Livestock Trypanosomoses and their Vectors in Latin America

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Foreword

It seems to me important in this preface to emphasise the various foci of interest that are to be found in this document and thereby draw the attention of readers from a variety of backgrounds.

This review of the literature on livestock trypanosomoses in Latin America is the first on this subject and will be useful to students, teachers and researchers who deal with this area. Together with the additional data derived from epidemiological surveys and questionnaires submitted to Veterinary Services, it provides an overview of the situation of trypanosomoses in Latin America in 1990-1995 that will serve as a benchmark for future studies and comparisons.

The overview of the diagnostic tools and control methods for trypanosomoses is very general and can be applied to America, Africa and Asia.

The study's appraisal of mechanical vectors and their harmful effects has highlighted and put figures on the harm and direct impact caused by Tabanids, which alone are sufficient justification for undertaking seasonal strategic control measures against these insects whose effect on livestock, while often localised and short-lived, has not thus far been given proper consideration.

In addition to their didactic value, the experimental studies can serve as examples. Some of these, as for instance the reassessment of antigen-ELISAs, underscore the need for research to remain independent, objective and impartial at all costs. It required a great deal of perseverance to convince the Establishment to question previously accepted ideas. Others, such as the epidemiological surveillance project in French Guiana, show that to study mechanically transmitted trypanosomosis, observations need to be conducted over several years to ensure accurate diagnosis. This is a new notion that contrasts with what prevails in Africa where *Glossina*-transmitted trypanosomoses are mainly governed by seasonal variations. The typical epidemiological mode for mechanically transmitted bovine trypanosomosis, which is analysed and described, can be used as a model not only for Latin America, but also Africa where mechanical transmission of trypanosomosis should not be disregarded. Such a detailed description of this particular epidemiological mode would not have been possible in Africa due to interference from cyclically transmitted trypanosomoses. This study will therefore provide African veterinarians with a new vision of trypanosomosis that is relevant to the sectors that lie at the boundaries of the *Glossina* distribution area. As *Glossina* populations recede, in particular thanks to PATTEC (Pan African Tsetse and Trypanosomosis Eradication Campaign), a new epidemiological mode based on purely mechanical transmission may become established in areas where *Glossina* have been eradicated requiring appropriate means of investigation and control.

This preface is also an opportunity for me to recall that mechanical transmission of trypanosomes curiously aroused some heated discussion when it was mentioned in Africa (in particular, on the PAAT forum in 1999) as if *Glossina* transmission, a long-standing institution, had been attacked by the revolutionary advocates of mechanical transmission!

While I find this image attractive and much to my liking, I must reassure those who may be anxious – this is not the case. Cyclical and mechanical transmission co-exists in highly variable proportions depending on the geographic area under consideration. Within the distribution area of *Glossina*, their role as main vector of bovine Trypanosomosis is not questioned. However, in these areas, and more importantly outside, the impact of mechanical transmission needs to be investigated.

Recent work conducted in Burkina Faso on a local strain has shown that *Trypanosoma vivax* can be transmitted with a very high incidence (60%-80% in three weeks) when there is contact between highly parasitæmic infected cattle, tabanids and unchallenged cattle. There is therefore evidence of mechanical transmission of *T. vivax* in Africa and this must be given proper attention. Just as the

African experience was able to benefit the New World when it became infected by trypanosomes, so the American experience should benefit Africa when *Glossina* will have regressed.

The data on mechanically transmitted trypanosomes can serve as a model on several continents, but also for all mechanically transmitted pathogens and as such be of interest to a broad range of readers.

Finally, physicians and epidemiologists that deal with Chagas' disease will find the scanty veterinary information that exist on the topic in this publication. Although ill-defined, it highlights the crucial role played by wild and domestic animals in the epidemiology of this major human disease that appears to be progressing northwards since it has already been notified in 8 southern US states.

Dr Bernard Vallat
Director General, OIE

In memoriam Pierre Morel, Hugues Raymond and Alain Provost, who so admirably shared their keen acarological, entomological and veterinary interests with others and significantly contributed to establishing my inclination and ability to embark into the area of research.

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Once the work has been done, I must add special thanks to the translator, and to the staff of the OIE Publications Department, for coordination of translation and for the editorial work of this English version that was made promptly and remarkably.

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Finally, I must add my grateful thanks to CIRAD and to OIE (World Organisation for Animal Health) for the technical and financial support of the present English edition of my manuscript.

Ma dédicace finale est pour Cécile Dumortier et Jean Desquesnes, mes parents, ainsi que ma sœur Chantal et mon frère Jean-Bernard, qui ont toujours été présents à mon esprit, malgré la distance imposée par ces exercices tropicaux !

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Livestock Trypanosomoses and their Vectors in Latin America

by Marc Desquesnes

Whenever livestock trypanosomoses are mentioned, Africa – their cradle – immediately comes to mind. Nevertheless, some of these diseases also occur on the American continent where they were mostly introduced by man along with stock animals (cattle, sheep, goats and horses) that were not native to the continent. The epidemiology of these diseases is far less documented in America than in Africa, and the goal of this publication is to provide a review of the literature supplemented by surveys and investigations conducted by the author and fellow researchers.

The review of the literature covers the history of the introduction and spread of trypanosomes in Latin America, their domestic and wild hosts and the sometimes new modes of transmission they have adopted on this continent. The vectors for mechanical transmission of *Trypanosoma vivax* in cattle and sheep and *T. evansi* in horses are studied in detail, particularly Tabanids whose direct impact alone provides grounds for seasonal control programs in a number of highly infested areas. The control of trypanosomoses relies almost exclusively on the use of two trypanocides to which many parasite strains are already resistant.

The investigations and epidemiological surveillance carried out for six years in French Guiana provided evidence that mechanically transmitted bovine trypanosomosis occurs against a subclinical enzootic background, in the form of periodic epizootic outbreaks (every three to five years) that are seasonal and most often multifocal which originate in trade of infected livestock or various resurgences deriving from seasonal factors that are highly adverse for the livestock. Most observations relating to mechanical transmission can be used as a model not only for trypanosomes in Africa, when *Glossina* control will have altered the epidemiological setting for trypanosomoses by abating cyclical transmission, but also for other mechanically transmitted pathogens (parasites, viruses and bacteria).

What is the outlook for livestock trypanosomoses in Latin America?

Trypanosoma vivax mainly affects cattle and sheep from Paraguay to Central America and is continuing its geographical progression across the continent giving rise to deadly epizootic waves in newly infected areas and occurring in the form of periodic epizootic outbreaks in previously infected areas.

Trypanosoma evansi, which is found from Argentina to Panama, has a huge wild and domestic reservoir as well as a vector-host-reservoir (vampire bats), mainly affects horses and dogs; it continues to spread within the subcontinent in the form of epizootic waves followed by enzootics with predominantly clinical expressions.

Trypanosoma equiperdum remains a mystery; very strict international regulations deter notification of the disease. Consequently, there is very little information available and it is not reliable.

Finally, *T. cruzi* is found in domestic and wild animals in Latin America with apparently very little clinical or economic impact. In contrast, its progression northwards has brought it in to the southern United States where epidemiological cycles have become established essentially in the fauna but also in dogs and, in a few cases, in humans (Chagas' disease). This would present a serious threat if the parasite were to encounter a vicarious vector capable of establishing a new link between the reservoir and humans.

CONTENTS

Introduction

Trypanosomoses are a set of human and animal diseases that are mainly found in intertropical Africa, South America and Asia.

In Africa, there are many trypanosomes that are pathogenic for mammals, including man, some of which cause sleeping sickness. The annual incidence of this disease is 250,000 to 500,000 cases, and it is thought to be responsible for more then 45,000 human deaths per year. Human and animal trypanosomoses are transmitted by various hæmatophagous biting insects, mainly *Glossina*, or tsetse flies, which are the intermediate hosts and the vectors of cyclical transmission. *Glossina* are present only in Africa (including Zanzibar); they have however occasionally been reported in the Persian Gulf: in 1903, close to Aden (a few *Glossina tachinoides* specimens) and, in 1984, close to Djizan, Saudi Arabia (some 20 *G. morsitans* and *G. f. fuscipes*) (J. Itard, unpublished paper). In inter-tropical Africa, animal trypanosomoses are one of the major obstacles to the development of livestock farming over more than 10 million km^2 where 50 million head of cattle are exposed to infection [1]. As a result, human and animal trypanosomoses are a major concern for the development and health organisations on this continent.

In Latin America, Chagas' disease, is also a major human disease that particularly affects poor populations living in dilapidated dwellings; of the 90 million people exposed to the risk, 20 million are estimated to be infected, the mortality rate being approximately 10% [2, 3, 4]. *T rangeli*, a non-pathogenic trypanosome belonging to the *Tejeraia* subgenus, is present in the same areas and on the same vectors, which can interfere with the diagnosis of Chagas' disease.

Although human and animal trypanosomoses in Africa, and Chagas' disease in America, have been known and subject to control measures since they were discovered just over a hundred years ago, they are still present and significant. Furthermore, trypanosomoses are not limited to these long-standing entities, since some trypanosomes have been able to become emancipated from their cyclical vectors and find other modes of transmission, in particular by mechanical or venereal means. This has enabled them to expand their geographical distribution and, as a result, cause emerging diseases. A number of trypanosomoses have been identified in Europe (*Trypanosoma equiperdum*), in Asia (*Trypanosoma evansi*) and on the American continent. In Asia, *T. evansi*-induced trypanosomosis mostly affects horses, cattle, and to an even larger extent buffaloes [5]. Its growing impact and geographical distribution is attracting increasing attention.

In Latin America, four species of trypanosomes are of medical and economic importance, or can interfere with the diagnosis of livestock hæmoparasitoses[1]: *Trypanosoma equiperdum*, *Trypanosoma vivax*, *Trypanosoma evansi*, and *Trypanosoma cruzi*. Only *T. cruzi*, in the Stercoraria group, is indigenous to America while man imported the other three salivarian species there, together with their main domestic hosts.

Trypanosoma cruzi, the agent of the Chagas' disease, is pathogenic for man and dogs; it is transmitted cyclically in the excreta of Triatomines and enters the body through bite wounds or the mucosal membranes of the eye or mouth; many domestic mammals, and more than 150 wild animal species act as its reservoirs. It is moderately or non-pathogenic for livestock, but can interfere with the diagnosis of animal trypanosomoses.

Trypanosoma equiperdum, which is responsible for dourine or '*mal de coït*', a chronic disease in horses, is transmitted by the venereal route; it has been sporadic in America, from Chile to Canada. This is

1 In this document, the term 'livestock' will be used in the broad sense, to include all farm animals together with Equidae that, in Latin America, are used mainly as working animals for herding cattle.

not a typically South American parasite – it originates from Africa, but is also found in Asia and Eastern Europe.

Trypanosoma vivax is mainly a parasite of ruminants (cattle, sheep, goats and buffaloes) causing anaemia and significant losses in production; *T. vivax* originates from Africa where it is principally transmitted by *Glossina*, while in the Latin America, it is mechanically transmitted by hæmatophageous biting insects. The disease is found in many countries, most often in the epizootic form. Depending on the country, the disease is called 'Secadera', 'Huequera', or 'Cacho Hueco'.

Trypanosoma evansi is the most widespread of livestock pathogenic trypanosomes in the tropical and sub-tropical areas (Asia, Africa, Latin America); it is responsible for 'Surra', in African camels in Asian buffaloes and in America it causes 'Mal de Caderas' in Brazil, 'Murrina' in Central America, and 'Derrengadera' in Venezuela. This parasite is especially pathogenic for Camelidae and Equidae causing enzootic disease, or deadly epizootics; it is mechanically transmitted by biting insects or bats (vampire), and has numerous wild and domestic animal reservoirs. It can also be transmitted by the peroral route to carnivores, which are often highly sensitive to infection.

Latin America, which includes Mexico, Central America, South America and several Caribbean islands (**Fig. 1**), is home to approximately a quarter of the world bovine population, i.e. 280 million head most of which are exposed to *T. vivax*, *T. evansi* and *T. cruzi* infections, while the horses, that are mainly used for herding cattle, are exposed to *T. evansi*, *T. equiperdum* and *T. cruzi*.

Figure 1 – Map of Latin America

While African trypanosomoses in animals are well known because they have been studied and subject to control measures for a long time in many countries, the same does not apply to American trypanosomoses. The epidemiology of these pathologies – which is recent as a result of vectors and sometimes hosts different to those in Africa –, their distribution – which is continuously developing (emerging diseases), and their impact – which is periodic rather than constant as in Africa – are still poorly understood on the American Continent. As such, they deserve to be thoroughly explored, particularly in view of their recent spread through huge livestock farming areas (Pantanal, Brazil), and introduction into countries that had so far been uninfected (Bolivia). The purpose of this study is to provide a review of the literature and update information on livestock trypanosomoses in Latin America. Although *T. equiperdum* is sporadic and the medical and economic importance of *T. cruzi* for livestock is apparently minor, *T. vivax* for domestic ruminants and *T. evansi* for horses are considered to be a major scourges for stock-farming on the sub-continent; this study will therefore focus mainly on those two species. In addition to the data in the literature, we shall also refer to the results of studies and experimentation we conducted in the three Guyanas.

Prehistoric development

Trypanosomoses in vertebrates are parasitic diseases caused by species of the *Trypanosoma* genus (Trypanosomatidae family). Some of the genera in Trypanosomatidae family found in plants (Phytomonas) or in insects (*Crithidia*, *Blastocrithidia*), and those found in vertebrates (*Trypanosoma* and *Leishmania*) are thought to have a common phylogenetic origin that goes back very far. According to the hypotheses initially formulated by LEGER (1904) [6], and generally accepted [7], the evolution of non-biting insects towards biting insects, and then from phytophagous to hæmatophagous insects, is thought to be concomitant with the evolution of intestinal Flagellata of insects into bloodstream Flagellata of vertebrates. This evolution is thought to be at the root of major changes in the biology of insect Trypanosomatidae. Recent studies conducted on the ribosomal RNA of Trypanosomatidae do not however confirm the hypothesis of co-evolution, but instead point to secondary adaptation of trypanosomes to their hosts and vectors [8].

Among the insects able to transmit the trypanosomes of vertebrates, the earliest Tabanidae and Culicidae fossils date back to the Triassic (first epoch of the Secondary period, 200 million years BC). The earliest fossil traces of *Glossina* spp. were found in Colorado, United States of America (USA), and date back to the Miocene (third epoch of the Tertiary period, from 25 to 5 million years BC); these have been described by COCKERELL [9] under the names *Glossina oligocenus* and *Glossina osborni*. However, it is thought that the Proto-Glossinidae appeared in the Triassic at the same time as most biting insects [10].

According to LAMBRECHT [10], the most likely explanation for the existence of *Glossina* fossils in North America, their absence in South America, and the current presence of these insects in Africa, is as follows: during the Triassic, the Proto-Glossidinae were present in North America and Africa, when a strip of land formed a bridge between the southern tip of North America and the northern tip of Africa, the only remnant of which we see today is the Floridian peninsula; in the Jurassic (180 million years BC), when the continents separated, *Glossina* continued to evolve separately in North America and Africa. Later on, during the glaciation, in the Cretaceous (from 170 to 140 million years BC), they were destroyed by the cold that advancing down the continent from north to south, but were unable to invade South America because at the time Central America was submerged. The American *Glossina* therefore became extinct and today only the African *Glossina* survive.

Other authors believe the connection between North America and Africa was Europe; this implies that to the European Continent was also home to Proto-Glossidinae, which were to disappear in much the same way as the American *Glossina* [11].

Needless to say, there are no fossil traces of Trypanosomatidae and one can only speculate as to their history. According to HOARE [7], among the Stercoraria, like *T. cruzi*, adaptation of the parasite to its vectors, biting louse in the sub-family Triatominae (Reduviidae), goes back a long way as it is very robust with nearly 100% of vectors capable of being infected. Most likely, stercorarian trypanosomes (dixenic) evolved from *Blastocrithidia* (monoxenic) when their phytophagous insect hosts became hæmatophagous. In contrast, the salivarian group is thought to be the result of a much later evolution from trypanosomes that developed cyclically in the hindgut of their vector insects, but could also be mechanically transmitted, in particular by *Glossina*. A number of hypotheses based on kinetoplast RNA analysis date the differentiation between *T. brucei* and *T. cruzi* back 100 million years [12].

In the case of salivarian trypanosomes, adaptation by *Glossina* to cyclical transmission is thought to be recent and sometimes vulnerable; its vulnerability being proportional to the complexity of the modifications in the life cycle. Hence, the infectivity rates of *Glossina* are 20% for *T. vivax*, the first evolutionary stage with development in the proboscis, 10% for *T. congolense*, the second evolutionary stage with development in the gut followed by migration to the mouth parts, and only 1% for the

very complex *T. brucei* (1 to 2/1,000 for *T. b. Gambiense* according to FREZIL [13]), a more complex evolutionary stage, with the metacyclic infectious form found in the salivary glands, after passing through the gut. This hypothesis is further reinforced by the lability of cyclical transmission by *Glossina*; indeed, a number of trypanosome strains that are cultured in laboratories for a long time, or in the absence of cyclical transmission, lose their capacity to infect *Glossina* [14]

It is not known whether *Salivaria* already existed in North America during the Miocene and later disappeared together with *Glossina*. If so, for unknown reasons they presumably were unable to return to the mechanical transmission mode and/or were unable to find sufficiently sensitive and abundant hosts. It does however appear more likely that the *Salivaria* differentiated only in Africa, well after the continents separated (Cretaceous); it is indeed in Africa that they have the widest range of hosts and of vectors, and thus were able to become firmly established. LAMBRECHT [10] dates this event to the middle of the Tertiary period (Oligocene), but their salivarian specialisation may have occurred at a later stage.

South America naturally harbours only Stercoraria, i.e. primitive parasites or ones directly descended from primitive forms, most of the species of which are similar to the trypanosomes of batrachians, reptiles and birds [8]. In mammals, the subgenera *Megatrypanum* and *Herpetosoma* include respectively some 30 and 50 non-pathogenic species, while the subgenus *Schizotrypanum* comprises only three parasite species of New World mammals, five parasite species of bats some of which are cosmopolitan, and *T. cruzi*, a parasite for humans and many mammals [7]. The presence of *Salivaria* in South America is therefore due to their introduction by man who brought them in infected domestic animals.

Origin of livestock trypanosomes in Latin America

Horses and cattle were brought into Latin America by man as of the 16th century, often from African countries, particularly regions where trypanosomoses due to *T. evansi*, *T. equiperdum*, and *T. vivax* are enzootic [7]. *Trypanosoma brucei* and *T. congolense* may also have been introduced into Latin America, but they have never been reported in the New World and appear to have been unable to maintain themselves probably due to the absence of *Glossina*.

Because its mode of transmission is venereal, *T. equiperdum* spread very early on and broadly in South Africa, Europe (17th century) and Asia; the horse trade is thought to have introduced it into America from North Africa. *Trypanosoma equiperdum* was eradicated from North America around 1950 [7]. The parasite has been found in Brazil, Venezuela and Chile (PINTO,1933, according to HOARE [7]), but has more recently been reported only in Bolivia and Paraguay [15].

According to HOARE [16], *T. evansi* was introduced into Colombia in the 16th century along with the Arabian horses that belonged to the cavalry of the Spanish conquistadores. HOARE also mentions its introduction into Brazil in the 19th century [7], specifying that the trypanosome was discovered on the island of Marajo (Amazon estuary) in 1827, then in Paraguay in the 1847, again in Brazil in 1850 in the Pantanal region, and in 1860 in the Mato Grosso, before spreading throughout Brazil and on into Bolivia, Guyana, Venezuela and Colombia. The geographic extension and establishment of the parasite have depended both on the movement of horses and the presence of other hosts, both wild and domestic (ruminants and carnivores). Epizootics due to *T. evansi* are described periodically from Argentina to Panama [17].

In 1919, LEGER and VIENNE [18] for the first time described a cattle trypanosomosis epizootic in French Guiana close to Cayenne. Based on their observations concerning the morphology, morphometry, pathogenicity and the life cycle of the parasite and because they were unable to equate it with any other, they suggested calling this trypanosome *Trypanosoma guyanense*. Later on, because this name had already been given to another trypanosome, it was renamed *T. viennei*, and then identified as *T. vivax*. According to CURASSON [19], the parasite was introduced around 1830, when zebus (*Bos indicus*) were introduced from Senegal into Guyana and the French West

Indies. But we now know that these imports had already occurred at the beginning of the 18th century, starting in 1733, directly from Africa (Senegal, coasts of Guinea) or the Cape Verde Islands and, indirectly, via the Caribbean islands then under Spanish or Portuguese rule [20]; *T. vivax* may therefore have been introduced into America more than two and a half centuries ago.

In 1926, bovine trypanosomoses are reported by FABRE and BERNARD [21] in Guadeloupe and, in 1929 by CAROUGEAU [22] in Martinique, which were attributed to *T. vivax* by MESNIL. *Trypanosoma vivax* was unable to become established in either Guadeloupe or Martinique; after several epizootics, the parasite was identified for the last time in Guadeloupe in 1939, by ROUBAUD, and in Martinique in 1943 (according to the Archives of the Institut Pasteur of Martinique, 1943). Presumably, the nature and/or scarcity of *T. vivax* vectors on these islands prevented its long-term establishment. A recent serological survey has confirmed that the parasite has disappeared from Martinique [23].

On the other hand, *T. vivax* rapidly became permanently established all over South America. Reports of the parasite cannot be used to establish a chronology because observations from one country to another are highly disparate. Nonetheless:

– in 1920, TEJERA described *T. vivax* in Venezuela [24];

– in 1931, PLATA [25, 26] reports an epizootic due to *T. vivax* on the Atlantic coast of Colombia, which was traced back to the importation of livestock from Venezuela;

– in 1939, NIESCHULZ, quoted by HOARE [7], records the presence of *T. vivax* in Surinam and Guiana;

– Johnson reports it in Panama in 1941 [27];

– in 1944, FLOCH and LAJUDIE discover that the parasite is also present in Northeastern Brazil, in the state of Para and on the island of Marajo [28]. In 1972, it is found again in the vicinity of Belem, on buffaloes [29];

– later on, surveys conducted by WELLS *et al.* [30-32] showed up its presence in Peru, Ecuador, Paraguay, Costa Rica and Salvador. According to these authors, *T. vivax* is found in nearly all the countries of Latin America, from Paraguay to Salvador, from 12° north latitude to the Tropic of Capricorn (23° south) [31].

Importance

The pathogenicity of *T. vivax* described in 1919 by LEGER and VIENNE [18] was very high (50% mortality). As far back as 50 years ago, KUBES [33] suggested that bovine trypanosomosis in America had considerable economic impact. VIRVIESCAS [34] estimated that 12,000 head of cattle died of trypanosomosis in 1931 and 1932 in Venezuela. However, objective data are not always sufficient to establish the actual economic and health impact of this disease.

The pathogenicity of *T. evansi* found in South America is very high in horses, particularly in Venezuela and Argentina where it is enzootic, and in Brazil where horses are regularly imported to replace the stock that is periodically decimated by the disease. Less is known about its importance in buffaloes, and very little in cattle, sheep and goats.

As emphasised by CLARKSON [15], WELLS [17] and VOKATY [35], the information available concerning trypanosomoses in Latin America is fragmentary. For these countries, it is of priority concern to determine the comparative economic impact of trypanosomoses between themselves and in relation to other hæmoparasitoses. Concomitant anaplasmosis and babesiosis can hinder the clinical diagnosis of livestock trypanosomosis. Furthermore, serological diagnosis of trypanosomosis is not species-specific and treatments can simultaneously act on several blood parasites. Hence, the prevalence and relative incidence of each particular parasite is difficult to assess in the field. The need to take stock of current knowledge about livestock trypanosomoses in Latin America and provide additional insight forms the basis for the present work.

CHAPTER 1: TRYPANOSOMES

Trypanosomes are flagellate protozoans that inhabit the blood plasma, the lymph and various tissues of their hosts. These are obligate parasites that multiply in their definitive hosts and sometimes in an intermediate host. They are mainly transmitted by hæmatophagous vectors: mammals (vampire-bats) or arthropods, sometimes ticks, but most often by biting insects that transmit cyclically (*Glossina*, reduviid bugs, horseflies) or mechanically (horseflies, *Stomoxys*, etc.).

The genus *Trypanosoma* belongs to the protozoan branch, order Kinetoplastida, Trypanosomatidae family. The trypanosomes that are pathogenic for livestock present in Latin America belong to the *Salivaria* section, sub-genus *Duttonella* for *T. vivax*, and *Trypanozoon* for *T. evansi* and *T. equiperdum*. Reference is also made to trypanosomes that are moderately or non-pathogenic for livestock and which can interfere with the diagnosis of livestock trypanosomoses in Latin American livestock: *T. cruzi* and a number of *Megatrypanum* spp., which belong to the Stercoraria section (**Fig. 2**).

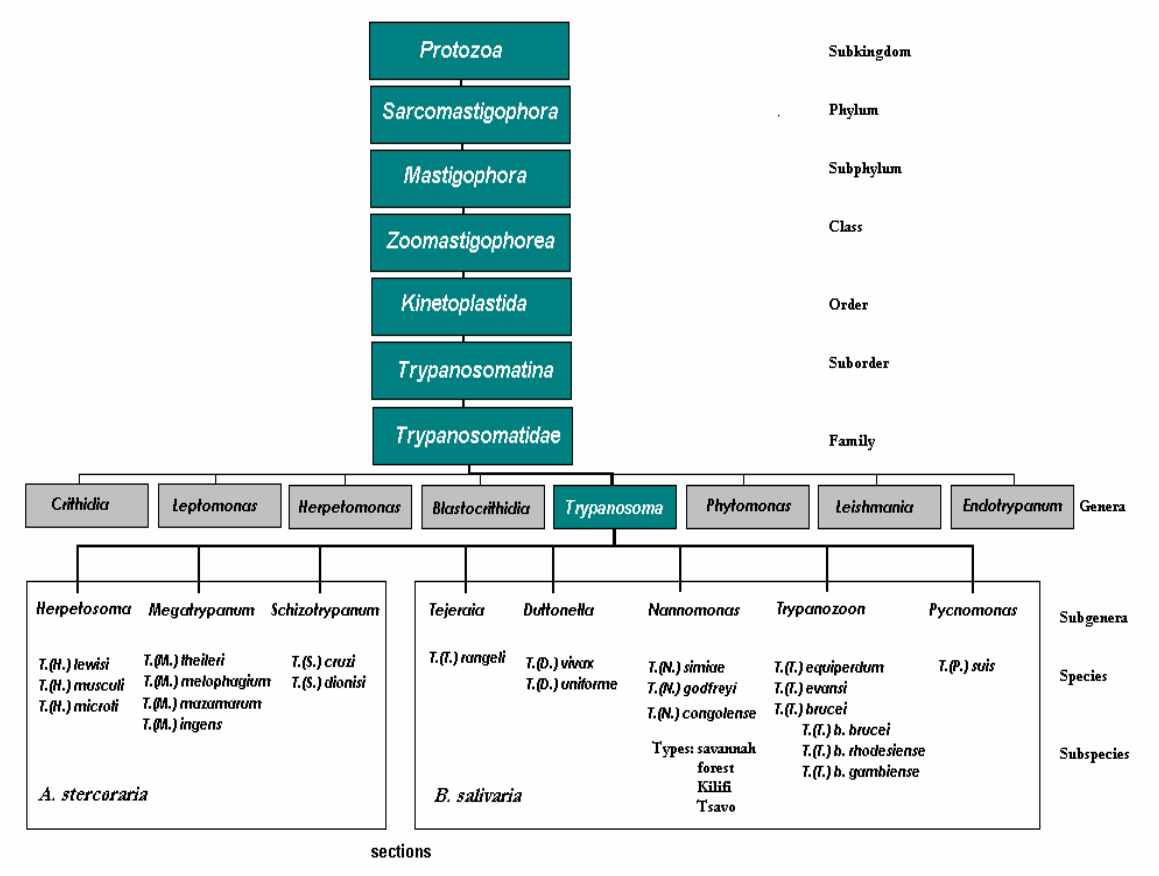


Figure 2 – Classification of the major mammalian trypanosomes (according to WHO [3])

Some extravascular forms, whose morphology sometimes differs from the bloodstream forms, have been described occasionally for *T. vivax* [36], regularly for *T. evansi* and in most cases for *T. equiperdum* and *T. cruzi*. The latter is even found in amastigote intracellular forms. In this paper, the morphology of parasites is illustrated solely for the purposes of routine diagnosis (by blood sampling) and hence only the bloodstream forms are described. The nomenclature used in this section is the one adopted by HOARE [7] but abbreviated species names are used herein as specified below.

1. NOMENCLATURE AND MORPHOLOGY

1.1. Trypanosomes that are pathogenic for livestock

1.1.1. *Trypanosoma* (*Duttonella*) *vivax viennei* LAVIER, 1921

Trypanosoma vivax was described for the first time in Africa by ZIEMANN in 1905. In 1919, LEGER and VIENNE discovered a trypanosome that is pathogenic for cattle in French Guiana [18] causing clinical signs that evoke what is known in Africa as 'Nagana'. Nonetheless, they suggested that the Guyanese parasite be named *Trypanosoma guyanense*. After some hesitation concerning the nomenclature [37, 38], in particular because this name had already been attributed to a bird trypanosome by MESNIL [39], ROUBAUD and PROVOST [40] decided to use the name *T. vivax* for this parasite because of its similar morphology (shape and average size: 22.5 μm) and biological features (pathogenic only for Bovidae).

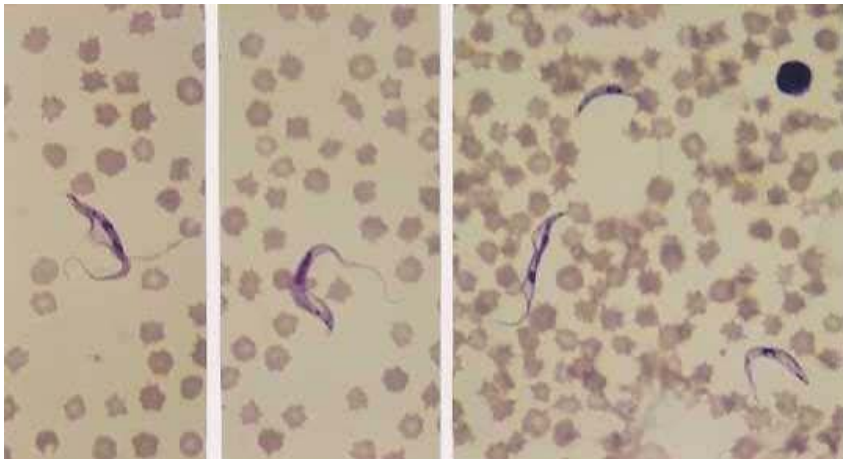
In 1938, ROUBAUD *et al.* demonstrated that this trypanosome had lost its ability to develop in *Glossina* (*Glossina palpalis*), which implies that the parasite had evolved in the absence of its African vector [40]. Later on, HOARE [41] suggested the name *T. vivax vivax* for the African trypanosome and *T. vivax viennei* for the American trypanosome, the latter differing from its African relative by the absence of intermediate hosts and the forms specific to those stages of development.

The WHO[2] International Committee [42] quoted by WELLS [17] had also suggested the name *Trypanosoma vivax viennei* (*T. v. viennei*) for the mechanically transmitted American parasite, and *T. v. vivax* for the African parasite that transmits cyclically. However, most authors agree to simply call both *T. vivax*; we will conform to that practise too.

Description: in the bloodstream form, *T. vivax* is a medium size trypanosome (total length: 18-31 μm; average: 22.5 μm; width 1.5-3 μm); compared to the African strains, the South American *T. vivax* is slightly smaller (16-26.5 μm). It has a free flagellum (7 μm), an undulating membrane that is generally stunted, a large kinetoplast (1 μm) often terminally positioned; the posterior region is rounded. The kinetoplast index (KI[3]) and the nuclear Index (NI[4]) are equal and slightly greater than one. In Africa, in its main hosts, *T. vivax* goes through a polymorphic cycle of development whereby the slender forms that are present as the parasitæmia intensifies give way, once they are recognised by the host's antibodies, to stumpy forms that present new surface antigens, etc. In America, *T. vivax* is mechanically transmitted and is no longer polymorphic, and only the slender forms of the parasite are found. However, its morphology is relatively variable. Typical and atypical forms of the parasite are shown in **Figures 3 and 4.**

Examination of a fresh sample shows that *T. vivax* has very lively movements, particularly the flagellum that spirals rapidly enabling the parasite to 'screw' into the medium and cross the microscope fields in a very characteristic manner. The morphometry of the *T. vivax* recently isolated in America is fairly consistent with the data in the literature – the average length of parasites found in French Guiana and Venezuela is respectively 20.3 μm and 21.5 μm [43]; the Brazilian parasite is smaller (18.7 μm), but nonetheless remains within the range established for *T. vivax* [44]. No differences in the morphology of *T. vivax* strains were observed between French Guiana and Venezuela.

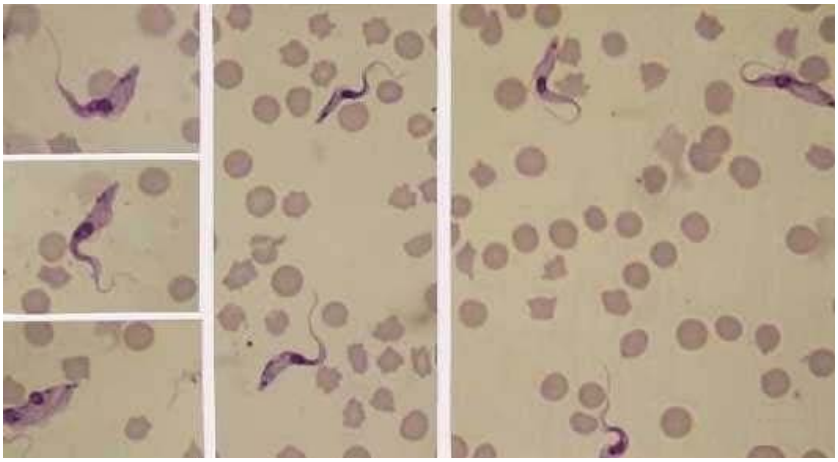
2 World Health Organization
3 KI = distance from the rear tip of the body to the centre of the nucleus/distance from the centre of the kinetoplast to the centre of the nucleus
4 NI = distance from the rear tip of the body to the centre of the nucleus/distance from the centre of the nucleus to the front end of the body



Caption: From left to right
(1) dividing forms with 2 nuclei, 2 flagellae and clearly visible undulating membrane
(2) form with 2 clearly visible kinetoplasts
(3) a form dividing by longitudinal stretching (left) and 2 adults

Figure 3 – *Trypanosoma vivax* from French Guiana: slender adult forms during division (M. DESQUESNES)

The parasites observed on the field samples collected in French Guiana were nearly always slender bloodstream forms (**Fig. 3**). However, occasionally in the field and generally during the first parasitæmic peak for experimental inoculations, the trypanosomes are polymorphic (**Fig. 4**). This polymorphism is quite unrelated to the polymorphism described in Africa and a more appropriate term would probably be 'pleomorphism' as the variations observed do not seem to abide by any rule. Indeed a single preparation may contain slender, medium and broad (1 μm to 4 μm) forms, mostly long (20-26 μm), but sometimes short (15-16 μm), whose posterior tip is either rounded or tapering, while the distance from the kinetoplast to the rear tip is itself variable and unrelated to the shape of the rear portion. Only the size of the kinetoplast appears to be stable in these observations. This pleomorphic appearance of the parasite is short lived, and rapidly gives way to a monomorphic parasite as soon as the second parasitæmic peak is reached. Similar observations have been reported by GARDINER and WILSON [45]; these non-specific forms are probably the result of changes in the parasite arising from very rapid replication, preservation in nitrogen and/or the host's immune response.



Caption: From left to right
(1) from top to bottom: division of kinetoplast, flagellum and nucleus with clearly visible undulating membrane
(2) stumpy (bottom)and slender (top) adult forms, faintly visible undulating membrane
(3) common adult form with medium developed membrane (top left) and intermediate form with anterior nucleus (top right)

Figure 4 – *Trypanosoma vivax* from French Guiana adult slender and stumpy forms in the process of division (M. DESQUESNES)

1.1.2. *Trypanosoma* (*Duttonella*) *uniforme* BRUCE *et al.*, 1919

Floch [39, 46] mentions the possibility of there being two trypanosomes in the '*vivax*' group and describes a small parasite (16.7 μm) with little motility, which he identifies as *T. vivax uniforme* (the same as the parasite described by TEJERA in Venezuela) and another larger one (22.5 μm) that is

highly motile, which he identifies as *T. vivax vivax*. In Colombia however, BETANCOURT [47] considers the size of New World parasites to be intermediate between that of *T. v. vivax* and *T.v. uniforme*. Recently, DAVILA *et al.* [44] described a Bolivian parasite with the same size characteristics as *T. uniforme*. Characterising and more specifically defining these two species will require additional research, especially in view of the scanty information on the latter.

1.1.3. *Trypanosoma* (*Trypanozoon*) *evansi* (STEEL 1885) BALBIANI, 1888

Trypanosoma evansi was discovered in India in 1880 by Griffith Evans. In Latin America, several other names were used – *T. equinum*, *T. venezuelense* (VOGES, 1901, LAVERAN and MESNIL, 1904, and MESNIL, 1910 quoted by HOARE [7]), and *T. hippicum* [48] – before being recognised as the single agent causing 'Murrina' under the name *T. evansi* [7]. However, in Argentina, the dyskinetoplastic variant responsible for 'Mal de Caderas' is sometimes still given the name *T. equinum* [14, 49] although this distinction is no longer accepted.

The International Committee of the WHO had recommended the name *T. brucei evansi* (*T. b. evansi*) (WHO, 1978, according to WELLS [17]), on the grounds that went back some time [7] whereby *T. evansi* was thought to be derived from *T. brucei* and that evolution from *T. brucei* to *T. evansi* arises from the loss of kinetoplast DNA maxicircles. It is also BRUN's *et al.* view [50] that *T. evansi* differentiated from *T. equiperdum* in the same way.

However, based on the International Nomenclature of Zoology, UILENBERG [51] and the participants in the '10th international meeting on *Trypanosoma evansi*' emphasise the fact that the name *evansi* was coined prior to the name *brucei* and that the designation *T. brucei evansi* is not suitable [52]. The ad hoc 'Non-Tsetse Transmitted Animal Trypanosomoses OIE Group' in the interim therefore prefers to refer to the nomenclature that was submitted to the International Commission on the Zoological Nomenclature in 1989 [52, 53, 54, 55], and thereafter recalled and confirmed at the 10th and 11th international meetings on *Trypanosoma evansi* [52, 54]: *Trypanosoma* (*Trypanozoon*) *evansi*.

Like most authors we use the abbreviated designation *T. evansi*.

Description: the morphology of the bloodstream form of *T. evansi* is similar to the bloodstream forms of *T. brucei*; it is a medium-sized trypanosome (15 and 34 µm in length; average: 24 µm). Its polymorphism, however, is very limited and most authors have described *T. evansi* as a monomorphic parasite (slender forms) [56] (**Figs 5 and 6**). The free part of its flagellum is short, from 3 to 5 µm (and non-existent in the stumpy forms); the undulating membrane is very well developed, creating 'pockets of light' when observed in a fresh sample under a phase contrast microscope; its movements are lively but it actually covers very little distance.

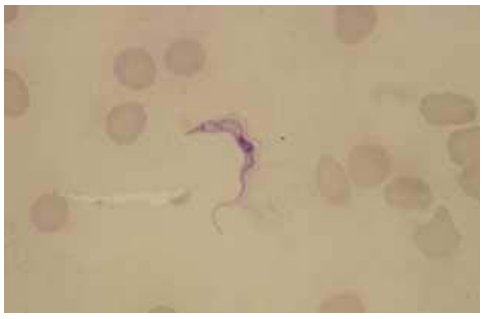


Figure 5 – *Trypanosoma evansi* (Venezuela): kinetoplastic form
(M. DESQUESNES)

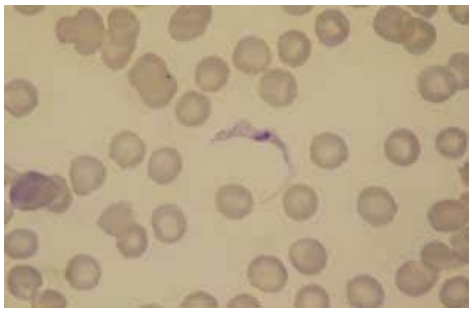


Figure 6 – *Trypanosoma evansi* (Venezuela): akinetoplastic form
(M. DESQUESNES)

When the kinetoplast is present, it is small (0.6 μm), positioned well away from the rear end, and very tapered in the characteristic slender forms. The KI and NI are equal or slightly greater than one.

Measurements made on recently isolated strains indicate mean lengths equal to or greater than those previously described in the literature – the length of parasites observed in French Guiana, Surinam and Venezuela is respectively 20.7, 27.5 μm and 26.5 μm [43]; the mean length of those observed in Brazil is approximately 25 μm for the strains isolated on dogs and coatis, compared to 17.2 μm for an equine strain [57]; cultivated on rodents, this strain can be 25 to 30 μm long [58]; these deviations are within the range defined for the species, and seemed to be related more to the host than the parasites; they are not therefore a criterion for discrimination.

Similarly, the proportion of individuals whose kinetoplast is visible varies from one strain to another: up to 20% for the Venezuelan strain (TVVG1) (**Fig. 6**), and less than 5% in dogs in French Guiana and Surinam [43]. Dyskinetoplasty in *T. evansi* has often been described [14], and although some authors have recently used it to differentiate *T. equinum* from *T. evansi* [49], it is not a reliably distinctive characteristic and hence no longer accepted [14].

1.1.4. *Trypanosoma* (*Trypanozoon*) *equiperdum*, DOFELIN, 1901

Trypanosoma equiperdum is very similar to *T. evansi* – their main differences relate to their mode of transmission and the fact that only *T. equiperdum* has maxicircles [50].

Just like *T. evansi*, *T. equiperdum* is sometimes dyskinetoplastic either naturally or, according to RIOU *et al.* [59], following various trypanocidal treatments. KAMINSKY *et al.* [60] have however shown that there is no connection between the presence of a kinetoplast and resistance to isometamidium.

The WHO had suggested the name *T. brucei equiperdum* (*T. b. equiperdum*) [42].

TRAVASSOS SANTOS DIAS (quoted by TOURATIER [55]) suggested that a special group for the classification of *T. equiperdum* be set up on the basis of its venereal mode of transmission and life cycle that takes place essentially in tissue. As pointed out by UILENBERG [51], and like most other authors, we use the abbreviated designation *T. equiperdum*.

Description: in the bloodstream forms, *T. equiperdum* is generally monomorphic and resembles *T. evansi* although it is sometimes smaller (20 μm); most often, its form is slender or intermediate with a free flagellum but it is sometimes stumpy and/or devoid of a kinetoplast. It is indistinguishable from *T. evansi* either on a smear or in a fresh sample.

1.2. Trypanosomes that are moderately or non-pathogenic for livestock

The trypanosomes in Latin America that are moderately or non-pathogenic for livestock belong to the Stercoraria section, sub-genus *Megatrypanum* HOARE 1964. Their prevalence is not negligible and can interfere with diagnosis, especially parasitological diagnosis. It is therefore important to be able to identify them.

1.2.1. *Trypanosoma* (*Megatrypanum*) *theileri*, LAVERAN, 1902

Trypanosoma theileri is a cosmopolitan parasite of Bovidae found in cattle (on all continents), buffaloes (Asia, South America), and antelope (Africa). It is generally considered to be non-pathogenic. Its prevalence in cattle is very high, at times 100% according to certain studies [61].

Description: this trypanosome varies in size and is generally much larger than the previous (69-109 μm) but sometimes comparable (25 μm); its width ranges from 1.4 to 5 μm. It has a free

flagellum (4 to 26 μm) and a well-developed undulating membrane. Its rear end is highly tapered and rigid.

In a fresh sample, it moves on the spot more slowly than the previous parasites but its convolutions are more marked. Because of its size and tapered shape, it is often identifiable in capillary tubes (WOO technique [62, 63]); however, a more appropriate identification in these circumstances is *Megarypanosoma* sp. because it cannot be distinguished from other megatrypanosomes using this technique. On a stained smear, the kinetoplast is seen to be large (1.1. μm) and located well away from the highly tapered rear portion of the parasite; the KI is high (2.5-5.8) and the NI is slightly greater than one.

1.2.2. *Trypanosoma* (*Megatrypanum*) *ingens*, BRUCE, HAMERTON, BATEMAN and MACKIE, 1909

Trypanosoma ingens was discovered in African antelope and cattle. Until recently, it had been reported only in Africa, Java and Malaysia [7]. In 1993, L. VAN VLAENDEREN found *T. ingens* in a cow in Surinam [64]. Identification was performed using morphometry (total length 62 μm) and the highly characteristic morphology described as follows by VAN VLAENDEREN: 'rear tip pointed; under-developed undulating membrane; deeply stained body of the parasite with granular structure in the cytoplasm making it impossible to distinguish the nucleus from the kinetoplast; presence of an unstained, transverse stripe across the middle of the body characteristic of *T. ingens*'. On a fresh sample, it cannot be differentiated from *T. theileri*.

1.2.3. *Trypanosoma* (*Megatrypanum*) *melophagium* (FLU, 1908) NÖLLER, 1917

This species, which is a parasite of sheep and found mainly in Europe, has been reported in North Africa, Canada, Australia [7], and Madagascar (UILENBERG, unpublished data), but also in Argentina (DIOS, 1928, quoted by HOARE [7]) and Colombia [65]. Its distribution essentially overlaps with the distribution of its vector, *Melophagus ovinus* (Hippoboscidae). *Trypanosoma melophagium* is not found in the inter-tropical zone except at higher elevations. In Latin America it is found in Mexico and the Andes. In a fresh sample, its morphology is similar to that of *T. theileri* although it is more motile than the latter. On a stained smear, it can be distinguished owing to its smaller size and higher kinetoplast index [7].

1.3. Other trypanosomes

1.3.1. *Trypanosoma* (*Schizotrypanum*) *cruzi* CHAGAS, 1909

Trypanosoma cruzi belongs to the subgenus *Schizotrypanum* in the Stercoraria section; its cycle takes place in the hindgut of a number of bugs in the Reduviidae family, subfamily Triatominae. It is a human parasite and the agent of American human trypanosomosis or Chagas' disease. It is also a parasite of many domestic and wild mammals. It is a long and firmly established, robust parasite in America since it has reservoir vectors (bugs) as well as a broad range of reservoir hosts (domestic and wild animals). *Trypanosoma cruzi* is classically found in the host (described below) in circulating bloodstream forms. Rather than replicating, this form invades the tissues and gives rise to an intracellular form of the Leishmania type that replicates in the amastigote, epimastigote and trypomastigote forms located in the reticuloendothelial cells, muscles (including the heart), liver, nervous system, etc. [7]; bloodstream and tissue forms alternate periodically.

Description: in mammals, bloodstream forms of *T. cruzi* are of two types: stumpy forms with an oval-shaped nucleus, a kinetoplast placed close to the rear end, and a long free flagellum (8-10 μm); and slender forms that have an elongated nucleus, a subterminal kinetoplast and a short, free flagellum (2-4 μm). There are numerous major morphological variations of *T. cruzi*. The parasite's total length ranges from 12 to 30 μm, with an average of between 16 and 22 μm.

In a fresh specimen, slender forms move rapidly while stumpy forms move slowly. On a smear, the parasite is seen to be C- or S-shaped; the kinetoplast is very large (1.2. µm) and sometimes forms a 'stain' that overlaps over the edge of the body. The undulating membrane is stunted and the nucleus is located in the central or front portion of the body (NI: 0.9 to 1.9 or more).

1.3.2. *Trypanosoma* (*Megatrypanum*) *mazamarum* MAZZA, ROMANA and FIORA, 1932

This species has been reported only in Argentina and Brazil on deer (*Mazama rufa* and *M. simplicornis*); it cannot be distinguished from *T. theileri* on the basis of morphology [7]. further confirmation of its separate existence is therefore needed. The many other species of trypanosomes found on the fauna of South America have not been reported in livestock. Hence, *Trypanosoma rangeli*, which is borne by reduviid bugs (Reduviidae: Triatominae), is a natural parasite for man, monkeys, dogs and opossums [7]; horses can be experimentally infected but no natural infections have been observed.

It is not thought that these parasites present any serious risk of interfering with the diagnosis of livestock trypanosomoses in Latin America.

2. MAMMALIAN HOSTS

Among the trypanosomes present in America, only *T. cruzi* is pathogenic for man. However, in the past, human infections with *T. vivax* have occasionally been described in Ghana (MACFIE, 1917, quoted by HOARE [7]) and Uganda [66]; none have been reported in America.

2.1. *Trypanosoma vivax*

In Latin America, *T. vivax* is found regularly in zebu (*Bos indicus*), taurines (*Bos taurus*) and their crossbreeds, sheep (*Ovis aries*) and domestic goats (*Capra hircus*). SHAW and LAINSON [29] and DIDONET-LAU and LAU [67] have reported them in water buffalo (or kerabau) (*Bubalus bubalis*) in Brazil.

Unlike the African *T. vivax*, which is pathogenic for horses and sometimes causes severe clinical signs [68, 69], the American *T. vivax* does not seem to infect this species [17], although a report published long ago by JOHNSON [27] (Panama) does mention a short-lived parasitæmic episode under experimental conditions. However, additional parasite screening should be performed on horses as they could be an excellent reservoir that is often in close contact with cattle (working horses used for herding purposes). The American parasite does not infect cats in contrast to what has been observed in Kenya [71]. Alpacas (*Lama pacos*) and llamas (*Lama glama*) are sensitive to experimental infection [14]; but field observations are lacking.

Whereas in Africa *T. vivax* has many wild hosts (artiodactyls, perissodactyls and even carnivores) in addition to its domestic hosts (cattle, sheep, goats, horses, camelids, donkeys and asses), in South America the only wild animal found to be infected is deer (*Odocoileus gymnotis*) in Venezuela by FERNANDEZ [72] and FIASSON *et al.* [73]. In French Guiana and Guyana, due to heavy pressure from hunting activities in the vicinity of stockbreeding areas, this species is unlikely to act as a reservoir for *T. vivax*. In Brazil, in the highly extensive stock-farming areas of Pantanal, deer may be a reservoir for the parasite (R.A.M.S. SILVA, unpublished paper). The trypanotolerance that is observed in Africa on wild animals [74, 75] has not been described in Latin America; to the best of our knowledge, there has been no research published on this topic. Failing any evidence that there is an effective wild reservoir in Latin America, it can only be assumed that the establishment of *T. vivax* is entirely dependent on its domestic hosts.

Finally, the trypanotolerant taurine breeds in Africa (N'Dama, Baoulé, etc.) are not found in America or else have been absorbed by cross-breeding [20]; local livestock is therefore wholly sensitive to the parasite.

2.2. *Trypanosoma evansi*

The domestic hosts in Latin America on which *T. evansi* is the most virulent are horses (*Equus caballus*), asses and donkeys (*Equus asinus*) together with their crossbreeds, cats (*Felis domesticus*), dogs (*Canis familiaris*), and water buffaloes (*Bubalus bubalis*) (*T. evansi* is non-pathogenic for African buffaloes (*Syncerus caffer*) [14]. Dogs may act as sentinel animals and/or a peridomestic reservoir for *T. evansi*. Water buffaloes can act as a reservoir or alternatively, as has been reported in Asia, contract an acute disease with a high rate of mortality [61].

Trypanosoma evansi may also infect sheep, goats and cattle, which are often asymptomatic carriers of the parasite [76], in contrast with observations made in Asia where cattle are sometimes highly susceptible. In experimental infection of sheep (Venezuelan strain), following a short parasitæmic phase with hyperthermia and nervous signs, the parasite becomes difficult to detect in the blood but the drop in haematocrit is peristent [43]. Some strains are virulent in cattle [77-79] while others are incapable of infecting them (RAISINGHANI *et al.* [80]). Although pigs can be infected, animal husbandry practises are such that they have little exposure to infection. Guinea pigs (*Cavia porcellus*) can harbour the parasite, specifically in Peru, where it is raised for meat. FERRIS [14] has detected *T. evansi* in llamas and alpacas in Chile by serology. Similarly, in Colombia, infected llamas have been found [54]. Recent experimental research has shown that *Lama guanicoa* is wholly receptive and susceptible to infection [81].

Vampire bats (*Desmodus rotundus*) are simultaneously hosts, reservoirs and vectors of *T. evansi*; their role in the epidemiology of *T. evansi* in South America is therefore crucial [16]. Many other wild animals had been found to be infected and could act as reservoirs for the parasite in Latin America as shown below. Capybaras (*Hydrochoerus hydrochaeris*) raised in free-ranging or semi-free ranging conditions are potentially a major reservoir [82]; a survey conducted in Colombia by WELLS *et al.* [15] showed that 24% of the animals are carriers of the parasite; in Venezuela, TORO *et al.* [83] found 25% of the animals to be antibody carriers while REVERON *et al.* [82] found that 70% were.

Infections have been detected in South American coatis (*Nasua nasua*) [84, 85], sometimes with high prevalence. The animals may exhibit severe clinical signs and sometimes die (R.A.M.S. SILVA, unpublished paper).

Infections have also been found on other animals: wild dogs (*Canis azarae*), red howler monkeys (*Alouatta seniculus* and *A. ursina*) in Venezuela [70], deer (*Odocoeilus virginianus chiriquensis* and *Mazama satori*) in Panama [56], and wild pig (collared peccary, *Tayassu tajacu*) (according to VAN VLAENDEREN [64]). The parasite has been identified in a New World mouse (*Oryzomys* sp.) in Brazil by NUNES *et al.* [86].

There are no reports of cat infection but infection of ocelots (*Felis pardalis*) has been described in South America [64], tigers in India [87], and leopards [88]. It is likely that most carnivores are receptive. *Trypanosoma evansi* has even been reported in chicken [89].

Finally, experimental infection of wild animals is indicative of the likelihood that many species are involved the epidemiology of *T. evansi* in South America: marsupials, chiroptera, primates, lagomorphs, Edentates, rodents, carnivores, perissodactyls and artiodactyls [17, 24, 90].

Unlike *T. vivax*, *T. evansi* can call on a huge wild reservoir and has been able to become established and spread through Latin America independently of livestock. However, the epidemiological importance of each species has not been determined and some may be epidemiological dead ends.

2.3. Other trypanosomes

Trypanosoma equiperdum occurs naturally only in Equines. Rabbits, mice, guinea pigs and other rodents together with dogs have been found to be susceptible under experimental conditions.

Trypanosoma theileri has been found only on cattle and buffaloes in Latin America.

Trypanosoma mazamarum has been found only in deer: *Mazama rufa* and *Mazama simplicornis*.

Trypanosoma melophagium has been described only in sheep.

Trypanosoma ingens has been found only in cattle in Surinam; considering its hosts in Africa (cattle, antelope and Tragulidae), Java, and Malaysia (*Tragulus javanicus*) [7], *T. ingens* may be present in wild hosts such as deer in South America too.

Trypanosoma cruzi is a parasite in man and many other animal species both wild and domestic. A non-exhaustive list of 150 wild animals (including the vampire bat *Desmodus rotundus*) and some ten domestic or peridomestic animals, which are found to be infected, has been compiled [3].

In particular, *T. cruzi* can be found in dogs, cats, cattle, goats, sheep, rabbits, and Equines [3, 91, 92, 93]. Among susceptible domestic animals, guinea pigs may play an important epidemiological role, particularly in Peru. In Paraguay, FUJITA *et al.* [92] suggest that cattle, pigs, dogs and cats provide reservoirs for *T. cruzi*. In French Guiana, studies conducted by DEDET *et al.* [94] show that *Didelphis marsupialis* and *Philander opossum* are frequently infected. Positive serological results for *T. cruzi* have also been found in dogs in Cacao [95]. *Trypanosoma cruzi* has never been reported in livestock in Guyana or French Guiana although unnoticed occasional contamination may have occurred. Hence, the parasite may interfere with the diagnosis of trypanosomosis in livestock, in particular serological diagnosis of *T. vivax*-induced trypanosomosis in ruminants and *T. evansi*-induced trypanosomosis in equines.

2.4. Affinity of these trypanosomes for their hosts in Latin America

Any study of animal trypanosomoses necessarily comes up against the lack of species-specificity of diagnosis, particularly serological diagnosis. The extent of this problem varies depending on the range of *Trypanosoma* spp. that can infect any given host. **Table I** lists the main species of trypanosomes and their mammalian hosts. Among the pathogenic trypanosomes, *T. evansi* and *T. cruzi* differ from other species by their broad range of hosts, both wild and domestic animals. *Trypanosoma equiperdum* is the only parasite that is practically entirely dependant on a single species. Finally, the status of *T. vivax* is intermediate, with a limited range of hosts and, to date, a very small potential reservoir among wildlife. Horizontally, **Table I** shows the various species of trypanosomes that can be found in a given host; vertically, it shows the various hosts for each *Trypanosoma* spp.

3. TRANSMISSION

Although mechanical transmission of *T. vivax* and *T. congolense* was observed very early on in Africa [96], as well as the inability of *T. vivax* in the French West Indies to fulfil its cycle in Tsetse flies [40], for a long time most authors considered trypanosomoses caused by *T. vivax*, *T. congolense* and *T. brucei* to be parasitoses that relied strictly on the cyclical transmission by *Glossina* and hence on their presence [97, 98]. In actual fact, we now realise that these parasites are mechanically transmitted by other hæmatophagous insects and that *Glossina* can also transmit them mechanically [99].

