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PROCEEDINGS OF FIRST SYMPOSIUM ON NEW WORLD TRYPANOSOMES

Georgetown, Guyana
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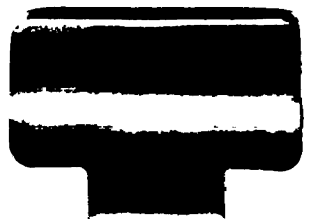INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
TECHNICAL COOPERATION AGENCY IN BARBADOS

AREA OF CONCENTRATION III: AGRICULTURAL HEALTH

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FOREWORD

From 1994 to the end of 1996, the Centre de Cooperation Internationale en Recherche Agronomique pour le Developpment – Elevage et Medecine Veterinaire Tropicale (CIRAD-EMVT) and the Inter-American Institute for Cooperation on Agriculture (IICA) collaborated in a project entitled *"Hemoparasite Network for the Guianas"*. This project was conducted in the Guianas, i.e. Guyana, Suriname and French Guiana, in partnership with the national and departmental veterinary services of these countries. Funding was provided by the French Interministerial Fund for the Caribbean (FIC).

The project sought to increase knowledge of the epidemiology, clinical and economic importance of hemoparasites, particularly trypanosomes, in these three countries, in order to develop effective control methods and thus improve the health and productivity of livestock.

Information was exchanged on New World Trypanosomes through publication of a technical newsletter, called *Trypnews*. From 1994 to 1996, interest and subscription in this newsletter grew steadily. Although the newsletter was not published from 1996 to 1998, we are very pleased that Dr. Rita Tamasaukas of the Romulo Gallegos University in Venezuela has recently begun publishing a new version of *Trypnews*. Dr. Desquesnes and Dr. Vokaty continue to serve on the editorial committee of the new *Trypnews*, along with other Trypanosome experts in South America.

During the lifespan of the Hemoparasite Network, epidemiologic studies of Trypanosomes and other hemoparasites were conducted in the Guianas, with the active participation of the national veterinary services. The CIRAD-EMVT specialised laboratory in arthropod-borne diseases in Cayenne, French Guiana served as the Hemoparasite Reference Laboratory for the Guianas. National veterinarians and technical staff received training in laboratory diagnostics, and laboratory equipment and materials to carry out their national surveys. The results of these studies are included in these Proceedings of the First Symposium on New World Trypanosomes.

The project culminated in the First Symposium on New World Trypanosomes, which was held in Georgetown, Guyana from November 20 to 22, 1996. During this Symposium, a small but august group of Trypanosome researchers from both the Old and New Worlds had a lively exchange of their research findings, and new friendships and collaborative efforts were forged. For example, Dr. Rita Tamasaukas of Romulo Gallegos University in Venezuela very kindly offered to take over primary editorial responsibility for *Trypnews*.

We wish to thank CIRAD-EMVT, IICA, the French Interministerial Fund for the Caribbean, and the governmental veterinary services of Guyana, Suriname and French Guyana for their enthusiastic support during the project. We also wish to thank all the participants in the Symposium for their keen interest, and for their patience in waiting for these Proceedings to be published. We also wish to thank Ms. Sophia Prescod of IICA Barbados for her painstaking efforts in preparing this document.

Much work remains to be done on the subject of New World Trypanosomes. This is evidenced by the serious outbreak of *T. vivax* in Brazil and Bolivia in 1996/97.

Dr. Alberto Davila of FIOCRUZ in Brazil and Dr. Rita Tamasaukas have announced that they are organising the *Second Symposium on New World Trypanosomes* at the Romulo Gallegos University in San Juan de los Morros, Guarico, Venezuela from October 13 to 15, 1999. Congratulations to them for this initiative. We offer them our cooperation and support.

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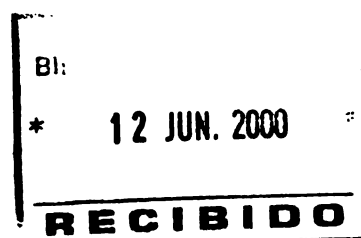
HISTORY OF *TRYPANOSOMA VIVAX* IN THE NEW WORLD

E. CAMUS
CIRAD-EMVT GUADELOUPE (F.W.I.)
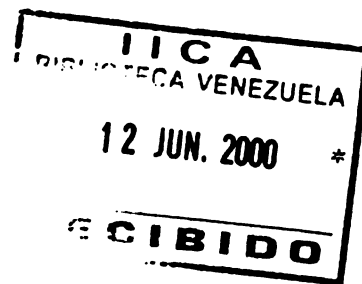
ABSTRACT

The history of diseases can be a tool to anticipate their evolution. *Trypanosoma vivax* was probably introduced in French Guiana, South America, by zebu cattle imported from Senegal in 1830. At the same time, animals were imported to Guadeloupe and Martinique, introducing heartwater and its tick vector from Africa and possibly also trypanosomiasis. However, the significant trade between Africa and Brazil before 1830 could also be responsible for the introduction of *T. vivax* into South America. The first diagnosis of *T. vivax* in the new world was made in 1919 in a cattle herd in French Guiana, where more than 50% of the animals died from trypanosomiasis. The disease was subsequently diagnosed and the parasite observed in Venezuela (1920), in Panama (1926), Guyana (1952), Brazil (1967), Equador (1977), Peru (1977). The disease was suspected because of seropositive cattle in Costa Rica, Salvador Paraguay (1977), Guatemala, Chile and Trinidad (1994). Most of the animals were cattle, except in Brazil where water buffaloes were involved. After 1930, the disease disappeared from Guadeloupe and Martinique and disappeared also from French Guiana between 1920 and 1935. For years the main suspected vectors were tabanids but *Cryptotylus unicolor* was demonstrated to be an experimental vector only in 1988. In 1907, an African strain of *T. vivax* was successfully transmitted by *Stomoxys*. The introduction, spread and disappearance of trypanosomiasis in different countries of The New World are discussed, together with preventive measures.

RESUMÉ

L'histoire des maladies peut permettre d'anticiper leur évolution. *Trypanosoma vivax* a probablement été introduit en Guyane Française avec des Zébus importés du Sénal en 1830. A la même époque, des bovins étaient importés aussi en Guadeloupe et Martinique, introduisant la cowdriose et son vecteur et peut être aussi la trypanosomiase. Cependant, les courants commerciaux entre l'Afrique et le Brésil avant 1830, peuvent aussi avoir été responsables de l'introduction de *T. vivax* fut observé en Guyane Française dans un troupeau bovin où plus de 50% des animaux moururent de trypanosomiase. La maladie et le parasite furent ensuite identifiés au Venezuela (1920), en Guadeloupe (1926), Martinique (1929), Colombie (1931), Suriname (1938), Panama (1941), Guyana (1952), Brésil (1967), Equateur (1977), Pérou (1977). La maladie fut suspectée à cause de bovins séropositifs au Costa Rica, Salvador et Paraguay (1977), Guatemala, Chili et Trinidad (1984). La plupart des animaux affectés étaient des bovins sauf au Brésil avec des buffles d'eau. Après 1930, la maladie disparut de Guadeloupe et de Martinique et disparut aussi de Guyane Française entre 1920 et 1935. Pendant des années le principal vecteur suspect a ét'le taon mais ce n'est qu'en 1988 que la transmission expérimentale de *Crytotylus unicolor* a été réussie. Très tôt en 1907, une souche africaine de *T. vivax* a été transmise par des *Stomoxys*. L'introduction, l'extension et la disparition de la trypanosomiase dans différents pays du Nouveau Monde sont discutées ainsi que les mesures préventives.

RESUMEN

La historia de una enfermedad puede ser una herramienta que puede anticipar su evolución. El *Trypanosoma vivax* probablemente fue introducido en la Guyana Francesa, Sur América, con el ganado zebú importado del Senegal en 1830. Al mismo tiempo, los animales fueron importados a Guadalupe y Martinica, introduciendo de Africa la enfermedad heartwater y sus vectores: las garrapatas, y posiblemente también la trypanosomiasis. Sin embargo, el importante comercio entre Africa y Brasil antes de 1830 pudo haber sido responsable de la introducción del *T. vivax* en Sur América. Es solamente en 1919 que el *T. vivax* se observó en los rebaños bovinos en la Guyana Francesa, donde mas del 50% de los animales murieron de trypanosomiasis. Luego se diagnosticó la enfermedad y el parásito fue observado en Venezuela (1920), en Panamá (1926), Guyana (1952), Brasil (1967), Ecuador (1977), Perú (1977). En Costa Rica, El Salvador y Paraguay (1977), Guatemala, Chile y Trinidad (1994) se sospechaba de la enfermedad por el ganado bovino que resultaba seropositivo. La mayoría de los animales eran bovinos, excepto en Brasil donde los involucrados eran búfalos de agua. Después de 1930, la enfermedad desapareció de Guadalupe y Martinica y también desapareció de la Guyana Francesa entre 1920 y 1935. Por años el vector mayormente sospechado fue un tabánido, pero solo en 1988 se logró demostrar experimentalmente la capacidad vectorial del *Cryptotylus unicolor*; a principios de 1907; una cepa africana de *T. vivax* se transmitió exitosamente por *Stomoxys*. La introducción, la extensión de la trypanosomiasis en diferentes países del Nuevo Mundo se discuten, junto con las medidas preventivas.

INTRODUCTION

The history of diseases helps to understand their evolution (Nicolle, 1933). The history of *Trypanosoma vivax* in the New World should be a good example for such a theory.

History starts with the introduction of the disease into countries or continents, then its spread through countries or continents. And sometimes the history ends with the disappearance of the disease.

From knowledge of disease history, practical information can be deduced concerning:

- a better understanding of their epidemiology;
- preventive measures to be taken to avoid or limit their spread;
- control measures to implement in countries where the disease is established.

INTRODUCTION OF TRYPANOSOMIASIS INTO THE NEW WORLD

The disease was probably introduced in 1830 with Zebu cattle imported from Senegal into French Guiana (Curasson, 1943).

Another possibility is introduction from Africa into Brazil before 1830 with Portuguese sailors (Ferris, 1984).

It can be noted that in 1830 also, heartwater and its *Amblyomma* tick vector were introduced into Guadeloupe with Zebu cattle imported from Senegal. *T. vivax* could have been introduced at the same time in Guadeloupe and in Martinique.

FIRST IDENTIFICATION OF *T. VIVAX* IN THE NEW WORLD

In 1919, Leger & Vienne observed the following clinical signs in a cattle herd in French Guiana: progressive anemia, weight loss, edema, posterior paralysis and 50% mortality.

The authors described the etiologic agent (named *T. guyanense*) and already suspected the role of tabanids in the transmission.

SPREAD OF *T. VIVAX* IN THE NEW WORLD

Based on clinical and parasitological observations, the diagnosis of trypanosomiasis was made in the following countries:

1920	Venezuela	Cattle	(Tejera, 1920)
1926	Guadeloupe	Cattle	(Fabre et Bernard, 1926)
1929	Martinique	Cattle	(Carougeau, 1929)
1931	Colombia	Cattle	(Plata, 1931)
1938	Suriname	Cattle	(Nieschulz et al., 1938)
1941	Panama	Cattle	(Johnson, 1941)
1952	Guyana	Cattle	
1967	Brazil	Water Buffalo	(Shaw & Lainson, 1972)
1977	Ecuador & Peru	Cattle	(Wells et al., 1977)

The disease was also serologically suspected in the following countries:

1977	Costa Rica	Cattle	(Wells et al., 1977)
1977	El Salvador	Cattle	(Wells et al., 1977)
1977	Paraguay	Cattle	(Wells et al., 1977)
1984	Guatemala	Cattle	(Ferris, 1984)
1984	Chile	Lama & Alpacas	(Ferris, 1984)

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| 1984 | Trinidad | Water Buffalo | (Ferris, 1984) |

Because of serological cross reactions with *T. evansi*, it could not be concluded with certainty that the diseases described were really due to *T. vivax*.

DISAPPEARANCE OF *T. VIVAX*

In Guadeloupe and Martinique, the disease, diagnosed during 3 or 4 years in 1930, was not reported subsequently.

A survey in 1986 (E. Camus, unpublished results) showed no antibodies on hundred sera from cattle and no parasite on more than 1000 smears.

Biting flies (tabanids) described in 1926 had apparently disappeared. Today, tabanids are almost absent in those two islands.

In French Guiana, a transitory apparent disappearance was observed between 1920 and 1935 and again more recently between 1990 and 1995 (Desquesnes & Gardiner, 1993).

VECTORS OF *T. VIVAX*

Tabanids have been suspected since 1919 (Leger & Vienne).

The experimental transmission of an American *T. vivax* strain was achieved only in 1988 with *Cryptotylus unicolor* (Ferenc et al., 1988) and then with *Tabanus nebulosus* (Otte et al., 1991) and *T. importunus* (Raymond 1990).

Very early in 1908, African strains of *T. vivax* were proven to be transmitted by *Stomoxys*.

Boophilus ticks were suspected (Lopez et al., 1979) but was never confirmed.

PROPOSED PREVENTIVE MEASURES

Observations indicate that:

- in all cases, the introduction of *T. vivax* in a new country was apparently linked with the introduction of infected cattle;

- the infection does not seem to spread naturally by vector movement from country to country.

It can be deduced that testing imported cattle with sensitive methods to detect *Trypanosome* carriers may reduce the risk of introduction:

- microhematocrit technique;
- serological tests (once the specifity of ELISA is improved);
- PCR, when it will become easier to use and less expensive.

CONCLUSION

Trypanosomiasis caused by *T. vivax* in the New World has a history of remarkable adaptation from a cyclical vector disease (tsetse fly) to a mechanical one. It is also an example of the spread of an infection by uncontrolled movement of cattle.

So far, no effective methods have been identified to control tabanid vectors.

A limited control of the disease could be achieved by controlling cattle movements and by applying trypanocides to infected animals and herds.

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ORIGIN AND DISTRIBUTION OF NEW WORLD LIVESTOCK TRYPANOSOMES AND THEIR AFFINITY FOR SOME MAMMALIAN HOSTS

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ABSTRACT

The origin and distribution of the New World trypanosomes of livestock are briefly presented. Non-pathogenic trypanosomes are *T. theileri* in cattle and buffaloes, *T. ingens* in cattle, and *T. melophagium* in sheep in the highlands. Only the *Megatrypanum* species can interfere with parasitological diagnosis in cattle and buffaloes. *T. vivax* is pathogenic in cattle, sheep, goats and buffalo, and although it has been found in deer, the existence of a wild reservoir has never been demonstrated. It has not been determined whether the South American *T. vivax* can infect dogs, cats and horses. *T. evansi* is pathogenic in horses, buffalo, dogs, cats and some wild fauna including vampire bats which act both as reservoir and vector, and it can also be found in cattle, sheep, goats and pigs. *T. evansi* has a large domestic and wild reservoir and is well established in Latin America. *T. equiperdum* is sporadic in Latin America and restricted to horses and donkeys due to its sexual transmission. *T. cruzi*, the agent of Chagas' disease in humans, is also pathogenic in some wild fauna, and is found in all domestic and wild mammalian hosts. The study of trypanosomiasis, mostly based on serological surveys, encounters problems of species specificity. Problems of sub-genus or species-specificity depend on the *Trypanosoma* species present in the area and the host under investigation. Infections with the four pathogenic *Trypanosoma* species described cannot be properly differentiated by antibody detection. Cross reaction should be carefully considered in sero-epidemiological surveys.

RESUMÉ

L'origine et la distribution des trypanosomes du bétail présents dans le Nouveau Monde sont brèvement présentées. Les trypanosomes non pathogènes sont *Trypanosoma theileri*, présent chez les buffles et les bovins, *T. ingens* chez les bovins et *T. melophagium* chez les ovins dans les hauts plateaux. Seuls les mégatrypanosomes des bovins et des buffles peuvent interférer dans le diagnostic parasitologique des trypanosomoses dans ces espèces. *T. vivax* est pathogène chez les bovins, ovins, caprins et bubalins; bien qu'il ait été trouvé chez les cerfs du Nouveau Monde, leur rôle de réservoir n'a jamais été démontré. Il n'a pas été observé que les souche sud américaines de *T. vivax* soient capables d'infecter les chat, chien et chevaux, contrairement aux souches africaines. *T. evansi* est pathogène chez les chevaux, buffles, chien, chats, et de nombreux animaux sauvages, y compris les vampires qui agissent comme réservoir et vecteurs du parasite. *T. evansi* peut également être trouvé chez les bovins, caprins, ovins et porcins ; il possède un vaste réservoir sauvage et domestique qui lui confère une solide et large implantation en Amérique du Sud. En raison de sa transmission vénérienne, *T. equiperdum* parasite exclusivement des équidés et sévit de manière sporadique en Amérique du Sud. *T. cruzi*, agent de la maladie de Chagas chez l'homme, est également pathogène pour une partie de la faune sauvage, et peut être trouvé chez presque tous les mammifères domestiques et sauvages. L'étude des trypanosomoses, principalement basée sur des enquêtes sérologiques, rencontre d'important problémes de spécificité et de sensibilité. La distinction des sous-genres et des espèces doit prendre en considération la totalité des espèces présentes dans une aire géographique donnée, et chez les hôtes étudiés. Les infections par les quatre trypanosomes pathogènes qui ont été décrits ne peuvent être différentiées par examen sérologique. Les réactions croisée peuvent considérablement biaiser les enquêtes sérologiques.

RESUMEN

Se presenta brevemente el origen y distribución del Trypanosomiasis del ganado en el Nuevo Mundo. Los trypanosomas no-patógenos son el *T. theileri* en vacunos y búfalos, *T. ingens* en bovinos y el *T. melophagium* en ovinos de la regiones montañosas. Solo las especies de *Megatrypanum* en bovinos y búfalos pueden interferir ligeramente en el diagnóstico parasitológico. *T. vivax* es patógeno en ganado vacuno, ovino, caprino y búfalos; aunque también se ha encontrado en venados, la existencia de un reservorio silvestre no ha sido demostrado. No se ha podido determinar si el *T. vivax* de Sur América pueda infectar a perros, gatos y caballos. El *T. evansi* es patógeno para los caballos, búfalos, perros, gatos y algunos animales silvestres incluyendo al murciélago vampiro (hematófago) el cual pueda actuar de las dos maneras como portador y vector. También se le puede encontrar en ganado vacuno, ovino, caprino y porcino. El *T. evansi* tiene muchos reservorios tanto como en animales domésticos como silvestres, lo cual ha sido muy bien establecido en América Latina. El *T. equiperdum* es esporádico en América Latina y se limita a los caballos y burros debido a su transmisión sexual. El *T. cruzi*, agente del Mal de

Chagas en humanos, es también patogeno en algunos animales silvestres. Se puede encontrar en todos los mamíferos domésticos y silvestres. El estudio de la trypanosomiasis, se basa mayormente en investigaciones serológicas, aunque se presenta el problema de la especificidad de especie. Los problemas de sub-género o de especificidad de especie depende de la especie de *Trypanosoma* presente en el área y los hospedadores bajo investigación. Las infecciones con las cuatro especies patógenas de *Trypanosoma* descritas no se pueden diferenciar apropiadamente por detección de anticuerpos, por lo que la reacción cruzada debe ser cuidadosamente considerada en una investigación sero-epidemiológica.

I. ORIGIN OF THE NEW WORLD TRYPANOSOMES FOUND IN LIVESTOCK

Horses, cattle, sheep and goats were introduced into South America during the 16th century, mainly from African countries in which *T. vivax*, *T. evansi* and *T. equiperdum* were endemic. If *T. brucei* and *T. congolense* were ever introduced into South America they could not be maintained, probably due to the absence of their preferred vector, the tsetse fly; indeed their existence has never been reported in the New World.

T. equiperdum originated in North Africa and had an early and wide distribution throughout South Africa, Europe, Asia, and America, due to commercial movement of horses and its venereal transmission. It was eradicated from North America around 1950 (Hoare 1972). *T. equiperdum* was found in Brazil, Venezuela and Chile, but has recently been observed only in Bolivia and Paraguay (Wells 1984).

T. evansi was introduced into South America in the 16th century by Spanish conquistadores bringing horses from Arabia. In the 19th century it was seen in Marajo Island in 1827; in Paraguay in 1847; in Pantanal in 1850; and in Matto Grosso, Brazil in 1860; subsequently it spread throughout Brazil and to Bolivia, Guyana, Venezuela and Colombia (Hoare, 1972). Its geographical spread and establishment were governed by the movement of horses and the existence of wild and domestic hosts, of which vampire bats seem to have a determinant role. Distribution of *T. evansi* extends from Argentina and Chile to Panama (Wells et al. 1977); however the distribution of the parasite is not clearly defined in the north.

