# INFECTIOUS DISEASES OF THE HORSE

Edited by T.S. Mair and R.E. Hutchinson

A peer reviewed publication

© World copyright by Equine Veterinary Journal Ltd 2009

ISBN 0-9545689-2-3

First published 2009

Equine Veterinary Journal Ltd.
Mulberry House, 31 Market Street, Fordham, Ely, Cambridgeshire CB7 5LQ, UK
Tel: 01638 720250 ▪ Fax: 01638 720868 ▪ Website: www.evj.co.uk

*The authors, editors and publishers do not accept responsibility for any loss or damage arising from actions or decisions based on information contained in this publication; ultimate responsibility for the treatment of patients and interpretation of published material lies with the veterinary surgeon.*

Front cover image:
A transmission electron micrograph of negatively stained Hendra virus.
*Supplied by the Australian Biosecurity Microscopy Group (Australian Animal Health Laboratory, CSIRO, Geelong, Australia).*

Typeset and published by:
*Equine Veterinary Journal Ltd, Mulberry House, 31 Market Street, Fordham, Ely, Cambridgeshire CB7 5LQ, UK*

Printed in Great Britain by:
*Geerings Print Ltd, Ashford, Kent, UK.*

# EQUINE TRYPANOSOMIASIS

P. Van den Bossche*‡, S. Geerts and F. Claes†§

*Departments of Animal Health and †Parasitology, Institute of Tropical Medicine, Antwerp, Belgium; ‡Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, South Africa; and §Department of Biosystems, KULeuven, Belgium.*

**Keywords:** horse; mule; donkey; trypanosomiasis; nagana; dourine; surra

## Summary

Equine trypanosomiases are acute or chronic infectious diseases caused by protozoan blood parasites. Depending on the parasite species involved 3 diseases can be distinguished: nagana, surra and dourine.

## Introduction

### Nagana

Trypanosomes causing nagana (or tsetse-transmitted trypanosomiasis) belong to the subgenera *Nannomonas* (*Trypanosoma congolense*, *T. simiae*, *T. godfreyi*), *Trypanozoon* (*Trypanosoma brucei brucei*, *T. b. rhodesiense* and *T. b. gambiense*) and *Dutonella* (*Trypanosoma vivax*). With the exception of *T. vivax* that can also be found outside the African continent, they occur in sub-Saharan Africa (**Fig 1**). In wild animals these parasites cause mild infections. In domestic animals, however, the infection can be fatal depending on the host and parasite species involved. Nagana is a major direct and indirect constraint to livestock and rural development in sub-Saharan Africa. Nagana in horses, is caused by an infection with *T. congolense*, *T. vivax* and/or *T. b. brucei*.

### Surra

*Trypanosoma evansi* is the causal agent of surra. There are numerous natural hosts of *T. evansi*. They include domestic animals such as horses, donkeys, mules, camels, goats, sheep, pigs, cattle and water buffalo but also a broad range of game animals (e.g. Indian elephant, capybara, lions, tiger and antelope) and pets (cats and dogs). Surra occurs mainly in north and northeast Africa, Latin America (except Chile), the Middle East and Asia and causes considerable economic losses as a result of a reduction in reproduction, working performance, milk yield and/or meat production of cattle, horses, pigs and buffalo (**Fig 1**).

### Dourine

Dourine is the result of an infection with *Trypanosoma equiperdum*. Solipeds are the natural hosts of *T. equiperdum*. Because of the difficulties in detecting and isolating *T. equiperdum*, there has been doubt about it being the causative organism of the disease. The parasite is cosmopolitan, but nowadays, western Europe, Australia and the USA are considered to be free from dourine (**Fig 1**).

## Aetiology

Trypanosomes, the causal agents of equine trypanosomiases, are elongated extracellular Protozoa with a forwards pointing flagellum for locomotion. They usually possess a kinetoplast, a specific structure that is situated near the base of the flagellum. Trypanosomes have been found in many species of mammals, reptiles, fish, birds as well as in vectors such as insects, ticks, leeches and vampire bats. Some trypanosomes can infect various animal species, while others are species-specific.

Transmission of trypanosomes can be the result of inoculation of infected blood (e.g. during vaccination campaigns) but they are usually transmitted cyclically, mechanically or sexually. Cyclical transmission occurs when the trypanosome undergoes a developmental cycle in the insect vector, the tsetse fly. In mechanical transmission, a

*Author to whom correspondence should be addressed.

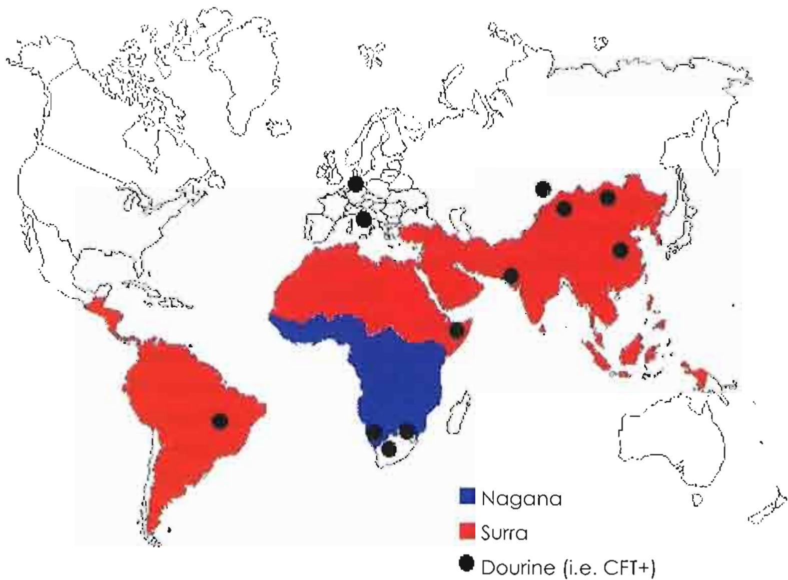


FIGURE 1: Distribution of nagana, surra and dourine.

haematophagous insect becomes contaminated with an infectious agent during normal feeding and the agent may persist on the mouthparts until the next feed without undergoing any biological development. On the African continent, cyclical transmission is the main mode of transmission for *T. congolense*, *T. brucei s.l.* and, to a lesser extent, *T. vivax*. Mechanical transmission is the only mode of transmission for *T. evansi*, but it can also occur with trypanosome species that are normally transmitted cyclically (i.e. *T. congolense* and *T. b. brucei*) and it is a common mode of transmission for *T. vivax*. *T. equiperdum* is transmitted sexually, during copulation, from the stallion to the mare or *vice versa* (Laveran and Mesnil 1912).