Pressure from the advocates of exclusively *Glossina*-borne transmission discouraged any investigation on non-cyclical transmission of livestock trypanosomes by horseflies and *Stomoxys* from being initiated [100], developed [101] or ascertained in Africa [102, 103] until recently. The predominance of *T. vivax* in *Glossina*-free areas [104], and its persistence in areas where *Glossina* have been eradicated argue in favour of this mode of transmission. Recent attempts to induce mechanical transmission of African trypanosomes experimentally using *Stomoxys* achieved a success rate of 11.5% for *T. brucei*, 3.4% for *T. vivax* and 0.9% for *T. evansi* [105]. With insects held in semi-

freedom, the incidence of *T. vivax* transmission is very high: 60% to 80% in 20 days [103]. In a study on mice, SUMBA *et al.* [106] achieve a 10% transmission rate for *T. congolense* via *Stomoxys nigra*. The epidemiological data are consistent with the experimental data, confirming the existence of mechanical transmission of the main African livestock trypanosomes. In America, although mechanical transmission appears to be patent, a number of hypotheses concerning cyclical transmission have been proposed [17, 97] albeit without any basis.

Host species	***Trypanosoma* spp.**					
	T. vivax	*T. evansi*	*T. cruzi*	*T. equiperdum*	*T. theileri*	*T. ingens*
Horses (*Equus caballus*)**, donkeys** and **asses** (*Equus asinus*), **and mules**	?	+++++	++	+++++		
Cattle (*bos taurus* and *Bos indicus*)	+++++	++	++		+++++	+++
Sheep (*Ovis aries*)	+++++	++	++			
Goats (*Capra hircus*)	+++++	++	++			
Water buffaloes (*Bubalus bubalis*)	+++	+++	++		+++++	?
Pigs (*Sus scrofa*)		++	++			
Domestic dogs (*Canis familiaris*)	?	+++++	+++++	+ *		
Domestic cats (*Felis catus*)	?	++	++			
Rabbits (*Oryctolagus cuniculus*)		?	++	+ *		
Humans (*Homo sapiens*)	?		+++++			
Deer *Odocoileus gymnotis*:	++	?	++		?	?
O. chiriquensis & *Mazama satorii*		+++	++		?	?
Alpacas (*Lama pasos*) **Llamas** (*Lama glama*)	+	++	++			
Guinea pigs (*Cavia porcellus*)		+++	++	+ *		
Vampire-bats (*Desmodus rotundus*)		+++++	++			
Capybaras(*Hydrochoerus hydrochaeris*)	?	+++++	++			
Coatis (*Nasua nasua*)		+++++	++			
Wild dogs (*Canis azarae*)		+++	++			
New World mice (*Oryzomys* sp.)		++	++			
Southern oppossum (*Didelphis marsupialis*)		?	+++++			
Opossum (*Philander opossum*)		?	+++++			
Howling monkeys (*Allouatta seniculus* & *A. ursinus*)		+++	?			
Wild pigs or peccaries (*Tayassa tajacu*)		++	?			
Squirrel monkey (*Saimiri sciureus*)			++			
Other wild species	?	Carnivores & rodents	150 species			

* experimentally susceptible

Key: The affinity of a trypanosome for its host is defined here as the receptiveness of the host to infection, and by the frequency of natural infections in the host; it is expressed as a number of crosses (+) ranging from 1 to 5; a question mark (?) is shown when the presence of a trypanosome in a host species is suspected or possible but has not been demonstrated; when nothing is specified, it means that the trypanosome has never been either found or suspected in that host.

Table I – Affinity of the major mammalian trypanosomes in Latin America for their hosts

3.1. *Trypanosoma vivax*

Outside of Africa, *T. vivax* has been found in Latin America, the Caribbean islands and Mauritius [45, 107]. In Latin America, ***T. vivax* is mainly transmitted by tabanids and *Stomoxys*** [61]. However, they are not its only potential vectors – *Culicoides* (Ceratopgonidae), i.e. biting insects of the *Haematobia* genus, certain Culicidae and others might also be involved [70, 108]. Most authors today consider *T. vivax* transmission by insects in America to be **strictly mechanical**.

The next section presents an overall study of the hæmatophagous insects that are vectors of trypanosomes in Latin America and describes their direct and indirect harmful effects. Some attention is given to special characteristics of horseflies that foster trypanosome transmission. Mechanical transmission of *T. vivax* has successfully been achieved with several species of Stomoxyinae, in particular the sub-species *Stomoxys nigra* [96, 105], and several species of American Tabanids: *Tabanus importunus* [109], *T. nebulosus* [110], and *Cryptotylus unicolor* [111, 112].

A list of the mechanical vectors for *T. vivax* in Cuba, compiled by CORDOVES *et al.* [113], also includes many Culicidae species, one Ceratopogonidae species (*Culicoides furens*) and one species of Simuliidae (*Psilopmia quadrivittatum*). RUIZ MARTINEZ [70] reports the possibility of transmission by certain *Culex*, *Mansonia* and *Aedes* species.

LOPEZ *et al.* [114] and CORDOVES *et al.* [113] have reported the presence of *T. vivax* in the livestock tick, *Boophilus microplus*. Trans-ovarian transmission is thought to be compatible with the life cycle (monotropic/monophase) of ticks but this mode of transmission has never been experimentally induced. *Boophilus microplus* has been shown to move from one head of cattle to another by MASON and NORVAL [115], but statistically this is of very little significance. Transstadial transmission of the parasites is therefore theoretically possible but probably unusual. Furthermore, in ticks, the interval between two bloodmeals is generally long which argues against mechanical transmission.

It would therefore be difficult to compile a comprehensive list of the potential vectors of *T. vivax*. We can conclude that the main vectors of *T. vivax* are horseflies and *Stomoxys* and, more generally, **any hæmatophagous biting insect that is likely to abound in a stockfarming area.**

Trypanosome transmission by **blood-sucking flies** is also possible – the latter are indeed attracted by the blood that flows from Tabanid bite wounds and can carry contaminated blood from one lesion to another and therefore from one animal to another. LAMBORN [117] has described cases of transmission of sleeping sickness being experimentally induced in this way with *Musca sorbens*. No models have so far been proposed to assess the incidence of this phenomenon in livestock.

Iatrogenic transmission of *T. vivax* by syringe also deserves to be mentioned and taken into consideration when planning mass prevention programmes. According to R.A.M.S. SILVA, in 1996-1997, on the Brazilian – Bolivian border a vaccination campaign against foot-and-mouth disease led to a *T. vivax* epizootic (L. TOURATIER, unpublished paper).

It should be noted that BETANCOURT [118] in Columbia, and GONZALEZ and ESPINOZA [119] and MELENDEZ *et al.* [120] in Venezuela, observed congenital transmission of *T. vivax* in cattle during epizootics. In Africa, OGWU *et al.* (1986, quoted by OKECH *et al.* [121]) have also reported similar cases in cattle under experimental conditions, likewise PIGNEUR [122] in Rwanda, and OKECH *et al.* [123] for cattle in natural conditions; EHLHASSAN *et al.* [124] have detected this mode of transmission in sheep. It has not been clearly established whether the transmission of the parasite occurs by the transplacental and/or the intravenous route when there is vascular breach during parturition. High parasitæmia observed immediately after birth argues in favour of infection before parturition (transplacental route) [118]. Congenital transmission may be very important in the epidemiology of trypanosomosis in Latin America. The offspring of subclinical carrier animals may cause the disease to reappear in herds that have largely returned to non-infected status [14].

3.2. *Trypanosoma evansi*

The transmission vectors for *T. evansi* are **hæmatophagous biting insects** and **vampire bats**. Contamination via the **oro-digestive** route is also possible.

In his review of pathogen transmission by Tabanids, KRINSKY [125] states that NIESCHULZ *et al.* had demonstrated the feasibility of transmission of *T. evansi* by a single insect, and that many species of Tabanids can, at least experimentally, transmit the parasite. Authors generally agree that Tabanid-borne transmission of *T. evansi* is solely mechanical [126].

Other studies have shown the possible role of the *Stomoxys* [127], *Haematobia* and *Culicoides* genera [89]. *Trypanosoma vivax* and *T. evansi* probably share the same hæmatophagous arthropod vectors.

Mechanical transmission by reduviid bugs was experimentally achieved by MANZ [128], on mice. However, since livestock and reduviid bugs rarely share the same habitats, this type of transmission is unlikely to have much epidemiological significance.

HOARE [7, 16] has described transmission of *T. evansi* by vampire bats (*Desmodus rotundus*) in detail. Vampires are simultaneously hosts, reservoir and mechanical vectors of the parasite. **They are infected by the oro-digestive route** during bloodmeals taken on an infected host (passage of parasite through the oral and/or gastric mucosal membranes), or **transcutaneously**, by been bitten by another infected congener. In the latter case, the parasite can infect both ways: from the biter to the bitten by contamination of the wound through saliva, and, perorally, from the bitten to the biter.

The outcome of the disease caused by the infection – lasting up to one month – is either the bat's death or recovery. In the latter case, it can continue to be an asymptomatic carrier for a very long time. After multiplying in the blood, the parasites are present in the bat's mouth cavity, from where they are **transmitted by biting another host** (vampire bat, livestock or wild animal). Intra-species contamination between vampire bats provides a reservoir that harbours the parasite even if the preferential host (horses) is absent. It is worth noting that the geographic distribution of vampire bats and of *T. evansi* trypanosomosis largely coincide (16), their habitat lies within the 10°C minimum winter temperature isotherms [129]. HOARE [7, 16] suggests that vampire bats play a predominant role in the transmission of *T. evansi* and that insects are very occasionally involved and only when they pullulate intensively. Vampire bats, whose range of action is approximately 5 km [130], are thought to contaminate herds and are then relayed by hæmatophagous insects to produce epizootic outbreaks.

The parasite's mode of transmission is referred to as 'mechanical'; in vampire bats, there are no non-infective forms and/or forms morphologically distinct from those found in the host. It follows that the parasite does not go through a biological cycle in vampire bats. But because these bats act as both vector and reservoir and because the parasite **multiplies in the vector**, there is justification for considering the vampire bat to be '**a biological vector**' of *T. evansi* of a different sort to the 'cyclical transmission biological vector', e.g. the typically African *Glossina* for the *Salivaria* group, and also different from the straightforward 'mechanical vector', i.e. hæmatophagous insects for *T. vivax* and *T. evansi*.

Oro-digestive contamination is not the preserve of vampire bats. It has also been reported in dogs and other carnivores (lions, hyenas), as a result of ingesting raw, parasite-infested meat [19]. This has been experimentally confirmed for *T. brucei* and *T. evansi* in dogs, cats, tigers and mice [87, 131, 132, 133]. The authors suggest that the parasite is capable of penetrating through the healthy mucous membranes of the mouth or gut. This is probably the main mode of infection for carnivores in America, particularly dogs.

Iatrogenic transmission of *T. evansi* is possible but its importance is not determined. Congenital transmission has been reported in dromedary camels [134]. Finally, although transmission via blood-sucking flies has never been described, it remains a possibility.

3.3. *Trypanosoma equiperdum*

Trypanosoma equiperdum is transmitted mainly by passing through the **genital mucosae** during coitus, but it can also cross the **eye and nose mucous membranes**, and so can be transmitted from mare to foal [4]. Just as with the other *Trypanozoon*, **congenital** transmission is possible but its impact has yet to be determined [13]. *Trypanosoma equiperdum* transmission via **hæmatophagous insects** has also been ascertained but its epidemiological significance can only be extremely slight since the parasitæmia is very short-lived [63].

3.4. *Megatrypanum* spp.

Trypanosoma theileri can transmit mechanically, cyclically or by the transplacental route [61].

Vectorial and iatrogenic mechanical transmission

Parasitæmia in livestock due to *Megatrypanum* spp. is often less than 10^3 parasites/ml. Some instances of high parasitæmia have been reported for *T. theileri* [7, 135-138], sometimes combined with *T. vivax* infections [43, 46]. With these exceptions, parasitæmia is generally low, which is not conducive to purely mechanical transmission either via insects or by iatrogenic means (animals treated using needles), but nonetheless remains possible.

Cyclical transmission by Tabanids

Transmission of *Megatrypanum* spp. is generally considered to follow the model of Stercoraria [126]. The mechanism is difficult to investigate because of the high prevalence of a Trypanosomatidae of the genus *Blastochridia*, whose epimastigote forms present in the hindgut of Tabanids cannot be differentiated from those of *Megatrypanum* spp. (or from the other *Trypanosoma* spp. in vertebrates).

Trypanosoma theileri is transmitted mainly by Tabanids [7] on which the typical Stercoraria cycle can be observed. REICHENOW (research from 1940 to 1952), quoted by HOARE [7], describes replication of the parasite in the host: the bloodstream adult forms transform into epimastigotes, undergo unequal binary fission that gives rise to intermediate trypomastigotes, and then transform into metatrypanosomes, the infective form found in the gut of Tabanids that contaminate the hosts with their excreta. The parasite penetrates through wounds caused by Tabanid bites. Transmission may occur as a result of the insects been crushed in the vicinity of the bite wounds or ingested by the cattle (peroral route). Transmission of *T. theileri* via **ticks** has also been described, in particular by *Hyalomma anatolicum anatolicum* [139].

Tabanid flies probably convey ***T. ingens*** but neither the latter's life cycle nor its vectors have been described.

Trypanosoma melophagium transmits cyclically in the form of metatrypanosomes that are present in the rectum of *Melophagus ovinus*. The parasite is released when the sheep bite (or scratch) themselves and the vector is crushed. The metatrypanosomes then gain an entry through the mucous membrane of the sheep's mouth.

3.5. *Trypanosoma cruzi*

Trypanosoma cruzi is not a livestock trypanosome as such, but it is sometimes found in domestic ruminants and horses. Its transmission mode is essentially cyclical through bugs that belong to the Reduviidae family: triatomines, or reduviid bugs, of the genera *Rhodnius*, *Panstrongylus* and *Triatoma*.

The metacyclic trypomastigote infective form (metatrypanosome) is present in the excrement of the bugs that contaminates bite wounds or the mucous membranes, particularly the eye. Parasite transmission can also occur when reduviid bugs bite one another. As such, they are both a reservoir and vector for *T. cruzi*.

Transmission of the infective forms found in the faeces of the bugs can also take the **per os** route [3]. Recent work indicates that, in mice that ingest infected bugs or their excreta, the parasite gains an entry through the intestinal mucosae and produces local immunity [140]. This may explain how a number of family infections arise from food contaminated by the excreta of the bugs [141]. Ingestion of infected bugs is also thought to be the cause of contamination of dogs and cats and possibly livestock. It has recently been shown that other trypanosomes in the sub-genus *Herpetosoma* are also orally transmitted in rodents including in the trypomastigote form: *T. lewisi*, *T. microti*, *T. evotomys* and *T. grosi* [142].

Triatomine insects are not the only cyclical vectors of *T. cruzi* – it would seem that a number of marsupials, in which infection is common including those in urban areas, are more than just a reservoir. Indeed, the life cycle described in the gut of triatomines has also been observed in ***Didelphis marsupialis*** (southern opossum) in which the trypomastigote-epimastigote-infective metacyclic trypomastigote cycle takes place in the anal scent glands [143]. The parasites extracted from the scent glands have the same features as the metacyclic forms in insects and are infective via the subcutaneous, intraperitoneal, per os, and transconjunctival routes. Because infective forms of *T. cruzi* are present in the excreta of marsupials, contamination between marsupials is highly likely making the opossum a true reservoir for the parasite. It also means that the parasite can be transmitted in the absence of insect vectors, e.g. in urban areas, through the **contamination of food** by opossum faeces. All mammals can be contaminated in this way by the oral route.

On the basis of these observations, *D. marsupialis* can be classified amongst the biological reservoirs/hosts/vectors for cyclical transmission of *T. cruzi*. It is remarkable that the typical stercorarian cycle described for insects occurs in an analogue way in the distal portion of a mammal's gut.

Just as with *T. evansi*, it was clear from a very early stage (DIAS [144]) that the bloodstream form of *T. cruzi* was transmissible. DIAS was able to achieve contamination of a cat by ingestion of infected rats. It can therefore be concluded that **ingestion of the raw flesh of infected animals** can cause infections in humans and animals, in particular dogs (oro-digestive transmission).

In man, **congenital** transmission *in utero* (transplacental), by nursing, or through **blood transfusion** is also important.

Failing conventional direct transmission via reduviid bugs, livestock may be contaminated by ingesting these insects themselves or their *excreta*, or feed contaminated by *Didelphis marsupialis* faeces. Cattle and horses rarely share the same habitat as reduviid bugs. For this reason and except in very unusual circumstances, contamination of cattle and horses by bugs would seem to be insignificant. However, small ruminants and pigs are more likely to share the same habitat as these vectors, in particular under conventional stockfarming conditions. The opossum is perhaps an important link in the chain of contamination, from the bugs to the livestock. A serological survey conducted by FUJITA *et al.* [92] in Paraguay, shows that 8% of cattle is infected and 10% of pigs. The study by ALCAINO *et al.* [145] in Chile finds 30% to 38% of goats to be carriers of antibodies. Hence, it would seem that the incidence of *T. cruzi* infection of livestock is by no means negligible.

Finally, the discovery of *T. cruzi* in dogs in the USA [146] shows that a new epidemiological pattern is furthermore being established by the spread of the parasite. This pattern relies on a rodent/carnivore cycle in which rodents probably act as reservoir (transmission from rodent to rodent by biting) whereas dogs are thought to be an epidemiological *cul-de-sac*. This epidemiological pattern is totally emancipated from cyclical transmission. This evolution does not preclude the

parasite finding new true (cyclical) vectors as it spreads geographically. If so, it may expand its range of hosts, perhaps even to include man.

3.6. Conclusions

The trypanosomes present in Latin America call on a variety of modes of transmission, as set out in **Table II**. Concerning livestock, the main modes are venereal transmission for *T. equiperdum*; mechanical transmission for *T. vivax* and *T. evansi* via hæmatophagous insects; biological mechanical transmission for *T. evansi* via vampire bats; peroral transmission for *T. cruzi*; and cyclical transmission via horseflies and *Melophagus* for *T. theileri* and *T. melophagium*. Vector insects play a crucial role in the epidemiology of trypanosomoses caused by *T. vivax* and *T. evansi*. Their direct and indirect effects, mechanism and conditions of transmission and their vectorial competence deserve to be investigated in detail. This investigation is presented in Chapter 3.

Modes of transmission: main agents (route of entry)	***Trypanosoma* spp.**					
	T. vivax	***T. evansi***	***T. cruzi***	***T. equiperdum***	***T. theileri***	***T. melophagium***
Mechanical						
– **by hematophagous biting insects:** horseflies, *Stomoxys* (bite)	++++	++++		+	+	
– **by bloodsucking insects:** *Musca* spp. (contamination of wound)	+	+				
Biological mechanical by Chiroptera: *Desmodus rotundus* (bite)		++++	?			
Cyclical						
– via insects:						
Reduviidae (transcutaneous, transmucosal, or peroral)			++++			
Tabanidae (transcutaneous route)					++++	
Melophagus ovinus (peroral route)						++++
Cyclical						
– via mammal:						
D. marsupialis (peroral contamination)			++++			
Peroral bloodstream forms: all hosts						
– (ingestion of infected animal)		carnivores	carnivores			
– (oro-nasal contamination)			++	++		
Iatrogenic (syringe needles)	++	++	+	+	+	+
Venereal (transmucosal)				++++		
Congenital (transplacental, intravenous)	+	+	+	++	+	

Key: the incidence and effectiveness of the various modes of transmissio are expressed by a number of crosses (+) from 1 to 4.

Table II – Transmission modes of the main mammalian trypanosomes in Latin America

CHAPTER 2: PATHOGENICITY, CLINICAL SIGNS, AND CHARACTERISATION OF LIVESTOCK TRYPANOSOMES IN LATIN AMERICA

Livestock trypanosomoses are classically either acute or chronic diseases that cause fever and are accompanied by anaemia, lacrimation, hypertrophy of the lymph nodes, œdema, loss of appetite, digestive and/or nervous disorders, followed by an unkempt coat, loss of weight and wasting; in acute cases, the outcome can be death. Apart from the genital signs that are specific to *T. equiperdum*, there are no specific symptoms. The latter's' intensity and nature vary according to the species and strain of parasite, and depending on the species, breed, immune status and nutritional condition of the host.

1. PATHOGENICITY AND CLINICAL SIGNS

1.1. *Trypanosoma vivax*

1.1.1. Natural infections

The pathogenicity of this trypanosome as described by LEGER and VIENNE [18] on cattle in French Guiana was very high. The main clinical signs observed at the time were progressive anaemia accompanied by a weight loss, sub-lingual and dewlap œdema, and lacrimation. In the most severe cases, fatal paresis of the hindquarters occurred. The overall mortality rate was high since in just a few months more than 50% of the 180 animals concerned had died and one third of the survivors were considerably weakened. Such a high degree of pathogenicity is probably indicative of the fact that these animals were encountering this parasite for the first time. 70 years later, CAMUS and MARTRENCHAR [147] observed less pronounced symptoms but weight losses were significant and were not always regained three months after treatment. The epidemiological surveillance system set up in French Guiana by the author *et al.* between 1991 and 1996 was rarely able to pinpoint the cause of the death of a bovine as trypanosomosis. What it did record were some considerable drops in production particularly on farms with low animal husbandry standards (inadequate feed and deworming), and especially farms that had recently imported bovines from metropolitan France. The clinical impact of trypanosomosis on farms with good animal husbandry standards was practically imperceptible outside of the dry season. In a case of this type, the mean haematocrit value in a batch of infected cattle dropped by only 3% [43]. On the other hand, during the dry season the impact was more noticeable on these farms. These field observations indicate that pathogenicity was closely dependent on the receptiveness of the hosts and hence their immunocompetence. The level of pathogenicity described by LEGER and VIENNE is currently observed only in animals that are imported from areas free of the parasite. Similarly, in Colombia, early reports by ZAPATA (1931 quoted by VIRVIESKA) and VIRVIESKA [34] mention very high mortality due to *T. vivax*, but later studies by WELLS *et al.* [140] describe more limited pathogenicity. Clinical signs are: abortion, high rate of stillbirths and significant drops in milk yield [148], drops in the haematocrit and in growth rates that are not later offset [149]. WELLS *et al.* [148] and MATEUS and GONZALEZ [150] stress that in America *Bos taurus* are more susceptible than *Bos indicus*. This observation cannot however be generalised as in Africa certain *Bos taurus* breeds are, on the contrary, trypanotolerant.

In Panama, there are clear clinical signs though the outcome is rarely fatal [27]. In Brazil, fatal cases have been reported in buffaloes [67]. During recent epizootics among cattle in Pantanal, Brazil and in Bolivia, the clinical signs were pronounced and the estimated mortality rate when untreated was 34% ([151, 152] and EULERT *et al.*, unpublished contribution). This indicates once again that *T. vivax* pathogenicity is very high only in newly infected bovine populations.

The 'haemorrhagic syndrome' described in East Africa [153], and the haemorrhagic signs observed in West Africa [154] have not been reported in America.

Inoculation chancre is a cutaneous reaction due to the multiplication of parasites on the site of the bite wound where polynuclear cells, lymphocytes and macrophages congregate. It occurs when trypanosomes invade the site locally and is no doubt connected with *Glossina*-borne transmission [155, 156, 157, 158]. In the case of mechanically inoculated bloodstream forms, there is no formation of a chancre; none have been reported in Latin America.

Extravascular locations of *T. vivax* – nervous system and aqueous humour of the eye – have been described in connection with African strains [36]. They have not been demonstrated for the American strain but possibly do exist.

Generally speaking, recent observations conducted in America report significant mortality only in newly infected areas. The clinical signs gradually fade and the disease becomes chronic and enzootic [159]. However, even without any symptoms, OTTE *et al.* (quoted by TOURATIER [55]) report significant drops in yield in some animals, ranging from 20% to 25%. The economic impact of the disease may therefore be significant. Furthermore, animals imported from parasite-free areas are highly receptive and susceptible to infection. The pathogenicity described in recent observations appears to be less strong than it was at the beginning of the century; whether this difference is attributable to improved animal husbandry practises, the existence and use of trypanocides, immunity arising from enzooticity, and/or a decrease in the virulence of the parasite as a result of transmission being strictly mechanical is a matter for speculation.

In addition to losses in condition and appetite, weakening and weight loss among cattle, sheep and goats [160, 161, 162, 163], the authors observe: a significant drops in erythrocyte [164], white blood cell [165], and neutrophil [166] levels, as well as lesions on enlarged lymph nodes examined post mortem. VALENCIA [167] reports diarrhoea and states that the haematocrit decrease is concomitant with a low haemoglobin level, lymphocytosis and monocytosis and that the anaemia is sometimes microcytic and sometimes macrocytic. VAN MEIRVENNE [168] records coagulation and complement system disorders, a drop in serum albumin, and immune complexes and immunoglobulins M with potentially pathogenic effects.

1.1.2. Experimental infections

Experimentally induced infections of ruminants using *T. vivax* strains that are present in America rarely cause the animals to die [166]. More often than not, clinical signs are moderate although there can be significant weight loss [164].

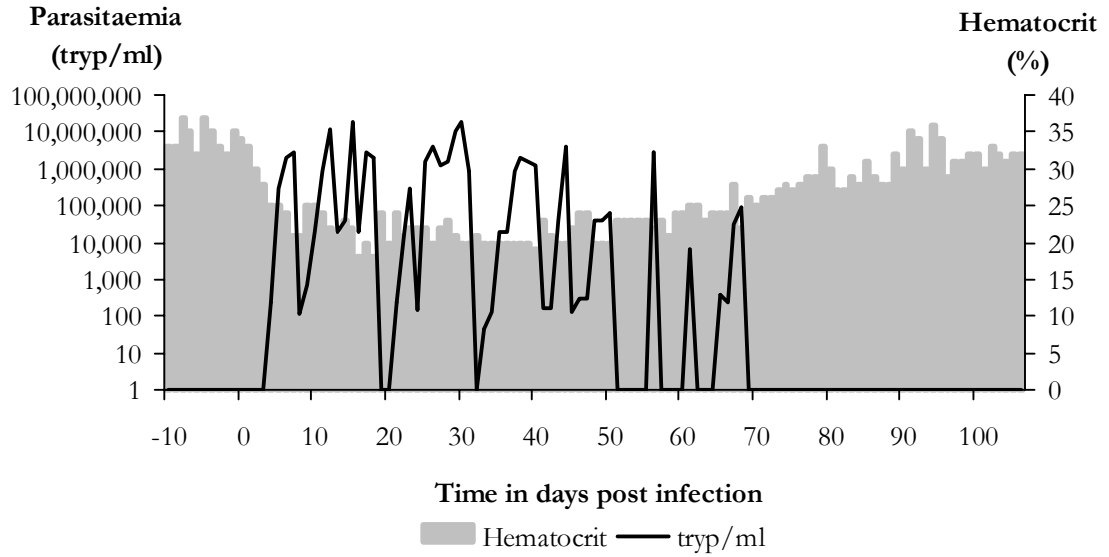
1.1.2.1. Sheep

Experimental infections of 13 Black Belly X Creole sheep aged between one and two years, using three strains of *T. vivax* from French Guiana (TVFG1, TVFG2, TVFG3) and one from Venezuela (TVVG1) by inoculation into the jugular vein of 10^5-10^6 parasites/ml. produced the following results [169]:

General remarks: using the same inoculum, clinical signs vary from one animal to another even when their physiological status is identical, but broadly speaking they are comparable from one strain to another.

The first phase of the infection lasts from three to four months – the symptoms observed are: fever, lack of appetite, lacrimation, listlessness, transient lack of motor co-ordination, ataxia, panting, weakening and weight loss. In pregnant females: complete or partial abortion, metritis, and high stillbirth rates together with low milk production. Vertical transmission was not observed. A number of individuals remain severely affected for more than four months making it necessary to undertake curative treatment. No fatal outcomes were recorded in these particular conditions.

In the second phase of the infection, or following a trypanocidal treatment – which turned out to be non-sterilising –, the haematocrit gradually returns to its initial value (**Fig. 7**), and the symptoms disappear. The trypanosome can only be detected during low-level and short-lived parasitæmic peaks (<10^5 parasites/ml). Woo's test may remain negative for several months although the infection persists (**Figs 9 and 10).**



Comment: this animal is able to control the parasitæmia three months after the beginning of the infection

Figure 7 – Hematocrit and parasitæmia profiles in a sheep infected with *Trypanosoma vivax*, that is able to control the infection

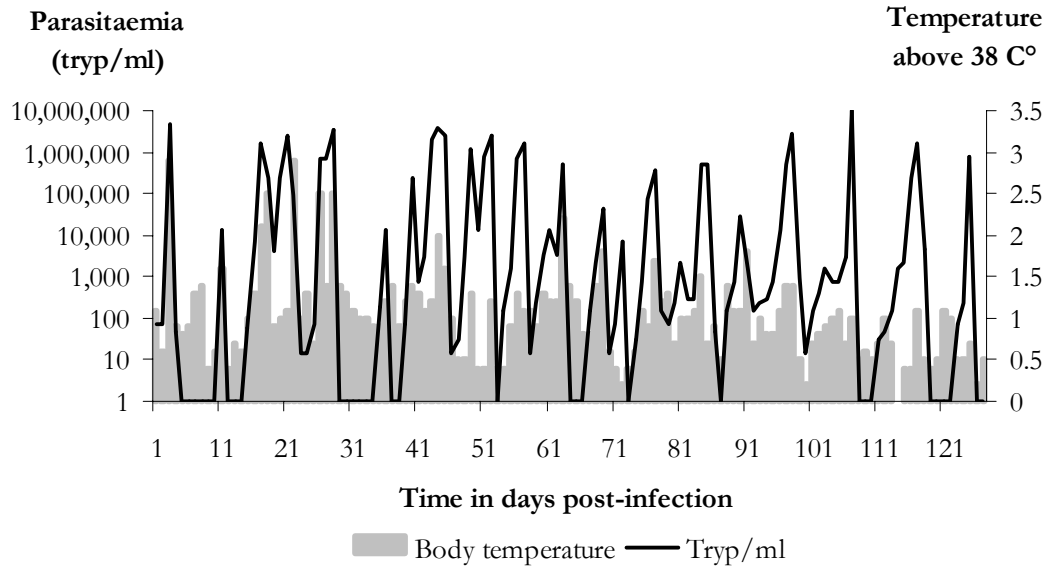
Remark: Throughout the document, a logarithmic scale is used for the y-axis that expresses the parasitæmia.

Parasitæmia: parasitæmia is always very high in the initial phases of the infection reaching or superseding 10^7 parasites/ml and can fluctuate widely or suddenly disappear (**Figs 7 and 8**). Thereafter, peak levels go down regularly (**Figs 7 and 9**). Parasitæmia control is variable from one animal to another and generally poor in pregnant females but satisfactory after three or four months for other animals.

Temperature: hyperthermia always occurs in the initial phases of the infection with temperatures reaching 40°C to 41°C. Peak temperatures coincide with peak parasitæmia as can clearly be seen on **Figure 8**. Later on, the peaks in temperature and parasitæmia gradually ebb.

Haematocrit: there is a significant drop in the haematocrit, which frequently goes from 35% to 20% and sometimes 15%. An average 30% to 40% decrease from the initial value is observed. The haematocrit value collapses very rapidly within three to ten days following infection and remains low some time – three to five months if no treatment is given. When curative therapy is administered, the haematocrit returns to its initial value or to that of control animals within four to eight weeks (**Fig. 7**).

Weight: during the initial phase of infection, weight loss ranges from 5% to 30%. Four sheep infected with the TVFG1 strain were put under observation for six months. The body weight index, at equal age, never returned to the same level as control animals. During the second phase of infection, either by spontaneous recovery or following treatment, return to initial weight occurred within two to four months.



Comments: To facilitate reading of temperature changes only temperatures above 38°C are charted. This animal is unable to control the parasitæmia even after four months of infection.

Figure 8 – Temperature and parasitæmia profiles in a sheep infected by *Trypanosoma vivax*

Repeat infections: when the parasitæmia is kept below the detection level (Woo's technique) over a long period, either naturally or following non-sterilising trypanocidal treatment, repeat infections occurred spontaneously or subsequent to specific stress conditions (**Fig. 9**). They are short-lived and the parasitæmia is low level. However, longer term repeat infections with high level parasitæmia have been induced by dietary restrictions over a period of two to four weeks (**Fig. 9b**). As of the 60th day of rationing, clinical episodes reoccurred – noticeable temperature and weakening with high parasitæmia.

Conclusions: experimental pathogenicity of *T. vivax* strains from French Guiana and Venezuela is clearly discernible in sheep; two animals of the thirteen had to be treated to avert a fatal outcome to the infection, while two others turned out to be highly susceptible and unable to control the parasitæmia during more than five months. The individual susceptibility of genetically very similar animals varies considerably. Pregnant females are always highly susceptible to infection (one abortion and two partial stillbirths out of four pregnancies). Some lambs were born prematurely while others died because the mother did not have enough milk. The economic impact of primary infections is therefore high in sheep.

Thereafter, following the clinical phase that lasted from two to four months, most of the animals became asymptomatic and aparasitæmic carriers of *T. vivax* whatever the strain used. When animal husbandry practises and feeding are adequate, carrier immunity apparently becomes established. This is facilitated by treatments with diminazene aceturate (that turned out to be non-sterilising; **Fig. 9**).

In animals whose carrier status is not detectable, the parasites and clinical signs reappear subsequent to long-term food rationing, in a way comparable to what is seen in the field during the dry season. This would suggest that sheep might act as a reservoir and source of contamination in the epidemiology of bovine trypanosomosis, particularly in view of the very perfunctory monitoring system for sheep farms that is seldom able to record and/or identify the signs of trypanosomosis due to *T. vivax*.

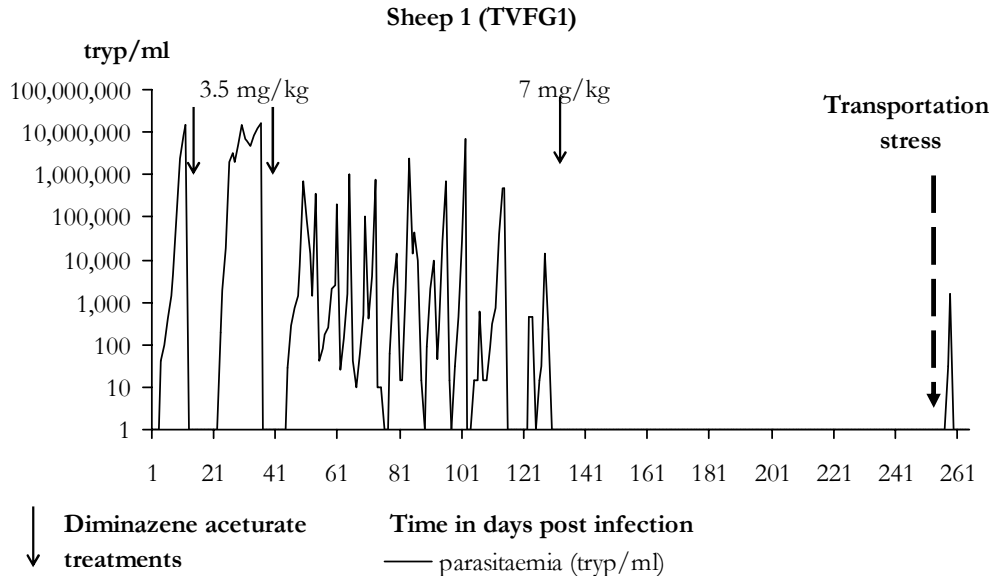


Figure 9a – Resumption of T. vivax *parasitæmia following transportation-induced stress in a sheep that has controlled parasitæmia for 160 days*

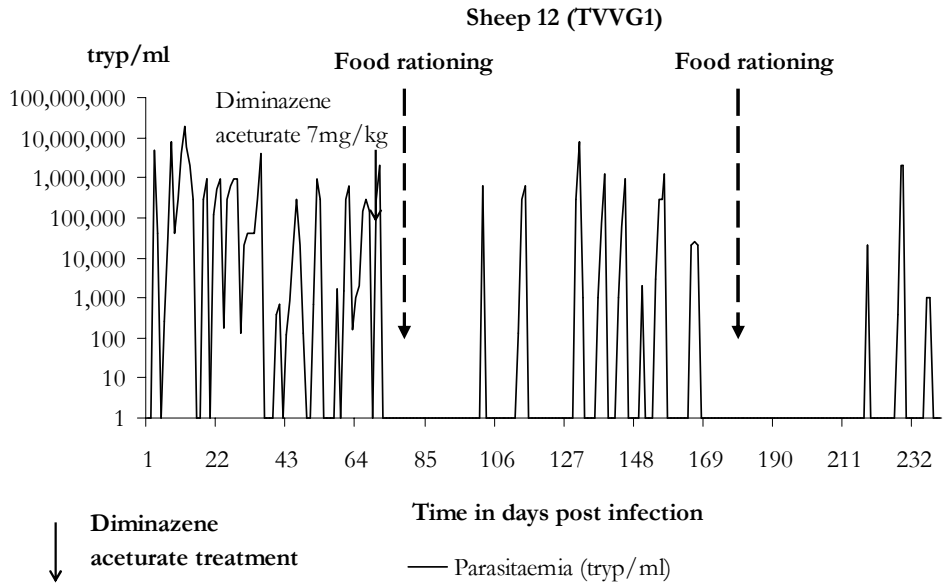


Figure 9b – Resumption of T. vivax *parsitæmia following food rationing in a sheep that had parasitæmia under control*

Figure 9 – Parasitæmic waves in two sheep infected with *Trypanosoma vivax* (TVVG1), parasitæmic resumption

1.1.2.2. Cattle

Experimentally induced infections in four Borans calves aged five months with the IL 4007 (TVFG1) strain [43] produced the same symptoms as those already described by CAMUS and MARTRENCHAR [147]; like in sheep, although identical inoculate are used, symptoms vary considerably from one animal to another ranging from transient listlessness to a complete halt in food intake along with permanent recumbency and nervous signs. In our experiments, when clinical signs indicated a possible fatal outcome, we applied treatment; this was required for one animal in four. These inter-individual variations should stimulate research on the analysis of resistance mechanisms in the so-called 'susceptible' breeds.

1.1.2.3. Comparison with African Strains

Although some African strains of *T. vivax* are moderately pathogenic (NYARKO, 1966, and HENNING, 1949 according to VOHRADSKY), many authors have recorded severe clinical courses that were often fatal (METTAM, 1934 and 1938, and STEWART 1936, 1937 and 1938, according to VOHRADSKY [154]) from which it can be inferred that *T. vivax* infections can be at least as severe as those of *T. congolense*.

In pregnant ewes [124], experimentally induced infections using a strain from Nigeria caused mortality of the mothers or abortion, intra-uterine transmission and non-viability in all the progeny. In sheep and goats, under experimental conditions, mortality was 25% with the *T. vivax* strains from Nigeria [36, 170] and 17% to 75% with those from Burkina Faso [171]. Their pathogenicity appears to be more marked than that of the American strains although it should be kept in mind that experimental conditions differ.

In crossbreed bovines (zebu × Baoulé) that were experimentally infected using strains from Ghana, the mortality rate was 75% [154]; with a strain from Burkina Faso, it reached 30% (BENGALY and DESQUESNES, unpublished paper). Isoenzymatic studies by karyotyping and DNA probes, conducted by DIRIE *et al.* [172] on African and South American *T. vivax* strains exhibited a great deal of similarity between the Colombian and West African strains. In spite of this, the epizootics described in Africa are generally far more deathly; the same applies to the results of experimental inoculations. Generally speaking, the South American strains are considered less pathogenic than the African ones [153]. This, however, needs to be confirmed by a comparison under identical research conditions. Considering the fact that in field conditions, 30% mortality rates are sometimes reached in the case of primary infection of cattle by American strains, further comparisons under identical conditions are needed to ascertain that assumption.

1.1.3. Discussion on apparent pathogenicity

Parasite pathogenicity:

Could it be that the highly pathogenic strains of *T. vivax* described by LEGER and VIENNE [18] and ZAPATA in South America have lost some of their pathogenicity?

FIENNES [173] stated that the virulence of the *T. vivax* strain may be alleviated in cattle by the sheer number of mechanical transmissions. A few years later, LEWIS (1954, according to HOARE [7]) observed that the first two passages of the strain are highly pathogenic but that thereafter they more often than not produce chronic infections; however, SFORZA (1940) did not record any decrease in pathogenicity after 50 passages of *T. vivax* on calves. It is therefore difficult to reach any final conclusion on this point.

Trypanotolerance:

For cattle, a distinction is made between four overall categories of susceptibility to trypanosomoses: 'trypanotolerant' animals – a category in which a number of African *Bos taurus* breeds fall (N'Dama, Baoulé, Muturu, Somba, etc.); susceptible animals, – zebu (*Bos indicus*); their crossbreeds whose susceptibility is intermediate; and finally improved European breeds of *Bos taurus* that are often highly susceptible [47, 174]. In Latin America, there are no trypanotolerant cattle breeds and the local zebu and European stock are fully susceptible to the disease. The Creole cow may possess the resistance genes of the African *Bos taurus* [20] since it is a cross between the latter and the zebu. In experimental infections with strains from French Guiana, individual variations observed in host susceptibility whether in the Borans of Kenya or the zebu and Creole sheep of Guiana should stimulate research into host genetics and isolation of the resistance genes (or susceptibility genes) in the so-called 'susceptible' populations (non-trypanotolerant).

Immune status of hosts

In addition to genetic features, the intensity of the disease essentially depends on the immune and physiological state of the host. Primary infections give rise to marked symptoms but repeat infections are less severe. The severity of the symptoms and the frequency of relapses depend in particular on the individual's nutritional status, degree of parasite infestation, whether or not intercurrent diseases are present, and pregnancy. It is not age dependent [7] although in calves [14] the progression of the disease is rare, but sometimes marked and fatal [175]. Diet plays a very important role in the course of the infection in sheep. Depending on its quality, animals range from being healthy carriers to noticeably diseased [43].

1.1.4. Conclusion

The severity of the symptoms due to *T. vivax* infection varies according to the pathogenicity inherent to the strain, the specific immune status (enzootic or new outbreak) and the non-specific immune status (animal husbandry standards on the farm, diet) of the host. It also varies from one individual to another (resistance or susceptibility genes?). The pathogenicity of strains can only be fully established under comparable conditions.

In the field, in America, mortality due to *T. vivax* in livestock is most often moderate [176], but high in the case of new outbreaks of infection [152]; the disease is characteristically epizootic on this continent.

In Africa, because of multi-specific infections, the situation is more complex. In some cases the mortality rates recorded are high regardless of the strain, whether the East African [177] or the Central [175] and West African [178], which are thought to be highly pathogenic [179]. Furthermore in Africa the often highly enzootic status of trypanosomoses produces a certain degree of immunity amongst animals. Under these circumstances, epizootic outbreaks (characteristic of a newly infected population) are unusual and seasonal enzootic developments are more commonly observed.

The clinical signs of *T. vivax* infection in South America are highly variable; they can be completely imperceptible, or cover the full range from transient fever to death, through varying degrees of growth loss, decreased output, lack of appetite, abortion and wasting. These manifestations are generally marked in the case of an epizootic occurring for the first time in a disease-free area, or one where the disease has not been recorded for several years; subsequently, these signs scale down until they disappear entirely as premunition immunity grows or the parasite clears spontaneously.

The virulence, and moreover the diversity, of the mechanically transmitted strains in America are no doubt less than those of the cyclically transmitted strains in Africa. This, in addition to inadequate animal husbandry standards, is possibly the explanation for the apparently more marked pathogenicity in Africa than America [153].

There is a striking opposition between the high genetic polymorphism of the cyclically transmitted vector-borne strains in Africa and the probable genetic 'oligomorphism' of the strains in Latin America whose transmission is exclusively mechanical. polymerase chain reaction (PCR) fingerprinting using random primers would be useful to support this hypothesis.

1.2. *Trypanosoma evansi*

In Latin America, horses are the farm animals that are most susceptible to infection followed by buffaloes. Pathogenicity for other farm animals is lower and poorly documented. Dogs are highly susceptible and act as sentinel animals and perhaps a reservoir.

1.2.1. Horses

Prevailing symptoms in horses are recurrent fever [180], with transient hyperthermia, anaemia and weakness, ophthalmia with lacrimation and conjunctivitis, rash, enlarged lymph nodes, weight loss and, classically, œdema of the intermandibular region, the limbs and the abdomen [181]. Intraocular haemorrhage [56] and nervous symptoms that occur when the parasite passes into the central nervous system and the aqueous humour [14] have been observed. The parasite multiplies at the puncture site sometimes causing a cutaneous reaction [182].

In the epizootic areas in Brazil, morbidity and mortality rates in horses are sometimes very high – respectively 97%, and 50% to 83% [183]. In the enzootic areas in Argentina, infections are sometimes asymptomatic [49] while others are clinically very severe [184]. *Trypanosoma evansi* pathogenicity in South America is therefore highly variable and, in this respect, comparable to that of Africa and Asia [17]. However, there are variations in the pathogenicity, perhaps even in the 'morphology' of the parasite, which have led to the disease being given several different names. *Trypanosoma equinum* is thought to be a dyskinetoplastic variant, which is less pathogenic than *T. evansi* in horses, and moderately pathogenic in mules [14].

More so than *T. vivax*, *T. evansi* has extravascular locations due to its nervous and muscular tropism. Experimental infections performed on rats (*Proechimys* sp.) have shown the parasite to be present in all the organs – the tissue is infiltrated by mononuclear cells [90].

1.2.2. Buffaloes (*Bubalus bubalis*)

Trypanosoma evansi is markedly pathogenic for buffaloes. In Indonesia, the infection has a clinical course that lasts from one to seven weeks with weakening, loss of appetite, anaemia and thrombocytopenia, along with myocarditis, hydropericardium with petechiae, interstitial pneumonia, liver congestion, congestion and necrotic foci in the spleen, enlarged lymph nodes, bone marrow hyperplasia and interstitial myositis [185]. In Vietnam, high rates of abortion have been reported by NGUYEN DANG KHAI (1995, quoted by Touratier [88]); in India, mortality is high in untreated animals (WALIA *et al.* quoted by Touratier [186]).

Using immuno-histochemical techniques, SUDARTO *et al.* [187] have shown the presence of parasites in the Virchow-Robin spaces of the brain of an infected buffalo suffering from meningo-encephalitis; muscular lesions (muscle fibre and blood vessel necrosis with mononuclear infiltration) have also been reported [188].

In Venezuela, GARCIA and ASO [189] have found spleen, liver and glandular enlargement together with lymphoproliferation, but, the economic impact of infections has not been assessed. LUCKINS [5] reports reproductive disorders in buffaloes and warns against immunosuppression induced by *T. evansi*, which could interfere with the use of certain vaccines. LOHR *et al.* [190] point out that the infection can foster the development of intercurrent diseases that are usually kept well under control.

1.2.3. Dogs

The discovery of *T. evansi* infections in dogs may indicate the undetected presence of the parasite in a given geographical area (sentinel animal), but the canine species is possibly also a reservoir for the parasite. In dogs, the infection is usually acute with intermittent fever (38°C-40°C), œdema of the head, abdominal wall and legs [181]; a number of more specific signs are sometimes detected: keratitis and haemorrhage in the anterior chamber of the eye [56]. Hypochromic microcytic anaemia and high leucopoenia have been reported, along with gradual weakening, lack of appetite and paresis of the hindquarters [181].

1.2.4. Cattle

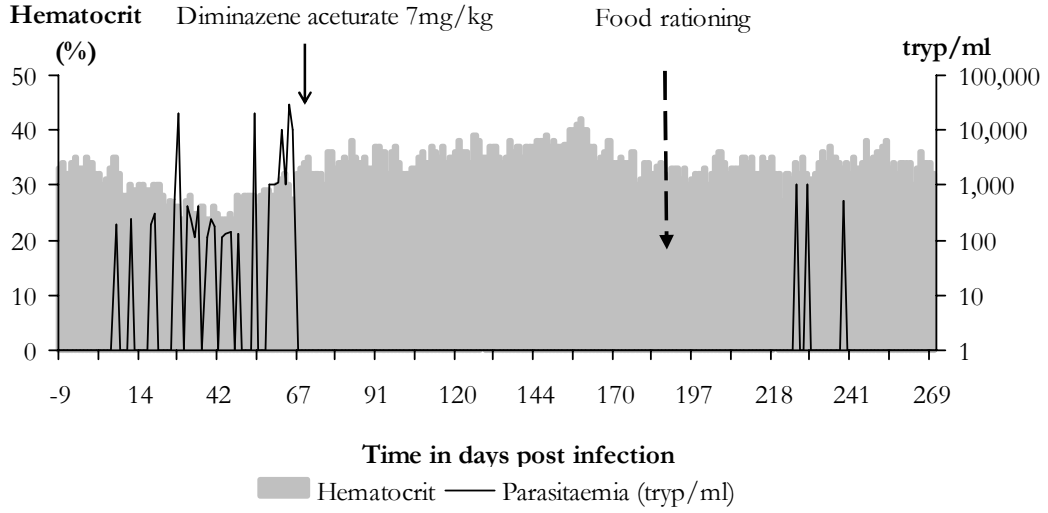
The impact of infection in cattle is fairly variable depending on the strains used. The strain isolated in South America by KAGERUKA and MORTELMANS [191] does not appear to be infective for five month-old Borans calves in spite of the fact that they came from a trypanosomosis-free area [43]. Its virulence in cattle may have been attenuated by the many passages on rodents.

Conversely, using an Indonesian strain, PAYNE *et al.* [77, 78, 79] report hyperthermia, haematocrit drops and loss of weight in experimentally infected calves and heifers and in naturally infected fattening stock. Other authors have recorded more severe symptoms and sometimes death [56]. In Thailand, cattle raised in natural conditions have been found to exhibit nervous signs, e.g. excitement, aggressiveness, seizures leading to death [192].

In addition, immunosuppression brought about by the infection adversely affects ruminant immunisation by vaccination[88] and fosters the development of intercurrent diseases.

1.2.5. Goats and sheep

Experimental infections in sheep using the *T. evansi* strain from Venezuela give rise to slight fever (40°C maximum) that coincides with the parasitæmic peak, widely fluctuating, low-level parasitæmia (< 10^5 parasites/ml), a 20% to 25% drop in the haematocrit value for two months post infection which then either returns to the initial value (**Fig. 10**), or fluctuates below the normal value [43]. During the temperature peaks, the animals are sometimes listless or, on the contrary, excitable and aggressive, as observed by TUNTASUVAN *et al.* [192] in cattle. In our experiment, following treatment with diminazene aceturate and once parasites were no longer detectable in the bloodstream, a regimen of dietary restrictions caused detectable parasitæmia (HCT) after 20 and 30 days (**Fig. 10**). Throughout the 300-day follow-up period, the animals remained carriers of the parasite exhibiting aparasitæmic periods of up to 160 days.



Comment: food rationing causes parsitæmia to reappear that can be detected using Woo's technique 30 days after the start of the rationing programme.

Figure 10 – Parasitæmia and hematocrit in a sheep infected with Venezuelan *Trypanosoma evansi* (TEVA1)

Diminazene aceturate treatments are commented on in Chapter 8.

This experiment established the limited but effective pathogenicity for sheep and their potential role as a reservoir for the parasite. A more thorough investigation of pathogenicity, specifically in

the tissues, would help to make a more accurate assessment of the medical and economic impact of these infections.

Under similar conditions, with the *T. evansi* clone RoTat 1.2 [193], the initial peak parasitæmia levels are 10^4 to 10^6 parasites/ml depending on the cases [43]; clinical signs are short-lived (approximately ten days) and vague: fever, lack of appetite, excitability. They are largely comparable to those observed with the Venezuelan strain.

Furthermore, the immunosuppressive effect speculated for cattle has been experimentally established for sheep – leukocyte and lymphocyte proliferation is reduced during the active infection by *T. evansi* and vaccination against *Pasteurella hæmolytica* in infected animals is less effective, including on those that were given sanative treatment [194, 195].

1.2.6. Conclusions

Trypanosoma evansi pathogenicity varies from one strain to another as does the receptiveness of different species of hosts and possibly between individuals belonging to the same species. The clinical impact is generally high in horses and dogs, in particular during epizootic outbreaks and more moderate in the enzootic areas; it is less strong in buffaloes and, under the local farming conditions, probably unnoticeable in sheep, cattle and goats. It is interesting to compare these observations with those made by GONZALEZ *et al.* [196] in goats. They found them to be a possible reservoir for the parasite with inconspicuous symptoms including a drop in the haematocrit. The impact of *T. evansi* infections on domestic ruminant production has been demonstrated but not widely investigated in Latin America. Their immunosuppressive effects in ruminants deserve attention as their effect on intercurrent diseases may be greater than the direct effects of the infection itself.

1.3. Other trypanosomes

Trypanosoma equiperdum: in most cases dourine is a chronic disease in horses that gives rise to moderate fever, gradual loss of weight, and characteristic genital œdema. Dependent œdema is also observed, together with hindquarter paresis, wheals, and occasionally pulmonary symptoms. The clinical outcome of disease is often fatal within 8 to 20 months. Although the disease has not been reported in Latin America for the last 15 years, caution is advisable as international sanitary regulations are such that they possibly discourage official notification of the disease.

Trypanosoma theileri is generally considered to be a non-pathogenic [7, 197] or, an opportunistic microorganism particularly in the case of *T. vivax* trypanosomosis [43, 46]. Although the exact succession of events cannot be established, some authors have recorded high parasitæmia during rinderpest vaccination campaigns (GALLIARD, 1925, according to HERBERT [198]; HORNBY and BAILEY, 1929, according to HOARE [7]).

In some cases, *T. theileri* infections in cattle are reflected in high parasitæmia along with clear clinical signs [135, 136], and anaemia and lymphocytosis [137, 138, 199]. However, in most of these cases, the authors state that the responsibility of the parasite in the clinical expression has not been fully established, and that another, undiagnosed pathology may be the cause of the outward signs [198]; reproducing this situation experimentally would be useful to ascertain this.

Trypanosoma cruzi: little is known about the pathogenicity of *T. cruzi* in livestock but it appears to be fairly low according to the few experimental findings available. ALCAINO *et al.* [145] report that no symptoms were visible in an experimentally infected kid although they did detect ventricular hypertrophy using ECG. The long-term effects of the infection deserve more thorough investigation. Here again, little research has been conducted either in the field, where no specific diagnostic tools are available, or under experimental conditions owing to the limited risk of human infection and/or a lack of interest in this work because its pathogenicity is presumed to be low and

because of the short economic life expectancy of farm animals. *Trypanosoma cruzi* is markedly pathogenic in dogs and produces cardiac signs with a potentially fatal outcome.

1.4. Clinical confusion

Laboratory diagnostic techniques for trypanosomoses are described in Chapter 4. At this point, it is useful to just briefly describe the instances of possible confusion between one trypanosomosis and another, and with other diseases encountered in Latin America.

In the event of suspected or confirmed outbreaks of *T. evansi* infections in horses, it is important to reach a definitive diagnosis in dogs. However, it is necessary to distinguish *T. evansi* from *T. cruzi*; there are no specific clinical signs but cardiac signs argue in favour of a *T. cruzi* infection while ocular signs point to *T. evansi* infections – in both cases the disease can rapidly be fatal. Generally speaking, only laboratory tests can discriminate the two.

1.4.1. Trypanosomoses in horses

Equine trypanosomosis caused by *T. evansi* can be clinically mistaken for equine infectious anaemia (EIA), referred to as '*pesta bova*' which, like '*Derrengadera*', can be transmitted by horseflies. In its chronic form, it causes feverish episodes, anaemia, dependent œdema and weight loss [200]. *Trypanosoma equiperdum* infections can also be confused with '*Derrengadera*' especially the cutaneous, ocular and nervous forms. On the other hand, genital œdema is more characteristic [55]. *Trypanosoma cruzi* infections in horses are very poorly documented and the clinical signs have not been precisely described; additional research is needed to determine the pathogenicity of *T. cruzi* for this species.

Some of these infections can be differentiated using laboratory diagnostic procedures but in the case of akinetoplastic strains of *T. equiperdum* or *T. evansi* [88], and mixed infections, discrimination may be unlikely or even impossible.

1.4.2. Trypanosomoses in ruminants

Unless there is an epizootiological context (for instance the spread of *T. vivax* trypanosomosis outbreaks on the Brazilian/Bolivian border in 1996-1997 during a vaccination campaign against FMD), it is difficult to diagnose blood parasitosis in ruminants without using laboratory tests. Indeed, the clinical signs of *T. vivax* trypanosomosis – anaemia, weight loss and weakness – are the same as for other blood parasitoses (*T. evansi* trypanosomosis, anaplasmosis, babesioses etc.), some helminth infestations, and even a number of viral infections. Although the presumptive evidence is sometimes strong, the diagnosis can only be established by laboratory tests and these are rarely performed. For this reason, it is worth briefly recalling the characteristics of other blood parasitoses in cattle, which may interfere with the clinical diagnosis and possibly even the laboratory diagnosis.

Theileria mutans found in Guadeloupe [201] and Martinique [23], was investigated by means of a serological survey in French Guiana [202]; all the serum samples were negative [43] and the parasite has never been reported there.

Symptoms in ruminants infected by *T. vivax* or *T. evansi* have been described above; the differential diagnosis for these two infections can only be determined by laboratory tests and should be considered in areas where both coexist.

1.4.2.1. Ehrlichia bovis

Bovine ehrlichiosis was described for the first time in Brazil in 1982, by MASSARD *et al.* [203]; it is transmitted by *Amblyomma cajennense* [204]. *Ehrlichia bovis* parasitises monocytes and macrophages and occasionally is found in lymphocytes [205]. After a one to three-week long incubation period,

the symptoms are essentially a fever that can last for more than a week, enlarged lymph glands, anorexia, apathy, weight loss and immunosuppression that is conducive to the development of intercurrent diseases [204, 206]. Blood smears can be used for diagnostic purposes but, because many animals are asymptomatic carriers [206], ehrlichiosis can be confused or coexist with other blood parasitoses.