T. vivax was first described in French Guiana by Leger and Vienne (1919), during an epidemic near Cayenne. According to Curasson (1943), it was introduced into the French Antilles and French Guiana in 1830 in Zebu cattle imported from Senegal. Identified in Martinique and Guadeloupe in 1926, it was observed for only 15 years, and disappeared. The literature mentions its presence in Venezuela (Tejera, 1920), Colombia (Plata, 1931), Suriname and Guyana, in 1939 (Hoare, 1975), Panama (Johnson, 1941), Marajo Island (Floch and Lajudie, 1944), and around Belem, in Brazil (Shaw and Lainson, 1972). Wells et al. (1977) recorded serological evidence of the parasite in Peru, Ecuador, Paraguay, Costa Rica and El Salvador. A recent suspected case in Argentina still needs to be confirmed (Bakos et al. 1992). The incidence of *T. vivax* extends from Paraguay to El Salvador (Wells et al., 1977); however its distribution in the north is not clearly defined.

T. theileri is a cosmopolitan parasite introduced to the Americas with cattle. *T. mazamarum* has been described in deer in Argentina and Venezuela (*Mazama rufa* and *M. simplicornis*), but whether it is really different from *T. theileri* is not known (Hoare, 1972). *T. ingens* has been described in Africa, Java and Malaysia (Hoare, 1972) and has recently been identified in Suriname (Van Vlaenderen 1996).

T. melophagium is a cosmopolitan parasite transmitted by an intermediate host, *Melophagius ovinus* (Hippoboscidae), and its geographical distribution is governed by the presence of this host. It is present in Europe, North Africa, Canada and Australia (Hoare 1972); in South America it is only present in the highlands, and was observed in Argentina and Colombia by Wells et al. (1986).

T. cruzi is the only pathogenic trypanosome originating in Latin America where it is endemic throughout the sub-continent, from Argentina and Chile to Mexico. While *T. cruzi* has been found in the southern United States: Texas, Oklahoma, Louisiana and South Carolina, and more recently in the Virginias (Barr et al. 1995); its distribution is not clearly defined in North America.

II   HOSTS OF THE MAIN NEW WORLD TRYPANOSOMES

The study of trypanosomiasis is affected by problems of diagnostic sensitivity and sub-genus or species-specificity. Problems associated with specificity arise due to the *Trypanosoma* species present in the area and the host under investigation. The main trypanosomes found in Latin America, and their hosts, are indicated in Table 1.

Hosts of the pathogenic trypanosomes

Cattle, sheep and goats are the preferential hosts of *T. vivax* in Latin America; but the parasite has also been found in buffaloes (Shaw and Lainson 1972; Didonet-Lau and Lau, 1988). In Africa, *T. vivax* is found in horses (Anosa, 1983) and cats (Bwangamoi et al., 1989). According to Ruiz Martinez (1971), the South American parasite does not grow in horses, however the potential role of horses as a reservoir of *T. vivax* should be investigated. Gardiner (1990) indicated that alpaca (*Lama pasos*) and llama (*Lama glama*) may be infected; and a human case was mentioned by Lavier (1927). *T. vivax* was found in deer (*Odocoileus gymnotis*) by Fernandez (1931) and Fiasson et al. (1948); and Silva suggested recently that deer could act as reservoirs of the parasite in Pantanal, Brazil (personal communication), however this has not yet been confirmed. Wild hosts of *T. vivax* in the New World would allow enzootic and definitive implantation of the parasite. To date, in Latin America, a wild reservoir of *T. vivax* has never been confirmed, nor has whether horses, cats and dogs could be infected with the parasite.

T. evansi and *T. cruzi* have the widest host range of trypanosomes in Latin America. Domestic and wild fauna act as a wide reservoir of these parasites.

Apart from humans, *T. evansi* can infect all the species mentioned above, including pigs and carnivores. It is mostly pathogenic in horses, buffaloes (Troncy et al. 1981) and carnivores (Boehringer and Prosen, 1961). Transmission of *T. evansi* by vampire bats has been described by Hoare (1965). Vampire bats are hosts, reservoirs and mechanical vectors of the parasite. The infection occurs during the blood meal on an infected host (through the oral mucosa). The disease can last for one month; ending in death or cure with a long term asymptomatic carrier state. Parasites multiply in the blood of the vampire bat and migrate to the buccal cavity, from where they are transmitted when the vampire bat bites. Contamination from bat to bat is possible and allows the parasite to survive even in the absence of horses, the preferred hosts. Vampire bats should be considered as biological vectors of *T. evansi* since the parasite can survive, multiply in bats and be transmitted for a long time. It is different from tabanids, the true mechanical vectors, and also different from the cyclical biological transmission of salivarian trypanosomes by tsetse flies in Africa or *T. cruzi* in triatomine bugs.

More than 150 species of wild and domestic fauna have been found infected by *T. cruzi*, including the main livestock species: horses, cattle, sheep, goats, pigs, and domestic species including dogs, cats, rabbits and guinea pigs. In humans in Argentina, Brazil, Chile, Bolivia and Venezuela, seroprevalence of *T. cruzi* varies from 0.5-2% in the cities to 20-63% in highly endemic areas; in dogs, seroprevalence rates vary from 4.5% to 100% and in cats from 0.5% to 61% (Anon., 1991). A recent survey in Chile indicated positive responses to *T. cruzi* antigens of sera from dogs (12-24%), cats (0-15%), goats (5-12%), rabbits (4-26%), and sheep (4.8%) (Correa et al. 1992). In Mexico, Alcaino et al. (1995) found 6.5-38.5% of positive samples to *T. cruzi* in goats. In French Guiana, Dedet et al. (1985) found 30.8% of opossum (*Didelphis marsupialis*) infected. A fatal clinical case was identified in a dog by Frenay and Desquesnes (unpublished). Only three acute human cases were confirmed in 1996 (Beaudet, cardiologist at Cayenne Hospital; personal communication).

T. equiperdum is the only parasite with high host specificity since it can only be found in equidae, including horses, donkeys and mules.

Table 1:   Hosts of the pathogenic trypanosomes in Latin America

Host species	*Intensity of affinity and/or pathogenicity					
	T. vivax	T. evansi	T. equiperdum	T. cruzi	T. theileri	T. ingens
Horses, donkeys, mules	?	++++	++	++++	?	?
Cattle (Bos taurus, B. indicus)	++++	++	++		++++	+++
Sheep	++++	++	++		++++	+++
Goats	++++	++	++		? ˙	?
Buffalo (Bubalis bubalis)		+++	++	++	+++	?
Pigs		++	++			
Dogs (Canis familiaris)	?	++++	+++++			
Cats		++	++			
Rabbits		?	++			
Humans			++++++			
Deer (Odocoileus gymnotis)	++		++		?	?
(O. chiriquensis & mazama satorii)		+++	++		?	?
Alpaca (Lama pasos)	+	++	++			
Llama (Lama glama)	+	++	++			
Guinea pig (Cavis porcellus)		+++	++			
Vampire bats (Desmodus rotundus)		++++	++			
Capybara (Hydrochoerus hydrochoeris)	?	++++	++			
Coati (Nasua nasua)		++++	++			
Wild dogs (Canis azarae)		+++	?			
New World mice (Oryzomys sp.)		+++	++			
Didelphis marsupialis		?	+++++			
Howler monkeys (Allouatta seniculus, A. urismus)		+++	?			
Wild pigs (Tayassa tajacu)		?	?			
Saimiri (Saimiri sciureus)		?	?			
Other species in wild fauna	?	carnivores	140 species	?	?	?

* least + ; greatest +++++

III   INTERFERENCE IN DIAGNOSIS

Interference in parasitological diagnosis:
There is potential interference of T. cruzi in the parasitological diagnosis of Trypanosoma sp. in the above mentioned hosts.   Megatrypanum sp. can also interfere with diagnosis in livestock.   Blood smears allow sub-genus identification, providing the parasitemia is not too low (>10^5).   Hematocrit centrifuge technique (HCT) does not always allow sub-genus identification, but Megatrypanum sp. can generally be differentiated from smaller trypanosomes. It is sometimes (but not always) easy to identify T. vivax by its motility characteristics. It is generally difficult to differentiate T. evansi from T. equiperdum under such conditions. Mouse inoculation can be used, but not on a regular basis for economical reasons. Even through mouse inoculation, isolation of T. equiperdum is not easy (Gardiner, 1990).

Interferences in serological diagnosis
T. theileri does not interfere in the antibody detection test (Plat and Adams, 1976; Luckins, 1977 Ferenc et al. 1990; Desquesnes and Gardiner, 1993).

In livestock, the interference of T. cruzi in the diagnosis of the other Trypanosoma sp. has been infrequently reported. In a study in Argentina, Monzon and Colman (1988) found approximately 35% of horse sera positive to T. cruzi and 95% positive in T. evansi, among which 2% gave higher titers for T. cruzi than T. evansi. T. equiperdum

can interfere in the diagnosis of *T. evansi* (Touratier, 1992). It is known that antibody-detection allows the detection of infections by pathogenic *Trypanosoma* sp., but does not allow the identification of the parasitic species involved. All four pathogenic *Trypanosoma* sp. may interfere in serological diagnosis regardless of technique used: i.e. immunofluorescence, hemagglutination, indirect-ELISA, etc.

Some of the seroepidemiological surveys carried out in Latin America do not take these interferences into consideration; for example the study on *T. evansi* in dogs by Greiner et al. (1994), in Brazil, and the study on *T. cruzi* by Correa et al. (1992) in Chile.

Since species-specific antigen detection tests (Nantulya and Lindqvist, 1989) are not reliable (Desquesnes, 1996), there is no tool that could be used with confidence to identify the species involved in a positive antibody-detection against any of the pathogenic *Trypanosoma* species present in Latin America.

As an example, positive results were recently obtained on indirect-ELISA *T. equiperdum* in horses and mules in Mexico, in the total absence of symptoms. With the same samples, positive tests were also obtained against *T. evansi* and *T. cruzi* (*T. vivax* was not performed). The source of these positive results could not be definitely ascertained.

Perspectives for a species-specific diagnosis
Attempts to develop species-specific antibody detection tests have been made by Ijagbone et al. (1989) for *T. brucei*. Further research is necessary to identify species-specific antigens; recombinant antigens would be the most suitable technology to produce standardized parasitic antigens. It is likely that PCR techniques will allow species-specific diagnosis, but sensitivity is lower than antibody detection; moreover, for species-specific diagnosis, several oligonucleotides are necessary, which will most probably decrease the sensitivity of the test (if a single PCR is processed in multiplex system) or increase its price (if several monospecific PCR are processed).

IV   CONCLUSION

When conducting seroepidemiological studies it is important to consider the different *Trypanosoma* species potentially present in the area and the hosts under investigation, since the antibody detection tests currently available are not species-specific.

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A REVIEW OF TRYPANOSOMES OF LIVESTOCK IN GUYANA

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ABSTRACT

Protozoa of the genus *Trypanosoma* shown to date to infect ruminants in Guyana are *Trypanosoma vivax* and *T. evansi*, as demonstrated by parasitological and serological techniques. *T. theileri*, because of its cosmopolitan distribution, is regarded as present in cattle in Guyana as well. Similarly, *T. evansi* and *T. equiperdum* are suspected of infecting equines in Guyana based on their distribution in this geographical area.

RESUMÉ

A ce jour, les protozoaires du genre Trypanosoma dont la présence a été montrée par examens parasitologiques et sérologiques chez les ruminants sont *Trypanosoma vivax* et *Trypanosoma evansi*. *Trypanosoma theileri*, parasite cosmopolite, est également présent chez les bovins. En outre, sur la base de leur distribution géographique, la présence de *Trypanosoma equiperdum* et *T. evansi* est suspectée chez les chevaux.

RESUMEN

Los protozoos del género *Tripanosoma* que infectan a los rumiantes en Guyana hasta la fecha son *Tripanosoma vivax* y *T. evansi*, como se ha demostrado en las técnicas parasitológicas y serológicas. *T. theileri*, a causa de su distribución cosmopolitana, también se considera presente en ganado de Guyana. Igualmente, se sospecha que *T. evansi* y *T. equiperdum* están infectando a los equinos de Guyana debido a su distribución en esta área geográfica.

INTRODUCTION

This review seeks to review the available information on trypanosomes of livestock in Guyana for the benefit of scientists and researchers throughout the country, thus laying the groundwork for further investigations into the effects of pathogenic trypanosomes on the country's livestock industry. This information will permit an assessment to be made of the possible effects of trypanosomes could exert on livestock trade with the Eastern Caribbean and elsewhere. This area has recently been shown to be free of *Trypanosoma vivax*, the cause of non-tsetse transmitted animal trypanosomes in the New World (Ferenc et al., 1990).

TRYPANOSOMES IN BOVINES

Documentation of trypanosomiasis in Guyana in general is rather sparse but early reports of this disease in cattle date back to 1915 (Seton-Milne, 1917), in which unidentified trypanosomes were found in the blood of a Shorthorn bull exhibiting high fever for a number of days. Other occurrences of infection of cattle with trypanosomes were reported in various government reports in 1956 and 1961 by Fletcher, as described by Craig (1975), where *Trypanosoma vivax* was mentioned as the causative organism.

The first quantitative account of trypanosome infection in cattle in Guyana was however given by Craig (1975), who found five out of 1,019 animals to be positive for *T. vivax* on the examination of thick blood films. Animals examined comprised a mixture of 791 local and 228 imported cattle, the latter being Holstein heifers imported from the United States of America at 4 - 6 months of age, that were between 5 - 18 months of age when bled. The presence of *Babesia spp.*, *Anaplasma marginale* and *Eperythrozoon spp.* were noted at the same time (Table 1).

Table 1: The prevalence of *Trypanosoma vivax* and other hemoparasites of cattle in Guyana

Organism	Native Cattle (791)		Imported Cattle (228)	
	No.	%	No.	%
Trypanosoma vivax	5	0.6	0	0
Babesia spp.	46	5.8	48	21.0
Anaplasma marginale	88	11.1	35	15.4
Eperythrozoon spp.	0	0	3	1.3

(Adapted from Craig (1975).

Two of the calves bled were three and seven months of age and were noted by Craig to offer little resistance once roped, in comparison to the other three animals, which were adults and vigorously resisted restraint. Trypanosomes were found only in local cattle in this study (Table 1).

Trypanosoma vivax was later identified in some of the same imported cattle about a year later when they were between 18 - 30 months old (K.M.G.Adam, 1976, Personal Communication). The animals had been moved to a newly cleared area, with a sandy-loam type of soil, just south of the coastal zone, and at the time were undergoing severe stress due to the shortage of water. A number of deaths occurred among these animals and although trypanosomiasis is not recognized as a disease entity in this country, the significance of *T. vivax* as contributing to these deaths cannot be ruled out, as this organism is a known pathogen in this geographic area (Wells et al. 1982; Silva et al., 1995).

The tsetse fly, *Glossina* spp., is the known vector of *T. vivax* in Africa (Soulsby, 1982), where biological transmission occurs. In Guyana the vector of *T. vivax* is unknown, nevertheless New World mechanical transmission of *T. vivax* by *Tabanus nebulosus* has been experimentally demonstrated (Otte and Abuabara, 1991), and it can be assumed that tabanids will similarly transmit trypanosomes in Guyana.

Cattle observed by Craig (1975) with patent parasitemias were from herds on the coast where the higher density of animals would predispose to interrupted feeding by hematophagous insects, a condition required for effective mechanical transmission. No parasitemic animals were found in the Rupununi Savanahs bordering Brazil in Guyana's south-west, and it was postulated that the lower density of animals there may prevent vector transmission. Present joint studies in which Suriname and Guyana are working in collaboration with CIRAD-EMVT in French Guiana in determining the serological prevalence of *Babesia*, *Anaplasma* and *Trypanosoma* spp. in cattle, have shown that to date 35% of the cattle tested are exhibiting seroreactivity to *Trypanosoma* spp. with the ELISA. It is envisaged that mixed infections of *T. evansi* and *T. vivax* will be encountered as the former organism is widely distributed throughout the tropics with cattle being included in its host range (Fraser, 1986). *Trypanosoma evansi* causes mild disease in cattle and is mechanically transmitted by *Tabanus* spp. (Levine, 1985).

Trypanosoma theileri was not seen by Craig (1975) in blood films but this large non-pathogenic species is cosmopolitan in distribution and is likely to occur in Guyana. It is biologically transmitted by *Tabanus* and *Haematobia spp.* and is characteristically rarely seen in blood films (Levine, 1985).

TRYPANOSOMES IN EQUINES

Craig (1975) cites a lone report of infection of horses in the Rupununi with *T. evansi* which dates back to 1944. More recently trypanosomes were observed in horses in the same part of the country and were considered to be *T. evansi* (E. Elanchazian, 1976, personal communication). The range of distribution of *T. evansi* includes Central and South America where the infection can be fatal in horses and dogs (Fraser, 1986).

Among the vectors of *T. evansi* in Central and South America is the vampire bat, *Desmodus rotundus*, (Levine, 1985) which fact Craig (1975) suggests, may be a possible explanation for the presence of *T. evansi* and not *T. vivax* in the Rupununi, the vampire bat and other wild mammals acting as reservoir hosts of *T. evansi*.

Trypanosoma equiperdum is the causative organism of dourine, an infection of equines which is endemic in Asia, Africa, South America and a small portion of the southern United States (Blood et al., 1983). There are no reports of this disease in horses in Guyana but, if it does exist, it will most likely be found in the Rupununi on the border with Brazil.

TRYPANOSOMES IN SMALL RUMINANTS

The existence of trypanosomes in small ruminants in Guyana was first brought to the attention of the veterinary authorities when blood which was taken from a moribund lamb was found to be heavily parasitised (Applewhaite, 1990). These trypanosomes were identified as *T. vivax* on morphological characteristics (A. G. Luckins, 1985, personal communication). The lamb was euthanised and a diagnosis of Haemonchus subsequently made. Despite diagnosing both trypanosomiasis and haemonchosis, the animals on the farm of origin were only treated for the latter, and appeared in good health when the farm was visited again at the time of the study.

Hematological examination (thick blood smears) of blood samples from 197 sheep and 77 goats on 12 farms showed that nine sheep (4.6%) and one goat (1.3%) were parasitemic at the time that they were bled (Table 2). Comparative examination of thick blood smears and the hematocrit centrifuge technique (HCT) (Woo, 1971) indicated good agreement (75%) between the two methods in the case of the ovine blood (Table 3).

Table 2:   Trypanosome positive sheep, goats and farms on coastal Guyana as determined by haematological methods

Species	No. blood samples	No. samples positive for trypanosomes	% samples positive	% farms positive
Sheep	197	9	4.6%	44
Goats	77	1	1.3%	20%

Table 3:   Comparison of thick blood smears and the HCT* in detecting circulating trypanosomes in sheep and goats

Species	No. of Farms	Thick Smears		*HCT	
		+	-	+	-
Sheep	4	4	104	3	105
Goats	1	-	15	-	15

* Haematocrit Centrifuge Technique

Sera from this study were subjected to the ELISA (enzyme-linked immunosorbent assay) test in collaboration with the Centre for Tropical Veterinary Medicine, Edinburgh (Applewhaite, unpublished), and seroreactivity was detected in 125 of 197 (63.4%) ovine sera, and in 27 of 47 (61.7%) caprine sera (Table 4).

Table 4: Seroprevalence of *T. vivax* in sheep and goats on farms on coastal Guyana

Sera		No. +ve (%)	Farms	No. +ve (%)
Sheep	197	125 (63.4)	9	9 (100)
Goats	47	29 (61.7)	*3	3 (100)

* Goat sera was not tested from 2 of 5 farms

In a later baseline survey of ovine health in the same coastal area in which the previous study was conducted, indirect fluorescent antibody (IFA) assay of 161 sera detected *Trypanosoma spp.* antibody in 103 (64%) of the animals. Thirty-eight of these (23.6%) were positive for *T. evansi* only, 11 (6.8%) were positive for *T. vivax* only and 54 (33.5%) were positive for both (Table 5).

Table 5: Detection of IFA antibody in ovine sera in Guyana

No. Samples Tested	No. Positive *Trypanosoma sp* (%)	No. positive *T. vivax* (%)	No. positive *T. evansi* (%)	No. positive both *T.vivax & T. evansi* (%)
161	103 (64%)	11 (6.8%)	38 (23.6%)	34 (33.5%)

(Adapted from Vokaty et al. - 1993)

CONCLUSION

Both parasitological and serological techniques have been used to confirm the presence and determine the prevalence of *T. vivax* in cattle and small ruminants, respectively, in Guyana (Craig, 1975; Applewhaite, 1990; Vokaty et al., 1993). Although there is a difference in specificity in the techniques employed by Applewhaite (unpublished) and Vokaty et al., (1993) there is good agreement in the rate of infection with *Trypanosoma* spp. (*T. vivax* and *T. evansi*) of sheep and goats by the two techniques, this being between 61.7% and 64%.