### Nagana

Nagana caused by *T. congolense*, *T. brucei s.l.* and/or *T. vivax* is the result of a bite of an infected tsetse fly (*Glossina* spp.) that injects the parasite along with its saliva before taking its blood meal. For a few days, the trypanosomes will multiply locally at the site of the bite thereafter they invade the lymphatic system and blood vessels and, especially in the case of *T. brucei s.l.*, invade other tissues and organs, including the central nervous system.

### Surra

*Trypanosoma evansi* does not undergo cyclical transmission, thus, no specific insect stages occur. Two different bloodstream forms can be observed. The long slender form is seen most of the time; the short and stumpy form is scarce but present. The morphology of this trypanosome species is indistinguishable from that of *T. b. brucei*.

### Dourine

The morphology of this parasite is identical to the bloodstream forms of *T. b. brucei*. Transmission of the parasite occurs during copulation. After entering the host through the genital tract, *T. equiperdum* first migrates to the bloodstream of the host but converts rapidly into a tissue parasite.

## Epidemiology

### Nagana

The epidemiology of nagana is complex and, because of the focal nature of the disease, varies spatially and is determined by a number of tsetse-related variables. Generally speaking, the probability of a host contracting trypanosomiasis depends on the rate at which it is fed upon by infected male or female tsetse flies (Rogers 1988). The trypanosomal infection rate

of a tsetse population is generally low with a large proportion of the flies being refractory to infection with trypanosomes. The attraction of tsetse flies to a host, such as a horse, and subsequently the proportion of tsetse that feed and challenge a host is the result of a number of visual and olfactory stimuli.

Although the horse population in tsetse-infested Africa is relatively small, the prevalence of nagana in horses can take considerable proportions. For example, at a gate-clinic in The Gambia, 61% of all horses presented were infected with trypanosomes (Dhollander *et al.* 2006). Using more sensitive molecular tools, Faye *et al.* (2001) and Pinchbeck *et al.* (2008) detected a much higher overall prevalence for any trypanosome species reaching up to 93% with a high proportion of mixed infections. In many tsetse-infested African game parks, horseback safaris cannot be sustained because of the high prevalence of trypanosomal infections.

### Surra

*Trypanosoma evansi* is transmitted mechanically by haematophagous insect vectors such as *Tabanidae*, *Stomoxys*, *Haematopota*, *Chrysops* and *Lyperosia*. Vampire bats, *Desmodus rotundus*, are also reported to spread the disease in Latin America (Stephen 1986). Little is known about the epidemiology of *T. evansi* in Africa since more attention is given to tsetse-transmitted trypanosome species. Epidemiological data of surra in equids are scarce apart for Ethiopia where a recent survey showed that equine trypanosomiasis is highly endemic in the highlands (F. Claes, unpublished data). From Eastern Africa, the disease probably spread further into the Middle East and later to Asia. Nowadays, surra can be found in feral camelids in Oman and occasionally in commercially bred camels and horses. Surra is endemic throughout central Asia (India, Mongolia, Kazakstan), southeast Asia and the Indonesian archipelago with the possible exception of Western New Guinea (Singh *et al.* 1995; Reid 2002; Clausen *et al.* 2003). Unfortunately most prevalence data are from camels, cattle and buffalo rather than equids. It is possible that the parasite is more prevalent in the former species that may act as a reservoir and subsequently causes outbreaks in the horse population. In South America also, little is known of the prevalence and spread of *T. evansi*. During a survey in Venezuela, 12% of horses were found seropositive. In Colombia, French Guyana, Argentina and the north of Brazil, no prevalences are recorded but the parasite seems enzootic and high mortality was observed in *T. evansi* outbreaks in horses in these regions (Desquesnes 2004). Only Chile seems to be free from *T. evansi*, possibly due to the natural barrier of the Andes.

### Dourine

Since the 19th century, dourine has occurred only sporadically in Europe. Around 1918, the disease was reported in Russia, Turkey, Hungary and Spain. During World War II, however, *T. equiperdum* was re-introduced into western Europe (Saurat 1946). After the war, the disease was eradicated in western Europe through stringent control measures. Nevertheless, sporadic cases of seropositive animals are still reported (e.g. in Italy and recently in Germany). The latest official reports of dourine were from China, Kazakhstan, Kyrgyzstan, Pakistan, Ethiopia, Botswana, Namibia, South Africa, Brazil, Italy and Germany.

## Clinical signs

### Nagana

Although horses are susceptible to infections with all 3 salivarian trypanosome species, the most common infections are those with *T. congolense* followed by infections with *T. vivax*. Infections with *T. b. brucei* are rare. On the other hand, mixed infections with different species are common.

The clinical signs of nagana in horses differ little from those seen in cattle. They can vary from lethargy and anorexia to no abnormalities detected on clinical examination. Parasitic peaks with large numbers of parasites in the peripheral blood are observed usually in the early phase of infection accompanied by pyrexia (rectal temperature may reach 40.5°C), tachycardia and tachypnoea. Animals with acute nagana are very depressed. Severe anaemia may develop with the packed cell volume (PCV) declining to low levels with increasing weakness and signs of ataxia. Small skin nodules (urticaria) and oedematous plaques may form on the flanks. Progressive oedema of the ventral parts such as sternum, belly, prepuce and especially the legs can be very pronounced but is not a common feature

(Pinchbeck *et al.* 2008). The mucous membranes of the eye, the gums or vagina may become icteric as a result of the rapid destruction of red blood cells.

Equine trypanosomiasis due to *T. b. brucei* (in a single or mixed infection) is often acute and can lead to death in 2 weeks to 3 months with little or no evidence of involvement of the central nervous system (Taylor and Authié 2004).

Infections with *T. congolense* or *T. vivax* have a more chronic nature. In the chronic phase of the infection the parasitaemia is usually low and parasites are difficult to detect using parasitological diagnostic tools. The anaemia progresses with mucous membranes becoming pale. Animals further lose condition and weight and become extremely weak and show signs of ataxia. In the chronic phase of infection, the subcutaneous oedema may extend to the head. Animals may become severely emaciated. In chronic *T. b. brucei* infections, the disease is often associated with nervous symptoms and the presence of the parasite in the cerebrospinal fluid and macroscopically visible lesions of the meninges and the brain leading to ataxia and paralysis (Neitz and McCully 1971; McCully and Neitz 1971). Up to 20% of the horses infected with *T. brucei* may develop keratitis and corneal opacity (Neitz and McCully 1971).

## Surra

There are 2 forms of this disease. An acute form, which occurs mostly in horses and camels and a chronic form, which is mostly seen in cattle and water buffalo.

