology: a suitable DNA probe can tell whether the amplified PCR product is indeed what was expected.

Chapter 4
Control

Control of vector-borne diseases, (virtually all forms of trypanosomosis are in this category, except dourine), can be based on:

- control of the causal agent
- control of the vector
- use of innate resistance of the host to the effect of the infection

The accent in this manual will be on drug control of the causal agent, but the other approaches will be briefly reviewed, in order to place drug control in a wider perspective and indicate its place in integrated control.

CONTROL OF THE TRYPANOSOME

Chemotherapy and chemoprophylaxis

Chemotherapy: generalities. Chemotherapy is the treatment of disease by the use of chemical drugs. Such drugs are *curative*. They disrupt or block one or more of the vital processes which are essential to the invading micro-organism. Certain compounds have specific effects on some enzyme system or block essential metabolic pathways, but the exact way in which they work is often not known or only incompletely understood, and this is true of most of the *trypanocides* (chemotherapeutic drugs which kill trypanosomes or inhibit their development).

It is important to realize that drugs alone will not cure trypanosomosis. Trypanosomes overwhelm the immune system of the host; they are immunosuppressive. Chemotherapy, by stopping the multiplication of the trypanosomes, helps the immune system to overcome the infection. Treatment will be more effective in a well-fed and rested animal, in which the immune system is not adversely affected by stress and lack of food.

Chemotherapeutic drugs are toxic to the trypanosome, because they interfere with one or more of its vital processes. An ideal drug is one which kills the parasite but, at the same time, causes no or minimal harm to host cells. However, cells of all living organisms have much in common and a drug which affects a certain metabolic process in a parasite often has a similar disruptive effect on the cells of the host. If this side effect is too severe, the drug is too toxic for use. When the minimum effective treatment dosage is close to the toxic level, the margin of safety is low and the drug must be used with great care. When the toxic dosage is many times the therapeutic level, the margin of safety is high and the dangers attendant upon its use are correspondingly less. But it should always be remembered that all drugs have a disruptive effect on one or another living process and, accordingly, they should always be used with care and at the recommended dose level only. The efficiency of the vital detoxifying processes of animals that are ill may be reduced and a drug may be much more toxic for an animal with damaged liver or kidneys than for a healthy animal. It should also be remembered that the few trypanocides which are still commercially available have been developed before the stringent tests for absence of chronic toxicity and carcinogenicity that nowadays are required before new drugs can be released on the market.[27]

One should distinguish between *local toxicity*, the irritant action at the site of injection, and *systemic toxicity*, the toxic effect on the whole system (usually because of the effect on one or more internal organs).

The toxicity of drugs differs in different species of animals. For example, the margin of toxicity to quinapyramine in dogs is quite low; in pigs it is many times higher. Horses are known to be the most sensitive of the domestic animals to drugs which are irritant at

[27] Ethidium bromide is a widely used compound in molecular biology, where it is handled with stringent security precautions because it is highly mutagenic and therefore potentially carcinogenic. The same compound has been used extensively in the treatment of bovine trypanosomosis since the 1950s. (The author has commonly seen the kettle, used in the morning to prepare the solution of ethidium bromide in hot water, become the tea-pot in the afternoon.)

the site of inoculation, and one must always be careful of the subcutaneous route in horses because of this sensitivity. Diminazene aceturate has given fatal reactions in camels, horses, donkeys and dogs at doses which are considered to be normal and harmless in cattle.

One of the earlier trypanocides, dimidium bromide, had been used throughout Africa in many thousands of cattle until signs of acute toxicity were reported in several countries. They were caused by an acquired sensitivity to light (photosensitivity), causing necrosis and sloughing of extensive areas of unpigmented skin, followed by infection and death.

In certain instances a combination of drugs may prove to be toxic whereas either may be used safely if administered alone. On the other hand, some compounds used at the correct dosage are non-toxic, but are excreted so slowly from the organism that repeated administration at relatively short intervals may result in accumulation until a level is reached which will result in the appearance of toxicity.

It is essential to read thoroughly all available information in leaflets issued by the manufacturers on possible toxic reactions, before administering a drug.

Chemoprophylaxis: generalities. Chemoprophylaxis, or chemo-prevention, is the prevention of disease by the use of chemical drugs. Logically, this implies a residual effect, as prevention depends on the persistence of the drug in the system of the animal. A chemoprophylactic drug is also curative, but a curative drug is not necessarily prophylactic. There is no essential differerence between the two categories of drugs, drugs that can be used for chemoprophylaxis just persist longer, and what has been said above about the toxicity of curative drugs also applies to the preventive ones. Preventive drugs are administered at defined intervals, and care must be taken not to administer them more frequently than prescribed. A combination with another drug can be dangerous. For example, the most commonly used chemoprophylactic drug, isometamidium, will cause weight loss in poorly nourished cattle if administered at short (monthly) intervals.

Cattle treated with diminazene aceturate after several isometamidium treatments suffer from hepatic damage and may even die.

Often, a depot of the drug is formed at the site of the injection where it is retained and slowly released into the circulation to maintain a concentration in the blood at a level at which no trypanosomes can exist. Other drugs are not maintained in the form of such a local depot, but are found loosely attached to blood proteins and become slowly available to act on the parasite.

There is another way to create a drug depot, even if the drug is normally only curative, by incorporating it into a suitable support. After subcutaneous implantation the drug will be gradually released so that an adequate prophylactic (and curative) level is maintained in the blood and tissue fluid.

The administration of drugs. Drugs are normally sold in powder form or compressed tablets, and are stable over long periods when kept dry. Some become unstable when dissolved and must be used within a short period. The manufacturer's instructions should always be followed and it is a good practice to use solutions only on the day they are made.

The drug is dissolved, or suspended when it is insoluble, in a suitable liquid (mostly sterile water) and administered by injection with a syringe. Depending on the drug, the injection will be given by the subcutaneous, intramuscular or intravenous route.

The subcutaneous route. Absorption after subcutaneous (SC) administration is the slowest of all three methods of injection; the drug enters the circulation slowly from the subcutaneous site. This can be an advantage for chemoprophylactic drugs.

Compounds which are systemically toxic are also given in this way, so that they do not enter the blood stream and exert their poisonous reaction in large quantities at a time. However, drugs which are locally irritant at the site of the injection are not given SC

since they remain in a concentrated form long enough at the site to cause skin necrosis (death of the skin). The skin sloughs away and a raw area prone to infection remains and may take weeks to heal, leaving a hairless scar. The skin of horses is particularly prone to such damage.

If the drug presentation injected causes pain, this will be most pronounced when the SC route is used, because the subcutis is well supplied with sensory nerves.

A short needle can be used for SC injections. It is inserted into the pouch formed by picking up a fold of loose skin. The drug is absorbed from the subcutaneous depot into the circulation via the extensive network of lymph vessels and blood capillaries which occur there.

SC injections can be made in cattle under the skin of the dewlap, on the side of the neck, or over the ribs behind the shoulder. In sheep and goats the best site is just behind the point of the elbow where there is less hair or wool. In pigs the most suitable site is under the skin behind the ear flap where it joins the neck. SC injections in horses and camels are best given on the side of the neck. Dogs and cats may be injected in a skin fold over the ribs behind the shoulder.

The intramuscular route. Muscles are richly supplied with blood vessels and absorption is much more rapid than from the subcutis and the curative effect faster. Intramuscular (IM) injections are also more suitable for locally irritant drugs and also for painful drugs, as there are fewer sensory nerves in muscles. Nevertheless, the mechanical pressure exerted by a large quantity of injected liquid can be quite painful.

Any accessible mass of muscle can be used, but one should avoid as much as possible the heavily muscled areas which make up the prime joints of meat and are of the greatest value in food animals. Not only are concentrated drug residues in such sites highly undesirable (but this is true for any part of the animal that is eaten), but muscles may show local damage after the injection. Very irritant

drugs can cause destruction of the muscle (necrosis) and its replacement by fibrous tissue. This process may be quite extensive after repeated injections and parts of the choicest meat cuts may be rendered inedible and unsuitable for sale. Muscles concerned with walking should also be avoided as the irritant effects may cause lameness.

For these reasons, the neck is the best site in all species (except for pigs, which are normally injected by the SC route only, as indicated above), although absorption of drugs from the neck muscles may be somewhat slower than from the hip or thigh. The neck is an area which is one of the least valuable in the beef carcass and even a great deal of trimming and wastage will not reduce its value too much. The injection should be made into the middle third of the neck, halfway between the cervical vertebrae (which can be felt beneath the skin) and the crest of the neck. In the case of irritant drugs, or a large volume to be injected, the dose may be divided into two equal amounts and injected on both sides of the neck. Although less desirable as far as carcass value is concerned, in animals pressed together in a crush it is not always practical to inject in the neck (and moreover inject in the correct site of the neck), and the rump is then commonly used.

A 4-cm (16 G) needle is recommended. The needle is thrust deeply into the muscle mass and must therefore be strong with a thick shaft. This may result in leakage of the drug along the track of the needle into the subcutaneous tissues when the needle is withdrawn. This should be avoided if the material is irritant by applying pressure and rubbing to the area immediately after the needle is withdrawn.

The intravenous route. The needle is passed into a vein and the drug is thus introduced directly into the circulation. This route is used for drugs which are highly irritant at the injection site, as drugs applied intravenously (IV) are rapidly diluted in the circulating blood. It is also used in urgent situations, when the action of the drug must be rapidly achieved. This is not normally the case in trypanosomosis, but in diseases with a very rapid course, heart failure, induction of anaesthesia, etc. It may also be used when large volumes of liquids

are administered as in shock or blood transfusions. The method requires a certain level of skill and practice. It is relatively time consuming and only rarely used in the treatment of trypanosomosis, apart from individual suramin treatment of surra, and certainly not for mass inoculation of cattle.

It should also be realized that drugs with a marked systemic reaction will have almost immediate access to the various organs and may cause a dangerous toxic effect; the likelihood of this happening is lessened by carrying out the injection slowly.

It is obvious that IV treatment should be carried out by a practised operator and only if the manufacturer's leaflet states that the drug is recommended for intravenous use. Shock (sometimes fatal) may exceptionally occur even when all precautions have been taken, although the likelihood of this is much less if the injection is given slowly.

The jugular vein in the neck is normally used for IV injections in ruminants (including the camel) and horses. It is a large vein, conveniently located just beneath the skin, in a groove (the jugular groove) running between the neck muscles from the angle of the jaw down to the front of the shoulder. The vein carries blood from the head towards the heart, and when the jugular vein blood flow is obstructed by placing one's thumb in the groove, or by using a thin rope or specially designed tongs, the blood accumulates above the obstruction and distends the vein. It thus becomes clearly visible beneath the skin, making it easy to thrust a needle into its interior. It is essential to have needles with a very sharp tip (disposable needles are best), and to push it into the vein with a decisive movement, otherwise the vein is likely to "roll" away from the needle. The needle is pushed well into the vein, so that it does not come out at every movement of the animal, but care must be taken that it does not pass right through the vein. To avoid this it is best to push the needle obliquely forward (towards the head of the animal) inside the vein, not perpendicularly in relation to it. Effective restraint of the animal is important. The tissues around the vein, and its walls, are very sensitive to irritant substances and leakage may result in necrosis and rupture of the vein, often with a fatal result.

Once the needle is in place and blood flows out, the syringe is attached and the plunger is first withdrawn slightly to check whether blood enters the syringe, a confirmation that the needle is correctly positioned in the vein. *After releasing the pressure on the vein*, the injection is then given slowly and carefully. After emptying the syringe, a little blood is sucked back into the syringe by slightly withdrawing the plunger and pushed out again, washing any (irritant) fluid out of the needle into the circulation. A 5-cm long needle, with a relatively large bore, is required for large animals such as cattle, camels and horses.

In dogs IV injections are usually made into the radial vein on the inner foreleg.

As stated above, IV administration of trypanocides is not commonly carried out in cattle and is mainly used for injecting suramin in camels.

Dosage of drugs. The correct dosage of a drug eliminates or controls the trypanosome, with no or acceptable toxic side effects to the host.

It is not only useless to give a drug at ineffectively low levels, but underdosing may have extremely serious consequences, as it may contribute to the development of drug resistance (see Drug resistance, p. 110).

Effective, safe dosage rates of drugs have been established in long series of experiments and trials in laboratory and domestic animals. The dosage is normally indicated as the amount of the active ingredient to be given per unit of body weight, usually in milligrams (mg) of the active compound per kilogram (kg) of live body weight (mg/kg). The drug is usually issued in powder or tablet form and dissolved or suspended in a suitable liquid (usually water). The prescribed concentration is expressed in grams (g) per litre or in milligrams per millilitre (mg/ml). Things would be simple if the commercial preparation consisted entirely of the active compound, but this is usually not the case as manufacturers add other ingredients (see Names of drugs, p. 97), which may cause confusion.

The percentage of active ingredient in the commercial presentation has to be known as the dosage rate is based on it.

The dosage can now be expressed in millilitres (ml) (= cubic centimetres, cc) of this liquid per kg (ml/kg), and the volume to be injected into an animal of estimated weight can be calculated.

The procedure is illustrated with the two following examples.