Mixed infection of cattle by *T. vivax* and *T. evansi* has been demonstrated by ELISA serology in which 35% of the animals sampled seroconverted (Desquesnes, 1995, personal communication). Despite the proven pathogenicity of *T. vivax* for cattle in South America (Otte et al., 1994), bovine trypanosomiasis is not recognized as a disease in Guyana and livestock, at least in coastal Guyana, are not treated for it. Anemia in ruminants in Guyana is usually attributed to gastro-intestinal nematodes and/or *Babesia* and *Anaplasma* spp., and the relevant therapy instituted against these organisms.

The high levels of infection of ruminants in Guyana by *Trypanosoma spp.* as indicated by serological tests, would, however, warrant a closer look at the aetiology of anaemia and emaciation in these species, especially when the effects of *T. vivax* in neighbouring Brazil and Venezuela are taken into consideration (Otte et al., 1994; Silva et al., 1995).

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THE EPIDEMIOLOGY OF SALIVARIAN TRYPANOSOMES IN GUYANA

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ABSTRACT

The veterinary services of Suriname, French Guiana and Guyana, supported by CIRAD-EMVT in Cayenne and IICA in Guyana, embarked on a collaborative project in 1993 aimed at increasing knowledge of the epidemiology, clinical and economic importance of hemoparasites in the participating countries. The main organisms of interest were *Trypanosoma vivax* and *Trypanosoma evansi* in the bovine population. Bovine blood samples were taken from abattoirs and farms located in six of the ten administrative regions in Guyana using sample sizes based on the size of the cattle population in each region. Demographic details were recorded for each animal sampled. Samples were analyzed by direct microscopic examination of the·buffy coat, examination of thin, stained blood smear and serum analyzed using the indirect Enzyme-Linked Immuno-Sorbent Assay (ELISA) technique on sera. Microhematocrit analysis revealed a trypanosome prevalence of 3.3%, while the thin blood smear yielded a prevalence of 0.6%. The ELISA for *Trypanosoma vivax*, which was the most sensitive of the three tests, indicated a seroprevalence of 34.4%. These results are similar to those of Vokaty et al (1995). It would be useful in the future to determine the clinical and economic impact of *T. vivax* on the bovine population.

RESUMÉ

En 1993, avec le soutien du CIRAD-EMVT de Cayenne, et de l'IICA Guyana, les services vétérinaires du Suriname, du Guyana et de la Guyane Française, ont monté un projet de collaboration visant à accroître les connaissances de l'épidémiologie et de l'importance clinique et économique des hémoparasites présents dans les Guyanes. Les parasites les plus importants sont *T. vivax* et *T. evansi* chez les bovins. Des échantillons de sang de bovins étaient collectés aux abattoirs et dans des fermes localisées dans 6 des 10 régions administratives du Guyana; la taille des échantillons ayant été prédéterminée en fonction de la taille des effectifs régionaux et de la prévalence des infections estimée au cours d'une pré-enquête. Des données complémentaires sur l'origine, le statut et l'état des animaux étaient également enregistrées. Les échantillons étaient analysés par examen direct de tubes capillaires (technique de Woo), examen du buffy coat à l'état frais (technique de Murray), de frottis sanguins colorés, et diagnostic sérologique par recherche d'anticorps en ELISA-indirectes sur antigènes de *T. vivax* et/ou de *T. evansi*. La technique de Woo a révélé 3,3% d'échantillons positifs, et l'examen des frottis seulement 0,63%. L'ELISA-indirecte *T. vivax* a été beaucoup plus sensible, révélant une séroprévalence de 34,4% d'infections, ce qui est en accord avec les travaux de Vokaty *et al.* 1995. Il serait très utile à l'avenir, d'établir l'impact clinique et économique de *T. vivax* sur la population bovine et déterminer ses modes de transmission et vecteurs au Guyana.

RESUMEN

Los servicios veterinarios de Surinam, Guyana Francesa y Guyana, sustentados económicamente por el CIRAD-EMVT en Cayenne y el IICA en Guyana, enbarcados en un proyecto cooperativo en 1993, ayudaron a incrementar el conocimiento de la epidemiología, la importancia clínica y económica de los hemoparásitos en los países participantes. Los principales organismos de interés fueron el *Trypanosoma vivax* y el *Trypanosoma evansi* en la población bovina. Se tomaron muestras de sangre de mataderos y fincas, localizadas en seis de las diez regiones administrativas de Guyana, utilizando la información disponible sobre la población bovina por cada región de Guyana para determinar el tamaño muestreal. En las diversas áreas donde se efectuaron los muestreos, se registraron los datos demográficos por cada animal muestreado. Las muestras de sangre fueron analizadas por examinacion microscopia directa del frotis de capa blanca, de frotis delgados sanguineos coloreados y el suero fue analizado por la técnica de ELISA indirecta. La prueba del microhematocrito reveló una prevalencia del *Trypanosoma* de un 3.3%, mientras que en los frotis sanguíneos delgados coloreados fue del 0.63%. La técnica de ELISA para *Trypanosoma vivax*, fue la mas sensible de las tres pruebas e indicó una seroprevalencia de 34.4%. Estos resultados concuerdan con los hallazgos de Vokaty *et al.* (1995). Seria útil en el futuro establecer el impacto clínico y

económico de estos organismos en la población bovina y determinar su vector y el modo de transmisión de los trypanosomas en Guyana.

INTRODUCTION

The similarities and differences between French Guiana, Suriname and Guyana have led to varying degrees of interaction between their governments and people, and over the years hemoparasite detection work has been undertaken independently in Suriname and Guyana. In 1993, two international organizations involved in the improvement of animal health, the Inter-American Institute for Co-operation in Agriculture (IICA) and Centre de Co-operation International en Recherche Agronomique pour le Development - Elevage et Medicine Veterinaire des pays Tropicaux (CIRAD-EMVT) jointly developed a collaborative project involving the veterinary services of Suriname, Guyana and French Guiana, "Hemoparasite Network for the Guianas".

This project had as its broad objective, the pooling of veterinary resources existing within the three countries in an effort to generate and exchange information on hemoparasites in livestock. The project aimed to increase knowledge of the epidemiology, clinical and economic importance of haemoparasites in the participating countries in order to develop effective control methods for the improvement of the health and productivity of livestock in these countries. To this end a hemoparasite reference laboratory for the Guianas was created at the CIRAD-EMVT Laboratory in Cayenne, French Guiana. *T. vivax* and *T. evansi* were of particular interest to the project.

Guyana is divided into ten administrative regions and it was decided to utilize these established boundaries (Figure 1) and to design a proportional sampling strategy. Over 1,800 blood and serum samples were collected from Regions 2, 3, 4, 5, 6, 9 and 10 (Table 1); and the suspicions of the veterinary authority about the presence of trypanosomes in the country have been confirmed.

Trypanosoma vivax and *Trypanosoma evansi*, the organisms most commonly found in the Guianas, appear to be mechanically transmitted by biting flies in South America. Large rodents are believed to be a reservoir of *T. evansi* (Hoare, 1965). In Brazil there is evidence that outbreaks are influenced by the presence of domestic carriers such as cattle, dogs, horses; wildlife reservoirs; increases in vector population (tabanids etc.); and local husbandry practices (Vokaty et al, 1994).

Applewhaite, L, (1990) found a seroprevalence rate of 63.5% in sheep on ELISA. In 1992 a baseline survey for *Trypanosoma* spp. in Mahaica/Berbice, a coastal region of Guyana found that 64% of the sheep population tested serologically positive on Indirect Fluorescent Antibody (IFA) test (Vokaty et al., 1993). There had been no large scale prevalence survey of *Trypanosoma spp.* in cattle in Guyana until the present survey began in January 1994.

Based on the size of the cattle population in the various regions of Guyana, a sample size of 400 animals per region was pre-determined using a 95% confidence interval and estimated prevalence of 37%. This was calculated using the formula of Cannon and Roe.

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Figure 1:   Guyana Administrative Regions
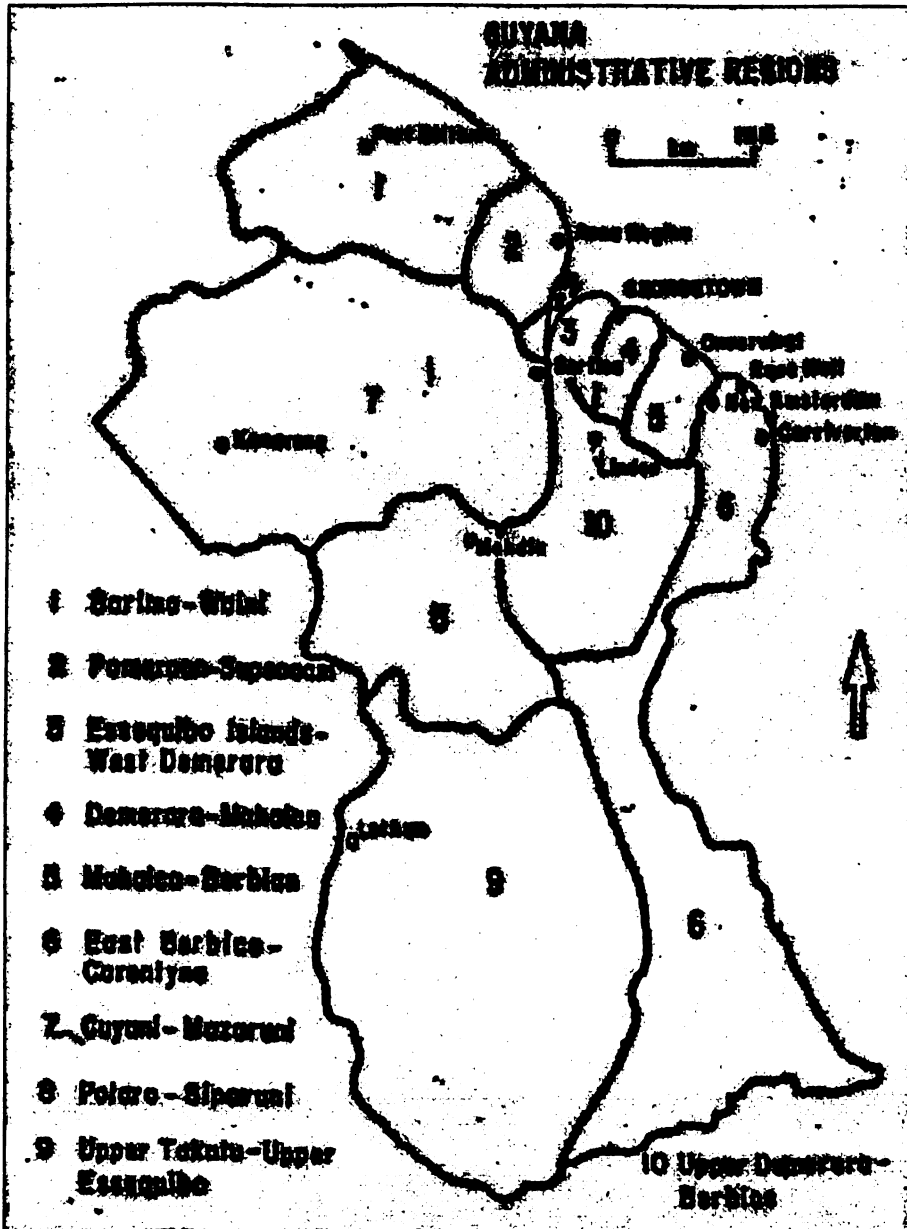
Table 1: Table showing number of bovine blood and serum samples taken in each region during the period 4 January 1994 - 30 September, 1996

REGION	NUMBER OF SAMPLES			
	MALES	FEMALE	UNKNOWN	TOTAL
2	55	185	-	240
3	55	213	-	268
4	35	213	-	236
5	163	138	2	303
6	141	254	2	397
9	90	210	-	300
10	10	52	-	62
Unknown	-	1	30	31
Totals:	549	1253	34	1837

MATERIALS AND METHODS:

Whole blood serum and samples were taken from randomly selected cattle at abattoirs and on farms and demographic data noted.

The blood samples were analysed via the microhaematocrit centrifuge technique and examination of the buffy coat, and by direct examination of thin blood smears. Serum samples were also tested using the Card Agglutination Trypanosome Test (CATT). Test kits were provided by the Tropical Medical Institute (IMT) in Belgium. This card test has since been discontinued due to inconsistencies in the results obtained, both here in Guyana and in French Guiana. Duplicate serum and thin blood smear samples obtained from each sample taken in Guyana were sent to Cayenne for comparison and ELISA testing. The results obtained for samples collected between 4 January, 1994 and 30 September, 1996 are presented in Table 2 and Table 3.

Table 2: Trypanosome results obtained on samples for the period (a) 4 January 1994 - 14 May 1995 and (b) 15 May 1995-30 September 1996 respectively[1]

No. of samples tested	Number positive and % prevalence					
	Microhaematocrit		Thin blood smears		ELISA	
	No.	(%)	No.	(%)	No.	(%)
957	32	3.34	6	0.62	329	34.3
880	5	0.56	-	-	-	-
1837	37	2.01	-	-	-	-

Table 3: Results of 957 samples taken between 4 January, 1994 and 14 May, 1995 showing variations in prevalence in the regions

Regions	Samples/Region	% Prevalence					
	No. of samples tested	Micro-haematocrit		Thin blood smears		Serology ELISA	
	Total	nos.	(%prev)	nos.	(%prev)	nos.	(%prev)
2	24	1	(4.17)	0	(0)	9	(37.50)*
3	104	6	(5.77)	4	(3.85)	35	(34.61)*
4	84	1	(1.19)	0	(0)	14	(16.67)
5	233	9	(3.86)	1	(0.43)	89	(38.20)*
6	184	12	(6.52)	1	(0.54)	82	(44.56)*
9	300	3	(1.0)	0	(0)	93	(31.00)
10	13	0	(0)	0	(0)	1	(15.38)
Unknown	15	0	(0)	0	(0)	6	(40.00)
Total	957	32	(3.34%)	6	(0.63%)	329	(34.38%)

* The above results indicate that the seroprevalence of trypanosomes in Guyana, after analyzing 957 of the 1,800 samples taken is approximately 34.4%. Region 6 (Corentyne), Region 5 (Mahaica/Berbice), Region 2 (Essequibo Coast) and Region 3 (West Demerara), have the highest seroprevalence.

DISCUSSION

The results obtained confirm that hemoparasites do exist in the cattle population in Guyana and the seroprevalence of 34.4% appears to be relatively high. This compares favourably with the seroprevalence of 30% obtained by Vokaty et al. (1995) in sheep in Mahaica/Berbice. It should be noted here that although the indirect ELISA for *T. vivax* detection will cross react with *T. evansi*, the figures suggest that trypanosomiasis may be endemic in some parts.of Guyana.

In Guyana and more specifically in the Rupununi (Region 9), farmers recalled symptoms indicative of *T. evansi* infections in horses which may have occurred 20 years ago, however, nothing has been observed or reported since, and no clinical cases of trypanosomiasis in cattle have ever been reported in Guyana. A small survey conducted in 1995 found no *T. evansi*.

To date the economic impact of trypanosomiasis on the cattle industry in Guyana has not been determined. It is

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[1] The samples are sorted by date because thin blood smears and ELISA test were not conducted after May 14, 1995.

hoped that a research project would be designed with the specific objective of establishing the economic impact and identifying the vector.

As a result of this project, bovine serum banks have been established in Guyana and Suriname which will also serve the purpose of serological surveillance for brucellosis, infectious bovine rhinotracheitis, bovine leukosis and foot and mouth disease in Guyana and Suriname.

ACKNOWLEDGMENTS

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EPIDEMIOLOGY OF *T. VIVAX* IN BOVINES AND OVINES IN FRENCH GUIANA

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ABSTRACT

Further to an epidemiological survey carried out in cattle in 1991-92, epidemiological surveillance in cattle and a complementary survey in sheep were carried out between 1993 and 1996, with parasitological and serological techniques (indirect-ELISA *T. vivax*). In sheep, 163 samples were collected in 1991-93; all were negative. Of the 164 samples collected in 1994, several showed subclinical infection by *T. vivax*, and others showed drastic clinical signs including abortion. The parasite was observed on one farm only. In cattle, 495 samples were collected in 1992-94. The parasite was not observed, but the mean seroprevalence rate was 7.8%. In 1994-95, during an outbreak of trypanosomiasis, 183 samples were collected. The parasite was observed on seven of the eight farms investigated, with a mean seroprevalence rate of 50%. In 1995-96, 863 samples were collected: the parasite was observed in a single animal among 309 samples from one farm, and numerous animals in an outbreak in imported cattle. The mean seroprevalence rate during that period was about 26%. It was observed that sheep and cattle can be infected clinically or subclinically depending on the management level of the farm, especially supply of water and food supplies. Incidence rates above 70% were observed during all seasons. During periods of low tabanid prevalence; crepuscular tabanids of the genus *Chlorotabanus* were suspected to be a vector of the disease. Self cure was observed in cattle at a mean rate of 15-20% a year. Inapparent circulation of the parasite was observed on a cattle farm with an adequate management level at a rate of 2% a month. Sheep may have an important role as a reservoir for *T. vivax* in cattle. Trypanosomiasis due to *T. vivax* is a permanent threat to cattle and sheep production in French Guiana.

RESUMÉ

A la suite d'une enquête épidémiologique sur les trypanomoses menée chez les bovins de Guyane Française, en 1991-1992, une enquête complémentaire chez les ovins et une épidémiosurveillance ont été mises en place chez les bovins et ovins, de 1993 à 1996, à l'aide de diagnostics parasitologiques et sérologiques (ELISA-indirecte *T. vivax*). Chez les moutons, 163 échantillons de sérum collectés entre 1991 et 1993 etaient tous négatifs; 164 échantillons sanguins collectés en 1994 ont montré des infections subcliniques dans quelques cas, et dans d'autres, des infections cliniques avec symptômes abortifs et amaigrissements prononcés; le parasite n'a toutefois été observé que dans une seule ferme. Chez les bovins, 495 échantillons ont été collectés en 1992-94; le parasite n'a pas été observé mais la séroprévalence s'élève à 7,8%. 183 échantillons ont été prélevés en 1995-96, durant une épizootie multifocale; le parasite a été observé dans 7 fermes sur 8, avec une séroprévalence moyenne de 50% sur 863 échantillons prélevés en 1995-96, le parasite n'a été observé que chez un seul animal parmi 309 dans une ferme. Chez de nombreux animaux dans une autre ferme d'animaux récemment importés présentant un foyer actif d'infection. La séroprévalence moyenne durant cette période a été de 26%. Il a été observé que les moutons et les bovins peuvent être porteurs du parasite, de manière clinique ou subclinique, selon la qualité des paramètres zootechniques, spécialement l'alimentation et l'abreuvement. Quelle que soit la saison, des incidences supérieures à 70% ont été enregistrées, même pendant la période d'activité minimale des taons; les espèces crépusculaire du genre *Chlorotabanus* sont soupçonnées de transmettre le parasite. Des taux moyens de stérilisation spontanée ont été évalués à 15-20% par an. La circulation inapparente du parasite a été observée dans une ferme de bon niveau zootechnique avec une incidence mensuelle d'environ 2%. Les moutons peuvent avoir un rôle important dans l'épidémiologie de la trypanosomose bovine. La trypanosomose à *T. vivax* est une menace permanente pour les élevages bovin et ovin de Guyane Française.

RESUMEN

Adicionalmente al examen epidemiológico llevado a cabo en el bovinos en 1991-92, se llevó a cabo una vigilancia epidemiologica en bovinos y un estudio complementario en ovinos, entre 1993 y 1996, utilizando técnicas parasitológicas y serológicas (ELISA-indirecta para *T. vivax*). En los ovinos, se colectaron 163 muestras entre 1991-93 y todas resultaron negativas. 164 muestras colectadas en 1994, demostraron una infección subclínica por *T. vivax* en algunos casos, y síntomas drásticos, incluyendo el aborto, en otros casos. Solo se observó este parásito en una sola finca. En el ganado bovino, se colectaron 495 muestras entre 1992-94. Aunque no se observaron parásitos, la seroprevalencia promedio fue de un 7.8%. 183 muestras se colectaron entre 1994-95, durante un brote de

trypanosomiasis. Observándose el parásito en siete de las ocho fincas estudiadas investigadas, con una seroprevalencia promedio de un 50%. 863 muestras se colectaron entre 1995-96, el parásito se pudo observar en un solo animal de las 309 muestras tomadas en una granja, y en numerosos animales durante un brote en ganado importado. La seroprevalencia promedio durante ese periodo fue alrededor del 26%. Se observo que los ovinos y bovinos podían ser infectados clínicamente o subclínicamente dependiendo del nivel de manejo de la finca, especialmente en lo referente a suministros de agua y alimento. Se observaron tasas de incidencia por encima del 70% durante todas las estaciones, durante el periodo de baja prevalencia de tábanidos; se sospechó que los tabánidos crepusculares del género *Chlorotabanus* pudieran ser el vector de la enfermedad. La auto-cura (self-cure) se observó en los bovinos con una tasa promedio de un 15-20% por año. Una inaparente circulación de los parásitos también fue observada en una finca bovina con un adecuado nivel de manejo, a una tasa del 2% por mes. Los ovinos pueden ser un importante reservorio epidemiológico del *T. vivax* para el ganado vacuno. La Trypanosomiasis debido al *T. vivax* es una amenaza permanente para el ganado de producción, bovino y ovino, en la Guyana Francesa.