The pathognomonic signs of surra are similar in the different host species but not always readily observed. In acute infections, animals suffer from high pyrexia, progressive anaemia, loss of general body condition and exhaustion, finally resulting in death. In chronic infections, subsequent waves of fever, associated with the parasitaemia peaks, are observed. Oedema, mainly of the lower legs, urticarial plaques and petechial haemorrhages on the mucous membranes can develop (Stephen 1986). The principal clinical signs of the acute form of the disease in horses are pyrexia associated with peaks in the parasitaemia, oedema of the belly, anaemia and muscular weakness. However, *T. evansi* is notorious for its variable pathogenicity so not all signs may be observed and differences may occur from one horse to another.

The mortality rate in horses can be very high (80–100%) (Stephen 1986). In cattle, water buffalo, sheep and goats the mortality rate is much lower (10–40%).

## Dourine

*Trypanosoma equiperdum* causes a chronic infection in horses that can persist for 1–2 years. The incubation period is highly variable. Clinical signs usually appear within a few weeks of infection but, in some cases, may not be evident until after several years. The appearance of clinical signs may be accelerated by stress in infected animals (Barrowman 1976).

An infection with *T. equiperdum* can generally be divided into 3 phases, although the clinical course can vary considerably under different conditions. The first phase is characterised by oedema, tumefaction and damage to the genitalia. In mares, the first sign of infection is usually a small amount of vaginal discharge, which may remain on the tail and hindquarters. Swelling and oedema of the vulva develop later and extend along the perineum to the udder and ventral abdomen. Vulvitis and vaginitis with polyuria may occur. Significant abortion losses can be observed in infected herds. In stallions, the initial signs are variable oedema of the prepuce and glans penis, spreading to the scrotum and perineum and to the ventral abdomen and thorax (**Figs 2** and **3**). Swellings may resolve and reappear periodically. Vesicles or ulcers on the genitalia may heal and leave permanent white scars (leucodermic patches). Conjunctivitis and keratitis are often observed in outbreaks of dourine and may be the first

FIGURE 2: Genital and ventral abdominal oedema in an Ethiopian horse affected by dourine.

signs noted in some infected herds. The second phase of the infection is considered to be pathognomonic for dourine. In this period typical cutaneous 'plaques' or areas of thickened skin can occur with variable sizes ranging from very small to the size of a hand and a thickness of one centimeter (Laveran and Mesnil 1912). These plaques usually appear over the ribs, although they may occur anywhere on the body, and usually persist for 3–7 days. Such plaques have also been observed sporadically in animals infected with *T. evansi* (Brun *et al.* 1998). The final phase of dourine is characterised by progressive anaemia and by disorders of the nervous system. Initially these signs consist of restlessness and a tendency to shift weight from one leg to another followed by progressive weakness and incoordination (**Fig 4**). Ultimately, paralysis (mainly of the hind legs), paraplegia and death occur (Stephen 1986).

At *post mortem* examination, gelatinous exudates can be found under the skin. In the stallion, the scrotum, sheath and testicular tunica may be thickened and infiltrated. In some cases the testes are embedded in a tough mass of sclerotic tissue. In the mare, the vulva, vaginal mucosa, uterus, bladder and mammary glands can be thickened with gelatinous infiltration. The lymph nodes, particularly in the abdominal cavity, are hypertrophied, softened and, in some cases, haemorrhagic. The spinal cord of animals with paraplegia is often soft, pulpy and discoloured, particularly in the lumbar and sacral regions.

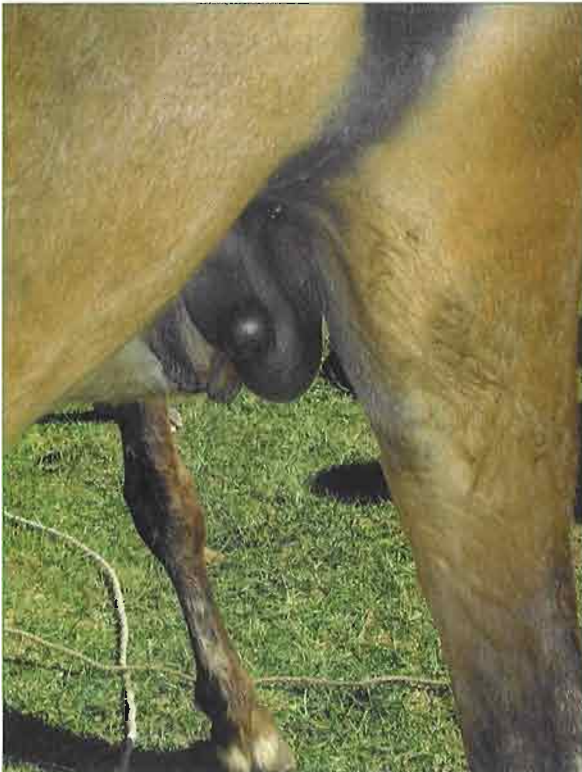

FIGURE 3: Genital oedema in an Ethiopian horse affected by dourine.

Acute equine trypanosomiasis must be distinguished from African horse sickness and anthrax. Repeated examination of blood allows for the differential diagnosis with other causes of severe pyrexia in horses such as equine babesiosis (*Babesia caballi*) and theileriosis (*Theileria equi*).

## Diagnosis

### Nagana

Diagnosing nagana is difficult because there are no specific clinical signs and parasitaemias are usually low making parasitological detection of the parasites difficult. As a result, parasitological detection methods have high specificity but low sensitivity (Paris *et al.* 1982). The body fluid most commonly examined is blood. Lymph, aspirated from a punctured superficial lymph node (usually not easily palpable), provides useful supplementary diagnostic material. The simplest parasitological diagnostic techniques are the examination of wet or Giemsa-

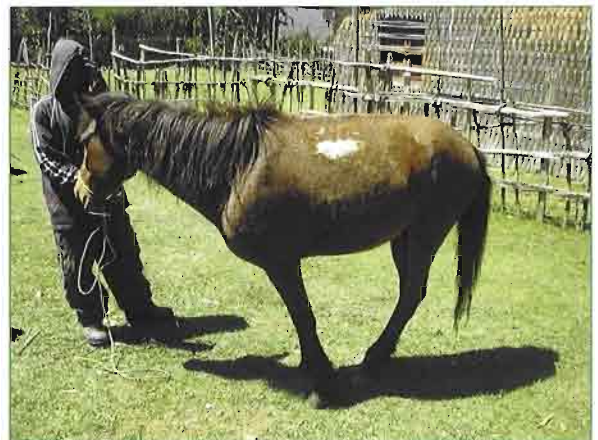

FIGURE 4: Apathy, depression and incoordination in a horse affected by dourine.