- A drug is to be administered at a dosage rate of 1 mg/kg. The commercial presentation is made up entirely of active ingredient (this is an imaginary situation). It is issued in 1 g powder sachets to be dissolved in water to make up 100 ml of solution. The solution thus contains 1 g/100 ml = 1 percent (weight/volume) and should be administered at a dosage rate of 1 ml/10 kg body weight, as 1 ml contains 10 mg of the drug and the dosage rate of the solid drug is 1 mg/kg. If this drug is issued in 10 g lots, each lot is dissolved in 1 litre of water to give the concentration of 1 percent, and the dosage rate of the solution is 1 ml/10 kg body weight, as above.
- The drug diminazene aceturate is sold by one company under the trade name Berenil® (see Names of drugs, p. 97 .and Table 4). It is issued in granulate form in sachets containing 1.05 g of active ingredient, but this corresponds to 2.36 g of powder. The manufacturer recommends dissolving one sachet (1.05 g of active compound) in 15 ml of water, giving a 7 percent solution of active ingredient, or 70 mg/ml. If the required dosage rate is 3.5 mg/kg, then the dosage rate of solution to be administered is 1 ml/20 kg.

There are also sachets containing 23.6 g of granules, containing 10.5 g of active compound. To obtain a dosage rate of 3.5 mg/kg, one sachet has to be dissolved in 150 ml of water to obtain a 7 percent solution. The dosage rate of 3.5 mg/kg is obtained as above, by using the solutions at 1 ml/20 kg.

All dosages are based on the live body weight. The weight of the animal to be treated should therefore be determined or estimated as accurately as possible.

The most exact method is by the use of a weighing-scale or balance, but in the field and in most treatment centres these are not available.

The use of a "weigh-band" is more practical, but less accurate, for cattle. This is a flexible tape graduated to read the weight directly when put around the chest behind the elbows. These tapes are designed and calibrated for modern breeds of cattle, but the conformation of the many different breeds in Africa varies greatly and weigh-bands should be recalibrated locally against the real weight (determined on a scale) of a number of animals of the local breed or type. Care must be taken that the tape is placed around the chest at the exact recommended point. In the case of zebu cattle the tape should be behind the hump. Weighing by weigh-band can be undertaken even by inexperienced operators. However, it slows down mass inoculations.

Weight is most often estimated by eye, but this is prone to inaccuracy and perhaps the greatest cause of errors in drug dosage. Skill is required, which can only be acquired by experience, to be gained for instance by attending sales where the animals are weighed on a scale.

In fat animals the proportion of body fluid (blood and tissue fluid) to total body weight is somewhat less than in the average animal. Because drugs are carried in the blood plasma and the tissue fluid, fat animals will have a slightly higher concentration of the drug per ml of body fluid. The tendency in such animals will thus be towards slight overdosing, which is not a serious error.

One should not underestimate the possible errors of weight estimates. Within 10 percent accuracy levels such errors are acceptable. Considerable underdosing results in ineffective treatment and the development of drug resistance, while considerable overdosing may be associated with toxic effects.

Names of drugs. To the user of drugs, two kinds of names are important:

(i) The *generic name* which is based on a shortened form of the chemical formula of the active principle and constitutes an internationally used official name. For example, a well-known trypanocidal drug is *isometamidium chloride.*[28]

(ii) The *trade name* (or brand name), used by a manufacturer for its commercial preparation. In addition to the active principle, commercial formulations may contain other ingredients (adjuvants) which may be inert substances to bulk up a drug active in small quantities, or to influence the solubility or the rapidity with which the drug is absorbed into the circulation. While there is only one official generic name, there may be several trade names and companies that prepare a product containing the active principle. (However, as long as the company that initially developed the drug has patent rights no other company is allowed to manufacture products with that active ingredient without the consent of the first company. Patent rights expire after a number of years.) Trade names for preparations containing isometamidium chloride as the active principle are *Samorin* and *Trypamidium.* Trade names are written with a capital letter. They are copyright and the property of the company that markets the drug; by international law the name may not be used to describe any other product. A trade name is often designated as follows: Samorin ®, indicating that this is a registered name. Instead of the sign ®, the designation ™ (trade mark) may be used. Unfortunately, there are many illegal drugs on the market, including trypanocides, often of inferior quality.

Use of drugs. The first successful attempts at the treatment of cattle suffering from "nagana" were made with tartar emetic (= antimony potassium tartrate) early in the twentieth century. The drug is extremely irritant at the site of injection and had to be given intravenously. Another disadvantage was that several injections

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[28] The complete chemical name for this drug is 8-(m-amidinophenyl diazoamino)-3-amino-5-ethyl-6-phenylphenanthridinium chloride, but this name is of interest only to chemists and pharmacologists.

were needed to achieve a cure. The drug suramin is also one of the earliest, but is only active against trypanosomes of the *brucei* group (subgenus *Trypanozoon*). For a long period, few other drugs were available and it was only after the Second World War that a few new compounds reached the market stage that were both effective and practical, able to effect a cure by a single injection.

In the past, pharmaceutical companies routinely screened large numbers of chemical compounds for their trypanocidal action (mostly against trypanosomes of the *brucei* group, easiest to maintain in laboratory animals, but of less importance in the context of AAT). Few of the promising ones however were developed up to the market stage, either because they were found to be too toxic in domestic animals, or because they did not show the expected efficacy in practical field situations. Sometimes the trypanosomes quickly developed resistance to the drug and some have disappeared from the market for this reason. However, the fact that at present there are very few effective products against African trypanosomosis commercially available is to a large extent because of economic reasons. It should be realized that the chemical industry is not actively seeking to develop new compounds, because the market is rather uncertain and not very profitable.[29] Several drugs have disappeared from the market, and it is significant that since the first edition of this manual, in 1985, only one novel type of trypanocide has been added to the list of commercially available veterinary products, and then only for use against surra in camels. For the control of African bovine trypanosomosis the only drugs currently in use are diminazene aceturate, homidium salts and isometamidium chloride, and all have been in use for 35 years or more. In this field guide we will consider only those compounds that are at present available, although they may not be available in each and every country concerned. It has recently been estimated that about 35 million doses of trypanocides are at present used each year in Africa.

Curative drugs. Curative drugs are mainly used where disease incidence is low and only a limited number of animals in a herd

[29] One should not blame the industry for this. Private industry can only survive by taking into account the cost/benefit principle.

contract the disease during the course of a year. This is often the case when the threat mainly occurs seasonally. Examples are the higher risk situations that may occur during the rains, as in the case of *T. evansi* in camels, of mechanically transmitted *T. vivax*, or in marginal tsetse areas. The trypanosomosis risk may also be higher during the dry season, for instance when herds enter tsetse belts during transhumance, either accidentally or deliberately (see Epidemiology, p. 34). Curative drugs are meant to cure individual infected animals, not to protect the whole herd or group for a longer period.

Curative treatment is most effective in herds that are inspected and treated at regular intervals. The more frequently the visits are carried out, the more effective the treatment regimen. For instance, if loss of condition and some mortality occur when cattle are visited and treated on a three-monthly basis, the situation may be corrected if the visits are carried out on a monthly or two-monthly basis.

Block treatment of all animals in a herd should not be carried out with curative drugs, at least not with one that possesses a residual effect, because of the high risk of drug resistance developing during the period that the drug reaches a subcurative level (see Drug resistance, p. 110). Table 4 gives the few curative drugs and the only prophylactic one that are available at present. All curative drugs also possess some residual effect, but in the case of diminazene aceturate and mel cy this is practically negligeable.

Prophylactic drugs. These are used where the risk is so high that the health of the herds cannot be maintained by individual application of curative compounds. In such a situation too many animals contract the disease too frequently. This is particularly the case in tsetse-infested areas. Although curative treatments may prevent a high death rate, the overall health of the affected herds is not satisfactory, with loss of condition, poor (re)productive performance, many clinical cases and some deaths, despite a relatively large number of treatments at each regular visit. Other circumstances which might warrant the use of prophylaxis may occur when the infected animals cannot be reached (for instance, inaccessibility during the rainy season, transhumant herds, trade cattle moving to distant markets and passing through tsetse belts, etc.).

TABLE 4

Commercial trypanocides*

Generic name	Trade names**	Solution for use	Dosage rate***	Route	Remarks
Suramin	Naganol	10%	10 mg/kg (1 ml/10 kg)	IV	Mainly used against *T. evansi* in camels
Diminazene aceturate	Berenil, Ganaseg, Trypazen, Veriben	7%	3.5-7 mg/kg (1-2 ml/20 kg)	IM	Mainly used in cattle and small ruminants
Homidium bromide	Ethidium bromide	2.5%	1 mg/kg (1 ml/25 kg)	IM	Mainly used in cattle and small ruminants. Should be dissolved in hot water. Potentially carcinogenic
Homidium chloride	Ethidium C, Novidium	2.5%	1 mg/kg (1 ml/25 kg)	IM	See above, but soluble in cold water
Quinapyramine methyl sulphate	Antrycide, Trypacide, Noroquin, Quintrycide	10%	5 mg/kg (1 ml/20 kg)	SC	Now mainly used against *T. evansi* and *T. brucei* in camels and horses
Mel cy	Cymelarsan	0.5%	0.25-0.5 mg/kg (1-2 ml/20 kg)	IM or SC	Registered only for use against *T. evansi* in camels
Isometamidium chloride	Samorin, Trypamidium	1% 2%	0.25-0.5 mg/kg (1.25-2.5 ml/50 kg) 1.0 mg/kg (2.5 ml/50 kg)	IM	Used mainly in cattle, as a curative at lower rates, as a prophylactic at higher rates. Also contains homidium, and is therefore to be considered as potentially carcinogenic as well

* Not all of these trypanocides may be available in every country and there is also no guarantee that production of all of them will be continued. The situation is rather fluctuating, mainly because of economical reasons.

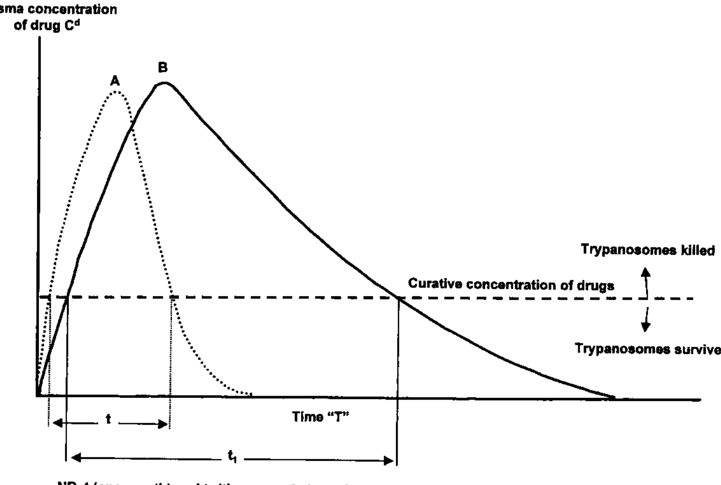
** The list of trade names is not complete, and names listed do not imply a qualitative judgement.

*** Dosage rate of solutions for use are given in brackets.

As has been said earlier, there is no fundamental difference between curative and prophylactic drugs, the latter just persist and protect long enough in the organism of the host to be of use in prevention. Figure 15 shows the difference for two imaginary drugs: drug A has a curative concentration lasting one month only, too short to be of use in chemoprophylaxis, while drug B, with a curative concentration lasting three months, can be used in prevention.

When considering prophylactic drugs, the notion of *challenge* or *trypanosome risk* is important. This means the amount or rate of infection (number of infected insect bites) to which the average animal in a herd is exposed in a given period of time. The challenge somehow affects the period of protection conferred by a prophylactic drug. While there is no adequate explanation for this phenomenon, it is a fact that the period of protection is shorter when the challenge is higher. It is as though each trypanosome "consumes" a minute amount of the drug and the total sum of these very small amounts of drug reduces the amount of active compound in the circulation. The reduction of the level of injected drug in the blood plasma and tissue fluid appears to result from the combined effect of the natural excretion of the drug and the level of challenge.

Figure 15
Plasma concentration curves for two trypanocides with different excretion rates



NB. t (one month) and t_1 (three months) are the protective periods

How can we measure challenge? When we consider tsetse-transmitted trypanosomosis and cattle, the challenge depends on the density of tsetse flies in the area and on the proportion of tsetse flies which are infected (the so-called infection rate in the fly population). The (apparent) fly density can be roughly estimated by counting flies caught on a screen during so-called fly rounds or by using traps. But this number is influenced by many factors. Also, the species of tsetse influences challenge because there are different host preferences. The infection rate is established by dissecting a large number of flies and recording the percentage that are infected. This is also not a simple matter; young (so-called teneral) flies have to be excluded as they have not yet had the chance to infect themselves and their inclusion would result in too low an infection rate. Non-pathogenic trypanosomes (for example, from reptiles) have to be distinguished in the fly from those causing AAT. The figure for challenge obtained by these methods at best gives a very rough, comparative indication, and because it is so inaccurate in absolute terms it is not commonly used any more.