INTRODUCTION

Following a seroprevalence survey of *T. vivax* in cattle carried out in 1991-92, epidemiological surveillance in cattle was implemented during the next four years to study the analytical epidemiology of bovine trypanosomiasis. A retrospective survey was carried out in sheep, and new sampling was initiated in 1994.

SHEEP SURVEY AND SURVEILLANCE

The survey included 163 animals from 17 farms sampled in 1991-93, and 164 animals sampled from 7 farms sampled in 1994. One of the farms found to be actively infected was sampled again 45 days later. The total number of samples taken in 1994 was 209. Results are partially reproduced in Table 1, where only typical situations are presented. Since the epidemic of 1988-89, *T. vivax* was identified for the first time in sheep in July 1994.

Table 1:   Parasitological and serological results on sheep farms between 1991 and 1996

N° of the Farm	Date of sampling	Number of samples	Positives in HCT *	% positives in HCT	Positives in indirect- ELISA *T. vivax*	% positives in indirect- ELISA *T. vivax*
Farm S1	04/91	9	0	0%	0	0%
Farm S1	13/09/94	12	0	0%	0	0%
Farm S10	03/92	11	0	0%	0	0%
Farm S10	16/09/94	30	0	0%	4	13%
Farm S12	04/91	21	0	0%	0	0%
Farm S12	28/07/94	31	3	9,6%	9	29%
Farm S12	13/09/96	37	8	21.6%	22	59%
Farm S14	12/91	10	0	0%	0	0%
Farm S14	24/08/94	34	0	0%	2	6%

*positives in HCT: confirmed as *T. vivax* infections by blood smear examination

Farm S1 was not infected. Farms S10 and S14 had inapparent infection between the two samplings. No clinical signs were recorded by the farmers. On S10, where there were no introductions of new sheep between the two samplings, infection occurred inapparently in this farm and the origin of the infection could not be established. On the other farm, positive samples came from recently acquired animals which were probably healthy carriers,

Farm S12 was infected in early 1994 and incidence reached 30% within 45 days. In this recent infection, HCT yielded a high prevalence of 22% and serology reached 60%. Treatments were established to control the infection and reduce the clinical signs of weight loss and abortion. It is probable that incidence would have reached 100% in the absence of treatment. On this farm, sheep had been bought from a herd found to have been infected during the

epidemic of 1988-89; resurgence of the active infection and contamination of naïve animals from these healthy carriers was suspected. Farm S12 had both cattle and sheep, and the cattle were found serologically positive.

BOVINE SURVEILLANCE

Parasitological examinations through HCT and stained blood smear, and serological examinations through indirect-ELISA *T. vivax* were carried out on 2,566 samples.

1992-1994
A total of 495 samples were collected from 10 farms. The period was considered as inter-epidemic since all parasitological examinations were negative, and there were no clinical signs of trypanosomiasis. Seroprevalence rates ranged from 0% to 50% with a mean of 7.8% which was a reflection of various epidemiological situations.

Farm B7 had a seroprevalence rate of 56% during the survey of 1991-92. In 1994 the seroprevalence in 53 animals below 15 months of age was nil; but seroprevalence in 112 animals above four years of age was 12%. It appears that there was no contamination of the young herd but that the parasite was still present in older animals. However the seroprevalence in the older animals had decreased indicating that self-cure occurred at a mean rate of about 15% a year, as no sterilizing trypanocidal treatment was available in French Guiana before 1996.

1994-1995
A total of 183 animals were sampled on eight farms. Four herds were sampled two to three times at several week intervals to evaluate the incidence of the infection. A total of 330 samples was collected during this period. The parasite was found for the first time in cattle in September 1994, on several farms, with obvious clinical signs, loss of weight and condition. This period was considered as an epidemic period. The mean seroprevalence rate was about 50%. Results are presented in Table 2, and discussed below.

Table 2:   Parasitological and serological results in bovine farms during epidemic period

N° of the Farm	Date of sampling and letter of the herd	N° of cattle in the farm and/or in the herd	Number of samples	Positives on HCT*	% positives in HCT	Positives in indirect-ELISA *T. vivax*	% positives in indirect-ELISA *T. vivax*
Farm B11	29/09/94 A	40/300	36	1	3%	1	3%
	18/10/94 A	40/300	38	0	0%	1	3%
	21/10/94 B	70/300	62	12	19%	15	24,2%
	28/12/94 B	70/300	69	0	0%	64	92,7%
Farm B12	14/09/94 A	20/70	15	4	26,6%	15	100%
	06/10/94 B	30/70	24	5	21%	11	45,8%
	02/12/94 B	30/70	22	11	50%	14	63,6%
Farm B13	06/09/94 A	20/80	13	3	23%	5	38,4%
	02/12/94 B	60/80	55	3	10%	48	87,3%
Farm B14	15/11/94	127	64	6	9,4%	24	37,5%
	02/03/95	127	15	4	27%	13	86,7%
	16/05/95	127	25	0	0%	25	100%
Farm S12/B15	13/09/94	30	13	0	0%	9	69,2%
Farm B16	10/11/94	30	24	1	0%	xxxxxxxxxx	xxxxxxxxxxx
Farm B5	28/01/95	44	30	1	1%	3	10%
Farm B9	07/02/95	200	15	1	6,6%	0	0%

*positives in HCT: confirmed as *T. vivax* infections by blood smear examination

On Farm B11, the infection was not circulating in herd A during the time of sampling. In herd B, the infection was active and circulating with a very high serological incidence of 68% within two months, during the period of the year

when the activity of tabanids is at its maximum. These observations confirm the fact that tabanids are short distance vectors.

On Farms B12 and B14 active infections were observed with high parasitological prevalence, reaching 50% on B12 and 100% on B14, within six months. These observations confirm the fact that tabanids are potentially highly efficient vectors.

On Farms S12 and B15 the seroprevalence rates reached 70% in cattle and 60% in sheep. These mixed farms are an excellent illustration of the epidemiological relationship of *T. vivax* in cattle and sheep.

Farm B9 had a recent infection, with positive results to parasitological tests, but negative results to serological tests. Treatment was applied early so the sampling was not done again. The origin of the infections could not clearly be established but two hypotheses were favoured, which may both be true. It is possible that a herd from area 1, with a low level of infection, had been spread all over the country by sale; the outbreaks observed corresponded to the distribution of the animals sold. Alternatively, the herds where the outbreaks were observed could have been previously infected in 1989, or have bought or exchanged cattle from infected farms, with clinical signs re-appearing because of the shortage of water and grass due to the dry season. In both cases, the infection spread throughout the herds due to high tabanid activity.

1995-1996
Eight hundred and sixty-three animals were sampled on nine farms. Two herds were sampled at intervals. In one case there was evidence of asymptomatic infections. In the other case there was some incidence of symptomatic infections which were subsequently cured with isometamidium chloride. *T. vivax* was only found on two occasions, once in an asymptomatic situation, and once during an outbreak in a herd of cattle imported from metropolitan France and naïve to *T. vivax*. Clinical signs of *T. vivax* infections were not observed or suspected in the rest of the country during this period.

Results of the 1995-1996 sampling are presented in Table 3 and discussed below.

Table 3: Parasitological and serological results in bovine farms during post-epidemic period

N° of the Farm and letter of the herd	Date of sampling	N° of cattle in the herd	Number of samples	Positives in HCT*	% positives on HCT	Positives in indirect-ELISA T. vivax	% positives in indirect-ELISA T. vivax
Farm B17	Jan-96	60	45	0	0%	0	0%
Farm B5	Jan-96	44	20	0	0%	0	0%
Farm B7	Jan-96	400	14	0	0%	0	0%
Farm B12 A	Jan-96	70	16	0	0%	5	31%
Farm B18	Feb-96	350	49	0	0%	0	0%
Farm B19	Mar-96	20	11	0	0%	2	18%
Farm B14	Apr-96	127	73	0	0%	26	36%
Farm B18	Apr-96	370	24	0	0%	0	0%
Farm B9	May-96 A	200	140	0	0%	16	11,4%
Farm B9	May-96 B	200	29	0	0%	2	7%
Farm B5	May-96	44	20	0	0%	0	0%
Farm B11	May-96	309	309	1	0,3%	92	29,7%
local herd	jul-96	309	59	1	1,7%	13	29%
asymptomatic	Oct-96	309	206	0	0%	82	39,8%
Farm B12	Jun-96	60	53	0	0%	0	0%
Farm B15	14 Jun-96	65	12	3	25%	9	75%
imported	18 Jun-96	65	53	9	17%	28	53%
and local	25 Jun-96	65	59	13	22%	26	44%
herds	08 Aug-96	65	45	0	0%	22	49%
	11 Sep-96	65	47	0	0%	22	47%
	10 Oct-96	65	33	0	0%	4	12%

*positives in HCT: confirmed as *T. vivax* infections by blood smear examination

Farm B11 had a high management level, and satisfactory control and surveillance of the animals. No clinical signs were recorded during the entire period of epidemiological surveillance. The total cattle population of the farm was sampled in May 1996 and the parasite was found in one animal, at a parasitological prevalence of 0.3%, and a serological prevalence of 29%. The infected animal was negative to indirect-ELISA at the first sampling, but positive at the second sampling, indicating recent infection. The seroprevalence of the herd increased from 22% in May to 40% five months later. Insect trapping could not be organized, but observations indicated the presence of stomoxes and crepuscular tabanid species of the genus *Chlorotabanus (Chl. mexicanus)*.

Farm B15 had a low management level and cattle and sheep had been found to be infected in the past. In June, imported cattle began to display acute clinical signs and a mean weight loss of about 40 kg per animal was recorded, with body temperatures above 39°C in half of the infected animals. Parasitological examinations indicated a high prevalence of active infections reaching 22% positive results by the end of June. Among the imported cattle, the incidence reached 70% within two months, coinciding with a period when the tabanid activity was at its minimum. Once again, insect trapping could not be organised, but observations indicated an abundance of a crepuscular species of tabanus from the genus *Chlorotabanus*.

CONSIDERATIONS ON TABANIDS AS VECTORS AND PARASITES

Annual variations in the population of four tabanid species important in livestock are indicated in Figure 1. Diurnal variation of activity is shown in Figure 2. Abundance was estimated from the number of insects caught in the Malaise trap between 6.00 a.m. and 7.00 a.m. Data on tabanids are from Raymond and personal communications.

Figure 1:   Seasonal variations in tabanids activity

a: *Tabanus importunus*
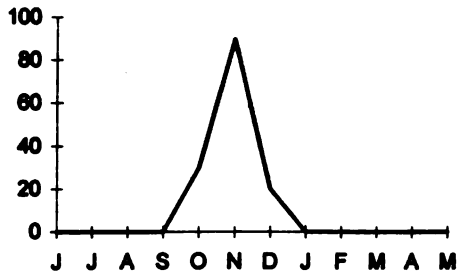
b: *Cryptotylus unicolor*
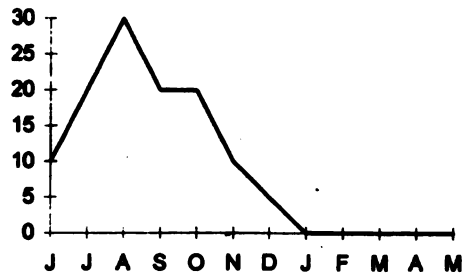
ç: *Phaeotabanus cajennensis*
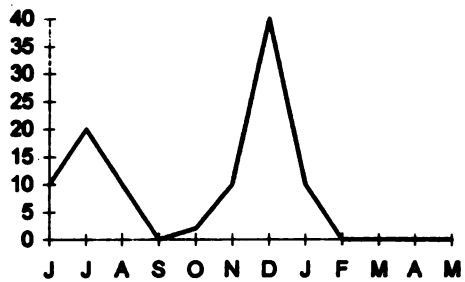
d: *Tabanus occidentalis dorsovittatus*
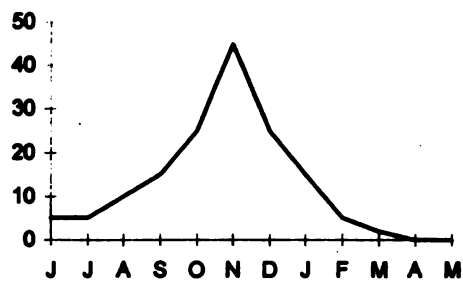
e: all *species*
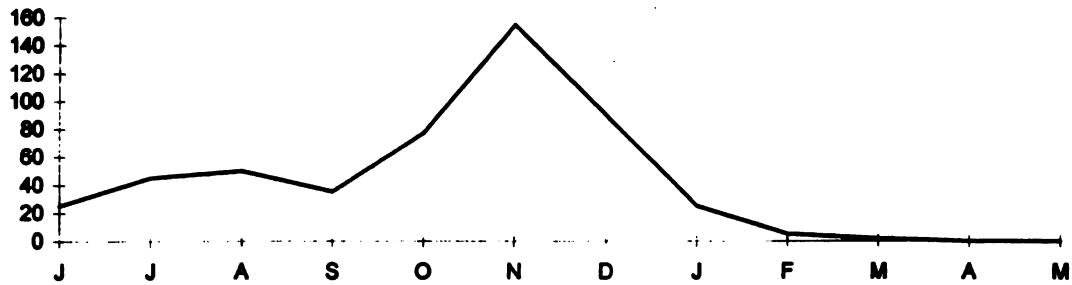
Figure 2: Diurnal variations of tabanids activity
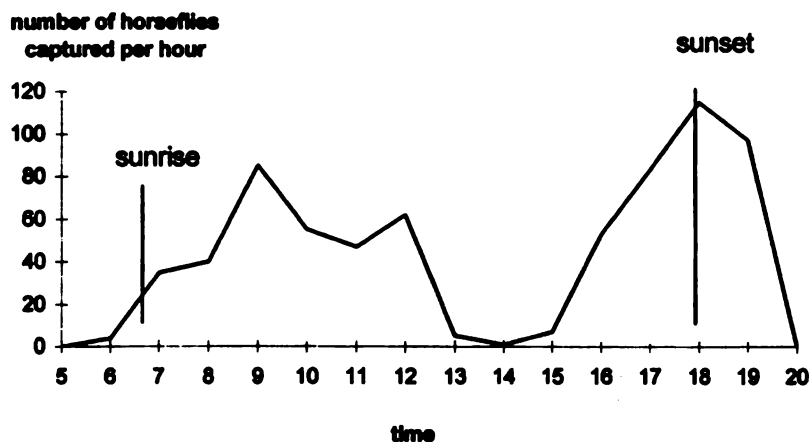
Weight loss caused by tabanids has been evaluated in a herd with satisfactory control of parasites and hemoparasites and adequate food and water supply. Monthly mean weight gains were measured over a period of 23 months.

It appears that weight gains are negatively correlated to tabanid activity, as illustrated in Figure 3. Data on weight gain are original data from de La Rocque and Desquesnes (unpublished); data on tabanid activity are from Raymond (1988 and 1989).

Figure 3: Monthly variations of tabanid activity and weight gains in a well controlled cattle herd
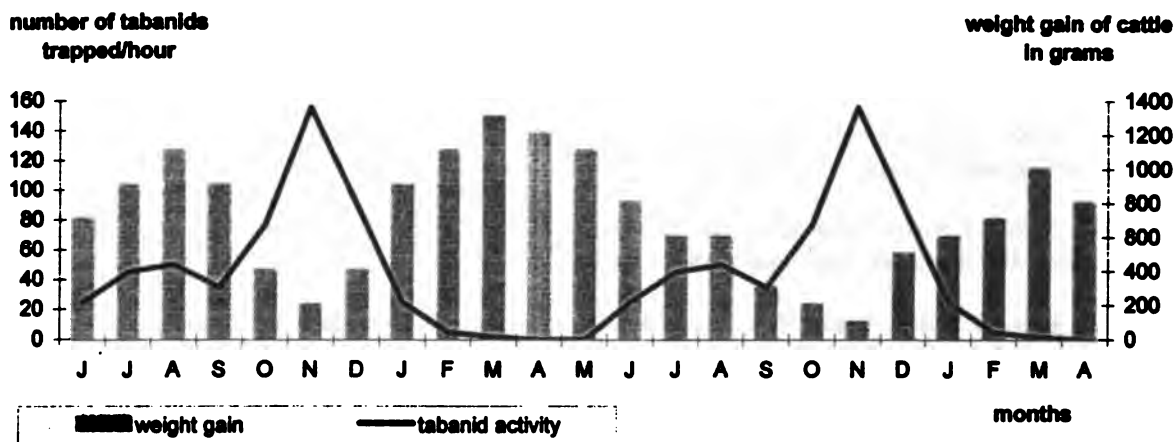
Tabanids are not only vectors of disease, they have a direct, highly significant effect on weight gain in cattle. Trypanosomiasis epidemics generally, but not invariably occur during the tabanid season, as discussed above.

CONCLUSIONS

Clinical signs

It appears that host factors are determinants of the course of *T. vivax* infection and, eventually, *T. vivax* disease. Cattle and especially sheep infected with *T. vivax* have variable clinical manifestations. Depending on the management of the farm, clinical signs may vary from nil, to significant loss of weight and condition, and even abortions in ewes. If the management level of a farm is satisfactory, clinical signs may be absent and the infection

inapparent even if the trypanosome is circulating: an incidence of 2-5% was observed in such conditions. Where the management of the farm, especially nutrition, is low, clinical signs are apparent, and may even be acute if the general immunity of the herd is nil, as in the case of imported cattle described above: an incidence above 70% was observed in such conditions. Up to 20% of cattle may still be positive three years after an outbreak, although clinical signs may be absent during these three years.

Transmission
Sheep and cattle can act as reservoirs of *T. vivax* for several years. *T. vivax* is transmitted from herd to herd by infected cattle or sheep, and is transmitted within the herd by biting insects. Incidence of infection may be high throughout the year, and in June may be dependent on species of tabanids that have crepuscular activity (*Chlorotabanus* species). Stomoxes could also play a role. Since incidence rates of infection of above 70% have been recorded throughout the year, it appears that mechanical transmission is never a limiting factor.

Self cure
In the absence of sterilizing trypanocide treatment, a herd of 23 cattle which were all positive in December 1994 presented six negative animals in May 1996, indicating a mean annual self cure rate of about 20%. Similarly, in farm B9, seroprevalence decreased from 56% to 12% between 90 and 93, indicating a mean annual self cure rate of 15%.

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ANAPLASMOSIS AND TRYPANOSOMIASIS AS SEEN IN SURINAME BETWEEN 1994 AND 1996

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ABSTRACT

Trypanosomiasis and anaplasmosis are recognized in Suriname and are currently being studied through the hemoparasite project of the Guianas, which started in 1994. To date a total of 1,842 animals have been sampled. The clinical effects on the animals are discussed. Distribution of trypanosomiasis and anaplasmosis in the districts are highlighted.

RESUMÉ

L'anaplasmose et la trypanosomose sont présentes au Suriname et sont actuellement à l'etude dans le cadre du réseau d'information sur les hémoparasitoses dans les Guyanes mis en place depuis 1994. A ce jour un total de 1842 animaux ont été prélevés. Les observations cliniques sont presentées et discutées. La distribution de l'anaplasmose et de la trypanosomose dans les différentes régions administratives du Suriname sont présentées.

RESUMEN

La trypanosomiasis y la anaplasmosis son conocidas en Surinam pero han sido mejor estudiadas a través del proyecto de hemoparásitos de las Guyanas, el cual se inició en 1994 con un total de 1842 animales muestreados a la fecha. Se discutirán los efectos clinicos sobre el animal. Se analizará detalladamente la distribución de la trypanosomiasis y anaplasmosis en los distritos del país.

INTRODUCTION

The Hemoparasite Information Network for the Guianas was a project initiated by IICA, CIRAD-EMVT and the veterinary services of Suriname, Guyana and French Guiana. To date 1,860 animals have been sampled in Suriname during the course of the project. Most of the samples were collected in the slaughterhouse where the animals underwent an antemortem examination and every carcass was inspected immediately after slaughtering, with the result that none of the sampled animals were visibly sick nor had high fever or extreme cachexia. Whenever a vehicle was available, farms were visited to obtain a medical history of the animals sampled in the slaughterhouse; however poor record keeping in the slaughterhouse made it impossible to trace the origin of 153 animals.

Suriname has ten districts, and these were divided into six regions, for the purposes of the study. The majority of cattle are located in the district of Wanica, around the capital. Nickerie district has the second largest number of cattle. Four hundred samples are to be collected from each of five regions, but in the sixth region where there are very few animals only 37 samples were collected. Blood collection is currently being carried out in Para.

The distribution of Babesia, Anaplasma and *Trypanosoma* species in the samples collected to date is shown in Table 1.