1.4.2.2. Anaplasma marginale

Anaplasmosis is an infectious, virulent, inoculable, non-contagious disease that affects ungulates. It is caused by a Rickettsia, an intracellular bacteria of the genus *Anaplasma* that parasitises erythrocytes. On a Giemsa-stained blood smear, the parasite shows as a purplish corpuscle located on the inner margin of the erythrocytes (**Fig. 11**). *Anaplasma marginale* is transmitted by ticks, or else mechanically by biting diptera (horseflies, *Stomoxys*). In certain geographical areas where tabanid pullulate intensively such as in Louisiana [207] and French Guiana [208], these insects are thought to play a predominant role in transmitting anaplasmosis. *Anaplasma marginale* is considered to be moderately or non-pathogenic for sheep, goats and water buffalo but pathogenic for cattle. Bovine anaplasmosis is more often than not enzootic; in this sort of context, because young animals are not very susceptible and adults are premunised, clinical signs are scanty and vague.

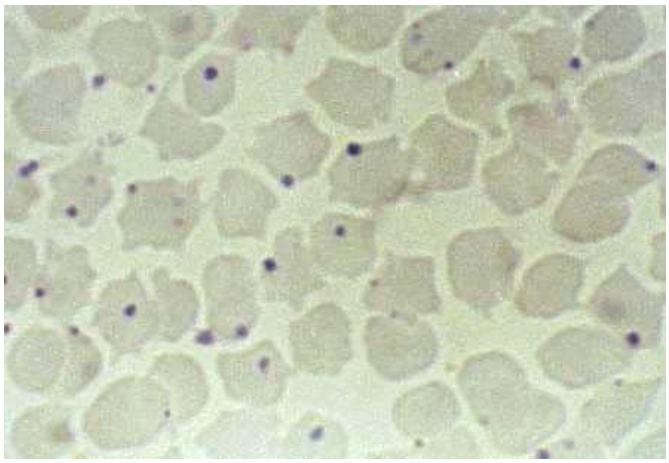


Figure 11 – *Anaplasma marginale* on a stained smear (M. DESQUESNES)

In Guiana for instance, its clinical incidence as confirmed by laboratory tests is very low in the local zebus. It is most often rife at the end of the dry season and is expressed as subacute or chronic forms that affect animals of approximately one year of age. The clinical signs are fever accompanied by inappetence, anaemia and jaundice, enlarged lymph nodes, ruminal atony, followed by constipation and weight loss. This clinical state is very similar to that of trypanosomosis caused by *T. vivax*. In cattle imported from Europe and when no prevention measures are implemented, acute anaplasmosis affects nearly 100% of the animals within a month following their arrival when they come into contact with local animals. The main manifestations are: fever with polypnea and hyperthermia (40°C-41°C), marked anaemia (chalk-coloured mucosae, haematocrit less than 15% and sometimes 10%) and very significant weakening. Animals that are not treated soon die. Bovine trypanosomosis too occurs in a very acute form among imported animals, which is why the two can easily be confused. Individual parasitological diagnosis is done by examining stained blood smears. The infection is easy to detect on imported animals as parasitæmia is extremely high (**Fig. 11**), but far less conspicuous in local livestock. Recommended treatment is with imidocarb and tetracyclines. When the anaplasmosis is enzootic it can exacerbate other diseases including trypanosomosis and hence make it difficult to establish a diagnosis. Conversely, immunosuppression due to *T. vivax* causes anaplasmosis to develop, which in turn exacerbates the trypanosomosis symptoms [209]. Anaplasmosis and *T. vivax*-induced trypanosomosis in cattle are therefore often clinically confused.

1.4.2.3. Tropical bovine babesioses

Babesioses are virulent, inoculable, non-contagious, infectious diseases that affect most domestic mammals [61]. They are caused by sporozoans of the genus *Babesia*, located in the red blood cells of the definitive host. Typically, babesias take the shape of a large (*B. bigemina*) or small (*B. bovis*) double pear on a blood smear along with many other intermediate shapes (**Fig. 12**). Babesiae are transmitted by ticks (transovarian transmission). In Latin America, *Boophilus microplus* is the main

biological vector and natural reservoir for tropical bovine babesioses: *B. bovis* and *B. bigemina*. Clinical signs of babesioses are anaemia together with haemoglobin release causing haemoglobinuria (*B. bigemina*) or release of vasoactive substances that can generate a circulatory shock with nervous or motor symptoms (*B. bovis*).

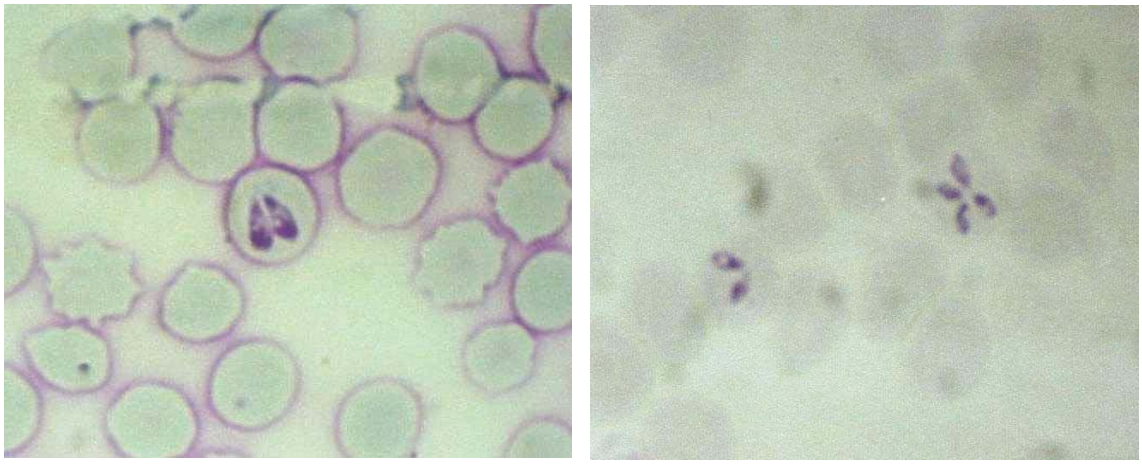


Figure 12 – *Babesia bigemina* (left) and *Babesia bovis* (right) on stained smears
(M. DESQUESNES)

In French Guiana, clinical babesioses confirmed by laboratory tests are very rare. *B. bigemina* babesiosis has been clinically observed in European cattle (*Brune des Alpes* breed) with typical symptoms: fever, anaemia (haematocrit < 15%), jaundice and brown colouring of urine (haemoglobinuria and bilirubinemia) (J. FAVRE, unpublished contribution). In zebus, there have been suspicions in peracute cases that were rapidly fatal but because these were not confirmed by blood smear examination they cannot be attributed to babesiosis with certainty.

B. bovis babesiosis has been a found on livestock imported from Europe that exhibit fever and predominantly nervous symptoms: unsteadiness, head pressing. Serological signs are less obvious: normal mucous membranes, haematocrit over 15%. Furthermore, the disease rapidly leads to death. Recommended treatment is based on imidocarb or diminazene aceturate.

In European cattle, the acute and peracute forms of babesioses can be confused with trypanosomosis; the same applies to zebus with the chronic forms of these diseases. Differential diagnosis is made even more difficult by the fact that the most commonly used treatment is the same for trypanosomosis and for babesioses (diminazene aceturate) and that the clinical improvements thus achieved work as 'therapeutic diagnosis' alternatively in favour of babesioses or of trypanosomosis depending on the user.

1.4.2.4. Conclusion

In the field, where cattle are raised in semi-extensive or extensive conditions as is generally the case in that part of the world, it is very unusual to call on diagnostic laboratory tests. Furthermore, stock farmers tend to apply treatment at an early stage which interferes with the diagnosis, particularly in view of the fact that some of the substances that are effective against trypanosomosis also work against babesiosis (diminazene aceturate), bacterioses and anaplasmosis (tetracyclines), or babesioses and anaplasmosis (imidocarb). The latter also appears to be active against all three blood parasites since its efficacy has been demonstrated against *T. brucei* [210], and observed on *T. vivax* [147]. Furthermore, these substances have general anti-bacterial and anti-parasitic effects that can give rise to transient improvement in many cases. Much confusion in the therapeutic diagnosis arises as a result of the clinical improvements observed after any treatment. In all cases, complete recovery can only be obtained if the choice and dose of the medication are entirely suited to the blood parasite that is responsible for the symptoms.

Bovine trypanosomosis due to *T. vivax* can clinically be confused with many other cattle diseases; when it is expressed by anaemia and weakening, it essentially needs to be differentiated from *T. evansi* trypanosomosis, bovine anaplasmosis (*Anaplasma marginale*), *B. bigemina* babesiosis and from a number of helminth infestations that cause anaemia such as hæmonchosis (*Hæmonchus contortus*), and in Brazil, bovine ehrlichiosis.

When it shows in the form of nervous symptoms, it needs to be differentiated from rabies and *B. bovis* babesioses, and possibly from poisoning by fodder due to nitrates (*Brachiaria* sp. *var.* Tanner), alkaloids (*Datura stramonium*), monofluoroacetic acid (*Palicourea* spp.), heterosides (*Asclepias currassavica*) etc. [211]. The only way to distinguish between these disorders is to conduct field studies and laboratory tests.

In buffaloes, it is particularly easy to confuse *T. vivax* and *T. evansi* infections because the receptiveness of the animals to both parasites is fairly similar.

In the South American context where all or part of these infections and/or disorders are possible, it is essential during epidemiological surveys to look at the full range of blood parasites present in order to assess the relative incidence of a given blood parasitosis, in particular trypanosomosis.

2. PARASITE CHARACTERISATION

2.1. General considerations

Many different characterisation techniques have been applied to trypanosomes, including:

- examination of parasite morphology [7];
- study of pathogenicity by host species [212];
- infectivity for *Glossina* [40, 162, 172, 213], mice [172, 214], guinea pigs [215], etc.;
- sensitivity to various trypanocidal drugs [216], *in vivo* [217, 218], and *in vitro* sensitivity [60, 219, 220];
- isoenzyme analysis, which for a long time was the main technique for intra- and interspecific characterisation [172, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231];
- pulsed field gel electrophoresis [232, 233];
- cross immunity tests *in vitro*: immunolysis test [234], cross immuno-electrophoresis [235];
- passive [235] or active [172] cross protection tests;
- more recently DNA characterisation using RFLP (restriction fragment length polymorphism) [236];
- DNA fingerprinting by PCR with arbitrary primers [237, 238, 239];
- the use of species-specific nuclear [236, 240], or kinetoplast DNA probes [4, 241];
- genomic analyses by nuclear and/or kinetoplast DNA sequencing [242, 243].

These analyses, which are often long-winded and costly, are unquestionably relevant in fundamental terms. Depending on the method, they can be used to investigate the phylogeny of parasites, their mode of reproduction (clonal [244], conjugate [245]), certain aspects of their pathogenicity (apathogenic, haemolytic strains, etc.) the specificity of their host species, sensitivity to trypanocidal drugs, their shared or specific antigenic and immunological features, etc. However, with the exception of the study of trypanocide resistance, these analyses have so far led to very few practical measures or applications.

In general, characterisation techniques require large quantities of parasites; there has therefore been limited work on *T. vivax*, which is extremely difficult to culture *in vitro* or to adapt to rodents. There are major obstacles in producing this strain on ruminants, especially the quality of parasite/blood cell separation [238]. PCR techniques, which require very small quantities of the parasite, are therefore well suited to *T. vivax* characterisation.

2.2. Characterisation of South American *Trypanosoma vivax* and *Trypanosoma evansi* strains

2.2.1. *Trypanosoma vivax*

The earliest work on characterising American *T. vivax* was performed by LEGER and VIENNE [18] on strains from French Guiana. They describe the marked pathogenicity of the strain in cattle and its dimensions that are comparable to those reported for the species in Africa (see Chapter 1). CAMUS and MARTRENCHAR [147] and DESQUESNES *et al.* [43] carried out additional investigations into the morphometry and pathogenicity of several strains isolated in French Guiana that confirmed the pathogenicity findings in respect of cattle and sheep.

FABRE [215] indicated that *T. vivax* in the French West Indies cannot be cultured in guinea pigs; DIRIE *et al.* [172] and DESQUESNES [43] note that the strains from Colombia, Venezuela and French Guiana cannot be cultured on mice. ROUBAUD *et al.* [40], HULL [162], and DIRIE *et al.* [172] have shown that the *T. vivax* from South America and from the French West Indies is incapable of infecting *Glossina*.

In Colombia, OTTE [246] recorded *T. vivax* resistance to isometamidium chloride (see Chapter 6).

In Africa for the purposes of characterising *T. vivax*, a distinction is generally made between the East and the West African strains [231]. MURRAY found identical profiles for the Nigeran and Colombian strains using isoenzyme analyses. DIRIE *et al.* [172] compared FOUR strains of *T. vivax* from Colombia to African strains – they report that the parasites cannot be cultured *in vitro* [248]; that the isoenzyme analyses and karyotyping brought out strong commonalities between the Colombian strains and the West African strains but that they do discriminate all the strains investigated; that the DNA probe described by DICKIN and GIBSON [249] hybridises fully with the Colombian and West African parasites whereas it does not recognise the parasite isolated in Kenya.

However, the most interesting findings come from cross-immunogenicity tests and DNA fingerprinting with arbitrary primers.

2.2.1.1. Cross-immunogenicity

The immunolysis tests conducted by DIRIE *et al.* [172] show that the immunogenicity in the Colombian parasites is lower than that of the West African parasites but that they have at least partially shared antigenicity since the sera of the animals infected with the Colombian strains do not lyse the West African parasites whereas the opposite is true.

Cross-protection experiments conducted by DIRIE *et al.* [172] with four Colombian strains showed total protection between the strains. Added to the other characteristics observed, the authors conclude that there is probably a single serodeme for these four strains in spite of the fact that they were isolated from locations that were very far from one another.

The cross-protection studies between a *T. vivax* strain in French Guiana and the Venezuelan strain (TVVG1) show only partial-cross protection; the strains appear to belong to similar but different serodemes [43].

Several serodemes therefore probably exist on the continent; movement of parasite strains, and hence of their potentially infected hosts, needs therefore to be controlled. International trading in South American livestock should be subjected to the requirement of a sterilising treatment against *T. vivax* parasites so as to avoid spreading the different serodemes throughout the continent. Attempts have been made to support the theory of low heterogeneity among the investigated strains through the study of the genetic characteristics of strains by means of PCR with arbitrary primers.

2.2.1.2. AP-PCR characterisation

Trypanosome characterisation with the arbitrary priming-PCR method was initiated by WAITUMBI and MURPHY [237]. It relies on the interpretation of specific patterns or fingerprints obtained by a PCR with short oligonucleotides of arbitrary base sequence. Out of the 13 primers tested, one 10-base oligonucleotide (IL0525) was able to differentiate all 37 isolates of *T. congolense*, *T. simiae*, *T. brucei* and *T. evansi* that were challenged. The method has also been applied to *T. vivax* by DIRIE *et al.* [230] who were able to distinguish all of the clones or isolates of *T. vivax* challenged with IL0525. The main conclusions that can be reached from these investigations are consistent with most of the data supplied by the other characterisation techniques, i.e. the similarity between the Latin American *T. vivax* strains and those from West Africa and their divergence from those isolated in Kenya.

The same oligonucleotides [43] was used to study three strains of *T. vivax* from French Guiana-TVFG1 (or IL4007), TVFG2 and TVFG3, and one *T. vivax* strain from Venezuela isolated in the state of Guarico (TVVG1). The fingerprints differ only very slightly from those of the Guyanan and Venezuelan *T. vivax* strains, all of which exhibit six bands or major doublets (**Fig. 13 a**, col. 6,9 and **Fig. 13 b**, col. 2-4, 8 and 10) with the exception of the TVFG3 strain which displays one additional high molecular weight band (1,350 bp) (**Fig. 13 a**, col. 10 and **Fig. 13 b**, col. 6-7). However, they are clearly distinguishable from the reference African strain, IL3568, (**Fig. 13 a**, column 11).

The fingerprints obtained using the strains from French Guiana and Venezuela are very similar to those obtained by a DIRIE *et al.* [238] using Colombian strains that were isolated on the coast (IL3842, Monteria, IL3841, Lorica). There are probably great similarities in the genetic material of these parasites.

In the experiment conducted by DIRIE *et al.* [238], the 13 African and Colombian strains were discriminated although the cross-protection tests between the Colombian strains showed no difference in immunity.

On the other hand, DESQUESNES [43] did observe differences in immunity between the *T. vivax* strains from Venezuela and from French Guiana whereas AP-PCR differentiation is practically impossible. This diagnostic tool is therefore not powerful enough to show up the immunological characteristics of the strains investigated.

With the strain sensitive to diminazene aceturate (TVFG3), a 1,350 bp product is visible which is not found with the other strains (TVFG1, TVFG2 and TVVG1) that are resistant to the substance. Additional investigation would be required to determine whether this band can act as a resistance marker.

The obvious benefits of these studies are that they are simple, rapid and specific and only require a minimal amount of parasites. However, their reproducibility needs to be tested.

Figure 13 a

Key: Gel electrophoresis photographed under UV light: (1) molecular marker pBR 322 DNA Msp I Digest; (6) TVFG1/sheep; (7) non-infected sheep DNA; (8) non-infected mouse DNA; (9) TVFG2/sheep; (10) TVFG3/sheep; (11) *T. vivax* IL3568/mouse; (12) molecular marker ϕ X 174 DNA Hae III Digest.

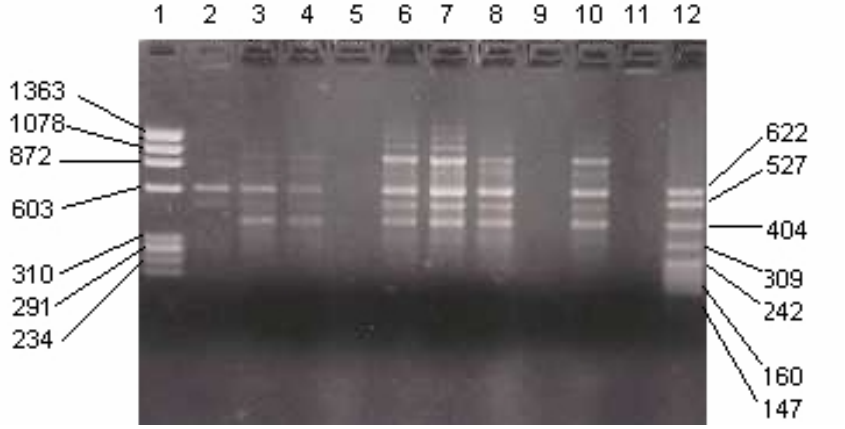


Figure 13 b

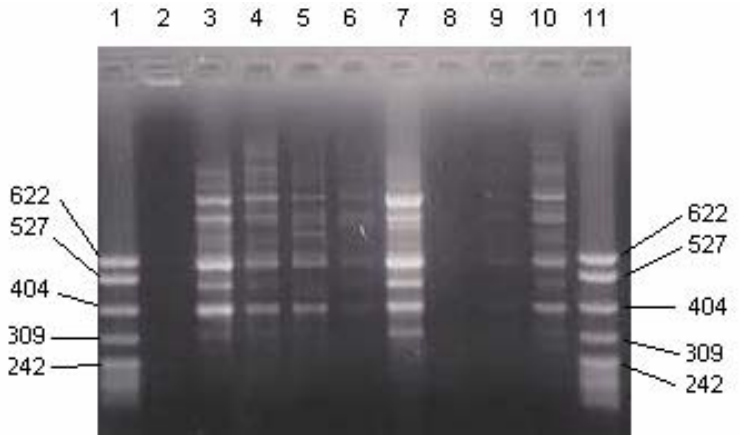
Key: Gel electrophoresis photographed under UV light: (1) molecular marker ϕ X 174 DNA Hae III Digest; (2) TVFG1/bovine 354; (3) TVFG1/sheep; (4) TVFG2/sheep; (5) non-infected bovine DNA; (6-7) TVFG3/sheep; (8) TVVG1/sheep (9) non-infected sheep DNA; (10) TVVG1/sheep; (11) distilled water. (12) molecular marker pBR 322 DNA Msp I Digest.

Figure 13 – PCR products obtained with the IL0525 oligonucleotide on various strains of *Trypanosoma* spp.

2.2.1.3. Genetic polymorphism of strains

In the experiments conducted by DIRIE *et al.* [238], the IL0525 primer was able to distinguish the *T. vivax* isolates, but the oligonucleotide's ability to discriminate between subpopulations within a single isolate was not tested as the strains under investigation had been isolated on only one occasion. If successive isolations of parasites originating from a single isolate exhibit different fingerprints, it can be inferred that the strain's structure is polyclonal, which shows up as a different dominant population for each parasitæmic wave.

The genetic material of parasites isolated during five successive parasitæmic waves in a sheep infected with TVFG2 was challenged using IL0525. The gel profile is reproduced in **Figure 14**; 600-630 bp, 850-900 bp and 1,050 bp products continued to be visible; a product of approximately 300 bp is visible in the first two isolations, disappears in the next two and becomes clearly visible once again during the fifth parasitæmic wave. The 488-500 bp product is only visible during the third and fourth parasitæmia peaks. Finally a product made up of approximately 750 bp does not become visible until the third parasitæmic wave. What the experiment has shown is that there are small genetic variations among isolates from a single strain [43]. However, because bands vary in intensity, one cannot be completely certain that a sequence is absent.



Key: Gel electrophoresis photographed under UV light: (1) and (11) molecular marker pBR 322 DNA Msp I Digest; (2) distilled water; (3-7) TVFG2/sheep no. 8; from 3 to 7 the isolation dates for TVFG2 are given in number of days post infection: (3) J9; (4) J17; (5) J34; (6) J50; (7) J 68; (8) distilled water; (9) TVVG1/sheep; (10) TVFG1/sheep; (11) marker pBR 322.

Figure 14 – PCR products obtained with the IL0525 Oligonucleotide on various isolates of a single strain of *Trypanosoma vivax* (TVFG2) cultured in sheep

Discussion-conclusion: under the same test conditions, a monoclonal strain would exhibit a constant fingerprint for all parasitæmic peaks since its genetic material is constant. Variability in the fingerprints of the TVFG2 strain from parasitæmia wave to another indicates that it is a polyclonal strain. The ability of the IL0525 probe to differentiate between all *T. vivax* strains therefore does not apply solely to strain differentiation but also to the differentiation of the temporarily dominant populations within one strain.

However, this interpretation partially relies on the 'quantitative' aspect of PCR that is still poorly understood, particularly in the case of arbitrary amplification.

It should be noted that the polymorphism observed in the TVFG2 strain is moderate, similarly to the strains from Guyana and Venezuela. A comparative study between the African and American strains would be helpful in verifying the theory that moderate polymorphism, or 'oligomorphism' is related to the mechanical transmission of Latin American strains, whereas high polymorphism is found in African isolates collected in areas infested by *Glossina*; if this is the case, their polymorphism is connected with the parasite's cyclical transmission (modification of genetic material in the insect), heavy parasitic pressure (multiple infections), and the abundance of reservoirs among wild animals in which strains selected differ from those typical of domestic livestock. This theory is indeed supported by the fact that genetic hybridisation with *T. vivax* appears to occur only under some very specific circumstances [244, 250] and exclusively in the intermediate host, i.e. *Glossina* [251]. A more thorough investigation of the strains isolated in Latin America would furthermore provide evidence that the other American strains of *T. vivax* are also only slightly polymorphic.

The greater the genetic polymorphism of a strain, the greater the probability that that strain will present a broad antigenic repertoire. Knowledge about the genetic polymorphism of an isolate would provide insight into its ability to evade the host's immune system and hence its pathogenicity. This would have implications for the control methods being considered – a strain with moderate polymorphism could be naturally controlled by a few additional non-sterilising treatments on the livestock in view of its limited antigenic repertoire; in contrast, it would be preferable to treat strains with high polymorphism using chemo-prophylactic methods with a sterilising effect; otherwise, the animal's immune system could be overwhelmed.

2.2.2. Trypanosoma evansi

The same investigative methods have been applied to characterisation of *T. evansi*. The morphometry of *T. evansi* was studied and discussed in Chapter 1; it appears to vary depending on the host, and strains cannot be differentiated [58]. Morphological characterisation focussing on the kinetoplast has been widely applied; in certain regions, and/or certain hosts, there are akinetoplast forms in relatively stable proportions. This is thought to be a means for differentiating strains and perhaps species. By adding up these observations, MONZON and COLMAN [49] show that *T. venezuelense* may just be a dyskinetoplastic variety of *T. evansi* [252] that is less pathogenic for mules that it is for horses. In Argentina the percentage of parasites that have a kinetoplast is low – three to 20% in the study conducted by MONZON *et al.* [253]. Dyskinetoplasty is observed in dogs infected with *T. evansi* in Brazil [229] whereas it is not seen in capybaras – isoenzyme analysis does not however show up any differences between these strains. Hence, it is thought to be a minor variation. As far as we know, no comparative studies of the pathogenicity of the American, African and Asian strains of *T. evansi* have been published. Research tends to focus on the most highly affected hosts which depends on the continent: dromedary camels in Africa, buffaloes in Asia, horses in America; however, observations on water buffaloes, cattle and sheep show that *T. evansi* pathogenicity is sometimes also marked among the ruminants which are generally believed to be 'moderately sensitive'.

Isoenzyme analyses show a large degree of homogeneity within the *T. evansi* species [14], particularly between the American and West African strains, which supports the theory of a geographic origin for the strains found in America. The full extent of characterisation research using isoenzyme studies [229, 254, 255], RFLP, PCR, nuclear probes [236], kinetoplast probes [two and 41], nuclear and/or kinetoplast DNA sequencing [243] and molecular karyotyping [256] of *T. evansi*, *T. brucei* and *T. equiperdum* points to a large degree of homogeneity within the *T. evansi* species [242, 257], and very close relationships between *T. evansi* on the one hand, and *T. brucei* and *T. equiperdum* on the other. The AP-PCR profile obtained with the IL0525 oligonucleotide on the Venezuelan strain (TEVA1) [43] is different from the profile obtained by WAITUMBI and MURPHY [237] with the African strain KETRI3260. Arbitrary primers recently developed in Thailand for the characterisation of *T. evansi* have produced profiles that enable all the tested isolates ('fingerprints') to be differentiated [239]; a study of the heterogeneity of the American strains of *T. evansi* could be undertaken with these primers.

2.3. Conclusions

The new tools for genetic characterisation of parasites are promising but not yet capable of exploring the full extent of the phenotypic characteristics relevant to epidemiology and parasite control.

Characterisation of many *T. evansi* and *T. vivax* isolates from Latin America could be conducted using arbitrary amplification, especially in view of the encouraging results obtained in the People's Republic of China where a relationship was established between the presence of a given PCR product and chemoresistance in *T. evansi* [88]. Primers that distinguish between all the isolates of a species can be used for fundamental research on movement of parasite strains. In the field, they could be used to trace movement of a parasite from one host to another [239].

However, one must remain conservative as to the significance of fingerprints obtained by arbitrary amplification because:

1) the quantitative aspects of arbitrary amplification are poorly understood and likewise the intensity of the bands; the presence of the major bands is constant whereas the minor bands may sometimes be present but invisible which is a potential source of error;

2) a number of phenotypic differences are not matched by variations in fingerprints with the oligonucleotides used (see above, absence of correlation between the cross protection test and arbitrary amplification);

3) conversely, certain genetic differences that are detected by arbitrary amplification may not have a phenotypic expression – in this case they are simply strain markers (no significance);

4) by working with strains or isolates rather than clones, it may be that the fingerprints obtained are fortuitously or randomly reproducible since the predominant population during collection may vary over time and according to the host.

More thorough standardisation of arbitrary amplification methods is necessary as well as new oligonucleotides with enhanced performance that are capable of diagnosing resistance to trypanocides, evaluating the genetic polymorphism of strains, characterising their pathogenicity and assessing their immunogenicity.

CHAPTER 3:
THE MECHANICAL VECTORS OF TRYPANOSOMES AND THEIR HARMFUL EFFECTS

A mechanical insect vector is defined as any hæmatophagous insect that is liable to bite several hosts in succession within a few minutes or hours; the residual blood and/or lymph that remains in the mouthpart possibly contains pathogenic agents (although these do not develop or multiply in the vector) and is inoculated through the saliva [258].

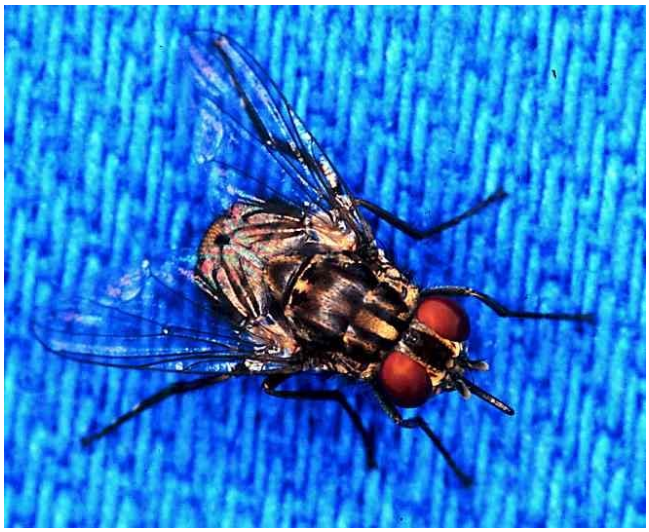
It would be difficult to compile a complete list of the potential mechanical vectors for trypanosomes, especially in view of the fact that local factors of overabundance may turn a species into a vector at a given point in time and space. By way of example, NOIRTIN *et al.* [116] reported a record of 60,000 bites by blackflies in one day on one cow. In a situation like this, Simuliidae could very well become vectors of diseases. **Any hæmatophagous insect that is liable to pullulate in a stockfarming area is therefore a potential mechanical vector for livestock trypanosomes**; however, epidemiological and experimental observations indicate that the most important are some of the members of the Stomoxyinae, Hippoboscidae, Culicidae and especially Tabanidae families. To properly understand the direct and indirect harmful effects of these insects for livestock, we need to study their morphology, life cycle and abundance.

1. THE MORPHOLOGY AND BIOLOGY OF MECHANICAL VECTORS

1.1. Stomoxyinae

Two genera of the subfamily Stomoxyinae (Diptera: Muscidae), are involved in the transmission of trypanosomes in the New World: *Stomoxys* and *Hæmatobia.*

1.1.1. *Stomoxys*



Stomoxys (stable flies) are biting hæmatophagous insects, regardless of gender. They are small and resemble the housefly (**Fig. 15**) apart from the appearance of the mouthpart which is of the biting type and comparable to that of *Glossina*, comprising a labium, labrum and hypopharynx. These are busy insects that are liable to switch hosts during a single bloodmeal and hence are excellent mechanical vectors.

Figure 15 – *Stomoxys calcitrans*
(S. MIHOK, with permission of author)

The larval stage is short – around five weeks; it occurs on the ground in organic debris (droppings, litter, silage or farm residues) which is both where oviposition and larval development take place. The larval phase lasts from 20 (*Stomoxys calcitrans*) to 40 days (*Stomoxys nigra*). Both the male and female adults need to take a bloodmeal before reproduction, which takes place three to five days after they emerge; the females lay some hundred eggs in small packets and require one meal every

time they lay. Adult life expectancy is estimated to be between two and four weeks and the total number of eggs produced is from 60 to 800 [259]. Stable flies are permanently active although there are clear seasonal peaks in the wet or dry periods, depending on the species, [260]. Adults are present both on pastures and in stables (hence the English name 'stableflies'). Generally speaking, stable flies are sedentary – they wait for the animals at locations situated along their itinerary that are suitable to the species; they have a high degree of affinity for cattle and horses and, in the absence of livestock, are able to cover more than 5 km in search of their prey. Stable flies essentially bite the lower parts of the animal but when the density is greater than 25 insects per limb, they are found on other parts of the body too. Together with tabanids, stable flies are considered to be the main vectors of animal trypanosomoses that are not transmitted by *Glossina*.

1.1.2. *Hæmatobia*

The genus *Hæmatobia* includes from two to eight species from the Old World whose taxonomy is not yet finalised; the main species is *Hæmatobia irritans* (L.) (horn fly); it was introduced into the New World in the 19th century and is presently cosmopolitan. Their overall morphology is similar to that of *Stomoxys* but they are smaller. *Hæmotobia* are biting insects that feed mainly on livestock, and both males and females are hæmatophagous. Their English name 'hornfly' refers to their predilection for the base of cattle horns. They have a short life cycle of around two to three weeks, which takes place on the ground in cattle dung, which is where the eggs are laid and develop. The larval stage lasts 10 to 16 days. Males and females take bloodmeals before reproduction, which occurs three to four days after the adults emerge; the female lays several dozen eggs either in small packets or spread out. The average life expectancy of adults is from one to two weeks and total egg production is 100 to 200 eggs per female. *Hæmotobia irritans* is generally sedentary but young females are capable of covering several kilometres in search of a host. Apart from these cases, *Hæmotobia* do not fly much and live in close proximity with livestock, essentially cattle, moving away only to lay their eggs. They feed 20 to 30 times a day, which works in favour of mechanical transmission of pathogens but do not often switch host which on the other hand works against this mode of transmission [259].

The incidence of *H. irritans* in transmitting livestock pathogens is poorly understood; the proliferation of this species on certain stock farms however has led to consider its potential role as a transmission agent of trypanosomes, particularly *T. vivax* in Surinam dairy farms (J. FAVRE, unpublished contribution).

1.2. Hippoboscidae

Hippoboscidae are a family of pupiparous hæmatophagous Dipterans comprising some hundred species. Both males and females are biting insects. They have a dorso-ventrally flattened body, short legs with claws, and often no or small, deciduous, atrophied wings giving them the appearance of louse (wherefrom the name 'louse flies'). Their limited mobility may be related to the relative specificity of the host. The main genera are *Hippobosca*, which are parasites of horses and cattle (*H. rufipes* and *H. equina*) and dromedaries (*H. camelina*); the genus *Lipoptena* is found on Cervidae and the genus *Melophagus* include the *Melophagus ovinus* – the transmission agent for *Trypanosoma melophagium*, a non-pathogenic sheep parasite [258].

Female hippoboscids lay their eggs on the hosts or very close to them. The larvae immediately pupate. These insects never move very far from their hosts and some Apteran species stay on them permanently. Hippoboscids are suspected primarily of contributing to the transmission of *Trypanosoma evansi* in Africa (*H. camelina*) [261]: their importance in Latin America is poorly understood. They are also intermediate hosts for *Dipetalonema dracunculoides*, a filarial nematode in carnivores [258]. Pupiparous insects that belong to related families (Nicteriibidae and Streblidae) may be the vehicles of trypanosomes in vampire bats.

1.3. Tabanidae

Tabanids (Diptera: Tabanidae) belong to the order of the Dipterans, suborder *Brachycera*. There are more than 4,000 species of horseflies that are found on all ecological sites with considerable harmful effects [126]. Their importance in the mechanical transmission of trypanosomes in Latin America is universally recognised. However, the relative roles played by *Stomoxys* and the other transmission agents still remains to be determined. Horseflies are also responsible for cyclical transmission of *T. theileri*.

A general overview of Tabanidae is provided by the example of tabanids in French Guiana largely based on work done by Hugues RAYMOND.

1.3.1. Morphology

An adult tabanid has the overall appearance of a fly (**Figs 16 and 17 a 1**); its mouthpart is suited to bites of the telmophagous type (see paragraph 2.1.1). Their size ranges from 5 to 25 mm, depending on the species and environment in which they develop. Their colour is highly variable, generally dull except for the eyes, which are large and bright.

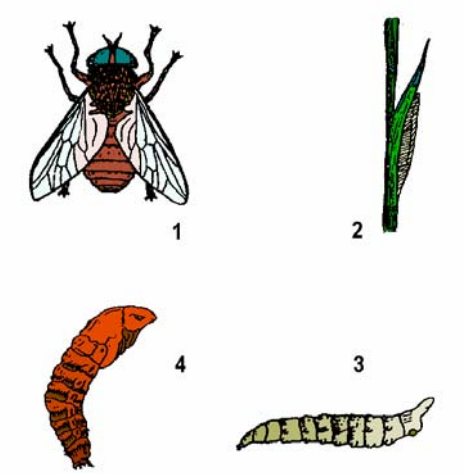
Figure 16 – *Tabanus importunus*, adult resting on a wooden post (H. RAYMOND)

Eggs are oblong in shape, around 2 mm long, and are deposited on grass in packets of variable shape, by groups of 200 to 500 (**Fig. 17 a 2 and b**). The larvae are spindle-shaped and ringed (**Fig. 17 a 3**) and they have no differentiated appendices. Their colour is dull and their size ranges from 2 to 30 mm depending on the stage. They live underground and are rarely seen. The pupa is cylinder-shaped, with a cephalic bulb and spiked body (**Fig. 17 a 4**); its size is the same as that of an adult.

1.3.2. Biology

1.3.2.1. General considerations

Life cycle: only the females feed on blood most often from domestic or wild animals but sometimes from birds or reptiles. The bloodmeal provides the nutrients necessary for vitellogenesis. Apart from a few autogenic species [262], Tabanids are closely dependent on their hosts to fulfil their life cycle. Mating occurs very early following adult emergence and then the females seek out a host. After a bloodmeal, oocyte development is accomplished within about one week. The female lays her eggs on twigs in the grass or on shrubs (**Fig. 17 b**), and then looks for a host to take another bloodmeal and begin a further reproductive cycle. In this way she takes up to four bloodmeals at one-week intervals or longer depending on whether or not she is able to find a host. In the meantime, she can survive by feeding in the same way as males from the nectar of flowers. The maximum lifespan of an adult female is one to two months.



Key: a.1: Adult horsefly; a.2: oviposition;
a.3: larva; a.4: nymph

Figure 17 a – Adult horsefly, oviposition, larva and nymph [263]

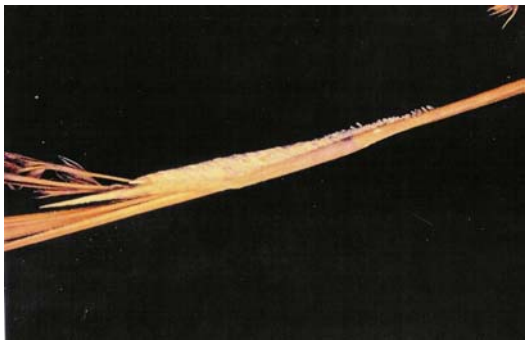


Figure 17 b – Tabanus importunus *oviposition on a twig (H. Raymond)*

Figure 17 – Morphology of the various stages in the life cycle of Tabanids

The larvae that emerge some ten days after oviposition have a long and complex cycle (nine to ten stages) either on water or underground. They are predatory or saprophagous; hydrobiontic or edaphic depending on the species. The shorter stages last ten days while the longest can last up to 300 days. The entire larval phase extends from less than 100 days to more than three years. This ability to quiesce explains why there are significant variations from one year to another. The larvae are highly disseminated and their density in the environment is only between 1.2/m² and 1.5/m². The ecological niches of the larvae are highly variable for all the species. When the environmental conditions are adverse (cold, immersion, drought etc.), larvae enter into a quiescent phase until conditions are once again propitious. Hence, in French Guiana, the rainy season triggers larval quiescence, which is released at the beginning of the dry season. Short development cycles are believed to be the reason for seasonal peaks of abundance. The pupa, which is sometimes aerial but more often underground, hatches between one and three weeks after nymphosis. Males feed on nectar, live in groups and are not commonly seen; they are not attracted to the traps used to capture females. The life cycle of Tabanids is illustrated in **Figure 18**.

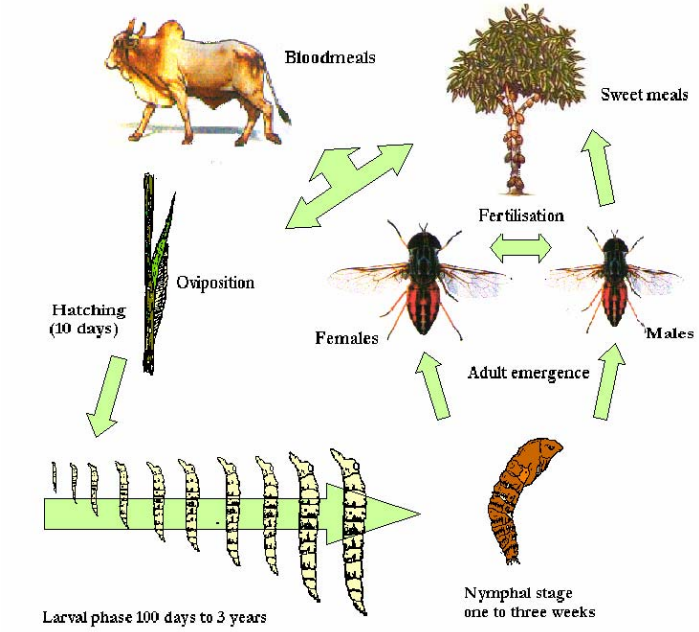


Figure 18 – Life cycle of Tabanids [263, 267]

Preferred hosts: although tabanids can feed on reptiles, birds and mammals, they are directed towards their hosts by olfactory (in particular carbon-dioxide) and visual stimuli and have a marked

preference for large, warm-blooded animals. If emergent females do not find any readily accessible hosts, they can cover several kilometres in search of them. Conversely, if they do emerge in a stockfarming area, they remain fully sedentary. It is generally thought that a distance of 200 metres is enough to prevent horseflies from passing from one stock farm to another (FOIL and RAYMOND, unpublished papers).

Horses are highly attractive hosts to tabanids (horseflies) (and are the most severely affected farm animals. Their attractiveness is probably due to their size and smell. Furthermore, they have finer skin than cattle. Horses are highly sensitive to horsefly bites and sometimes react violently. A horse in the midst of cattle attracts horseflies more than the latter; however, cattle are also sensitive to Tabanids' harmful effects. The latter's economic significance is the overriding reason for the interest that has been taken in these insects.

Ecology: horseflies, depending on the species, are present in all types of habitats: savannas, riparian areas, coastal areas and forests. They are found in abundance along creeks, in savannas, at the edge of savannas and wooded areas as well as in the vicinity of stock-raising areas where they multiply intensively as a result of the availability of hosts [264].

Circadian rhythm: in French Guiana, most species have a bimodal cycle and attack at dawn and sunset on clear dry days [265]. Some species are active only at these times, e.g. *Cryptotylus unicolor*, which is totally inactive during the daytime. Others such as *Tabanus importunus*, are very noticeably active in the morning and evening but nonetheless continue, albeit less intensively, throughout the day. Yet others, such as *Phæotabanus cajennensis*, reach peak activity at noon. All species of horseflies tend to be most aggressive in the morning and at sunset (**Fig. 19**).

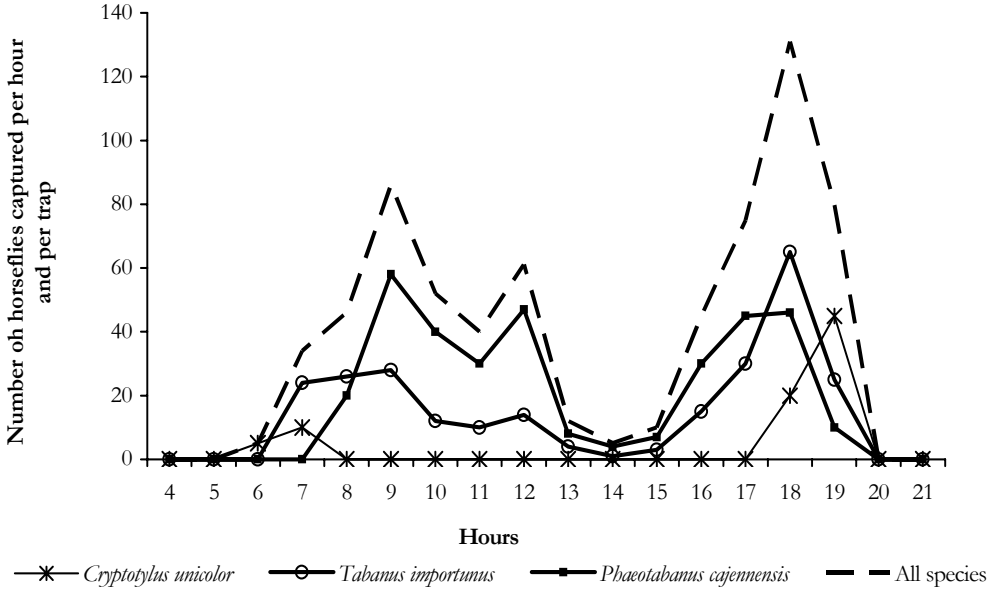


Figure 19 a – Daytime variations [268]
Key: the y-axis shows the number of horseflies captured per hour and per Malaise trap, using CO_2 as bait; The x-axis shows the time of day of capture. The insects were captured in November in a savanna area.

Peak activity varies a great deal according to the species – in Africa, the most common species are most often seen after midday and in the afternoon (*Tabanus tæenolia*, *Atylotus agrestis*) [261]; MIHOK, unpublished paper; DESQUESNES and DIA, unpublished paper).

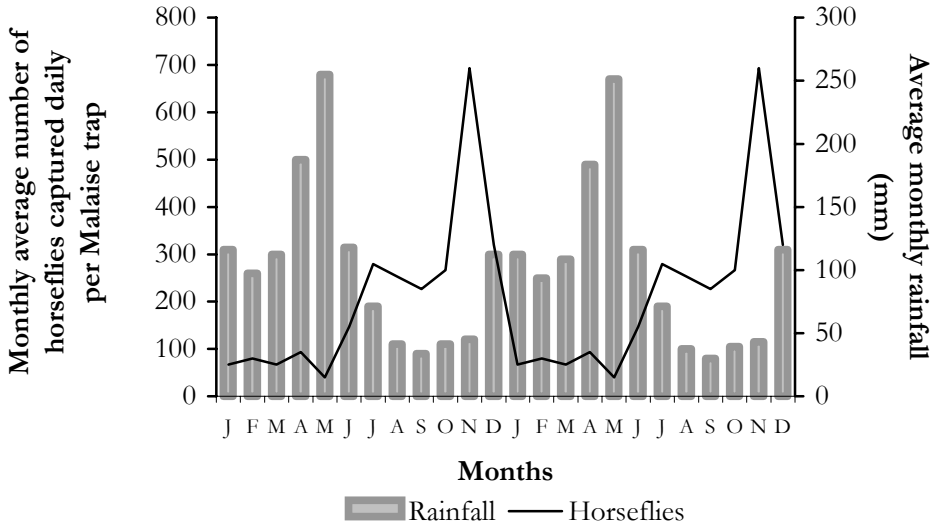


Figure 19 b – Seasonal variations [43]
Key: on the y-axes – left, the monthly average number of horseflies captured daily per Malaise trap; right, average monthly rainfall between 1980 and 1984; on the x-axis, the months of the year.

Figure 19 – Fluctuations over time of Tabanid activity in the savanna of French Guiana

1.3.2.2. Abundance

Seasonal variations: tabanids activity is seasonal; in tropical areas, the peak occurs during the rainy season or the dry season depending on the case. In French Guiana, some species such as *Tabanus occidentalis* (var. *dorsovittatus*) are found all year round but most are highly seasonal with a patent peak during the dry season, particularly towards the end, in November [265]. Seasonal variations in the activity of tabanids in French Guiana that are of veterinary relevance are illustrated in **Figure 19 b**. According to RAYMOND [266], Tabanid activity begins in June for the earliest species (*Ph. cajennensis*), in October for later species (*T. importunus*) with a peak as of the middle of the dry season, from October to December. It ends in January for the species with short-lived activity (*T. importunus* and *Cryptotylus unicolor*) and March for the later species (*T. occidentalis dorsovittatus*). As soon as the rains begin, activity drops off suddenly and recedes to almost none in January. If there is little rainfall during the first rainy period (December-February), some activity may persist up to March but it is less intense. During the rains, the larval phases are extended enabling insects to survive until the following dry season. Horseflies are able pullulate starting from the middle of the dry season thanks to these shortened cycles during that period. If there is little or occasional rainfall during the wet season, some species extend their period of activity throughout the year. **Figure 19 b** shows tabanid activity and annual rainfall – the inverse relationship between the two parameters is clearly visible.

In contrast, in the Pantanal, Brazil, Tabanids are active during the first part of the rainy season [183, 269], from September to November, but they also remain very abundant until the end of the rainy season in March [151]. It is remarkable that the *T. importunus* species is present both in French Guiana and Pantanal but active during the dry season in one case and the wet season in the other.

Annual variations: while seasonal variations can be predicted, the same does not apply to annual variations. Many interacting parameters are involved sometimes favouring one species, sometimes another. The larval phases may last between two months and three years [262]; emergence and disappearance of adults occur very rapidly and all stages are sensitive to weather changes. Taking one species at a time, some predictability can be achieved by detailed investigation, but so far it has not been possible to establish a reliably predictive model of pullulation for Tabanid species as a whole [109].

Main species of veterinary relevance: these are numerous and vary depending on the geographical area but most belong to the genera *Chrysops*, *Hæmatopota* and moreover *Tabanus* which are the biggest and most abundant specimens.

In French Guiana, FLOCH and FAURAN [270] have described about 50 species of Tabanids; H. RAYMOND made a qualitative inventory, recording the presence of 84 species of horseflies [266], but the quantitative inventory of Tabanids conducted in northern French Guiana also by RAYMOND [271] has shown that there are probably seven species that are of special significance for stock farming. Abundant and large: *Tabanus importunus* (also predominant in Brazil) and *T. olivaceiventris*; hyper abundant: *Tabanus occidentalis* (var. *dorsovittatus*) and *Phæotabanus cajennensis*; abundant and crepuscular: *Cryptotylus unicolor*, *Chlorotabanus mexicanus* and *Ch. inanis*.

1.3.2.3. Natural regulation of horsefly populations

General considerations: Tabanid population around stock farms is very high due to the abundance of the hosts be they horses or cattle. However, various parasites and predators to some extent naturally regulate Tabanid populations over the various stages of these insects' life cycle. 75% of the eggs laid by *Tabanus importunus* can be parasitised by Microhymenoptera [109]. Although this mode of regulation is not enough to fully control these populations, it does have an effect, which must be considered when contemplating any insecticide treatment that is liable to destroy Microhymenoptera populations. Horsefly larvae have a number of enemies: fungi, nematodes, Tachina and birds. So far, it has not been possible to assess their impact or control them.

There are two types of predators for adult horseflies in French Guiana and Guyana: wasps and birds.

Sand wasps: sand wasps belong to the Hymenoptera order, family Sphecidae, (subfamily Nyssoninae, Bembicini insect tribe). In French Guiana they are called '*mouches-lézards*' and in Spanish-speaking countries '*insectos policia*' while in English-speaking countries they are referred to as 'horse guard wasp' or 'cowfly tiger'.

They are predators of Dipterans, especially horseflies; their area of distribution goes from Argentina to the USA and their preferred habitats are coastal savannas [216]. This wasp hunts in the vicinity of livestock by hovering and darting very quickly when it sees its prey; it captures horseflies when they land on cattle or horses, kills them with its poisonous sting, and then carries them to a burrow dug in the sand (**Fig. 20 a**) where it has already laid eggs. The wasp's larvae feed on horseflies and other Dipterans captured by the adult. A wasp can capture from three to 9 insects a day and nurture up to 10 larvae. One larva can devour up to 40 catches. At most, one adult can eliminate some 300 horseflies during its lifespan. Unfortunately, these wasps are not very active in the evening when horseflies reach their peak activity, and furthermore they are not abundant in November when horsefly prevalence is at its highest [272].

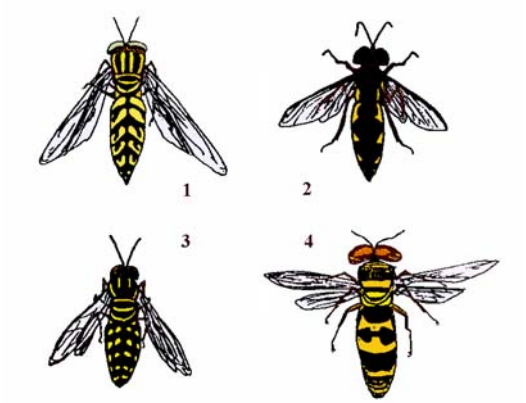
Several species have been identified in French Guiana [268]:

– *Stictia signata* (**Fig. 20 b 1**), the most abundant, is active from July to January with a maximum in August to September; between 5 and 15 individuals may be found hunting close to every head of cattle.

– *Stictia heros* and *Stictia maculata* (**Figs 20 b 2 and 3**) are not abundant (less than one wasp per head of cattle), active from November to January with a peak in December.

– *Rubrica surinamensis* **(Fig. 20 b 4)** is active from August to January with a peak in October. Placing sand heaps on pastures that are not naturally sandy may encourage sand wasps to settle in stock farms.

Figure 20 a – Stictia signata *digging its burrow in river sand (photograph by H. RAYMOND)*



Key: 1 – *Stictia signata*; 2 – *Stictia heros*; 3 – *Stictia maculata*; 4 – *Rubrica surinamensis*

Figure 20 b – The four main species of sandflies in French Guiana [263]

Figure 20 – The sandwasps of French Guiana

Insectivorous birds: many insectivorous birds are also horsefly enemies [273], but few are truly effective. The egret (*Bubulcus ibis*) catches horseflies when they land on the livestock to sting. Some livestock herds are continually escorted by these white birds – three or four egrets per head of cattle and sometimes more. When water is scarce in the pastures at the end of the dry season, the egrets migrate towards the marshes, leaving the livestock to its own fate.

– Chickens and, even more so, guinea fowl are excellent horsefly and tick predators [273] as they feed on them; livestock quickly becomes accustomed to their presence and their role – it is therefore doubly beneficial to associate these species at the times of day that Tabanids are active.

– Finally the smooth-billed ani (*Crotophaga ani*), a common black bird in Guyana the size of a blackbird, captures horseflies not directly on the cattle but rather on the wooden posts of fences or paddocks where the insects frequently alight just after a bloodmeal.

There is evidence that to maintain a Tabanid population, only 2% of the females need to fulfil the normal cycle [259]. The implication is that horsefly parasites and predators do not significantly impact the survival of horsefly populations; they can, however, contribute to reducing the seasonal parasitic load.

1.4. Other insects

Mosquitoes are Nematoceran Diptera that belong to the family of the Culicidae of which there are 3,000 species spread out all over the world. Males feed on plant juices but the female is hæmatophagous. She has mouthparts of the solenophagous type (suitable to 'capillary feeding') that enables her to puncture small superficial blood vessels so as to take a bloodmeal; the sting is not very painful. The female lays some 100 to 400 eggs in an aquatic oviposition site that varies according to the species (temporary or permanent site); the larvae and nymphs are aquatic and the larval stage lasts 10 to 20 days depending on the species and conditions. The lifespan of adults is between three weeks and more than four months.

Mosquitoes occasionally kill livestock when they attack on a massive scale [274], probably due to anaphylaxis caused by the toxicity of the saliva. In addition to their crucial role in transmitting Rickettsia (*Eperythrozoon ovis*) and many animal arboviruses (yellow fever, dengue, equine encephalitis, etc.), mosquitoes, particularly the genera *Culex*, *Mansonia* and *Ædes*, have sometimes been suspected of been involved in the mechanical transmission of trypanosomes, specifically

T. vivax in sheep in French Guiana (J. FAVRE, personal data) and Cuba [113], and in cattle in Venezuela [70].

Phlebotomidae (sandflies) are Nematoceran Diptera that have recently been elevated to the rank of the family of Phlebotomidae [258]; the genus *Lutzomyia* could be involved in trypanosome transmission. Bloodmeal analyses have shown a high degree of affinity for cattle in several species present in Latin America [275]. In Colombia, *Lutzomyia longipalpis* prefers to feed on cattle but also feeds on horses and dogs [276].

Ceratopogonidae ('biting gnats') are very small Diptera (0.6-5 mm). They are vectors of several animal arboviruses (Blue Tongue, Akabane virus, etc.) and of filiar nematodes and also produce seasonal cutaneous hypersensitivity in livestock. Certain species such as *Culicoides furens* are suspected of transmitting *T. vivax* [113].

Simuliidae (blackflies) are Nematoceran Diptera that look like small houseflies (1-5 mm), and are vectors of equine encephalitis in Venezuela, which, in the case of massive attacks, can cause abrupt deaths in livestock [274]. Some species such as *Psilopmia quadrivittatum* are also thought to be vectors of *T. vivax* [113].

Based on these observations, together with the ability of these insects to pullulate occasionally, it seems likely that some species of Culicidae, Phlebotomidae, Ceratopogonidae, Simuliidae, and a number of other hæmatophagous arthropods (fleas, louse, etc.) play a local and/or temporary role in the mechanical transmission of *T. vivax* and *T. evansi*. The importance thereof, however, has not actually been assessed or generalised.