Another way to determine challenge in an area is by using the so-called *Berenil index* (BI). Diminazene aceturate (of which Berenil is a trade name[30]) is a commonly used curative drug that is rapidly excreted. Its preventive effect is short (although some authors have claimed that it may exert a prophylactic effect lasting for up to three weeks). Blood samples of no fewer than ten cattle in the area where the level of challenge is to be established are examined over a year at weekly intervals by the most efficient method which can be practically used, and the infections are recorded. Cattle found to be infected are treated with diminazene aceturate. The number of treatments (= infections recorded) over a year reflects the challenge in the area, and is expressed as the average number of infections each animal contracts over a year. The following example explains the principle.

[30] The term *Berenil index* has become familiar to personnel concerned with trypanosomosis. However, a better name might be *Diminazene index*, as the company that developed diminazene aceturate and introduced the trade name Berenil® does not hold patent rights to the compound any more and other trade names now also exist for formulations of the drug.

In a herd of 25 head of cattle a total of 29 cases was detected in the course of a year, an average number of infections per animal of 1.16 (29 divided by 25). The BI therefore is 1.16.

The method is realistic and practical since the animals graze naturally throughout the area and the results are not influenced by various experimental conditions. Nevertheless, the BI found should not be taken as the absolute number of infections contracted, which is underestimated. The BI is influenced not only by the (relatively short) persistence of an effective level of the drug after treatment, but also by the susceptibility of the cattle concerned. It has been found that in mixed herds of susceptible zebu cattle and trypanotolerant N'Dama cattle the BI found in the former is significantly higher than in the latter. Thus, a BI determined in one breed of cattle does not necessarily apply to a different breed in the same area. Also, the sensitivity of the detection method used is of influence; none will detect 100 percent of the infections, but some are more sensitive than others (see Laboratory methods, p. 60).

A disadvantage of the method is certainly also that it is slow and labour intensive. The longer the period of observation, the greater the accuracy. A year is the minimum, so that any seasonal variation is taken into account, but a period of two years provides a much more reliable picture.

A Berenil index of three or less is regarded (in cattle that are not trypanotolerant) as relatively low, requiring only curative treatment of infected cases as they are recorded. For this purpose, in an ideal situation, monthly or at least two-monthly inspections should be carried out. A BI of four to six indicates medium to high challenge and the disease may theoretically still be controlled by curative drugs if inspections can be regularly undertaken at monthly intervals throughout the year. However, a prophylactic regimen may be more indicated. A higher Berenil index reflects high to very high challenge and will definitely require prophylactic treatment if the disease is to be controlled efficiently. However, at that level it may not always make economic sense to continue to keep cattle.

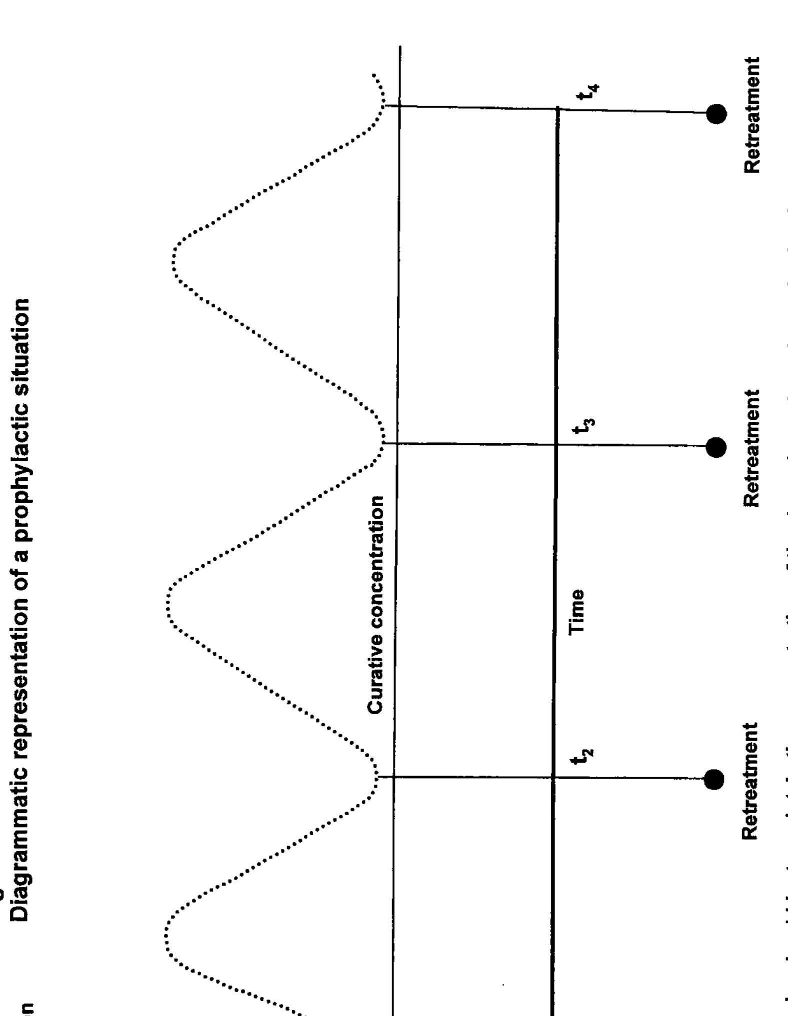
In the first edition of this field guide reference was still made to three drugs for the prevention of animal trypanosomosis, but two have since been discontinued (because of drug resistance and other problems). The only prophylactic drug that remains available is isometamidium chloride (Samorin®, Trypamidium®) at doses of 0.5 to 1 mg/kg (see Table 4). The drugs that have been discontinued are pyrithidium bromide (Prothidium®) and quinapyramine chloride. The latter was an almost insoluble salt which, mixed with quinapyramine methyl sulphate (curative), was marketed as Antrycide Prosalt. Although both drugs were initially very promising, serious problems of resistance and to some extent important local or systemic reactions have led to their abandonment.[31]

When using chemoprophylaxis the concentration of the drug in the body fluids should ideally not drop below the curative level in any of the animals in the treated herd, so that the establishment of trypanosomes and the development of disease are prevented at all times. This objective is achieved by a planned series of treatments at appropriate regular intervals.

Figure 16 is a diagrammatic representation of a prophylactic regimen. The intervals between treatments are equal and so spaced that the concentration of the drug in the plasma is never permitted to drop to a level which is ineffective in controlling the parasite. The interval is chosen so that the drug level in the plasma is boosted by the next treatment before it reaches the limit of the effective concentration. This principle is of utmost importance if the drug is to remain effective, that is to say if the development of drug resistance is to be prevented.

[31] Quinapyramine chloride may still be manufactured by one or more firms for inclusion into commercial "Prosalt" mixtures, but it cannot really be recommended any more in the prophylaxis of AAT.

Figure 16
Diagrammatic representation of a prophylactic situation



The aim should be to maintain the concentration of the drug above the subcurative level by means of a series of treatments at planned regular intervals

The chosen interval between treatments may vary with the existing situation, depending on the persistence of the drug (on its rate of transformation or excretion) and on the challenge. Higher levels of isometamidium give longer periods of protection but, as stated above, the level of challenge also interferes in a manner which is not completely clear. In the face of a high challenge the dosage of the drug should be increased or the intervals between treatments shortened. However, short interval (monthly) administration of isometamidium is dangerous, particularly in cattle under nutritional stress. The concurrent administration of diminazene aceturate is particularly dangerous in this situation (see Chemoprophylaxis: generalities, p. 89).

Recent laboratory methods of determining the concentration of isometamidium in the blood with an ELISA, using antibodies to the drug conjugated with a protein, (see Drug resistance, p. 110) may assist in establishing suitable treatment intervals.

In recent years research has been carried out on slow release devices (= sustained release devices) (SRD) containing homidium or isometamidium in polymers which are implanted subcutaneously. The intention is to maintain an adequate and more regular level of the drug in the blood and tissues for longer periods than can be attained by depot-forming injections. In laboratory experiments involving cattle, SRDs containing homidium or isometamidium have given protection against tsetse-transmitted infection with *T. congolense* lasting about three times as long as protection afforded by IM injections, using identical quantities of these drugs. It should however be remembered that these drugs have mutagenic properties (i.e. they cause gene mutations), making them potentially carcinogenic.

Notes on the various drugs. See also Table 4. Names in brackets are some of the trade names of commercial formulations that are, or have been, commonly available. No attempt is made to be exhaustive.

Suramin (Naganol). A white powder, very hygroscopic (= readily taking up water from the air), which must be kept in tightly closed containers.

Suramin is effective against trypanosomes of the subgenus *Trypanozoon* (*T. evansi*, *T. brucei*), not against *T. congolense* and *T. vivax*. It is irritant at the site of injection and is normally given intravenously. Toxicity may sometimes be evident in horses, even at recommended dosage rates, while other animal species are much more tolerant. Resistance to suramin of *T. evansi* is widespread.

Diminazene aceturate (Berenil, Ganaseg, Trypazen, Veriben). This is a yellow powder that produces a clear yellow solution in water.

It is normally injected intramuscularly, as some swelling may occur after subcutaneous administration. It is not normally used in horses, because even after IM injection the reaction is sometimes severe. Toxicity and even deaths have been reported in camels, donkeys and dogs treated at the recommended dosage.

The tissues at the site of treatment are stained by the drug and 14 days should elapse after treatment before slaughtering animals. Diminazene aceturate is less effective against trypanosomes of the subgenus *Trypanozoon* (such as *T. evansi* and *T. brucei*) than against *T. congolense* and *T. vivax*.

Diminazene is also active against *Babesia* infections (babesiosis or redwater). This is sometimes an advantage in situations where one does not have the opportunity to diagnose and differentiate between possible causes of a febrile disease associated with anaemia. For a long time resistance to diminazene has been less of a problem than for other drugs, but it is no longer unusual.

Quinapyramine methyl sulphate (Antrycide, Trypacide, Noroquin, Quintrycide). Production of this compound has been taken up again, after an interruption towards the mid-1970s because at that time it had been found to cause serious drug resistance problems in cattle trypanosomosis in Africa. Its use in cattle is now strongly advised

against, because drug resistance to it develops readily and can be associated with cros resistance to all the other trypanocidal drugs in use. Moreover, there are toxicity problems in cattle. Quinapyramine is now produced mainly for the treatment of surra in camels and horses, in particular where there is resistance of *T. evansi* to suramin.

It is dispensed as a pale cream powder, producing a clear solution in water. It is administered by subcutaneous injection.

Under certain conditions, which have never been fully explained, the drug causes systemic toxic effects in cattle. Heat, fatigue, fear, etc. aggravate this. Toxicity in horses and dogs is also well known. Acute toxicity in dogs may be avoided if the drug is preceded by a dose of a tranquillizing drug some 20 minutes before treatment and dogs should only be treated when cool and rested.

The activity of quinapyramine against *T. vivax* is less pronounced than that against other species. *T. simiae* infection in pigs may also be treated, using very high dosage rates (20-40 mg/kg) but the intervention has to be very fast, as the course of the disease is so rapid.

Homidium bromide (Ethidium bromide) and *Homidium chloride* (Ethidium C, Novidium). Both are crimson powders dispensed as tablets that produce a deep red solution. Contrary to the chloride, the bromide requires warm water to ensure that it is completely dissolved. At normal dosage rates no toxicity problems have been reported. Deep intramuscular injection is recommended as both drugs are irritant at the site of injection. Horses are the most susceptible to the irritant effect and should be treated only by the intravenous route, taking great care to avoid leakage into the tissues surrounding the jugular vein. The dosage rate of 1 mg/kg indicated in Table 4 is for the treatment of *T. congolense* and *T. vivax*. *T. brucei* is less susceptible and the dosage for this species should be increased to 1.5 or even 2 mg/kg.

As stated before, molecular biologists use Ethidium bromide in their colorations with great care because it is highly mutagenic,

and therefore potentially carcinogenic; this fact appears to be as yet little known in the veterinary world. It is not uncommon to see the hands of inoculators, unprotected by gloves, deeply stained with the drug.

Resistance to homidium is also well known.

Isometamidium chloride (Samorin, Trypamidium). This drug belongs to the same chemical group as homidium, the group of the phenanthridinium compounds.[32] It is a dark red powder, producing a brownish red solution in water.

It is normally administered by deep intramuscular injection as it is irritant at the site of injection. Preferably, the neck should be used to avoid damage to the more valuable areas of the carcass. Pressure should be applied to the site immediately after the needle is withdrawn to prevent leakage into the subcutaneous tissue. *It should not be administered by the subcutaneous route.* As far as systemic toxicity is concerned, isometamidium has a wide margin of safety. In camels intramuscular administration may give a severe local reaction, especially at the high dosage rate required for the cure of *T. evansi* infection (1-2 mg/kg). In horses it is not advisable to exceed a dosage of 0.5 mg/kg.

Depending on the dosage rate isometamidium can be used (in cattle) as a curative drug (0.25-0.5 mg/kg) or as a prophylactic (0.5-1 mg/kg). The protective period after prophylactic doses varies from two to more than four months depending on dosage and challenge. In situations of medium challenge 0.5 mg/kg given every two months may protect as well as 1 mg/kg applied every three or four months, but under high challenge the higher dosage rate should be used. When challenge is intense, 1 mg/kg may have to be applied every three or even two months, but such intensive treatment is not recommended for extended periods.

[32] The drug should be handled with care; it is likely to be carcinogenic since it contains homidium, and also belongs to the same chemical group.