BABESIOSIS

Although clinical babesiosis does exist in Suriname and is regularly seen in Holstein dairy cattle, we were not able to collect blood from an animal with parasitemia. Suspicious bloodsmears were seen in all districts but babesiosis was difficult to confirm as there were only one or two parasites per smear.

ANAPLASMOSIS

Anaplasmosis was seen in all breeds of cattle although Holstein cows were the most sensitive to illness. The parasite exists in all regions, however without statistical analysis of the results it was difficult to confirm the apparent slight difference in the distribution of the parasite. One or two cows on each farm, usually creole crossbreeds, were found to be positive for anaplasmosis. These were usually well fed animals without any clinical signs, and with a hematocrit between 15 and 20, but sometimes up to 30.

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Table 1: Prevalence of *Babesia*, *Anaplasma* and *Trypanosoma* species in cattle sampled

Region	District	Total no. of samples	*Babesia*	*Anaplasma*	*T. vivax*	*Trypanosoma* spp.	*T. theileri*
I	Paramaribo Wanica	451	1?	13	3	13	4
II	Para Brokopondo	123	1?	2	1	3	2
III	Commewijne Marowijne	357		8	2	9	14
IV	Coronie Nickerie	352	1?	4	2	15	10
V	Sipaliwini	397	1?	8	2	14	12
VI		37		1			
Unknown Origin		153		1	2	2	

Two cases of anaplasmosis were observed:

a) On 3 March 1994, ten cows (approximately 7/8 Holstein) were sampled on a dairy farm and found to be negative both on the CCT test[2] and stained bloodsmears. On 14 August 1996 and on 17 August, 1996 the farmer lost two recently purchased pregnant Holsteins (two and three years old), with signs of possible anaplasmosis. On 6 September 1996 three out of ten animals on the farm tested positive for anaplasmosis.

b) In May 1994, three Holstein calves were found to be highly infected with *Anaplasma marginale* (hematocrits: 15 and 20). They were treated with imidocarb and tetracycline and eventually recovered.

TRYPANOSOMIASIS

Trypanosomes were found in all districts except Sipaliwini, however this may have been due to the small number of animals sampled in this district.

The majority of positive samples were collected during the rainy seasons, peaking in the middle of June when rainfall is at its highest. During the more moderate rainfall months of December to January, a smaller peak was noticed. In 1996 the rainfall in February was heavier than normal and more trypanosomes were found in February with an extension into March which was quite unusual.

One to three (1 to 3) trypanosomes were found in the buffy coat with the CCT test, however it was difficult to determine the species because the parasites were not detected in smears made from the buffy coat. *T. theileri* was commonly seen in all districts except for Sipaliwini. *T. vivax* was often seen in blood samples but clinical signs of trypanosomiasis were not observed in cattle.

Twice animals with high parasitemia of trypanosomes were found during blood collection on farms:

a) On 19 April 1994 *T. vivax* was detected in a creole/zebu crossbreed without clinical signs of disease. The other cows on the farm were half Holstein. The affected cow was the most active cow of the group and the owner did not notice anything amiss with the animal.

b) On 26 January 1996 numerous trypanosomes were found in a blood sample of a zebu on a large zebu farm. There were approximately 50 trypanosomes in the buffy coat and 0-2 trypanosomes per field in

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[2] Capillary Centrifuge or WOO Test

the direct examination of full blood. The animal did not seem to be sick and had a normal body score and a hematocrit of 30.

The study was unable to confirm trypanosomiasis as a disease entity in Suriname. Although it may have an effect on productivity, nutrition is still the most limiting factor of productivity of cattle in Suriname.

SEROPREVALENCE OF *T. VIVAX* AND OTHER HEMOPARASITES IN CATTLE IN FRENCH GUIANA

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ABSTRACT

An epidemiological survey was carried out in cattle in French Guiana, with parasitological techniques, namely the WOO test and blood smear examination, and nine serological ELISA tests for antigen and/or antibody detection for several *Trypanosoma* sp., *Babesia* sp., *Anaplasma marginale* and *Theileria mutans*. The serological techniques provided by ILRI are still under evaluation. Five cattle producing areas were identified in the coastal area of the country, and 2953 cattle were sampled between 1991 and 1992. Four herds, numbering 200 head, were regularly sampled for the observation of seasonal variations. Of 509 observations on capillary tubes (Woo test), 7.6% showed trypanosomes identified as *Megatrypanum* species. Since *T. vivax* was not observed, it was concluded that the survey was conducted during a post-epizootic period. *A. marginale* was observed in 28% of the 442 stained blood smears observed, and *B. bigemina* in 3 samples. The 2953 serum samples tested found *Trypanosoma* sp. antibodies present in 22% of the cattle, with 12% positive for antigens of *T. vivax*, 20% for *T. brucei* and 18% for *T. congolense*. The last two tests were considered as cross reacting with *T. vivax* since neither *T. evansi* nor *T. congolense* are present in French Guiana. There was no seasonal variation, but dairy cattle had higher prevalence rates than beef cattle, probably due to different management practices. Seroprevalence of *A. marginale* antigens was 61%, in agreement with the epidemiological findings, since anaplasmosis is highly endemic in the country. Similarly, high seroprevalence rates were found with tests for *Babesia* sp.: 62% for *B. bovis*; 22% antigens and 67% antibodies for *B. bigemina*. Antibody detection for *Theileria mutans* was also performed, but all were negative, confirming the fact that this parasite is not present in the country. Re-evaluation of the antigen-ELISA for *Trypanosoma* sp. appears to be necessary.

RESUMÉ

Une enquête épidémiologique a été menée en Guyane Française chez les bovins, à l'aide de techniques parasitologiques (test de Woo, technique de Murray, examen de frottis colorés) et de neuf tests sérologiques (détection des les antigènes et/ou les anticorps) pour *Trypanosoma* spp *Anaplasma marginale*, *Babesia bigemina*, *B. bovis* et *Theileria mutans*. Les techniques sérologiques ont été fournies par l'ILRI et sont en cours d'évaluation. Cinq aires d'élevage ont été définies sur la côte du pays; 2953 bovins ont été prélevés entre 1991 et 1992, et quatre troupeaux présentant un total de 200 têtes ont été prélevés régulièrement afin d'observer d'éventuelles variations saisonnières. Parmi 509 examen directs des tubes capillaires, 7,6% présentaient des trypanosomes, toujours en très faible nombre, et identifiés pour la plupart à *T. theileri*. *T. vivax* n'a pu être observé pendant toute la durée de l'enquête, mais le parasite ayant été retrouvé par la suite, la période d'enquête a été considérée comme inter-épizootique. *Anaplasma marginale* a été observé dans 28% des 442 frottis testés, et *B. bigemina* dans 3 cas seulement. Les 2953 sérums testés ont révélé les séroprévalences suivantes : anticorps anti-trypanosomes 22%, antigènes de *T. vivax* 12%, antigènes de *T. brucei* (détection des antigènes de *T. evansi*) 20%, et antigènes de *T. congolense* 18%. Les séroprévalences indiquées par les deux derniers tests sont considérés comme des réactions croisées avec les antigènes de *T. vivax* car les deux parasites n'ont jamais été signalés en Guyane Française. Aucune variation saisonnière des infections n'a été enregistrée, mais les vaches laitières avaient des séroprévalences plus élevées que le bétail d'embouche, probablement du fait des conduites d'élevage très différentes. La séroprévalence des antigènes d'*Anaplasma marginale* a été de 61%, ce qui est en accord avec les observations épidémiologiques réalisées sur le terrain: l'anaplasmose est hautement enzootique, et les cas cliniques sont rares. De même de très fortes séroprévalences sont enregistrées pour les babésioses (*B. bovis* 22% ; *B. bigemina* : 22% d'antigènes et 67% d'anticorps), et sont conformes aux observations de terrain. La détection des anticorps dirigés contre *Theileria mutans* s'est avérée négative dans tous les cas, ce qui confirmait l'absence de ce parasite. La ré-évaluation des tests de détection des antigènes de Trypanosomes (antigènes-ELISA) est apparue impérative.

RESUMEN

Se llevó a cabo un estudio epidemiológico en bovinos en la Guyana Francesa, utilizando técnicas parasitológicas, incluyendo la prueba de WOO y el frotis sanguíneo, y nueve pruebas de ELISA para la detección de antígenos y/o anticuerpos para varias especies: *Trypanosoma sp., Babesia sp., Anaplasma marginale y Theileria mutans*. Los kits de serologia suministrados por el ILRI están todavía bajo evaluación. Se seleccionaron cinco áreas de ganadería en la zona costera del país. Muestreándose 2953 ejemplares de ganado vacuno entre 1991 y 1992, y cuatro manadas, para un total de 200 cabezas muestreadas regularmente para la observación de una posible variación estacional. Entre 509 observaciones de tubos capilares, el 7.6% mostró Trypanosomas identificadas como especie de *Megatrypanum*. Ya que el *T. vivax* no fue observado, se concluyó que la investigación fue llevada a cabo durante un período post-epizoótico. El *A. marginale* se detectó en un 28% de los 442 frotis sanguíneos coloreados observados, y *B. bigemina* solo en tres muestras. Del examen de 2953 muestras de suero se indica que los antígenos de *Trypanosoma sp.* se encontraron presentes en un 22% de los bovinos, con un 12% de positividad para antígenos de *T. vivax*, 20% para *T. brucei* y un 18% para *T. congolense*. Las últimas dos pruebas se consideraron una reacción cruzada con el *T. vivax* ya que ni el *T. evansi* ni el *T. congolense* están presentes en la Guyana Francesa. No hubo variaciones estacionales, pero el ganado lechero tuvo una mayor prevalencia que el ganado de carne, probablemente por los diferentes sistemas de manejo. La seroprevalencia de los antígenos para *A. marginale* fue de un 61%, estando en concordancia con los hallazgos epidemiológicos, ya que la anaplasmosis es altamente endémica en el país. Igualmente, se encontró alta seroprevalencia de *Babesia sp.*: 62% para *B. bovis*, 22% de antígenos y 67% de anticuerpos para *B. bigemina*. La detección de anticuerpos para *Theilera mutans* también se realizó, pero todas las pruebas resultaron negativas, confirmando el hecho de que este parásito no está presente en el país. La re-evaluacion de la técnica de Ag-ELISA para *Trypanosoma sp.* aparentemente es necesaria.

INTRODUCTION

French Guiana has approximately 8000 cattle, including 70% zebu, 15% cross bred and 15% European breeds. Trypanosomiasis, together with the other hemoparasites, anaplasmosis and babesiosis, was suspected to be one of the greatest constraints to cattle production. An epidemiological survey was carried out by CIRAD-EMVT in 1991-92 to establish the relative importance of trypanosomosis due to *T. vivax*, anaplasmosis, and babesiosis.

MATERIALS AND METHODS

The livestock producing areas of French Guyana are indicated in Figure 1. Samples were collected from four areas, from the border with Suriname in the west, to the border with Brazil in the east. The cattle population in each area and the number of samples collected are indicated in Table 1. Samples were collected from 2953 cattle. Four herds, totalling 200 animals, were sampled every three to four months over an 18 month period to evaluate seasonal variations.

Parasitological examination, HCT and blood smear examinations were carried out on 509 samples. When HCT was positive, DG/BCM and mouse inoculation were also performed. Nine serological tests under evaluation, provided by ILRAD (now ILRI), were processed for each sample; half of the tests were processed at ILRAD, Kenya, and the other half at CIRAD-EMVT-Guyane. The tests processed are indicated in Table 2.

Figure 1: Cattle producing areas in French Guiana
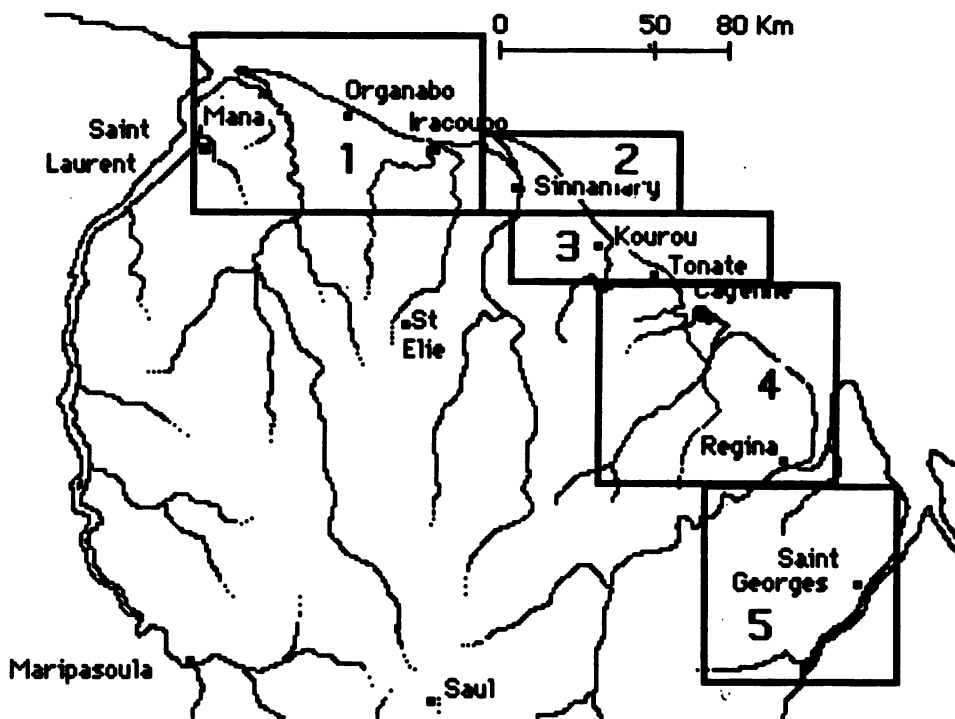
Legend: area 1: St Laurent; area 2: Sinnamary; area 3: Kourou; area 4: Cayenne; area 5: St Georges.

Table 1: Sampling of the cattle population per area

Area	Number of cattle	Number of samples	% of sampling
1 St Laurent	2006	419	20
2 Sinnamary	1323	616	47
3 Kourou	2883	1380	47
4 Cayenne	2034	477	23
5 St Georges	273	61	22

Table 2: Serological tests processed on 2953 cattle serum samples

T. vivax antigen detection[1]	Ag-ELISA *T. vivax*	(monoclonal antibody TV27/9.45.35)
T. evansi antigen detection[1]	Ag-ELISA *T. brucei*	(monoclonal antibody TR 7/47.34.34)
T. congolense antigen detection[1]	Ag-ELISA *T. congolense*	(monoclonal antibody)
Trypanosoma sp. antibody[2]	indirect-ELISA *T. vivax*	(rodent adapted strain)
Babesia bovis antibody[2]	indirect -ELISA *B. bovis*	(australian isolate)
Babesia bigemina antibody[3]	sandwich ELISA	(monoclonal antibody F4/86.19. and parasitic lysate)
Babesia bigemina antigens[3]	Ag-ELISA	(monoclonal antibody F4/86.19.)
Anaplasma marginale antigens[3]	Ag-ELISA	(monoclonal antibody Am w)4/36.13.
Theileria mutans antibody [1]	indirect -ELISA *Th. Mutans.*	

1 method of NANTULYA and LINDQVIST, 1989.   3 method of ILRI under evaluation (unpublished).
2 method derived from the method of FERENC *et al.*, 1990.   4 method of KATENDE *et al.*, 1990.

RESULTS

Of the 509 samples analysed by HCT, 7.7% were positive for what were presumed to be *Megatrypanum* species based on their size, morphology and motility. Parasitological examination of the 509 samples did not reveal any pathogenic trypanosomes. Thirty-nine mice were inoculated with the positive samples. No parasite could be isolated during three months of observations. *A. marginale* was observed in 28% of 442 blood smears, and *B. bigemina* in 3 of the 442 samples.

Results of the serological tests are indicated in Table 3.

Table 3: Percentages of positive results to serological tests for 2953 cattle

Antigen (Ag) or Antibody (Ab) detected:	percentage of positive Samples			
T. congolense Ag	18%			
T. brucei Ag (detection of *T. evansi*)	20% ???	)*Trypanosoma* sp. Ag	) Ag and/or Ab to	
T. vivax Ag	12%	) 26%)	) *Trypanosoma* sp.	
		))	) 37%	
Ab to *T.vivax* (detection of *Trypanosoma* sp. infections)	22%	) Ag and/or Ab.)to *T. vivax*) 29%)	))))	
Ab to *Babesia bovis*	62%	*	) *Babesia.* sp.	
Babesia bigemina Ag	22%	) *B. bigemina*	) 84%	
Ab to *Babesia bigemina*	67%	) 73%	)	
Anaplasma marginale Ag				61%
Ab to *Theileria mutans*				0%

No seasonal variations could be detected in any of the parasite species under study. Results with *A. marginale* and *Babesia* sp. indicated enzootic stability. Antibodies to *Theileria mutans* were not detected, confirming that the parasite is absent from the area investigated. For *T. vivax*, detailed results by area are indicated in Figure 2.

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Figure 2: Seroprevalences of cattle trypanosomosis due to *T. vivax* in the 5 cattle producing areas of French Guiana

seroprevalence rates of trypanosomiasis in cattle
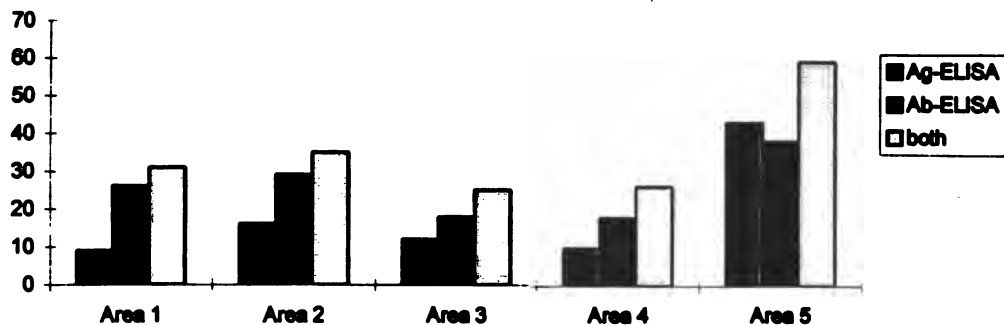
	Area 1	Area 2	Area 3	Area 4	Area 5
Ag-ELISA	9	16	12	10	43
Ab-ELISA	26	29	18	18	38
Both	31	35	25	26	59

DISCUSSION

There was no correlation between HCT and the four serological tests for *Trypanosoma* species, confirming the fact that positive samples to HCT were most probably due to the presence of *T. theileri*. The 7.7% prevalence of *T. theileri* infection is comparable to the 10.9% observed by van Vlaenderen in Suriname (1996), and the 6.7% observed by Monzon *et al.* in Argentina (1993). *T. evansi* was not observed, and has never been observed or suspected in French Guiana. *T. vivax* was also not observed, although it was observed during an epidemic, in 1988 (Lancelot, 1989), and in 1994-95 (Desquesnes, unpublished). During this survey, clinical signs of trypanosomiasis were not observed, and it was considered as an inter-epidemic period.

Of the samples positive on Ag-ELISA, 58% were negative on indirect-ELISA, which is very improbable during inter-epidemic period. Forty-two per cent of those positive Ag-ELISA were positive on indirect-ELISA, which would indicate active infection of the animals. Seventy-five percent of the samples positive on indirect-ELISA were negative on Ag-ELISA, which would indicate cured animals, or false negative results to Ag-ELISA, which was demonstrated later by Desquesnes and de La Rocque (1995).

Twenty-two percent of the samples were positive to *T. brucei* antigens, and 18% to *T. congolense* antigens. These results have been explained in subsequent research on the specificity of the Ag-ELISA (Desquesnes, 1996). The results were not considered reliable in the present study and were therefore not published in the initial paper of Desquesnes and Gardiner (1993).

Indirect-ELISA did not indicate any seasonal variation in prevalence, which is to be expected during an inter-epidemic period. Seroprevalence in zebu and zebu-crossed was 31%, higher than that of European cattle which had a seroprevalence of 9%. This was probably due to the geographical distribution of the disease during the epidemic of 1988-89 rather than a breed factor. Among European cattle, seroprevalence was higher in dairy cattle (50%), than in beef cattle (9%). This was probably due to the fact that treatments are avoided in dairy cattle because of regulations on milk residues.

Among the four tests used, indirect-ELISA *T. vivax* for antibody detection of *Trypanosoma* species was shown to be the only reliable test for trypanosomes. Based on indirect-ELISA, the seroprevalence of cattle trypanosomiasis

during this inter-epidemic period ranged from 18% in Areas 3 and 4, to 28% in Area 5, close to Brazil. Since clinical signs were not observed for more than three years, it is suspected that these positive results come from asymptomatic carriers.