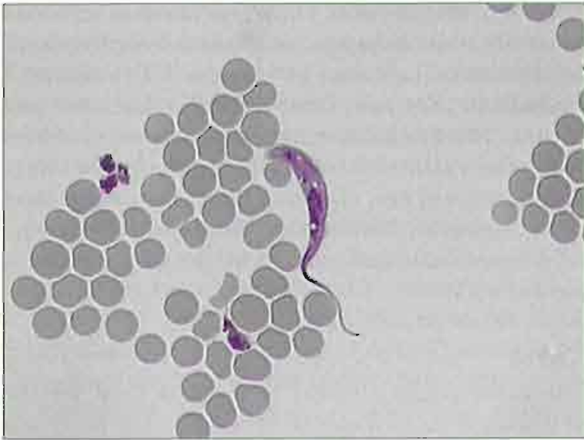

FIGURE 5: Giemsa-stained thin smear of blood infected with Trypanosoma vivax.

stained thick or thin films of fresh blood. Wet blood films are simple, inexpensive and give immediate results. Depending on the trypanosome size and the parasite's movements, a presumptive diagnosis can be made of the trypanosome species involved:

*Trypanosoma vivax:* Large, extremely active, traverses the whole field very quickly, pausing occasionally.

*Trypanosoma brucei:* Various sizes, rapid movement in confined areas.

*Trypanosoma congolense:* Small, sluggish, adheres to red blood cells by the anterior end.

The Giemsa-stained thin smear permits accurate determination of the species of the parasites involved. Trypanosome species can be identified by the following morphological characteristics:

*Trypanosoma vivax:* 20–27 μm long, undulating membrane is not obvious, free flagellum present at the anterior end, posterior end rounded, kinetoplast large and terminal (**Fig 5**).

*Trypanosoma brucei:* is a polymorphic trypanosome species. Two distinctly different forms can be distinguished, i.e. a long slender form and a short stumpy form. Often, intermediate forms, possessing characteristics of both the slender and stumpy forms, are observed. The cytoplasm often contains basophilic granules in stained specimens (**Fig 6**).

The long slender form is 17–30 μm long and about 2.8 μm wide, undulating membrane is conspicuous, free flagellum present at the anterior end, posterior end pointed, kinetoplast small and subterminal. The short stumpy form is 17–22 μm long and about 3.5 μm wide, undulating membrane is conspicuous, free flagellum absent, posterior end pointed, kinetoplast small and subterminal.

*Trypanosoma congolense:* 8–25 μm (small species), undulating membrane not obvious, free flagellum

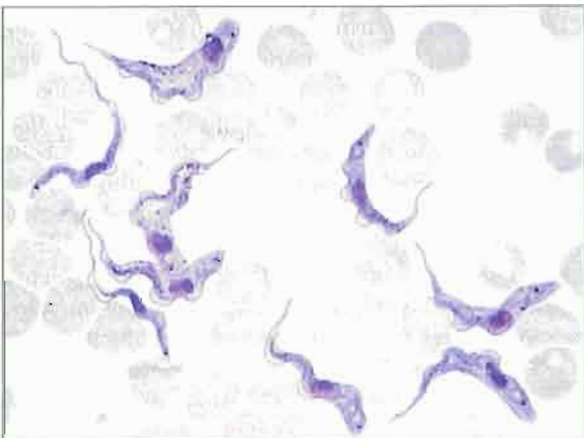

FIGURE 6: Giemsa-stained thin smear of blood infected with long slender and stumpy forms of Trypanosoma brucei brucei.

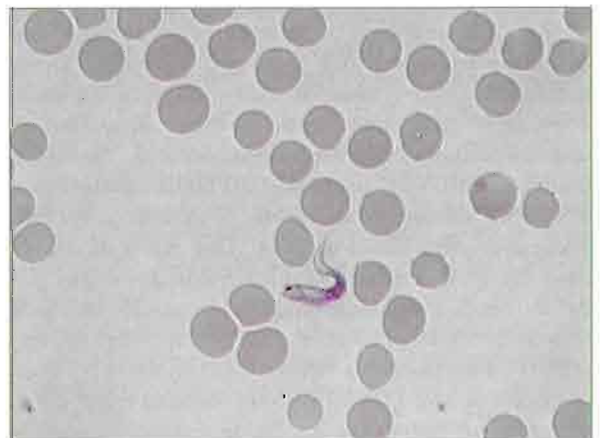

FIGURE 7: Giemsa-stained thin smear of blood infected with Trypanosoma congolense.

absent, posterior end rounded, kinetoplast is medium sized and terminal, often laterally positioned (**Fig 7**). Although *T. congolense* is considered to be monomorphous, a degree of morphological variation is sometimes observed. Within *T. congolense*, different types or subgroups exist (savannah, forest, kilifi, tsavo) that have a different pathogenicity. However, these types can only be distinguished using polymerase chain reaction (PCR).

The diagnostic sensitivity can be improved by increasing the volume of blood to be examined and concentrating the trypanosomes. The microhaematocrit centrifugation technique or Woo-method (Woo 1970) is more sensitive but identification of trypanosome species is difficult. Alternatively, the buffy coat and the uppermost layer of red blood cells can be extruded onto a clean microscope slide and covered with a cover slip (buffy coat technique or Murray method: Murray *et al.* 1977). The methods are particularly useful in that the PCV can be assessed after centrifugation.

Xenodiagnosis or the subinoculation of blood into rodents such as mice or rats, can be used to detect *T. brucei* and *T. congolense* infections (Uilenberg 1998). However, since rodents are refractory to *T. vivax* and not all *T. congolense* and *T. brucei* infections become established in the new host, this method has serious limitations. Mixed trypanosomal infections may also remain undetected. The mini-anion exchange centrifugation technique (mAECT), the most sensitive parasitological test, is seldom applied in animals due to its high cost (Lumsden *et al.* 1979).

As an alternative to parasitological tests, DNA-detection based on PCR is used frequently. These molecular tests usually have high diagnostic sensitivity and specificity. Several *Trypanozoon* specific primers have been designed; including TBR primers, pMUTEC primers, ORPHON primers and ESAG6/7 (Moser *et al.* 1989; Wuyts *et al.* 1994; Pereira de Almeida *et al.* 1998; Holland *et al.* 2001). Specific PCR tests have also been developed for detecting *T. congolense* and *T. vivax*, (Majiwa and Otieno 1990; Morlais *et al.* 2001) Moreover, several molecular tests, such as PCR-RFLP analysis (Geysen *et al.* 2003) and ITS-1 PCR (Davila and Silva 2000; Claes *et al.* 2004) are available that differentiate between trypanosomes of the *Trypanozoon* subgroup, *T. congolense* and *T. vivax.*

The development of anti-trypanosomal antibody detection techniques has been a major improvement in the serodiagnosis of nagana. The indirect fluorescent antibody test (IFAT) (Luckins and Mehlitz 1978) and different ELISA systems (Luckins 1977; Greiner *et al.* 1997; Hopkins *et al.* 1998) have been evaluated and are being used in the field. Most tests, however, have been developed for bovine trypanosomiasis and need to be adapted for use in horses.