Isometamidium should never be used at the same time as other trypanocidal drugs and at least a fortnight should elapse between treatment with isometamidium and any other drug, particularly diminazene aceturate. Also, after diminazene treatment, there has to be an interval of at least two weeks before isometamidium can be administered.

Mel cy (Cymelarsan). This is an arsenical drug, related to melarsen (which is used in human sleeping sickness). The name mel cy is derived from the chemical formula Melarsen oxide, with cysteamine as a lateral chain.

It is dispensed as a dry powder, highly soluble in water. The drug was developed in the 1980s for use against surra (*T. evansi* infection) in camels, as the other compounds in use against this disease are either frequently associated with resistance problems (suramin, quinapyramine) or are not always sufficiently effective against this trypanosome species (isometamidium). Mel cy is administered preferably intramuscularly, but the subcutaneous route is also possible. There are often transient local and systemic reactions. It has also been tested against *T. evansi* in domestic buffaloes and *T. evansi* and *T. brucei* in other animals. The dosage rate originally recommended by the manufacturer for camel surra (0.25 mg/kg) proved to be too low in these other animals and even for camels a dose of 0.5 mg/kg may be more realistic. Mel cy so far appears to have been registered only for use in camels.

Drug resistance. Drug resistance, also called drug fastness, may be defined as a loss of sensitivity by a strain of an organism to a compound to which it had previously been susceptible. It implies failure of treatment or prevention, and if no other active drugs are available the animal has to rely on its immune defences alone to combat the disease.

Drug resistance is known in many pathogenic micro-organisms, as well as in larger parasites. The results of its appearance can be catastrophic where an animal (or human) population has come to depend on the regular administration (or external application in the case of ectoparasites) of the compound, and no other effective

treatment is available. Prolonged, regular use of a drug (or pesticide) apparently inevitably leads in the end to the development of resistance by the micro-organism or parasite in question.

The discovery of the first compound active against *T. brucei rhodesiense* was rapidly followed by reports of resistance to that drug. Sooner or later, newly developed trypanocidal drugs have failed to cure some cases of human or animal trypanosomosis after a period of use. Every time that new drugs are released by the manufacturers and used in the field, drug resistance has appeared. It is especially by *misuse* of trypanocides, by their mistaken and inefficient application, that resistance develops.

Resistance to chemicals used against other micro-organisms, helminths, insects and ticks is believed to be caused by the selection of "naturally" resistant individual organisms, which were present in small numbers before the compound was used, or which arise by continually occurring natural mutations during its use. Susceptible organisms are killed by the chemical, but resistant natural mutants survive and multiply, giving rise to a resistant population.

In the case of trypanosomes the evolution of drug resistance may not always follow this process. Trypanosomes often appear to actively *develop* resistance; it is as though they are capable of learning the trick of avoiding the toxic effects of the compound. Thus, the process may not always be one of selection of pre-existing resistant trypanosomes. We have already seen that trypanosomes have an amazing ability to change in the face of the successive defensive reactions of the host's immune system, and chemoresistance appears to be another example of the ability of the trypanosome to adapt in the face of unfavourable changes in its environment. The level of drug resistance may vary considerably among different isolates, and the sensitivity of trypanosomes to drugs may also vary at different times in the course of an infection.

Drug resistance in trypanosomes may be a combination of such adaptation and of selection.

In addition to classical drug resistance in trypanosomes, a change in behaviour of the parasites may produce the same practical result. It is commonly observed that trypanosomes, belonging to a so-called resistant strain, disappear quickly from the blood stream following treatment, and the only (but very important) difference with a non-resistant strain is that they reappear after a period of varying length. They have apparently acquired not so much a classical drug resistance but the ability to hide in parts of the organism of the host in which the drug cannot penetrate sufficiently to rid the animal of all the trypanosomes in its body (for example the chambre of the eye or parts of the brain). It is unknown how this "behavioural" resistance arises.

In this section we shall only discuss classical drug resistance further, but the result of behavioural resistance is similar.

The exact mechanism of drug resistance is insufficiently known and this is not surprising when one remembers that the precise mode of action of the trypanocides is also unclear. It is possible that sometimes penetration of the drug into the trypanosome is decreased because of changes in the surface of the parasite cell or that the enzyme process that is disrupted by the drug in susceptible trypanosomes has become insusceptible to the action of the drug. Whatever the mechanism involved, *the fundamental fact of extreme practical importance is that drug resistance in trypanosomes often arises or is accelerated as a result of their exposure to a sublethal level of the compound used. It is therefore clear that such an event, so often the result of the careless use of drugs, must be avoided.* Naturally resistant strains (as opposed to the exceptional naturally resistant individual parasite) of a pathogenic trypanosome apparently do not exist in an area where drugs have never previously been used. This apparently indicates that non-resistant trypanosomes have a (perhaps only slightly) better chance of surviving than resistant ones (but this changes of course when a trypanocide is used). Indeed, resistance may in the long run spontaneously disappear or at least greatly decrease after the use of the trypanocide is stopped, as the non-resistant individuals (which may for example

have survived in domestic or wild animals that were not treated) in the end gain the upper hand.

As resistance often appears to be a direct result of the exposure of parasites to a sublethal (subcurative) dose of the drug, every attempt must be made to avoid that prime cause. Figure 15 is a graphic diagram of the plasma concentration curves of two drugs. A is a curative drug and B a prophylactic one. The horizontal line indicates the lowest effective (curative) level of the drug. (For purposes of convenience this level is represented as being the same for both drugs.) It is now obvious why the use of prophylactic drugs for purely curative treatment is to be avoided. The length of time during which a subcurative level occurs in the blood is much longer with such compounds than with purely curative drugs with a short residual effect; prophylactic drugs thus give occasion to new infections to become established and develop resistance during this long period of a subcurative concentration. When drug B is used in mass prophylaxis, a subcurative level can be avoided by retreating the animals at appropriate intervals, so that the level always remains above a sublethal one (see Figure 16).

If we accept the fact that drug-resistant strains are most likely to arise during the course of a regime that permits subcurative exposure to occur, and that the greater the fraction of the cattle population in which subcurative drug concentrations exist, the greater the likelihood of this unwanted contact between parasite and drug, the most favourable circumstances for the development of resistance are:

(i) *Under high challenge and widespread use of a curative drug with an appreciable persistence in the system.* In such circumstances there will be many cattle at any one time with subcurative plasma concentrations of the drug. Block inoculation of whole herds with such curative drugs particularly encourages the development of resistance. This is the reason why diminazene, which is quickly eliminated from the organism, can be used for block treatment.

(ii) *During the course of a prophylactic regime with intervals which are too long, which have been incorrectly assessed.* The plasma drug levels drop into the subcurative range before each reinoculation.

(iii) *In all cases of serious underdosing.* If the error is great, the drug may never reach the effective level, or only for short periods of time. Underdosing may result from incorrect weight estimation, from using the wrong amount of drug when making up the injectable solution, or when the drug has not completely dissolved before use. Also, the dosage of the drug is often based on that used in cattle, but the bioavailability (the levels available in the blood and tissues) of drugs is sometimes different in other animals; for example the bioavailability of trypanocidal drugs in goats is lower than in cattle, for the same dosage per kg of body weight.

(iv) *Where a prophylactic cover is interrupted while the challenge remains high.* This situation may occur for example when transport has broken down, when the drug is temporarily out of stock, or when there are delays in the presentation of the herds by the owners. Whole herds may thus be exposed to infective tsetse bites in the presence of subcurative drug levels.

In order to delay the development of resistance (complete avoidance of its development is probably impossible), the following are recommended.

(i) Avoid underdosing.

(ii) Reduce the application of trypanocides by integrating their use with other control methods. This lessens selection pressure by the drugs. There is definitely a strong correlation between the scale and frequency of treatments and the development of resistance.

(iii) Systematic mass treatments exert a strong selection pressure for resistance. Limit curative treatment wherever possible to individual clinical cases.

(iii) Avoid the use of quinapyramine in cattle as resistance to this drug induces cross-resistance to isometamidium, homidium and diminazene (see Table 5).

When resistance first appears in the course of a drug regime it is confined to the parasites present in relatively small numbers of animals, but the numbers will increase rapidly, particularly if it is

not detected and subcurative levels persist or increase. The trypanosomes become more and more efficient at dealing with the trypanocide and each retreatment will lead to an increased level of resistance.

As long as drug fastness remains confined to the domestic host it is called an *individual resistance.* Resistance becomes firmly established in the blood forms of the parasites, as a genetical characteristic, and will survive direct passages through a series of the same or different hosts. It has also been shown that drug fastness survives cyclical passage through the tsetse fly and is passed on by the infected insect during subsequent blood meals. In this manner resistance becomes disseminated throughout an area, and is present in as many host species as are favoured by the tsetse fly. It is then known as *area resistance.* This is a most serious event presenting a different problem. It must also be remembered that the hosts of tsetse are not only domestic animals but also various wild game species which will in turn infect future generations of tsetse.

Individual resistance can be dealt with by the use of an alternative drug to which the parasite remains susceptible. Once the resistance is widely disseminated (area resistance) it will be necessary to use in that area, for at least a year, a drug to which the trypanosomes remain sensitive. During that time the resistant strain becomes "diluted" as it were and is replaced by the normal susceptible strains; it thus tends to disappear.[33] As stated above, non-resistant trypanosomes may have a biological advantage over resistant ones, and some scientists have indeed found that resistant strains are less virulent and less fit, so that they tend to disappear if put in competition with normal susceptible strains. However, it has also been shown that drug resistance may persist in the field for at least four years.

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[33] In acaricide resistance, for example, the resistance factor may persist for decades in field populations of ticks after use of the compound in question (or a related one of the same resistance group) has ceased. If an acaricide of this group is then used again, the factor, although diluted after such a long time, is selected out quickly again because the sensitive individuals are killed by the compounds, and the resistant ones will reconstitute a resistant population.

The secret of the management of drug resistance is prevention. Signs of resistance must be immediately dealt with to prevent the spread of the drug fast strain and the change from individual to area resistance. This requires efficient monitoring and the regular examination of blood preparations of all clinical cases and, if none are found, of at least 10 percent of the animals of herds selected at random.

The phenomenon of *cross-resistance* is of utmost importance. *Cross-resistance is resistance to a drug that has arisen as a result of previous exposure of the trypanosome strain to a different drug.* Cross-resistant drugs are often members of the same chemical family. The resistance that appears to the drug used is known as *primary resistance*, that which appears to a different compound is called *secondary resistance.*

In general, continued exposure to a drug that has caused resistance, or an increase of its dose, boosts the resistance to a higher level and this mistake is to be avoided. Also, exposure to a different drug, to which there is secondary resistance, has the same effect and it is therefore necessary to know the resistance relationships of all the available compounds. It is also important to know that secondary resistance may even, in turn, lead to a lack of sensitivity to a third drug. This may be illustrated by the following example.

A strain of *T. congolense* that has acquired primary resistance to homidium compounds may show secondary resistance to quinapyramine methyl sulphate. If treated with the latter compound this secondary resistance will become enhanced and may reach a level that could create resistance to diminazene aceturate, a drug that is commonly used in *sanative actions*. A drug that will eliminate parasites that have developed resistance to another compound is known as a *sanative drug*.

Table 5 shows the cross-resistance among the five compounds in use for the treatment of tsetse-transmitted trypanosomosis in livestock. The five columns in the left half of the table show the resistance score when the drugs are given at the basic curative rates, while the four columns on the right record the score when the drugs are used at higher dosage rates (except for quinapyramine which is too toxic). The sanative action of

some of the drugs when administered at increased doses can be read in this right half of the table. However, once resistance to a drug is present it is as a general rule not a good strategy to increase its dose; even when there may be some short-term benefits, the selection pressure is increased and the level of resistance will certainly rise sooner or later.

TABLE 5
Cross-resistance between trypanocidal drugs*

Trypanosomes resistant to	Cross resistance to: At curative doses					At increased doses**			
	QP	M	PB	IM	DA	HM	PB	IM	DA
QP	+	+	+	+	+	+	±	-	-
HM	+	+	+	+	-	+	+	-	-
PB	+	+	+	+	-	+	+	-	-
IM	+	+	+	+	-	+	+	-	-
DA	+	-	-	-	+	-	-	-	+

QP = Quinapyramine; HM = Homidium; PB = Pyrithidium bromide; IM = Isometamidium; DA = Diminazene aceturate.
+ = resistant; - = not resistant; ± = some strains resistant.

* Pyrithidium bromide is shown in the table even though it is no longer commercially available, just to illustrate the pronounced resistance problems associated with it.

** Quinapyramine is too toxic at increased doses and therefore does not figure in the second half of the table.

A careful examination of Table 5 reveals several important facts.

(i) Quinapyramine resistance confers cross-resistance to each of the other compounds, at least to some degree. Because of its

low toxicity margin it cannot be used at higher dosage rates and the drug has lost its popularity for use against tsetse-transmitted AAT.

(ii) Cross-resistance between homidium, pyrithidium and isometamidium is prominent, and it should be remembered that these drugs belong to the same chemical family (phenanthridinium group).