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PRELIMINARY SERO-SURVEY FOR ANTIBODIES TO *TRYPANOSOMA CRUZI* IN HUMANS IN TRINIDAD, GUYANA AND SURINAME

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ABSTRACT

When 397 blood bank samples from different parts of Trinidad, and 196 samples from Suriname, were assayed by the ELISA technique for specific antibodies *Trypanosoma cruzi*, none were found to be positive. At the same time, blood samples taken by a cardiologist in South Trinidad from patients showing clinical signs suggestive of Chagas disease were positive on ELISA (2.0%), while workers with a history of exposure to forested areas were negative. From a randomly selected sample of 228 healthy persons in Guyana's North West District, only one person (0.4%) was positive for antibodies. Follow-up studies in high risk individuals, planned for 1997, are presented. Individuals living near to foci of triatomine bugs, such as the Amerindians in Guyana and the Bush Negros in Suriname, as well as cardiac patients with symptoms suggestive of Chagas disease, will be sampled. Stratification of blood bank samples in the three countries will be tested to evaluate the risks of transmission via blood transfusion.

RESUMÉ

397 échantillons de sérums issus de la banque de sérum du Trinidad et 196 échantillons du Suriname ont été testés par ELISA-indirecte pour la recherche d'anticorps spécifiques dirigés contre *Trypanosma cruzi*; tous les échantillons ont été négatifs. Durant la même période, des échantillons de sang prélevés par des cardiologistes du sud du Trinidad, chez des patients présentant des signes cliniques évoquant la maladie de Chagas, 2% des échantillons étaient positifs, alors que des travailleurs exposés dans des zones de forêt étaient négatifs. A partir d'un échantillon de 228 personnes sélectionnées au hasard dans la région Nord-Ouest du Guyana, seulement une personne s'est avérée positive pour la recherche d'anticorps (0,4%). Des études ultérieures, chez les individus à haut risque des trois pays, planifiées pour l'année 1997, sont présentées. Les populations habitant près des foyers fréquentés par les triatomes sont particulièrement visées (5 villages de noir-marrons du Suriname) ainsi que des groupes d'amérindiens du Guyana et des patients cardiaques dont les symptômes suggèrent la maladie de Chagas seront prélevés. Un échantillonnage stratifié des produits des banques de sang des trois pays sera testé afin d'évaluer les risques de transmission de la maladie de Chagas à l'occasion des transfusions sanguines.

RESUMEN

Cuando fueron ensayadas 397 muestras de bancos de sangre de diferentes partes de Trinidad y 196 muestras de Suriname por la técnica de ELISA para anticuerpos específicos de *Trypanosoma cruzi*, en ninguno se encontró positividad. A la vez, las muestras de sangre tomadas por los cardiólogos en el Sur de Trinidad de pacientes que mostraban signos clínicos que sugerían el mal de Chagas fueron positivas por el ELISA (2.0%), mientras que en los trabajadores con un historial de alta exposición a áreas forestales fueron negativas. De muestras tomadas aleatoriamente de 228 personas sanas en el Distrito Nor-oeste de Guyana, solo una persona (0.4%) fue positiva para los anticuerpos. Se hace una presentación para mas estudios planificados para 1997, a fin de tomar muestras de grupos de alto riesgo en tres países, La población que vive cerca de los focos frecuentados por insectos Triatominos, tales como la población del Bosque Negro de Suriname, grupo sonic Amerindio de Guyana y algunos pacientes cardiacos con signos que sugieran el mal de Chagas serán muestreados. La estratificación de las muestras de los productos de los bancos de sangre de los tres países será llevado a cabo para identificar cualquier riesgo potencial de la transmisión del mal de Chagas en los bancos de sangre.

INTRODUCTION

In most South American countries, there is an established and recognized focus of Chagas' disease endemicity (Schmunis, 1991), which has resulted in attempts to manage this disease in the human populations. There is not much published data on the status of Chagas' disease in Guyana, Suriname and Trinidad and Tobago. Guyana and Suriname form part of the north-east edge of the South American continent, while Trinidad and Tobago is only 15 miles off the Venezuelan coast. Absence of data in these three countries, prevents any intervention even if it was warranted.

Fistein (1966) and Fistein (1981) identified *Trypanosoma cruzi* as an infection in triatomine bugs, and antibodies against this in humans in Trinidad. However, Omah-Maharaj (1991) was unable to demonstrate *T. cruzi* specific antibodies in the human population of Trinidad and Tobago. Recently, however, Aziz and Parsad (1993) indicated that a focus of Chagas' disease endemnicity does exist in Trinidad, and reported the presence of antibodies and *T. cruzi* trypomastigotes in patients presenting at a cardiac clinic.

These data have not been confirmed by any other research workers as far as we know, but these unpublished data indicate a need for follow-up in order to clarify the status of Chagas' disease in this country. The common finding of infected wild triatomines, *Eratyrus mucronatus*, *Panstrongylus geniculatus* and *Rhodnius pictipes* in Trinidad alone indicated a need for clarification of the Chagas' endemnicity situation in the island state of Trinidad and Tobago and also in the mainland countries of Guyana and Suriname.

If there are indeed significant foci of Chagas' disease in these countries, then there is a need to know if this adversely affects the integrity of the nations' blood banking. Since blood transfusion is one potential means of infection with *T. cruzi* (WHO 1991), it is important to measure the extent of any contamination of the blood supplies.

The preliminary data presented originates from sero-diagnostic studies which were conducted based on the following hypotheses:

I.   Chagas' disease may be endemic in Trinidad and Tobago, Guyana and Suriname, but has not been identified.

II. If Chagas' disease is endemic, blood-banking may be at risk.

III. Some cardiac patients (e.g. with apical aneurysms) may possibly be suffering from Chagas' disease.

MATERIALS AND METHODS

During 1994, 1995 and 1996, serum samples were collected from two classes of subjects.

Blood Bank donors in Trinidad and Tobago and in Suriname
In Trinidad and Tobago, 397 blood samples were collected from six blood collection sites located throughout the country, including seven from Point Fortin Hospital and 259 from the National Transfusion Service in Port-of-Spain. Numbers were based on the numbers of potential subjects for blood donation. Two hundred blood donors were sampled from the Paramaribo Blood Transfusion Service (Red Cross), the only one available in Suriname. The serum samples were transported to CAREC laboratories where they were stored pending the laboratory tests.

High Risk Subjects
In 1994, three hundred and twenty- seven serum samples were taken from high risk persons in Trinidad. These consisted of 247 presenting at Dr. Parsad's cardiac clinic in San Fernando, Trinidad, 32 oil field workers and 28 hunters, as well as 20 at risk patients. In Guyana, 228 hunters and 20 at risk patients were sampled, and 228 serum samples were collected from apparently healthy persons in the Mabaruma North West District, many of whom live in thatched accommodation, where triatomine bugs are known to be prevalent.

All sera were screened for antibody by the Indirect Hemagglutination Assay (IHA), using antigen obtained from Dr. Elsa Segura, Chagas' laboratory, Argentina. Confirmation was made by assaying all "positives" by an ELISA procedure obtained from Gull ® Laboratories, Salt Lake City, to demonstrate IgG antibodies.

RESULTS AND DISCUSSION

Blood Bank Donors: None of the samples from blood banks in Trinidad (Table 1) or from Suriname (Table 2) were confirmed as having positive proof of exposure (IgG antibodies) to *T. cruzi*. Three out of 259 samples initially showed reaction when assayed by the IHA screening tool, but these were not confirmed by the ELISA, a more specific and sensitive tool.

Table 1: Chagas' Disease Serology
1994 – Blood Bank, Trinidad

LOCALITY	NUMBER TESTED	SCREENED POSITIVES		CONFIRMED POSITIVES	% CONFIRMED POSITIVE
		IHA ±	IHA ±	ELISA +	
Point Fortin Hospital	7	2	0	0	0
Sangre Grande Hospital	15	0	1	0	0
San Fernando Hospital	87	7	1	0	0
National Blood Transfusion Service	259	22	3	0	0
Eric Williams Medical Sciences Complex	20	0	0	0	0
Tobago Hospital	9	1	0	0	0
Total	397	32	5	0	0

Table 2: Chagas' Disease Serology
1994 - Suriname

SOURCE	NUMBER TESTED	NUMBER POSITIVE			% POSITIVE
		IHA ±	IHA +	ELISA +	
Suriname Blood Bank	196	3	0	0	0

These were small numbers of samples tested in relation to the total quantity of blood samples collected in a blood bank system over a period of one year (1994). Perhaps in order to satisfy ourselves in screening for Chagas' antibodies, at least 0.05 to 0.10% of blood collected should be screened, and this should be done over as wide a geographical range as possible. This geographical spread was achieved for Trinidad and Tobago, but perhaps not for the Suriname blood collection. The bottom line however, is that no sample appeared to be positive for *T. cruzi* antibodies. On this evidence therefore, there is not sufficient reason to start routine screening blood bank products for this parasite.

High Risk Subjects: Serological results from a sample of persons at high risk of contracting Chagas' disease, because of residence in thatch accommodation where triatomines may abound, are shown in the Guyana samples (Table 3). Table 4 also shows the results for patients presenting with cardiac symptoms in southern Trinidad.

Table 3: Chagas' Disease Serology
1994 – Guyana

SOURCE	NUMBER TESTED	NUMBER POSITIVE			% POSITIVE
		IHA ±	IHA +	ELISA +	
Guyana (North West District) Health Patients	228	2	0	1	0.4

Table 4: Chagas' Disease Serology
1994 - Cardiology Clinic, Trinidad

LOCALITY	NUMBER TESTED	SCREENED POSITIVES		CONFIRMED POSITIVES	% CONFIRMED POSITIVE
		IHA ±	IHA ±	ELISA +	
Cardiac Patients	247	14	2	5	2.0
Oilfield Workers	32	2	0	0	0
Hunters	28	0	0	0	0
Other Patients	20	0	0	1	5
TOTALS	327	16	2	6	1.8

In Guyana, while two of the screened 228 serum samples were positive by IHA, only one (0.4%) proved to be positive for IgG antibodies, proving past exposure to Chagas. In Trinidad samples, there were two risk factors - occupation and clinical manifestation (Table 4). In oil field workers and hunters who have a strong link with the forest and therefore a risk of infection, there was no confirmation of antibodies in any of the 60 samples assayed by either test.

Among the cardiac patients of Trinidad, there were five confirmations from the 247 tested giving a 2% positivity rate in this risk group in 1994. This must be considered at least preliminary evidence of a causative relationship between exposure to *T. cruzi* and clinical manifestations such as enlarged hearts with apical aneurysm, which could result in congestive heart failure. Indeed, follow-up in the succeeding years showed that these particular patients were consistently positive in 1995 and 1996 (Table 5).

Table 5: Chagas' Disease Serology
Cardiologist's Routine Samples – Trinidad

YEAR	NUMBER TESTED	ELISA RESULTS		% CONFIRMED POSITIVE
		±	+	
1994	47	1	2	4.3
1995	39	0	2	5.1
1996	20	1	5	25.0

None of these samples were tested by IHA method

These data provide evidence that high risk groups should be investigated to confirm that there is some risk of *T. cruzi* infection in these three countries, and that there may be some morbidity associated with exposure to *T. cruzi*. However, before there is any loss of confidence in blood bank products due to risk of contamination by Chagas' disease, there is need for a well executed survey. This should include blood bank collected samples from as wide a geographical distribution as possible from within these three target countries. At the same time, high risk patients (based on the residence, occupation or clinical condition) should be assayed to determine if any clinical condition may be associated with any of these risks.

With regard to the confirmatory ELISA tests, it is important to point out that the manufacturers concede that the presence of IgG antibodies is not sufficient evidence to distinguish between past and present infection. Secondly, patients' sera with a high titre to *Leishmania* spp. may exhibit a positive reaction in the Chagas' IgG ELISA test. Test results must therefore be viewed together with clinical data in order to make a judgement on individual cases.

The present data seem to confirm to some extent, the suspicion and findings of Fistein (1966) and Fistein (1981) as well as Aziz and Parsad (1993) that there are some *T. cruzi* exposed persons in Trinidad. It does not appear however that the numbers have been large enough to affect the supply of blood to the blood banks. We are in the process of following up on these preliminary studies by an investigation in Trinidad and Tobago, Guyana and Suriname.

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MEXICO FREE FROM DOURINE NEGATIVE REPORTS OF DOURINE IN CHIHUAHUA

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ABSTRACT

In April 1995 a number of horses in Chihuahua, Mexico were found to be positive for dourine, and an epidemiological study was conducted to establish the prevalence of seropositive animals in the region. Three thousand equine samples were analyzed, and results showed a seroprevalence of 0.14/100. Seropositive animals did not present any clinical signs, even when immunosuppressed, and the causal agent was not isolated. The sera apparently positive for *Trypanosoma equiperdum* were retested to confirm the diagnosis. Four sera were found to be positive to *T. cruzi*, indicating the possibility of cross reactions. The results suggest that the disease continues to be exotic in Mexico and epidemiological surveillance is being maintained through equine serological monitoring in Chihuahua and other areas of the country.

RESUME

En avril 1995 des chevaux du Chihuahua, au Mexique, ont été trouvés séropositifs pour la dourine, et une étude épidémiologique a été menée afin d'établir la prévalence des animaux séropositifs dans la région. Trois mille échantillons de chevaux ont été analysés, montrant une séroprévalence de 0.14/100. Les animaux séropositifs ne présentaient aucun signe clinique, même après immunosuppression, et l'agent causal n'a pu être isolé. Les sérums apparemment positifs pour Trypanosoma equiperdum ont été retestés afin de confirmer le diagnostic. Quatre sérums ont été trouvés positifs vis-à-vis de T. cruzi, indiquant une possible réaction croisée. Les résultats suggèrent que la dourine n'est pas présente au Mexique, mais une épidémiosurveillance est maintenue par des examens sérologiques dans le Chiuhuahua ainsi que d'autres régions du pays.

RESUMEN

En abril de 1995, varios caballos en Chihuahua, México, se encontraron positivos a Dourine y un estudio epidemiológico se llevó a cabo con el fin de establecer la prevalencia de animales seropositivos en la región. Las muestras de 3000 equinos se analizaron y los resultados confirmaron una seroprevalencia de 0.14/100. Los animales seropositivos no mostraron ninguna señal clínica aún inmunosuprimida y el agente causal no fue aislado. Los sueros aparentemente positivos a *Tripanosoma equiperdum* fueron analizados nuevamente para confirmar el diagnóstico. Se encontraron cuatro sueros positivos a *T. cruzi* lo cual indica la posibilidad de reacciones cruzadas. Los resultados sugieren que la enfermedad continúa siendo exótica en México y se ha mantenido una vigilancia epidemiológica a través de un monitoreo serológico equino en Chihuahua y en otras áreas del país.

INTRODUCTION

On 21 April 1995 sixty horses were assembled at the quarantine control point of Palomas in Chihuahua State for export to the United States of America. Six of the horses tested serologically positive for dourine using the Complementary Fixation Test (CF). The horses which tested positive came from the Nabuquipa, Gómez Farías, Guerrero, Chihuahua and Madera municipalities of the state, and were quarantined, retested and subsequently slaughtered under the supervision of the Mexican veterinary services.

METHODOLOGY

Serological sampling was introduced in the zones of origin and contact of the affected animals during the period 11 May to 27 May, 1995. A total of 187 samples were taken from 12 municipalities in the regions, including samples from two abattoirs; laboratory results confirmed the diagnosis of the first six sera, and four more samples also tested positive. Nevertheless, in the field study no horses were found displaying clinical symptoms nor lesions associated with the disease.

A programme of random serological sampling was launched across the state to establish the prevalence of seropositive animals in the region. A minimum sample size was established for each of the fourteen Rural

Development Districts in the state.

RESULTS AND DISCUSSION

In the epidemiological study, a total of 3001 samples from donkeys, horses and mules were collected and analyzed and a seroprevalence of 0.14/100 was found. Of the 43 sera apparently positive for *T. equiperdum*, 18 were forwarded to the National Institute for Diagnosis and Epidemiological Referral of the Ministry of Health for confirmation of the diagnosis. However, at that Institution 12 sera were found to be positive to *T. cruzi* which indicated the possibility of cross reactions.

The lack of clinical evidence, together with the possibility of crossed reactions of *T. equiperdum* with other trypanosomes that are antigenically indistinguishable, indicates that the disease continues to be exotic in Mexico. This conclusion was reinforced by experimental immunosuppression in two horses which apparently tested positive to *Trypanosoma* but displayed no clinical symptoms of the disease. In addition it was not possible to isolate the causal agent, which is needed to establish a definitive diagnosis in keeping with the technical criteria of the OIE.

Currently epidemiological surveillance continues to be maintained throughout the country. In addition there is a ruling in force issued by the Mexico-USA Commission for the Prevention of Foot and Mouth and Other Exotic Animal Diseases (CPA) that all horses which are transported from the state of Chihuahua for reproductive purposes should test serologically negative for trypanosomiasis.

EPIDEMIOLOGICAL DIAGNOSIS OF TRYPANOSOMIASIS OF CATTLE BY *TRYPANOSOMA VIVAX* IN VENEZUELA[3]

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ABSTRACT

In Venezuela, production of milk and meat is inefficient due to a number of factors including low nutritional value of pasture, low reproductive efficiency, disease and high mortality rates in calves and adults. The hemoparasites, *Trypanosomia vivax*, *Anaplasma marginale* and *Babesia bigemina* are widespread, and responsible for major economic losses in milk, meat and dual purpose cattle production systems. This study was carried out in the south of Aragua State and the north-west of the Guárico State, where *Trypanosoma vivax* has been detected in both cattle and sheep production systems. The average absolute seroprevalence of *T. vivax* in the animals sampled was greater in adults than in calves, although the differences were not statistically significant. The absolute seroprevalence was higher among females (87.56%) than among males (12.44%), although significant statistical differences were not confirmed when corrections were made for the number of samples. The seroprevalence of bovine trypanosomiasis was higher in dual purpose (beef and milk) systems of production. Seroprevalence was also significantly higher on farms where no type of trypanocide treatment had been applied on any occasion; and lower where the trypanocide drug, diminazene, was used. This data is preliminary and the total number of samples corresponding to the different seasons (dry and rainy) in the given regions is still to be processed.

RESUMÉ

Au Venezuela, la production de lait et de viande bovine est affectée par de nombreux facteurs incluant la faible valeur alimentaire des pâtures, un faible taux de reproduction, des maladies et de fortes mortalités chez les veaux et les adultes. Les hémoparasites, *Trypanosoma vivax*, *Anaplasma marginale* et *Babesia bigemina* sont largement répandus et responsables de pertes économiques majeures dans les systèmes de production laitière, mixte et de viande. La présente étude a été menée dans le sud de l'Etat d'Aragua et le nord-ouest de l'Etat de Guarico, où *Trypanosoma vivax* a été détecté chez les bovins et ovins. La séroprévalence moyenne de *T. vivax* était plus élevée chez les adultes que chez les veaux, bien que les différences ne fussent pas statistiquement significative. La séroprévalence moyenne était plus élevée chez les femelles (87,56%) que chez les mâles (12,44%), mais elle n'était pas significativement différente après correction sur le nombre d'échantillons. La séroprévalence de la trypanosomose était plus élevée dans les systèmes de production mixtes (lait et viande), elle était également plus élevée dans les élevages n'ayant recours à aucun trypanocide que dans ceux utilisant l'acéturate de diminazène. Ces données sont préliminaires, et la totalité des échantillons prélevés dans la région pendant les différentes saisons (sèche et humide) est en cours d'analyse.

RESUMEN

En Venezuela, la producción de leche y carne es ineficiente debido a un número de factores que incluyen el bajo valor nutritivo de la pastura, enfermedades y alta tasa de mortalidad en terneras y adultos. Los hemoparásitos, *Tripanosoma vivax*, *Anaplasma marginale* y *Babesia bigemina* están ampliamente difundidos y son responsables de grandes pérdidas económicas en leche, carne y sistemas de producción de ganado de doble finalidad. Este estudio se llevó a cabo en el Sur del Estado Aragua y el Noroeste del Estado de Guárico donde *Tripanosoma vivax* se ha detectado tanto en sistemas de producción de ganado como de ovejas. El promedio de seroprevalencia absoluta de *T. vivax* en los animales del muestreo fue más alta en los adultos que en las terneras, aunque las diferencias no eran significativas estadísticamente. La seroprevalencia absoluta era más alta en las hembras, (87.56%) que entre los machos (12.44%), aunque no se confirmaron diferencias estadísticamente significativas cuando se hicieron las correcciones para el número de muestras. La seroprevalencia de Tripanosiamiasis bovina era más alta en sistemas de producción de doble finalidad (carne y leche). La seroprevalencia también era significativamente más alta en las granjas donde no se había aplicado ningún tipo de tratamiento tripanocide nunca; y más baja donde se utilizó la droga Tripanocide, diminazene. Estos datos son preliminares y el número total de muestras correspondientes a las diferentes estaciones (lluviosa y seca) en dichas regiones todavía tienen que procesarse.