1.5. Biting insect breeding

Research on biting insects is made much easier when they can be bred in laboratory conditions. Tsetse have been artificially raised in several research centres in Europe, e.g. CIRAD in Montpellier, and in Africa, e.g. CIRDES (Burkina Faso) where sterile males are produced on a massive scale to thereafter be released under control programmes conducted in West Africa. Breeding mosquitoes is very easy and many research centres do this, in particular the *Institut Pasteur* (IP) and the *Institut de Recherche et de Développement* (*IRD* – Research and Development Institute) in metropolitan France and overseas, but also in a number of universities as recently listed in a survey conducted by Dr Dominique Cuisance:

(http://www.agriculture.gouv.fr/spip/IMG/pdf/invent_insect_medico.pdf)

Using observations relating to the biology and ecology of *Stomoxys* [277], a number of stable flies breeding have been established [278]. For example such a breeding was established in Reunion Island by the GDS (Animal Health Group) to produce parasitoids for the purposes of a bio control programme against *Stomoxys*, i.e. the main vectors of anaplasmosis (T. HUE, personal contribution) (**Fig. 21 b**).

Unfortunately, similar schemes for Tabanids, which are far more difficult to breed, cannot be set up. Some attempts to raise Tabanids have been made in a number of laboratories but none have so far been able to complete the full reproductive cycle. Adult female horseflies can be captured and fed in cages placed on cattle and then individually housed in pots covered with gauze. A wetted piece of paper is placed in the pot together with a wooden rod to complete this device for maintaining adults (**Fig. 21 a**). The insects' wings are preferably removed so as to prevent them from becoming exhausted and injuring themselves by flying around the pot. Insects should furthermore be placed in darkness as much as possible to avoid stress. They can be given a sweet diet by placing a sugar lump on the gauze for a few hours every two days (H. RAYMOND, personal contribution). In this way oviposition and hatching are achieved [43]. This experiment in French Guiana did not include raising the larvae. Other authors in Africa, specifically in Mali, captured the larvae and pupae in the natural environment, often in the mud close to water holes

and rivers, and raised them in a humid environment made of sand, glass beads and/or non-nutrient agar gel. They were able to obtain adults by feeding the larvae with a piece of cow liver placed in the middle once a week [279]. This method of breeding is difficult to manage – the larvae have to be raised individually because in most cases they cannibalistic. Since partial phases of Tabanid breeding have been successfully accomplished, a complete cycle might be feasible in captivity. However, because of the very particular conditions (individual housing due to cannibalism of larvae) breeding these insects on a massive scale is not feasible. This is a hindrance to research on Tabanids.

Female adults being fed on a bovine

Females being maintained in a cup under laboratory conditions

Figure 21 a – Partial raising of tabanids (*Tabanus importunus*) (M. DESQUESNES)

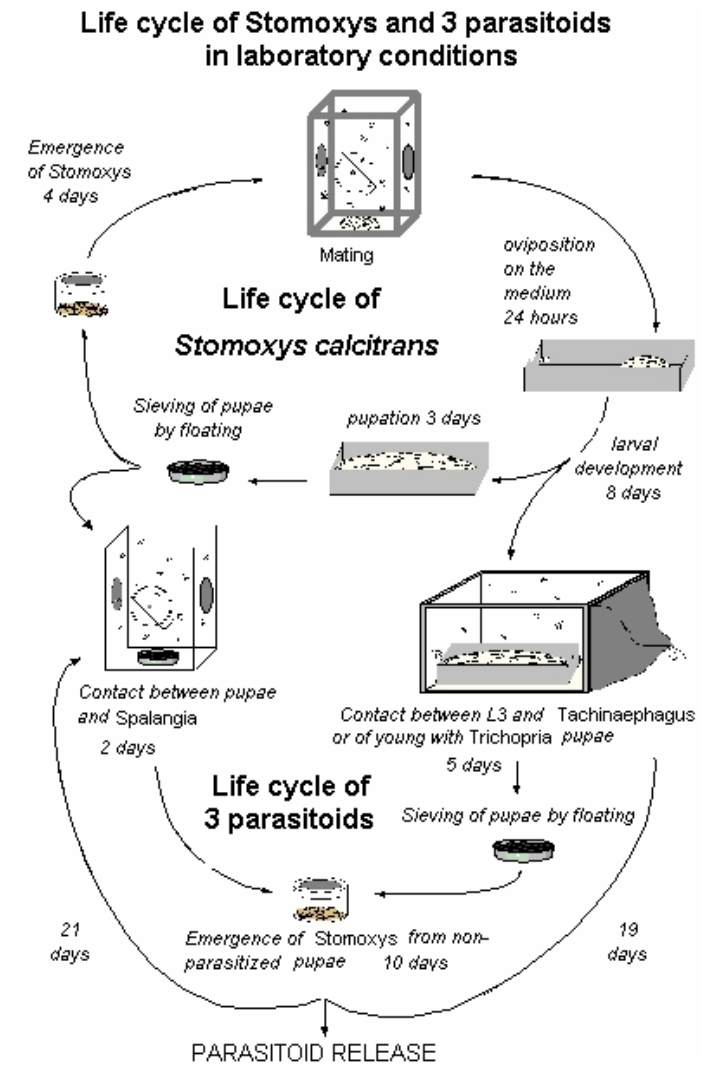


Figure 21 b – Life cycles of *Stomoxys* and parasitoids in laboratory conditions (T. HÜE)

2. DIRECT AND INDIRECT HARMFUL EFFECTS OF HÆMATOPHAGOUS INSECTS

Hæmatophagous insects produce direct harmful effects due to biting and blood-sucking, and indirect harmful effects deriving from with the transmission of pathogens. We shall illustrate the direct harmful effects of hæmatophagous insects through the example of Tabanids, the primary transmission agents of livestock trypanosomes in Latin America. The economic impact of these insects is reviewed. All these harmful effects, both direct and indirect, are elements to be considered when assessing the rates of return of the potential control schemes described in Chapter 6.

2.1. Direct harmful effects

2.1.1. Mode of action

The Tabanids that parasitize livestock in Latin America are a buzzing species that alight and take off frequently causing **disturbance to the animals both visually and acoustically** [280]; the other hæmatophagous insects, in particular *Stomoxys*, fly more quietly but cause visual disturbance because of their abundance.

Horsefly bites are **painful** for several reasons:

1) the mouthparts are **large**,
2) the biting apparatus is of the telmophagous type; the bite causes **multiple breaches** in the skin tissue that form a micro-haematoma (then absorbed by sucking),
3) as soon as the blood reaches the proboscis, the insect injects a little saliva.

Horsefly saliva has **anti-coagulant** properties but is also **irritating** and causes an inflammatory skin reaction.

Tabanids generally bite the finer parts of the livestock's skin where the hair is short – limbs, head or belly [208]; each species has its special areas of affinity [281]. The insect pumps the blood until satiation: complete repletion is achieved in approximately three minutes. During the meal, it excretes a little of its gut contents. At the end of the meal, burdened by the additional load, the horsefly takes a short, clumsy flight to a temporary shelter (shrub, fence post, etc.) and excretes another portion of the meal so as to cast off some weight and fly further away to a suitable shelter for oviposition. **Blood depletion** brought about in this way is considerable – the largest species can remove up to 700 milligrams in the course of one meal [280].

Because of the anti-coagulant properties of the saliva and the large size of the mouthparts, Tabanid bite wounds often cause a little bleeding which increases the **blood loss**, attracts flies and fosters bacterial proliferation (harmful effects secondary to skin wounds).

2.1.2. Consequences

Energy losses: in response to visual and acoustic disturbance and to the pain caused by the bite, hosts generally twitch their skin, swish their tails, shake their heads and stamp [280]. These defensive reactions produce significant losses of energy [282]. In the Camargue area, BOY and DUNCAN [283] established that horses spend up to 60% of their time ridding themselves of insects during the horsefly season. Wound scratching wounds further increases those energy losses.

Skin wounds and secondary infections: being bitten many times causes skin wounds that can easily become infected. Further wounds may arise when itching causes the animals to scratch. This in turn attracts not only ordinary flies (*Musca* spp.) that also disturb the animals and themselves transmit infections (including trypanosomes) but, more importantly in Latin America, screwworm

flies, *Cochliomyia hominivorax*, whose carnivorous larvae invade the wounds, enlarging them dangerously. Cochliomyiasis can have disastrous consequences, especially when they cause deep skin lesions on the limbs of horses. The cost of veterinary drugs used to disinfect and remove insects from the wounds caused by a horsefly bites is an element that needs to be considered.

Allergic reactions: recurrent seasonal hypersensitivity attributed to Ceratopogonidae is found in horses, cattle and sheep and massive Culicidae attacks are sometimes fatal for livestock [274, 284].

Curtailment of time spent feeding: livestock that are disturbed by horseflies and kept busy scratching wounds are distracted from their normal activities, especially feeding. In the Camargue area, BOY and DUNCAN [283] established that during the horsefly season the time horses spent on feeding dropped from 80% to 40%.

Furthermore, under a tropical climate, animals spontaneously stop eating during the hottest hours (11:00 am to 4:00 pm) when they rest in the shade. Their usual feeding time is therefore at dawn and dusk. In tropical America most Tabanids that are detrimental to animal health are themselves crepuscular [280]; their daytime activity is from 6:00 am to 10:00 am and from 5:00 pm to 7:00 pm. When the density of horseflies is very high, rather than feeding, livestock tend to gather close to one another for the purposes of mutual protection, or else lie down to avoid being bitten by horseflies on the lower parts of their bodies and limbs which are main targets. When cattle have access to the edge of a forest, they take shelter there. Those who have access to ponds or puddles, immerse themselves to escape the insects. All this behaviour interferes with normal feeding patterns. In forests, not only do the animals not feed properly, but are also driven to eating unusual, sometimes poisonous plants [211].

Horseflies sometimes bother livestock to the extent that they prevent any daytime feeding whatsoever. In some countries such as Madagascar, free ranging livestock naturally feed at night. In the Amazon, animals feed at night but inadequately because nighttime feeding makes them more vulnerable to predators (pumas, and more importantly jaguars) [280]. In French Guiana, large cats often wound cows with calves. Most cattle farms suffer up to 10 depredations per year – most often calves – by wild carnivores. In these circumstances, feeding at night does not make up for the deficit caused by horseflies during the day.

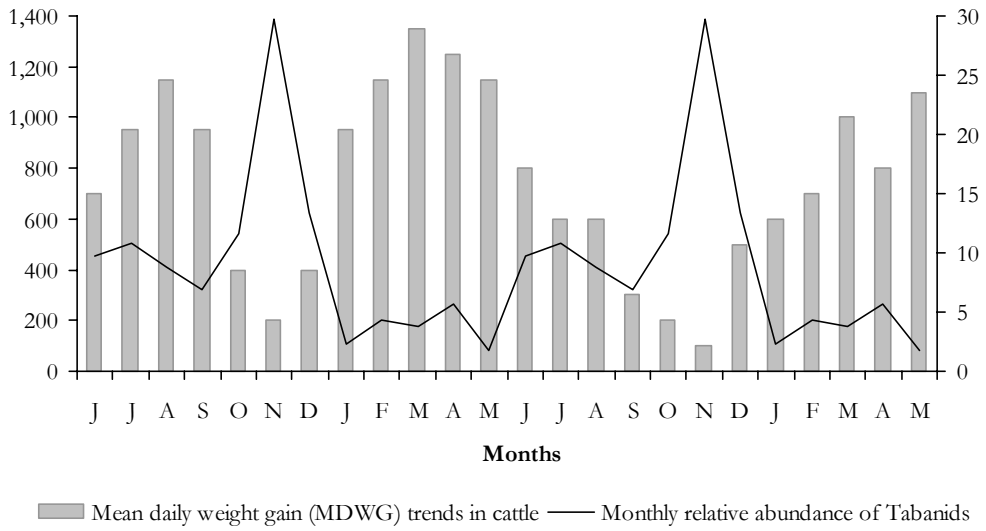
Blood depletion and loss: the volume of blood removed from a head of cattle by horseflies is estimated to be 200 millilitres per day [285], the equivalent of 400 *T. importunus* bites. This volume is increased in the case of superabundance and, additionally, by the amount that bleeds from wounds caused by bites.

2.1.3. Overall assessment

Weight gain changes: as a result of protection strategies that distract livestock from feeding, discomfort due to skin lesions, increased energy expenditure in response to disturbance and bites, depletion and blood loss, mean weight gain may slow down, become stationary, or even be reversed. The effect of horseflies alone is enough to make animals lose weight. FOIL and HOGSETTE [259] estimate that weight losses directly related to the presence of Tabanids can reach 45 kg per head of cattle per year. In the USA, a considerable drop in milk yields and decreased fat content have been observed in dairy cows. [286]. Similarly, *Stomoxys* can cause mean daily weight gain to decrease by between 160 and 220 grams in cattle in a moderately infested area (50 to 100 insects per animal) [287].

Observations recorded in French Guiana [263] show mean daily weight gain (MDWG) trends for cattle throughout the year on a farm where grass and feed supplies are always adequate and preventive treatment against hæmoparasitoses is regularly applied. The average annual MDWG on this farm is 745 grams. MDWG drop becomes apparent in November during the period of maximum Tabanid activity and in this way can be distinguished from the indirect effects of Tabanids (transmission of hæmoparasites) and from dietary factors (diminished pasture quality

during the dry season). Even under these conditions, the decline in MDWG is very noticeable during the horsefly season dropping from 1,000 grams between February and May to 200 grams in October and November (**Fig. 22**). The average MDWG during the season of horsefly activity is 418 grams (from September to January), i.e. 327 grams less than the annual average (745 grams). In French Guiana, when no protective measures are implemented, a horse in good condition can lose up to a third of its weight during the season of horsefly activity. Even under the protection of '*boucans*' (see Chapter 6), the mean annual loss is estimated to be 40 kg per animal [263].



Comments: the MDWG shown were recorded on a farm where adequate prevention measures are taken against anaplasmosis and animals are properly fed, enabling the drop in MDWG during the dry season to be attributed mainly to Tabanid activity. Superimposing the monthly MDWG value for the cattle and horsefly abundance suggests there is an inverse relationship between these two parameters, both of which are affected by rainfall. Indeed, plentiful rainfall is detrimental to horseflies, which rapidly disappear following the first rain in December, at a time when weight gain is fostered by regrowth in pastures and the halt in disturbance from horseflies.

Figure 22 – Mean daily weight gain (MDWG) trends in cattle (according to DESQUESNES) **(expressed in grams) and Tabanid activity** (according to RAYMOND, 1988) **(relative abundance) over the year**

Immunocompetence: the direct harmful effects of hæmatophagous insects diminish the immunocompetence of the hosts. This is later discussed as a factor that fosters greater receptiveness to the infectious agents transmitted by those insects.

2.2. Indirect harmful effects

Hæmatophagous insects cause indirect harm by transmitting pathogens (bacteria, Rickettsia, viruses, protozoa and helminths) from one host to another; this occurs when a bloodmeal is taken on a first host, interrupted and then resumed on a different host. The insects are sometimes cyclical vectors or intermediate hosts, but most often they transmit these pathogens mechanically.

2.2.1. The general mechanism for transmission

2.2.1.1. Host switching

When a hæmatophagous insect interrupts its bloodmeal due to the reactions of the host, it continues to attempt to bite until the host stops reacting (less sensitive area of skin, or extreme fatigue in the host) thus allowing the insect to gorge itself and reach either partial or total satiation.

Field and experimental observations have shown that when a meal is interrupted regularly before the digestive tract is replete (estimated by candling the insects), whenever possible, the insect repeatedly reattempts to bite until the digestive tract is approximately half replete. Beyond that point, its appetite recedes and further attempts to bite are deferred for several days until the first food bolus is digested [43]. This is why visual and olfactory traps that attract the insects seeking a host generally only capture fasting insects or ones that contain remains of meals taken several days before [261, 288, 289]. Under experimental conditions, by allowing an insect to begin a meal and then keeping it at a distance from the host, its appetite is maintained and experimental transmission can be achieved, e.g. up to 30 minutes after the first meal in the case of equine infectious anaemia [290]. These artificial conditions are not found in the field – if a host is accessible, attempts to feed will continue until the insect's appetite is satisfied, generally within less than 15 minutes. Taking together the biological and experimental data and considering that horseflies are able to cover more than 6 km and transmit the disease for approximately 30 minutes after the first meal, HAWKINS *et al.* [290] suggest that horseflies may act as vectors over a considerable radius. This 'additive' approach does not take into account the natural behaviour of the insects which is to satisfy their appetites at as close quarters and as quickly as possible rather than refraining from eating or wandering through the countryside unnecessarily!

2.2.1.2. Transmission through mouthpart contents

Pathogens are transmitted when insects inoculate saliva before beginning to suck blood. The cloning technique for trypanosome strains [291] has shown that inoculation with a single parasite is enough to infect a suitable host. The volume of blood in the mouthparts of *Tabanus fuscicostatus* was measured by FOIL *et al.* [292] using an ELISA method; it was estimated to be ten nanolitres. Considering that the amount of blood contained in a horsefly proboscis varies, depending on the species, from approximately 1 to 12 nanolitres (FOIL, personal contribution), a parasitæmia in the range of 10^5 to 10^6 is therefore necessary and sufficient to make an insect infectious. Clearly, the higher the number of parasites inoculated the more likely the infection – the infective power of a vector for a host is therefore related to the level of the parasitæmia. In horseflies, the trypanosomes are thought to be able to survive in the mouthparts from a few minutes to half an hour whereas *Stomoxys* are thought to survive less than nine minutes [261]. Experimental transmission can only be achieved if the interval between two meals is a very short – three minutes in experiments conducted with *T. vivax* and *T. evansi* in *Stomoxys* [105]. These conditions argue in favour of almost immediate transmission and, hence, very short distances.

2.2.1.3. Transmission through regurgitation of intestinal contents?

FERENC *et al.* [112] have suggested that hæmatophagous insects regurgitate part of the intestinal contents at the beginning of the meal; this could increase the risks of contamination for the host and allow transmission of pathogens even when the parasitæmia is below 10^5. The authors point out that trypanosomes can survive in horsefly gut for approximately 5 h to 7 h during which time the insect may be able to transmit the parasites by regurgitation. Little is known about the importance of regurgitation in horseflies; in *Stomoxys*, it is thought to occur only it under experimental conditions, not in natural ones [261]. This has not been reported for horseflies but live *T. congolense* were found in the excreta of mosquitoes (*Anopheles arabiensis*) [293]. The length of time during which an insect might carry live parasites may therefore vary depending on the species and on the trypanosome. But just as with horseflies and *Stomoxys*, these observations demonstrate a parasite's capacity to survive in an insect rather than its capacity to be transmitted.

Indeed, feeding patterns in hæmatophagous insects, particularly the appetite pattern, are such that meals are almost immediately resumed after interruption (and possibly after the insect has been contaminated) as long as satiation is not achieved, or, in the case of partial repletion, resumed several days later, i.e. far longer than the survival time of trypanosomes in the vector.

If regurgitation takes place in natural conditions, it might increase the infectivity of the insect, perhaps making it infective even at parasitemic levels of less than 10^5 in the first host. However, it

is unlikely that deferred trypanosome transmission can take place in this way since the survival time of parasites in the gut is far shorter than the interval that separates the two meals.

By way of conclusion, wherever the infected biological material originates from (mouthpart contents or intestinal regurgitation), horseflies and *Stomoxys* are immediate, short distance vectors.

2.2.2. Diseases transmitted

Horsefly involvement has been suspected in many diseases and sometimes demonstrated – the list in **Table III** is not comprehensive. The data derives mainly from reviews of pathogen transmission by hæmatophagous insects written by WELLS [97], KRINSKY [294], RODHAIN and PEREZ [258], FOIL and HOGSETT [259] and BRAVERMAN [274].

Infective agents	**Transmission**	
	Mechanical	**Cyclical**
Viruses		
Equine infectious anemia (EIA)		horseflies, mosquitoes?
Blue-Tongue	Tabanidae	*Culicoides* spp.
Vesicular stomatitis	and	
Equine encephalitis	Stomoxyinae	Simuliidae, Culicidae
Foot and mouth disease		
Rickettsias		
Coxiella burnetii (Q fever)	Tabanidae,	
Eperythrozoon ovis	Culicidae and	
Anaplasma marginale	Stomoxyinae	
Bacteria		
Bacillus anthracis (anthrax)		
Clostridium chauvoei and *C. perfringens*		
Pasteurella multocida and *P. tularensis*	Tabanidae	
Pasteurella bollingeri (hem. sept. buffalo)		
Francisella tularensis (tularemia)	and	
Brucella abortus, *B. suis*, *B. melitensis*		
Listeria monocytogenes	Stomoxyinae	
Erysipelothrix rhusiopathiae		
Leptospira spp.		
Protozoans		
Trypanosoma theileri	Tabanidae	Tabanidae
Trypanosoma vivax and *T. uniforme*	Stomoxyinae	
Trypanosoma evansi	and	
Trypanosoma equiperdum	Hippoboscidae	
Trypanosoma melophagium		*Melophagus ovinus*
Trypanosoma cruzi		Reduviidae
Helminths		
Dirofilaria immitis	Tabanidae	Culicidae
Brugia guyanensis and *B. beaveri*	Stomoxyinae	Culicidae
Stephanofilaria stilesi		Stomoxyinae
Dipetalonema dracunculoides		Hippoboscidae

Table III – Infective and parasitic diseases of livestock (and carnivores) that are mechanically (and/or cyclically) transmitted by hæmatophagous insects in Latin America [125, 126, 258, 274, 295]

In Latin America the main diseases transmitted by horseflies are trypanosomoses induced by *Trypanosoma vivax* and *Trypanosoma evansi*, anaplasmosis (*Anaplasma marginale*), bovine leucosis and

equine infectious anaemia (EIA). Leptospirosis, brucellosis, equine encephalitis and ehrlichiosis also deserve to be mentioned [295].

2.2.3. Proven mechanical transmissions

Although there have been advocates of the existence of cyclical transmission of *T. vivax* through horseflies [17, 108], no observations or evidence of the life cycle have been afforded. The epimastigote forms of *Blastocrithidia* spp. and *Crithidia* found in Tabanids [7, 28] cannot be differentiated from the *Trypanosoma* spp. in mammals; hence earlier suggestions and observations must be viewed with caution.

It follows that the normal mode of transmission for *T. vivax* and *T. evansi* in America is mechanical transmission by hæmatophagous insects. For *T. vivax*, experimental evidence has been provided for three common Tabanid species: *Tabanus importunus* [109], *Cryptotylus unicolor* [111, 112], and *Tabanus nebulosus* [110]. Transmission is obtained by interrupting the insect's meal and artificially switching host. The interval between two attempts to feed is always very short; if that is not the case, the insects refuse to feed.

Experimental transmission of *T. vivax* and *T. evansi* was achieved with several species of Stomoxyinae (*Stomoxys nigra*, *Hæmatobosca squalida*) at respective success rates of 3.4% and 0.9% [296] and a maximum interval of three minutes between meals. Transmission of *T. equiperdum* by hæmatophagous insects has been demonstrated but is not thought to have any real epidemiological significance because the parasitæmia is very short lived [63].

In Africa, recent work conducted by DESQUESNES and DIA [103, 571, 572] demonstrate that *Trypanosoma vivax* and *T. congolense* are mechanically transmitted by very common Tabanid species on this continent (*Atylotus agrestis* and *A. fuscipes*). Mechanical transmission is therefore not peculiar to the American and Asian trypanosome strains but rather a general characteristic of livestock trypanosomes. It would appear that there is a close connection between effectiveness of transmission and very high-level parasitæmia. This would explain why *T. vivax* is so easily mechanically transmitted in cattle, and *T. evansi* in dromedaries since the parasitæmia scores for these host/parasite pairs are very high.

2.2.4. Requisites and probability of transmission

Although theoretically and/or experimentally, any pathogen present in the blood may be mechanically transmitted, the statistical incidence of the phenomenon needs to be examined for each 'pathogen/host/vector' system as this affects the epidemiological impact [153].

Limiting factors: a **strictly** necessary condition for trypanosomes to be mechanically transmitted by hæmatophagous insects that has been established by observations of the transmission mechanism is that the individuals and/or species must be in close proximity on the stock farm or at a watering point.

Contributing factors: furthermore, the general principle of mechanical transmission relies on several factors relating to the parasite, host, vector and their mutual relationships. This results in a greater or lesser probability that mechanical transmission that we shall attempt to outline for *Trypanosoma* spp.:

– Infective hosts: the higher the level of parasitæmia in the infective host (immuno-suppressed or recently infected animal) and the more sensitised is its skin (interruption of meal), the higher the risk;

– Potential vector: the risk is high when the following conditions hold true: large insect and large mouthparts (residual blood), pain is caused by the bite (switch of host), the insect is harassing, abundant and mobile; *Stomoxys* and moreover horseflies meet those criteria, especially the *Tabanus*

importunus species: it is a large insect whose bite is painful, capable of ingesting up to 0.5 millilitres of blood, seasonally highly abundant in and around stock farms and very harassing (hence its name). The abundance of vectors is a parameter that directly influences the probability of transmission.

– The potential host: host immunocompetence affects the consequences of an infective bite; immunosuppressed hosts (inadequate food and drink, intercurrent diseases, large amounts of blood depleted by horseflies) and unchallenged animals that are introduced into at an infected area tend to be less immunocompetent. High density and promiscuity of the hosts heighten the risk of transmission (host switching);

– The relationships between hosts and vectors are essential; the higher the affinity of a vector for its host and the more intense the activity of the insects at the times and places where their hosts assemble, the higher the risk of contamination. One of the direct effects of horseflies is to induce livestock to gather together for the purpose of mutual protection thereby establishing conditions conducive to mechanical transmission.

2.2.5. Epidemiological implications

2.2.5.1. Domestic cycle

These parameters imply that the capacity of insect vectors to mechanically transmit is related to the infectious and physiological status of the infective and the potential hosts as well as to insect pullulation. Pullulation can bring about significant seasonal variations in the incidence of mechanically transmitted trypanosomoses. Even during periods when hæmatophagous insects are largely inactive, if the hosts are highly receptive, the incidence of infection can nonetheless be very high as has been observed in *T. vivax* epizootic outbreaks on cattle in Colombia [297] and on livestock imported from metropolitan France into French Guiana [43]. Similarly, new outbreaks of *T. evansi* in horses in Brazil [269] have given rise to morbidity rates for infections of up to 97% [269].

The safety time and space interval beyond which trypanosomes are unlikely to be mechanically transmitted from one animal to another is in the order of a few minutes and approximately 100 metres. Mechanical vectors therefore tend to transmit trypanosomes within the same herd but are not effective links between different herds unless the latter come in very close contact, e.g. shared watering points during the dry season. Special attention needs to be paid to these high-risk locations in epidemiological terms.

Under sedentary livestock raising conditions, mechanically transmitted trypanosomes are introduced mainly through the introduction of carrier animals. In the case of *T. evansi*, vampire bats can also bring the parasite into the farm (see below). *Trypanosoma vivax* has indeed continued to spread to the Brazilian-Bolivian border as a result of livestock trading opportunities opened up by the construction of new roads [219]. Where highly extensive stock-raising techniques prevail, infections may furthermore occur when herds share grazing land and/or watering points.

In areas where transmission is strictly mechanical, once hosts have acquired a certain degree of resistance (carrier status not apparent), mechanically transmitted trypanosomoses often follow a silent course for long periods (several months and years) and then clinically reappear as a result of seasonal immunosuppression as has been observed in huge enzootic areas in Venezuela (*T. vivax* and *T. evansi*) and on the Guyana Plateau [43]. When this occurs, vertical transmission of parasites in the host can also become important and cause further epizootic outbreaks.

2.2.5.2. Wild and domestic cycles

Mechanical transmission requires the host to be in the close vicinity; hence, hæmatophagous insects do not provide a good direct link between the wild and the domestic cycles.

For *T. evansi*, HOARE [7] pointed out that the connection between the wild and the domestic reservoirs was provided mainly by vampire bats (which can remain infective throughout their lifespan), but that transmission between domestic animals occurs mainly through biting insects. Furthermore, a number of hosts such as dogs may be a link between these two reservoirs because wild animals can orally contaminate them, and they can thereafter contaminate livestock via insects. This phenomenon is not seen in French Guiana as is illustrated by the fact that *T. evansi* is present among wild animals and domestic dogs while it is not present on livestock [43]. Surinam is in an intermediate situation where *T. evansi* is present on wild animals and dogs and has also been detected on cattle [299].

Similar observations indicating that there is little interaction between the wild and the domestic cycles have been made regarding camel trypanosomosis in Mali. In this case, camels appear to be the main reservoir and source of contamination for other camels although many wild and domestic species are also infected [300]. This is inferred from the very short distance of mechanical transmission (a few metres), which implies that contamination can only occur between animals that graze or drink together whether or not they belong to the same species.

For *T. vivax*, the discovery of wild deer infections in America indicates that insects can sometimes provide the link between the domestic and wild reservoirs since there is no other known mode of transmission from livestock to wild animals. Under highly extensive stock raising conditions such as in Brazil, domestic and wild animals tend to graze together presumably establishing the link between the two cycles.

2.2.6. Conclusions on the role of hæmatophagous insects

So far, in French Guiana, epizootics due to *T. vivax* in local cattle have always been observed during the dry season [46]. Two epidemiological factors coexist during this season: livestock **immunosuppression** deriving from inadequate water and grazing (both quantity and quality) and from the direct harmful effects of Tabanids, and **peak activity of mechanical vectors of *T. vivax***. Horseflies have both direct harmful effects by causing immunosuppression in the livestock and indirect ones, as vectors of parasites. As such, they play a **dual role as both contributing factor to and transmission agent for bovine trypanosomosis**. The same also applies to *T. evansi*-induced trypanosomosis in horses.

Other blood parasites such as *Anaplasma marginale* together with viruses and bacteria sometimes not only call on the same mechanical vectors but also act as contributing factors (by bringing about immunosuppression) to trypanosome infection.

3. ECONOMIC IMPORTANCE OF HÆMATOPHAGOUS INSECTS

The economic impact of Culicidae, Ceratopogonidae, Simuliidae and Phlebotomidae on livestock has not been assessed but in addition to the impact of the diseases they may transmit, they generate skin pathologies, allergic reactions and toxic anaphylaxis that may entail a significant economic cost in certain situations.

3.1. *Hæmatobia irritans*

In the USA, economic losses caused by *Hæmatobia* are estimated to be US$800 million, 60 million of which are spent solely on insecticides [259]. Comparative studies of calf weight gains with and without insecticide treatment on the cows argue in favour of treatment since the weight of the calves in the treated batches was 5.6 kg more than in the untreated batches; the mean parasitic loads for treated and untreated animals were respectively 1 and 58 flies per head [301]. Research has often highlighted the overall benefits of insecticide treatment for the purposes of controlling all hæmatophagous insects indiscriminately. But little has been done to distinguish between the

causative species and assess their share of responsibility in transmitting blood parasites. Specifically, there has been no assessment of the impact of *Hæmatobia* control on trypanosome transmission.

3.2. *Stomoxys*

A comparison between breeds has shown that before the age of one, Brahman zebus and Brahman crossbreeds are not very susceptible to *Stomoxys* whereas European breeds of equivalent age experience a 220 gram drop in their MDWG even when insect loads are low (approximately 20 insects per animal); beyond the age of one, their susceptibility is equivalent but at higher parasitic loads (> 90 per animal) and their MDWG declines by 160 grams [287]. Some authors have proposed a model for calculating losses attributable to stable flies [302].

In 1993 in the USA, in some areas the economic impact of *Stomoxys calcitrans* on cattle came to US$10 per head; these figures include the direct losses caused to livestock and the cost of the control measures implemented [259]; for the country as a whole, the overall loss was estimated to be US$100 million per year.

In Latin America, the direct impact of stable flies have not been measured and it is not easy to speculate on its extent. Stable flies are thought to be responsible for transmitting *T. vivax* during bovine trypanosomosis outbreaks but further evidence is needed, especially in view of the fact that some Tabanids are active almost all year round.

3.3. Tabanids

In the USA in 1965, the annual economic losses attributable to Tabanids were estimated to be US$40 million [259]. Estimated losses deriving from horseflies on cattle and horses are provided below on the basis of observations made in French Guiana and Brazil.

3.3.1. Cattle

Assessment of losses

To assess losses, the following parameters need to be considered:

- drop in MDWG caused directly by horseflies;
- medical expenditure on insecticides and disinfectants to treat skin wounds;
- cost of preparing the '*boucans*' and handling the animals;
- preventive and curative treatment for anaplasmosis;
- preventive and curative treatment for trypanosomosis.

It is especially difficult to assess anaemia and emaciation caused by trypanosomosis due to *T. vivax* since response is highly variable from one animal to another and, for the herd as a whole, they depend very much on the herd's immune status in relation to this parasite. In a recently infected herd, losses are thought to come to several dozen kilos per animal during the dry season. In a habitually infected herd, the parasite may cycle through and infect animals without being noticed. Only a few cases display clinical signs of infection. Weight gains were monitored in a habitually infected zebu stock farm – the mean loss was estimated to be 3.3% of bodyweight over a two month-cycle [43].

Overall economic assessment

An estimation of the mean economic impact of horseflies on the cattle-raising industry in French Guiana is provided. The analysis was applied to beef cattle born locally whose average weight is 330 kg [263]. **Table IV** shows the losses deriving respectively from horseflies, anaplasmosis, and trypanosomosis.

Setting aside hæmoparasitoses, horseflies are thought to be responsible for an average annual loss of approximately 5.3% of bodyweight (BW). Anaplasmosis is thought to cause an additional 0.8% loss in BW, and trypanosomosis a further 3.3% loss in BW or more.

Horseflies alone	
– MDWG loss of revenue: 13.5 kg/animal/year, at 3 €/kg	i.e. 41 €/animal/year
– veterinary drugs (topical and general therapy)	7.6 €/animal/year
– additional manpower : monitoring, handling, application, setting up *boucans*	4.6 €/animal/year
Total 53.2 €/animal/year, equivalent to 17.5 kg BW/animal/year	**i.e. 5.3% of BW/year**
Anaplasmosis alone	
– estimated average loss in yield : 2 kg/animal/year, i.e.	6.1 €/animal/year;
– veterinary drugs, average expenditure	1.5 €/animal/year;
Total 7.6 €/animal/year equivalent to 2.5 kg BW/animal/year	**i.e. 0.8% of BW/year**
Trypanosomosis alone	
– losses due to a trypanosomosis outbreak, average 10 kg/animal/outbreak	30.5 €/animal/outbreak
– veterinary drugs, average expenditure in the event of an outbreak 3.0 €/animal	3 €/animal/outbreak
Total 33.5 €/animal/outbreak, equivalent to 11 kg BW/animal/outbreak	**i.e. 3.3% of BW/outbreak**

Caption: *estimates relating to fattening cattle whose average weight is 330 kg, expressed in Euros (at 1992 meat prices), kg of body weight (BW) and percentage of BW*

Table IV – Estimated losses in cattle due to horseflies, anaplasmosis and trypanosomosis in French Guiana

In total, the losses generated directly and indirectly by horseflies yearly come to 20 kg-31 kg of BW/animal/year of, i.e. €60-€95 or 6% to 9.4% of BW. In French Guiana as a whole (8,000 head of cattle), for the 4,000 beef cattle that are generally well tended to, the average loss comes out as €240,000 due to Tabanids and anaplasmosis (permanent scourges), plus an additional occasional loss of €135,000 due to trypanosomosis (epizootic outbreaks).

If one extrapolates these figures to the three Guyanas, 16,000-25,000 tonnes of meat are lost every year (for a population of 800,000 head).

3.3.2. Horses

It is far more difficult to assess the economic impact of Tabanids on horses:

– violent reactions to insect bites prevent any riding activities throughout the dry season although it is the most suitable time;

– a horse in good condition can lose between 10% and 30% of its bodyweight depending on whether or not it is protected by '*boucans*';

– the impact of equine infectious anaemia (EIA) transmitted by horseflies may be considerable: in 1982, nearly 90% of the horse population in French Guiana had to be slaughtered because of EIA infection;

– New epizootic outbreaks due to *T. evansi* in horses take a severe toll: in Pantanal, Brazil, morbidity is nearly 100% and mortality is between 10% and 80% [269]; a ranch with a stock of 800 lost 83 horses at an estimated cost of US$38,000 [303].

4. CONCLUSIONS

The role of Tabanids as mechanical vectors of disease was for a long time underestimated particularly on the African Continent where it was overshadowed by the role of *Glossina*. The fact that trypanosomosis distribution goes beyond the geographical range of the tsetse-fly in Africa and its spread over Latin America have led to reassessing the importance of these vectors. Trypanosome transmission by Tabanids has been demonstrated for several species. But observations of frequent trypanosomosis transmission outside of the peak Tabanid season is an indication that horseflies have a very strong vectoring capacity or/and that very effective vicarious vectors are also involved (*Stomoxys*?).

Tabanids play a twofold role in the epidemiology of trypanosomosis: direct effects – blood depletion and harassment leading to immunosuppression of the livestock, and indirect effects: mechanical transmission of trypanosomes. Hence, Tabanids are thought to be involved in triggering clinical manifestations of trypanosomes in carrier animals as well as in transmitting parasites as inferred from the fact that clinical trypanosomosis and abundance of Tabanids are generally concomitant in Latin America and in the *T. evansi*-infected areas of Africa [261, 300].

In Brazil, *T. evansi* causes considerable losses among horses; SEIDL *et al.* [303] estimate that 27%-91% of these losses might be averted if an appropriate control strategy was adopted.

Estimates conducted in French Guiana show that the annual impact of the direct harmful effects of Tabanids on cattle-raising is approximately 5% of BW lost between October and December. The indirect harmful effects of Tabanids (anaplasmosis and trypanosomosis) are estimated to cause a drop of between 1% and 4% in BW. According to these estimates, the direct harmful effects of Tabanids are greater than the indirect harmful effects and that on its own is justification for implementing horsefly control programmes. Total annual impact of horseflies is between 6% and 9% of BW.

In the case of French Guiana, these data have established that treating cattle by insecticide spraying every ten days during the period of maximum Tabanid activity is cost-effective (see control methods). However, there is no certain evidence that controlling Tabanid proliferation also curbs hæmoparasite transmission. Be that as it may, reducing the direct effects of Tabanids relieves the livestock and enables it to offer greater resistance against blood parasites in general and trypanosomosis in particular. Tabanid control is therefore even more cost-effective in herds already infected by trypanosomes. Techniques for horsefly control and conditions relating to effectiveness thereof are discussed in Chapter 6 together with the other control methods for trypanosomoses.

CHAPTER 4: DIAGNOSIS

Clinical diagnosis of trypanosomoses in mammalian hosts is difficult because the symptoms are evocative of the full range of hæmoparasitoses or even other livestock diseases (see Chapter 2) and are not species-specific; laboratory diagnostic procedures are therefore necessary to confirm clinical suspicions and conduct epidemiological surveys. Trypanosome detection in vectors and its relevance will be discussed at the end of the chapter.

1. PARASITOLOGICAL TECHNIQUES

1.1. Sampling and preservation

Trypanosome detection can be conducted on a sample of blood, lymph node, cerebrospinal fluid, genital secretions, organ smear, etc. and depending on the test is performed on a fresh preparation or on fixed or frozen biological material. On cattle, depending on practitioners' preferences, availability of restraint facilities and the required sample size, the material is collected from the jugular or caudal vein (5 ml-10 ml vacuum tube) or from a vein in the ear (capillary tubes for parasitology). For medical diagnostic purposes, ear sampling is advisable because it is more sensitive [19, 304] whereas, for epidemiological surveys, this method does not provide a large enough volume for blood banking purposes. Use of anti-coagulant-containing tubes is recommended although a capillary tube can be prepared using a dry tube immediately after the sample has been collected.

Samples must be refrigerated prior to being treated but tubes must not be cooled too quickly to avoid clumping of the whole blood (possible even with anti-coagulant).

1.2. Direct microscopic exploration

This consists in looking for the parasites on a fresh or a fixed sample, either directly in the biological material (most often, blood) or following enrichment. The blood sample must be microscopically examined soon after it has been collected (2 h-4 h).

1.2.1. Direct examination of a fresh preparation

In Africa, a phase contrast microscope is widely used for direct exploration of genital secretions (*T. equiperdum*), CSF, lymph node fluid or fresh blood despite this method's low sensitivity. It sometimes enables parasites to be identified on the basis of morphological and motility criteria. A direct parasite count system has been described to provide a rapid estimation of parasitæmia for the purpose of monitoring experimental infections [305].

1.2.2. Blood or lymph node smears

This is the definitive diagnosis. Material aspired from an enlarged lymph node (most often the prescapular lymph node) or a blood sample, collected preferably with anti-coagulant, is used to prepare a methanol-fixed, Giemsa-stained smear that is observed under a microscope (× 1,000) – the subgenus to which the trypanosomes belong is identified on the basis of morphological and morphometric features. In routine practice, the identity of the species can often be inferred from the epidemiological context. Smear tests are therefore relatively specific but their sensitivity is very low, in the region of 10^4-10^5 parasites/ml.

A variant of the smear test is examination of a thick drop [306] partially spread over the strip using another strip. The strip is quickly dried and then immersed in distilled water to lyse the red blood

cells and eliminate the haemoglobin. It is then dried and stained for 30 minutes using 4% Giemsa in PBS. This is a commonly used test for diagnosing human and animal trypanosomoses.

1.3. Microscopic exploration following concentration

1.3.1. Haematocrit centrifuge technique (HCT or Woo's test)

This technique involves differential centrifugation of heparinised blood for 5 minutes at 13,000 rpm in a haematocrit tube [62, 63]. The tube is placed in the slot of a counting slide and observed preferably with a long focal lens (**Fig. 23 a**). The capillary tube is partially rotated so as to examine the inside of the tube and thoroughly explore the buffy coat. With this technique, approximately 70 µm of blood can be explored. HCT is quick and inexpensive and additionally shows whether the animal is anæmic. Depending on the species, its sensitivity varies from 500 (*Trypanosoma brucei*), 1,250 trypanosomes/ml (*Trypanosoma vivax*), to 6,525 trypanosomes/ml (*Trypanosoma congolense*) [307]. Subgenera can sometimes be identified in this way. Under controlled conditions, the positivity threshold is 200 ±110 *T. vivax*/ml on average, with a sensitivity of 100% when the parasitæmia is > 700/ml, 80%-46% between 700 and 60 parasites/ml, practically nil below that [305]. For *T. vivax*, LEEFLANG *et al.* [309] indicate that the test is more sensitive with three-minute centrifugation without affecting the haematocrit value.

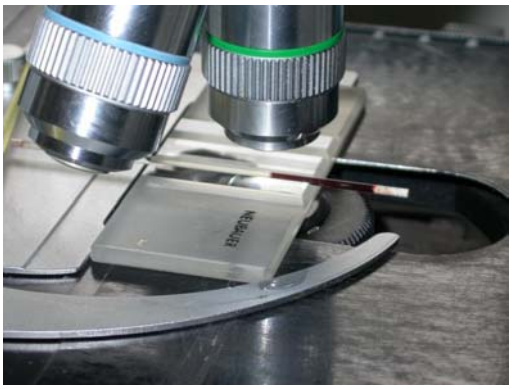


Figure 23 a – WOO's test: direct examination of the buffy coat through the capillary tube

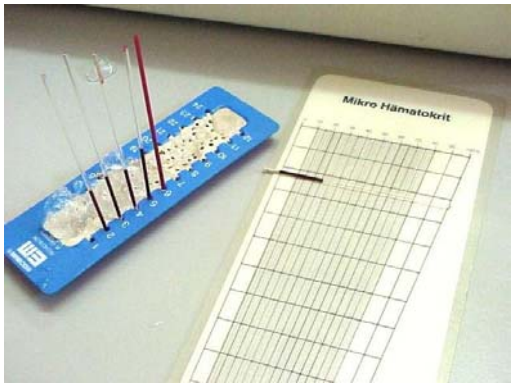
The **double centrifugation technique** is a variant of HCT that consists in first centrifuging a volume of 1.5 millilitres of blood and then performing another HCT on the buffy coat produced by the first step [309]. This method's sensitivity is able to detect less than 100 trypanosomes/ml of blood [310]; however, recovering the buffy coat is a difficult operation and gives rise to inter-operator variations. As a result, this technique is more demanding and affords only a slight improvement in sensitivity while its reproducibility is not as good.

1.3.2. Buffy coat method (BCM)

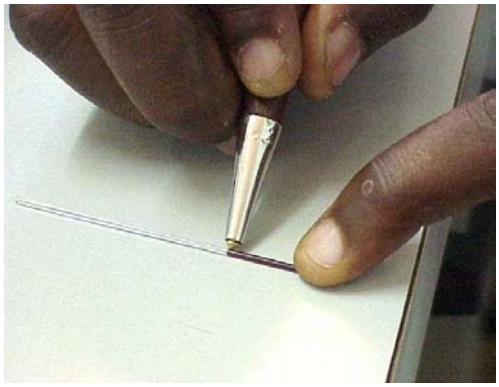
The BCM is another variation on the HCT method in which the capillary tube is cut so as to extract the biological material located at the white blood cell/plasma junction (**Fig. 23 b**). The fresh specimen is placed between a slide and cover slip and viewed through a dark ground microscope (the method is referred to as DG or BCM/DG) [311]. The same method can be applied to material that has been fixed/stained [312]. Because high-speed centrifugation causes morphological changes, morphometric features of the parasites cannot be examined with this technique. The subgenus, however, can be identified. The relative sensitivity of HCT and of BCM has been the topic of controversy between authors and depending on the parasite species. Both methods yield similar results. HCT must be performed indoors (it is important to avoid any movement of the tube in the slot due to draughts) whereas BCM can be performed outdoors. Because they involve measuring the haematocrit both methods can detect anaemic conditions that are often associated with trypanosomoses.

1.3.3. Quantitative Buffy Coat Method – QBC

This is an HCT that uses a capillary tube coated with acridine orange and potassium oxalate to make the parasites fluoresce; in addition, the tube is fitted with a float on a level with the buffy coat that flattens the parasites against the tube walls at the end of the centrifuge phase. As a result of these additions, it is easier to detect the parasites under a microscope.



1)



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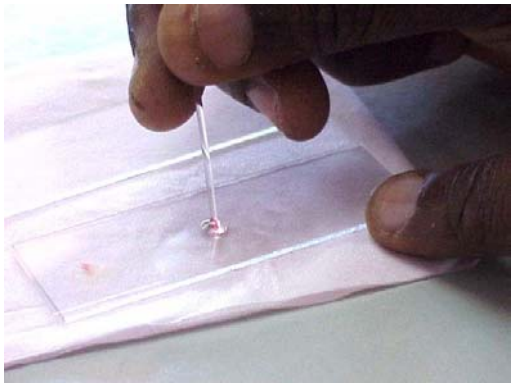


3)

4)

Figure 23 b – Buffy coat method: hæmatocrit, buffy-coat extraction and observation after being spread on a slide

Figure 23 – Microscopic examinations of the buffy coat (M. DESQUESNES)

An unpublished review conducted by FREZIL in 1995 [313] pointed to the disparities between results obtained using QBC. According to some authors, the detection limit is one trypanosome per 110 µl [314], while others find it no more sensitive than HCT [315, 316]. Four years later, the same authors [317] maintain that QBC is more sensitive than HCT and mAECT in detecting *T.b. gambiense*. TRUC *et al.* [318] find QBC and mAECT to be equally sensitive. QBC sensitivity is decidedly controversial. Its relevance to diagnosing sleeping sickness in humans is still under investigation. In veterinary medicine, the cost of the tubes is prohibitive – €3.08/tube according to FREZIL [313] and €1.05 according to BAILEY and SMITH [317], as against €0.08 for normal capillary tubes. QBC is therefore not contemplated for veterinary use.

1.3.4. DEAE-cellulose column filtration

This technique relies on the electrical properties of DEAE-cellulose at a given pH whereby blood cells are bound but not trypanosomes [319]. Unfortunately, while differences in surface charges between blood cells and trypanosomes are high in rodents, they are very low in ruminants making the technique far less effective. In practice, this method is used more for isolating parasites on large capacity columns than for diagnostic purposes.

On the other hand, a technique derived therefrom – miniature anion exchange centrifugation (mAECT) – is useful for diagnostic purposes and its sensitivity is as good or better than that of HCT [320] although it is a lengthier and more costly procedure. Its use in human medicine is widespread.

1.3.5. Silicone centrifugation system

This technique was described by NESSIEM [321]. It consists in depositing 1 ml of blood on a silicone mixture with a density of 1.075 and centrifuging it at 150 g for 5 minutes. The red blood cells sediment at the bottom of that tube whereupon the parasites can be retrieved from the supernatant for direct microscopic examination. This method's sensitivity is equivalent to that of the HCT and mAECT techniques.

Percoll [322] or Ficoll gradients (DESQUESNES, unpublished paper) can also be used successfully. Due to their high cost, these techniques are more commonly used for isolating parasites than for diagnostic purposes.

1.3.6. Cell lysis

Hypotonic [323] or detergent-based [324] red blood cell (RBC) lysis is more difficult to implement and lengthier than HCT while it affords only a slight improvement in sensitivity; it is impractical in the field [323] and therefore not commonly used.

1.4. Parasite culture

In vitro: the only sensitive technique for diagnosing *T. theileri* is *in vitro* culture, which can be performed in several media: NNN, blood agar medium, NOLLER's medium, etc. [7]. The prevalence of *T. theileri* in cattle varies **according to the technique used** between 5% by parasitology to 100% by hæmoculture. Since this parasite's pathogenicity is low, this diagnosis is not often performed, but such a disparity in findings shows how important the technique used is. *Trypanosoma cruzi* is grown on the same media but under very stringent conditions of safety because it can infect humans by the conjunctival route [3].

The 'kit for *in vitro* isolation' (KIVI) is an isolation medium that is currently being assessed for human and animal trypanosomoses in Africa [315, 316, 318]; in spite of its lower sensitivity compared to direct visualisation techniques (HCT and QBC), it could be used to isolate strains in America.

For *T. evansi*, BALTZ *et al.* [325] have developed culture media that yield variable results depending on the strain. In these media, *T. equiperdum* produces bloodstream forms of the parasite [14]. HIRUMI *et al.* [326] have described a serum-free medium for the bloodstream forms of *T. evansi* that is effective in maintaining the infectivity of the parasites for mice.

Trypanosoma vivax culture is sometimes possible but far less easy to implement and less productive than the other trypanosomes [248, 327, 328]. *In vitro* trypanosome cultures are complicated, lengthy and costly procedures. As such, they are used for isolating strains and producing parasites but very little for diagnostic purposes.

In vivo: the most widely used technique is intraperitoneal inoculation of infected biological material into mice, mainly the NMRI, Balb/C, CF1 and C3H strains. This is a method of choice for a sensitive diagnosis of *T. evansi* infections since most parasite strains are highly virulent in mice and develop within three to five days. Nonetheless, extending explorations for 40 to 60 days post infection is recommended. *Trypanosoma cruzi* can also be cultured in this way providing stringent precautions are taken while manipulating the infected animals because of the risk of human contamination.

Initial attempts to infect rabbits [40] and rats [329] with *T. vivax* have failed. After many attempts, mice infections with certain strains of *T. vivax* were obtained, specifically in Nigeria [330] and Uganda [132]. Infectious strains for rodents [214] have been used to more extensively study *T. vivax*

variant antigens [331, 332], which were previously assumed to be similar to those of *T. congolense* and *T. brucei*.

For isolating strains in the field, better sensitivity is obtained by irradiating the animals or inducing immunosuppression with cyclophosphamide (200 mg/kg) [291]. In Burkina Faso, field strains of *T. vivax* were isolated from irradiated mice for routine use in spite of the limitations. On the other hand, strains in French Guiana and Venezuela, were unable to be cultured even on splenectomised or immunosuppressed (dexamethasone) mice [43]. Further research on irradiated or immunosuppressed (cyclophosphamide) mice has yielded positive results (DESQUESNES, unpublished paper). The *T. vivax* strain in French Guiana can now be cultured in laboratories that do not have irradiation facilities and therefore can be offered to South American laboratories for producing the antigens needed for ELISA. Generally speaking, culturing capability for *T. vivax* strains in rodents is low or nil.

Finally, it is worth noting that the diagnosis of dourine (*T. equiperdum*) was achieved by performing intrascrotal injections on rabbits; the parasite can be seen in the serous material found in the testicular œdema five days after inoculation [61]. In rats, the initial inoculations generate low parasitæmia but after a period of adaptation the parasite begins to multiply adequately [14]. In view of this difficulty in the initial stages, the method is suitable for isolating parasite strains that are difficult to isolate otherwise, but not for diagnostic purposes.

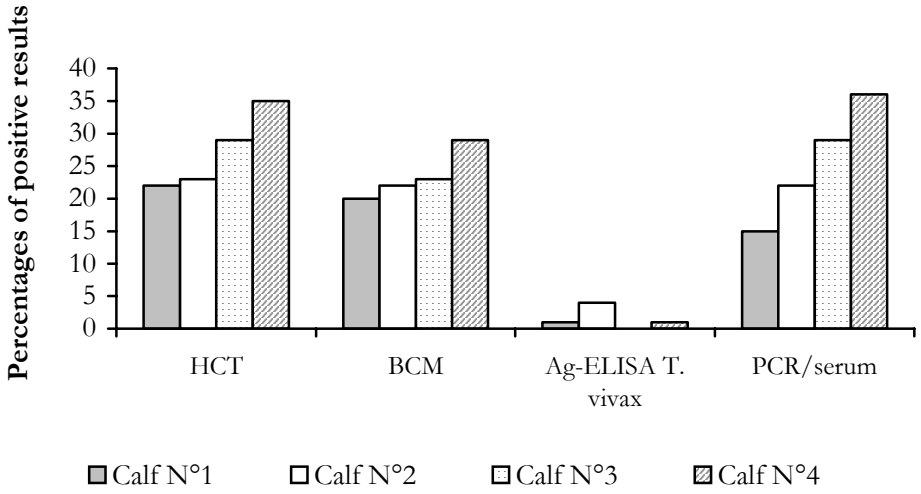
Excepting the case of *T. evansi*, *in vivo* culture is not a widely used diagnostic tool (time-consuming, costly, uses live animals) although it is useful for isolating strains and large-scale production of parasites for antigen preparation (ELISA).

1.5. Specificity and sensitivity

Specificity: trypanosome subgenera are identified on the basis of the morphological and morphometric criteria proposed by HOARE [7], by means of microscopic examination of stained blood or lymph node smears. To do so, several parasites of characteristic shape and size must be observed which is possible only when the parasitæmia is greater than 10^5. In addition, *T. vivax* can be differentiated from *T. uniforme* based on size. It is sometimes possible to identify parasites by HCT or, better still, BCM: *T. theileri* based on its large size and its stiff and highly tapering posterior tip; *Trypanozoon* which have a broad, highly refringent, undulating membrane; *T. vivax* which moves across the microscopic field (BCM) or 'swims by screwing into the medium' (HCT). While these observations can be valuable in a familiar epidemiological context, they are not always enough to identify the species [333]. Even in ideal conditions, it is impossible to distinguish *T. evansi* from *T. equiperdum* and *T. brucei*.

Sensitivity: the most sensitive technique for diagnosing *T. evansi* infections is by inoculating mice; in a survey conducted on a 156 samples from horses in the province of Formosa (Argentina), MONZON *et al.* [334] detected 88.2% infections by mouse inoculation as against 71.1% with HCT and 63.4% with BCM. The other parasitological diagnostic techniques used are even less sensitive. In any case, HCT and BCM are still the quickest and most economical and do not require using live animals. For *T. vivax*, HCT is also more sensitive than BCM [64, 335, 336] (**Fig. 24**).

In addition to the inherent sensitivity of the techniques, their field performance mainly depends on whether the infection is acute or chronic; in the latter case sensitivity is a very low. The main application for parasitology is during epizootic outbreaks where it can be usefully supplemented with serology. Other than under these circumstances, its sensitivity is very poor.



Key: for each test – HCT [63], BCM [311], Ag-ELISA [367] and PCR [368] on serum, the percentages of positive results obtained from daily monitoring (50 days) of calves experimentally infected with *T. vivax* are given in the order of the animal number, from left to right. Test sensitivity from highest to lowest is: HCT, BCM, PCR, and Ag-ELISA; however, the results of the first three tests are fairly similar [336]; only the antigen-ELISA stands out due to its very weak sensitivity.

Figure 24 – Sensitivity of four tests for detecting *Trypanosoma vivax* in four experimentally infected calves

2. DIAGNOSTIC TOOLS USING ANTIBODY SEROLOGY

Although antibody detection cannot establish whether the infection is active or took place in the past, it is extremely valuable as an epidemiological tool to assess the prevalence of infections. Most of the tests have been designed for African parasites: *T. congolense*, *T. brucei* and *T. vivax* and then adapted for *T. evansi* and *T. equiperdum* [61]. Common antigens shared by the trypanosomes have been known and/or suspected for a long time and antibody detection is therefore not considered to be species-specific.

2.1. Immunoglobulin M screening

Agglutination test: this consists in reacting the suspect serum with a suspension of lysed parasites (by sonication) in a capillary tube and then examining the micro-agglutinations, either directly or under a microscope. The diagnosis can be done in the field but the antigen is highly unstable. Although it is used for *T. brucei* infections, its results with *T. congolense* and *T. vivax* are disappointing [61].

CATT test®: this is a variant of the agglutination test that uses whole, stained parasites (freeze-dried); direct observation of agglutination is easy but sometimes subjective. Initially developed for diagnostic purposes in humans (Testryp CATT®), it was later applied for screening *T. congolense* infections in pigs and *T. evansi* infections in ruminants and pigs. Its sensitivity is comparable to the Complement Fixation and Indirect Hæmoagglutination tests [337]. A CATT test/*T. evansi*® has been developed from an Indonesian clone with the variable antigen type (VAT) that has been named RoTat 1.2, common to many *T. evansi* strains (isoVAT) [338]. Its sensitivity is better but the ubiquity of isoVAT requires further investigation [193]; its specificity is poorly defined. When applied to the detection of *T. equiperdum* infections, the test is positive only in animals that show clinical signs [14]. The reproducibility of the CATTtest/*T. evansi*® is as yet unsatisfactory and cross-reactions with *T. vivax* have been observed [43].