(iii) Isometamidium at increased dosage rates will eliminate strains that are resistant to any of the other compounds. It will also act as its own sanative, eliminating at higher dosage rates strains that have become resistant to it at the lower curative levels; it is unique in this respect and as a result may be called a *universal sanative.*

The sanative dosage of isometamidium is usually taken as 1 mg/kg. It has been used at 2 mg/kg, but at this dosage rate there is a severe local reaction with muscle destruction and replacement fibrosis. This, if the drug is used over an extended period to deal with an area resistance, would lead to distortion and stiffening of the neck muscles. (As noted the area is of minimum commercial value.) The dose of 2 mg/kg is therefore only used under special circumstances and after careful consideration. A resistance problem requiring the use of isometamidium at the highest level is likely to be the result of misuse of the compound to which the primary resistance arose, mostly through continued use after the drug fastness was detected.

(iv) Diminazene aceturate is also a most useful sanative drug and cross-resistance to drugs other than to quinapyramine has not been reported.

It is known that resistance can also develop against the new drug mel cy (Cymelarsan), which in turn may result in cross-resistance to some other arsenical drugs, and, as has been shown in at least in one experiment, to partial cross-resistance to diminazene aceturate.

Sanative pairs of drugs are two drugs between which there is no cross-resistance and either may therefore be used to treat trypanosomes that have become resistant to the other. The adoption of this principle allows to make in advance a contingency arrangement so that if resistant strains appear after a period of use of

one drug, the second drug of the curative pair can be used. However, such a “sanative” treatment regimen should not be applied haphazardly, with a change of drug at every visit, as this may well result in strains resistant to both drugs. Before the drug switch is made there must be good evidence of the presence of resistance and the second drug should be used for a year or so before a switch in the reverse direction can be considered. It is also important to integrate other control measures in order to reduce the number of treatments with the second member of the sanative pair and thus delay the development of resistance to it.

How can one detect drug resistance? The manufacturer of a newly developed compound should run a series of tests before it is commercialized. One of the aims is to check its tendency towards developing drug-resistant strains of trypanosomes, by deliberately encouraging the process. The classical experimental method is to infect a laboratory animal with the trypanosome species to be examined; once the parasite is established the animal is treated with the drug, given deliberately at a very low level, insufficient to eliminate the parasite. The experiment is meant to imitate the classical situation of subcurative exposure conducive to the development of resistance. The process is enhanced by transferring the strain to another susceptible animal which is also treated as soon as the parasites are established. Retreatment at higher dosage rates and further repetition of the process will accelerate the development of resistance. Any compound to which resistance appears quickly in the laboratory is obviously not suitable for the field.

When drug-fast strains first appear in the field, further treatment with the drug will cause the resistance to increase and there is a danger of it reaching a level which results in cross-reaction to drugs that would have been effective if they had been used directly at the first appearance of the drug-fast strain. It is therefore of prime importance to detect drug-fast strains as early as possible, before resistance generalizes and develops into an area resistance. As stated before, there is some evidence suggesting that drug-fast strains of trypanosomes may be somewhat milder in their pathogenicity and may not produce pronounced clinical signs of the disease. A

somewhat benign form of trypanosomosis as is sometimes caused by drug-resistant trypanosomes may initially complicate the early detection of developing resistance.

T. congolense is perhaps most likely to develop resistance, but there are also numerous instances of resistance in *T. vivax*, even to the "sanative" drug diminazene aceturate. Many strains of members of the *brucei*-group (subgenus *Trypanozoon*) also have developed resistance to one or more compounds; the veterinary world is particularly concerned with the widespread resistance to drugs against surra (*T. evansi*) in camels and other animals, in Africa as well as in Asia and Central and South America. The appearance of unexpected cases of trypanosomosis during the course of a drug regime should always be considered with suspicion.

The identification of samples of individual animals is invaluable for the rapid detection and confirmation of drug resistance. An identified individual animal may thus be found infected within the period during which it should have been protected. In order to confirm or to refute the presence of resistance, the parasite must once again be exposed to the drug and its blood be examined within a short period, say 10-20 days after this renewed treatment. If at all possible, it is best to transport such suspect animals to a tsetse-free centre, where they can be monitored without risking to induce an area resistance. However, much valuable time may thus be lost, and efficient field monitoring should detect resistance at an early stage, before it is widespread.

To confirm suspected resistance at an early stage, positive blood preparations may be taken from as many cattle as possible, pooled, and inoculated into a susceptible calf. The animal is then monitored regularly and treated with the trypanocide at the recommended dose as soon as the parasitaemia starts. Persistence or reappearance of the infection indicates that there were drug-resistant trypanosomes in the initial pooled inoculum. A group of susceptible ruminants, preferably from outside the tsetse area, is then infected with the breakthrough trypanosome strain and, on becoming parasitaemic, treated with various dosages of the trypanocide in order to assess the drug sensitivity of the

strain. The animals should be examined regularly at short intervals and monitoring should continue over a prolonged period (at least three months, unless the animals become positive before) in order to determine the curative dose which will provide a permanent cure.

Instead of ruminants, laboratory rodents (usually mice) can be used to screen for drug resistance. They offer the advantage of being far cheaper, but they can only be used for those species and strains of trypanosomes that will grow in them. Also, infections in mice require far higher dosages of trypanocide than in cattle. The advantage of ruminants is that all isolates from cattle will grow in them and that the results are directly applicable to the field.

In recent years some progress has been made with the development of assays to determine drug sensitivity of trypanosomes grown in *in vitro* cultures, but this is not yet applicable to field isolates, and it is definitely not a routine procedure, even in a well-equipped laboratory. As indicated in Prophylactic drugs, p. 101, other laboratory tests can determine the concentration of trypanocide in the blood, and if it is above a certain level and the animal is nevertheless parasitaemic, this helps in confirming resistance. Drug detection is carried out with an ELISA using antibodies to the drug conjugated with a protein (as drugs alone do not induce the formation of antibodies.

All these methods are time consuming and expensive, and the appearance of drug-fast strains calls for urgent remedial action. If drug resistance is strongly suspected, sanative measures should be commenced in the field even while experimental confirmation takes place at the same time.

Once drug resistance has been confirmed, knowledge of the range of possible cross-reactions between the drug (or drugs) in use and other compounds is required. If resistance occurs in an area where curative drugs such as homidium or isometamidium at 0.25 mg/kg are used, diminazene aceturate often controls the situation. For this, it is used to block inoculate the herds in the area, in order to eliminate the developing drug-fast strains. It must be remembered

that block inoculation with other curative drugs that have an appreciable persistence may encourage resistance and should be avoided under any circumstance.

If the trypanosome risk is low and a curative regime is in use, one may use the alternative member of a curative pair of drugs (see above).

Where resistance appears in a group of cattle in a high risk area and a prophylactic isometamidium regime is in force, block inoculation with diminazene aceturate is practised if the cases breaking through the drug cover are still few. This is when resistance has been discovered in its early stages and can still be regarded as an individual resistance. Diminazene aceturate will not control the situation where challenge is high and the resistant strain is widely distributed and transmitted by tsetse flies (area resistance), because the rate of reinfection with resistant trypanosomes is too great.

Diminazene aceturate may sometimes be used to prevent the development of drug-fast strains. In some cases where challenge is high and prophylaxis is practised, poor presentation of the animals for treatment or the influence of some other factor may make the appearance of drug-fast strains almost inevitable. In such cases a block treatment with diminazene aceturate may be carried out at regular intervals, usually every six months, to eliminate any strains that are likely to have developed resistance to the prophylactic drug. For example if in such an area isometamidium is administered every three months at 1 mg/kg, a diminazene aceturate block inoculation would be carried out every six months, one month before a routine isometamidium treatment: day 0 isometamidium – after three months isometamidium – after five months (from day 0) diminazene aceturate – after six months isometamidium – after nine months isometamidium – after 11 months diminazene aceturate – after 12 months isometamidium – and so on. The diminazene aceturate is given two months after the isometamidium because the concentration of the latter will be fairly low and the former will be eliminated before the next isometamidium treatment, thus removing any danger of combined toxicity.

There are situations where trypanosomes are resistant to all commercially available drugs, including diminazene aceturate, homidium and isometamidium. In such a case of multiple drug resistance, trypanocides cannot offer a solution and other measures should be considered (see p. 125-133). The use of integrated control should help to prevent such situations from occurring in the first place.

Other causes of apparent drug failure. Often, apparent drug failure in the course of chemotherapy under field conditions is wrongly blamed on drug resistance and the real cause is to be found in incorrect procedures, such as:

(i) The challenge has been underestimated and a curative regime introduced that has failed to control the disease situation satisfactorily, with intervals between inspections being too long. Such a situation is due to an incomplete treatment cover and not to drug-resistant strains. Reassessment of the situation may even indicate that the challenge is so high that regular prophylaxis is needed. A similar situation may occur if the challenge increases over time and there is no efficient monitoring system to detect such changes. The increased challenge thus remains obscure until advanced clinical cases are apparent.

(ii) The appearance of cases of trypanosomosis in the course of a prophylactic regime may be caused by similar reasons. A regime of isometamidium at 0.5 mg/kg every three months may have to be changed to 0.5 mg/kg every two months, or 1 mg/kg every three or four months. Wherever possible, exact information on the challenge and its seasonal changes should be obtained so that control measures may be logically and correctly planned. It is possible to determine the Berenil index in a small group of cattle grazing in the same area as the affected herds. It is recommended to start using the prophylactic drug already at a higher dosage rate or at shorter intervals and to make the final adjustment later, when the challenge has been accurately established. It is certainly better to give a higher dose than may really be necessary and reduce it later

than to create subcurative levels by underdosing and thus encourage the development of resistance.

(iii) Disease cases resulting from underdosing may also be blamed on drug failure. Underdosing is usually due to incorrect weight estimation but can also result from careless bulk mixing of drugs.

(iv) The drug used is of inferior quality or its efficacy has expired. Some unscrupulous people try to sell illegal drugs, or even coloured solutions containing no active compound; the best guarantee against such malpractices is to buy from official traders and only drugs in their original packing, originating from well-known pharmaceutical companies. Every drug deteriorates with time, and for this reason each batch has an expiry date after which the manufacturer does not guarantee its efficacy. It is important not to use drugs after their expiry date, which is indicated on the packing material.

All the above deficiencies in drug administration will of course eventually lead to genuinely resistant strains if allowed to proceed unchecked. Thus, it is of paramount importance to involve personnel fully in the planning and implementation of drug regimes. Animal health assistants should be familiar with the theory and the principles that ensure the successful control of trypanosomosis.

The importance of regular monitoring of the disease situation cannot be sufficiently stressed. Examination of blood preparations should be a matter of routine, irrespective of the trypanosomosis risk and the treatment conditions.

Vaccination

So far all attempts at developing a vaccine against trypanosomosis have failed. With the rapid advances in molecular biology the situation may change at some point in the future, but so far this approach has been stranded by the almost unlimited ability of trypanosomes in the host to change their surface antigens frequently. With new antigens appearing, antibodies elicited against previous types of antigens are no longer effective and the immune system has to start all over again to produce new antibodies, until it becomes exhausted. Also, the antigen repertoire is different between different strains, types and subspecies of the same trypanosome species.

Moreover, African tsetse-transmitted trypanosomosis is often a mixed infection of two or even three different species. The subject of vaccination will therefore not be discussed any further in this guide.

VECTOR CONTROL

Although the subject of tsetse control is addressed extensively in the FAO publication series "Training manuals for tsetse control personnel" (Vols 4 and 5), the control of trypanosomosis cannot be considered in isolation of the control of its vectors, and is part of an integrated approach towards control. The general principles and possibilities of tsetse control, which have also been mentioned in the Introduction, are therefore briefly reviewed.

Vector control has traditionally been based on specialized large-scale operations (insects recognize no boundaries between farms, districts, or even countries). Recently, there has been a tendency towards smaller-scale methods which can be applied by farmers themselves, but this approach is still in its infancy. This section is limited to tsetse flies; the control of mechanical vectors such as stable flies and tabanids is beyond the scope of this field guide.

Past methods

Some methods have been based on *ecological* control. *Ecology* is the science of animals or plants in relation to their environment.

Tsetse flies require rather precise conditions of temperature and relative humidity, which are determined by the presence of woody vegetation. The different tsetse species may conveniently be grouped into three broad categories according to preferred vegetation type: forest, riverine and savanna type of tsetse. *Bush clearing* may deprive savannah species from the resting places and shade needed to maintain these conditions. The method has been used to diminish the density of fly, to stop fly advances, or to isolate an area from the main tsetse belt before clearing it from the fly. The crossing of such a barrier can only be prevented by ruthless clearing of all trees and bush over a width of several kilometres. However, some risk remains as tsetse flies may be carried over large distances in or on vehicles, and may also follow animal herds. Such barriers require

Later, extensive use has been made of spraying insecticides from aircraft, either fixed wing or helicopters (the latter being more expensive to use but more suitable for covering difficult terrain). It is beyond the scope of this book to go into details of aerial insecticide spraying (Sequential Airspray Technique, SAT) against tsetse flies, which is a specialist's job. SAT efficacy depends on factors such as droplet size, wind and inversion conditions.

A particular concern with large-scale insecticide application is the pollution it may cause, as most insecticides are harmful to aquatic life (including fish, frogs and other amphibia), while the earlier compounds, such as DDT, also affect terrestrial animals, including birds. The effect of pollution caused by a one-time application is usually quite temporary, but repeated use at frequent intervals of insecticides with a long residual activity is more harmful.