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[3] Translated from Spanish

BACKGROUND

In Venezuela, production of milk and meat is inefficient due to a number of factors including low nutritional value of pasture, low reproductive efficiency, disease and high mortality rates in calves and adults. The haemoparasites, *Trypanosoma vivax, Anaplasma marginale* and *Babesia bigemina* are widespread, and responsible for major economic losses in milk, meat and dual purpose cattle production systems.

Studies have shown that trypanosomiasis represents a serious constraint to cattle production, especially in traditional cattle rearing states such as Guárico, Apure, Zulia and southern Aragua, where the prevalence ranges from 19% in the western plains region, to 58%. In the north-east of Falcón state (Duno 1992). Toro (1990) has estimated its prevalence as 21% at the national level.

Bovine trypanosomiasis is a contagious infection, spread by hematophagous insect vectors, which attacks the blood cells of its hosts. The chronic or sub-clinical forms of this disease weaken cattle, and, when compounded by other stress factors such as poor nutrition, improper management and climatic changes; induce a progressive deterioration in production and reproduction of herds. Acute and sub-acute infections are less frequent, displaying clinical signs of fever, anemia and mortality. All of these lead to enormous losses in both milk and meat production.

The majority of studies on *Trypanosoma vivax* have been conducted with African strains or isolates of this species, which are very different from American strains or isolates and have a notable difference in mode of transmission to the vertebrate host. African *T. vivax* is transmitted biologically by a species of the *Glossina* insect, which is non-existent on the American continent. The mechanism of transmission of the American *T. vivax* is less clearly understood, but it is believed to be mechanically spread by insect species such as *Tabanus* and *Stomoxys* or arthropods such as *Boophilus*. Knowledge about the African *T. vivax* should be cautiously applied to the American *T. vivax*, and only as a basis for designing and developing local studies with the American strains and under local conditions.

This study was carried out in the south of Aragua State and the north-west of the Guárico State, where *Trypanosoma vivax* has been detected in both cattle and sheep production systems, with low to moderate seroprevalence in Aragua; and moderate to high seroprevalence in Guárico depending on the season (dry or rainy). In addition, the farms in this area are stable units with good facilities and possess the most valuable requirements for long term field work *i.e.* collaboration, patience and comprehension of their owners and workers.

Knowledge of bovine trypanosomiasis, as for other disease complexes, is of vital importance for full understanding and proper treatment of this disease in our country. Hence the purpose of this study is to review a significant portion of the bibliographic documentation which exists on this disease, at the global and regional levels and to report the results of the studies carried out by my colleagues and myself on this topic.

DIAGNOSIS OF BOVINE TRYPANOSOMIASIS

Bovine trypanosomiasis caused by *T. vivax* presents problems for clear diagnosis, given that the majority of cases are limited to presumptive clinical diagnosis or to chance detection of the parasite by direct laboratory methods (stained smears, thick drops, micro centrifuge) which have limitations in accuracy for confirming sub-clinical infections.

The ELISA technique offers a good option for the diagnosis of trypanosomiasis, since the large volume of samples to be processed makes it difficult to utilize elaborate techniques, and immunologic procedures to evaluate the presence of different VATs from the parasite, etc., with traditional techniques of limited sensitivity and specificity are impractical. All these considerations seek to overcome the difficulties in the diagnosis of *T. vivax*, since conventional parasitological techniques (fresh smears, thin stained smears, thick fresh and stained smears, Woo, double centrifuge, QBC) present problems in the detection of trypanosome infections, since these are not frequently found in peripheral circulation and it is very difficult to cultivate the parasite in laboratory animals. Moreover, the traditional serological tests present the disadvantage that adhesion of unspecified serum components to the parasites mounted on the slide holders (in IFA) make it difficult to distinguish between positive and negative serum (in IFA) at high dilutions. When thick smears are used as antigens, the excessive presence of erythrocytes obstructs easy observation of the parasites and even though red blood cells are washed in PBS, centrifuged and resuspended with

bovine albumin, many parasites do not become concentrated in the sediment of red blood cells. On the other hand, the preparation of a large number of blood smears to prove standard antigens is difficult; requiring low temperatures for storage and transport, and occupying large amounts of freezer space.

Indirect serological methods also have their limitations, since they only offer data on exposure to the parasite, and do not facilitate the distinction between acute and chronic infection. Acute infections are only detected (with a high level of accuracy) by direct tests in which parasites are observed in the phase of parasitemia; while chronic infections have the limitation that the processing of the samples must be conducted within 24 hours of collection. Because there is often a considerable distance between the farms and the laboratory, it is almost impossible to process the samples collected within this time by the mentioned conventional techniques and obtain reliable results.

The combination of direct and indirect methods is therefore indispensable for accurate interpretation of the results obtained in field surveys with this type of hemoparasite. Utilization of the Quantitative Buffy Coat test (QBC), a technique developed for the field diagnosis of malaria in humans, has the potential for field diagnosis of the hemoparasites which affect cattle, and particularly trypanosomiasis. This test is simple, and detects levels of parasitemia much lower than other direct, serological, immunological or ADM probe (Becton Dickinson, 1993) methods, as well as producing precise and reliable results in a few minutes. Among the serological and immunological methods are the IFA; and in particular the ELISA, which with its two versions (Ag-ELISA and Ab-ELISA), offer a valuable technique for epidemiological diagnosis of this disease.

EPIDEMIOLOGY OF BOVINE TRYPANOSOMIASIS IN VENEZUELA

Geographic Distribution

Toro (1990) reports serological prevalences of bovine trypanosomiasis due to *T. vivax* in Venezuela as 19.0% in the Zulian region; 21.1% in the Andean region; 20.8% in the west-central region; 33.5% in the central region; 22.7% in the savannahs; 3.4%* in the east and 23.9% in Guyana, for a national average total of 20.8% (*additional results by IFA). Included in his study are geographic conditions, climatic factors, data on grasses, management, preventive measures and estimated seroprevalence.

Tamasaukas (1992a; b; 1993) and Tamasaukas and Roa (1991-1992) report that according to data from the Ministry of Agriculture and Livestock Production (MAC, 1984) there was an estimated cattle population of 1,308,719 in Guárico State, and there were a total of 9,495 head on the 19 farms sampled during the survey. The absence of reliable records, as well as seasonal herd migration in the region during the period due to the planting of agricultural produce, made it difficult to determine the exact distribution [sex and age] of cattle on these farms.

Over 80% of the farms reported evidence (especially clinical) of bovine trypanosomiasis, as indicated in analysis of the information provided by PRODETEC-Valle de la Pascua (1991, unpublished data), by Arias *et al.* and from the Programme of Agricultural Cooperation CONVENIO MAC-PDVSA (Chicco and Linares, 1992). It was found that 73.6% of farms were larger than 500 ha., 21.1% between 100 ha. and 500 ha. and 5.35% were less than 100 ha. Arias *et al.* (1983) reported different categories of producers (with respect to small, medium and large sized farms) under the same classification used in the studies of Tamasaukas (1993) and Tamasaukas and Roa (1991-1992).

The seroprevalence of bovine trypanosomiasis caused by *T. vivax* was low during part of the dry season and during transition from the dry season to the beginning of the rains, with 3.9% in the central region; 4.9% in the south of Aragua, and 2.9% in the northwest of Guárico; however there was in fact a high seroprevalence in sheep, in one of the three farms in the south of Aragua (17.45%).

Age Distribution

Drager and Mehlitz (1978), Murray *et al.* (1982) and Perrone *et al.* (1992) among others, report a lower incidence of trypanosomiasis in calves and animals less than one year old. However, Duno (1992), among others, reports that there is no relation between age and susceptibility or resistance to trypanosomiasis.

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The average absolute seroprevalence of *T. vivax* in the animals sampled was:

0 to 12 months	16.3%
13 to 24 months	8.5%
older than 25 months	75.2%

No statistically significant differences were observed when corrections were made for the number of samples. These values were different from those obtained by Duno (1992) of 61.54%, 56.03% and 56.77% in the corresponding age groups, however other studies have drawn similar conclusions with respect to the absence of any relation between the age of the animals and their susceptibility to the disease. The results differed from those of Robson and Askar (1972) who reported that adults were more susceptible than the young, due to their greater movement between pastures, thus exposing them more to the effect of insect vectors.

Perrone *et al.* (1992) found that the average prevalence of bovine trypanosomiasis on a farm in Guárico State was higher in adults than in the young; and Reid *et al.* (1970) indicated the same in their study on cattle and antelopes. However, they did not indicate whether the differences were statistically significant, nor did they suggest any reason for this higher prevalence among adults.

Distribution by sex
Toro *et al.*(1982) and Murray (1989) noted that the levels of infection are similar in males and females; while Robson and Askar (1972) report that adult males display higher levels of infection than females, possibly due to the greater stress to which they are subjected, since they are used in ploughing activities. Mawena (1986) in his study on goats, reported that males withstand the infection better than females.

This study showed that the absolute seroprevalence was higher among females (87.56%) than among males (12.44%), although significant statistical differences were not confirmed when corrections were made for the number of samples. We concluded that there is no relationship between sex and degree of susceptibility or resistance to trypanosomiasis, as suggested by Wain *et al.* (1970), Toro *et al.*(1982) and Murray (1989); and in contrast to Robson and Askar (1972).

Production system: meat, milk, dual purpose
Several authors report that in bovines, the stress of pregnancy, parturition and lactation can influence the recurrence or exacerbation of the disease (Murray, 1982; 1989: ILRAD, 1989; Poivey *et al.*, 1983). Toro *et al* (1980), García *et al.* (1990) and Duno (1992) in Venezuela, and Desquesnes (1992) in French Guiana, report in their studies that bovine trypanosomiasis is encountered at higher levels in dairy cattle. A similar finding is reported by Paling *et al* (1987) in his study on the epidemiology of *T. vivax* in Kenya, Africa. Mawema (1987) also reports observing high parasitemia of *T. vivax* in gestating goats.

Tamasaukas (1993), Tamasaukas and Roa (1991-1992) and Tamasaukas and Gonzalez (1994) report in different studies that the seroprevalence of bovine trypanosomiasis was higher in dual purpose systems of production on the farms surveyed in the northern, central and south of the east of Guárico and the north-west of the same state. The results show that there was a significantly positive association of seroprevalence on dual purpose (beef and milk). These findings differ from those of Duno (1992) who encountered greater significance on dairy farms. Similarly, Murray *et al.* (1982) and Poivey *et al.* (1983) reported that in cattle, the stress of pregnancy, birth, lactation and nursing could cause recurrence and exacerbation of trypanosomiasis. Moreover, García *et al.* (1990) report a high prevalence of trypanosomiasis (90%) in milking cows, with a 25% rate of active infections, which confirms the high susceptibility of lactating animals.

Agroecological zones
There were very few references to the possible relationship between bovine trypanosomiasis and the agroecological zone where cattle production is located. The first reference is by Otte (1991), who in his epidemiological study on bovine trypanosomiasis on the Atlantic coast of Colombia examined a number of interrelated factors including proximity to rivers, proximity to swamps, topography, and existence of natural forest. The results therefore indicated their influence on the parasitic levels encountered. The second reference is by Chollet (1992), who reports that in the north of Cameroon, a region where savannahs and gallery forests infested by *Glossina spp* predominate, bovine

trypanosomiasis due to *T. vivax* presents a serious constraint to cattle rearing.

Taking as reference the work of Gómez *et al.*, (1982) with different topographical and edaphic characteristics were delimited, defined and classified in Guárico. These units were located in four agro-ecological zones, (E, F, L, and J) as follows:

E - dry tropical forest - 44 units
F - humid foothill forest - one unit
L - very humid foothill forest - two units
J - very humid lower mountain forest - one unit

Gómez *et al.* (1982) report that in Guárico State two well defined seasons can be observed: the rainy season between March and November, and the dry season between December and April. The humid season lasts from three to six months occurring in almost all the state and from six to nine months in the mountainous zones and alluvial plains. Temperature is affected by both latitude and altitude, with latitude having the greater effect. Rainfall is high all year round, with average values of 2,100 mm and 2,300 mm.

Topography
Otte (1991) found no significant association between undulating topography and prevalence of bovine trypanosomiasis. Tamasaukas (1992a; b; 1993; and Tamasaukas and Roa, 1991-1992) reported that there was a significant positive statistical correlation between undulating relief and seroprevalence and suggested that the relationship observed in their studies could be explained by (a) the indiscriminate application of insecticides on these farms to control pests in agricultural crops, which may have induced resistance in the vectors of trypanosomiasis (b) the movement of vectors towards hilly unforested zones, where producers relocate their cattle during the planting season, while in the flat zones they are utilized for vegetable cultivation during the rainy season.

Type of cultivation
Seroprevalence was highest on farms where cereals were grown, however no relationship was found to be associated with this factor, nor with the cultivation of other agricultural products. There was a positive, significant association with the cultivation of sorghum, which might be due to the fact that this crop is so susceptible to infestation by insects that producers apply pesticides indiscriminately, resulting in resistance developing in tabanids.

Breed
Tamasaukas, (1992b; 1993;) Tamasaukas and Roa, (1991-1992) observed a significant positive association between seroprevalence and crosses between *Bos indicus* x *Bos taurus*, suggesting that this is due to the greater susceptibility of *B. taurus* cattle to trypanosomiasis. This coincides with the conclusions of Desquesnes and Gardiner (1993), although pure *Bos indicus* cattle did present high values of seroprevalence on one farm. Otte (1991) in his study reports that the "type of cattle" only became significant in the selection regression method, after it had been included in the "problem of flies" model. He noted that the breed of cattle probably did not have a direct relationship to the parasite level, but that it could be indirectly associated with some other factor, since analyzing it by multiple regression the result was negative, but by simple regression it was positive, leading to the conclusion that this factor is significant in more complex models only to modify the effects of some other factor already introduced into the analysis.

Anan *et al.* (1991) in Nigeria report a lower susceptibility to *T. vivax* in Zebu cattle (3.%) in comparison with Holstein Fresian (44%).

Presence of arthropod vectors
Tamasaukas (1992a; b; 1993) and Tamasaukas and Roa (1991-1992) reported a significant positive relation between seroprevalence and the presence of arthropod vectors (particularly tabanids); Otte (1991) also indicates this in his work. Hence, it can be suggested that tabanids were involved in the transmission of bovine trypanosomiasis where farms possessed areas or habitats favourable to their development and proliferation, especially in the rainy season.

Previous history
With respect to the history of the presence of bovine trypanosomiasis, they established a significantly positive

association in those farms which confirmed having evidence of the disease on some previous occasion. Therefore it was concluded that this disease is endemic in the region.

Application of trypanocides
Seroprevalence was significantly higher on farms where no type of trypanocide treatment (neither preventative nor curative) had been applied on any occasion; and lower where the trypanocidal drug, diminazene, was used.

Diagnostic Tests
Tamasaukas (1995a; b) reports that in her study on the evaluation of the QBC test (the Becton Dickinson system) in farms in the central region of Venezuela (encompassing the south of Aragua and the north west of Guárico) the presence of active *T. vivax* infections was detected in all the samples collected, and morphology clearly observed. Other hemoparasites, such as *Anaplasma marginale* and *Babesia bigemina* (in experimental infections, and field samples); *Ehrlichia spp.* (in canines and horses); and microfilaria (in canine blood samples), were also identified.

CONCLUSIONS

It should be noted that this data is preliminary and the total number of samples corresponding to the different seasons (dry and rainy) in the given regions is still to be processed.

It was concluded that the disease presented low prevalence in the northwest zone of Guárico State during the dry season, thus requiring studies during the rainy season to complete the epidemiological agroecological diagnosis of bovine trypanosomiasis on the said farms, complemented with direct tests to determine active infections (i.e. QBC) and other serological tests (i.e. Ac-ELISA), as recommended by Tamasaukas (1995c).

Tamasaukas and Roa (1994, unpublished data) registered similar findings on the same farms surveyed in previous years in the Chagaramas and Espino Municipalities in Guárico State, with a low seroprevalence by IFA of *T. vivax* (10% in the dry season) without observing significant differences between the different age groups or sex groups. It was noted that there are major changes in the environmental and agroecological conditions of the farms during this season in comparison with those observed in the rainy season.

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CATTLE TRYPANOSOMIASIS IN VENEZUELA: TRANSMISSION WITHOUT PARTICIPATION OF BLOODSUCKING FLIES

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ABSTRACT

Cattle trypanosomiasis in the New World is mainly caused by *Trypanosoma vivax*, an economically important hemoparasite found throughout the tropical regions of Central and South America. For years it has been stated that *T. vivax* is mechanically transmitted in the New World by bloodsucking flies like tabanids and *Stomoxys* sp., however, due to its high prevalence (25.5% in Venezuela) and enzootic condition, doubts have been raised in recent years about 'mechanical transmission' as the unique method of *T. vivax* dissemination. Thus, other means of transmission, or even a cyclical vector, could be responsible for the establishment of *T. vivax* in a continent without *Glossina* flies. In Venezuela, *T. vivax* was found to be present in titers of a five month old calf over a four month period, suggesting the transplacental route as an alternative means of *T. vivax* transmission to ensure the survival of the parasite in the New World. *Trypanosoma theileri*-like flagellates were found in the hemolymph of *Boophilus microplus*, a new finding for Venezuela. Since stages of *T. theileri* have been reported in hard ticks from other countries it is possible that these ixodids may play a role in the transmission of this elusive trypanosome to cattle.

RESUMÉ

La trypanosomose du bétail dans le nouveau Monde est principalement due à *Trypanosoma vivax*, qui est un hémoparasite économiquement important dans des régions tropicales d'Amérique Latine. Depuis longtemps il a été établi que *T. vivax* est, dans le Nouveau Monde, transmis mécaniquement par des insectes piqueurs comme les tabanides et les stomoxes; quoiqu'il en soit, du fait de sa forte prévalence (25,5% au Venezuela) et sa situation enzootique, des doutes ont émergé ces dernières années sur la la transmission mécanique comme unique moyen de transmission et de dissémination de *T. vivax*. Ainsi, d'autres moyens de transmission, et même la transmission cyclique pourraient participer à l'établissement de *T. vivax* sur un continent dépourvu de glossines. Nous avons observé un cas de transmission transplacentaire chez un veau né depuis 5 heures. La transmission transplacentaire est une alternative à la transmission mécanique, qui permet le maintien du parasite dans le Nouveau Monde. D'autre part, nous avons enregistré la présence de trypanosomes *Theileri*-like chez *Boophilus microplus*, pour la première fois au Venezuela. Puisque des formes de *T. theileri* ont été signalées chez ixodes dans d'autres pays, il est tentant de suggérer que les ixodes peuvent jouer un rôle dans la transmission de cet insaisissable parasite des bovins.

RESUMEN

La trypanosomosis de los bovinos en el Nuevo Mundo es causada mayormente por el *Trypanosoma vivax*, siendo una enfermedad hemoparasitaria importante a lo largo de la región tropical de Centro y Sur América. Por años se ha dicho que el *T. vivax* se transmite mecánicamente en el Nuevo Mundo por moscas hematófagas como los tábanos y *Stomoxys sp.;* sin embargo, debido a la alta prevalencia (25.5% en Venezuela) y su condición enzoótica, en años recientes han aparecido dudas sobre la "transmisión mecánica" como el único método de diseminación del *T. vivax*. Así, otros mecanismos de transmisión o inclusive un vector cíclico podrían ser los responsables del establecimiento del *T. vivax* en el continente sin las moscas *Glossinas*. Reportamos un caso de *T. vivax* de transmisión transplacental en un becerro de cinco horas de nacido con títulos por cuatro meses. La vía transplacental se sugiere como una forma alternativa de transmisión del *T. vivax* y asegurar la sobreviviencia del parásito en el Nuevo Mundo. Adicionalmente, comunicamos la presencia del *Trypanosoma theileri* en *Boophilus microplus*, un nuevo hallazgo para Venezuela. Ya que los estadios del *T. theileri* han sido reportados en las garrapatas en otros países, este hallazgo sugiere o asoma la posibilidad de que estos ixodidos puedan jugar un papel en la transmisión de este esquivo *Trypanosoma* de los bovinos.

INTRODUCTION

Trypanosoma vivax, *T. theileri* and *T. evansi* are the main species of *Trypanosoma* able to infect and multiply in the bloodstream of cattle in the New World. In Venezuela, *T. vivax* was first reported by Tejera (1920) just one year

after the pioneer paper of Leger and Vienne (1919) which showed the presence of this protozoan in the Americas for the first time. *T. vivax* has spread throughout Central America, as far north as El Salvador; in South America, as far as Paraguay (Wells et al. 1982); and even into Cuba (Cordoves et al. 1982) and through some islands of the Lesser Antilles (Ferenc et al. 1990).