The test is robust when it is used in a species and/or a region with a monospecific *T. evansi* infection as is the case with buffaloes in Vietnam [339] and camels in the Sahel region. It has the advantage of being easy to implement in the field without any special equipment. However, its sensitivity and specificity need to be further investigated especially in the case of potential application America as the test reacts with *T. vivax*, *T. evansi* and *T. equiperdum*.

Indirect hæmagglutination (IHA): the method described by GILL [340] for *T. evansi* has been adapted for *T. vivax* by CLARKSON *et al.* [160]. Tanned red blood cells (RBCs) are sensitised with the trypanosome's soluble antigens and placed on micro-plates with successive dilutions of suspect sera (from 1/20 to 1/40,000). Agglutination is observed visually and the endpoint is set to the highest dilution that agglutinates. While this test is practical for individual diagnosis, it has a number of drawbacks: preservation of the tanned RBCs (3-4 days), frequent non-specific agglutinations (false positives) and impracticality for large-scale studies. Seroconversion under experimental conditions occurs at a late stage (28 days) and there are significant cross-reactions between species whereby *T. vivax* infections are detected using *T. evansi* antigens [14].

Enzyme linked immunosorbent assay (ELISA) IgM: depending on the authors, IgM concentrations observed during experimental infections are either stable [330] or not [43]. The value of the method has therefore yet to be established.

2.2. Complement fixation (CF) reaction

The complement fixation (CF) reaction was initially developed for diagnosing dourine and Surra [61] and later adapted for other animal trypanosomes, e.g. *T. congolense*, *T. brucei* and *T. vivax* [341]. The parasite antigen is reacted with decomplemented serum and the complement is then added; if recognition occurs (the animal has antibodies), the complement binds to the antibody/antigen complex. Otherwise, it does not bind. The reaction is made visible by the introduction of hæmolysin-coated sheep erythrocytes. If the complement does not bind, the RBCs are lysed and the test is negative.

The method is impractical and has little species-specificity, sometimes even producing stronger interspecific (or heterologous) than intraspecific (or homologous) reactions, particularly in the case of successive infections with the various African species [341]. CF is not able to differentiate between infections due to *T. equiperdum* and those due to *T. evansi* [14]. Seroconversion is fairly rapid for *T. vivax* but slow for the other species; finally, positive reactions after sanative treatment persist for a long time – the decline after two months is only 50%.

Although in horses there are complement-inhibiting factors that can interfere with CF, it is nonetheless recommended for diagnosing dourine [342]; it is the preferred method of the US Federal Veterinary Service for testing horses imported from Mexico as well as the method chosen by the European Union (recommended by OIE). It is not species-specific.

2.3. Indirect immunofluorescence assay (IFA)

Indirect immunofluorescence assays have been used in humans and then animals for diagnosing trypanosomoses, particularly those caused by *T. vivax* [166]. The parasites can be produced directly in a splenectomised animal from which a sample is taken during the parasitemic peak. The smear is fixed using acetone (± ethanol) and stored at –20°C. Better still, the parasites can be isolated and stored at 4°C by fixation in 80% frozen acetone/0.25% formalin in a saline solution [343]. The serum under investigation is incubated, then washed and an anti-immunoglobulin antibody (conjugated to fluorescein) for the species being tested is added. Under ultraviolet light, the green-yellowish colour is visible. The positivity threshold for the test is determined with reference to the maximum serum dilutions beyond which fluorescence is no longer visible.

Seroconversion sometimes takes a long time, 60 to 90 days in sheep and goats for *T. evansi* infections [344], but overall sensitivity is good. However, its specificity is weak and the titres obtained with homologous antigens are not always higher than those with heterologous antigens. Animals infected with *T. vivax* or *T. congolense* produce up to 85% positive results using IFA for *T. brucei*. Cross-reactions between *T. evansi* and *T. cruzi* have been reported in horses [49] and between *T. evansi* and *T. vivax* in cattle [345]. In borderline cases, the interpretation is subjective and reproducibility has sometimes been questioned [345]. IFA was very widely used particularly for epidemiological surveys and has only been replaced by indirect ELISA techniques (IgG detection) from the 1980s.

2.4. Immunoglobulin G detection by enzyme linked immunosorbent assay (ELISA)

2.4.1. General considerations

The ELISA method described by ENGVAL and PERLMANN [346] has been adapted for diagnosing many infectious diseases [347] including *T. cruzi*-induced trypanosomosis in humans [348] and *T. evansi* [349], *T. equiperdum* [350], *T. vivax* [345, 351, 352], *T. congolense* [353, 354] trypanosomoses and others in animals.

The principle of indirect ELISA is based on sensitisation of ELISA polystyrene microplates using soluble antigens (5-30 µg/ml) extracted from the parasite by sonication and ultra-centrifugation. The specific immunoglobulins for the specimens under investigation bind to the antigens and are visualised by antiglobulins for the species (often marked with peroxidase) and the substrate/chromogen complex (hydrogen peroxide/ABTS for peroxidase). Reading is done visually or with a spectrophotometer at the appropriate wavelength for the chromogen (405 nm for ABTS) [345]. Alternatively, a chemiluminescent reaction (luminal oxidation) visualised by photography can be used [355].

Comparisons between the IFA, IH, CF and ELISA work out in favour of the latter in view of its excellent reproducibility, low-cost and high processing capacity which makes it a highly suitable tool for epidemiological surveys [345, 349, 356].

Antigenic standardisation however remains a serious problem, variations being inherent to *in vivo* parasite production. Laboratories have so far produced their own antigens, which makes the technique relatively variable. Furthermore, choice of the positivity threshold is sometimes arbitrary and needs to be defined and validated according to international and/or regional standards. Several recommendations on this topic have been published [43, 354, 357].

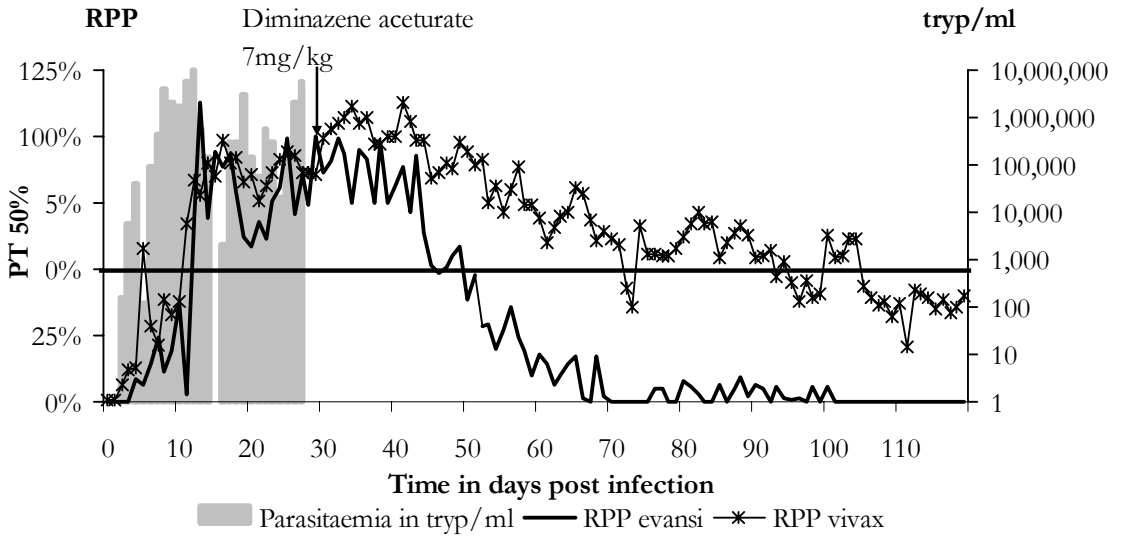
2.4.2. Performance of indirect-ELISA

These methods have good specificity for genera and even subgenera that are pathogenic for animals. No cross-reactions have been observed with *T. theileri* [345, 358]. However, among the pathogenic trypanosomes, specificity for subgenus is poor – multicross reactions between *T. vivax*, *T. congolense*, *T. brucei* spp., *T. evansi*, *T. equiperdum* and *T. cruzi* have been described [345, 353, 359, 360, 361]. Indeed, these cross-reactions can be used for diagnostic purposes (see paragraph 2.4.3.).

Research into the isolation of a non-protein antigenic fraction of *T. brucei* that does not cross-react with the sera of animals that are immunised against *T. congolense* and *T. evansi* [362] will perhaps enable species-specific diagnostic tools to be developed. Furthermore, the production of recombinant antigens would improve the specificity of this type of antigen and facilitate its synthesis and standardisation.

The indirect-ELISA *T. vivax* test has very satisfactory **sensitivity**; an evaluation of 13 sheep experimentally infected with *T. vivax*, sensitivity was 90% for the 1,226 samples tested. Positive

seroconversion occurs 7 to 15 days post infection and thereafter the optical density scores remain high throughout the duration of the infection. However, for two to three months following sterilising sanative treatment, the test yields positive results. By way of example, a serological follow-up profile is shown in **Figure 25** [43]. In this experiment, negative seroconversion under a homologous system (on *T. vivax* antigens) takes approximately 80 days. Homologous indirect-ELISA sensitivity in sheep experimentally infected with *T. congolense* (savanna and forest) or *T. evansi* is highly comparable [363].



Comment: relative percentage of positivity (RPP) is an expression proportional to the optical density of the specimen; in a homologous system, it goes above the positivity threshold (PT) from 8 to 15 days post infection, and goes back below that threshold approximately 80 days following sterilising treatment; in a heterologous system, it goes above the positivity threshold some 15 days post infection and goes back down again earlier than in a homologous system, approximately 3 weeks after treatment.

Key: PT = positivity threshold for indirect-ELISA *T. vivax* and *T. evansi*; the vertical arrow indicates treatment with diminazene aceturate (7mg/kg IM) [43]

Figure 25 – Indirect-ELISA *Trypanosoma vivax* (homologous) and indirect-ELISA *Trypanosoma evansi* (heterologous) reactions and parasitæmia of a sheep infected with *Trypanosoma vivax*, then treated with diminazene aceturate

Antibody persistence following sterilising treatment is two to four months in the case of recent infections by *T. vivax*, *T. evansi* [43] or *T. congolense* [364] in sheep, and three to four months in cattle [573]. It is shorter (30 days) in experiments conducted by FERENC *et al.* [345] involving calves infected with *T. vivax*, and much longer (more than ten months) in those conducted by AUTHIE on *T. congolense* (unpublished paper). Prolonged persistence might be the result of the cumulative effect of several infections. On these grounds, some authors have suggested that tests be interpreted as a function of age [365].

For a single antigen batch, test reproducibility is very good, but it can vary from one batch to another and even more so from one parasite strain to another. Interlaboratory reproducibility will not be satisfactory until recombinant antigens are available which may have the further benefit of providing a species-specific diagnostic tool. However, while recombinant antigens can improve reproducibility and specificity, this inevitably implies a loss in sensitivity. Depending on the particular circumstances, one technique will be preferable to the other. In the meantime, the widespread distribution of trypanosomal clones that are well characterised for the diagnoses should be encouraged so that laboratories all use comparable antigens. Y486 (*T. vivax* Zaria) or IL1180

(*T. congolense* Serengetti) clones could be offered in Africa and others (*T. vivax* and *T. evansi*) should be defined for Latin America.

2.4.3. Heterologous indirect-ELISA systems

Although serological cross-reactions between *Trypanosoma* spp. preclude a species-specific diagnosis, they are sometimes so pronounced that they can in fact be used for diagnostic purposes as substitutes, especially for a number of trypanosomes that are difficult (*T. vivax*) or dangerous (*T. cruzi*) to culture.

Trypanosoma vivax infections can be detected with equal sensitivity in a homologous, indirect-ELISA *T. vivax* system and a heterologous, indirect-ELISA *T. evansi* system [360]; in these experiments, the cross-reaction is not symmetrical and detection sensitivity of *T. evansi* infections with indirect-ELISA *T. vivax* is very weak. Additionally, negative seroconversion of animals infected with *T. vivax* is far quicker under the heterologous system (*T. evansi* antigens) than under the homologous system (*T. vivax* antigens) (**Fig. 25**). In fact, monospecific *T. vivax* and *T. evansi* infections can be discriminated by examining the average ratio of the optical densities of samples from the two systems:

MRv/e = the mean ratio of RPP indirect-ELISA *T. vivax*/RPP indirect-ELISA *T. evansi*°

This ratio is practically always less than 0.32 in *T. evansi* infections and greater than 0.32 in *T. vivax* infections [43]. However, this type of interpretation requires complete inter-laboratory standardisation as the results depend on the antigens and the reference samples used.

T. cruzi infections in humans can also be detected by indirect-ELISA *T. evansi* with a sensitivity equivalent to that of a homologous system (*T. cruzi* antigens) [361].

Trypanosoma evansi is antigenically a very rich parasite – in Latin America, indirect-ELISA *T. evansi* is probably able to detect infections induced by *T. vivax*, *T. evansi*, *T. equiperdum* and/or *T. cruzi* with equal effectiveness.

In experimental monospecific infections with *T. congolense* (savanna type 'S'), *T. vivax* and *T. evansi*, species can be discriminated by comparing the scores obtained from the three indirect-ELISA *T. congolense* (S), *T. vivax* and *T. evansi* tests as the homologous reaction is nearly always stronger than the heterologous one. But in the case of mixed infection or heterologous reinfection, serological reactions cannot be interpreted and it is difficult to discriminate [363]. Consequently, for field samples, a species-specific individual diagnostic tool is not achieved by sensitising the microplates with crude antigens. However, interpretation on the scale of a whole population does give an estimation of the relative proportions of the various species present.

Comment: a dot-ELISA system on nitrocellulose has been developed for detecting antibodies against *T. evansi* [366]. It provides a rapid individual diagnosis and requires little equipment. Sensitivity and specificity are stated to be close to those of indirect-ELISA.

2.5. Specificity, sensitivity and predictive value of antibody detection

Specificity: with the exception of *T. theileri*, which does not produce a cross-reaction with the trypanosomes that are pathogenic for cattle, to interpret an epidemiological survey one needs to consider the interference between the various trypanosomal pathogens that may be present in the area of investigation since detection of immunoglobulins directed against trypanosomes is not species-specific. In Latin America, the interference between *T. vivax*, *T. evansi* and *T. cruzi* in ruminants and between *T. evansi*, *T. cruzi* and *T. equiperdum* in Equidae, must therefore be considered. Persistence of antibodies for two to four months after parasite removal is also a factor that must be borne in mind when interpreting results, short of which the specificity of the test

could be undermined. As has been shown for African trypanosomoses in ruminants [363], an interpretation specific to the species for ELISA *T. vivax* and *T. evansi* tests could be developed on the basis of fully standardised antigens and reference sera.

Sensitivity: all antibody detection tests are very sensitive, and ELISA most of all. The latency of immune response requires a two-week post infection time lag for positive seroconversion. If the infection is suspected to be recent, it is advisable to perform an additional parasitological test (HCT) or repeat the test two weeks later.

Predictive value in respect of active infection: immunoglobulin kinetics are such that following sanative treatment a 3-month interval must elapse before negative seroconversion (the time lag is shorter under a heterologous system). When tests are performed 4 months after treatment, the predictive value of an indirect-ELISA concerning the activity of an infection is very high. Prevalence of infections in a population can be therefore be measured by sampling and antibody serology. By combining this data with the history of trypanocidal treatments on that population, active infection will sometimes be detected together with a good approximation of its prevalence.

Among the methods currently available, only indirect-ELISA provides an estimate of prevalence among animals that have recently come into contact with the trypanosomes.

However, in horses infected by *T. evansi* and treated with quinapyramine sulphate, persistence of antibodies detected by ELISA was much longer (Monzon *et al.* [575])

3. ANTIGEN SEROLOGY DIAGNOSIS

Antibody detection by means of ELISA is highly sensitive but, as previously pointed out, not species-specific. For the purposes of sero-epidemiological surveys, it is useful to determine the species involved. Furthermore, a positive reaction to antibody detection does not mean that the infection existed at the time the sample was taken; positivity can persist between one and four months depending on the techniques used. Ideally an antigen detection test that is both sensitive and specific would solve these problems and allow comprehensive, detailed investigations to be conducted on the epidemiology of trypanosomoses. Unfortunately, as will become apparent, none of the available methods has so far proved fully satisfactory.

3.1. Review of methods

Hyperimmune sera can be used to detect parasite antigens in samples tested with the indirect-ELISA technique [360], but standardisation of the method is difficult because plates are sensitised using the tested samples.

To overcome this drawback, antigen detection can be done by means of the immunocapture-ELISA technique that uses poly-or monoclonal antibodies; the advantage of the latter is that they can be synthesised on a large scale and are more reproducible than the former. In any case, the ELISA plates are sensitised with the antibodies; once non-specific sites are blocked, the sera to be tested are incubated, the plates are washed, the same antibody or antibodies, conjugated to an enzyme, are incubated and then visualised by means of an appropriate substrate/chromogen complex.

In the case of polyclonal antibodies, there is an additional step with a conjugate directed against the species producing the polyclonal antibodies.

3.2. Polyclonal antibodies

Trypanosoma evansi and *T. congolense* antigen detection by immunocapture was first developed by means of polyclonal antibodies produced by immunisation using crude trypanosome extracts [370].

Antigens were detected within 10 to 14 days p.i. and disappeared within 20 days of sanative treatment being applied. However, species-specificity was not satisfactory and reagent production was highly technical and elaborate. This work was supplanted by the development of monoclonal antibodies which were far it easier to synthesise on a large scale and whose performance was expected to be much better. A comparison of immunocapture-ELISA for *T. evansi* using polyclonal and monoclonal antibodies has come out in support of the latter [371].

3.3. Monoclonal antibodies

The development by NANTULYA *et al.* [372] of three types of monoclonal antibodies using procyclic forms (invariable antigens) of *T. vivax*, *T. brucei* and *T. congolense* (S) has practically brought to the aforementioned investigations to a halt. Since no antigenic cross-reactions were observed between the three types with parasite lysates [372], the authors concluded that the monoclonal antibodies were fully specific and developed the three immunocapture-ELISA (or antigen-ELISA Ag-ELISA) tests specific for *T. vivax*, *Trypanozoon* and *T. congolense* (savanna type).

These Ag-ELISAs were evaluated by NANTULYA and LINDQVIST [367], showing excellent performance in terms of specificity and sensitivity under experimental conditions. In field application [373], the tests' sensitivity was 96.2% and specificity 100%. A lot of further investigation was subsequently performed, none of which challenged findings relating to the reagents' sensitivity and specificity [369, 374, 375, 376, 377, 378, 379, 380]. An agglutination test for detecting *T. evansi* infections called Suratex was then developed [379] using the monoclonal antibodies specific for *Trypanozoon*. Thereafter, BOSOMPEM, ASSOKU, NANTULYA *et al.* [381, 382] referred to the development of new dot-ELISA based on these monoclonal antibodies and intended for the detection of trypanosomes in insects [381, 382]. However, Ag-ELISA was used for the first time in South America in 1991 on samples taken from cattle in French Guiana [383], yielding positive *T. brucei* and *T. congolense* results. As a result, the tests had to be re-evaluated first in the South American context and then in Africa.

A reassessment of Ag-ELISA – involving 2,953 serum samples collected from cattle-raising farms in French Guiana, 130 from cattle imported from metropolitan France upon their arrival in French Guiana (negative controls), 160 from African cattle (ILRI, Nairobi, Kenya) and 408 from sheep (CIRAD-EMVT, French Guiana) that were experimentally infected with various strains of *T. vivax* and *T. evansi* – gave rise to results that were at odds with previous findings; these results are summarised in **Table V** [43, 336, 383, 384, 385, 386, 387] (**Fig. 26**). It proved very difficult to convince the scientific community of the soundness of these results that strongly contradicted those that were initially published by the team that had developed these tests.

	Number of specimens tested	**Ag-ELISA *T. vivax***	**Ag-ELISA *T. brucei****	**Ag-ELISA *T. congolense***
Trypanosome-free cattle (imported from Metropolitan France)	130	8% false positives	6% false positives	10% false positives
Cattle possibly infected by *T. vivax* (cattle from French Guiana)	2,953	12% positives	20% false positives	18% false positives
Cattle infected with *T. vivax* from French Guiana (TVFG1 or IL4007) (experimental infections)	160	3.8% true positives	4.4% false positives	4.1% false positives
Sheep infected with *T. vivax* from French Guiana (TVFG1 or IL4007) (experimental infections)	338	2% true positives	26% false positives	22% false positives
Sheep infected with *T. evansi* (experimental infections with the Asian strain RoTat 1.2)	70	4.3% false positives	60% true positives	7.1% false positives

* use for detecting antigens against *Trypanozoon*

Table V – Specimens tested, positive results for the three Ag-ELISAs, and meaning

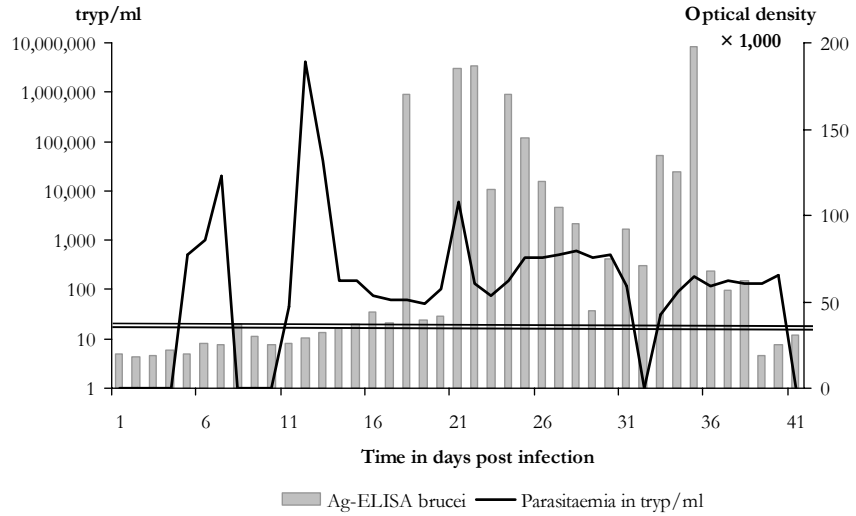


Figure 26 a – Ag-ELISA T. brucei

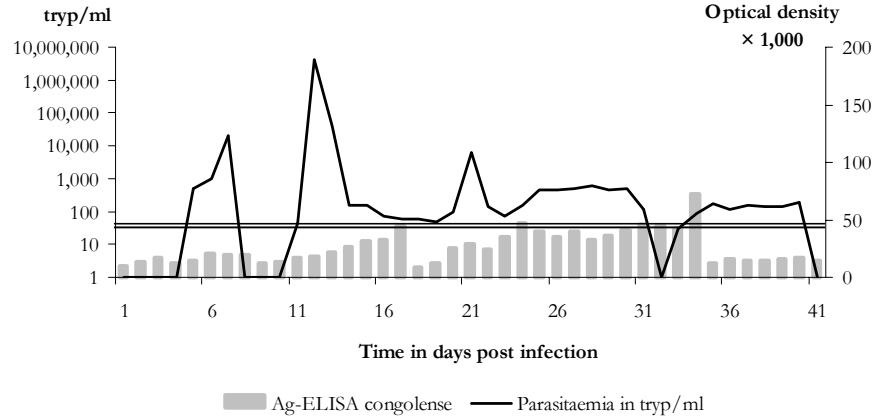


Figure 26 b – Ag-ELISA T. congolense

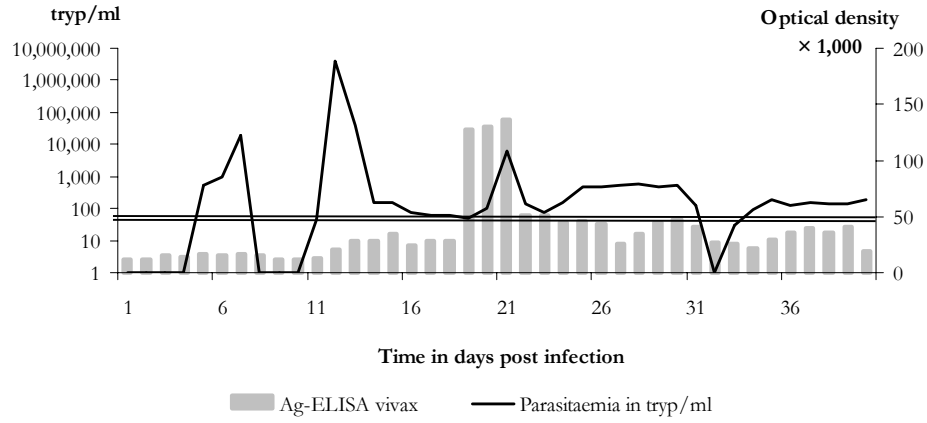


Figure 26 c – Ag-ELISA T. vivax

Key: the positivity threshold is set at 50 OD for Ag-ELISAs; it is represented by a horizontal line. The samples are positive for Ag-ELISA when the OD histogram is above that threshold.

Figure 26 – Parasitæmia and optical density scores by Ag-ELISA *Trypanosoma* spp. of a sheep infected with *Trypanosoma evansi*

In any case, the results obtained in French Guiana corroborated the lack of specificity and sensitivity recorded by a number of authors in Africa:

1) positive Ag-ELISAs for *T. congolense* in areas free of Glossinae [388];
2) positive Ag-ELISAs for *T. congolense* and *T. brucei* and negative Ag-ELISAs for *T. vivax* on animals whose parasitology was positive for *T. vivax* alone [389];
3) negative Ag-ELISAs for *T. vivax* for animals that were naturally or experimentally infected by *T. vivax* [389, 390].

Following this work and other investigations conducted in Africa, the validity of Ag-ELISAs was called into doubt during an international meeting (CTVM, IAEA, ILRI, KETRI, etc.) that was held in Nairobi in December 1996. The results presented by participants showed poor performance for these tests [391] and reached similar conclusions, which led to discarding immunocapture-ELISA.

Antigen detection by ELISA continues to be the method of choice for studying animal trypanosomoses and therefore research teams should attempt to produce new, more sensitive and specific monoclonal antibodies than so far developed. However, although this type of test does allow active infections to be detected with much higher specificity, they necessarily imply decreased sensitivity due to the fact that they rely on a single or a restricted number of monoclonal antibodies, and hence, of circulating target antigens(s).

4. POLYMERASE CHAIN REACTION (PCR)-BASED DIAGNOSIS

Detecting a specific DNA segment provides a species-specific diagnosis of active infections by trypanosomes; sensitive, specific primers have been described for the main trypanosomes that are pathogenic for mammals. After a review of the polymerase chain reaction (PCR) techniques on DNA, the technique's sensitivity is examined and various methods for preparing the samples are discussed with a view to improving test sensitivity in detecting trypanosomes in cattle.

4.1. A review of the techniques

4.1.1. General principles of PCR

DNA amplification by polymerase chain reaction, more commonly referred to as PCR, is able to show up the presence of DNA segments made up of known, or partially known, sequences of bases [392]. This reaction was made possible by the discovery of Taq polymerase, a thermostable enzyme extracted from *Thermophilus aquaticus* that polymerises nucleic acids. To achieve polymerisation a mixture made up of matrix DNA, Taq polymerase, deoxyribonucleic phosphate acids (dNTP), an appropriate buffer and two oligonucleotides that flank a specific portion of DNA is subjected to a series of heat cycles. The heat cycles comprise three phases: a denaturation phase (at approximately 94°C) that causes the DNA double helixes to open; a phase during which the oligonucleotides attach to the complementary sequence of matrix DNA (50°C approximately), and an extension phase during which the nucleic acids polymerise (at approximately 70°C). Starting in the initial phases, the products from the previous polymerisation step also serve as a matrix for the subsequent polymerisations. By repeating the heat cycle some 30 times a great many DNA portions of identical sequence and weight are synthesised. Under ideal conditions, and in the absence of inhibitors, the detection of a single DNA molecule is possible indicating how highly sensitive the method can theoretically be [393, 394].

The product of the polymerisation is visualised by agarose gel electrophoresis of DNA with the addition of ethidium bromide, an intercalating agent for DNA so that the DNA can be seen under ultraviolet light (transillumination in the orange range). In these conditions, a band of DNA can be seen whose weight is specific to the amplified DNA sequence. It is also possible to visualise the PCR product and confirm its specificity using a DNA probe of known sequence, labelled with an enzyme or radioactive isotopes.

A single, weakly specific oligonucleotide can also be used for amplification by means of a so-called arbitrary or random primer: this procedure is referred to as random or arbitrary priming. In this case amplification occurs from images that partially mirror the DNA sequences where the internal sequence of the amplified products is generally unknown.

When PCR is applied to diagnostic purposes, it can reveal the presence of the DNA of an organism, e.g. a parasite. It is comparable to antigen detection techniques in as much as it shows up the presence of the parasite and indicates that the infection is active. Indeed, once the parasite dies, the persistence of free DNA in the host's circulation is short-lived – one or two days at most [384]. Finally, the PCR technique can be used indiscriminately in the host or the vector. Because of its characteristics, PCR is likely to beneficially replace circulating antigen detection techniques.

4.1.2. PCR applied to trypanosomal detection

4.1.2.1. The oligonucleotides

Oligonucleotides that are specific for *T. vivax* (TVW1 and TVW2) and for *Trypanozoon* (TBR1 and TBR2) have been described by MASIGA *et al.* [395]. They rely on highly repetitive messages of parasitic DNA, which enhances their sensitivity. Other oligonucleotides specific for *T. vivax* were subsequently described [396, 397].

MOSER *et al.* [390] have described the TCZ1 and TCZ2 oligonucleotides for the detection of *T. cruzi*.

DIALL [300], followed by IJAZ *et al.* [399] have described oligonucleotides that recognise *T. evansi* but do not react with the *T. brucei* spp.

So far, no biological material has called the specificity of these reagents into question. For trypanosomal detection therefore, the use of PCR without a DNA probe is reliable until proof to the contrary. However, it should be noted that in the case of *T. cruzi*, cross-reactions with *T. rangeli* have been observed [400]; some new oligonucleotides (BP1/BP2) that were recently described react both with *T. cruzi* and *T. rangeli*, but their products have different molecular weights. Species discrimination is therefore thought to be possible [401]. *Trypanosoma evansi* cannot be differentiated from *T. equiperdum* because the primers described for the former react with the latter erratically. In addition, the DNA probes and enzymatic digestions do not establish a clear-cut separation between these two species [236].

Some primers are more sensitive than others; hence, PCR on serum using *T. brucei* primers [241] has yielded positive results when the parasitæmia was equal to at least 10^3 parasites/ml, whereas with *T. evansi* primers [300], results were positive only when the parasitæmia was greater than 10^4 parasites/ml. In Latin America, *T. brucei* primers are therefore recommended for the diagnosis of *T. evansi*; however, no techniques so far are able to distinguish *T. evansi* from *T. equiperdum*.

More recently primers specific for kinetoplasts that amplify the internal spacers transcribed from ribosomal DNA (ITS of rDNA) have been described [402, 403]. The value of this technique is that the PCR product obtained has a specific and different weight for each species of trypanosome [404, 405], which means that all the trypanosomes can be detected and identified in a single reaction (multispecific PCR). Additional work is being done to enhance these techniques [406].

4.1.2.2. Sample preparation

A PCR performed directly on blood is always negative due to the existence of reaction inhibitors (haemoglobin). As a general rule, the biological samples must be prepared for PCR usually by DNA extraction using the phenol-chloroform method. However, this technique is awkward, toxic and costly whereas more rapid and economical methods have now been developed. When used directly on fragments of a blood smear for detecting *T. evansi*, the method is claimed to be more sensitive

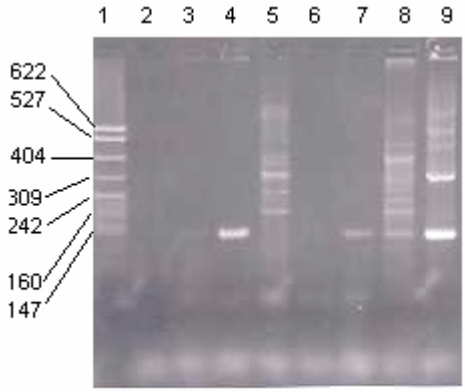
then inoculation of mice [407]. However, in the case of trypanosomes, test sensitivity is considerably enhanced by first enriching the blood sample (buffy coat technique) along with resin treatment or a commercial DNA purification kit.

Several techniques have been described; the simplest is to perform the test directly on plasma although sensitivity is low (approximately 500 trypanosomes per ml of blood) [408]. Better sensitivity is achieved with a buffy coat sample extracted from a haematocrit tube [409]. The blood lysis technique [300] is less sensitive (500 parasites/ml) and produces a large number of non-specific bands because of the plethora of host DNA in the sample. Sensitivity with the sediment of centrifuged plasma [408] is better than with the buffy coat (10 trypanosomes/ml) but not as good as with resin treatments (1 parasite/ml) (**Fig. 27**).

PANYIM *et al.* [393], for *T. evansi*, and PENCHENIER *et al.* [394] for *T. brucei*, state that a single trypanosome can be detected by PCR or by treating 1 ml of blood using resin (Ready AMP®). Bulk resin Chelex 100® (in a 5% suspension) can usefully replace Ready AMP® for cost reasons with comparable sensitivity [410].

The Quiagen® kit for DNA purification is very effective (1 parasite/ml) but expensive (€2/sample). A new DNA purification kit (DNAZol BD-polyacryl carrier, MRC®) is now available at a far lower cost (€0.05) (DESQUESNES, unpublished). Its sensitivity however remains to be assessed.

Fingerprint
of PBR 322 marker



Key: type of sample by column (C) from left to right:
C1: PBR 322 molecular marker;
C2: negative control;
from C3 to C8: samples with parasitæmias of 600 *T. vivax*/ml;
C3: plasma;
C4: plasma sediment;
C5: lysed blood;
C6: heparinised blood;
C7: buffy coat;
C8: purified DNA;
C9: positive control (plasma with 2.10^6 *T. vivax*/ml).

Figure 27 – Comparison of products obtained using the various preparation techniques for PCR

Collecting samples in 70° alcohol (DESQUESNES, unpublished) or on filter paper [411, 412] is also possible. This can be followed by treatment with Chelex 100® providing a very practical means for preserving and shipping samples when no cooling facilities are available. A review of the PCR techniques applied to trypanosomes was recently published by DESQUESNES and DAVILA [406].

4.2. Conclusions on PCR

The main benefit of PCR is its species-specificity. In this respect, it is superior to any other technique and an essential epidemiological tool capable of identifying various species that are present in the populations surveyed (hosts and vectors).

Ghost DNA (DNA from a dead parasite) is very short-lived [336]. Hence, detection of trypanosomal DNA is a rough indication of active infection.

Several techniques for preparing blood samples (buffy coat, filter paper, sediment of centrifuged plasma, resin treatment, DNA purification) are able to enhance the sensitivity of PCR diagnosis (1 to 10 trypanosomes/ml of blood), making it more sensitive than the parasitological diagnostic techniques (100 to 1,000 trypanosomes/ml of blood). In the field, this generally translates as the detection of twice as many positive samples with PCR compared to parasitology [397, 413]. Although it is higher, this sensitivity is not enough to detect all infections – as is apparent from experimental infections during aparasitæmic periods [414], and, in the field, when prevalence derived from PCR is compared with antibody seroprevalence. One illustration is a survey on *Trypanosoma* spp. pathogens for cattle, in the Sideradougou area of Burkina Faso, where respective prevalences of 8.5%, 18% and 80% were found by parasitology, buffy coat PCR and indirect ELISA serology [450]. While PCR is twice as sensitive as parasitology, the serological results indicate that the actual prevalence of infections is far greater.

Although in theory PCR sensitivity is very high (detection of one DNA molecule in a sample) and can usefully be used for detecting pathogens that are present in great numbers in the blood, it has limitations and is indeed inherently inappropriate when the infective agents do not circulate in the bloodstream or else circulate at rates of less than one per volume of treated sample material. This is often the case with trypanosomes; aparasitæmic periods are a biological limitation to the application of PCR on blood and its derivatives [406].

For the detection of active infections, antigen detection techniques are more promising in terms of sensitivity than PCR because the quantity of circulating antigens will always be larger than the number of parasites from which they stem. However, a great many antigens need to be captured for the method to retain its theoretical superiority. Concerning the host, PCR sensitivity for detecting circulating trypanosomes is promising and efforts to develop techniques for preparing the samples that will further enhance sensitivity should be continued. The specificity of PCR is a major step forward. Finally, recent multispecific diagnosis in a single PCR opens up opportunities for more widespread PCR application in epidemiological surveys by reducing the cost four- or fivefold. [406].

5. DIAGNOSIS IN VECTORS

5.1. Biological vectors

Once infected, vampire bats (*Desmodus rotundus*), which are biological vectors of *T. evansi*, become permanent carriers of the parasites. Furthermore transmission by mutual biting between congeners ensures that the parasite circulates through an infected colony and is maintained. From an epidemiological standpoint, it is therefore useful to identify infected bat colonies and be in a position to examine the reservoirs and potential sources of contamination for horses.

Nets are used to capture bats at night (see Chapter 6). Vampire bats are diagnosed using the same techniques as those already described for mammals but no data has been published on this topic in the scientific literature.

5.2. Cyclical vectors

Vectors that transmit cyclically, such as *Glossina* in Africa or reduviid bugs and opossums in Latin America, also become permanent carriers after being infected by trypanosomes. In Triatomines infected by *T. cruzi*, the parasite circulates and is maintained in the population by mutual biting. Triatomines are a true reservoir for this parasite [258]. There have been no reports of a similar occurrence in opossums, but mutual contamination between opossums is likely as the marsupials' infected faeces contain forms that are infective perorally and by the transconjunctival route [143]. A proper understanding of the epidemiology of trypanosomoses requires knowledge of the prevalence of infected and/or infective vectors.

The specificity for species of parasitological diagnosis in *Glossina* is very low [416]; it is more often performed by PCR with the specific primers [409, 410, 417]. PCR techniques have been used for screening trypanosomes in the mouthparts, salivary glands and/or gut of *Glossina* [409, 418].

In reduviid bugs, parasitological diagnosis is also insensitive and non-specific, sometimes leading to confusion between pathogenic and non-pathogenic species (*T. cruzi* and *T. rangeli*). On the other hand, PCR techniques are highly sensitive [398, 401, 419, 420] and some are species-specific [400]. Reduviid bugs can be caught by hand or in traps; the diagnosis is performed on the excreta or the gut contents following dissection of the distal part of the digestive tract [258]. Opossums, which are captured at night using cage traps containing fruit as bait, are diagnosed with the same techniques as mammals, mainly parasitological diagnostic methods [94].

5.3. Mechanical vectors

The presence of trypanosomes in hæmatophagous mechanical vector insects can be diagnosed by parasitological means. Except in the unusual case of recent ingestion of pathogenic trypanosomes by the insects, this examination is neither very specific nor sensitive [7]. Horsefly gut often contains Trypanosomatidae in various forms (amastigote, choanomastigote, epimastigote, trypomastigote, and metatrypanosomes) that are consistent with *Crithidia* forms of insect development or *Trypanosoma theileri* or *T. theileri*-like forms [294] that cannot be differentiated. A more specific and sensitive diagnosis can be achieved using PCR on the mouthparts or gut of horseflies [410]. However, the practical value of such a diagnosis needs to be assessed.

The study of mechanical transmission by hæmatophagous insects (Chapter 3) has shown that they transmit the trypanosomes immediately after being contaminated, when a bloodmeal taken on an infected host is interrupted prior to satiation, and the insect resumes its meal a few seconds or a few minutes later on another host. Unlike true vectors such as *Glossina*, reduviid bugs, opossum and vampire bats, deferred transmission does not occur with hæmatophagous insects that transmit mechanically – they are vectors while they are taking the meal. In this case, the only way to identify an 'infectious insect' in meaningful epidemiological terms would be to detect trypanosomes (or trypanosomal DNA) in the mouthparts of an insect that is captured precisely when it switches host.

The most common method for capturing mechanical vector insects is to use visual decoys sometimes in combination with an olfactory attractant that partially mimic the hosts. The insects are captured when they are **in search of a host** on which to feed [289]. Most of the time, insects that are searching for a host have either fasted or else have some digested blood in their digestive tract [261, 288]. Since hæmatophagous insects take bloodmeals at several days interval and trypanosome survival is estimated to be just a few hours (5 h to 7 h, according to FERENC *et al.* [112], insects captured in this way are unlikely to be infective. They are simply a short-lived epidemiological dead end.

Detecting trypanosomes in the gut means that the insect has fed on an infected host; because the parasite is thereafter sequestered once and for all, this type of observation has little epidemiological relevance. It can only serve to indirectly detect infections in wild animals. For that purpose, it is advisable to use ultraviolet traps to attract females that have recently taken a bloodmeal [289].

Detecting trypanosomes in mouthparts means that the insect is potentially infective which might be epidemiologically significant. However, this is extremely unusual in view the techniques used for collecting insects. To identify an 'infectious insect' in any epidemiologically meaningful way, the insects would need to be captured by hand on the livestock or by means of a trap place in the midst of the herd. Compared to a direct blood sample taken from the livestock – which is after far simpler and more informative – no additional benefit is derived from this complex operation.

Detecting trypanosomes in hæmatophagous mechanical vector insects is therefore of very low practical value. Its only valid purpose, if insects were captured using ultraviolet traps, would be to

detect infection in wild hosts that are difficult to capture, i.e. using the insect simply as a blood sampler, as it were. Practitioners need to keep these considerations in mind to avoid needless use of PCR, which, although it is a fascinating tool, is sometimes inappropriate to the epidemiological context in the field.

6. CONCLUSIONS ON DIAGNOSIS OF TRYPANOSOMOSES IN LATIN AMERICA

Diagnosing trypanosomoses raises problems of sensitivity and specificity that vary according to the parasites that may be present in the geographic area under consideration, and according to the hosts.

The trypanosomes found in Latin America are listed together with their hosts in **Table I** (Chapter 1). For each of the host species, clinical, diagnostic and epidemiological investigation needs to consider the possible presence of various *Trypanosoma* spp.

Parasitological diagnosis, although it is thought to be insensitive, is actually more sensitive than recently developed antigen detection techniques by immunocapture-ELISA *Trypanosoma* spp. test. These techniques turned out to be unusable due to their very low sensitivity and lack of species-specificity. Antigen detection by immunocapture nonetheless remains an important goal for which new, more specific and sensitive monoclonal antibodies need to be developed. There must be no mistake however – the sensitivity of an antigen detection test based on a single monoclonal antibody cannot be high because only one antigen among the range presented by the parasite is captured. Strategically – on the molecular scale – this type of test can aim at high specificity but its sensitivity is necessarily low.

The characteristics of the CATT/*T. evansi*® and the indirect-ELISA *T. vivax* tests for IgM screening are not reliable. On the other hand, IgG screening by means of indirect-ELISA *T. vivax* or/*T. evansi* are highly satisfactory both in terms of sensitivity and genus-specificity for pathogenic *Trypanosoma* spp. An appreciation of changes in the quantitative response of samples during infection is more often than not enough to infer whether the infection is currently active or was so previously and hence to establish the immune status of the animals and sometimes their infectious status. Specificity for species remains a major problem in certain epidemiological contexts where multiple infections may exist. A comparative interpretation of positivity scores does however provide an estimate of the relative importance of species on the scale of whole populations. Once again, although the development of recombinant antigens may raise hopes that highly effective and fully standardised tests will soon be available, one must not forget that indirect-ELISA based on a single antigen could, if the antigen is properly selected, be highly specific but its sensitivity would be well below conventional indirect-ELISA sensitivity which relies on a broad spectrum of antigens (all the parasite's soluble antigens). One technique would then be preferred over the other depending on the particular goals of the investigation. Several techniques for preparing samples for PCR provide a sensitive and specific diagnosis that is relatively cheap, e.g. sediments of centrifuged plasma or DNAzol BD®. These can usefully supplement the set of diagnostic tools described above since PCR affords high species-specificity. Sample treatment with commercial bulk resins, and the development of multispecific PCR can potentially also provide inexpensive diagnostic tools.

In all cases, the choice of diagnostic tool must always consider the user's goals, the technical constraints and the economic limitations.

For **diagnosing trypanosomosis in individual animals or herds** (veterinary goal), parasitological diagnostic tools are recommended: HCT, combined with BCM and stained smear test when the HCT is positive. PCR on sediment of centrifuged plasma or purified DNA can enhance sensitivity providing the test is performed rapidly. Similarly, antibody serology (CATT test, indirect-ELISA or IFA) is useful in non-enzootic areas. In a recently infected herd, two serological assays 15 days apart should be performed. In addition to these tests, in the case of *T. evansi*, inoculating mice or *in*

vitro culture may be helpful particularly if the purpose is to detect *T. equiperdum* infection [325]. However, chances of success are limited.

For epidemiological surveys, estimating the prevalence of infections can be performed by means of indirect-ELISAs; in addition, parasitological or PCR-based diagnosis should be performed to confirm the species present, perform a differential diagnosis in respect of other hæmoparasitoses, and, through the haematocrit value, provide information on the clinical impact of trypanosomoses. In farm animals, interference by *T. cruzi* in serological tests is probably low but has never been properly assessed for want of an appropriate test. In enzootic areas with mixed infections, interference between *T. vivax* and *T. evansi* must be considered.

In areas and/or species with a monospecific infection, HCT, CATT/*T. evansi* or indirect-ELISA are sufficient.

For animal health surveillance (*T. equiperdum*), test sensitivity is a major concern; indirect-ELISA is thought to be the best technique but because of cross-reactions, specificity must be confirmed by other techniques (CF, IFA, etc.). In fact, until such time as oligonucleotides specific for *T. equiperdum* become available, the distinction between *T. evansi* and *T. equiperdum* will be difficult to establish. DNA probes (236) have been described for the purpose of differentiating the species; but this work has not been conclusive.

A priority in the area of diagnostic tools for livestock trypanosomoses is the development of sensitive, species-specific ELISA for detecting antigens and antibodies. The counterproductive experience with immunocapture-ELISA highlights the need for co-operation between research institutes in different countries and continents in view of the sheer scale of these projects. A prior strategic assessment is also needed. Continued work on improving the preparation of samples for PCR and the development of multispecific PCR should also be encouraged, as only PCR is capable of reliably identifying the species present.

CHAPTER 5:
OVERALL EPIDEMIOLOGY AND THE EPIDEMIOLOGY OF *TRYPANOSOMA VIVAX* IN FRENCH GUIANA

As stated by VOKATY [35]: 'in contrast with trypanosomoses in Africa, very little has been published on the geographic distribution, vectors, reservoirs, clinical and economic importance of non-tsetse transmitted trypanosomoses in the New World'.

Many observations made prior to 1950 have not been confirmed and very little recent objective data is available. A number of geographical areas have received more specific attention that has led to a number of enzootic and epizootic areas being defined, but the distinction between the two is sometimes blurred. This section provides a study for each country based on the data in the scientific literature and information collected by means of a questionnaire sent out by EMVT in 1995-1996 to researchers and representatives of official animal health bodies in all the countries of Latin America (including the Caribbean countries). This information is referred to herein as unpublished data. The data was collated in the form of abstracts published in the *Proceedings of First Symposium on New World Trypanosomes* [421].

A description of the epidemiology and economic importance of various *Trypanosoma* species is first provided, followed by a particular study of the analytical epidemiology of *T. vivax* in French Guiana; the latter could be used as a model for mechanical transmission of *T. vivax* in Latin America.

1. GENERAL EPIDEMIOLOGY OF *TRYPANOSOMA VIVAX* AND *TRYPANOSOMA EVANSI*

1.1. Colombia

There are 28 million head of cattle and 2.5 million horses [422] in Colombia; most livestock movements take place in the eastern part of the country close to the Venezuelan border in both directions (ESPINOZA, unpublished paper).

1.1.1 *Trypanosoma vivax*

Based on a serological survey of trypanosomosis covering the whole country, WELLS [30] found 48.2% of the samples to be positive. The development of indirect immunofluorescence assays (IFA) [423] and the use of capillary centrifugation techniques [312] have enabled a relatively good appreciation of the epidemiological situation in respect of trypanosomoses in Colombia to be achieved [148, 424, 425]. *Trypanosoma vivax* is enzootic throughout the country at elevations below 1,000 metres [426]. At higher elevations, its presence is sporadic no doubt due to livestock movements from the enzootic area and short-term pullulation of hæmatophagous insects, especially Tabanids [150]. On the Atlantic coast, OTTE *et al.* [149] have observed a connection between the prevalence of *T. vivax*, the vicinity of marshes, and the abundance of Tabanids, particularly the *T. nebulosus* species. In an epizootic outbreak, MATEUS and GONZALES [150] describe a 30% morbidity rate and a 6% to 7% mortality rate combined with an abortion rate of approximately 3% and very protracted recovery periods. Observations made by OTTE *et al.* [427] on the northern coast of the country, indicate an increase in incidence of infections at the end of the rainy season (October) when Tabanids are abundant (*T. claripennis*); in this study, parasitological prevalence (HCT) is highly variable ranging from 0% to 23%, sometimes 61%, depending on the farm. WELLS [17] states that seroprevalence is also highly variable depending on the herd, ranging from 15% to 90%. In another study, which found overall regional seroprevalence to be 51% and an overall incidence of nil, in individual herds, WELLS *et al.* [424] recorded seroprevalences of

between 31% and 81% – a sure sign of the instability of the enzooticity and epizooticity of the infections.

There is an overall agreement between authors on the relative importance of the disease which is classified the third most serious blight on livestock-raising in Colombia, coming just after fluke and the other hæmoparasitoses and their vectors [148]. They also agree on the fact that the epidemiology is poorly documented and that many points require further clarification [428]. OTTE *et al.* [297] were able to observe very few biting insects in a herd where the apparent incidence of infection was 100%; this observation argues in favour of a very high vectorial capacity of the insects. Some of the infections are totally asymptomatic while others are fatal [172]; finally, the task of interpreting data is complicated by the insensitivity of parasitological diagnostic tools and lack of specificity of serological diagnostic tools [429].

1.1.2. *Trypanosoma evansi*

This affects horses and occurs in the form of epizootic outbreaks throughout Colombia. WELLS *et al.* [148] described an epizootic in the northern plains in 1968. Capybaras, that were regularly found to be infected [82], and perhaps dogs are considered to be reservoirs for the parasite. Tabanids and vampire bats are thought to be the vectors [17]. The economic impact of trypanosomosis in horses is not documented but is no doubt considerable since mortality is high during epizootics. The prevalence of infections here has not been established by a serological survey. There was a major epizootic in 1990-1991 among horses in the eastern plains [55]. No study of the prevalence or impact of *T. evansi* has ever been conducted on domestic ruminants.

1.2. Venezuela

There are 18 million head of cattle, 1.1 million sheep, 1.3 million goats and 0.15 million buffaloes in Venezuela; most livestock movements occur in the western part of the country close to the border with Colombia in both directions (ESPINOZA, unpublished paper).

1.2.1. *Trypanosoma vivax*

In a parasitological survey in farms where symptoms of trypanosomosis had been seen, CLARKSON *et al.* [160] found 10% to 15% of the animals to be infected. TORO *et al.* [83, 430] used passive hæmoagglutination and capillary tube agglutination to conduct an epidemiological survey in cattle: average seroprevalence was 25.5% and the infection is distributed all over the country regardless of age, breed or stock raising technique. Prevalence in states varies from 17% in Guarico State to 40% in the neighbouring state of Apure. The seroprevalences by state are shown in **Figure 28 a**, and the enzootic, epizootic and uninfected areas are shown in **Figure 28 b**. Results provided by ESPINOZA (unpublished paper) are very different from those given by TORO [431], which is only to be expected when the epidemiological situation is highly unstable.

In the state of Guarico, more recent studies using IFA [432, 433] have shown an average prevalence of 35% in cattle; discrepancies between one municipality and another range from 17% to 55%. A study by PERRONE *et al.* [434], shows up large differences between serology and parasitology findings: while the parasite is very rarely observed, the prevalence of antibodies is 10% in young stock and 80% in animals over 36 months. DUNO *et al.* [435], in a survey of 60 farms in the state of Falcon (northern coast), report that only 1% of cattle were found to be carriers of the parasite (HCT) whereas 57.8% were carriers of antibodies (IFA). No correlation was found between seroprevalence and age or gender, but seroprevalence in the dairy herds was significantly higher (66%) than in the beef cattle herds (50%); these observations are identical to other findings in French Guiana [358]. A study by ROSSI *et al.* [359] using indirect-ELISA *T. vivax* in cattle yields highly variable seroprevalence, ranging from 0% to 87% depending on the region involved, and generally greater than 35% in the States of Zulia, Falcon and Guarico.

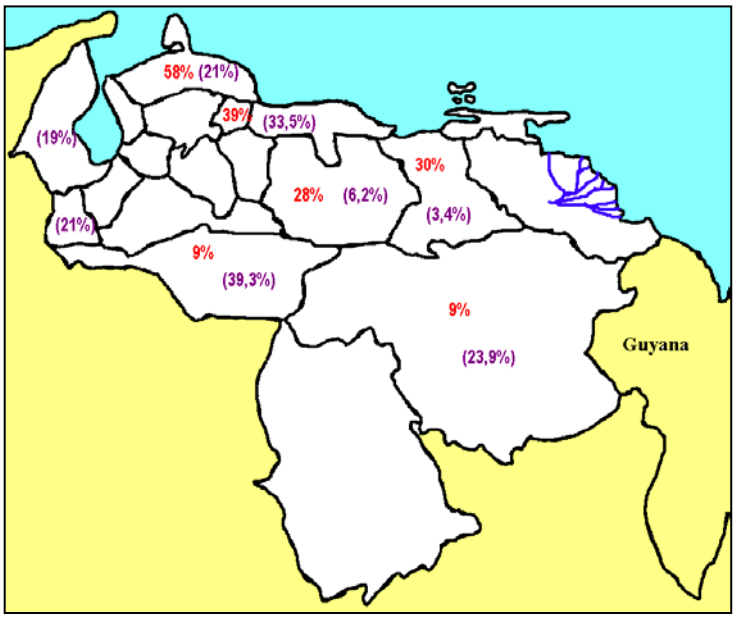


Figure 28 a – Regional seroprevalences of T. vivax *among cattle in Venezuela according to ESPINOZA (unpublished data) and TORO [431] (seroprevalences according to TORO in brackets)*

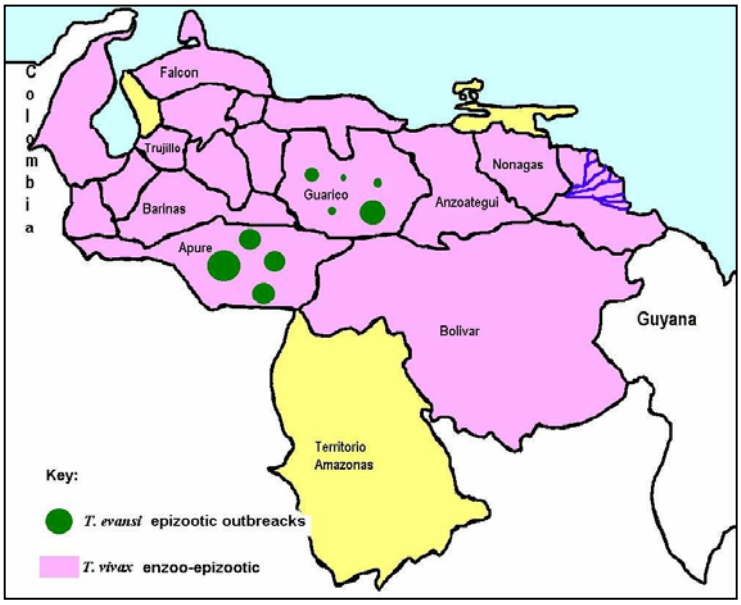


Figure 28 b – Distribution area of T. vivax *and* T. evansi *in Venezuela according to ESPINOZA (unpublished data)*

Key:
T. evansi epizootic outbreaks
T. vivax enzoo-epizootic

Figure 28 – Distribution area and regional seroprevalences of *Trypanosoma vivax* and *Trypanosoma evansi* in Venezuela

1.2.2. *Trypanosoma evansi*

A recent parasitological study has shown that 12% of horses are carriers of the parasite, while serology (IFA) gives a positive result for 69% of the animals [189]. An indirect-ELISA *T. evansi* test was first used in horses very recently – it was reported by REYNA *et al.* in 1992; very high prevalences were detected: 82% in wild horses, and 57% in stock bred horses. Only racehorses were not infected. In the state of Apure, in south-east Venezuela, a serological study on capybaras was positive in 14% of the young stock and 83% of adults [82]; another study carried out by ARIAS *et al.* [436] reports a seroprevalence of 50% using IFA compared to 9% by parasitological means. It is suspected that wild animals, particularly capybaras, play a significant role in the epidemiology of equine trypanosomosis in Venezuela. However, the relative role played by cattle, dogs and capybaras as reservoirs has not been fully elucidated. trypanosomosis caused by *T. evansi* is considered to be enzootic throughout the plains of Venezuela [17]. **Figure 28 b** shows the enzootic, epizootic and uninfected areas for each state.

Although there is no epidemiological surveillance network in Venezuela to monitor the situation, *T. vivax* and *T. evansi* are considered to be enzootic in most of the country.

1.3. Brazil

1.3.1. The Guyanese region

The Northern part of Brazil, whose southern boundaries are the Amazon and Rio Negro rivers, is part of the Guiana; this region is clearly separated from the rest of Brazil by the Amazonian forest. Its cattle population is approximately 150,000 (**Table VI** and **Fig. 29**).