A different way of using insecticides is to apply these on domestic animals, so that flies settling on such animals are killed. Synthetic pyrethroid formulations are applied by spraying, dipping or used as a "pour-on" formulation, which is more expensive but does not need any pump, spray-race or dip. When such live bait animals are used without any other form of tsetse control, difficulties arise with the persistence of flies in areas where the treated animals do not go. Nevertheless, locally the flies may be reduced in density to a tolerable level. The frequency of application depends on how long the insecticide remains active on the animal (the length of its residual activity); the effect is shorter during the rains. Depending on the particular insecticide used, ticks (and other ectoparasites) may also be reduced (although the frequency of application needed for the effective control of ticks is greater than that required for tsetse control).[34] This method of insecticide application is much less polluting than spraying the environment.

So far, the use of insecticides has not produced insecticide resistance in tsetse flies, as far as is known. This can presumably be attributed to the low selection pressure for resistance and the fact that each female tsetse fly only produces few descendants during her life span.

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[34] This is in fact fortunate: intensive tick control on indigenous animals with a high level of resistance to tickborne diseases (and tick infestation) is not only often unnecessary, but also undesirable, as endemic stability to tickborne diseases may be lost by unnecessary tick control.

Sterile male technique

It has been known for a long time that male tsetse flies that have been rendered sterile by gamma irradiation or by certain chemical compounds will mate with females, but these will not produce offspring, as females normally mate once only after hatching, contrary to males. With the continuous release of sterile males in large numbers it is possible to eradicate tsetse flies from a particular area. For the recent successful campaign on Zanzibar (see Introduction) a ratio of more than 100 sterile males against one wild one was used at some stage.

The method is very specific and not polluting in itself,[35] but the effect on the population only becomes apparent after a period, as opposed to control by instantly killing insecticides. Because of this and to allow the sterile males to be competitive, a substantial fly suppression has to precede the application of SIT (Sterile Insect Technique), which is reserved for the final "mopping up" of the remaining population. The released males will seek out the females even in places where the insecticide cannot penetrate.

The males to be sterilized have to be mass reared in the laboratory. Also, the numbers of males that can be obtained are limited by the low rate of reproduction of tsetse flies and the fact that they have to be fed at least twice a week on blood. Where tsetse eradication is envisaged in places where several tsetse species occur in association, as is often the case, mass rearing has to be carried out for each individual species separately.

Use of traps or screens

The application of insecticide on livestock has been mentioned in Use of insecticides, p. 126. Another approach is the use of traps or insecticide-impregnated targets (screens). The attractivity of traps and targets for tsetse flies depends on their shape, size, colour and colour pattern, and this differs from species to species. The catch can be increased, in many cases considerably, by certain substances which have an attractive odour for the flies, e.g. acetone, phenolic

[35] What is meant is that the sterile male technique involving a radiation source is not in itself polluting, but this does not hold for the use of sterilizing chemicals or nuclear waste.

molecules, bovine or buffalo urine. This type of attractivity is also called *olfactory* or *olfactive*. The choice for the best substance depends very much on the species of tsetse fly one is dealing with, and can even be seasonally different. Savannah tsetse flies are generally more attracted by the odours studied so far than riverine species.

Some of the drawbacks of traps and screens are:

- Community-based fly suppression efforts are difficult to sustain. The motivation of the owners may diminish after the tsetse population has gone down.
- Traps and screens may be stolen for the cloth they contain. This is particularly serious when they are part of a barrier against (re)invasion.
- During the rainy season the rapidly growing vegetation may camouflage the trap or screen, which thus loses its visual attractivity for the flies.

Preliminary observations indicate that certain types of traps and odour substances will also attract mechanical vectors of trypanosomosis, such as tabanids and stable flies. This may be of help in reducing mechanical transmission of the disease. More research on this point is needed.

About eradication and control

What is meant here by methods of *control* are methods that reduce the fly density, and therefore the trypanosomosis challenge, to a "tolerable" level, a level at which cost-effective animal production is possible, but it does not necessarily aim at eradication of tsetse. Indeed, for a long time the various ways of controlling tsetse flies were intended to eradicate them from land that was destined for human and/or livestock settlement. Especially the application of insecticide from aircraft opened the possibility of large-scale eradication campaigns and the sterile male technique was also very promising in this respect. However, at present it is more and more recognized that eradication is often not sustainable and that expensive large-scale campaigns can only be justified in certain circumstances:

(a) where tsetse populations that are isolated from the main belts are concerned;
(b) where this is not the case, but the maintenance of permanent effective fly-proof barriers between the area freed from tsetse and the infested land can be assured. Effective barriers can be created (bush clearing, traps and screens, associated with strict surveillance and movement control), but their perfect maintenance is costly and difficult to ensure permanently.

It should be said that human settlement in the freed area is sometimes enough to discourage the fly from coming back (permanent bush clearing for agriculture, elimination of wild host animals). This is valid where savannah species of tsetse are concerned, which usually cannot maintain themselves in a densely settled area (but there are exceptions where they have turned to domestic animals and humans as their sole source of food).[36] Riverine species have a wide range of hosts and live in dense vegetation along rivers, so that they are not eliminated just by human activities.

(c) Where plans (economical as well as social) have been made for the use of the land to be freed from tsetse *and where the implementation of such plans is assured.*

There are no universal answers that will apply to all the various situations that exist, depending on species of tsetse, type and value of livestock, wild hosts, veterinary infrastructure, foreign exchange situation of the country, etc. Extension services have an important role to play, for initial information and motivation and then, as fly numbers go down and the owners lose motivation and interest, for continuously reminding them that any let-up in control will inevitably lead to a return of the old situation.

[36] The unrelenting human pressure on land associated with the population explosion in Africa may eventually get rid of most of the problem of savannah tsetse species, but at the cost of uncontrolled land degradation.

It goes without saying that wherever control of tsetse flies is attempted in areas to be settled, without prior human occupation, land-use plans are just as essential as in the case of eradication.

INNATE RESISTANCE TO TRYPANOSOMOSIS

It is well-known that genetically determined innate resistance to many diseases occurs in animal populations that have been subject to natural selection by exposure to disease pressure over many generations. This is also true for trypanosomosis. Taurine (humpless) breeds of cattle were the first to be introduced into Africa. They populated what is now the Sahara, but were pushed back further south when this area became a desert thousands of years ago. At present, they persist in the subhumid and humid northern parts of sub-Saharan Africa where they live and produce in tsetse areas. Such taurine breeds are now mainly confined to West Africa, from Senegal to Nigeria, but they used to occur as far to the east as the central Sudan (Nuba Mountains) and even western Ethiopia. N'Dama cattle (which originate from Guinea) have rather long horns, while breeds with short horns comprise, for example, the Baoulé (Burkina Faso and northern Côte d'Ivoire) and the Muturu (Nigeria). They are "dwarf" cattle (although a N'Dama cow can weigh as much as 200 kg, similar to the size of many of the smaller zebu breeds).

It should be added that trypanotolerance is not limited to taurine cattle, as it has been found that some of the zebu breeds in East Africa, such as the Orma Boran and the Masai zebu, also have a higher resistance to trypanosomosis than other breeds such as the "normal" Boran zebu, Indian breeds of zebu and other "exotic" (i.e. introduced) breeds such as European taurine breeds. However, the resistance of West African taurine breeds appears to be considerably more pronounced.

Apart from cattle, breeds of sheep and goats (and even of horses) living in tsetse areas are also relatively trypanotolerant. This is particularly true of the Djallonke sheep and dwarf goats in West Africa. Knowledge of the resistance of small ruminants is still fragmentary and far more is known about resistance in cattle.

There have been attempts to introduce West African trypanotolerant cattle to human populations who had no cattle previously, and to other parts of Africa, with some success, notably in the Central African Republic, Gabon and the Democratic Republic of Congo (ex-Zaire). However, livestock owners who are used to larger cattle, are not readily attracted to the smaller trypanotolerant breeds.[37] There are also limits to their trypanotolerance and when challenge is high even such animals may show clinical trypanosomosis. Their resistance is particularly effective in the face of riverine species of tsetse, which usually occur in lesser numbers and have a lower infection rate with pathogenic trypanosomes than the savannah species. Nevertheless, there are situations where such cattle live under continuous, but moderate, exposure to savannah species as well. Animals of such breeds not exposed to infection before and introduced as adults to tsetse-infested areas, may well become ill and even die. Cattle that stand up to challenge in a particular region may suffer from disease when introduced into another area, and any such move has to be well monitored and supervised.

Trypanotolerant breeds are certainly of great benefit, particularly where riverine species of tsetse flies are concerned, and where people are used to them.[38]

INTEGRATED CONTROL

Drug prices and drug and pesticide resistance are on the increase, and we should use all available methods that can be applied in each particular situation and are cost-effective. Combining different control methods against a parasitic disease is called *integrated disease control* or *integrated disease management* and is generally not intended to achieve eradication of the parasite in question.[39] Such a cost-

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[37] Often without realizing that you can keep more of them on the same stretch of land. Most cattle owners go for size, and even in areas where trypanotolerant taurine cattle have been bred for ages, crosses with larger zebu are commonly preferred in marginal zones with a low tsetse density.

[38] With the rapid advances in biotechnology it may one day be possible to transfer the genes responsible for their trypanotolerance to more productive breeds. But that is another story.

[39] Eradication has to be technically feasible, financially affordable, socio-economically beneficial, desirable in agro-ecological terms, and it will have to be permanent; all these conditions are rarely met.

effective combination of techniques, adapted to each particular set of circumstances, is very relevant for the control of African animal trypanosomosis. It cannot be sufficiently stressed that the cost-benefit principle is an essential aspect of all methods. Apart from this crucial point, all of the available methods have advantages and disadvantages and the various techniques act in a complementary way; an advantage of one may offset a disadvantage of another.

The sequential use of insecticidal spraying or of insecticide-impregnated targets, followed by the sterile male technique, has already been discussed.

A combination of chemoprophylaxis against the disease and insecticidal application on the cattle against the vector may greatly improve the trypanosomosis situation.

Where drug resistance is a problem, the accent will be put more on vector control and/or on the use of trypanotolerant livestock.

Many more examples of integrating two or more control measures could be given. It is essential to realize that drugs alone are not the answer to AAT. Flexibility and adaptation to each situation are important. For instance, the association of trypanotolerant livestock and systematic chemoprevention is likely to be uneconomical; it is more logical to reserve curative trypanocides for the limited number of animals that may require treatment.

Finally, one must always keep in mind the necessity for gaining the confidence of the owners. Right from the beginning, before drawing up a plan, the control options should be thoroughly explained and the stockowners should be given a full opportunity to discuss the situation. Gaining and keeping their full confidence in this manner will enable extension and/or control personnel to work in a more relaxed and efficient manner.

Chapter 5

Non tsetse-transmitted trypanosomoses

As we have mentioned, there are strains of two African trypanosome species that have adapted to mechanical transmission by biting flies in various animal species, or to life in tissue fluids and venereal transmission in horses and donkeys. Much of what has been written on AAT in other chapters also applies to these forms of trypanosomosis, and in this chapter we shall briefly mention some of the peculiarities of the parasites involved, their distribution and, where relevant, disease and control aspects.

Trypanosoma evansi

T. evansi has almost certainly arisen from *T. brucei brucei* by adaptation to mechanical transmission by biting flies, and it remains very closely related to *T. brucei*. It has lost the capability of being cyclically transmitted by tsetse flies and in the process has become almost monomorphic, the great majority of the parasites being indistinguishable from the long slender forms of *T. brucei*. It has spread far outside the tsetse regions of sub-Saharan Africa and is at present known to occur throughout the Sahel region of Africa, in North Africa, most Near and Far East Asian countries (including southern Siberia, China and Indonesia) and many countries in Latin America, from Argentina in the south to at least Venezuela and Colombia in the north. The situation in central America is not well defined, but in the past the parasite has been reported from at least one country, Panama.

T. evansi is the cause of the most important parasitic disease of camels, and is also highly pathogenic for horses (and mules and donkeys) and for dogs. It is also of considerable economic importance in Asian (domestic) buffalo, and to a less extent in some countries in cattle as well. Many other host species, wild as well as domestic, have been found naturally infected. The most common name for this disease is

surra, of Indian origin, now generally accepted, but in Africa and Latin American countries there are various other names (see below).

The disease in camels[40]

Apart from the name surra, which originates from India, the disease is known in many Arabic-speaking parts of Africa as el debab, and many other local names exist, e.g. gufar in the Sudan. It probably occurs wherever camels are reared.

Surra in camels is usually a chronic wasting disease, and the general description of the symptoms of African animal trypanosomosis in Chapter 2 applies.

Striking clinical symptoms develop a month or so after infection, with acute bouts of fever, associated with dullness, lack of appetite and lachrymation, coinciding with peaks of high parasitaemia. Gradually the animal loses condition, the hump shrinks and progressive weakness becomes noticeable. Oedemas may occur. Pregnant animals often abort. The disease is usually fatal, sometimes rather quickly, a few weeks or more often a few months after the onset of the disease; the evolution is much more often chronic, and may last as long as two or three years. In chronic cases it is usually very difficult to find parasites in the blood by direct means.