## Surra

The same parasitological techniques used for the diagnosis of nagana can be applied for the diagnosis of *T. evansi*. For serodiagnosis, the RoTat 1.2 VSG might serve as a marker for *T. evansi*. Several antibody detection tests have been developed and applied in the field based on this antigen; mostly used is the CATT/*T. evansi* test, a card agglutination test (Bajyana Songa and Hamers 1988). Other tests, based on the same antigen, are the ELISA, the LATEX/*T. evansi* and the immune trypanolysis (Verloo *et al.* 2001).

For molecular diagnosis, kinetoplast DNA (kDNA) probes based on mini-circle sequences have been developed (Gibson *et al.* 1983; Borst *et al.* 1987). Unfortunately, this method is not suitable for dyskinetoplastic *T. evansi* strains. To overcome this problem, a species-specific PCR based on the RoTat 1.2 VSG gene is available (Claes *et al.* 2004).

## Dourine

Clinical signs of dourine can provide strong indication of the disease, as can its chronic evolution. However, since differential diagnosis with nagana and surra is very difficult, confirmatory diagnosis is needed. It is very difficult to detect *T. equiperdum* in the body fluids of infected horses. Therefore, diagnosis of *T. equiperdum* infection is based on serological evidence. Although antibody and antigen ELISAs have been developed for *T. equiperdum* (Alemu *et al.* 1997), the only internationally recommended serological test remains the complement fixation test (CFT) (Watson 1915), which does not distinguish between *T. equiperdum*, *T. evansi* and *T. brucei* (Robinson 1926; Hoare 1956; Richardson and Kendall 1957). Indeed, possible cross-reactions with *T. evansi* and *T. brucei* may occur and consequently the latter

parasites cannot be distinguished from *T. equiperdum* unless the samples originate from *T. evansi* and *T. brucei* free regions. Unfortunately, countries where dourine is currently reported often lie within the distribution area of *T. evansi*.

Currently, the main difficulty with diagnosing dourine is to distinguish *T. equiperdum* from *T. evansi*. Some authors mention the absence of maxicircles in the kDNA of *T. evansi* to be a major difference between *T. evansi* and *T. equiperdum* (Riou and Saucier 1979; Frasch *et al.* 1980). However, since dyskinetoplastic strains exist in both *T. evansi* and *T. equiperdum*, the validity of this characteristic can be questioned. So far, neither isoenzyme analysis (Lun *et al.* 1992), nor southern blot analysis (Hide *et al.* 1990; Zhang and Baltz 1994) or microsatellite markers (Biteau *et al.* 2000) can distinguish *T. equiperdum* from *T. evansi*. Recent characterisation studies indicated a heterogeneity in *T. equiperdum* isolates. In general, most *T. equiperdum* are very similar to *T. evansi* while two strains (OVI and BoTat 1.1) resemble *T. b. brucei* (Claes *et al.* 2003).

## Treatment and prevention

### Nagana

A number of trypanocidal drugs can be used to treat horses infected with a *T. congolense*, *T. vivax* and/or *T. b. brucei* infection (Connor and Van den Bossche 2004; Holmes *et al.* 2004) (**Table 1**). All these drugs are toxic and have a narrow safety margin.

Isometamidium chloride is the drug that is tolerated best (Connor and Van den Bossche 2004; Dhollander *et al.* 2006; Auty *et al.* 2008) on condition that it is administered correctly, i.e. deeply i.m. and avoiding any leakage of the drug to subcutaneous tissues which may cause sloughing of the skin. This is because the drug causes coagulative necrosis at the injection site, which is encapsulated after some weeks by fibrous tissue (Kinabo and Bogan 1988). For curative and prophylactic purposes the recommended dose is 0.5 mg/kg bwt i.m. It is advised not to exceed this dosage (Uilenberg 1998). The prophylactic cover is highly variable and depends on many factors. Dehoux *et al.* (1996) mention a protection period of 2.5 months in a moderately tsetse-infested environment. Isometamidium chloride can also be injected i.v., which is well tolerated by the horses if administered correctly without any leakage (Dehoux *et al.* 1996). Diminazene aceturate mainly has a curative effect, although the drug provides a short protection period of about 18 days (Faye *et al.* 2001). It is effective against *T. vivax* and *T. congolense* at 3.5 mg/kg bwt i.m. and against *T. brucei* at 7 mg/kg bwt. Although Uilenberg (1998) does not recommend to use the drug in horses because of occasional severe reactions at the injection site, Dhollander *et al.* (2006), who treated more than 1000 *T. vivax* and *T. congolense* infected horses in The Gambia, did not observe any side effects at 3.5 mg/kg bwt. Horses in good condition received the drug in one dose, whereas in animals with a high parasitaemia or severe anaemia the drug was divided over 2 or 3 portions with a 4 h interval. Awan and Johnston (1979), however, reported adverse effects (prostration, staggering gait, appetite loss and marked reactions at the injection site) after treatment at 3.5 mg/kg bwt in 7 horses infected with *T. brucei*.

Quinapyramine is used mainly for the treatment of *T. b. brucei* (and *T. evansi*) in horses and camels.

**TABLE 1: Currently available trypanocidal drugs for use in horses**

| Compound | Dosage (mg/kg bwt) | Route | Use | Active against | Side effects |
|---|---|---|---|---|---|
| Isometamidium | 0.5 | i.m./i.v. | C, P | Tc, Tv, Tb, Te | (+) |
| Diminazene | 3.5 | i.m. | C | Tc, Tv, Te | + |
| Quinapyramine sulphate | 3-5 | s.c. | C | Tc, Tv, Tb, Te | ++ |
| Quinapyramine sulphate and chloride (prosalt) | 3-5 | s.c. | P | Tc, Tv, Tb, Te | ++ |
| Homidium bromide or chloride | 1 | i.m./i.v. | C, (P) | Tc, Tv, Te | + |
| Suramin | 7–10 g/a* | i.v. | C, (P) | Te | (+) |

Tb = *Trypanosoma brucei*; Tc = *T. congolense*; Tv = *T. vivax*; Tv = *T. evansi*; C = curative; P = prophylactic; * = g/animal, to be repeated after a week.