Trypanosoma vivax was first reported on the island of Marajo [28], and then found in the Amapa Federal Territory [437]. SERRA FREIRE [438] and PEREIRA and ABREU [439] report the presence of *T. vivax* respectively in buffaloes and cattle, and in buffaloes and sheep. DIDONET-LAU and LAU [67] also report fatalities in buffaloes. SERRA FREIRE *et al.* [440] conducted a parasitological survey in the Amapa Federal Territory and found prevalence to be 20% to 40% among buffaloes; in another survey in this area conducted by SERRA FREIRE [438] the percentage of buffaloes infected was 8.9% and 7.6% for cattle. Most of the animals in this region come from Mato Grosso and the state of Para where the prevalence of infections is high [440]. ***Trypanosoma evansi*** was discovered very early on the island of Marajo [7] and is no doubt still present in this area of Brazil mainly in buffaloes. Very little information is available about stock raising in the state of Roraima; this northern region in Brazil is thought to be infected by *T. vivax* and *T. evansi*. The impact of the disease in this part of Brazil is poorly documented.

1.3.2. Central and Southern Brazil

1.3.2.1. Trypanosoma vivax

In addition to the Guiana Highlands, *T. vivax* has been reported in Mato Grosso [7] and the part of the state of Para located to the south of the Amazon (Belem). *Trypanosoma vivax* infections in buffaloes have been described; it is considered to be moderately pathogenic but the buffaloes are suspected of being a major reservoir for the parasite [29, 441]. According to SERRA FREIRE *et al.* [440], 31% of buffaloes are infected by *T. vivax* in the state of Para.

In cattle, a serological survey (IFA) conducted in Mato Grosso by WELLS *et al.* [30] has shown that 54% of the 666 animals tested were positive. More recently, SILVA *et al.* [151] described an epizootic in the north of the Pantanal; this epizootic is a sign that the disease is progressing southwards in Brazil. The economic impact of trypanosomoses in Brazil is little-known and poorly documented. It is increasingly attracting attention as is apparent from the study conducted by STEVENSON [159], a KETRI[5] consultant, under a joint EMBRAPA[6] and IICA[7] undertaking. The main conclusions are that its clinical impact is significant and there is a need for epidemiological surveys concerning this disease to which 5 million cattle in the Pantanal are exposed. It is spreading towards the southern and central regions of Brazil, putting some 40 million head of cattle at risk (R.A.M.S. SILVA, unpublished paper). In 1996-1997, new outbreaks of trypanosomosis caused by *T. vivax* were reported by SILVA *et al.* [298] at the borders between Brazil, Bolivia and Paraguay indicating that the disease is progressing westwards and southwards. The authors attribute the disease's geographic extension to the fact that roads have been built that run through the Amazonian forest generating trade in live animals. It is thought that the recent cases reported in Bolivia by SILVA *et al.* [298] were brought about in this way. The progression of *T. vivax* therefore continues.

5 Kenya Trypanosomosis Research Institute (Nairobi, Kenya)
6 Empresa Brasileira de Pesquita Agropecuaria
7 Inter-American Institute for Cooperation in Agriculture

1.3.2.2. Trypanosoma evansi

FRANKE *et al.* [442] conducted a survey in the Pantanal (Mato Grosso), where the enzootic establishment of *T. vivax* relies partly on wild animals. Antibodies by indirect-ELISA were detected in 9.6% of horses, 4.2% of cattle, 18.6% of dogs and 14% of capybaras. However, the test used is unable to discriminate these responses from those raised against *T. vivax* or *T. cruzi* [359]. NUNES and OSHIRO [84] found the parasite in dogs, coatis and capybaras. A survey conducted by STEVENS *et al.* [229] recorded seroprevalences of 27% and 58% in wild and semi free-ranging capybaras. In 1994, SILVA *et al.* [181, 183, 269, 443] reported an epizootic in the Pantanal with very high morbidity and mortality rates in horses, respectively 97% and 83%; four dogs from which samples were taken on that occasion were also found to be infected. Their role as a reservoir needs to be considered.

Trypanosoma vivax and *T. evansi* are spreading within Brazil with considerable clinical and economic consequences in the newly infected regions.

1.4. The Guianas

The Guianas are located on the Guiana Highlands (natural region) in central-northern South America and extend over 1.5 million km². From east to west, the Guiana Highlands comprise the northern Amazonia part of Brazil, French Guiana (French overseas *département*), Surinam, Guyana and the western part of Venezuela (**Fig. 29**). Many of its geoclimatic and sanitary characteristics are shared with the rest of Latin American, especially Central America and the two uppermost thirds of South America. **Table VI** shows the surface areas and human, bovine and equine populations of the Guianas.

	Guianan part of Venezuela	Guyana	Surinam	French Guiana	Guianan part of Brazil	Natural Guianan region
Surface area in km²	415,000	214,000	163,265	90,000	620,000	1.5 million km2
Human population	500,000	865,000	355,000	150,000	350,000	2 millions inhabitants
Cattle population	200,000	280,000	89,000 to 170,000	8,000	150,000	808,000 head
Equine population	?	3,500	1,500	800	?	?
1 bovine par n inhabitants	1/2.5 inhb	1/3 inhb	1/2 inhb	1/20 inhb	1/2.5 inhb	1/2.5 inhb

Table VI – Human and animal populations and surface areas in the Guianas
(according to GIACOTTINO [446], DAVILA and SILVA 2000 [422] and information from Veterinary Services)

The countries of the Guiana Highlands that lie on the Guiana Shield have many similar geographic, soil and climatological characteristics. The equatorial forest grows naturally throughout the Guianas except along the coast where a narrow band made up of savanna and mangroves stretches over a breadth of between 10 km and 150 km. Guyana has an interior savanna – Rupununi – located in the southern part of the country close to the Brazilian border; it's drier climate and savanna type vegetation makes it an extensive stock farming region that is isolated in the midst of a primary forest.

Detail of the Guiana Highlands:

Key: the area in grey on the map of South America (top), and the area outlined in red on the bottom map are the boundaries of the Guiana Highlands.

Figure 29 – Maps of South America and detail of the Guiana Highlands

In French Guiana, there is little farming activity; only one head of cattle per 20 inhabitants. In contrast, in the Independent Guianas, as well as Venezuela and Brazil, farming is the mainstay of the economy; there are approximately one head of cattle per three inhabitants in these independent states. In the coastal regions, farming is largely concentrated in an area of polders and consists essentially in sugar cane plantations, rice growing and stock farming. The cattle population, composed mainly of Brahman zebus, is 8,000 head in French Guiana, 170,000 in Surinam and 280,000 in Guyana. In Guyana, other types of stock farming are also growing: 70,000 goats, 120,000 sheep and 3,500 horses. In Surinam, there are 7,000 goats and 2,000 sheep. Stock farmers in the Guianas face a number of similar animal health constraints, principally: cochliomyosis (*Cochliomyia hominivorax*), hæmoparasitoses due to *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma vivax*, *T. evansi*, equine infectious anaemia, horseflies, ticks and vampire bats. However, the relative significance of trypanosomoses remains to be established.

In 1994, an information network on livestock hæmoparasitoses in the Guianas (hæmoparasite Information Network, HIN) was set up [444, 445] by CIRAD-EMVT and IICA[8], in which CIRAD-EMVT served as reference laboratory [247]. This network conducted any epidemiological survey in Guyana, Surinam and French Guiana. The results of these surveys are reported below, after a reminder of some historical data on trypanosomoses in the Guianas.

8 Inter-American Institute for Cooperation in Agriculture

1.4.1. Historical data

It was in French Guiana that ***T. vivax*** was identified for the first time on the American Continent [18]. Thereafter there were regular reports of the parasite all over the territory, starting in 1935, 1940-1942, 1946, 1953, etc. [46] to date at regular intervals of between two and five years (FAVRE, unpublished paper). A serological survey carried out in 1984 (IFA) [447] reported a 35% rate of positives for the whole of French Guiana.

NIESCHULZ [7] diagnosed *T. vivax* as early as in 1939 in Surinam. Although some unpublished information has circulated since NIESCHULZ published his report, practically nothing on the existence of trypanosomoses has been published concerning Surinam. During the many years of civil strife, information was not collected in the field, nor were laboratory diagnostic tests performed.

According to HOARE [7], *T. vivax* was introduced into Guyana by livestock from French Guiana at the end of the last century but was not formally identified until 1955. In 1975, six cattle out of 1,019 tested were diagnosed to be infected with *T. vivax* by smear examinations [448]. A parasitological study (HCT) by APPLEWHAITE [449] reports a 4.6% infection rate in 197 sheep tested and a 1.3% infection rate in 77 goats tested. More recently, a serological survey of 161 sheep in the coastal region of Mahaica/Berbice by IFA in respect of *T. vivax* found 40.4% of the animals to be positive [450].

Trypanosoma evansi has never been identified in French Guiana; a number of positive serologies have been observed in cattle [451, 452] by IFA and by the CATT®test [453]. There have been nor reports of clinical suspicions of *T. evansi* infections however.

Likewise, no clinical episodes of *Derrengadera* in livestock have been reported in Surinam. However, around 1980, LIEUW-A-JOE suspected a clinical case of trypanosomosis in a hunting dog, one of whose symptoms was intraocular haemorrhage (unpublished data). The presence of *T. evansi* in Surinam has a recently been reported with PCR but the parasite has not been isolated [64, 299].

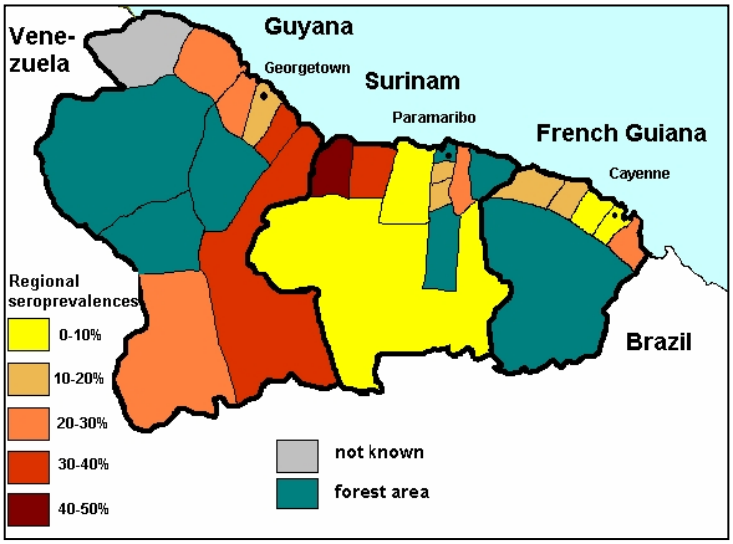
It is thought that *T. evansi* was introduced into Guyana from Brazil [7] in the last century, but its existence has not been reported since 1970. At that time, a trypanosomosis epizootic in horses had been reported by stock farmers and veterinarians in Rupununi (Guyana Veterinary Services Archive) in the south of the country; no symptoms have been seen since. The situation in the coastal area is unclear. A serological survey in sheep (IFA) yields 23.6% positives for *T. evansi* alone; however, because of cross-reactions with *T. vivax*, one cannot conclude as to the presence of the parasite with any certainty [450].

1.4.2. Current status of trypanosomosis due to *Trypanosoma vivax*

A cross-sectional survey was conducted on approximately 3,000 head of cattle in French Guiana (37% of the bovine population) located in five different areas. The samples were taken from the stock farms between 1990 and 1992 and supplemented with a longitudinal survey (1,000 samples) for the purpose of assessing seasonal variations [358]. Surveys were conducted in Surinam and Guyana between 1994-1996 in the slaughter houses of the capitals and in farms located in regions that did not serve these slaughterhouses; samples were collected from 1,819 head in Surinam out of a total of some 97,600 animals distributed over 10 administrative regions, and from 1,113 heads in Guyana out of a total of 267,400 animals distributed over 10 administrative regions [43].

The parasitological diagnosis (HCT) came out positive for 2.7%, 6.7% and 7.6% of the cases respectively in Guyana, Surinam and French Guiana. These were mostly *T. theileri* but *T. vivax* was identified on one smear from Surinam and three from Guyana. Mice inoculations came out negative.

National seroprevalence (indirect-ELISA *T. vivax*) were 42 ± 3%, 31 ± 2% and 22.2 ± 1.5% respectively in Guyana, Surinam and French Guiana. Regional seroprevalences are provided in **Figure 30.** These seroprevalences are high and increase from west to east – they confirm the importance of trypanosomosis due to *T. vivax* in the Guianas.



The relative importance of other hæmoparasitoses (anaplasmosis, babesioses) was assessed at the same time [454]. Trypanosomosis induced by *T. vivax* would seem to be the hæmoparasitosis that has the most noticeable clinical manifestations and medical and economic impact [43]. No seasonal variations were recorded in the herds monitored in French Guiana and, since no parasites were detected, the conclusion drawn was that the survey had taken place during an inter-epizootic period.

Figure 30 – Regional breakdown and seroprevalence of bovine trypanosomosis in the three Guianas

The survey did not show up any specimen of *T. vivax* in French Guiana although the serology returned a 22% prevalence of *T. vivax* infections, i.e. the serological 'scar' of the last epizootic observed in 1987-1988 [324]. In the past, *T. vivax* epizootics in cattle in French Guiana occurred periodically every three to five years; whether or not the parasite is present on the farms in the interval is not known nor what its potential reservoir is or whether it is reintroduced by importing infected livestock. Additional studies have shown that trypanosomosis exists in an insidious enzootic form with periodic multiple epizootic outbreaks that very rapidly cause seroprevalence to climb from an average 20% to an average 80%; the prevalence gradually returns to former levels as the infections slowly disappear over a period of two to five years [43].

In view of these seroprevalences observed in the Guianas (22%-42%), their epidemiological status in respect of cattle trypanosomoses would appear to be fairly comparable; the higher prevalences observed in Guyana and Surinam probably simply reflect the scarcity of trypanocides in those countries whereas, in French Guiana, diminazene aceturate treatment is fairly common.

1.4.3. Current status of trypanosomosis due to *Trypanosoma evansi*

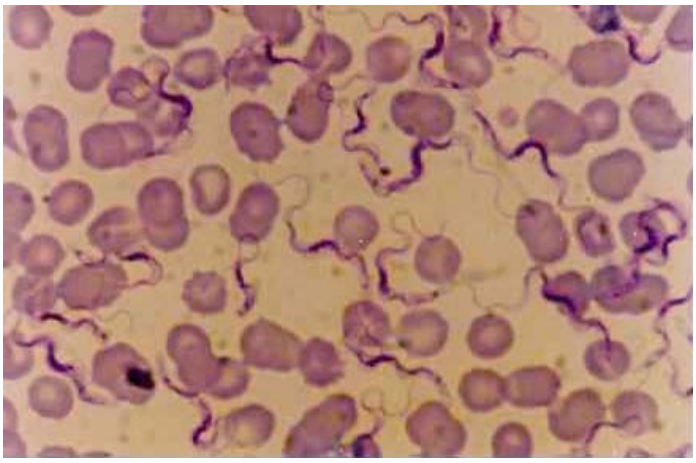
Trypanosoma evansi has not been isolated in the Guianas, but in the past its presence was reported in Guyana. As the parasite is enzootic in Venezuela [17] and probably in the north of Brazil, it was also investigated in the Guianas at the same time as the other surveys.

1.4.3.1. French Guiana

The first indications that *T. evansi* was present in French Guiana came from IFA tests performed on cattle [451, 452] and from CATT® tests performed on dogs and cattle [54]. Mice inoculations using samples collected from suspect animals (20 cattle, 11 peccaries and 10 horses) remained negative. A serological survey on 300 horses returned 3% positives (indirect-ELISA) [43] but mice inoculations performed with material from the seropositive cases remained negative. Because all the horses are from Guiana or an area free of *T. evansi* infection (metropolitan France), the undetectable carrier state is not considered feasible. The positive serologies are therefore more likely to be false

positives due to the low species-specificity of the indirect-ELISA. The presence of *T. evansi* in horses in Guiana has not therefore been ascertained.

On the other hand, out of the twenty observations conducted on clinically suspect dogs, one smear sample, performed post-mortem by a practitioner in Cayenne (D. FRENAY), revealed an abundance of trypanosomes. The animal was a hunting dog that was born and had always lived in Guiana; it exhibited acute clinical symptoms with cardiac signs that were fatal in three days. It was on this smear examination that a parasite of the subgenus *Trypanozoon* (**Fig. 31**) was identified for the first time in French Guiana. It was of medium size (28.7 µm), had a 0.64 µm kinetoplast in the subterminal position (KI = 1.43) and an 8.36 µm free flagellum. Its most likely identity was *T. evansi* [43].



Remark: the level of parasitæmia (>10^8), the morphology (tapered posterior tip, small kinetoplast, located well away from the tip) and the morphometry of the parasite (kinetoplast size < 0,7µm, total length > 26µm, well-developed undulating membrane) bear a close resemblance to those of *T. evansi*, but the clinical signs observed were mainly cardiac, and could be clinically confused with an acute *T. cruzi* infection. Differential diagnosis is possible only by examination of the parasite.

Figure 31 – ***Trypanosoma evansi*****, in a dog, French Guiana** (original, FRENAY, LA ROCQUE and DESQUESNES) (magnification × 1,000)

Unfortunately, it proved impossible to isolate the parasite and no further cases have been reported since in spite of the practitioner being on the lookout. The most likely explanation is that this hunting dog was contaminated by the peroral route from infected game and acted as a sentinel animal, indirectly showing that *T. evansi* is present among wild animals in French Guiana.

Surveys on wild animals will be required to ascertain this hypothesis; in addition, it is essential to identify parasite species in dogs because *T. cruzi* positive serologies have also been recorded in Cacao [95] as well as a clinical case of Chagas pancarditis (DELAVENNA, 1995, according to RACCURT [95]).

Clearly, sufficiently sensitive and specific diagnostic tools are lacking, but so far no objective evidence of the presence of *T. evansi* in livestock has been provided. In any case, the situation is continually shifting, the presence of the parasite in wild animals is likely and the fact that it can occasionally contaminate dogs makes it a real, permanent threat for livestock in French Guiana.

1.4.3.2. Guyana

Southern region: under the Hæmatoparasite Information Network activities in the Guianas, samples that were collected from cattle in the Rupununi region and tested positive for Woo's test were inoculated into mice (C3H). No trypanosome was isolated.

A batch of 130 samples of cattle sera were tested using indirect-ELISA *T. vivax* and indirect-ELISA *T. evansi* tests to compare the responses in the two systems; 32% of the samples were positive, and all give a ratio of RPP *T. vivax*/RPP *T. evansi* greater than 0.5. This result does not argue in favour of the presence of *T. evansi* in cattle in Rupununi (see chap. 4, § 2.4.3.) [43].

Although one cannot exclude that *T. evansi* may be present in wild animals, or that re-infestation could occur via Brazil, the parasite has apparently not been able to become established in Guyana. It would otherwise probably have given rise to clinical signs in horses that are very widely used for herding livestock, as well as distinct homologous serological responses by indirect-ELISA *T. evansi*. However, one should remain very cautious, and a serological survey in horses would provide more information.

Coastal area: no parasites were detected by a survey conducted on 1130 cattle in this area either. A batch of 260 samples of bovine sera was tested in indirect-ELISA *T. vivax* and *T. evansi* systems – 29.6% of the samples were positive with *T. vivax*/*T. evansi* ratios of more than 0.5. Recent observations and the scientific literature therefore do not mention the presence of *T. evansi* in Guyana but the situation requires further clarification.

1.4.3.3. Surinam

Attempts to detect *T. evansi* by PCR using specific oligonucleotides, according to the method described by DIALL [300] were made in as part of the epidemiological survey conducted in the Guianas. Of the 41 blood samples in the batch, six were PCR positive indicating that *T. evansi* DNA was present in the samples [64, 299].

At the end of 1995, LIEUW-A-JOE, ROZENBLAD and BANSEE observed clinical signs in three hunting dogs that belonged to the same pack (unpublished paper) within three weeks of one another. A blood smear was performed. The author at CIRAD-EMVT examined the sample and found medium-sized parasites (27.5 µm), of various shapes, with a small kinetoplast (0.6 µm) in the subterminal position (KI = 1.28). The size data and overall features observed match those generally described for *T. evansi* stock. The only buffy coat smear available was confirmed to be PCR positive with the specific oligonucleotides for *T. brucei* described by MASIGA *et al.* [368].

What is surprising is that clinical manifestations have never been observed in horses. Just as in Guyana and French Guiana, the status of *T. evansi* in Surinam requires further investigation, but the clinical cases that have been observed in dogs (sentinel animals) and PCR diagnostic tests performed in cattle leave us in no doubt as to the presence of the parasite. Wild animals probably harbour *T. evansi* and contaminate hunting dogs by ingestion. Its presence in cattle is likely to be attributable to vampire bats, but infections have never been found in horses.

1.4.4. Conclusions on the epidemiology of livestock trypanosomoses in the Guianas

To date, only *T. cruzi* and *T. vivax* have been isolated throughout the Guianas. *Trypanosoma evansi* is observed regularly only in Brazil and Venezuela but it has been identified on a smear in French Guiana and Surinam. There has been no detailed investigation of the presence of *T. cruzi* in domestic animals. Most studies show the presence of the parasite in wild animals. In actual fact, diagnostic tools are not sufficiently specific or sensitive to conduct an epidemiological survey on trypanosomosis due to *T. cruzi* in livestock. Developing PCR for diagnosing this infection might be helpful in conducting such a survey. In French Guiana, *T. cruzi* is thought to interfere with the serological diagnosis of trypanosomoses in horses.

The epidemiological status of *T. vivax* in the Guyanese region would seem to be fairly uniform both in terms of prevalence (20%-40%) and of general epidemiology. In most cases it operates as an enzootic infection with a clinical expression in the form of epizootic outbreaks.

In contrast, the epidemiology of *T. evansi* appears to be more variable.

In Venezuela, the parasite is thought to be present all over the plains and affects both wild and domestic animals – *T. evansi* has frequently been found on capybaras, cattle and horses.

In Brazil, its preferred domestic host is thought to be buffalo.

In Surinam, there is an intermediate situation – the hunting dogs were probably infected by game while cattle are thought to be infected by vampire bats. There are no suspicions of the parasite in horses.

In French Guiana, the parasite was found on only one hunting dog from which it can be inferred that the parasite may be in circulation in wild animals. So far, there is no evidence of livestock being infected by this parasite.

In Guyana, an epizootic in horses was reported in the 1970s but the parasite does not appear to have become established in domestic animals and has not been reported since; the contamination may have originated from animals that had come from Brazil (Boa Vista region).

Unless the stock farming system is radically altered, or there is a major ecological change, the epidemiological status of *T. cruzi* will probably remain stable in view of its very long-standing establishment. On the other hand, the epidemiological status of *Salivaria* is likely to change. The epidemiology of *T. vivax* is thought to depend very closely on movements of carrier animals and the enzoo-epizootic form is inherently unstable. Finally, since *T. evansi* may possibly be carried to Surinam and French Guiana by movement of wild animals, it is feasible that domestic animals will become infected especially through vampire bats. A cautious approach must therefore be adopted with regard to this parasite, especially in view of the recent episodes described in Brazil that show how severely it affects horses, entailing very high morbidity and mortality rates of more than 80% in previously unchallenged animals [181, 183, 269, 443].

1.5. Other Latin American countries

1.5.1. *Trypanosoma vivax*

Serological surveys have shown that the distribution of *T. vivax* extends from **Paraguay** to **Salvador** [30, 32]. However, its southernmost boundary remains to be defined because Paraguay denies that it is infected in spite of the data from serological surveys (FAO mission reported by TOURATIER, unpublished data). To the north, very little information is available in Central America.

Chile has a cattle population of 3.4 million, 4.8 million sheep, 0.35 million horses and 72,000 camelids (llamas and alpacas). FERRIS [455] has published positive serologies (IFA) for *T. vivax* antigens in llamas and alpacas, but later on, using the same samples, found higher titres for *T. b. brucei*. He concluded that the infection was more likely to be due to *T. evansi* than *T. vivax* in spite of alpacas and llamas being sensitive to both [14]. According to MONTOYA BECERIA, these responses turned out to be false positives since they were negative when a homologous conjugate was used; on the other hand, he does not exclude that there was interference from *T. cruzi*. *Trypanosoma vivax* therefore has not been ascertained for Chile (MONTOYA BECERIA, C., unpublished paper).

There are 53 million head of cattle and 30 million sheep in **Argentina**. *Trypanosoma vivax* has not been isolated in this country but BAKOS *et al.* [456], in a zebu, recently observed a highly motile parasite whose morphological features were those of *T. vivax*. However, the parasite's identity needs to be established with greater certainty.

Trypanosoma vivax is present in **Ecuador** and **Bolivia** [15]. A serological survey conducted by WELLS *et al.* [30] provided the following seroprevalences (the figure between brackets is the number of samples tested): 40% (15) in **Paraguay**, 22.5% (310) in **Ecuador**, and 14.3% (49) in **Peru**. However, the IFA technique used for this survey is unable to discriminate between infections due to *T. vivax* and those due to *T. evansi* [14]. In the eastern part of **Bolivia** (Santa Cruz administrative unit), four cases of the parasite were identified on smears from cattle in the first quarter of 1996, and one case in a dog, which deserves particular attention (AGUIRRE BOVAYES,

unpublished paper). It was also identified on 159 cattle in the provinces of Velaco, Nuflo de Chavez, Guayaros and Chiquitos and in 25 cattle in the province of Laguna Concepcion [298]. It is thought that these new outbreaks in Bolivia are attributable to the introduction of infected livestock from Brazil via the new land routes. More recently (*Second Symposium on New World Trypanosomes*, San Juan de Los Morros, Venezuela, October 1999) EULERT *et al.* (unpublished) reported that in one year (1998-1999) multiple epizootic outbreaks of *T. vivax* affected the whole country with considerable clinical and economic impact. Dàvila and Silva report that in Bolivia the parasite is advancing on average by 1.3 km per day [422]. Mechanical transmission of the parasite to previously unchallenged livestock and trading in live animals are thought to be responsible for this rapid spread.

Trypanosoma vivax was identified in **Panama** only once in 1940, by JOHNSON [27], whereas the prevalence is already 5% to 50% depending on the herd. This late discovery is a reflection of the dearth of early investigations. No recent data has been found in the literature and the Veterinary Services of Panama acknowledge that they do not have the information, particularly as they can only call on parasitological diagnostic tools. In any case, the animal health authorities consider the impact of trypanosomoses to be very high (Dr EVANS, unpublished paper).

While parasites have not been isolated, 15% of cattle in **Salvador** are serologically positive and 23% in Costa Rica [30]. The existence of *T. vivax* has not been ascertained in these countries by isolation of the parasite.

Trypanosoma vivax is present in **Cuba** where there are many potential mechanical vectors as reported by CORDOVES *et al.* [113]. It's probable disappearance from the **French West Indies** since the 1940s, assumed by MOREL [457], has recently been confirmed for Martinique by ALONSO *et al.* [23], who found no serological trace of the parasite using IFA.

A survey by indirect-ELISA conducted in the **Caribbean** countries by FERENC *et al.* [345] showed a very low seroprevalence on those islands with only two samples being highly positive in Montserrat and St. Martin. Data has been provided to us privately reporting that trypanosomes that are pathogenic for livestock are not found in **Puerto Rico**, the **US Virgin Islands** (BOKMA B.H.) or **Jamaica** (LAZARUS C.).

According to documents drafted by CARAPHIN[9]: 'CARAPHIN Animal Health' [458, 459], there are no livestock trypanosomes in the following countries:

Antigua & Barbuda, the Bahamas, Barbados, Belize, Dominica, Granada, Montserrat, British Virgin Islands, St Kitts-Nevis, Santa Lucia, St Vincent & Grenadines, Trinidad and Tobago.

In **Mexico**, there are 35 million cattle, 6 million sheep, 9.3 million goats and 5.4 million horses. According to IRASTORZA, J. M. E. (unpublished paper), *T. vivax*, *T. evansi* and *T. equiperdum* have never been reported. In 1995 although there were no clinical signs, positive antibody serologies (CF) for *T. equiperdum* were found in the state of Chihuahua on horses and mules intended for export to the USA. The same samples tested for *T. cruzi* with hæmagglutination inhibition also turned out positive (RUBI, E., unpublished paper). The positive animals were slaughtered but infection has never demonstrated. Furthermore, investigations conducted on 3,000 Equidae in that state were never able to isolate the pathogen or discover clinical signs of dourine. Mexico has declared its dourine-free status since April 1996 [460]. Interference from *T. cruzi* or *T. evansi* may be the cause of serological cross-reactions. *Trypanosoma cruzi* has been found in circulation in Mexico even in urban areas; a recent serological survey has shown that 8.8% to 24.2% of dogs are infected [461]. Once again, the lack of specificity of antibody detection tests is a major handicap.

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Furthermore, the stringent (and justified) sanitary barrier imposed by the USA may work against official notification of the presence of trypanosomes on livestock in Mexico.

In some Latin American countries – Uruguay, Nicaragua, Honduras and Guatemala –, neither the scientific literature nor requests for information were sufficient to establish or update the data concerning the epidemiological status of trypanosomes.

Figure 32 shows the distribution area of *T. vivax* in Latin America derived from the data in the literature and information that was conveyed to us privately.

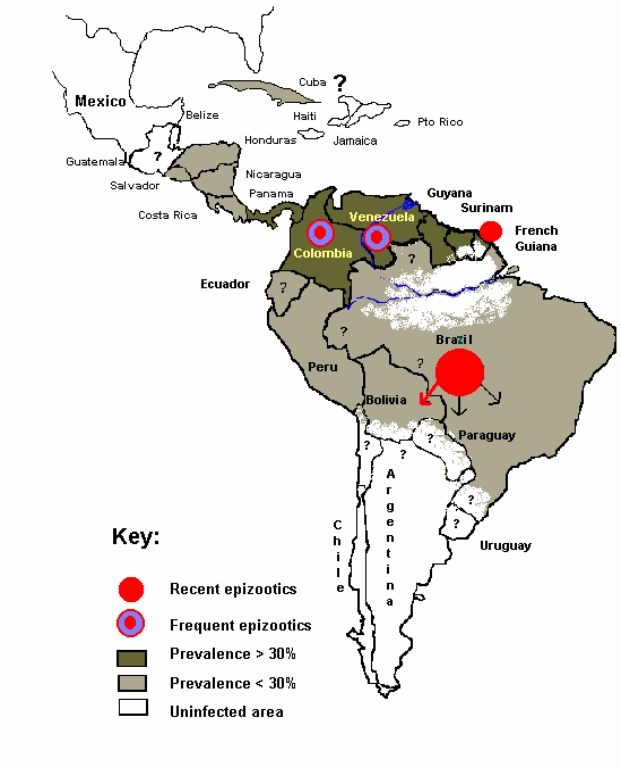

Figure 32 – Distribution area of *Trypanosoma vivax* in Latin America [462]

1.5.2. *Trypanosoma evansi*

According to HOARE [16], the occurrence of *T. evansi* coincides with that of vampire bats. Under this assumption, *T. evansi* is present all over South America, from Argentina to Panama. Its northernmost boundary, however, remains to be defined.

Trypanosoma evansi has not been reported in **Chile** (MONTOYA BECERIA, unpublished paper), but positive *Trypanosoma* sp. serologies have already been observed [455].

There are two million horses in **Argentina** (MONZON, unpublished data). IFA antibody detection has shown a 13% to 20% rate of positive animals in the Province of Chaco [463]. Another survey conducted from 1983 to 1987 in the province of Formosa found 20% of the animals to be positive [49] while, during an epizootic and using HCT alone, 90 out 137 animals tested were found to be infected [184]. The authors specify that 57,000 horses are at risk in this province.

Trypanosoma evansi has been reported in **Bolivia, Paraguay and Peru** [15], but very little recent data is available. In Bolivia, 17 cases of equine trypanosomosis were identified in the northeastern part of the country in the Beni administrative unit (AGUIRRE BOVAYES, unpublished data). *Trypanosoma evansi* has not been reported in northern **Panama**, but information there is vague.

Finally, the parasite has not been found in either the **Caribbean** countries or **Mexico** according to IRASTORZA, J. M. E. (unpublished data). Nonetheless, HOARE [7] does mention the existence of *T. evansi* in Mexico and all over Central America. The situation remains to be elucidated.

There is a great deal of uncertainty about the distribution areas of both *T. evansi* and *T. vivax* in Central America and Mexico.

Figure 33 shows the distribution area of *T. evansi* in Latin America derived from the data in the literature and information that was conveyed to us privately.

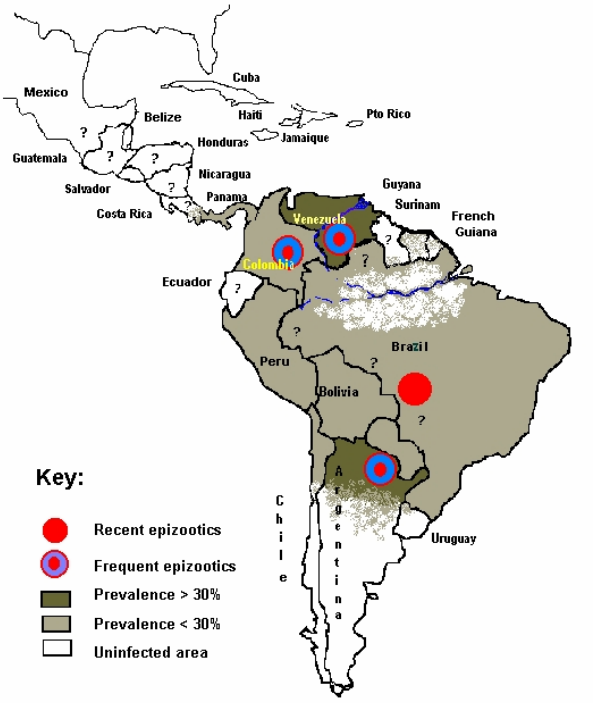

Figure 33 – Distribution area of *Trypanosoma evansi* in Latin America [462]

2. GENERAL EPIDEMIOLOGY OF OTHER TRYPANOSOMES

Trypanosoma equiperdum occurred in a larger area in America than *T. evansi* as is apparent from the fact that the disease was reported in Chile and Canada at the beginning the century. By 1976, *T. equiperdum* was reported only in Paraguay and Bolivia [15]. The actual distribution of *T. equiperdum* is not known – sometimes it can be clinically confused with *T. evansi*; furthermore, trade restrictions discourage the disease from being notified.

Trypanosoma theileri is cosmopolitan; its prevalence in Latin America is poorly documented. Recent surveys conducted in Surinam and French Guiana by parasitological diagnostic means (HCT and DG/BCM) reported prevalences of respectively 11.9% [64] and 7.6% [350]. Another survey conducted in Argentina gives a prevalence of 6.7% [199]. In actual fact, many infections go unnoticed when parasitological means are used to directly observe the parasite, even after enrichment (HCT). Using hæmoculture, FOIL indicates that practically all the cattle tested in Louisiana are carriers of the parasite (unpublished data). Similarly in Africa, 71% of the animals tested using hæmoculture were positive [179] and up to 100% in Nigeria [7]. Most authors that use HCT find only 5% to 15% of the animals to be infected whatever the country or type of investigation being conducted.

It is therefore thought that the extent of the *T. theileri* carrier state has been considerably under-estimated because of the poor sensitivity of the most commonly used diagnostic tool. Because experimentally infected animals remain carriers for more than a year (LAVERAN and MESNIL,

1912, and HORNBY, 1953, according to HOARE [7], in stock farming areas, due to the combination of the long-lasting carrier state and reinfection by horseflies, the prevalence of infections is likely to be close to 100%, i.e. consistent with the findings of hæmoculture.

Trypanosoma ingens was recently observed for the first time in Latin America in Surinam [64]; the author notes that out of the 513 samples included in his parasitological study of cattle in Surinam, 56 contained *T. theileri* and only one *T. ingens*. That study therefore indicates that *T. ingens* is comparatively uncommon compared to *T. theileri*. Its distribution in America is not known.

In **Latin America**, ***T. cruzi*** is the most widespread pathogenic trypanosome geographically speaking. Initially, it was described from Argentina to Mexico [258], in humans, and wild and domestic animals, particularly dogs.

An expert report published by WHO on Chagas' disease [3] mentions that the studies conducted in Argentina, Brazil, Chile, Bolivia and Venezuela yield highly variable rates of infection by *T. cruzi* that range, in humans, from 0.5% to 2% in large cities, to between 20% and 63% in highly endemic areas. In dogs, the rate ranges from 4.5% to 100% and, in cats, from 0.5% to 60.9%. In Chile, serological studies in the provinces of Elqui, Limari and Choapa revealed the presence of antibodies for 12% to 24% of dogs, 0% to 15% of cats, 5%-12% of goats, 4%-26% of rabbits and 4.8% of sheep [91]. In Paraguay, FUJITA *et al.* [92] carried out a survey showing that antibodies raised against *T. cruzi* were found in cattle (8%), pigs (10%), dogs (36%) and cats (37.5%). In French Guiana, investigations into prevalence of *T. cruzi* infections conducted by DEDET *et al.* [94] showed a high rate of infection among certain wild species, particularly peridomestic species such as *Didelphis marsupialis* (42.8% of the animals around the village of Cacao were carriers of the parasite) and *Philander opossum* (6%). Because appropriate diagnostic tools are lacking, the prevalences in livestock cannot often be determined. **Figure 34** shows the distribution area (in grey) of *T. cruzi* on the basis of the various sources mentioned in this document. Distribution of the human disease is somewhat more limited. Interference from *T. cruzi* in diagnosing livestock trypanosomosis must be suspected in this area – in other words, *T. cruzi* may interfere with the diagnosis of trypanosomosis in livestock wherever there are *T. vivax* and *T. evansi* infections.

Trypanosoma cruzi **is also found in the southern United States** and has been found on dogs in Texas, Oklahoma, Louisiana, the two Carolinas, and the state of Georgia [146, 464, 465]. Several vectors have been reported in the USA: *Triatoma gersaecheri* in Texas; *Triatoma sanguisuga* and *T. lecticularia* in South Carolina and Georgia, etc. [464]. The very few cases of native Chagas' disease were recorded in California, Texas and Tennessee most often by means of post mortem PCR [466, 467]. Many wild and domestic animals were found to be infected or carriers of antibodies, sometimes with high prevalence as; armadillos, badgers, coyotes as well as cattle and sheep were found to be carriers of antibodies in Texas and Louisiana; in South Carolina and Georgia, nearly 50% of raccoons (*Procyon lotor*) were serologically positive as well as some opossums (*Didelphis virginiana*) [464].

One must remember that cyclical transmission of *T. cruzi* has been described in opossums (*Didelphis marsupialis*) [143]. Hence, in the USA, *T. cruzi* has a huge wild and domestic reservoir together with two cyclical vectors (louse and opossum). Infections in dogs are seen more and more often. It is presumed that the main cycle occurs in louse and wild animals (raccoons and opossums), and domestic animals are infected by ingesting louse or, in the case of dogs, by ingesting infected prey. There are very few reports of human contamination and this is thought to be attributable to the long interval that elapses in the USA between the time when the vectors take their meals and the time they defecate making it unlikely for a bite wound to be contaminated, i.e. the most common mode of human contamination in South America. Furthermore, living and hygiene conditions in the USA are far less likely to foster contact between hosts and vectors then those that prevail in Latin America.

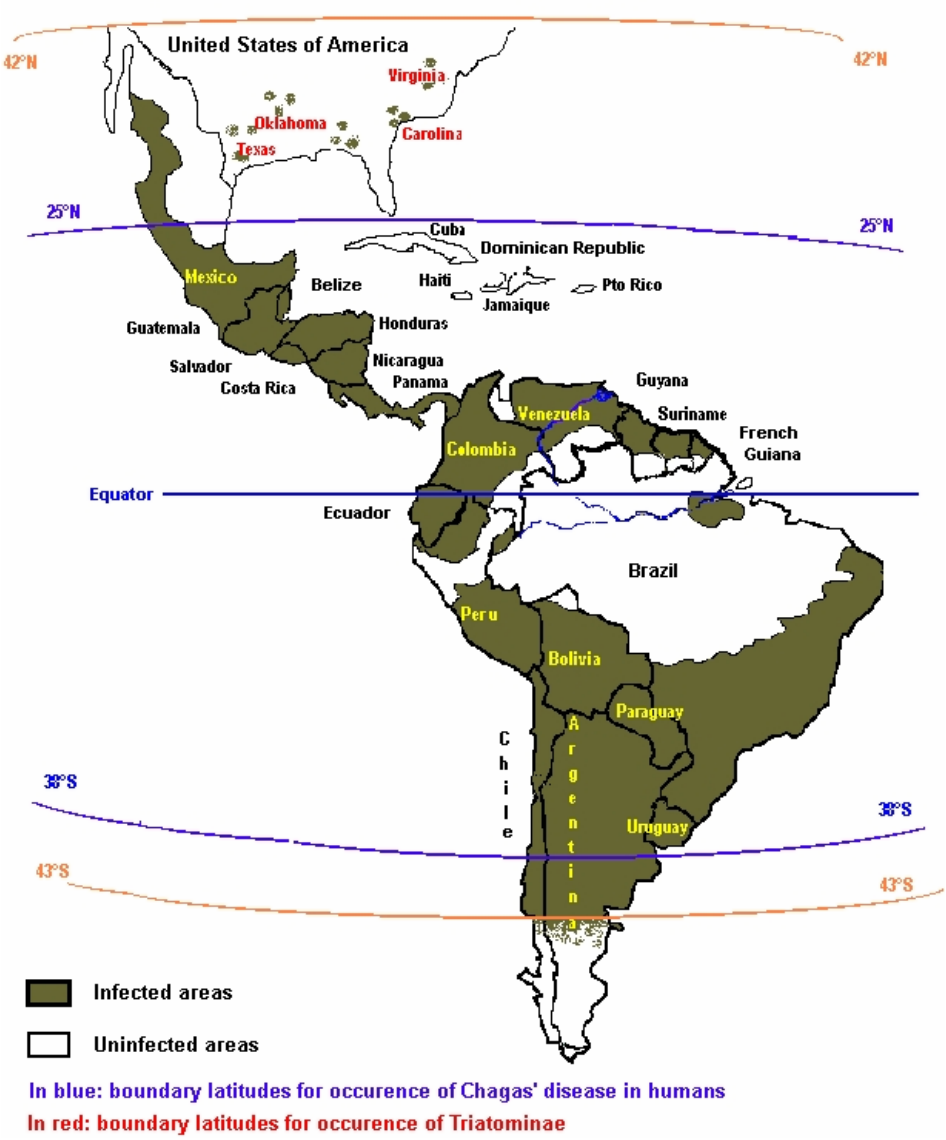

Figure 34 – Distribution area of *Trypanosoma cruzi* in Latin America (DESQUESNES [462], according to the WHO [3] and other sources quoted herein)

In any case, this progression in the distribution and establishment of *T. cruzi* should be taken very seriously. *Trypanosoma cruzi* is already capable of transmitting cyclically in the USA, by ingestion of food contaminated by opossum or louse excreta and by entry of the metatrypanosomes present in the faeces of louse through bite wounds or mucosal membranes. Furthermore, *T. cruzi* may be able to find vicarious vectors in the course of its progression possibly establishing a new epidemiological link to humans.

3. ECONOMIC IMPORTANCE

3.1. *Trypanosoma vivax*

VIERVIESCAS [34] had already realised how considerable the economic impact of trypanosomosis in cattle in Venezuela was at the beginning of the century. The mortality rate there reached 40% [70]. However, evidence that *T. vivax* was the responsible parasite was not always conclusive. The 'huequera' syndrome described by ZAPATA in 1931, and attributed to *T. vivax*, was probably the upshot of multiple pathological factors [468]. In 1954, FLOCH [46] reported that *T. vivax* was the

main cause of mortality in livestock in French Guiana. More recently, a more accurate assessment of the impact of trypanosomosis in ruminants has been achieved in some countries thanks to newer techniques and better knowledge of animal health factors.

In Venezuela, *T. vivax* is considered to be a major blight for stock farming but it is extremely difficult to establish the economic importance of bovine trypanosomosis because the incidence and prevalence of *T. vivax* are highly variable across time and space. Furthermore, the clinical manifestations of other hæmoparasitoses and their treatment considerably interfere with diagnosis [160]. In Colombia, in an enzootic area where only young animals are affected, OTTE *et al.* [297] evaluated losses due to *T. vivax*. In their study, they find that the mean daily weight gain (MDWG) of animals drops from 390 to 200 grams/day; incidence is 100%. The loss due to introduction of *T. vivax* into a dairy herd made up of 179 Holsteins and Holstein cross-breeds [468] in 1976 was estimated to be US$5,650, i.e. €23/animal. MATEUS *et al.* [426] estimated losses in six herds (1,380 head) due to an outbreak of trypanosomosis in Colombia to come to US$100,000, i.e. €55/animal. OTTE *et al.*, quoted by TOURATIER [55], mention that production in infected, asymptomatic, cattle nonetheless drops by between 20% and 25%. This is crucial data as it means that, to properly assess the economic impact of livestock trypanosomosis, one needs to consider all of the infected animals and not just those that with symptoms.

In northern Brazil, according to LANHAM *et al.* [441], the impact of *T. vivax* on buffaloes is considerable too. In the Mato Grosso, there are increasing fears, particularly in the Pantanal where a *T. vivax* epizootic recently occurred. With its 5 million head of cattle, the Panatanal would suffer severe economic losses if trypanosomoses of ruminants were to spread. The progression of these outbreaks towards Bolivia has already started [298]. DÀVILA and SILVA estimate that *T. vivax* could cause losses coming to US$160 million if one considers the 11 million head of cattle that are at risk at the Brazilian/Bolivian border [422].

In French Guiana, the average economic loss due to *T. vivax* during bovine trypanosomosis outbreaks is estimated to be 3.3% of body weight on average in infected herds, i.e. nearly €33.05/animal or, assuming that half of the population is affected, €134,000 [263].

By applying these estimates or those quoted by TOURATIER [55] to the huge stock farming areas in Venezuela, Colombia, Brazil and the Guianas, where infection prevalence is between 20% and 60%, the conclusion one reaches is that the economic importance of livestock trypanosomoses is considerable in Latin America. Variations in the clinical impact and the low mortality observed in enzootic areas often conceal the economic effects of this disease.

Following the fulminating epizootic flare-ups resulting from new infections, *T. vivax* has a strong tendency to go into the enzootic state and function in the form of less drastic, but nonetheless harmful foci for stock farmers.

3.2. *Trypanosoma evansi*

In Africa, camel-raising is severely affected by *T. evansi* infections [300, 469]. In Asia, economic losses caused by Surra in camels, horses and buffaloes are very significant, particularly in its chronic forms [470, 471]. Mortality is sometimes high in buffaloes, especially in Vietnam (NGUYEN DANG KHAI, 1995, quoted by TOURATIER [88]), where abortion rates also reach 60% [339].

Information relating to Latin America is disparate. The earliest data relied solely on impressions and could not be confirmed due to lack of investigative tools.

In Brazil, in the Pantanal (Mato Grosso), until 1939, no form of treatment was available and working horses regularly had to be brought in from elsewhere as replacements due to the high mortality attributable to *T. evansi* (R.A.M.S., SILVA, unpublished data). Recent epizootics in that region have caused considerable losses – for one ranch with 800 head [303] losses amounted to

€30,500. If the disease continues to spread, some 150,000 horses will be at risk [183, 269]. In Argentina, MONZON *et al.* [184] point out that 57,000 horses are exposed in the region of Formosa alone, but no economic data is available.

In cattle, infection is often imperceptible but nonetheless causes disorders in reproduction and immunosuppression that undermine efforts to establish strong immunity by means of vaccination schedules (foot-and-mouth disease and haemorrhagic septicaemia) [88]; under these circumstances, the economic impact of *T. evansi* on cattle is very difficult to ascertain but is far from being insignificant.

In Latin America, the impact of *T. evansi* infections on ruminants is little-known and most of the studies have dealt with trypanosomosis due to *T. vivax*. Because serological diagnostic tools are not species-specific, the impact of these infections in ruminants (including buffaloes) cannot be assessed for the time being. On the basis of observations made on other continents, and as already pointed out in the pathogenicity section, additional investigation is needed.

4. CONCLUSIONS ON OVERALL EPIDEMIOLOGY

There has been growing interest in trypanosomoses in Latin America in the last 20 years, especially since the 'Working Group on *Trypanosoma evansi* Infections' was set up in 1983 [472]. This group meets on an annual basis and publishes a report in the OIE[10] Scientific *and Technical Review* [52, 53, 54, 55, 88, 453, 472, 473, 474, 475]. In 1991, as a result of the group's successful work, its scope of investigation was broadened with the establishment of the 'OIE Ad hoc Group on Non Tsetse-Transmitted Animal Trypanosomoses' (NTTAT) [453]. This group has been instrumental in bringing about huge progress in the area of information on NTTAT, specifically by establishing links between researchers that deal with these trypanosomoses, both through the channel of OIE publications and under the warm and friendly impetus of the group's permanent secretary, Dr Louis TOURATIER, as well as thanks to the 'First International Symposium on NTTATs' organised in Annecy in October 1992. Since then, in addition, information and communication networks have been made available on the Internet (Embrapa/Fiocruz, PAAT, FAO, TRYPLINK, etc.).

Trypanosoma vivax, *T. evansi*, *T. cruzi* and *T. equiperdum* have been reported in practically all Latin American countries, but in many cases on the basis of serological diagnostic tools that are not capable of discriminating the species involved. Knowledge about the distribution, prevalence and medical and economic impact is therefore limited. Improving the diagnostic tools is a priority if better knowledge of livestock trypanosomoses in this region of the world is to be achieved.

The epidemiology of *T. vivax* and *T. evansi* in Latin America is found in the epizootic or enzoo-epizootic forms with domestic animals being the main reservoir for *T. vivax* and wild animals being involved in the epidemiology of *T. evansi*. In any case, these diseases and their major clinical and economic impact are visible in the form either of epizootic outbreaks that are causing the parasite to spread geographically by colonising new areas (in particular in Brazil) or of cyclical epizootic waves in previously infected areas. The periodicity of the epizootic outbreaks coincides with the time required for parasites to be eliminated from the host populations. This mechanism means that trypanosomoses are likely to be a permanent feature in South America and that they will continue to have a persistent, albeit variable, economic impact including in enzootic areas.

10 OIE – World Organisation for Animal Health

5. ANALYTICAL EPIDEMIOLOGY OF *TRYPANOSOMA VIVAX* IN FRENCH GUIANA

Previous studies have focussed very little on the analytical epidemiology of trypanosomosis due to *T. vivax*. The establishment of a CIRAD-EMVT branch in French Guiana in 1990 provided an opportunity to conduct an epidemiological survey on bovine hæmoparasitoses in this administrative unit that paid particular attention to trypanosomosis caused by *T. vivax*. The main objectives of the study were to determine the relative importance of *T. vivax* in cattle compared to other hæmoparasitoses, and ascertain the existence of *T. evansi*. Later on, an epidemiological surveillance system was established in French Guiana that provided additional information on the analytical epidemiology of trypanosomosis in cattle and sheep. The observations recorded there can be used as a model for interpreting similar situations encountered in South America.

5.1. Epidemiological surveillance system for sheep and cattle in French Guiana

Following a serological survey carried out on cattle in 1990-1992, which did not show up *T. vivax* as such but did disclose an antibody seroprevalence of 22%, an epidemiological surveillance system was set up in conjunction with the Departmental Veterinary Service (SVD – *Service Vétérinaire Départemental*). The details of these studies and observations were published in the form of a PhD thesis [43], the salient features of which are recalled here. Investigations were carried out using systematic sampling, and – where trypanosomosis was clinically suspected – the techniques used were HCT, smear tests, indirect ELISA *T. vivax* and confirmation by PCR in some cases.

5.1.1. Epidemiological surveillance of sheep in French Guiana

There are approximately 1,000 sheep in French Guiana; between January 1991 and March 1993, samples of serum were collected from 189 sheep on 17 different farms distributed all over French Guiana. These were tested retrospectively for antibodies raised against *T. vivax*; seroprevalence was nil.

The epidemiological surveillance system established was able to detect an active outbreak in July 1994. Simultaneously, several herds presented serological traces of infection (164 samples tested), but without any detectable clinical signs. The parasitological and serological prevalences of this outbreak were respectively 22% and 59%, with a serological incidence of 30% after 45 days, during a season when Tabanid activity is still limited while that of *Stomoxys* is a medium to low. It would seem that the parasite is able to replicate very effectively in sheep and that they may play an active role in the epidemiology of *T. vivax*. Transmission within the herd was probably done by crepuscular Tabanids of the genus *Chlorotabanus* spp., but it cannot be excluded that *Stomoxys* were responsible, or even mosquitoes, which were also highly active at the time. It was impossible to establish the origin of infection with any certainty: repeat infections stemming from previously infected sheep or cattle, or else accidental introduction of a carrier animal.

From the seroprevalences observed in other sheep farms, it would seem that the parasite circulated between 1992 and 1994 without there being any sufficiently visible clinical signs to alert farmers. This observation confirms the covert role played by sheep in maintaining, multiplying and disseminating *T. vivax*.

5.1.2. Epidemiological surveillance of cattle in French Guiana

A total of 2,600 examinations were performed between June 1992 and October 1996 whereby three epidemiological periods were distinguished:

An inter-epizootic period: from December 1990 to June 1994 the disease had not been diagnosed and no parasite had been observed; the prevalence of antibodies gradually receded in all of the

herds that had been found to be infected during the 1987-1988 epizootic. Serological monitoring indicates that the self-cure rate among cattle is approximately 15% per year. No trace of the parasite being in circulation was recorded.

An epizootic period: from July 1994 to June 1995, *T. vivax* was found in sheep in the month of July, and in a combined herd where the seroprevalence in cattle was 69%. The subsequent survey conducted in the field and notification by farmers led to active infection being confirmed on seven cattle farms. Samples were collected on a regular basis and indicated an average serological incidence of 35%; the seroprevalence at the end of the dry season was close to 100%. All the farms that were found to be infected by *T. vivax* had recently acquired animals belonging to a herd that had been sold off in its entirety. This surveillance programme was hence able to detect a multifocal epizootic due either to contamination by a herd that was sold and/or to repeat infections originating from animals that were carriers of the parasite. Tabanid abundance between July and September was low, and then very high from September to December. *Stomoxys* were present at all times but their abundance varied.

A post-enzootic period: from July 1995 to October 1996, ten farms were monitored. The period is referred to as post-enzootic because following the multifocal epizootic, only one clinical outbreak was observed under some rather special conditions, i.e. livestock imported from metropolitan France. On the farms that raise local cattle, no clinically observable outbreak of trypanosomosis was found. Some farms are free of trypanosomosis while others have maintained a serological trace from the previous epizootic. Two farms are highly informative in respect of the epidemiology of *T. vivax* in cattle.

Local zebus, good animal husbandry practices: in 1994, most of the cattle here were infected by *T. vivax* (seroprevalence 93%). A sample was collected from the whole herd in 1996. Of the 309 animals, only one was found to be a *T. vivax* carrier by HCT, smear and PCR (low parasitemia) without any symptoms. Antibody serology was negative indicating a recent infection. The sample taken from that case a month and a half later was indeed positive for indirect ELISA *T. vivax*. The parasite can therefore circulate within a cattle herd imperceptibly, outside of the peak period of horsefly activity. The only insects observed at dawn during this period were *Chlorotabanus mexicanus* and *Ch. inanis*.

Antibody seroprevalence in the batch to which this animal belonged went up from 22%-29% in three months, i.e. a monthly average incidence of 4%. Average haematocrit values for this batch dropped from 36.5% to 33.3% (significant at $p < 0.001$) in 45 days. No clinical manifestations were seen on this farm in spite of it being one of the most closely monitored in French Guiana. No trypanocidal treatment was applied and, three months later, further samples collected from the entire herd indicated a seroprevalence of 40%. The average monthly incidence was therefore approximately 3%. This episode of subclinical trypanosomosis that had no other immediate consequences would have gone unnoticed if no laboratory diagnostic tools had been used. This is therefore a case of subclinical enzootic trypanosomosis.

Imported herd, poor animal husbandry practices: this farm was also infected by *T. vivax* in 1994. It had imported 23 head of cattle from metropolitan France at the end of 1995 and beginning of 1996. Samples were taken from the cattle when they arrived using sterile needles but they were not given any particular prophylactic treatment, specifically not by injection. An outbreak of *T. vivax* trypanosomosis was observed in June 1996; both the locally bred animals (**Fig. 35 a**) and the recently imported animals (**Fig. 35 b**) were affected and some animals were visibly emaciated; the recently introduced animals were probably contaminated by the local livestock. Parasitological tests on various series of samples collected in June showed that up to 25% of the animals were active carriers. During this period the main hæmatophagous insect found on the farm was *Chlorotabanus* sp. Because mechanical transmission by iatrogenic means was excluded, this species was assumed to be responsible for the transmission of *T. vivax*.

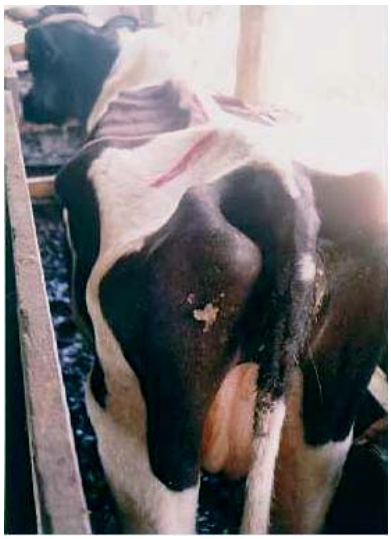

Figure 35 a – FFPN cow that was introduced into the herd several years before, that had recently exhibited symptoms of trypanosomosis and very noticeable emaciation (acute clinical reactivation of a chronic infection)

Figure 35 b – Batch of recently imported Salers heifers, infected by T. vivax *due to contact with local animals that are chronic carriers (M. DESQUESNES)*

Remark: some animals have lost up to 45 kg of bodyweight since the trypanosomosis outbreak began a month earlier.