The most important mechanical vectors of surra in camels are probably tabanids; hippoboscids (*Hippobosca camelina*) are also suspected, but so far there is no formal proof of their vector role.

Surra in domestic buffalo, cattle and small ruminants

Outbreaks of acute disease may occur in buffalo[41] and cattle in areas where the disease is introduced for the first time.

Even in endemic areas *T. evansi* may cause anaemia and loss of condition in buffalo, and although the infection is usually not fatal,

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[40] The word camel is used here in the orginal (Arabic) sense, and encompassess both the one-humped camel (or dromedary) and the two-humped bactrian camel.

[41] Only domestic buffalo (*Bubalus bubalis*) are concerned, the wild African buffalo (*Syncerus caffer*) is not affected by *T. evansi*.

the disease may assume real economic significance. Draught animals are particularly prone to the effects of the infection.

Cattle are rarely seriously affected, but the parasite may have a negative influence on fertility and dairy breeds may not attain their production potential.

Although experimental infections of small ruminants may cause disease, in the field sheep and goats are rarely seriously affected.

Surra in horses

Horses are extremely susceptible to infection with *T. evansi*. It has even been possible to cause fatal infection by inoculating one single trypanosome. The disease often runs an acute course, lasting from a few weeks to one or two months; more chronic cases are also found and the animal may survive even a year or more. In long-lasting cases, cerebral symptoms often develop.

Mules are said to be as susceptible as horses, but donkeys are more resistant.

Trypanosoma vivax

This species is readily transmitted mechanically, even in Africa, wherever sufficient biting flies occur, particularly (but not exclusively) tabanids and stable flies (*Stomoxys*). In the past *T. vivax* has also occurred on the island of Mauritius, in the absence of tsetse flies, but has apparently been eradicated there. *T. vivax* invaded the western hemisphere a long time ago, possibly with the introduction of West African cattle in the Caribbean region in the eighteenth and nineteenth centuries, and has since then established a firm foothold in the Americas. It has been found in the past as far south as Paraguay, and nowadays is known to occur at least from Bolivia in the south to El Salvador, in Central America, in the north, but the limits of its distribution are not well known. Cuba has also been reported to be infected.

The disease caused by the mechanically transmitted *T. vivax* does not appear to be essentially different from that caused by the tsetse-transmitted parasite.

Trypanosoma equiperdum

The venereal disease dourine is confined to equines, members of the horse family. Horses are most susceptible, donkeys much less so. (Mules are not really concerned, because they are not normally used for breeding, so they do not become infected.)

As transmission of dourine does not require insect vectors that are influenced by climatic factors, the disease may, in principle, been found anywhere. It has indeed spread in the past as far north as Canada and Russia, and as far south as Chile and South Africa. The present distribution of dourine is discussed in Distribution, p. 38. The disease has been eradicated from many parts of the world, mainly by serological testing and slaughter of positive animals. The complement fixation test has been used for this purpose in the past. Although it is still mandatory in some countries, modern, more sensitive tests are now preferable, e.g. the IFA test, or the ELISA. One great problem is the fact that none of these tests differentiates between *T. equiperdum* and *T. evansi* (or even *T. brucei*).

The symptoms of the disease are variable. Classically, there are three stages. During the first stage the genitalia become swollen and in mares there is a discharge from the vagina, and loss of pigment in the mucosa of the vulva or penis; this depigmentation occurs in patches. Slight fever and a loss of appetite may be noticeable. After a month or so the second stage starts with round urticarial eruptions, plaques (patches), on the neck, the chest, the flanks and the rump. A plaque looks "as if a coin has been inserted under the skin". They are visible for a few days and then disappear, but may come back. In the third stage a paralysis sets in, involving various muscles, and spreading to the hind legs, causing incoordination. Complete paralysis of the four legs may finally occur.

This is the classical evolution of the disease but, often, particularly in long-standing endemic regions, the symptoms are rather mild and not all stages are always apparent. It has for example been reported that the typical cutaneous plaques are not normally observed in dourine cases in South Africa. There also appear to be differences in virulence between strains. Even in cases terminating fatally, the disease may last for a year or even two years.

The diagnosis of dourine is not always easy. Clinical symptoms, if typical, can be of great help in an area where the disease is known to occur. Trypanosomes are not normally found in the circulating blood, but can often be detected in fresh preparations or stained smears from the mucous discharge from the vagina or tissue fluid taken from the swollen genitalia or the urticarial plaques. In atypical cases it may be impossible to detect the parasite. Mice, rats, rabbits and dogs are susceptible to infection with *T. equiperdum*, once it is established in laboratory animals, but animal inoculation is of little use as a routine method of diagnosis because it is very difficult and often impossible to obtain a first passage.[42] Serological tests have been decisive in eradication campaigns, with positive animals being slaughtered or at least prevented from breeding. However, it must be realized that these tests, while extremely valuable in areas where *T. equiperdum* is the only pathogenic trypanosome present, do not distinguish between this and other members of the subgenus *Trypanozoon*.

TRYPANOSOMES OF LIVESTOCK IN LATIN AMERICA

As stated at the beginning, we will not deal with the American trypanosome *T. cruzi*. Although it infects a large number of host species, including humans, it probably has little economic significance for livestock. As stated before, the economically important livestock trypanosomes have been introduced: *T. evansi*, *T. vivax* and *T. equiperdum*. *T. vivax* was probably introduced with cattle imported from West Africa, perhaps as early as the eighteenth century, while *T. evansi* and *T. equiperdum* may well have arrived much earlier with horses of the Spanish colonizers.

As no tsetse flies occur in the western hemisphere, it is generally accepted that the transmission of *T. evansi* and *T. vivax* is essentially mechanical, by biting flies as well as iatrogenic. Congenital transmission may sometimes also play a role. Special mention should be made of the transmission of *T. evansi* by vampire (blood-sucking) bats. The bats become infected when ingesting blood from a

[42] After adaptation to life in the circulating blood of laboratory animals, *T. equiperdum* becomes indistinguishable from *T. evansi* and from the long slender forms of *T. brucei*.

parasitaemic animal. The trypanosomes multiply in the bat, and the parasite is again transmitted, through the mucosa of its mouth, during subsequent bloodmeals. Thus, these bats are not only vectors but also reservoirs of infection. Apart from vampire bats, *T. evansi* is found in various other wild animals in Latin America, such as the capybara (giant rodent), wild carnivores, monkeys, and deer, which all may be reservoirs of infection for domestic livestock, including llamas and alpacas.

The disease caused by *T. evansi* in horses is known as *mal de caderas* in Brazil, as *derrengadera* in Venezuela and as *murrina* in several other Latin American countries. *T. evansi* infection in Latin America is probably in the first place an economic problem in horses (and donkeys).

T. vivax has also been found occasionally in some wild animals, such as deer, in the western hemisphere, and these may, in principle, act as reservoirs of infection for domestic livestock. Nevertheless, it is believed that domestic animals (cattle, buffalo, sheep and goats, and also llamas) are themselves the main reservoirs of infection. Some local names for the disease are *secadera*, *huequera* and *cacho hueco*.The economic importance in Latin America has not been properly assessed. *T. vivax* is mainly of economic importance for cattle, but losses have also occurred in domestic buffaloes in Brazil.

Dourine (*T. equiperdum*) has occurred in the Americas from Chile in the south to Canada in the north. It has since been eradicated in North America, but its present distribution in the western hemisphere is not well known. Since 1975 it has only been reported in Bolivia, but it is a disease which sometimes leads a secret existence.

Information on the economic impact, the epidemiology and the distribution of trypanosomoses of domestic animals of Latin America is still insufficient, although progress has been made in some countries, for example, Colombia, Brazil, Venezuela and French Guyana.

Practical tips for field personnel

Collecting blood (Figure 17)
One essential precaution is to change pins between animals, so as not to transmit infections with the blood from one animal to another. Batches of used pins, as required, have to be sterilized (by boiling or treatment with a disinfectant). Avoid hollow needles, which are particularly dangerous for mechanical transmission of trypanosomes and other haemoparasites.

Many parasites are more concentrated in small vessels, so that low parasitaemias are easier to detect in so-called "capillary blood". This is true not only for certain intracellular haemoparasites, such as *Babesia bovis*, but to some extent even for *Trypanosoma congolense*. The skin of the outer ear is cleaned of dirt and grease; if a liquid disinfectant is used, the skin surface should be completely dry before stabbing it with a sharp office pin, taking care to avoid visible veins; repeated stabbing may be necessary for sufficient blood to appear. One can also stab the tip of the tail, which is sometimes easier, particularly in a crush. The drop of blood is transferred to a slide (or taken up directly with the slide). If the skin is not dry, the blood will spread out on it and not make a suitable drop.

In many cases blood from a vein is satisfactory and often easier to collect. After cleaning and letting the skin dry, stab a visible vein on the outer ear with a sharp pin, or take a drop of blood from a tube in which jugular vein blood has been collected for other purposes, such as the preparation of serum. Some people prefer to take blood from the main vein under the tail, but this also depends on the way in which the animals are restrained.

Figure 17
Taking a blood sample

B - From the ear by piercing a marginal vein

Collecting a drop of blood
on to the tip of a slide

Collecting lymph

Choose a swollen lymph node, preferably the prescapular, which is the easiest to manipulate. Fix it firmly against the skin with one hand and insert with the other hand a large-bore needle, such as is used for IV injections, to a depth corresponding to the centre of the node. Some of the secrets of obtaining good lymph samples are:

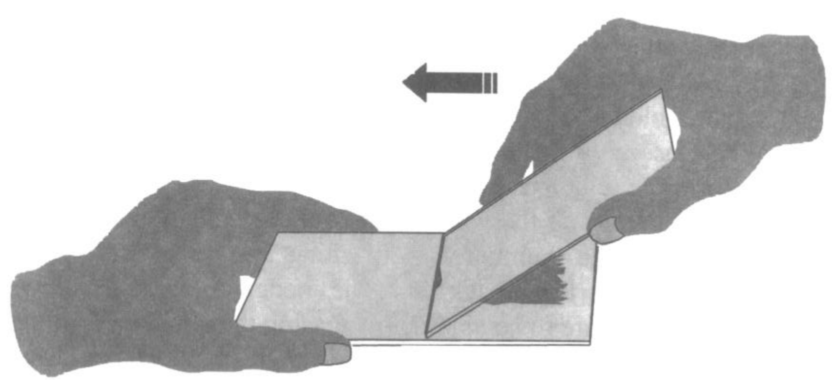
1) Avoid unnecessary movements, such as poking the needle around in the node, as this will result in bleeding and a useless sample (blood or lymph which is too diluted with blood).
2) When inserting the needle, do not close its posterior end, so as to allow the lymph to enter the needle.
3) Before and during the withdrawal of the needle, its rear end is closed with the thumb or by attaching a syringe, so that the lymph cannot flow back into the node. A syringe allows negative pressure to be applied, increasing the amount of lymph in the needle, but a clean syringe has to be used for each sample, as lymph is likely to enter the nozzle and contaminate the next sample.
4) The contents of the needle are expelled on to a slide, by attaching a syringe and pushing on the plunger. A smear is made, fixed and stained, as is indicated below for blood smears.

Making blood smears

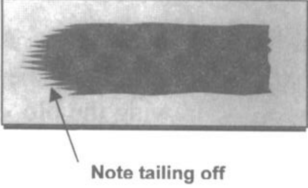
Blood smears should be even and thin, so that the red cells form one single layer. The thickness of the smear is influenced by the angle formed between the spreading slide used for drawing the blood over the second slide: the more acute the angle, the thinner the smear.

It is a good habit to make smears that are not only suitable for the diagnosis of extracellular parasites such as trypanosomes, but for intracellular organisms as well. The specific weight of white cells, as well as of red cells infected with protozoa, is slightly less than that of normal red cells and they tend to concentrate along the sides and in the tail of the smear. For the diagnosis of intracellular parasites and for establishing the white cell formula, it is therefore important that the smear does not reach the end and the edges of the slide, so that the tail and the sides can be properly examined with the microscope (Figure 18). The quantity of blood should be small, so

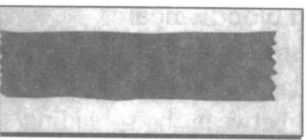
Figure 18
Preparing blood films



CORRECT



INCORRECT



that the tail of the smear tapers off before reaching the end of the slide, and one should cut (with sturdy scissors) one or two corners of one end of the spreading slide, so that the smear is less large than the slide on which it is made. It is also important that the edge of the spreading slide is smooth, and that the spreading movement is steady, in order to get an even smear. Another essential requirement for making good thin and even smears is that the slides are clean and free of grease. Do not use a slide twice for stained blood smears, as it is extremely difficult to clean them properly, and it is less expensive to buy new ones; one even risks finding stained parasites contained in rests of the first smear. (Such slides used for stained smears can be used after more normal cleaning for helminthological work or for examining fresh blood for trypanosomes.)

After the smear is made, it is essential to protect it from flies, which are attracted to the fresh blood, and will suck holes in the film, and may even cause pseudo-parasites to appear in the smear. Smears should also be protected from direct sun and other sources of excessive heat. The faster they are fixed and stained, the better, as old films stain badly and may even be useless, particularly in hot climates.