Quinapyramine dimethylsulphate has therapeutic activity whereas a mixture of dimethylsulphate and chloride (3:2 w/w) has a prophylactic effect against *T. congolense*, *T. brucei* and to a lesser extent *T. vivax* (Uilenberg 1998). The dosage is 3–5 mg/kg bwt subcutaneously (based on the sulphate in case of the mixture). Unfortunately, quinapyramine is poorly tolerated by horses and severe albeit transient (in 1–3 h) side effects usually occur (Awan and Johnston 1979; Connor and Van den Bossche 2004; Auty *et al.* 2008). The most commonly observed side effects are restlessness, salivation, sweating, fasciculations, diarrhoea and abdominal discomfort. Sometimes the animals become recumbent and roll. Therefore, it is recommended that: 1) the animals should be well rested before treatment: 2) the dose be split into 2 or 3 portions, which are injected with an interval of 4–6 h (particularly in weakened animals); and 3) the injection site is massaged to minimise local reactions and nodule formation (Connor and Van den Bossche 2004). Quinapyramine has been withdrawn from the market for use in cattle because of problems with the fast development of resistance and cross-resistance to all other trypanocides (Geerts and Holmes 1998). Although the drug is currently available only for use in horses and camels, the remark about (cross)-resistance development remains valid.

Finally, homidium bromide (ethidium bromide) or homidium chloride can also be used in horses. However, these drugs are highly mutagenic and are therefore not recommended. If no other drugs are available they can be used at 1 mg/kg bwt deeply i.m. or i.v. for the treatment of animals infected with *T. congolense* or *T. vivax*. Since horses are very susceptible to the irritant effect at the injection site, Uilenberg (1998) recommends to inject the drug i.v. avoiding any leakage into the tissues surrounding the jugular vein. After i.m. injection Stephen and Mackenzie (1958) observed some development of oedema in the ventral and inguinal regions, which disappeared after 2 weeks. Both drugs are known to have a short prophylactic effect (about one month) in cattle, although this has not been documented in horses.

Up to now drug-resistant strains of *T. congolense*, *T. b. brucei* or *T. vivax* from horse origin have not yet been reported most probably because scientists did not look for them. However, there was some suspicion of resistance of *T. vivax* to diminazene in The Gambia (Dhollander *et al.* 2006). To delay the development of drug resistance the same principles should be applied as for other livestock species (see Geerts and Holmes 1998). As mentioned above, the regular use of quinapyramine should be avoided. If isometamidium is used several times a year in a strategic prophylactic scheme it should be alternated with diminazene (as a sanative pair).

A programme of integrated disease control combining tsetse control techniques and trypanocidal or trypanoprophylactic drugs usually yields greater benefits than a single method alone (Holmes 1997). Particularly in areas with heavy tsetse challenges it is important to cut the thick vegetation around the horse stables and to use insecticide treated targets or insecticide sprays or pour-ons (Van den Bossche and De Deken 2004). Although synthetic pyrethroids are highly effective against tsetse flies even at low concentrations, they may not prevent horses from some contact with the flies and thus possible infection. Sprays or pour-ons also reduce nuisance by other biting insects, which can be quite important in the tropics.

## Surra

Most commonly used drugs to treat *T. evansi* infections are diminazene aceturate, isometamidium chloride, quinapyramine sulphate and suramin (**Table 1**). Suramin is recommended since it has a low toxicity in equids (Luckins 1994). As an alternative drug melarsomine (cymelarsan) can be used. This drug was developed initially for the treatment of surra in camelids but evidence shows that at a dose of 0.25 mg/kg bwt it is not toxic for horses and effective in clearing *T. evansi* infections. Trypanocidal drug resistance also occurs in *T. evansi*. Chinese *T. evansi* strains resistant to isometamidium have been reported (Brun and Lun 1994).

Due to the large spectrum of transmitting insect vectors and wild animal reservoir species, control at that level is almost impossible. Therefore, the control of surra is mainly based on diagnosis and treatment of infected animals.

## Dourine

There is no officially approved drug to treat horses suffering from dourine, although some older publications mention experimental treatment of horses with suramin and neoarsphenamine

(Novarsenobenzol; Ciuca 1933) or quinapyramine sulphate (Vaysse and Zottner 1950). Nowadays, international regulations of the World Organization for Animal Health (OIE) impose the slaughtering of CFT positive horses. Nevertheless, Zhang *et al.* (1992) and Brun and Lun (1994) reported on the *in vitro* sensitivity of different *T. equiperdum* strains to treatments with suramin, diminazene aceturate, quinapyramine sulphate or melarsomine. However, *in vivo* treatment failure may occur as a result of cryptic infections. The effectiveness of new drugs such as cymelarsan for treatment of dourine has not yet been evaluated. Drug resistance of *T. equiperdum* strains in the laboratory has been observed (Zhang *et al.* 1993), but resistant field cases are rare (or not studied).

## References

Alemu, T., Luckins, A.G., Phipps, L.P., Reid, S.W.J. and Holmes, P. (1997) The use of enzyme linked immunosorbent assays to investigate the prevalence of *Trypanosoma equiperdum* in Ethiopian horses. *Vet. Parasitol.* **71**, 239-250.

Auty, H., Mundy, A., Fyumagma, R.D., Picozzi, K., Welburn, S. and Hoare, R. (2008) Health management of horses under high challenge from trypanosomes: A case study from Serengeti, Tanzania. *Vet. Parasitol.* **154**, 233-241.

Awan, M.A.Q. and Johnston, R.S. (1979) Some observations on an outbreak of trypanosomiasis in horses in the Republic of Zambia. *Bull. Anim. Health Afr.* **27**, 177-179.

Bajyana Songa, E. and Hamers, R. (1988) A card agglutination test (CATT) for veterinary use based on an early VAT RoTat 1/2 of *Trypanosoma evansi*. *Ann. Soc. Belge Méd. Trop.* **68**, 233-240.

Barrowman, P.R. (1976) Experimental intraspinal *Trypanosoma equiperdum* infection in a horse. *Onderstepoort J. vet. Res.* **43**, 201-202.

Biteau, N., Bringaud, F., Gibson, W., Truc, P. and Baltz, T. (2000) Characterization of Trypanozoon isolates using a repeated coding sequence and microsatellite markers. *Mol. Biochem. Parasitol.* **105**, 185-201.

Borst, P., Fase-Fowler, F. and Gibson, W.C. (1987) Kinetoplast DNA of *Trypanosoma evansi*. *Mol. Biochem. Parasitol.* **23**, 31-38.