Figure 35 – Outbreak of trypanosomosis due to *Trypanosoma vivax* in European cattle
(M. DESQUESNES)

Among the imported animals, serological incidence of trypanosomosis was 70% within a few weeks. For the herd as a whole (both local and imported animals), on one occasion temperatures of between 39.5°C and 41.4°C were found on 13 animals of the 21 that had been found to be parasite carriers. In the batch of Salers heifers that had been imported in December 1995, weight losses of up to 45 kg per animal in one month (average weight of batch 320 kg) were recorded, i.e. 14% of bodyweight (**Fig. 35 b**).

5.2. Conclusions and summary concerning trypanosomosis due to *Trypanosoma vivax* in French Guiana

5.2.1. Findings

The surveillance systems set up for sheep and cattle, clinical parasitological and entomological observations in the field, an analysis of the data before, during and after epizootic outbreaks of trypanosomosis have enhanced our knowledge of the epidemiology of the disease as well as providing answers to many questions or proposed mechanisms to better understand the epidemiology of trypanosomosis due to *T. vivax* in French Guiana.

Is the parasite present on the farms during inter-epizootic periods?

Because immunocapture-ELISA is not satisfactory, no direct answer can be provided. The kinetics of antibodies is such that negative seroconversion occurs within three months after sterilising sanative treatment. It is hence highly likely (unless sterilising self-cure has recently occurred) that an animal with antibodies more than six months after the epizootic outbreak is still actively infected.

In one herd, most of which had been infected in 1988-1989, antibody seroprevalence was 56% one year after the epizootic outbreak; three years later, the antibody rate in animals aged less than

16 months was nil, and 12% in animals aged over four. These findings indicate that infected cattle can remain carriers for several years and that during that time the parasite does not necessarily circulate (0 incidence in young animals).

In another herd, in a batch comprising 23 animals that exhibited positive seroconversion in December 1994, 74% of the animals were still covert carriers 16 months after the outbreak of the infection as derived from antibody serology.

Both in cattle and sheep, *T. vivax* can be present on farms and nonetheless go entirely unnoticed for several months or years.

What are the reservoirs for Trypanosoma vivax*?*

The involvement of sheep in the epidemiology of *T. vivax* trypanosomosis became apparent during the epidemiological surveys carried out on this species. Depending on the farm, infected sheep were either symptomatic or asymptomatic. Both sheep and cattle are reservoirs for the parasite.

Can the parasite be self-cured or spontaneously removed?

In one farm, without any trypanocidal treatment, the seroprevalence of 112 breeding stock dropped from 56% to 12% between 1990 and 1993, i.e. an annual self-cure rate of 15%. On another farm, of the 23 animals that had positively seroconverted in December 1994, six were found to be negative in May 1996.

The average annual rate of self-cure was therefore from 15% to 20%.

Does the parasite circulate covertly?

Between 1990 and 1994, no clinical case of trypanosomosis was either reported or detected in any species. In 1993, the serological trace of *T. vivax* was discovered in three sheep farms that had been found to be trypanosomosis-free in 1990.

On a cattle farm where no symptom of trypanosomosis was visible, *T. vivax* was discovered in one animal out of the 309 tested in a batch whose positive seroconversion rate was 4% per month.

Laboratory tests therefore provided evidence that *T. vivax* can circulate in sheep and cattle farms without being suspected by farmers.

Does the parasite circulate outside of the peak transmission periods (Tabanid season)?

In the absence of clinical signs on farms with adequate animal husbandry practices, *T. vivax* can circulate covertly at a fairly low monthly incidence (4%) during the rainy season. During that period, a clinical outbreak of trypanosomosis occurred on unchallenged animals on a farm with inadequate animal husbandry practices (feeding). In this case, the disease became clinical and incidence reached 70% within a few weeks. If the animals had not been treated, incidence would have no doubt reached 100% for this batch and probably for the entire herd.

These observations in conjunction with those made during epizootic outbreaks imply that trypanosomosis can circulate at all seasons. During the rainy season, the most likely transmission agents are *Stomoxys* and the crepuscular species of Tabanids, *Chlorotabanus mexicanus* and *Ch. inanis* possibly with the involvement of mosquitoes too.

Can the infection be detected by parasitological diagnostic means?

It would seem that in the case of clinical trypanosomosis, direct examination is easy and effective, returning up to 25% positive results.

On the other hand, when the trypanosomosis is asymptomatic, parasitæmia is very low and fluctuates. A good illustration of this is a farm where the parasitological prevalence was 0.3% whereas the seroprevalence was 31%.

Except in symptomatic outbreaks, parasitological examination is therefore insufficiently sensitive and must be supplemented or even be replaced by antibody serology (indirect-ELISA).

Does the incidence of infection vary according to the season?

During the epizootic prior to 1989, symptomatic trypanosomosis was reported only during the second part of the dry season (October to December) and it was assumed that the animals had become infected during the dry season.

During the 1994-1995 epizootic outbreak, the serological incidence among the monitored herds was close to 100% in just a few weeks; the pullulation of Tabanids and tendency of livestock to assemble in response to attacks by biting insects provides a ready explanation for this high incidence. Seroconversions in this instance were recorded from July to March.

In 1996, a major outbreak with very high incidence was observed during the month of May and June in an imported herd. Very few hæmatophagous insects were found on a farm at the time.

Incidence of infection can reach 100% in a few days whatever the season. Vector load, even when it is low, is therefore not likely to be a limiting factor for infection; similar observations have also been made in Colombia by OTTE *et al.* [297].

Does the incidence of symptomatic trypanosomosis vary depending on the season?

While the disease can be transmitted in any season, receptiveness of animals fluctuates and reaches a peak during the dry season. The increase in symptomatic trypanosomosis cases during the dry season (season of activity for horseflies) is thought to be more due to the poor overall condition of the animals (inadequate food and water, parasitism of horseflies) then to high rates of parasite transmission.

During the dry season in epizootic periods, the incidence of symptomatic trypanosomosis is always very high, close to 100%. During the rainy season, it depends on the overall condition of the animals and the other animal husbandry parameters, especially feeding.

All in all, trypanosomosis due to *T. vivax* is a permanent threat for cattle and sheep farms – the infection circulates imperceptibly when animal husbandry practices are satisfactory, whereas in the opposite case, epizootic outbreaks occur with noticeable clinical manifestations. The impact of trypanosomosis on goats could not be assessed because the goat population is very small (500 animals) and widely dispersed.

5.2.2. Summary of the data collected

Trypanosomosis affects European cattle, zebus and their crossbreeds, as well as sheep; in the latter, clinical signs are often vague and surveillance is very scanty. Hence, farmers can rarely be warned. Cattle and sheep are reservoirs for the parasite.

There has not so far been any evidence establishing a direct connection between the periodicity of the epizootics in cattle (every three to five years approximately) and the climatic or entomological parameters studied.

The animals' immunocompetence may be undermined when the balance between the proportion of carrier and non-carrier animals is broken and when animal husbandry practices are inadequate. This may partially explain the periodicity. Indeed, the rate of self-cure was estimated to be between 15%

and 20% per year. As for covert circulation of the parasite, it too is highly variable since, depending on the farm, inter-epizootic incidence ranges from 0% to 4% a month with no clinical signs.

One theory which might explain the three to five-year interval observed between each multifocal epizootic is that all of the herds gradually move from an infected status caused by each epizootic to a less desirable immune status, i.e. when less than 20% to 40% of the animals are still carriers of the parasite (and therefore immune). By applying an annual self-cure rate of 20% to a 100% infected heard, the rate of covert carrier state would revert back to less 40% from three to four years after the epizootic outbreak, which partially explains why the interval between two outbreaks is three, four or five years.

Parasites come into farms as a result of infected livestock being introduced. Transportation stress maybe one of the reasons that parasitemia rises sharply in these animals making them potentially contaminant. The consequences of parasite transmission would in that case depend on the immune status of the recipient herd. The disease would go unnoticed if most of the animals are carriers of *T. vivax* or, on the contrary, highly apparent if most of the animals are disease-free. The intensity of symptoms varies according to the specific immune status of the animals and the animal husbandry standards on the farm. As such they can vary over the full range, from slow imperceptible circulation to symptomatic outbreaks with severe medical and economic consequences, via intermediate, low-level forms or sporadic cases within a herd.

The relative impact of Tabanids and other hæmatophagous insects on livestock gives an indication of their importance both as **harmful insects,** as **contributing factors** in bringing about clinical expression of hæmoparasitoses (direct impact), and as **transmitting agents** for trypanosomoses (indirect impact). The study conducted in French Guiana shows that the direct economic impact of Tabanids is greater than their indirect impact and that a very low vectorial load is enough support transmission of *T. vivax*. Tabanid control must therefore be construed as **the control of the main contributing factor** to the clinical expression of trypanosomosis and anaplasmosis (see section on other hæmoparasitoses).

While this form of control cannot prevent trypanosome transmission, it can aim to alleviate the direct harmful effects and hence avert clinical expression of hæmoparasitoses.

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CHAPTER 6:
CONTROL OF TRYPANOSOMES AND THEIR VECTORS

Livestock trypanosomoses in the New World occur as epizootic outbreaks, which in some areas are occasional and in others take place against an enzootic background.

In the occasional epizootic outbreaks, the fastest and most suitable control method is to use trypanocides to suppress the parasite on the host. The particular choice and delivery method for the trypanocide must however be carefully planned.

In the enzootic areas, the ideal control method would be to suppress the parasites by immunising the hosts against the trypanosomes. Failing that, chemoprophylactic methods should be used when strains are highly pathogenic and hosts are unable to control the infection. The alternative to controlling parasites is to control the vectors but with the techniques currently available this is only partially possible.

We review the available trypanocides below, placing the emphasis on the susceptibility of local strains to the most widely used substances in the New World: diminazene aceturate and isometamidium chloride. The methodology for studying the effects of trypanocides is discussed and strategies for use deriving from their properties are suggested. The outlook for research in the area of immunisation against the trypanosomes and vector insects is examined, together with control of parasite transmission and vector management techniques.

1. CHEMICAL CONTROL OF TRYPANOSOMES

1.1. Available trypanocides

1.1.1. Diminazene aceturate

Diminazene aceturate (Berenil®, Veriben®, Ganaseg®, etc.) is an aromatic diamidine that was described for the first time in 1955. Its mode of action on parasites is thought to be the result of the blockage of glycolysis and of DNA synthesis. Local tolerance in ruminants is fairly good; diminazene aceturate is generally injected via the intramuscular route but the subcutaneous route is also possible [63]. Treatment by diminazene aceturate to control bovine babesioses and trypanosomoses in cattle, sheep and goats, at doses of between 3.5 and 7 mg/kg rapidly became widespread [476]. It is also thought to be active against *T. theileri* at doses of 7 mg/kg (REITER *et al.*, 1987).

In ruminants: in cattle, sheep and goats, the indication for diminazene aceturate is sanative treatment of trypanosomosis, especially when it is caused by *T. vivax*, but it has no prophylactic effect. For this reason, it is recommended in Africa in areas where parasite loads are low, or for the purposes of controlling epizootic or sporadic outbreaks. Where it has been used on herds in Brazil, Colombia, Venezuela and French Guiana, clinical signs have generally disappeared to the satisfaction of farmers.

For a long time, no resistance to diminazene aceturate was observable, but starting in 1960, resistant strains of trypanosomes were identified in many African countries: Nigeria [477, 478, 479, 480, 481, 482], Chad [483], Uganda [484], Tanzania [216, 485, 484], Kenya [486], etc., and, later on, in South American countries too.

In Latin America, HULL [162] and BETANCOURT [47] have reported resistance of *T. vivax* infections to a 3.5 mg/kg dose of diminazene aceturate in Colombia. WELLS *et al.* [148] suggest that a dose of 7 mg/kg should secure parasite control but no experimental evidence of this has

been provided. In French Guiana, although the substance does indeed caused symptoms of bovine or sheep trypanosomosis to regress in the field, the two strains that were isolated during the 1989 (TVFG1) and 1994 (TVFG2) epizootics are resistant to treatment at the dose of 7 mg/kg [169] in sheep and calves, and indeed in one case even with up to 10 mg/kg [43]. Once the parasites disappear from the bloodstream for periods ranging from 10 to 130 days after treatment, they reappear either spontaneously, or subsequent to transportation stress or food rationing. The same applies to a Venezuelan strain (TVVG1) isolated in the state of Guarico [43]. In contrast, treatment at a dose of 7 mg/kg of diminazene aceturate on a Guyanese strain (TVFG3) isolated during an inter-epizootic period was found to be sterilising.

Many of the observations relating to the effectiveness of diminazene aceturate were based on clinical recovery of the animals and the disappearance of parasites from the bloodstream following treatment rather than on evidence that it is able to eliminate the infection.

In contrast, more recently, in Venezuela, ESPINOZA has suspected resistance to diminazene aceturate directly in the field (unpublished paper). The resistances observed in Venezuela may have been brought about by widespread use of diminazene aceturate and its many derivatives for the control of babesioses and/or trypanosomoses: Babenil®, Batrival®, Diminazeno Mck®, Ganaseg®, Pirobenz®, Tibabenox® Veriben®, etc. (ESPINOZA, unpublished paper).

In sheep that were experimentally infected with a *T. evansi* strain isolated in Venezuela (in the state of Apure), diminazene aceturate treatment (7 mg/kg IM) temporarily removed the parasites but did not sterilise the animals [43].

Other species: resistance of *T. evansi* to diminazene aceturate in horses has often been observed [487]; it is sometimes very strong – ZHANG *et al.* [218] have shown one field strain to be resistant to an 89 mg/kg dose in mice. Furthermore, the tolerance of horses to diminazene aceturate is low and it is therefore not recommended for treating them against trypanosomosis due to *T. evansi*. SABANSHIEV (quoted by PEREGRINE and MAMMAN [476]) has found that diminazene aceturate eliminates *T. equiperdum* at a dose of 5 mg/kg. However, the recommendation for animals infected by *T. equiperdum* is slaughter rather than treatment. In addition, some strains are resistant to the substance [218]. A number of *T. evansi* and *T. equiperdum* strains selected for their resistance to diminazene aceturate are also resistant to melarsomine in spite of the fact that the two compounds are not chemically related. In contrast, these strains become more susceptible to quinapyramine [218].

Multiple resistances to diminazene aceturate, suramin and isometamidium chloride have been found in strains isolated from dogs in Brazil [218]. The manufacturer (M. WATRIN, Roussel Uclaf, unpublished data) strictly advises against using diminazene aceturate on dogs and cats, as they are highly sensitive to the substance. However, its use on dogs in Europe does not appear to raise any particular problem (UILENBERG, unpublished data).

Persistence: diminazene aceturate is somewhat persistent in milk. It is detected at a concentration of 50 ng/ml 72 h after IV injection of 2 mg/kg [476], and at concentrations of respectively 4.56 ng/ml and 8.76 ng/ml in milk 21 days after IM injection of 3.5 mg/kg and 7 mg/kg in dairy cows [488]. In serum, diminazene aceturate can persist up to three weeks after IM treatment (7 mg/kg), and, *in vitro*, depending on observations, either displays trypanocidal activity (in cattle) or does not (in sheep) [155]. Hence, in spite of its persistence, its trypanocidal activity does not appear to be satisfactory. According to this data, a withholding period for milk and meat of more than 21 days is advisable; however, in France, recommended withdrawal times are of only 20 and three days respectively for meat and milk (M. WATRIN, Roussel Uclaf, unpublished data).

When used in conjunction with isometamidium chloride, diminazene aceturate is thought to be highly liver-toxic in undernourished animals [489] which no doubt explains why late treatment often gives rise to a fatal outcome.

1.1.2. Isometamidium chloride

Isometamidium chloride (Trypamidium®, Samorin®), like homidium, belongs to the family of phenanthridines. Since it was first marketed in 1961, isometamidium chloride has been recommended for the prevention and treatment of cattle and sheep trypanosomoses, in particular those due to *T. vivax* [216].

Ruminants: in cattle, at a dose of 1 mg/kg of bodyweight, isometamidium chloride affords protection for 17 to 28 weeks [217], five months on average. That is why it is recommended in Africa in enzootic areas and/or periods of very high parasite load.

Isometamidium chloride is therefore the therapy of choice in most cases, especially in the case of *T. congolense* and *T. vivax* infections that are resistant to diminazene aceturate [490]. In French Guiana and Venezuela, the diminazene aceturate-resistant strains are fully susceptible to isometamidium chloride (0.5 mg/kg IM), which affords protection for more than 4.5 months [43, 169].

However, some strains are resistant to isometamidium chloride, e.g. a Nigerian *T. congolense* strain that was resistant to homidium was also found to be resistant to isometamidium chloride. Initially, this was a rare occurrence [490] but is apparently becoming more frequent [401]. In Colombia, OTTE [246] found *T. vivax* to express resistance against isometamidium chloride.

ELISAs for detecting isometamidium chloride developed by PEREGRINE *et al.* [492] and EISLER *et al.* [493] can be used to assess the resistance of strains in the field [494, 495] by screening for relapse of infection post treatment. These techniques have not been evaluated in America. Preliminary results obtained in Africa are not fully convincing.

Other species: isometamidium chloride treatment against trypanosomosis due to *T. evansi* in horses is generally neither effective nor well-tolerated [217, 218]. Additionally, cross-resistances with diminazene aceturate and suramin sometimes occur [218].

In sheep experimentally infected with a Venezuelan *T. evansi* strain, the efficacy of that treatment was not demonstrated based on antibody persistence more than four months after treatment [43].

For treatment of *T. equiperdum* infections, although the parasites are susceptible *in vitro*, *in vivo* tests have not been satisfactory [218]. The recommendation is to slaughter the animals rather than treat them. Isometamidium chloride can be used on dogs but with the same reservations as with diminazene aceturate.

Persistence: according to pharmacokinetic studies conducted by Rhône Merieux (undated), isometamidium chloride has long-term persistence in plasma at low concentrations (15 μg/ml) that are nonetheless effective. Excretion in milk is very low (2 μg/ml). EISLER *et al.* [494] have established that the half-life of isometamidium chloride is 21 days in cattle and detect the product **for more than 70 days**. There is no withholding period for milk. Based on maximum allowable concentrations of residues set out by the JECFA[11] at its sittings on 9 and 18 June 1992, French legislation requires a withdrawal period of only 30 days prior to slaughter. It should be emphasised that this legislation is not consistent with the proven persistence of the trypanocides that lasts several months as shown by various observations [496], and as has been confirmed by detection of the chemical using the ELISA technique [497]. Since trypanocidal activity lasts more than four to five months, a withholding time of at least five months should be recommended. Research has been conducted on isometamidium chloride implants [498]. At equivalent doses, they are stated to remain active for longer than the injected product, which would mean that the withholding period

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should be extended. Because withholding times are often disregarded, this type of dosage form should be avoided.

No mutagenic or teratogenic effects have been detected (VAN GOOL, unpublished data). However, the mutagenic activity of other phenantridines has been demonstrated and so there are grounds to be dubitative in respect of isometamidium chloride. In addition, liver toxicity of isometamidium chloride has recently been shown in cattle, in particular in the case of repeat treatments at monthly intervals in alternation with diminazene aceturate. Marked toxic effects are observed in undernourished animals [489]; further investigations need to be carried out in this area as this association (alternate trypanocide treatment plus malnutrition) is very frequent in the field. Once again, fatal outcomes observed following late or repeated treatments might be due to the toxicity of these products under those circumstances.

1.1.3. Homidium salts

Homidium chloride (Novidium®) and homidium bromide (Ethidium®) are closely related derivatives of isometamidium chloride. Their sanative effect is good and they are prophylactic for one to three months depending on the authors. They are recommended for treating cattle, sheep and goats against trypanosomoses due to *T. vivax* and *T. congolense* [499]. Ethidium® is used as sanative treatment against bovine trypanosomosis in Venezuela (ESPINOZA, unpublished data).

Resistances to homidium are frequent and were discovered in the field at an early stage [500]; in Nigeria, ten years after extensive use of the product began, resistance to homidium was sometimes found in combination with resistance to two other trypanocides tested: diminazene aceturate and isometamidium chloride [490, 501]. Similar findings were made in the Central African Republic, Nigeria, Kenya, Côte d'Ivoire and Burkina Faso, according to CODJIA *et al.* [501]. Homidium was therefore found to be liable to generate and/or be associated with multiple resistances to the main trypanocides used on ruminants, in particular for *T. vivax* and *T. congolense* control.

Toxicity: molecular biologists and geneticists are familiar with homidium bromide under the name of ethidium bromide, which is known to be extremely toxic. Indeed, ethidium bromide is an intercalating agent for nucleic acids that is used, inter alia, to visualise DNA in agarose gel under ultraviolet lights. Furthermore, metabolic activation of ethidium bromide gives rise to the synthesis of a powerful mutagen that is used to produce bacterial mutations [502]. There is evidence that this transformation can occur in mammals [503]. In biology laboratories, it is mandatory to wear a mask and gloves for while handling this product.

Using such an agent in the field is ill-advised. Clearly, it is essential to conduct a long-term toxicity study for personnel that manipulate the product (often without any precautions since the assumption is generally that a drug cannot be harmful), for livestock inoculated and for consumers of beef – this has never been done. Unfortunately, habit, the absence of immediately obvious toxicity, and the narrow choice of trypanocides available on the market have made ethidium a 'must' for many practitioners and researchers in Africa. Since no epidemiological data on the long-term toxicity in humans or animals is available, the precautionary principle should be applied and the drug should not be used in the field. Additionally, its persistence in certain cattle organs such as the liver and kidneys is long-term [504] and presents a real risk in respect of human consumption. For the prophylaxis of trypanosomoses, sustained release devices with ethidium bromide have been evaluated in rabbits (KAGERUKA *et al.*, quoted by TOURATIER [88]). Considering the product's toxicity, these types of delivery method are highly dangerous. It is quite obvious that ethidium bromide would never obtain a market authorisation (MA) in France. One may justifiably wonder why it is still distributed and even promoted in other countries.

1.1.4. Suramin and/or Quinapyramine

Suramin (suramin sodium, Naganol®) was first synthesised in 1922. It is a uræmic compound that is effective against trypanosomes of the subgenus *Trypanazoon* that is specially recommended for the

treatment of trypanosomoses in horses and camels by the intravenous route because local tolerance is low. It is sometimes associated with quinapyramine. Suramin is ineffective against *T. vivax* and *T. congolense* [505].

Quinapyramine sulphate (Trypacide sulphate®, Antrycide®) is used for chemotherapy while the mixture of quinapyramine sulphate and chloride (Trypacide Prosalt®, Antrycide Prosalt®) is used for chemoprophylaxis mainly against trypanosomoses in horses and camels. Quinapyramine is injected subcutaneously. Horses and dogs are poorly tolerant to the substance and anaphylaxis and local reactions secondary to the injection are observed.

In Venezuela, quinapyramine seems to be the most effective curative and prophylactic trypanocide against *T. evansi* infections in horses [216], with a protective effect that lasts up to four months. In combination with suramin in the form of 'quinapyramine suraminate', at a dose of 4 mg/kg-50 mg/kg, protection lasts from 6.5 to 26.5 months [56]. Both these substances act by inhibiting the synthesis of parasite DNA and proteins [63] but combinations of the two were soon discarded because of their toxicity.

In cattle, quinapyramine has also been suggested as treatment for *T. vivax* and *T. congolense* infections [505], but is contraindicated because it produces cross-resistances with all the other trypanocides: diminazene aceturate, homidium and isometamidium chloride [506]; furthermore, the local reaction at the point of injection is a limitation to its use [14]. Finally, because it is eliminated slowly, it encourages the establishment of resistance. Dogs are poorly tolerant to quinapyramine [216].

1.1.5. Melarsomine

Melarsomine, better known under its registered trade name Cymelarsan®, was first synthesised in 1983 and marketed in 1992. It is one of the few trypanocides to have been developed in the last 35 years [507]. It is a trivalent arsenical, 'bis (amino ethylthio) – 4 melamino-phenylarsine dihydrochloride'. Its trypanocidal activity is produced by blocking trypanothione reductase, an enzyme involved in the regulation of the parasite's redox reactions (thio/sulfide) (Rhône Mérieux, undated). It is active only against *T. evansi* and *T. brucei*. Its metabolism is rapid and, as such, its action is purely sanative. Cymelarsan® is recommended in camels at a dose of 0.5 mg/kg by deep IM injection. Specifically, it is effective against parasites that are resistant to suramin and quinapyramine. Mérial (formerly Rhône-Mérieux) is planning to register Cymelarsan® for horses, cattle and buffaloes [88]. However, Cymelarsan® is only partially effective against trypanosomosis due to *T. evansi* in horses (ROSS, undated).

1.1.6. Other trypanocides

Trybizine hydrochloride (T46): this new trypanocidal drug belongs to the family of the diaminotriazines and is currently being evaluated in Shanghai against *T. evansi* in cattle and buffaloes. The effective doses are 0.5 mg/kg in cattle and 1.5 mg/kg in buffaloes. However, toxicity occurs in cattle at doses of 3 mg/kg (SHEN JIE *et al.*, quoted by TOURATIER [88]). In an evaluation of its activity against *T. brucei* in mice, the drug was found to be effective, specifically against strains that were resistant to melarsomine and/or diminazene aceturate, but its toxicity becomes apparent at a fairly early stage [508]. In the case of nervous invasion by *T. brucei* in rats, the treatment was not curative indicating that the drug has trouble penetrating the blood/meningeal barrier [509]. Studies on this drug and derivatives must be continued.

Mérial is no longer investigating new trypanocides [88], but other investigations are being conducted, in particular with a view to identifying active molecules that draw on the specific characteristics of glycolysis or the existence of enzymes that are specific to trypanosomes. For instance, the molecular transporter of trypanosomal glucose also recognises D-fructose whereas the human receptor for erythrocytes does not. This specificity could be put to use to foster internalisation of trypanocidal molecules [510]. Furthermore, a number of essential enzymes in

trypanosomes are not present in mammals (similar to ecotin and phosphoenolpyruvate kinase) making them therapeutic targets of choice [511].

The prospects of any new trypanocide being released, at any case in the short-term, are poor and it therefore a matter of concern that resistances are growing while the armamentarium is dwindling because some of the older compounds are no longer being produced or because they have been shown to be toxic. Research on new trypanocides should be strongly encouraged.

1.2. Studies on the effects of trypanocides and their consequences

To achieve optimal use of trypanocide properties, overcome their shortcomings where needed and deliver them rationally on the basis of the particular features of the strains present in the field, it is crucial to have a thorough knowledge of the actual effects of trypanocides during the course of infections.

Often, studies of trypanosome susceptibility/resistance to trypanocides draw somewhat categorical conclusions, e.g. the strain is either susceptible or resistant. The studies are performed *in vitro*, and generally consist in measuring the chemical resistance to trypanocides (actual chemical resistance), or else *in vivo* to assess their sanative capability. The latter must be precisely described in terms of chemical resistance, compartment resistance, full susceptibility, intermediate situations, etc. *In vitro* observations cannot always be transposed to the natural hosts since biological interactions and the drug's diffusion are not accounted for. Similarly, studies conducted in laboratory rodents cannot be extrapolated to natural hosts. In as far as possible (on the basis of technical and economic constraints), efficacy studies should be conducted on the natural hosts of economic interest or, failing that, on very similar models (sheep for cattle, donkeys for horses).

Furthermore, the duration of any prophylactic effect detected should be assessed.

1.2.1. Effects of trypanocides

Evaluations are conducted *in vivo* on the experimentally infected natural host that is placed under the protection of mosquito netting – treatment can have the following effects and significance:

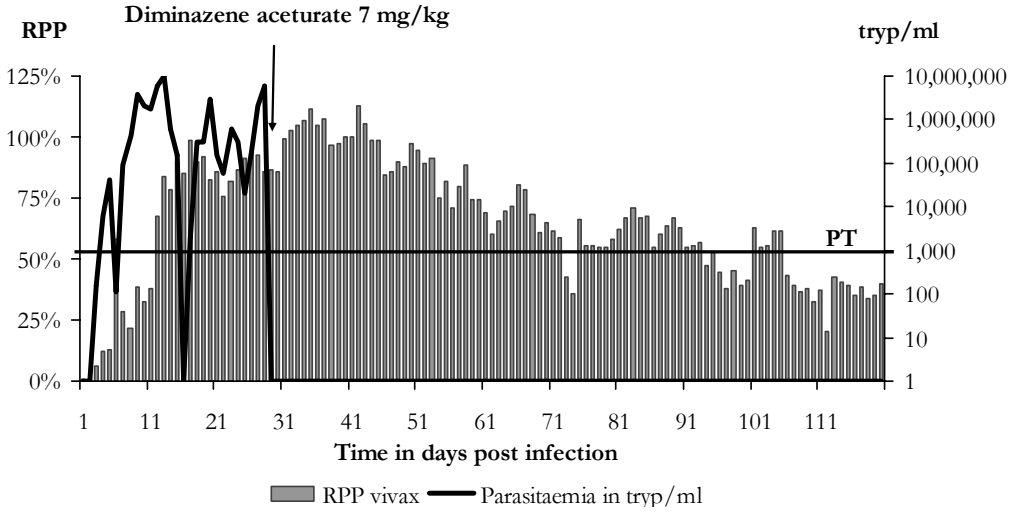
– **No effect**: the treatment does not remove the parasites and does not produce any clinical improvement. In this case, the parasites are truly chemically resistant and the substance is of no use. Another trypanocide must be tested for application in the field. This is unusual but possible because some field strains possess or acquire very high degrees of resistance [218].

– **Sterilising sanative effect (elimination of the infection)**: the parasites disappear the day after the treatment; clinical signs recede, as well as serological signs (antibodies) and no further parasitic resurgence is observed. For this to be ascertained, a three- to four-month parasitological and serological follow-up is needed to show that the parasites and antibodies have disappeared durably, meaning that there is no antigenic stimulation and hence that treatment has had a sterilising effect. Even in this case, reservations should be expressed since an extravascular location may continue to harbour the parasite from where it does not exercise any antigenic stimulation (aqueous humour of the eye) and can invade the bloodstream once again a few months or years later. Although theoretically possible, this phenomenon is probably minor.

  On the basis of such observations, one can conclude that the **strain is susceptible and the treatment is both sanative and sterilising**, as was the case for instance with diminazene aceturate on the TVFG3 strain (*T. vivax* from French Guiana) (**Fig. 36 a**) [43].

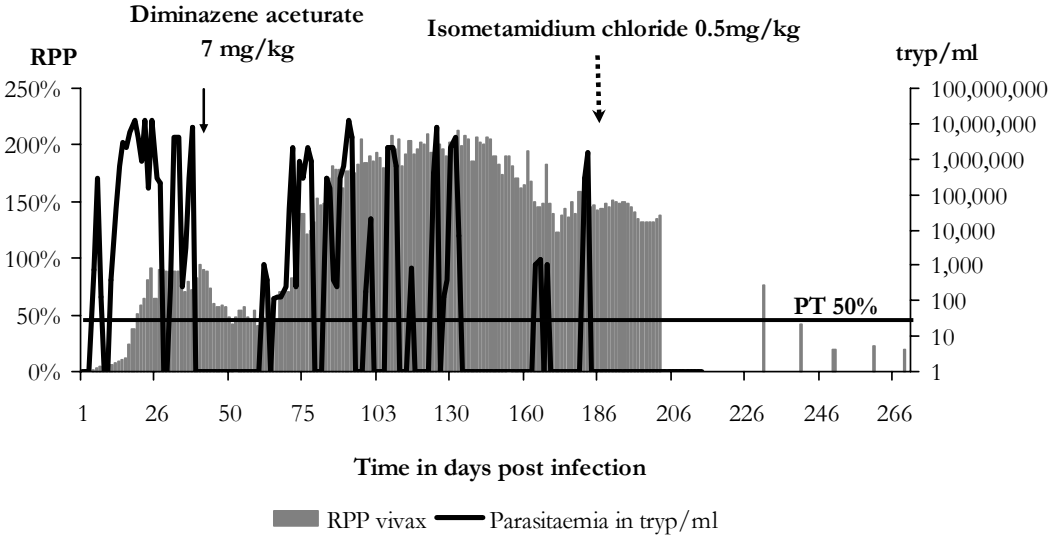
– **Temporary, non-sterilising effect:** the parasites cannot be detected the day after the treatment, clinical signs recede, but serological signs persist. Subsequently, both the parasites and the clinical signs reappear. This can occur in two situations: the strain was fully susceptible to the product and the parasites in the bloodstream were killed; however, the parasites in the

extravascular compartment either eluded the product, received an inadequate dose or survived and subsequently re-invade the bloodstream. These parasites retain the pathogenicity of the initial strain and, consequently, subsequent parasitæmia is high and clinical signs recur.



Remark: RPPs overshoot the positivity threshold 8 to 15 days post infection; the regular and complete decline of RPPs indicates that the treatment is sterilising; the RPPs go below the PT approximately 80 days after treatment.

Figure 36 a – Parasitæmia, diminazene aceturate treatment and response by indirect-ELISA T. vivax *in sheep No. 11 infected with TVFG3 (*T. vivax *from French Guiana)*



Remark: parasite resistance to diminazene aceturate (7 mg/kg IM) is confirmed by parasitological means and indirect-ELISA; however, isometamidium chloride treatment causes parasites to disappear durably and the level of specific antibodies to drop. At these doses therefore, the strain is resistant to diminazene aceturate but susceptible to isometamidium chloride.

Figure 36 b – Parasitæmia, diminazene aceturate and isometamidium treatments and response by indirect-ELISA T. vivax *in sheep No. 9 infected with TVFG2*

Key: ELISA results are expressed in RPPs (relative percentage of positivity), parasitæmias are represented on a logarithmic scale; PT = positivity threshold for indirect-ELISA *T. vivax*, as depicted by a horizontal line.

Figure 36 – Parasitæmias, trypanocidal treatments and responses by indirect-ELISA *Trypanosoma vivax* in sheep infected with *Trypanosoma vivax* from French Guiana

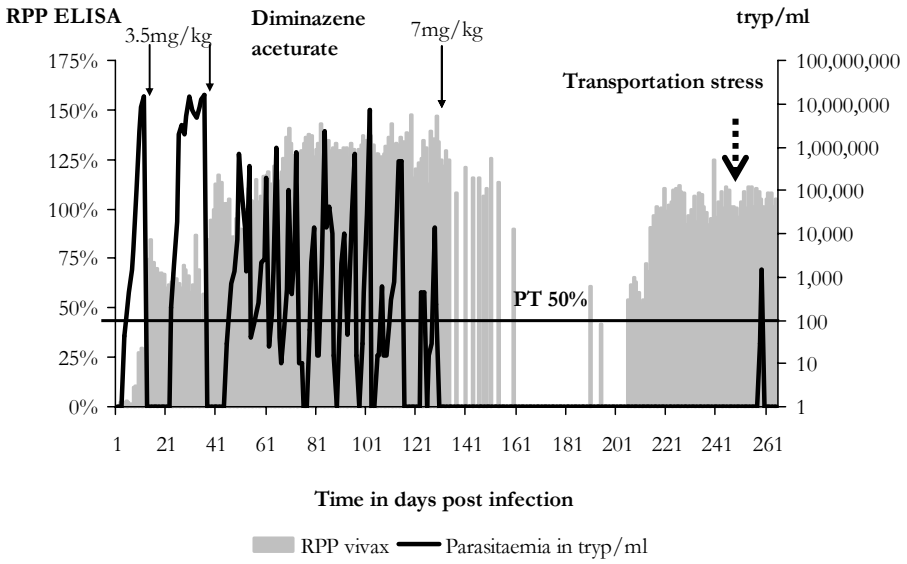
The strain is susceptible in this case, but the effect of treatment is temporarily sanative effect but non-sterilising.

Compartments of this type have been observed in buffaloes for *T. evansi* (Virchow-Robin spaces in the brain) [187] and goats for *T. vivax* (central nervous system and aqueous humour of the eye); furthermore, in mice, evidence has been provided that trypanosomes that reappeared spontaneously after diminazene aceturate treatment were nevertheless susceptible to the drug [36].

The bioavailability of diminazene aceturate has been studied in goats by PEREGRINE *et al.* [512]; its concentration in the CSF and the lymph was respectively only 23% and 33% of plasma concentration. The trypanosomes present in these compartments are able survive treatment because they are exposed to inadequate doses of the trypanocides. These resistances occur sporadically and do not lead to use of the trypanocide being discontinued.

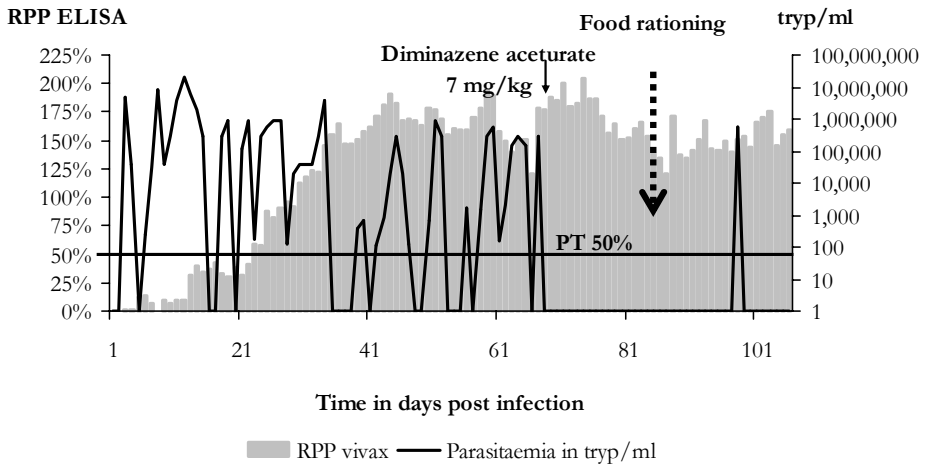
A temporary, non-sterilising effect is also observed when part of the parasite population whose pathogenicity is similar to that of the initial strain is resistant to the drug. In this case, the effect of the treatment will simply be to select the resistant parasites as has been shown experimentally by BURUDI *et al.* [513].

These effects (temporary, non-sterilising) have been recorded for diminazene aceturate treatment against *T. vivax* strains from French Guiana (TVFG1 and TVFG2) and against *T. vivax* (TVVG1) and *T. evansi* (TEVA1) strains from Venezuela (**Figs 36 b**, **37 a** and **38 b**, initial treatments) [43, 386]. In these cases, the treatment is ineffective.



Remark: Diminazene aceturate resistance at doses of 3.5 mg/kg and 7 mg/kg (IM); after the 3rd treatment, the RPP drops and then increases once again showing that the infection persists. A detectable parasitæmic wave occurs 130 days after treatment following transportation stress.

Figure 37 a – Parasitæmia, diminazene aceturate treatments and response by indirect-ELISA T. vivax *in sheep No. 1, infected with TVFG1 (*T. vivax *from French Guiana)*



Remark: Diminazene aceturate resistance at a dose of 7mg/kg; the imperceptible carrier state is revealed by persistence of antibodies (RPP stable after treatment), and the resurgence of detectable parasitæmia induced by food rationing for 20 days.

Figure 37 b – Parasitæmia, diminazene aceturate treatment and response by indirect-ELISA T. vivax *in sheep No. 12 infected with TVVG1 (*T. vivax *from Venezuela)*

Key: Results of ELISA *T. vivax* are expressed as RPP; PT = positivity threshold for indirect-ELISA *T. vivax*, depicted by a horizontal line; parasitæmia is represented on a logarithmic scale

Figure 37 – Parasitæmias, diminazene aceturate treatments and responses by indirect-ELISA *Trypanosoma vivax* in sheep infected with *Trypanosoma vivax* from French Guiana and Venezuela

– **Lasting, non-sterilising effect**: the parasites generally disappear one day after treatment, clinical signs recede, but serological signs persist. Subsequently, parasite resurgence in the blood can be observed but the parasitæmic levels are lower than before, and minor clinical signs are sometimes apparent. This is an indication that the strain has been partially eliminated – part of the parasite population that is susceptible to the drug has been destroyed while the rests has eluded or resisted treatment. The parasites that survive are possibly a subpopulation whose pathogenicity differs from that of the initial strains, parasites that express the resistance genes are incidentally debilitated, and the parasitemic waves are of lower intensity than prior to the treatment (**Fig. 37 b**). The conclusion is that **this strain is partially resistant and the treatment is durably sanative but non-sterilising**. It could also be that the treatment is followed by partial carrier state immunity, particularly in view of the consequent massive release of parasitic antigens. This situation has been observed with the TVFG1 strain from French Guiana with a 7 mg/kg dose of diminazene aceturate on sheep, whereas at the 3.5 mg/kg dose, pathogenicity of the resurgences is undiminished [43] (**Fig. 37 a**).

  In practice, effects can cover the full range of intermediate effects between **temporary, non-sterilising** and the **lasting, non-sterilising effect** with correspondingly dwindling clinical manifestations. It is difficult in these cases to ascertain whether a selection of more resistant and less pathogenic parasites has occurred or whether animals that have gradually become immunised are better able to control a strain whose pathogenicity is constant. To do so, the pathogenicity of the resurgent parasites in new animals would need to be assessed. The fact is that two to three diminazene aceturate treatments in succession under experimental conditions on resistant *T. vivax* strains from French Guiana have often led to increasingly satisfactory control of the infection ending up with the symptomless carrier state (**Fig. 37 a**, treatment 3) [43]) Similar situations have been found in the field. Repeated release of antigens as a result of treatment may produce immune stimulation that leads to better control of infection.

– **Lasting effect with improvement in clinical signs, elimination of the parasites and lasting protection**: this situation can arise if trypanocides have a prophylactic effect. The

parasites disappear the day after treatment, the clinical and serological signs recede, no resurgence of the parasites is observed, and reinoculation with the initial strain has no effect throughout the period of protection as measured by repeated inoculation of the same animals. Isometamidium chloride treatment at a dose of 1 mg/kg has been observed to afford protection for more than 4.5 months against the *T. vivax* strains from French Guiana and Venezuela [43, 386]. In Africa, the protection is effective against some strains of *T. congolense* only for one month [495].

1.2.2. Methodology for studying the effects of trypanocidal drugs in hosts

For trypanocidal drugs to be tested, trypanosome-free animals need to be selected. In practice, these animals are taken from a disease-free area or a farm where parasitological and serological antibody tests are negative. The animals are stabled under the protection of mosquito netting, experimentally infected using a syringe (or via *Glossina* in Africa); parasitological examinations (HCT) are used to monitor the infection. If therapy is applied too early, it might be prematurely concluded that a drug is effective. The infection should be allowed to become established over two to four weeks before applying treatment to enable the parasite to invade the host's various biological compartments.

After treatment, parasitology alone can detect resistance if parasite resurgence is early and intense (**Fig. 37 a**: treatments 1 and 2, **Fig. 36 b**: treatment 1). It is not an appropriate detection method if the resurgence is low-level, short lived (**Fig. 38**) and/or late (**Fig. 37 a** – third peak, **Fig. 37 b**) especially if tests are performed only twice or three times a week as is usually the case. If parasitology alone is used, even daily checks over a period of 3 months are sometimes not sufficient to demonstrate the resistance of parasites to treatment (**Fig. 37 a**).

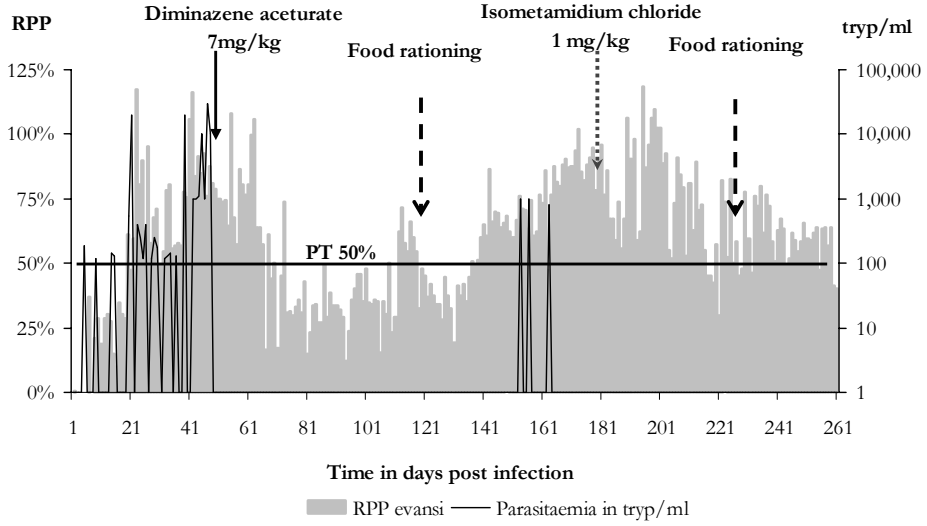


Figure 38 a – Sheep No. 14

After **isometamidium chloride** treatments, 60 days of food rationing did not induce parasitæmia but the fact that antibodies persist indicates that the strain is resistant to the treatment. Longer term monitoring would be required to confirm this.

The other parasite detection techniques (mouse inoculation, PCR, etc.) can be used in combination with the parasitological examinations [414], but they too are sometimes incapable of demonstrating the persistence of parasites, e.g. if the parasitemic level is below their detection limit or even inexistent, such as when trypanosomes are harboured in the body's extravascular compartments.

For any *in vivo* protocol, it is therefore essential to make use of antibody detection techniques – it is not until the specific antibodies disappear that the parasite can be considered to have been eliminated (two to four months after treatment).

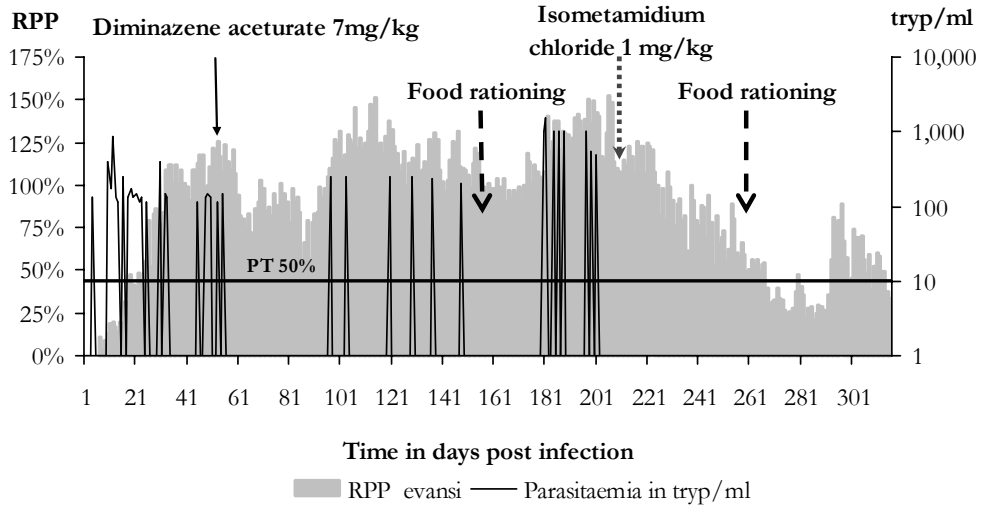


Figure 38 b – Sheep No. 15

Key: the response by indirect-ELISA *T. evansi* is expressed as a relative percentage of positivity (RPP); the positivity threshold (PT) of 50% is depicted by a horizontal line.

Remarks: Following **diminazene aceturate** treatments, the parasites disappear and the RPP drops, in one case below the PT (sheep No. 14), which could lead to drawing the conclusion that treatment is effective. In actual fact, after a period of food rationing lasting some 60 days, the RPP rises and parasites are perceptible once again.

Figure 38 – Parasitæmias, treatments and responses by indirect-ELISA *Trypanosoma evansi* in two sheep infected with *Trypanosoma evansi* from Venezuela (TEVA1)

1.2.3. Application

In Africa, in the huge hyper-enzootic areas, this degree of precision in assessing the trypanocidal effects is not often required because the main goal of curative treatments is clinical improvement. However, an estimation of the length of time a prophylactic is effective is useful. The 'block treatment' system developed by EISLER *et al.* [493, 498] can be used to assess how long isometamidium chloride affords protection in the field. This method, applied to detect resistance to isometamidium chloride cannot be applied to evaluate resistance to diminazene aceturate since it has a very short term effect, and, consequently, any infection detected even as early as one week after a treatment can be due to a new infection.

In Latin America, where livestock trypanosomes are transmitted by strictly mechanical means, a precise assessment of the situation is required because it is possible to maintain a 'trypanosome-free' status in isolated farms that carefully manage introduction of new animals (sterilising trypanocidal treatment). These farms need to choose a truly sterilising treatment whereas in an open farm or one that is largely infected, the use of non-sterilising substances is enough to maintain the enzootic status and hence the carrier immunity of the animals.

In the case of the *T. vivax* strains from Guyana, Venezuela and Colombia, diminazene aceturate has been shown to bring about clinical improvement but it does not eradicate the infection and in some cases encourages carrier immunity ([43, 47] ESPINOZA, unpublished data). Isometamidium chloride is sterilising and prophylactic for several months against the strains from Venezuela and French Guiana. Diminazene aceturate should therefore be recommended in largely infected herds (maintaining the enzootic situation through the asymptomatic carrier state) whereas isometamidium chloride should be recommended for isolated farms that wish to remain trypanosomosis-free.

1.3. Discussion on the strategy for using trypanocides

Use of trypanocides must be based on economic grounds and an epidemiological strategy. In addition, species tolerance influences the use of trypanocides; isometamidium chloride is more suitable to controlling *T. vivax* in cattle while suramin is more appropriate against *T. evansi* in horses.

1.3.1. *Trypanosoma vivax* control

Enzoo-epizootic area: in Africa, the parasitic strains vectored by *Glossina* are genetically highly polymorphic as a result of the cycles and genetic hybridisation that occur within the vector [251], their natural diversity – the result of a highly varied wild and domestic reservoir – and the co-existence of several pathogenic species (*T. vivax*, *T. congolense* and *T. brucei*). In enzootic areas, cattle's immune system is often overwhelmed by these multiple infections involving several species at once. Repeated chemotherapy or chemoprophylaxis is required on an almost continual basis.

The situation in Latin America is different. Only *T. vivax* is notably pathogenic for cattle. Clinical signs are marked in new outbreaks and then recede as the disease shifts from the chronic to the enzootic state through carrier immunity [159]. Carrier immunity control of infections is probably related to the moderate variability of mechanically transmitted strains. In enzootic areas, animals are therefore exposed to multiple infections that are generally monospecific. Because isometamidium chloride treatment is normally sterilising, it does not foster immunisation of the livestock [514] and is therefore not indicated. In contrast, diminazene aceturate treatment – particularly where there is high resistance to this drug (Guyana, Venezuela, Colombia etc.), or when it is used at low doses (3.5 mg/kg) – does foster the switch to carrier immunity by only partially destroying the parasites. It is therefore generally preferable in enzootic areas on this continent to use occasional chemotherapy, namely diminazene aceturate.

Sporadic area: in Africa, when trypanosomosis is sporadic (on the edges of the areas infested by *Glossina*), chemotherapy is generally recommended using, for instance, diminazene aceturate to control passing or highly seasonal infections (most often the rainy season). It is assumed and expected that the treatment will be sterilising.

In Latin America, because there is no wild reservoir for *T. vivax* and because the range of mechanical vectors is narrow, parasite eradication in isolated farms can be contemplated. In the geographic areas where most herds are disease-free, and as long as the outbreak of trypanosomosis is identified at an early stage, the only way to suppress the infections and prevent them from spreading is to systematically treat the exposed animals with a sterilising substance. The South American *T. vivax* strains are often resistant to diminazene aceturate and, since no other short-acting drug is available, and simultaneous treatment of the entire exposed population is unfeasible, the appropriate treatment is a time-release sterilising drug. In practice, these drugs are the same as those used in Africa and referred to as 'chemo-prophylactics', namely, isometamidium chloride.

In some South American countries where livestock is raised in extensive conditions, wild ungulates and deer in particular, may act as a reservoir as suggested by SILVA (paper posted on the Internet, 'Tryplink'). Similarly, further evidence that horses do not harbour *T. vivax* should be sought – if that were not the case, treating ruminants would not enable *T. vivax* to be eradicated.

In unstable areas, where there are repeat epizootic outbreaks, or where farms are not isolated, or else if eradication is not possible for technical reasons, these measures could be pointless and on the contrary might foster parasite resistance to isometamidium chloride. In this case, it would be advisable to use diminazene aceturate and allow the disease to become established as enzootic. In these circumstances, only animals that are clinically affected by the disease should be treated; the other animals will switch from the infected state to the chronic, most often, asymptomatic state naturally.

1.3.2. *Trypanosoma evansi* control

Trypanosoma evansi has a huge wild reservoir in the New World making any attempt to eradicate the parasite futile.

Isometamidium chloride and diminazene aceturate are not effective against *T. evansi* in horses. The effectiveness of melarsomine, suramin and quinapyramine needs to be evaluated for South

American strains. The first results obtained for Brazilian [218] and Venezuelan (MARTINEZ, 1971 quoted by FINELLE [216]) strains have not detected any resistance.

Equine trypanosomosis due to *T. evansi* is found in Latin America in the epizootic (outbreaks in the Pantanal) or enzootic (plains of Venezuela) forms, depending on the area.

When an epizootic outbreak of trypanosomosis occurs, considering the high pathogenicity of the strains, susceptible animals should be protected using chemoprophylactic drugs, e.g. quinapyramine.

In enzootic areas, the trypanocides used should have the same properties as diminazene aceturate for *T. vivax* in ruminants. Suramin and melarsomine may be proposed – however, their effects should be evaluated under experimental conditions.

Host/parasite combinations that are not highly pathogenic for the host such as *T. evansi* in cattle are not, as a general rule, considered under antitrypanosomal control programmes. While their direct economic and medical impact is low, their epidemiological consequences can be high in as much as this is one of the mechanisms whereby parasites can become established in new domestic reservoirs. No treatment against *T. evansi* is reported or recommended for cattle. As most *T. evansi* strains are resistant to isometamidium chloride [279] and not very susceptible to diminazene aceturate, cattle that do receive treatment (against *T. vivax* infection) may, if they are also infected by *T. evansi*, become reservoirs for this parasite and therefore a permanent threat for the equine population. However, although cattle can harbour *T. evansi*, they are not a good reservoir because their moderate susceptibility causes low-level parasitemias that is not conducive to mechanical transmission. In the final analysis, they are more an epidemiological dead end than a reservoir. One must remain cautious however and remember that this is the situation as observed today in Latin America in contrast with the state of affairs in Asia where *T. evansi* strains are highly pathogenic for cattle. It could be that one day strains of this type will also appear in America.

Quinapyramine treatment which could be used in ruminants against both *T. vivax* and *T. evansi* is definitely to be advised against in these species because it generates cross-resistances with other trypanocides commonly used to control *T. vivax*; furthermore, diminazene aceturate-resistant strains are often resistant to quinapyramine too [476].

The only way to simultaneously treat cattle against the *T. vivax* and *T. evansi* would be to combine two trypanocides such as isometamidium chloride and melarsomine. In addition to possible problems of toxicity, incompatibility and multiple resistances that might derive therefrom, the cost of this association would be prohibitive. Consequently, one can assume that ruminants will remain a reservoir for *T. evansi* on a lasting basis. Because horses and cattle are often raised together (working horses for herding purposes or ranches that breed both), the enzootic establishment of *T. evansi* will probably continue its progression through Latin America with the disastrous economic consequences that have been observed in newly infected areas.

Remark: dogs can also be reservoirs and because they cohabit with stock animals and are not frequently treated, they contribute significantly to maintaining and spreading *T. evansi*.

2. PROSPECTS FOR IMMUNOLOGIC CONTROL OF TRYPANOSOMES

Immunising hosts against parasites is one of the most elegant methods of controlling parasitoses. In the case of trypanosomoses, these principles come up against the difficulties that are inherent to their constitution and biology. Several areas of research are explored.

2.1. Immunisation against parasites

Immunisation of mammals against pathogenic trypanosomes has been obtained by infection followed by curative treatment for *T. congolense* [515] and *T. evansi* [516], but protection is generally effective only against homologous challenges with the same variant and when the quantity of parasites inoculated is small. It is low or inadequate in the case of heterologous re-inoculations [517]. Some degree of protection is however conferred by one or several infections/treatments even against a heterologous challenge (DESQUESNES and THÉVENON, unpublished). This area of research on trypanocide-controlled premunition deserves further investigation. In the field, animals probably gradually acquire partial immunity subsequent to several natural infections, possibly followed by treatment. Although the level of protection achieved in this way is not as high as for anaplasmosis or babesioses, this protective mechanism should not be disregarded.

Indeed, spontaneous immunisation of mammals against trypanosomes does occur during the course of infections. This is reflected in particular by the synthesis of immunoglobulins that bring about the destruction of parasites. Trypanosomes however use cyclical variation of surface antigens to escape these immune responses.

Under experimental conditions, gradual immunisation that leads to the aparasitæmic carrier state was observed with *T. vivax* strains from French Guiana and Venezuela; the immunity however is only maintained if the animals are properly fed. Parasitæmic resurgence and clinical signs can be induced by food rationing (see Chapter 2). Carrier immunity against a strain is therefore possible but vulnerable. Furthermore it only affords partial protection against other strains.

In Africa, for a long time the possibility of vaccination against trypanosomes was set aside in view of the considerable variability of surface antigens, the multiplicity of strains and their speed of circulation, the range of species present (*T. congolense*, *T. vivax*, *T. brucei*), and the recombination of genes in single species. Vaccines that are to this extent multivalent cannot conceivably be developed unless a sufficiently immunogenic and protective antigen is found.