Staining of trypanosomes

Giemsa staining, after fixation by methanol, gives the best result, but it takes more time than more recent fast stains. This is a disadvantage for individual cases in the field, but much less when staining large numbers of smears in the laboratory.

Giemsa staining. Thin blood smears (or lymph smears) are fixed with pure[43] methanol for at least two minutes (there is no maximum time limit), and then stained in Giemsa's solution, diluted 1:20 with buffered water, during some 30-60 minutes. The time needed to obtain the best results varies according to the make and sometimes even to the batch of Giemsa. (The make of Giemsa is important, some are much better than others.)

[43] By pure we mean laboratory quality and not contaminated with water.

The diluted stain is made up just before use, while the smears are being fixed, by letting the concentrated Giemsa flow from a measuring pipette[44] into a wide measuring cylinder holding the buffered water, and immediately swirling the cylinder slowly around to obtain an equal mixture. When adding the concentrated solution to the water, the tip of the pipette should be near the surface of the water without touching it. Avoid violent shaking and sharp shocks.

It is best to use special staining jars, in which the slides are maintained in a vertical position by grooves. There are jars for small or large numbers of slides. It is often convenient to use two such jars, one for methanol (which can be used several times, provided the methanol is protected against evaporation and against contamination) and one for the diluted Giemsa. (The interior of some staining jars can be taken out and transferred with the slides from the fixing to the staining jar.)

After fixation with methanol, the slides are transferred to the staining jar into which the diluted stain is then poured carefully until its covers the edges of the slides entirely. When preparing the stain and during the staining process, avoid shocks and contact with metal (e.g. forceps) of the diluted Giemsa, as this may induce precipitation of the stain.

After the required time for staining a flow of water is directed into the staining jar to flush out the staining solution with its covering film consisting of fine precipitate. Never allow smears to dry with staining solution adhering, as stain precipitate will cover the smear and make examination difficult or even impossible.

After washing, the slides can be put into a vertical position for drying (a wooden board with appropriate grooves is convenient). Drying may be accelerated by using an electrical hair drier, and this is particularly useful in a humid atmosphere, but avoid the use of excessive heat. Also avoid drying smears on filter paper, as this can damage the smear and moreover result in transferring cells and

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[44] The use of a measuring pipette is much more accurate than counting drops, the size of which depends on the orifice from which they fall.

parasites from the smear to the paper and later to another smear. (This is not a hypothetical occurrence; because of this the author has found nucleus-containing red cells of birds in blood smears of cattle.)

The water used for the dilution of the concentrated Giemsa must be buffered, to a pH of 7.0-7.2 (7.2 giving the best result). Unbuffered tap water will not do. There are various formulas of salt mixtures which in solution will give the required pH. Salt tablets that give the pH wanted when diluted in a litre of water are commercially available and are most convenient. One can also make up one's own salt mixture, for example, a mixture of di-sodium mono-hydrogen phosphate and potassium di-hydrogen phosphate:

Na_2HPO_4	3.0 g (anhydrous) or 7.5 g (hydrated: Na_2HPO_4 - 12 H_2O)
KH_2PO_4	0.6 g
Water	one litre

The water used should be clean, if necessary filtered. Buffered water should not be kept for long periods before use and if at all it should be stored in a dark bottle, to avoid the growth of algae (which may even be the cause of pseudo-parasites in stained smears).

One can buy a concentrated solution of Giemsa ready for dilution, or make up one own's concentrated solution from powder. The latter procedure is cheaper and preferable, because the shelf-life of the solution is limited, especially in a hot climate, while the powder, if kept dry, can be kept almost indefinitely, and the concentrated solution can be made up in limited quantities, as required.

Mix 3.8 g of Giemsa powder with 250 ml of pure glycerol and 250 ml of pure methanol. Leave the mixture in a well-closed bottle containing glass beads for at least 48 hours and agitate frequently during that time. The mixture can be kept for many months. Filter through filter paper before use. Dilution with buffered water is carried out just before staining.

In some countries May-Grünwald-Giemsa staining (which includes fixation) is commonly used, but it is more expensive, does not give a better result for the staining of parasites and has the great

disadvantage that there are often stain deposits on the smear, particularly in hot climates. (It does give a slightly better result for the differentiation of white blood cells, but not for blood parasites, including trypanosomes.)

Other staining methods

Smears of individual animals may be stained more quickly for rapid results with fast stains such as Diff-Quik, RAL 555, Field's stain or CAM's Quick-stain, after methanol fixation.

Making brain smears

Although brain smears are not commonly required for the diagnosis of trypanosomosis, they are often essential for the differential diagnosis of diseases causing central nervous symptoms, which may be due to infections by *Trypanozoon, Babesia, Theileria*, but also of course rabies, plant poisoning, listeriosis, etc.

Grey matter is used for smears, as it contains many capillaries. Opening the skull in order to have access to the brain is often a major undertaking in the field, and may be dangerous as brain material, potentially infected with rabies, is likely to spatter around. It is safer to take cerebellar material with a sharp spoon (curette) through the foramen magnum,[45] or by making a hole in the skull with a large nail and a hammer, through which cerebral material can be taken with a large-bore needle and a syringe.

A small quantity of grey matter (corresponding to the size of one or at most two match heads) is crushed between two slides, which are pulled one across the other while pressure is maintained, resulting in two brain smears.

The smears are fixed and stained in the same way as blood smears.

Artefacts and other pseudo-parasites

Thrombocytes in smears often mislead novices, as in the process of drying and disintegrating they may assume various shapes, and even ressemble small trypanosomes. When a thrombocyte lies across a

[45] The hole in the skull through which the spinal cord joins the medulla oblongata.

red cell, it is sometimes taken for an intracellular haemoparasite, such as a *Babesia*.

Stain deposits may prevent a proper examination of the smear and are sometimes mistaken for *Anaplasma* or for *Eperythrozoon*.

Flies may deposit micro-organisms on a fresh smear, which may be mistaken for haemoparasites, and pseudo-parasites may also result from yeast or algae in the water used in the staining procedure.

Sample size considerations

Compiled by Dr Joachim Otte (Animal Health Service, FAO)

At an early stage of study design the question of how large a sample one needs must be considered. Clearly, one wants to avoid making the sample so small that the estimate is too inaccurate to be useful. Equally, one wants to avoid a sample that is too large so that the estimate is more accurate than is required, thus wasting valuable resources. Accurate determination of the sample size required for a study can be quite complicated and most complex studies will require the assistance of a statistician. Experience shows that the theoretical calculations provide background information rather than sample sizes for practical use as other than purely statistical considerations are likely to determine sample size.

For less complex studies based on simple random sampling one of the following formulae should provide suitable estimates of the required size of the sample. The formula to use will depend on whether a categorical (discrete, qualitative) or numerical (continuous, quantitative) variable is being measured.

Sample sizes for estimating proportions in large populations

To determine the sample size necessary to estimate a population proportion, for example the prevalence of a given disease in a population or the proportion exposed to a suspected risk factor, the investigator must provide an educated guess on the anticipated proportion of diseased or exposed, P, and must specify how close to the true prevalence the estimate, p, should be.

The average of a series of estimates of the prevalence of a disease, p1, p2, etc. will be almost exactly equal to the true prevalence, P, and the distribution of the estimates will tend to normality. Thus, based on the values from the normal distribution (z-values) 68 percent of the estimates will differ from the true prevalence by less than the quantity *(PQ/n) (where Q=100-P and n=size of the sample), called the standard error of the estimated prevalence (SE). Similarly, 95 percent of the

estimates would differ from the true value by less than 1.96 times the standard error and 99 percent of the estimates would be expected to lie within 2.58 standard errors of the true value. Therefore, we might, for example, say that we would like to be 95 percent sure that our estimate lies within 1 percent of the true prevalence, P, i.e. we are requesting a 95 percent confidence of achieving a precision of *1 percent.

By transformation of the above formula for the standard error of a proportion to yield n, and by substituting d / z for SE we receive the following formula for the calculation of sample sizes:

n = P x (100 - P) x z2/d2

where
P is the anticipated prevalence
d is the desired precision
z is the appropriate value from the normal distribution for the desired confidence

level of confidence		
90 percent	95 percent	99 percent
z = 1.645	z = 1.960	z = 2.576

Thus, suppose the available evidence suggests that approximately 40 percent of the cow population will have antibodies to leptospira and the investigator wishes to be 95 percent sure that the estimate will lie be within 4 percent of the true prevalence, sample size would be:

n = 40 x (100 - 40) x 1.962/42 = 576.24

The resulting value should be rounded up at least to the nearest integer as some samples are likely not to yield useable results.

If the sample size is a large proportion of the population, say greater than 10 percent, then sample size can be readjusted to n' using the following formula:

$$n' = \frac{n}{1 + n/N}$$

where N is the total size of the population.

Usually one can make a rough estimate of what the highest expected prevalence of the disease might be in the population of interest. The appropriate sample size then is likely to be too big. If P is likely to lie between 35 and 65 percent, the advance estimate can be quite rough since the product PQ varies little for P lying between these limits. If, however, P is near 0 or 100 percent, accurate determination of n requires a close guess about P. If we do not have the slightest idea what prevalence to expect, one can use the sample size corresponding to the least favourable case, a prevalence of 50 percent, which has the largest sampling variation.

Sample sizes for various levels of precision and expected prevalence

Expected	**95 percent confidence**		
prevalence	* 10 percent	* 5 percent	* 1 percent
10 percent	35	138	3.457
20 percent	61	246	6.147
30 percent	81	323	8.067
40 percent	92	369	9.220
50 percent	96	384	9.064

Sample sizes needed to estimate a population mean

In analogy to what has been said for the calculation of the sample size needed to obtain an estimate of the prevalence of a disease at a predetermined level of precision, the investigator needs to supply an estimate of the standard deviation, s, or variance, s2, of the variable of interest in the target population and specify how close to the true mean the sample estimate, i.e. the tolerated difference, d, should be. In the absence of previous estimates, s can roughly be estimated from a knowledge of the highest and lowest values in the population and a rough idea of the shape of the distribution. If h = highest - lowest, then s = 0.29 h for a uniform (*) distribution, s = 0.25 h for a symmetrical distribution shaped like an isosceles triangle (D), and s = 0.21 h for a skew distribution shaped like a right triangle (*).

The laws of probability allow us to be 95 percent sure that the true population mean lies within the interval:

Sample mean ± 1.96 x standard error of the sample mean.

In other words, we can be 95 percent sure that the difference between the sample mean and the true mean is not greater than 1.96 x SE. For a simple random sample, the standard error of the sample mean is:

SE = s/*n

where: s = Standard deviation
n = Sample size

We thus have to solve the following equation for n:

z x s/*n = d

n = (z2 x s2)/d2

Thus, if we wanted to estimate the average weight of mature cows in a given region, and want to be 95 percent sure that our estimate is within ± 5 kg of the true mean (i.e. d = 5) and available evidence suggests that the standard deviation is 20 kg, the sample size required, assuming we will have to sample less than 10 percent of the population, would be:

n = (1.962 x 202)/52

n = 61

Thus, 61 cows would have to be weighed in order to obtain an estimate of the mean cow weight with a 95 percent chance of the true mean being within the interval of the estimate ± 5 kg. (For a 99 percent probability, 1.96 should be replaced by 2.58. To be 99 percent sure, 105 cows would be needed.)

If the calculated sample size is a large proportion of the population, say greater than 10 percent, then sample size can be readjusted to n' using the formula given in the preceding section.

In using the above formulae, it is assumed that the sampling unit is the same as the unit of concern. When using cluster or multistage

sampling, an upward adjustment of sample size may be required to obtain the desired precision of the estimate. The size of the adjustment required depends on the design effect, D, which is in turn determined by the within cluster correlation coefficient and the number of sampling units per cluster.

$$D = 1 + (b - 1)\,roh$$

where:
b is the average number of animals per cluster
roh is the intra-cluster correlation coefficient

Analysis of survey results based on cluster sampling have shown that for most animal diseases the intra-cluster correlation coefficient does not exceed 0.2 and as can be seen from the following table 27 clusters of ten animals each should ensure a prevalence estimate with a 95 percent confidence of achieving a precision of 10 percent at an expected prevalence of 50 percent.

The effect of intra-cluster correlation and cluster size on sample size:

roh	Animals per cluster	D	Animals required	Clusters required
..05	10	1.45	140	14
..05	20	1.95	188	10
..05	30	2.45	236	8
..10	10	1.9	183	19
..10	20	2.9	279	14
..10	30	3.9	375	13
..20	10	2.8	267	27
..20	20	4.8	461	23
..20	30	6.8	653	22

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This new edition of the well-known field guide on African animal trypanosomosis adheres as much as possible to the original style and, particularly, to the intention of the author of the first edition in that it is essentially meant to be a guide for field control personnel. Its scope has been extended somewhat beyond that of the African continent, as trypanosomes of African origin have spread to the Americas as well as to Asia, and even to Europe, but the main emphasis remains on Africa. More attention is also given to methods of control of the disease other than those using chemotherapy and chemoprophylaxis, as it is being realized that drugs alone are not a sustainable answer, and have to be integrated into a multidisciplinary and flexible approach to control of the disease.

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