Brun, R. and Lun, Z.-R. (1994) Drug sensitivity of Chinese *Trypanosoma evansi* and *Trypanosoma equiperdum* isolates. *Vet. Parasitol.* **52**, 37-46.

Brun, R., Hecker, H. and Lun, Z-R. (1998) *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). *Vet. Parasitol.* **79**, 95-107.

Ciuca, A. (1933) La dourine. *Bull. Off. Int. Epiz.* **7**, 168-172.

Claes, F., Agbo, E.E.C., Radwanska, M., Baltz., T., De Waal, D.T., Goddeeris, B.M. and Büscher, P. (2003) How does *Trypanosoma equiperdum* fit into the Trypanozoon group? A cluster analysis by random amplified polymorphic DNA (RAPD) and the multiplex-endonuclease genotyping approach. *Parasitol.* **126**, 425-431.

Claes, F., Radwanska, M., Urakawa, T., Verloo, D., Magnus, E., Majiwa, P.A.O., Goddeeris, B.M. and Büscher., P. (2004) Development of a PCR based on the *Trypanosoma evansi* RoTat 1.2 VSG DNA sequence. *Kinetoplastid Biol. Dis.* **3**, 3-15.

Clausen, P.H., Chuluun, S., Sodnomdarjaa, R., Greiner, M., Noeckler, K., Staak, C., Zessin, K.H. and Schein, E. (2003) A field study to estimate the prevalence of *Trypanosoma equiperdum* in Mongolian horses. *Vet. Parasitol.* **115**, 9-18.

Connor, R.J. and Van den Bossche, P. (2004) African animal trypanosomoses. In: *Infectious Diseases of Livestock*, Vol 1, 2nd edn., Eds. J.A.W. Coetzer, and R.C. Tustin, Oxford University Press, Cape Town. pp 251-296.

Davila, A.M. and Silva, R.A., (2000) Animal trypanosomiasis in South America. Current status, partnership, and information technology. *Ann. N.Y. Acad. Sci.* **916**, 199-212.

Dehoux, J.P., Diaw, M. and, Buldgen, A. (1996) Observation d'une flambée de trypanosomose équine due à *Trypanosoma vivax* en zone urbaine au Sénégal. *Tropicultura* 14, 35-36.

Desquesnes, M. (2004) *Livestock Trypanosomoses and their Vectors in Latin America.* World Organisation for Animal Health, Paris.

Dhollander, S., Jallow, A., Mbodge, K., Kora, S., Sanneh, M., Gaye, M., Bos, J., Leak, S., Berkvens, D. and Geerts S. (2006) Equine trypanosomosis in the Central River Division of the Gambia: A study of veterinary gate-clinic consultation records. *Prev. vet. Med.* **75**, 152-162.

Faye, D., Pereira de Almeida, P.J.L., Goossens, B., Osaer, B., Ndao, M., Berkvens, D., Speybroeck, N., Nieberding, F. and Geerts, S. (2001) Prevalence and incidence of trypanosomosis in horses and donkeys in the Gambia. *Vet. Parasitol.* **101**, 101-104.

Frasch, A.C.C., Hajduk, S.L., Hoeijmakers, J.H.J., Borst, P., Brunel, F. and Davidson, J. (1980) The kinetoplast DNA of *Trypanosoma equiperdum*. *Biochim. Biophys. Acta.* **607**, 397-410.

Geerts, S. and Holmes, P.H. (1998) *Drug Management and Parasite Resistance in Animal Trypanosomiasis in Africa*, Food and Agriculture Organization, Rome.

Geysen, D., Delespaux, V. and Geerts, S. (2003) PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in cattle. *Vet. Parasitol.* **110**, 171-180.

Gibson, W.C., Wilson, A.J. and Moloo, S.K., (1983) Characterization of *Trypanosoma* (Trypanozoon) *evansi* from camels in Kenya using isoenzyme electrophoresis. *Res. vet. Sci.* **34**, 114-118.

Greiner, M., Kumar, S. and Kyeswa, C. (1997) Evaluation and comparison of antibody ELISAs for serodiagnosis of bovine trypanosomosis. *Vet. Parasitol.* **73**, 197-205.

Hide, G., Cattand, P., LeRay, D., Barry, D.J. and Tait, A. (1990) The identification of *Trypanosoma brucei* subspecies using repetitive DNA sequences. *Mol. Biochem. Parasitol.* **39**, 213-226.

Hoare, C.A. (1956) Morphological and taxonomic studies on mammalian trypanosomes. VIII. Revision of *T. evansi*. *Parasitol.* **46**, 130-172.

Holland, W.G., Claes, F., My, L.N., Thanh, N.G., Tam, P.T., Verloo, D., Büscher, P., Goddeeris, B. and Vercruysse, J. (2001) A comparative evaluation of parasitological tests and a PCR for *Trypanosoma evansi* diagnosis in experimentally infected water buffaloes. *Vet. Parasitol.* **97**, 23-33.

Holmes, P.H. (1997) New approaches to the integrated control of trypanosomosis. *Vet. Parasitol.* 71, 121-135.

Holmes, P.H., Eisler, M.C. and Geerts, S. (2004) Current chemotherapy of animal trypanosomiasis. In: *The Trypanosomiasis*, 1st edn., Eds: I. Maudlin, P.H. Holmes and M.A. Miles, CABI Publishing, Wallingford. pp. 431-444.

Hopkins, J.S., Chitambo, H., Machila, N., Luckins, A.G., Rae, P.F., van den Bossche, P. and Eisler, M.C. (1998) Adaptation and validation of the antibody trapping ELISA using dried blood spots on filter paper, for epidemiological surveys of tsetse transmitted trypanosomosis in cattle. *Prev. vet. Med.* 37, 91-99.

Kinabo, L.D.B. and Bogan, J.A. (1988) Pharmacokinetic and histopathological investigation of isometamidium in cattle. *Res. vet. Sci.* 44, 267-269.

Laveran, A. and Mesnil, F. (1912) *Trypanosomes et Trypanosomiasis*, Masson et Co., Paris.

Luckins, A.G. (1994) Equine trypanosomiasis. *Equine. vet. Educ.* **6**, 259-262.

Luckins, A.G. and Mehlitz, D. (1978) Evaluation of an indirect fluorescent antibody test, enzyme-linked immunosorbent assay and quantification of immunoglobulins in the diagnosis of bovine trypanosomiasis. *Trop. An. Health Prod.* **10**, 149-159.

Luckins, A.G. (1977) Detection of antibodies in trypanosoma-infected cattle by means of a microplate enzyme-linked immunosorbant assay. *Trop. An. Health Prod.* **9**, 53-62.