In Latin America, on the other hand, *T. vivax* strains appear to be fairly homogeneous ([172] and Chapter 2, §2.2.1.). Weakly pathogenic but immunogenic strains might be discovered or selected, opening up the possibility of developing live vaccines that would at least reduce symptoms were the parasite to become established in new areas.

Another vaccinal approach has been explored by researchers (N. MURPHY, unpublished data) based on antigens in the flagellar pocket, which serves as the internalisation site for external elements. These investigations deserve to be carried further.

2.2. Immunisation against the disease

If host immunisation against variable antigens to control the parasites is unfeasible, immunising them against invariant antigens might be explored. A comparative assessment of immune responses to infection by *T. congolense* in trypanotolerant cattle (*Bos taurus*, N'Dama) made by AUTHIE *et al.* [518] prompted the authors to analyse the properties of the two proteins of major interest, namely HSP 70 (heat shock protein, molecular weight 69 KDa-70 KDa) [518, 590] and CP 33 (cysteine protease, molecular weight 33 kDa) [518, 519, 520]. HSP 70 induces the synthesis of IgM and IgG in resistant animals whereas only IgM are synthesised in susceptible animals. In the case of *T. congolense* primary infections, CP 33 (or congopain) induces higher immunoglobulin (IgG1) levels in resistant animals than in susceptible animals.

These two proteins are thought to be behind some of the pathogenic effects of trypanosomes that only trypanotolerant animals are able to control. The principle of the research undertaken by AUTHIE *et al.* is to immunise the animals against the 'disease' rather than the 'infection' by inducing specific immunity using recombinant congopain. An evaluation of *T. evansi* recombinant

cysteine protease produced on *Pichia pastoris* has been completed under the First Joint INCO-Trypadvac Research project, which came to an end in March 2004. However, vaccine immunisation in the field is as yet unfeasible.

This project is based on the assumption that the pathogenic effects of trypanosomes rely mainly on cysteine protease. The protection that such a system can afford is limited because it proceeds solely from the inhibition of a single parasitic protein whereas other molecules that are either constituted or generated by the trypanosomes cause their pathogenic effects. One can therefore surmise that immunisation of this type would provide only partial protection. Indeed, initial trials showed no protection at all in the acute phase but better recovery in the chronic phase [521]. Other pathogens generated by the trypanosomes should be targeted, identified, produced and associated to cysteine protease to enhance this mode of protection. The outlook for immunological control will therefore need to be reassessed upon completion of INCO-Trypadvac so as to define future areas of research.

2.3. Considerations relating to research strategy

Three strategies have been adopted in respect of immunological control:

– immunisation against surface antigens does not appear to be useful in contexts where challenges are relatively heterologous. To some extent, premunition by infection and treatment can be developed in Latin America where the strains are highly homologous. This strategy could also include attempts to attenuate the pathogenicity of local strains with a view to selecting a potential vaccine.

– immunisation against the antigens of the flagellar pocket is fairly promising; this research should be continued.

– immunisation against cysteine protease which is currently being evaluated, will certainly need to be combined with other proteins that are pathogenic for trypanosomes, namely those that are active during the invasive phase of the disease and cause destruction of red blood cells. Molecules of this type have yet to be identified.

Finally, once the work on cysteine protease has been completed, further investigation into antigens of the flagellar pocket – an organelle that can be used in an immune mechanism but also in a toxic mechanism for internalising molecules with trypanocidal activity – will be necessary.

3. PROSPECTS FOR IMMUNOLOGIC CONTROL OF TABANIDS

3.1. Background

The initial observations concerning immunisation of mammals against internal antigens of hæmatophagous arthropods were conducted by TRAGER on ticks in 1939 [522, 523], but little else was done in this area until the 1970s. Immunisation of mammalian hosts against their hæmatophagous parasites based on extracts of the parasite's internal antigens, particularly those in the gut, is a recently developed concept. It was applied to mosquitoes [524, 525], *Stomoxys* [526], ticks [527, 528, 529, 530], *Glossina* and horseflies [43, 531] with variable success.

Attempts of this type have been made on many occasions since the 1990s although none have yielded a vaccine with the exception of the '*Boophilus microplus*/bovine' developed by Australian [528, 532, 533, 534] and by Cuban [530, 535] researchers using recombinant antigens. Recent observations indicate that cross-protection between various ticks might be achieved, which may lead to the possibility of developing an anti-tick vaccine with a broader spectrum [536].

Further steps have been achieved with other ticks and with *Lucilla cuprina* [530, 537, 539] but have not reached the application phase. The mechanisms involved with *Lucilla cuprina* are different since the insect is in close contact with the host's immune system.

3.2. Considerations on research strategy

Most of the work done on insects has been disappointing; in particular immunisation has not been followed by any visible effect or increase in mortality. Indeed, some unexpected effects such as an extended lifespan in horseflies [43] or an increased nymphosis rate in mosquitoes were observed [525]. In any case, the experimental protocols are complex and the biological material not amenable to experimentation particularly the insects that cannot be raised in laboratories (horseflies).

The peritrophic membrane of insects is one of the causes most commonly mentioned to explain the difference in immunisation between mites and insects.

Because the contact time between hæmatophagous insects and their hosts is often short, control methods targeted on interrupting the vector while it is feeding or altering its bolus cannot be reasonably considered. At best, one might be able to use immune stimulation to obtain levels of resistance equivalent to those naturally acquired by the host as a result of natural exposure to bites.

Strategies that rely on the host's blood compartment could be considered but they too come up against a number of obstacles:

– systemic diffusion of hormones or insecticides presents the problem of residues in meat and milk;

– immunisation against commensal organisms in the insect gut presents the problem of common antigens shared by these organisms and mammals;

– the inhibition of digestive enzymes requires excessively large quantities of inhibiting agents.

During trials of bovine immunisation against horseflies, apparent coagulations of blood were observed in the gut of horseflies that had fed on a naturally 'resistant' bovine ('positive control effect'). The large proportion of insects (up to 30%) that died within a few hours of the bloodmeal was such that an animal that was initially intended to act as the control had to be withdrawn from the experiment. The case history showed that this animal had not received any treatment for more than three months. These observations perhaps pave the way for a new area of research – genetic selection of naturally resistant animals or genetic engineering of identical traits in animals that do not express them naturally. A pre-requisite to continuing this type of research on Tabanids is the development of a standardised means for raising horseflies in laboratory conditions.

4. CONTROL OF TRYPANOSOME TRANSMISSION

This paragraph reviews the various methods directed at controlling trypanosome transmission on livestock in Latin America as well as methods directed against the vectors.

Iatrogenic transmission of trypanosomes can be avoided by taking simple precautions when conducting collective prophylaxis (single-use needles).

Animals that are brought into a farm or country should be treated using a sterilising trypanocide even if the herd is infected to avoid introducing new strains whose genetic material and phenotype are different from those already present on the farm or in the country in question. Transmission of *T. vivax*, and perhaps *T. evansi*, from mother to offspring can be controlled by means of sterilising sanative treatment of mothers during pregnancy or of offspring at birth.

Although in some countries trypanocides are still used, the slaughter of animals infected by *T. equiperdum* is compulsory; naturally, reproduction should be prohibited.

To avoid peroral transmission of *T. evansi* and *T. cruzi* to cats and dogs, meat and offal from potentially infected animals (game, horses, etc.) should not be fed raw.

Shelters for livestock made of straw or haulm should be avoided so as to discourage contiguity between hosts/vectors of *T. cruzi* (Triatominae). When an outbreak of trypanosomosis due to *T. evansi* originates from the vampire bats, the animals can be taken into shelter at night to avoid being attacked by bats.

5. CONTROL OF TRYPANOSOME VECTORS

In the section relating to the transmitting agents for livestock trypanosomes in Latin America, many species of hæmatophagous insects that play or are suspected of playing a local and/or temporary role were mentioned. Some of these vectors are only occasional or else their role is just speculated – the scope of this study is confined to the transmitting agents whose role and importance have been demonstrated: vampires, *Stomoxys*, *Hæmatobia* spp. and Tabanids.

5.1. Vampire bat control

Not much has been done against vampire bats to control trypanosomoses; however, in the case of epizootic outbreaks due to *T. evansi* in Latin America, this would be justified because it reduces movement of the parasite between the host and the reservoir-vector. This can be achieved by capturing the bats at night using a 'Japanese net' according to the technique described by LIHNART *et al.* [540]. The animals so captured are coated with an anti-coagulant such as chlorophacinone with an excipient of the lanolin type and then released. The product is passed on to the whole colony by licking and contact. Treatment of one animal destroys some 15 congeners. When this process was used, an average reduction in the number of bites on the livestock of up to 91% was recorded using 'diphenylacetyl-2 indanedione-1,3' [541]. An alternative is to directly treat the cattle with an anti-coagulant that is harmless to cattle (e.g. diphenadione) but to which vampire bats are highly susceptible. In Mexico, THOMPSON *et al.* [542] observed a 93% drop in bites by *Desmodus rotondus* after administration of a single intra-ruminal dose on cattle that left no trace of the anti-coagulant in either the milk or meat.

5.2. Control of stables flies and hornflies

Eliminating the preferred oviposition sites of stables flies (organic debris, litter, etc.) is difficult to apply on farms.

Various methods for capturing adults have been tried with different types of traps (visual and olfactory baits); in Africa, the 'Vavoua' trap (**Fig. 39**) seems to give the best results [105], particularly if carbon-dioxide and octenol baits are used. Large numbers of insects are captured in the evening reaching scores of 1,500 *Stomoxys* per trap in 1 h [543]. In Australia, trapping efficiency has been evaluated on a new trap ('Australian trap)' which is stated to be as effective as insecticides [544]. Further field investigations must be conducted in America.

Insecticide spreading causes pollution and as such is inadvisable. Insecticides should therefore be used essentially by spraying the livestock as described below for horseflies. Chemical control of *Hæmatobia irritans* by self-treating dust bags of Coumaphos on livestock have generated significant weight gains [545], but many instances of insecticidal resistances have already been recorded for organophosphorous compounds [546] and pyrethroids particularly in Mexico and Argentina [547, 548]. Fipronil (Topline®) a new insecticidal/acaricidal molecule that was recently put on the market appears to be very promising; in Brazil, protection rates of more than 60% have been recorded up to five weeks after pour-on treatment applied to cattle [549]. However, its persistence makes for considerable withholding periods.

The use of parasitoids (*Spalangia* sp., *Tachninaephagus Trichopria* sp.) to control *Stomoxys* is being tested in Reunion Island under the POSEIDOM Project (T. HUE, unpublished data).

Cost-effectiveness of control methods can be assessed [302]. This is a necessary step because in some cases treatment does not generate any significant weight gain. Furthermore, it has never been demonstrated that the *Stomoxys* and *Hæmatobia* control is in any way cost-effective in terms of the control of trypanosomosis.

5.3. Tabanid control

In most cases, Tabanids have a marked seasonal activity – as such, temporary control methods against these insects when they are most abundant can be contemplated. In French Guiana, the direct harm caused by Tabanids alone is sufficient justification for establishing annual, short term, control schemes (seasonal chemical control) [43]. Furthermore, in the event of outbreaks of bovine or equine trypanosomosis, although horsefly control cannot prevent parasite transmission (there is evidence that transmission occurs even when vector density is low), it is beneficial because it alleviates the direct harmful effects of horseflies on livestock and hence enables the animals to better control infections caused by the trypanosomes. In any case, they can be used in combination with non-chemical methods to enhance efficiency.

5.3.1. Non-chemical methods

Special on-site arrangements: heaps of sand can be placed on pastures that are not naturally sandy to encourage sand wasps, which prey on horseflies, to settle. Water must always be in adequate supply not only for the well-being of the livestock but also because it encourages sedentarisation of horsefly eating birds (egrets). Putting domestic fowl (chickens, guinea fowl) in the same enclosures as a livestock is also helpful in diminishing the horsefly load.

The effects of clearing scrub, or on the contrary setting up hedges, are unclear. These measures can be recommended only in the light of a detailed study of the ecology of the species present. When the control technique is aimed at a particular species, special ecological measures, drainage or on the contrary temporary flooding, can give satisfactory results [258]. However, although such measures are able to control a target species they may encourage population explosions in another [259].

Setting up 'Boucans': *boucans* are the technique that is traditionally used in French Guiana that consists in forming a smoke screen where the livestock take shelter by means of very low burning fires made from leaves and branches piled into a container that is about one metre high (used oil tank with lid removed). The smoke repels horseflies. The livestock soon realise the benefit of the *boucan* and take shelter there. The drawback is that once the grass in that area has been consumed, the animals stop eating because they do not want to face the horseflies. *Boucans* therefore have to be moved on a regular basis, which requires considerable additional manpower. One recommended method is to feed fodder to the livestock at a spot that is under the permanent protection of smoke. This implies producing the fodder, an unusual practice in French Guiana and more generally in tropical regions. Furthermore, the method is not universal since a number of species such as those of the genus *Chrysops* are attracted by smoke [258].

Control by trapping: lures for trapping insects have been developed and are used in Africa to reduce the abundance of tsetse flies, particularly the riverine species as traps can be set up in strategic places due to their localisation [550]. *Glossina* in the palpalis group are particularly attracted to the biconical, two-coloured (black and blue) trap developed by CHALLIER and LAVEISSIERE [551]. Many other black and blue traps and screens have been derived from this model [552]. Some authors have demonstrated the cost-effectiveness of controlling *Glossina* by traps in the Central African Republic [553]. Visual and olfactory baits can also be coated with insecticides [554] or sterilising drugs [552] and are grouped under the term 'toxic attractant systems'. So far, these types of techniques have been applied only against *Glossina*.

For horseflies, some similar traps have been experimented: Manitoba [555], Vavoua (**Fig. 39**), Malaise (**Fig. 40**) or Nzi traps [556] (**Fig. 41**). They are effective and can be used to capture insects for the purpose of experimentation.

The best results for capturing horseflies in French Guiana were obtained using the Malaise trap that was carefully engineered by FRENAY [273]. Prior studies on the attraction of wood and panels showed that the best characteristics are obtained using two black panels that intersect at a 90° angle, with a 1 metre long edge. RAYMOND *et al.* [557] incorporated these features into the Malaise traps they used in French Guiana.

Figure 39 – Vavoua trap

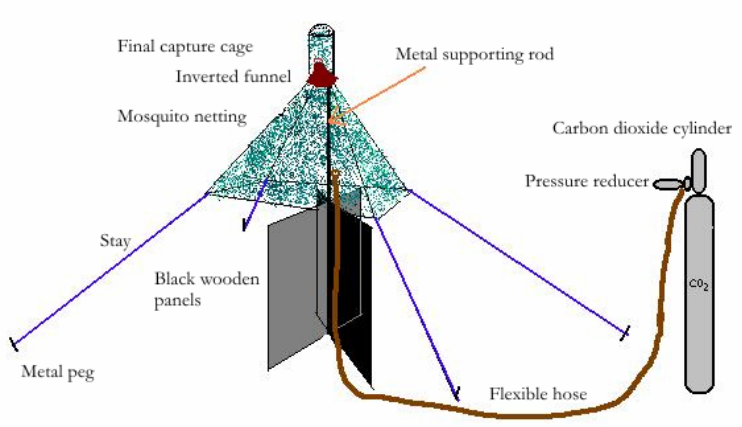


Figure 40 – Malaise trap

A mosquito net whose overall shape is conical (upward pointing) is placed over the panels about 80 centimetres above ground topped by an inverted funnel and a mosquito netting cage or plastic bag (**Fig. 40**). The efficiency of these traps can be further enhanced by releasing carbon dioxide (CO_2) or allowing dry ice to sublimate half way up the trap [558]. Indeed, CO_2 is naturally expired in large quantities by mammals and acts as a powerful attractant to Tabanids and other Diptera. When insects detect the gas in the air, they fly up the diffusion flame towards the source and end their approach upon sighting the black panels. Without CO_2, the trap can catch up to 200 horseflies in 1 h, and up to 350 specimens in one day. With CO_2, up to 450 insects can be captured in 1 h and up to 950 in one day [559].

In general, horseflies tend to be more attracted by cattle than by traps [558]. In French Guiana, Malaise traps attract very few *Chlorotabanus inanis*, *Ch. mexicanus* or *Cryptotylus unicolor* [264]. FOIL and HOGSETTE [259] report that more *T. fuscicostatus* are captured with this trap than with natural bait but that in most other species the opposite holds true – hence, relative percentages of *T. wilsoni* captures are 13% with the trap compared to 87% on cattle.

CO_2 is used for research but its application to control purposes would require less costly substances to be found. Studies have shown that octenol (± metacresol), ammonia, combined octenol/phenol and cattle urine can act as attractants [105, 560, 561]. HAYES *et al.* [562] state that they captured three times as many horseflies with an octenol/CO_2 – baited trap compared to a non-baited trap and nearly twice as many with octenol alone; they recommend that evaluation of octenol-baited trapping be conducted on a larger scale.

The Nzi trap developed by Steeve MIHOK (ICIPE) comprises an entrance with a blue visual attractant where the insects land and a black penetration cone that leads into a mosquito netting

cul-de-sac (secondary attraction by light), which is conically shaped and points upwards thereby naturally guiding the insects towards the funnel (cut plastic bottle) and then into a bottle and finally into a plastic bag.

The trap is quickly installed (5 mn), and requires three metal stakes and a flexible rod.

Nzi traps are nearly always more effective than conventional (pyramid-shared, NG2G, etc.) particularly in conjunction with olfactory bait (acetone, octenol, cow's urine, etc.).

A new device called 'Tetra' trap developed by DESQUESNES is currently being evaluated (DIA *et al.* [574]) in Africa and could also be evaluated in Latin America. The facade of the 'Tetra' trap is derived from the Nzi model but in addition to the lower entrance it also has an upper entrance, which is made by tilting the upper part of the front blue panel of the Nzi trap backwards. Furthermore, the entrance cone to the trap covers an angle of 90° and is repeated four times enabling the number of aspects to be multiplied by four (**Fig. 42**). The performance of the Tetra trap evaluated in Burkina Faso is considerably better for capturing Tabanids than the Nzi trap and comparable as regards *Stomoxys* (DIA *et al.* [574]). The most effective for *Stomoxys* is the Vavoua trap.

Figure 41 – Nzi trap

Figure 42 – Tetra trap

In Africa, by positioning the traps strategically close to water troughs, fewer traps are needed and their efficiency is enhanced [563]. This approach is applicable mainly to areas where the horsefly period of activity occurs during the dry season, e.g. the Guianas, but not in areas where their peak activity occurs in the wet season such as in the Pantanal, Brazil.

More evidence of the cost-effectiveness of trapping techniques is needed before their implementation by South American livestock raisers can be contemplated.

Genetic control: the release of sterile males for the purpose of insect control has been successful especially *Cochliomyia hominivorax*, and, to some degree in *Glossina* [564], but it is not feasible in the case of horseflies because insect density is too high and they cannot be raised under laboratory conditions. Furthermore, mating takes place before each reproductive cycle, which undermines the effectiveness of the technique.

5.3.2. Chemical methods

5.3.2.1. General considerations

Because horseflies are highly mobile insects and very stealthy temporary parasites that lay their eggs in a variety of sites that are widely dispersed, it is very difficult to control them. However, their usually marked seasonal activity makes it feasible to carry out cost-effective, short term, annual chemical control [43]. The benefits of this form of control are felt both in terms of direct effects – by reducing the damage caused by horseflies (skin wounds, blood depletion, loss of appetite and of MDWG) – as well as indirect effects – by reducing the overall vector density, the animals' overall condition is better-preserved and likewise their immunocompetence.

Insect repellents: substances that repel insects have been developed for human use, but no sprayable repellents for stock animals are available. A 10% diethyltoluamide emulsion has been tested on dairy cows; there was evidence that protection lasted only approximately 30 h but the quality and quantity of the milk produced was enhanced [565]. Persistence is too short-lived for field application to be considered.

Insecticides: insecticides can be spread in the environment, applied to special stands or directly on the animals.

For species of veterinary interest, larval biotopes are highly diverse and widely distributed. Chemical control of the larval phases is therefore unfeasible [280]. Insect destruction in farm buildings and on animal husbandry equipment with persistent preparations of the type used for domestic purposes is possible but has not been evaluated.

There are many delivery techniques and several insecticidal (and acaricidal) preparations they can be used directly on livestock: ear tags, dips, sprays and pour-ons. Only the two latter methods appear to be suitable for Tabanid control.

5.3.2.2. Pour-ons and sprays

Pour-on preparations have successfully been used against *Glossina* in several African countries [566, 567]. Against Tabanids, they are recommended in horses but are very costly for treating cattle (€1.08/animal) and spraying is generally preferred (€0.6/animal). Trials conducted in French Guiana by RAYMOND and FAVRE [568, 569, 570] using various pour-on or spray preparations of deltamethrine have given very encouraging results. Their insecticidal activity against *Tabanus importunus* lasted more than ten days during which the abundance of insects was significantly reduced; other trials conducted with pour-on deltamethrine have yielded a 68% reduction in the abundance of *Cryptotylus unicolor*. The authors recommend careful spraying in the most vulnerable areas to horsefly attacks – limbs, head and belly – using an association of deltamethrine and piperonyl-butoxide (BUTOX 50®, 50 ppm of active ingredient: 2 l/animal) and to avoid applying the treatment during peak sand wasp activity periods. In an isolated farm, after three years of spraying the livestock every ten days during the horsefly season, their abundance was significantly diminished; a positive indication that the treatment has long-term effects in closed environments [43].

5.3.2.3. Targeted chemical control

People that handle animals during the horsefly infestation period have often noticed that Tabanid loads vary enormously but persistently from one animal to another.

It has been established by counting the number of insects that land on the animals that the load on one cow may be twice as much as on another, and the same applies to horses. Furthermore, the load of a single horse placed in the midst of cattle is five to ten times larger [43]. Strong attraction is permanent and ostensible feature of some animals that farmers identify very easily. This

phenomenon is probably the outcome of a combination of several parameters: size, shape, colour of coat, metabolism (infection by trypanosomes increases the attraction of cattle for *Glossina* probably due to the fever caused by infection), and the host's particular smell. Analysing these parameters is no doubt a complex matter but might lead to identifying attractant chemical substances that could be used to trap insects.

In any case, an immediate practical application that can be inferred from these observations is to systematically and continuously treat hyper-attractive animals with insecticide, turning them, as it were, into 'biological traps for chemical treatment'. Pour-on preparations are persistent and easy to apply and, as such, are particularly well suited for this type of application.

HARVEY and BRETHOUR [545] had suggested that expenditure on chemical control against hæmatophagous insects could be reduced by treating only part of the livestock population – they recommended 5% to 20% of the animals in a herd. The assumption is that the insecticides are passed from one animal to another by contact. Treating hyper-attractive animals in precedence – 10% of the batch – would be equivalent to treating the whole herd and destroys the largest amount of insects. This method of targeted control can be recommended to farmers.

5.3.2.4. Cost-effectiveness of chemical control

In French Guiana, a study was conducted on fattening cattle whose average weight was 330 kg that estimated the annual loss due to the direct impact of horseflies: 5.3% of bodyweight, i.e. 17.5 kg or a €53 per animal at a price of €3 per kilo of body weight. This loss occurs during the six to eight weeks when horseflies proliferate at the end of the dry season.

The cost of Tabanid control, as described by RAYMOND and FAVRE [568, 569, 570] by spraying the cattle every ten days during the Tabanid season, including manpower, is approximately €732 (€122 × 6 treatments) for a batch comprising 100 head.

Assuming that this control method decreases the harmful effects of horseflies by 50% (up to 68% in the case of *Cryptotylus unicolor*, according to RAYMOND and FAVRE [569]), the return for a herd comprising 100 productive animals would be approximately 100 × 9 kg × €3, i.e. €2,744/year for the entire herd. According to these estimates, on a stock farm of this type, chemical Tabanid control would generate a profit of about €2,000. Not only would chemical control of horseflies be profitable in this case, it would have the additional benefit of controlling *Boophilus microplus* ticks which are very common in the area investigated.

Furthermore by lowering the intensity of the horsefly load, the overall condition of the animals is improved and hence their immunocompetence, which in turn contributes to immune control of infections due to *Anaplasma marginale* and trypanosomes. This additional benefit is difficult to assess but certainly not insignificant.

5.4. Conclusions

So far, little research work has been done on Tabanids and *Stomoxys* and the available means of control are highly inadequate. In Africa, research dealing with trypanosomosis control in camels is beginning to focus on trapping methods for insects that make use of attractants ('Development of Sustainable Technologies for the Control of Camel trypanosomoses and Its Vectors', ICIPE, draft proposal, 1996).

The work done by RAYMOND and FAVRE [568, 569, 570] and findings in the field after several years of chemical control using sprays have shown that Tabanid populations can be reduced by seasonal chemical control. However, in the areas where Tabanid activity is permanent, trapping techniques are needed because of the prohibitive cost of ongoing chemical treatment; **targeted chemical control** could usefully supplement **baited trapping** techniques.

Spraying livestock with insecticides reduces the populations of *Stomoxys* and horseflies but is does not eradicate them. It has been established that to maintain a Tabanid population only 2% of females in that population need to fulfil the normal life cycle [259]; such a small fraction of the population can easily be maintained by feeding on the wild fauna and do necessarily need to feed on the stock animals they infest.

Furthermore, it has been observed that *T. vivax* can be transmitted in spite of a very low vectoral load; as Tabanid control only reduces insect populations rather than eliminating them, it probably only impedes transmission of trypanosomes rather than preventing it. On the other hand, it does alleviate the direct harmful effects of these insects in the livestock sufficiently to prevent livestock receptiveness to trypanosomoses from increasing. In this respect, Tabanid control schemes aimed at **reducing the direct harmful effects** of the insects amount to **controlling the main contributing factor** to the clinical expression of trypanosomoses rather than actually stemming transmission of the parasites. It does incidentally hinder transmission and reduce the clinical and economic impact of trypanosomosis.

6. OVERALL CONCLUSIONS ON CONTROL

Depending on the epidemiological context, a variety control strategies can be recommended on the basis of the various elements described above.

In the enzoo-epizootic areas, both against *T. evansi* in horses and *T. vivax* in cattle, systematic trypanocidal treatment should be applied during the peak trypanosomosis season, in particular when the latter is concomitant with food scarcity and abundance of mechanical vectors as has been observed in French Guiana. Non-sterilising trypanocides are beneficial (diminazene aceturate). Vector control may be contemplated in the very particular cases described above.

In sporadic areas, to maintain the disease-free status of herds, the entire population should be treated using isometamidium chloride as soon as an infection is discovered.

In any case, any animal that is introduced into a herd should be treated with a trypanocide that is believed to have a sterilising effect (10.5 mg/kg diminazene aceturate or 1 mg/kg isometamidium chloride).

SUMMARIES AND CONCLUSIONS

1. CHOOSING THE APPROPRIATE TECHNIQUES FOR DIAGNOSING LIVESTOCK TRYPANOSOMOSES IN LATIN AMERICA

The poor sensitivity of parasitological techniques (HCT, BCM, smear tests) confines their application to epizootic outbreaks and to individual diagnosis of the disease. In these cases, clinical suspicions may have to be confirmed by several successive samples. These examinations, which also provide the haematocrit value, are easy to perform, quick and economical – they should be used as the primary diagnostic means.

Although the various livestock hæmoparasitoses may be clinically mistaken for one another, antibody detection tests have been shown to be a reliable means for **differentiating between genera** as well as the exclusive detection of **pathogenic subgenera of trypanosomes**. Indirect-ELISA ***Trypanosoma*** spp. tests are the best available diagnostic tools for conducting serological and epidemiological surveys on trypanosomoses. Their sensitivity is good and they provide a reliable estimate of the prevalence of infections. However, they are not very species-specific and cannot be used to determine whether the infection is active. There are some very marked **serological cross-reactions** between *T. vivax* and *T. evansi* (and potentially *T. cruzi*) in domestic ruminants, and also between *T. evansi*, *T. equiperdum* and *T. cruzi* in horses. When conducting epidemiological surveys based on serology, these interferences must be taken into consideration. Interpreting tests by means of the maximum positivity score is nonetheless a means for determining the relative importance of *T. vivax* and *T. evansi* in sectors where mixed infections exist. Experimental work conducted on sheep in Burkina Faso has shown that in the case of monospecific infections, a species-specific diagnosis can be achieved by interpreting the maximum positivity scores. It is therefore recommended that indirect-ELISA *T. vivax* and *T. evansi* be conducted simultaneously. Reference strains of *T. vivax* and *T. evansi* for South America adapted to rodents should be identified so that a standardised test can be developed and made available to all the laboratories on the sub-continent.

Whereas in a familiar epidemiological context (date of treatment, trends in antibody rates between two samples are known), an animal's carrier state in respect of the parasite can be serologically inferred with a relatively high degree of accuracy, antigen or DNA detection tests are more suitable for sensitively diagnosing **active infections**. It has been demonstrated that the Ag-ELISAs developed in Africa for the purpose of detecting *T. vivax*, *T. brucei* and *T. congolense* are poorly specific and poorly sensitive. They are no longer used. It would be wise to develop new, more sensitive and more specific monoclonal antibodies.

PCR considerably improves the species-specificity of diagnostic tools with a detection limit of between one and ten parasites/ml or of blood depending on the technique. Its sensitivity is comparatively better than parasitological methods but nonetheless insufficient to detect infections in aparasitæmic animals. Furthermore, it is costly. Considering that animals often carry trypanosomes but are aparasitæmic, PCR is not an appropriate diagnostic tool for trypanosomoses in an enzootic environment – it does however provide improved sensitivity and is the most species-specific of the methods available. Ideally, tools for establishing medical diagnoses or conducting epidemiological studies must provide sensitive antibody and antigen detection that establishes whether infections are due to *T. vivax*, *T. evansi*, *T. equiperdum* or *T. cruzi*. To develop such powerful tools, cooperation between several research bodies in Latin America and possibly even Africa and Asia would be advisable. In the meantime, parasitological means and indirect-ELISA *Trypanosoma* spp. can be usefully supplemented by PCR detection of species-specific DNA.

The analysis of the ratios of parasitic to serological prevalence plays a crucial role in assessing epidemiological situations.

2. BOVINE TRYPANOSOMOSIS IN FRENCH GUIANA, AN EPIDEMIOLOGICAL MODEL – THE CONTROL OF PARASITES AND VECTORS

2.1. Epidemiological model

Only *T. vivax* has been identified in livestock in French Guiana and none of the studies conducted have ever confirmed the existence of *T. evansi* except one case of a hunting dog.

Bovine trypanosomosis due to *T. vivax* operates in the enzoo-epizootic mode, most often as clinical epizootics against an unnoticeable enzootic background. The known reservoirs for the parasite are cattle and sheep and its vectors are horseflies and *Stomoxys*. Outbreaks of clinical trypanosomosis usually occur in the second part of the dry season (October-November); the contributing factors that occur concomitantly at this time of year are inadequate food and water, reemergence of intercurrent diseases such as anaplasmosis and the direct harmful effects of Tabanids. Under the combined effect of these factors, resurgence stemming from imperceptible carriers, or from their introduction into uninfected herds, causes infectious outbreaks that are spread very rapidly by hæmatophagous insects – in a matter of just a few days when animals are highly receptive. Morbidity is close to 100% in most of the outbreaks observed.

In this instance, the trypanosomosis is clinical and leads to significant decline in weight gain and sometimes even weight losses. Some cases of abortion and mortality have been related to trypanosomosis but, generally speaking, the most severe manifestation is prolonged loss of weight that is recovered very slowly. If animals are not treated, after two or four months of clinical progression, the symptoms spontaneously recede as the animals become immunised and the animal husbandry parameters improve at the beginning of the rainy season. Subsequently, some of the animals spontaneously eliminate the parasite (annual self cure: 15%-20%) while others go into the permanent carrier state which is generally asymptomatic until the next time an infectious episode occurs.

Trypanosomosis in ruminants is therefore a permanent threat for livestock in French Guiana. Depending on the stock farming technique, it can take on the following forms:

- **seasonal epizootic outbreak** (dry season) – in local, largely uninfected herds, the clinical signs are marked and the parasite circulates rapidly;
- **sporadic epizootic outbreak** – in imported herds with highly pronounced clinical signs and rapid circulation of the parasite whatever the season;
- **asymptomatic enzootic form**: on largely infected farms with good animal husbandry practices; the trypanosomosis is subclinical and the parasite circulates slowly and continuously;
- **clinical enzootic form**, on largely infected farms with inadequate animal husbandry practices; part of the population is naturally resistant and has imperceptible carrier status while other animals are chronically and adversely affected with the occasional acute clinical case particularly when non-immune animals are introduced into the herd.

Because parasitological methods are insufficiently sensitive and serological methods insufficiently specific, it is difficult to establish whether the wild fauna that lives in the vicinity of stock farms works as a reservoir for *T. vivax*. In contrast, evidence has been provided that sheep are a reservoir for bovine trypanosomosis, both on the basis of laboratory experiments and in the field. This species should therefore be taken into consideration when planning control programmes (as well as goats no doubt).

2.2. Control of trypanosomoses and their vectors

The two *T. vivax* strains isolated in 1988 and 1994 are resistant to diminazene aceturate but sensitive to isometamidium chloride which provides protection for more than 4.5 months at a dose of 0.5 mg/kg. Furthermore, there is evidence that although diminazene aceturate does not eliminate the trypanosomes, it does encourage carrier immunity. As such, diminazene aceturate is most suitable to enzootic situations while isometamidium chloride is more suitable to the non-enzootic environment.

In largely infected herds, if premunition is the chosen option, clinical signs need to be controlled by diminazene aceturate treatment (7 mg/kg IM). This therapy always leads to at least temporary regression of the symptoms and although it is non-sterilising it does appear to foster earlier control of the disease when applied repeatedly. Proper feeding and animal husbandry practices, in particular Tabanid control, are often enough to bring the trypanosomosis down to a manageable level without any noticeable medical or economic repercussions. If the entire herd appears to be affected by the disease but the symptoms are not alarming, systematic treatment at a dose of 3.5 mg/kg can be performed to foster carrier immunity. For outright clinical cases, a 7 mg/kg dose should be maintained.

In largely uninfected herds, bovine trypanosomosis can be controlled by treating the whole herd with isometamidium chloride at the beginning of the dry season (September), and any new animal that is brought in. Eliminating *T. vivax* from a farm cannot be contemplated unless there are no other potentially infected farms in the vicinity. Isometamidium chloride should be administered to all ruminants at a dose of 1 mg/kg (IM); the treatment should be performed twice at a three-month interval because in field conditions, injections are sometimes incomplete. Following treatment, the herds should be monitored by means of antibody detection diagnostic tools. Two samples collected at a three-month interval will establish whether the herd has been restored to disease-free status. Tabanid control should be maintained due to significant direct harmful effects of these insects and has been shown to be cost-effective in French Guiana.

Tabanid proliferation can be controlled by spraying animals with deltamethrine (1 ppm) every ten days during the season of peak insect activity (generally between October 15 to December 15), i.e. five to seven treatments a year. Outside the high season, only the hyper-attractive animals should be treated (with pour-on preparations). Conventional animal husbandry measures should not be neglected: *boucans*, sand heaps, shelters that livestock are encouraged to enter during the intense horsefly activity hours in the dry season (supplying food and water in shelters).

Since livestock is often free ranging, *T. vivax* eradication in French Guiana cannot be recommended. However, eradicating it from a few secluded herds would be a good step towards conducting a feasibility study on this option.

3. CHOOSING THE APPROPRIATE STRATEGIES FOR CONTROLLING TRYPANOSOMOSES IN LATIN AMERICA

Until such time as a species-specific (*T. equiperdum*) serological diagnosis becomes available, there can be no well-directed control of ***dourine***. Screening imported animals is arbitrary since it is based on the complement fixation reaction (CF), which is not species-specific. The presence of *T. cruzi* in Mexico may well be the reason for the positive *T. equiperdum* CF results that preclude any exports of horses to the USA. As matters stand today, recognisable clinical signs entail notification of the outbreak and the animals are generally slaughtered. Using a poorly specific diagnostic tool that is sufficiently sensitive (CF, indirect-ELISA or IFA) could be instrumental in limiting slaughter. It is difficult to detect healthy carriers because of interference arising from *T. evansi* and *T. cruzi* (perhaps *T. vivax*?) in horses. Consequently, dourine control continues to call on obsolete procedures that are unable to track the actual dissemination of the organism. In this particular case, due to the sanitary and economic implications that arise from notifying the disease, legislation would seem to deter

animal health authorities from volunteering information. Radical measures sometimes have unwarranted and no less radical effects.

Derrengadera (*T. evansi*) control is limited to direct intervention on sites where active outbreaks of equine trypanosomosis occur, considering the huge wild and domestic reservoir for the parasite and the fact that it is transmitted by both cosmopolitan hæmatophagous insects and a wild vector/reservoir (vampire bats) that are very difficult to control. In epizootic areas, systematic use of trypanocides is recommended when there is an active outbreak and appears to be cost-effective. In enzootic areas, serological (indirect-ELISA *T. evansi*) or parasitological (HCT and mouse inoculation) detection, control of horseflies and vampire bats as well as treatment of clinically affected animals are possible, but it is difficult to determine whether these measures are cost-effective. Finally, the economic impact of *T. evansi* infections should be investigated in ruminants.

Secadera (*T. vivax*) control in Latin America relies essentially on trypanocidal treatments. In epizootic areas – where there has been no evidence that an effective wild reservoir exists –, the eradication of *T. vivax* from a farm or a region can be attempted using isometamidium chloride on all ruminants provided that the strains are not resistant, as has been observed in Colombia. For epidemiological reasons, international trade in livestock coming from infected areas should be conditional upon sterilising treatment against *T. vivax* (various serodemes depending on the geographic area) and also against *T. evansi* in countries where the parasite is not present.

In enzootic areas, stringent abidance by adequate animal husbandry practices (feeding, water, worming and tick removal), in combination with protection of livestock against horseflies (*boucans*, shelters and insecticidal sprays) and occasional treatment of the most sensitive animals (diminazene aceturate) are enough to reduce the impact of bovine trypanosomosis to an economically acceptable level.

The investigations conducted in French Guiana have shown that the direct effects of Tabanids alone provide sufficient economic justification for implementing seasonal chemical control schemes against these insects. These observations cannot be generalised to the whole continent because Tabanid impact and the cost-effectiveness of control is connected to insect density and as such must be assessed locally.

Whatever the control methods used against mechanical vectors of trypanosomes, they can only reduce the population. It has been shown that even when hæmatophagous insects are not abundant, *T. vivax* can be transmitted to an entire herd of cattle within a few days whatever the season. Parasite circulation depends more on the parasitemic level of infective hosts and the receptiveness of potential hosts than on the abundance of vectors. Hence, during the dry season, seasonal horsefly population explosions are not the only factor involved in trypanosome transmission. By increasing livestock receptiveness, their direct harmful effects are instrumental in bringing about clinical manifestations of trypanosomosis. Horsefly control should be construed as the control of the main contributing factors to symptomatic trypanosomosis rather than a method for keeping the vector in check.

Because horseflies are highly attractive to horses, this data is probably also applicable to trypanosomosis induced in horses by *T. evansi*. However, further investigations on this species are needed to confirm this assumption.

Chagas' disease (*T. cruzi*) is a major human disease in Latin America since 90 million people are exposed and 20 million are infected [2]. Its annual incidence is 15,000 cases [95] and mortality rate is approximately 10% [3]. Recent studies have emphasised the importance of two types of peridomestic reservoirs for *T. cruzi* that had so far been underestimated: pets (dogs, cats, guinea pigs and other rodents) and farm bred animals (horses, cattle, sheep, goats, pigs, rabbits and guinea pigs) [93]. Indeed, the peridomestic cycles are precisely those that present the highest risk of human contamination [258]. Pathogenicity of *T. cruzi* in livestock requires further investigation but is assumed to be low. The effective involvement of livestock in the epidemiology of Chagas' disease is

very difficult to estimate because of the sometimes high prevalence of true livestock trypanosomes (*T. vivax*, *T. evansi* and *T. equiperdum*). Livestock infections probably play only a minor role in the epidemiology of the human disease and livestock is probably an epidemiological dead end. However, in disadvantaged rural populations, close cohabitation between species might foster peridomestic zoonotic spread of the parasite. Disease control relies essentially on hygiene and education of the populations at risk. Veterinary practitioners who deal with pets in endemic areas and veterinarians that work in the disadvantaged areas where there is little separation between human and animal habitats should be warned. A positive diagnosis in an animal may be an indication of risk for humans.

4. THE SPREAD OF LIVESTOCK TRYPANOSOMOSES IN AMERICA OVER TIME AND SPACE – OUTLOOK

The **geographic spread** of livestock trypanosomoses is closely related to modes of transmission and generally coincides with the spread of their main vectors.

Trypanosoma cruzi occurs in livestock but its incidence is poorly known because no adequate diagnostic tools exist. In view of its epidemiology, it can be assumed that livestock is not highly exposed to infection. However, according to recent investigations, prevalence is not insignificant. Its occurrence in livestock deserves special attention and further investigation. Although the human disease is typically South American (25° N-38° S), the parasite is spreading northwards in the USA to California and Virginia where the main epidemiological vehicles would appear to be dogs and wild animals (opossums, raccoons, etc.). It is likely that *T. cruzi* in North America fulfils its complete cycle in *Didelphis virginiana* [464] in much the same way as has already been described for South America in *Didelphis marsupialis* [143]. In view of the parasite's huge domestic and wild reservoir and its various transmission modes (including the peroral route), it is reasonable to predict that *T. cruzi* will maintain and extend its area of establishment northwards. In addition, the long incubation period for Chagas' disease and its weak clinical incidence makes it a covert scourge that tends to be under-estimated and belatedly discovered. Finally, just as *T. evansi* in vampire bats, it could be that *T. cruzi* is able to call on new vectors in North America and thereby continue its geographical progression, and possibly eventually find an epidemiological link leading to human infections. It is advisable to call the attention of the United States sanitary authorities to this threat.

Because ***T. equiperdum*** can be venereally transmitted, it is a potentially cosmopolitan parasite – as witnessed by the fact that it was introduced into North America and Canada in the last century. However, its mode of transmission and its strictly equine infectivity have meant that the parasite was eradicated from areas that benefit from extensive medical management, namely North America. The distribution of *T. equiperdum* in America is practically kept secret due to the restrictions on trade that arise from notification of the disease. Naturally, it is difficult to estimate its capacity to spread.

Because of ***T. evansi***'s multiple modes of transmission – mechanical transmission by Tabanids, mechanical and 'biological' transmission by vampire bats and peroral contamination of carnivores – its distribution is potentially cosmopolitan. Furthermore, its capacity to infect highly diverse hosts means that it can become established in all domestic animals as well as the wild fauna and become well-entrenched. It was introduced into North America and Australia at the beginning of the century but was able to be eradicated before establishing itself in the wild fauna. Although the present area of distribution of *T. evansi* in America coincides more or less with the distribution of vampire bats, it is able to spread by calling solely on hæmatophagous insects as vectors or via per oral transmission and therefore go beyond those boundaries. In spite of Mexico having declared its livestock to be trypanosomosis-free, the USA monitors animal movements at their borders very stringently. Within the infected perimeter, *T. evansi* continues to advance in particular in Brazil where outbreaks in newly infected areas are particularly deadly for horses.

In America, ***T. vivax*** is transmitted by mechanical vectors and hence its distribution could potentially be cosmopolitan. However, it has no or very few reservoirs apart from domestic ruminants and as such its establishment there is less stable than in Africa (as indicated by frequent epizootic situations) and more vulnerable than that of *T. evansi*. However, *T. vivax* is advancing within its area of distribution at the Brazilian/Bolivian/Paraguayan borders with considerable economic repercussions. In just one year, all of Bolivia has been infected through trade in animals that are carriers of the parasites. Many epizootic outbreaks have been observed that were attributed to Tabanids. It could also spread towards Mexico – it's presence and progression in Central America is very poorly documented.

Although all four of these parasites are capable of spreading, particularly northwards, ***T. evansi*** is the livestock parasite that has the highest potential in terms of **geographic expansion** and **stable enzootic establishment**. Its presence in Mexico cannot be discounted.

Although ***T. vivax***'s capacity to establish itself **spatially** is less powerful than *T. cruzi* or *T. evansi* (because it has no effective wild reservoir), its specific epidemiology in America deriving from its mechanical mode of transmission and its immunogenicity means that it triggers a disease that may extend **over time** in the form of **periodic epizootic outbreaks** (epidemiological seesaw). To eradicate *T. vivax* would require massive use of trypanocides that is likely to generate resistances at any time. It is not contemplated.

It should therefore be expected that *T. vivax* and *T. evansi* will continue their geographical progression with considerable economic impact in newly infected areas. In previously infected areas, they are an ongoing threat for farmers who must be in a position to manage periodic epizootic flare ups that can suddenly occur.

The situation of *T. cruzi* should be closely monitored inasmuch as it may become an emergent disease in the USA.

The situation with *T. equiperdum* remains a mystery as nobody wishes to talk about it!

5. TRYPANOSOME TRANSMISSION IN AMERICA AND AFRICA – AN EPIDEMIOLOGICAL COMPARISON

The specific epidemiology of bovine trypanosomosis in Latin America is a model for strictly mechanical transmission of trypanosomosis, which is not often observed in the field in Africa where trypanosomoses are mainly transmitted cyclically by *Glossina*. Nonetheless, knowledge about the epidemiological aspects of trypanosomoses that are transmitted strictly mechanically is very important for African epidemiologists as they will no doubt increasingly be confronted with this mode as *Glossina* infestation gradually subsides under the combined effects of changes in their ecological environment and control schemes, namely the PATTEC[12]. It is perhaps therefore useful in the conclusions to the present document to recall the characteristics of mechanically transmitted bovine trypanosomoses and compare them with those of bovine trypanosomoses that are cyclically transmitted by *Glossina*. The shift from one epidemiological mode to the other will probably be disturbing for farmers, veterinarians and epidemiologists alike in spite of prior knowledge. **Figure 43** shows a theoretical model of trends in parasitological and serological prevalences of *T. vivax* in cattle populations that are exposed to mechanical vectors (**Fig. 43 a**) or to cyclical vectors (**Fig. 43 b**), as described below.

In areas where transmission of *T. vivax* is strictly mechanical such as Latin America, bovine trypanosomosis occurs in the form of periodic multiple epizootic outbreaks against a subclinical enzootic background. Because mechanical transmission is vulnerable, the epidemiological situations

[12] Pan African Tsetse and Trypanosomosis Eradication Campaign

are unstable. Following an 'epizootic wave' (**Fig. 43 a**, phase 1) infections are gradually eliminated by self-curing and treatment (initially, treatments against trypanosomosis in the case of an epizootic outbreak, and later possibly against babesioses). During this period that might be referred to as the 'inter-epizootic period', concomitant immunity becomes established and maintains very low parasitemia. It is very difficult to detect parasites in the bloodstream of animals (parasitological prevalence is nil) but the presence of the parasite in the population is nonetheless confirmed by the non-insignificant prevalence of antibodies for several years (**Fig. 43 a**, phase 2). After several years have elapsed, most of the population becomes susceptible (seroprevalence down to 20%-30%) and when other circumstances arise (seasonal immunosuppression that leads to high parasitemias in a number of carriers and vector proliferation) a further 'epizootic outbreak' (**Fig. 43 a**, phase 3) is triggered. This phenomenon, which we can refer to as 'an epidemiological seesaw' is typical of mechanically transmitted bovine trypanosomosis and has been observed in several South American countries (French Guiana, Colombia, Venezuela) [43]. The trypanosomes that trigger the epizootic outbreaks are either already present in the herds (aparasitæmic resurgence following stress, namely: drought and hyper-abundance of hæmatophagous insects) or are brought in by the introduction of infected animals. That is why the disease often occurs in the form of multiple outbreaks (concomitant resurgences under the effect of climate, or dissemination of the disease by a batch of carrier animals that are sold individually to different farmers). This alternation between 'epizootic clinical' and 'inter-epizootic subclinical' phases is characterised by long silent periods during which the parasite is not visible followed by very widespread clinical explosions.

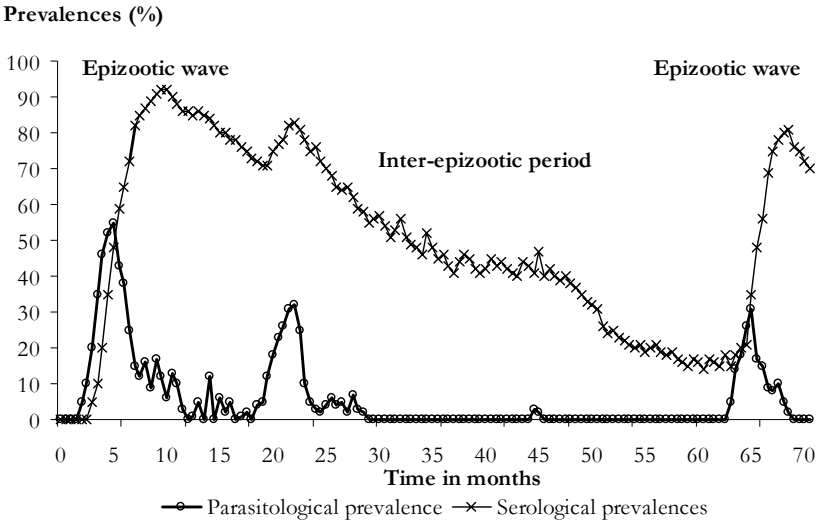


Figure 43 a – Strictly mechanical transmission by Tabanids and Stomoxys *(Latin America)*

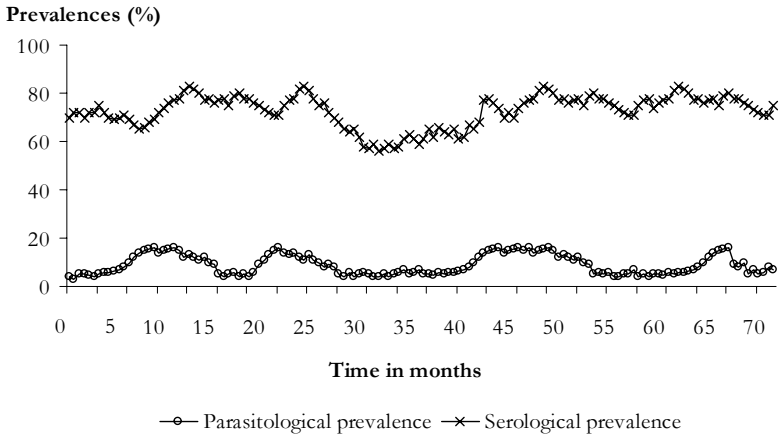


Figure 43 b – Stable enzooty with seasonal variations

Figure 43 – Model of trends in parasitological and serological prevalences in cattle populations

For mechanical transmission to be achieved, a number of conditions must be fulfilled simultaneously (high parasitemia in carriers, abundance of vectors, availability of recipients) and hence its probability is relatively low which explains why transmission is not observed every year. However, when these conditions are fulfilled, the incidence of mechanically transmitted infections is very high (> 70% per month) and trigger explosive outbreaks. Furthermore the mostly unchallenged animals all express the disease making it very ostensible.

In Africa in areas where the disease is cyclically transmitted by *Glossina*, these contrasts do not arise and trypanosomosis is a permanent blight that is constantly detectable. Although the incidence of infections and parasitological, serological and clinical prevalences vary on a seasonal basis (**Fig. 43 b**), there are never any wholly silent periods as is the case in America because *Glossina* are permanent carriers of several of the parasite species, some of which are highly pathogenic (*T. congolense* savanna type). Furthermore, mechanical transmission also occurs in areas infested by *Glossina* because these areas are also attractive to ecologically less demanding hæmatophagous insects. In addition, *Glossina* themselves are mechanical vectors. Mechanical transmission therefore occurs in addition to cyclical transmission and by contributing to the intensification of *T. vivax* maintains a high infection prevalence of this peculiar species.

On the edges of the *Glossina* distribution area or in areas where *Glossina* populations have been destabilised and vary enormously in size, a mixed situation prevails that is not amenable to modelling. The use of trypanocides does not necessarily decrease, indeed sometimes on the contrary, in areas where *Glossina* populations are dwindling due to the destruction of their habitats to make way for crops, or due to insecticides. This unexpected situation probably arises from the fact that a stable enzootic situation has given way to an unstable enzootic situation where premunised animals are no longer the majority, which leads to small-scale epizootic outbreaks with strong clinical expressions because many cases are primary infections. Under these circumstances, treatment is often necessary. Such a situation is not desirable and should not last, soon reverting to a better situation where only mechanical transmission is possible. Unfortunately several factors – *Glossina* dissemination during the rainy season and consequent repopulation, transhumance of livestock to infested areas, mechanical transmission outside the *Glossina* area – can cause these situations to last thereby considerably damaging the reputation of *Glossina* control campaigns. Indeed, once the cyclical vector has apparently been overcome, if the disease continues it is difficult to convince rural populations that control measures against *Glossina* must be maintained, since they draw the conclusion that they are ineffective against trypanosomoses.

In situations like this, there must be adherence to strict rules relating to (1) chemoprophylaxis on animals that about to begin transhumance and (2) sanative treatment at high doses when they return. This is the only way to avoid establishing foci for mechanical transmission when the transhumant animals return particularly if they come into contact with sedentary animals that are fully receptive.

Finally, it is worth noticing that Tabanid activity in western Africa has a peak of only two months at the end of the rainy season [574]. Hence, fortunately, the high risk period for mechanical transmission is short-lived. In some cases of intense, localised pullulation, transmission of trypanosomes may occur through *Stomoxys*.

6. CONCLUSIONS

Very little is known about livestock trypanosomoses in Central America and Mexico. Conducting epidemiological surveys in this scantily investigated geographical area should be a major short-term priority.

The medical and economic impact of livestock trypanosomoses in Latin America is growing. There is reason to fear that the spread of epizootic outbreaks due to *T. vivax* and *T. evansi* to the huge

areas where livestock are extensively raised in Central South America will become a major concern in the 10 coming years. Controlling these outbreaks will be made all the more difficult by the extensive stock raising conditions, lack of experience amongst farmers in respect of these new introduced diseases and the very narrow range of (effective) trypanocides available. A priority area of research should be the development of new trypanocidal molecules and the development of sensitive and species-specific diagnostic tools. Unfortunately, there is little indication that pharmaceutical companies will be taking an interest in the development of new trypanocides. Trypanosomosis due to *T. vivax* should be considered as an emergent disease in all newly infected areas (e.g. Bolivia in 1998-1999) and as a re-emerging disease in the enzootic areas where periodic epizootic flare-ups occur. It will probably become a priority disease in South America in coming years particularly in Venezuela, Bolivia and Brazil.

In Africa, apart from trypanotolerant breeds, it is unlikely that domestic ruminants will be able to control trypanosomoses considering the often high virulence of parasitic strains, the existence of numerous genetic variants within species, the concomitant presence of several pathogenic species and frequently highly inadequate animal husbandry conditions.

In Latin America, with only one, moderately pathogenic, species – *T. vivax* – following a period of clinical manifestation, trypanosomosis in ruminants more often than not becomes asymptomatic. Partial and gradual self-cure occurs. Consequently, the epidemiology of *T. vivax* is typically a periodic epizootic. Trypanosomosis control in ruminants on these two continents therefore follows quite different patterns – while only *Glossina* control and the use of trypanocides appear to be appropriate to control trypanosomoses in Africa, controlled premunition or identification (or selection) of a moderately pathogenic *T. vivax* strain for use as a live vaccine could be contemplated in America. These research avenues should be explored.

In Africa, a huge *Glossina* eradication programme has been implemented in many countries under the aegis of PATTEC. While this project may be able to free a few stock farming areas from the yoke of *Glossina*, there is reason to fear that some trypanosomes, in particular *T. vivax*, will continue to be present. For epidemiologists who have long-standing experience with cyclically transmitted trypanosomosis in Africa, the recent epidemiological impressions given in areas where *Glossina* have been eradicated may be misleading. Indeed, in the African experience, trypanosomosis is considered to be eradicated when cross-sectional surveys do not record any parasites in livestock or following one year-long monitoring. This is a hasty and misleading conclusion in the case of mechanically transmitted trypanosomosis. We have extensively described its various epidemiological features including the peculiarity that it may be invisible for several years – even by the standards of large-scale epidemiological surveys – and can reappear in the form of epizootic outbreaks which, in Africa, one might easily but wrongly interpret as being a reinvasion or temporary incursion by *Glossina* that had gone unnoticed.

If *Glossina* control becomes effective, it is probable that mechanical transmission will replace cyclical transmission. However, concomitance between mechanical vector abundance and livestock receptiveness may not be as true in Africa as it is in South America. It is therefore difficult to predict the impact of mechanical transmission of trypanosomes in Africa in the absence of *Glossina*.

Trypanosome eradication by means of trypanocides no longer appears to be possible in view of increasing resistances to these drugs. Eradicating mechanical vectors is not conceivable because it would require the eradication of many species of insects, which is practically impossible and in any case unacceptable. Trypanosomoses due to *T. vivax* and *T. evansi* therefore have a rosy future ahead of them in America (and in Africa). Since they cannot be eradicated, steps will have to be taken so that they do not take an excessive toll on livestock raising. Instances of concomitant immunity observed in the field or under controlled conditions indicate that these diseases could achieve the status of highly enzootic infections with little clinical incidence, just like anaplasmosis or babesioses. However, the inevitable epizootic outbreaks that will regularly occur should be controlled mainly by

use of trypanocides. The extent of their medical and economic impact will depend on how rapidly they are identified.

Having reviewed the topic in a manner I hope is constructive and reached the end of this paper, I appreciate the extent of the work that remains to be done and can only express the hope that this study will convince decision-makers that investment in research on the topic of livestock trypanosomoses in Latin America is necessary.

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