Lumsden, W.H.R., Kimber, C.D., Evans, D.A. and Doig, S.J. (1979) *Trypanosoma brucei*: miniature anion-exchange centrifugation technique for detection of low parasitaemias: adaptation for field use. *Trans. R. Soc. Trop. Med. Hyg.* **73**, 312-317.

Lun, Z.R., Allingham, R., Brun, R. and Lanham, S.M. (1992) The isoenzyme characteristics of *Trypanosoma evansi* and *Trypanosoma equiperdum* isolated from domestic stocks in China. *Ann. Trop. Med. Parasitol.* **86**, 333-340.

Majiwa, P.A. and Otieno, L.H. (1990) Recombinant DNA probes reveal simultaneous infection of tsetse flies with different trypanosome species. *Mol. Biochem. Parasitol.* **40**, 245-253.

McCully, R.M. and Neitz, W.O. (1971) Clinicopathological study on experimental *Trypanosoma brucei* infections in horses. Part 2. Histopathological findings in the nervous system and other organs of treated and untreated horses reacting to nagana. *Onderstepoort J. vet. Res.* **38**, 141-176.

Morlais, I., Ravel, S., Grébaut, P., Dumas, V. and Cuny, G. (2001) New molecular marker for *Trypanosoma* (*Duttonella*) *vivax* identification. *Acta. Trop.* **80**, 207-213.

Moser, D.R., Cook, G.A., Ochs, D.E., Bailey, C.P., McKane, M.R. and Donelson, J.E. (1989) Detection of *Trypanosoma congolense* and *Trypanosoma brucei* subspecies by DNA amplification using the polymerase chain reaction. *Parasitol.* **99**, 57-66.

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

Neitz, W.O. and McCully, R.M. (1971) Clinicopathological study on experimental *Trypanosoma brucei* infections in horses. Part 1. Development of clinically recognizable nervous symptoms in nagana-infected horses treated with subcurative doses of antrypol and berenil. *Onderstepoort J. vet. Res.* **38**, 127-140.

Paris, J., Murray, M. and McOdimba, F. (1982) A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. *Acta. Trop.* **39**, 307-316.

Pereira de Almeida, P.J.L., Ndao, M., van Meirvenne and N., Geerts, S. (1998) Diagnostic evaluation of PCR on dried blood samples from goats experimentally infected with *Trypanosoma brucei brucei. Acta. Trop.* **70**, 269-276.

Pinchbeck, G.L., Morrison, L.J., Tait, A., Langford, J., Meehan, L., Jallow, S., Jallow, J., Jallow, A. and Christley, R.M. (2008) Trypanosomosis in The Gambia: prevalence in working horses and donkeys detected by whole genome amplification and PCR, and evidence for interactions between trypanosome species. *BMC vet. Res.* 4, 1-7.

Reid, S.A. (2002) *Trypanosoma evansi* control and containment in Australasia. *Trends Parasitol.* **18**, 219-224.

Richardson, U.F. and Kendall, S.B. (1957) *Veterinary Protozoology*, Oliver & Boyd, Edinburgh.

Riou, G.F. and Saucier, J.M. (1979) Characterization of the molecular components in kinetoplast-mitochondrial DNA of *Trypanosoma equiperdum. J. Cell. Biol.* **82**, 248-263.

Robinson, E.M. (1926) Serological investigation into some diseases of domesticated animals in South Africa caused by trypanosomes. *Repts Direct vet. Educ. Res., Dept. Agric., S. Afr.* **9**, 11-12.

Rogers, D.J. (1988) A general model for the African trypanosomiasis. *Parasitol.* **97**, 193-212.

Saurat, P. (1946) La dourine: son existence actuelle en France. *Rec. Méd. Vét.* **122**, 7, 289-309.

Singh, V., Chaudhari, S.S., Kumar, S. and Chhabra, M.B. (1995) Polyclonal antibody-based antigen-detection immunoassay for diagnosis of *Trypanosoma evansi* in buffaloes and horses. *Vet. Parasitol.* **56**, 261-267.

Stephen, L.E. (1986) *Trypanosomiasis. A Veterinary Perspective*, Pergamon Press, Oxford.

Stephen, L.E. and Mackenzie C.P. (1958) *Trypanosoma vivax* infection in a mare: treatment with ethidium bromide. *Vet. Rec.* **70**, 293-294.

Taylor, K. and Authié, E.M.L. (2004) Pathogenesis of animal trypanosomiasis. In: *The Trypanosomiasis*, 1st edn., Eds. I. Maudlin, P.H. Holmes and M.A. Miles, CABI Publishing, Wallingford. pp 331-353.

Uilenberg, G.A. (1998) *Field Guide for the Diagnosis, Treatment and Prevention of African Animal Trypanosomosis.* Food and Agriculture Organization, Rome.

Van den Bossche, P. and De Deken, R. (2004) The application of bait technology to control tsetse. In: *The Trypanosomiasis*, 1st edn., Eds: I. Maudlin, P.H. Holmes and M.A. Miles, CABI Publishing, Wallingford. pp 525-532.

Vaysse, J. and Zottner, G. (1950) Contribution à l'étude de la chimiothérapie et de la chimioprévention de la dourine par l'antracyde. *Bull. Off. Int. Epiz.* **34**, 172-179.

Verloo, D., Magnus, E. and Büscher, P. (2001) General expression of RoTat 1.2 variable antigen type in *Trypanosoma evansi* isolates from different origin. *Vet. Parasitol.* **97**, 183-189.

Watson, E.A. (1915) Dourine and the complement fixation test. *Parasitol.* 8, 156-183.

Woo, P.T.K. (1970) The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta. Trop.* **27**, 384-386.

Wuyts, N., Chokesajjawatee, N. and Panyim, S. (1994) A simplified and highly sensitivity detection of *Trypanosoma evansi* by DNA amplification. *Southeast Asian J. Trop. Med. Public Health* **25**, 266-271.

Zhang, Z.Q. and Baltz, T. (1994) Identification of *Trypanosoma evansi, Trypanosoma equiperdum* and *Trypanosoma brucei brucei* using repetitive DNA probes. *Vet. Parasitol.* **53**, 197-208.

Zhang, Z.Q., Giroud, C. and Baltz, T. (1993) *Trypanosoma evansi: in vivo* and *in vitro* determination of trypanocide resistance profiles. *Exp. Parasitol.* 77, 387-94.

Zhang, Z.Q., Giroud, C. and Baltz, T. (1992) *In vivo* and *in vitro* sensitivity of *Trypanosoma evansi* and *Trypanosoma equiperdum* to diminazene, suramin, MelCy, quinapyramine and isometamidium. *Acta Trop.* **50**, 101-110.