The seroprevalence of *T. vivax* in cattle in Venezuela was estimated as 25.5% after evaluation of 1,884 serum samples by capillary aggulutination (CA) and passive hemagglutination (PH) techniques (Toro Benitez et al. 1980). These samples were collected from bovine herds located in nine states across the country. Previously, the seroprevalence for *T. vivax* was determined as 20.6% using only the CA technique (Toro Benitez, 1976). These epidemiological studies show that *T. vivax* infection is endemic in Venezuela in cattle of all breeds and ages, and on all types of farms, suggesting that *T. vivax* has developed an efficient method of transmission in Venezuela and also in other New World countries without the presence of *Glossina* spp., its natural vector in Africa.

In general, parasitology textbooks point out that *T. vivax* is propagated in the New World by 'mechanical transmission' by biting flies like tabanids and *Stomoxys* sp., although until 1990 there was a lack of field tests and epidemiological evidence to prove this form of transmission. Lately, a field assay carried out in French Guiana demonstrated that *T. vivax* was transmitted between zebu calves by *Tabanus importunus* horse flies (Raymond 1990), and a similar study was conducted in Colombia by Otte and Abuabara (1991) which proved that the neotropical horsefly *Tabanus nebulosus* was able to transmit *T. vivax* from an animal with high parasitemia to a susceptible host. Thus, these field tests provided experimental evidence for the mechanical transmission of *T. vivax* in the New World. Nevertheless, doubts still exist that this is the only method of transmission, due to the high seroprevalence and endemic condition of *T. vivax* in this continent. The existence of a biological vector of *T. vivax* in the New World cannot be ruled out, (Otte and Abuabara, 1991), and according to Wells et al. (1982) 'the method of transmission of *T. vivax* in the New World is unknown'. Otte (1991) shares this opinion after conducting an eight year study on cattle trypanosomiasis in Colombia.

Since there is no consensus about the mechanism and route of transmission of *T. vivax* in the New World, the possibility of alternative methods of transmission, or the existence of a multifactorial process for efficient propagation, must be considered. One possible means of *T. vivax* dissemination in this continent is the transplacental route, and authors in Africa have demonstrated that *T. vivax* is able to migrate across the placental tissues in pregnant ruminants (Ikede and Losos, 1972; Ogwu and Nuru, 1981). In South America, cases of transplacental transmission of *T. vivax* have been reported from Colombia (Betancourt 1978) and from Venezuela (Meléndez et al., 1993). Data from the Venezuelan case is presented in this paper.

Another cattle trypanosome found in Venezuela is *T. theileri* which is often considered a non-pathogenic species and probably occurs in cattle worldwide. Tabanid flies transmit *T. theileri* cyclically to bovine hosts and only under certain conditions do cattle show parasitemia and clinical symptoms of disease. Soulsby (1972) pointed out a probable relationship between persistent *T. theileri* infection and lymphocytosis, lymphopoiesis, and possibly leukosis (leukemia) in cattle; unfortunately further research was probably not undertaken in this field. Nonetheless, a recent clinical and fatal case of bovine trypanosomiasis due to *T. theileri* was reported from Iran in a cow with typical symptoms of disease plus marked lymphocytosis and leukocytosis (Seifi 1995).

Several reports have charged hard ticks with participation in the *T. theileri* life cycle after the detection of this hemoparasite or a *T. theileri*-like flagellate in tick hemolymph. Evolutionary stages of trypanosomes have been described from *Rhipicephalus* sp. and *Boophilus decoloratus* in Ethiopia (Bergdorfer et al. 1973), and *T. theileri*-like trypanosomes were found in *Amblyomma americanum* from Oklahoma, USA (Krinsky and Burgdorfer, 1976).

In Sudan, *Hyalomma a. anatolicum* was regularly found to be infected with various developing stages of *T. theileri*-like flagellates (Morzaria et al., 1986). *Hyalomma* was able to transmit this protozoan when applied to a susceptible calf in an experiment considered to be the first case of biological transmission of a trypanosome by a tick. Twice, *T. vivax* has been found in the hemolymph or internal organs of *Boophilus microplus* in the New World (López et al. 1979; Cordovés et al., 1982), though in both cases, the transmission of *T. vivax* by this tick was considered uncertain. In Venezuela, *T. theileri* has not been reported from hard ticks and is seldom diagnosed from cattle blood smears. This paper reports the presence of *T. theileri*-like flagellates in hemolymph samples collected from *B. microplus* while studying the epidemiology of cattle hemoparasitosis in the West Central Region of Venezuela.

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MATERIALS AND METHODS

During the last six years, longitudinal and cross-sectional studies were carried out on the epidemiology and immunodiagnosis of cattle babesiosis and anaplasmosis, and studies on dynamics of *Babesia* spp. sporokinetes in *B. microplus* (n=653) were microscopically examined. Samples collected were capillary block, venous blood for separating serum samples and tick hemolymph microdrops. Blood and hemolymph smears were fixed with methanol and later stained with Giemsa, whereas serum samples were processed for IFA tests following James et al.(1981). These microscopical evaluations showed some cases of *T. vivax* and *T. theileri*-like infections respectively.

RESULTS

Trypanosoma vivax in a newborn calf

A five hour old Carora breed calf was found infected with *T. vivax* trypomastigotes (2-4 /field) with a mean length of 22.5u. Morphological identification was done following the photomicrograph method of Shaw and Lainson (1972) and comparing it with measurements previously published. A follow-up of parasitemia and anti-*T. vivax* antibody levels was carried out over a four month period. *T. vivax* was detected only four times in the calf blood smears during this period on days 0, 35, 80 and 125 after the first blood collection. The infected calf grew normally and clinical symptoms of bovine trypanosomiasis were not observed during the sampling period. Antibodies were detected by IFA on days 0, 21, 35, 50 and 80 after the first serum sampling with low titers (1:80 - 1:160). Other calves from the same farm were also evaluated by the IFA test but tested negative to anti-*T. vivax* antibodies.

T. theileri-like flagellates in *B. microplus* hemolymphs

These trypanosomes were found in the hemolymph of three *B. microplus* which were subsequently labeled cases A, B and C.

Case A: Epimastigotes of a *T. theileri*-like trypanosome were observed in hemolymph of an engorged *B. microplus* on day 8 of oviposition. This tick was obtained from a Brown Swiss cow with serologic and clinical pathologic diagnosis of bovine leukosis, from "Torrellero Farm" in Lara State.

Case B: Many epimastigotes of a *T. theileri*-like flagellate were found in the hemolymph of an engorged *B. microplus* on day 10 of oviposition. This tick was detached from another Brown Swiss cow from the same farm. Positive hemolymph was diluted with saline solution 0.85% and epimastigotes showed greater motility.

Case C: This tick was collected from a Carora breed cow at finca "Miramar", Las Yaguas, Lara State, and on day eight of oviposition its hemolymph was highly positive to epimastigotes and a few trypomastigotes. Measurements of these stages were made with a microscope using a calibrated ocular and all the results are given below in microns. Epimastigotes (n=10): 10.2; Middle of nucleus to anterior end (NA): 10.7; and Nuclear index (NI=PN/NA): 0.95. Trypomastigotes (n=2): L: 31.75; F: 5.5; NA: 11.25; NI; 1.33.

DISCUSSION

The high seroprevalence of *T. vivax* in cattle herds located in tropical and subtropical regions of the New World suggests that this parasite has developed one or more mechanisms for transmission which have ensured the propagation of *T. vivax* among large and small ruminants on this continent. Since there is no general agreement regarding how *T. vivax* is transmitted in Central and South America, the challenge is to prove that besides mechanical transmission by bloodsucking flies, there may be other effective methods of propagation for this hemoparasite. The case of transplacental transmission of *T. vivax* in the five hour old calf ratifies the possibility that this protozoan is able to migrate across the placental tissues to infect a fetus and thus assure its survival in a new generation of hosts. This perinatal host-parasite contact may lead to a condition of immunological hyporesponse or tolerance against an agent transmitted transplacentally (Carlier and Truyens 1995), which would explain situations of endemic stability often detected in some tropical areas.

The search for a possible biological vector of *T. vivax* in the New World as proposed by Otte and Abuabara (1991), is a task that can now be carried out more rapidly and accurately using modern technology such as DNA probes or PCR on arthropod samples like hemolymph and blood meals. The presence of *T. theileri*-like trypanosomes in

B. microplus is a new finding in Venezuela, although this finding had been previously confirmed in *B. decoloratus* from Burgdorfer *et al,* (1973) and in *B. microplus* from Brazil in 1988. Since *T. theileri* is an elusive though cosmopolitan trypanosome of cattle, it is possible that in addition to the cyclical transmission carried out by tabanid flies, hard ticks may play a role in the life cycle of this ubiquitous trypanosome.

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MECHANICAL TRANSMISSION OF AGENTS OF LIVESTOCK DISEASES BY TABANIDS

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ABSTRACT

The magnitude of mechanical transmission of agents of livestock disease by hematophagous arthropods in nature is difficult to assess. Tabanids have been associated with the transmission of over 35 pathogenic agents of animals, and the majority of diseases associated with tabanids are mechanically transmitted. Studies on tabanid host-seeking and feeding behaviour can serve as a model for the evaluation of mechanical transmission of agents. We have used animal retroviruses as models for mechanical transmission, and our studies have been oriented toward describing the factors that contribute to the probability of transmission. Studies on factors that affect interrupted feeding, mixed feeding, and the quality of agents transferred between hosts have allowed us to identify important vectors and also to propose spatial barriers to reduce transmission. Techniques for measuring the amount of blood residue on the mouth parts of the tabanids and other Diptera following an interrupted blood meal have been developed. Blood residues on the mouth parts of horse flies, stable flies, and mosquitoes have been estimated to be 10^{-5}, 10^{-8}, and 10^{-9}, respectively. Using these studies in association with the titer that different agents reached in the blood of infected animals, we can predict the probability of mechanical transmission.

RESUMÉ

L'importance de la transmission mécanique des agents pathogènes par les arthropodes hématophages est difficile à estimer sur le terrain. Les tabanides ont été incriminés dans la transmission aux animaux de plus de 35 agents pathogènes, et la majorité des maladies associées aux tabanides sont transmises mécaniquement. Les études sur la recherche des hôtes par les taons, et le comportement alimentaire de ces insectes peuvent servir de modèle pour la transmission mécanique des agents pathogènes. Nous avons utilisé les rétrovirus des animaux comme modèle de la transmission mécanique, et nos études ont été orientées vers la description des facteurs qui contribuent à la probabilité de transmission. L'étude des facteurs qui provoquent l'interruption des repas sanguins, des repas sur divers hôtes, et la quantité d'agents pathogènes transférés entre les hôtes ont permis d'identifier d'importants vecteurs et aussi de proposer des barrières spatiales pour réduire la transmission. Les techniques permettant de mesurer la quantité de sang résiduel sur les pièces buccales des tabanides ou d'autres diptères à la suite d'un repas sanguin interrompu ont été développées. Les résidus de sang sur les pièces buccales des taons, des stomoxes et des moustiques ont été estimés respectivement à 10^{-5}, 10^{-8} et 10^{-9}. Ces résultats, associés à la connaissance de la quantité d'agents infectieux présents dans le sang des animaux permettent de prédire la probabilité de transmission mécanique.

RESUMEN

La magnitud de la transmisión mecánica de agentes patógenos por los artrópodos hematófagos en la naturaleza es difícil de asegurar. Los tabánidos han sido asociados con la transmisión de mas de 35 agentes patógenos de los animales, y la mayoría de las enfermedades que están asociada con tábanos son transmitidas en forma mecánica. Estudios sobre los hospedadores de los tábanos y del patrón de alimentación de estos últimos, pueden servir como modelo para la evaluación de la transmisión mecánica de los agentes. Hemos usado retrovirus animales como modelos de transmisión mecánica, y nuestros estudios se han orientado hacia la descripción de los factores que contribuyen a la probabilidad de transmisión. Estudios sobre factores que afectan la alimentación interrumpida, alimentación mixta, y la cantidad de agentes transferidos entre los hospedadores nos han permitido identificar vectores importantes y también proponer barreras espaciales para reducir la transmisión. Se han desarrollado técnicas para medir la cantidad de residuos sanguíneos en las cavidades bucales de los tábanos y otros Dípteros siguiendo una alimentación sanguínea interrumpida. Los residuos de sangre en las piezas bucales de la mosca de los caballos, moscas de establo, y de mosquitos han sido estimados en 10^{-5}, 10^{-8}, y 10^{-9}, respectivamente. Usando estos estudios en asociación con los pequeños títulos de los diferentes agentes alcanzados en la sangre de los animales infectados podemos predecir la probabilidad de la transmisión mecánica.

Mechanical transmission is important in the epidemiology of many agents of livestock disease but the magnitude of mechanical transmission of agents by hematophagous arthropods in nature is difficult to assess. By definition, in mechanical transmission there is no development or multiplication of the agent in the vector. Unlike agents that are biologically transmitted by arthropods, there are multiple routes of infection for mechanically transmitted agents. Mechanical transmission of blood-borne agents between hosts can be facilitated by man or by hematophagous arthropods. For agents in livestock blood, there are vampire bats, needles, surgical procedures, transfusions and animal husbandry procedures like tattooing, rectal palpation, shearing and ear tagging.

When considering the study of the prevalence or incidence of the agents in which mechanical transmission is important, the contribution of iatrogenic transmission (by the veterinarian or producer) should be evaluated. When trying to control the spread of agents, controlling iatrogenic transmission is imperative. For example, if needles or surgical instruments are used multiple times without cleaning, then other control measures will be ineffective. Control measures are difficult to enforce if the agent being considered is not recognized as the cause of an important, acute disease. If the disease cannot be related to mechanical transmission by man and insects, recommended control measures will not be followed.

However, successful management of mechanically transmitted agents that are recognized as important by a community can be accomplished. For example, the prevalence of equine infectious anemia virus (EIAV) in the United States has been greatly reduced by efforts to reduce transmission. The factors that have led to this success are:-

(1) the association of tabanids with the disease has been recognized for almost 100 years; an acute disease, swamp fever, occurred soon after the observation of high tabanid activity;

(2) a simple, sensitive, accurate diagnostic test (the agar gel immunodiffusion test) was developed and widely accepted;

(3) a community decision was made to control EIAV transmission.

Control of the movement of infected animals, isolation or slaughter of infected animals (the only host for the virus), and public education on iatrogenic transmission have been the key elements in control programs (Foil and Issel, 1991). These important elements do not exist for New World trypanosomes.

At least two communications during this symposium indicated that some authorities do not support the concept that mechanical transmission of T. evansi or T. vivax by insects is important. We should address the issues that would establish credibility of the importance of insect transmission. Transplacental transmission of T. evansi and T. vivax has been reported, but the extent of this type of transmission has not been determined (Melendez et al., 1993). Small animal reservoir hosts for T. evansi exist including vampire bats which can be both host and vector of T. evansi (Hoare, 1972). The mechanical transmission of T. evansi by tabanids has been extensively studied in different countries since the turn of the century and the evidence incriminating tabanids as vectors is conclusive. In Indonesia, Nieschulz (1929) and others were able to transmit T. evansi by a single tabanid, but the probability of transmission dropped precipitously when flies were not fed until 24 hours after the initial feeding. Moutia (1928) demonstrated mechanical transmission of T. evansi with Stomoxys nigra from guinea pigs to guinea pigs and dogs.

T. vivax is the only species of tsetse-transmitted trypanosome that has become permanently established outside of Africa. Mechanical transmission by tabanids was only recently demonstrated by Raymond (1990) who successfully infected a calf with T. vivax by the interrupted feeding of 44 Cryptotylus unicolor over an 8 - 10 day period. Otte and Abuabara (1991) were able to transfer T. vivax infection with 17 - 19 flies (three Tabanus spp.) to cattle after feeding flies on steers with a parasitemia of 5×10^5/ml. Mihok et al. (1995) fed flies from ten taxa of African Stomoxyniae on cotton soaked with blood containing trypanosomes (four species at 10^7/ml) and then on mice within three minutes. T. vivax was transmitted at 3.4% and T. evansi at 0.9%, while a simulation using 25 gauge needles transferred T. vivax at a 100% rate.

Therefore, transmission data clearly establishes that insect transmission of T. evansi and T. vivax is possible. However, establishing the magnitude of insect transmission in the overall epidemology is lacking. Examples of future studies that might be relevant for the New World trypanosomes can be taken from studies on bovine leukemia. Although true vertical transmission of bovine leukemia virus (BLV) probably does not occur, transmission via milk

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and in utero transmission have been described. The prevalence of these modes of transmission has been difficult to estimate because of the presence of colostrum-derived antibodies. Fewer than 20% of calves of BLV test-positive dams are infected before birth. Horizontal transmission is more important than transmission via milk under natural conditions; the majority of cattle become infected after the age of 18 months (Evermann et al, 1987; Ferrer, 1980).

For BLV, the most sensitive portal of entry is intradermal or intracutaneous inoculation and the most infectious bovine tissue, secretion or excretion is blood. The volume of blood required to transfer infection can be as low as 50 - 100 nl (Foil et al, 1989). Epidemics have been described during winter housing periods (Wilesmith et al, 1980), but there is evidence for a role of insects in BLV transmission (Bech-Nielsen et al, 1978). Manet et al, (1989) found that there was a significant correlation between the density of tabanids and the incidence of BLV, and the highest rates of seroconversion correlated with residue the seasonal activity of tabanids.

Foil et al. (1989) were able to demonstrate BLV transmission by horse flies (*T. fuscicostatus*) initially fed on cattle with BL; as few as 10 - 20 flies transmitted BLV to goats and dairy calves, respectively. Using mouthpart residues estimated (10 nl) for *T. fuscicostatus*, groups of 10 - 20 flies would be predicted to transfer from 50 - 200 nl of blood to recipients, and 100 nl of the donor's blood was show to be infectious for calves and goats. These studies help to confirm that tabanids can be involved in horizontal transmission of BLV among cattle. The relative importance of insects in the epidemiology of BLV is probably most related to geographic area.

Although tabanid transmission of agents is often used as a model, it is important to understand the relative importance of other insects. There are many geographic areas or instances where other modes of transmission occur even when tabanids are present. Tabanids have been associated with the transmission of over 35 pathogenic agents of animals (Foil, 1989). Studies on tabanid host-seeking and feeding behaviour will be presented to explain this phenomenon. However, the titer of infectious agent, the persistence of the agent and the infectiousness of the agent at the portal of entry are major factors in the ultimate probability of mechanical transmission. Similarly, the number and type of insects feeding on hosts is important.

We can obtain some information on the relative importance of different insects as mechanical vectors from transmission trials where the titer of the organism has been reported. The viremia or parasitemia of the donor most often determines the numbers and types of vectors required to transfer infection. Feline leukemia virus (FLV) has been transmitted by a single mosquito in trials where donor titers reached 10^9 infectious doses (ID)/ml. Equine infectious anemia virus (EIAV) reaches 10^6 ID/ml in the febrile donor and a single horse fly *(Tabanus fusicostatus)* has been shown to transfer the infections. When bovine leukemia virus exceeds 10^4 ID/ml, transmission by tabanids becomes possible. Transmission of BLV by tabanids using donors in the range of 10^3 ID/ml has not been demonstrated in limited trials (Foil and Issel, 1991). As agents approach 10^8 like FLV, single stable flies can transfer infection. When agents exceed 10^8, for Venezuelan Equine Encephalities virus and Rift Valley fever virus, vessel feeders (solenophages) like bedbugs and mosquitoes, as well as small arthropods like ceratopogonids and mites, can transfer infection.

Information on the quantity of blood remaining on the mouthparts of insects after an interrupted meal also can provide a starting point for evaluating the importance of different insects. Certainly, the number of insects and the quantity of blood transferred between hosts is important. The more efficient mechanical vectors are telmophagous, that is, pool feeders. The mouthparts of tabanids (*Tabanus fuscicostatus*) are known to have approximately 10 nl of blood meal residue following an interrupted feed, and as agents approach 10^6 infectious particles (IP) per ml of blood, transmission by individual tabanids occurs (Foil *et al,* 1987). The amount of blood on a 22-gauge needle following an intramuscular stick also is approximately 10 nl. Clearly, as agents approach 10^5 IP, groups of 10 flies, or at least 1 in 10 flies can transfer the infection. For deer flies, *Chrysops fuliginosus*, there is 6 nl of blood on the mouthparts after an interrupted feed (Knaus et al, 1993). Foil et al, (1983) reported transmission of EIAV with six deer flies *(Chrysops flavidus)*. Weber et al, (1988) estimated by measurements made from electron micrographs that the internal mouthparts of stable flies would retain 0.03 nl of blood, and this estimate is consistent with the transmission trials for EIAV (that is, 200 flies could transfer 6 nl of blood or a potential of 6 IP). Tabanids and stable flies are considered potential vectors of EIAV, but mosquitoes are not. Transmission studies using mosquitoes, including *Psorophora columbiae, Aedes taeniarhynchus* and *Aedes sollicitans*, in groups ranging 40 to over 300 have been negative. Based on estimates of between 10^{-9} and 10^{-10} ml of blood transferred to a second host by a mosquito following an interrupted feeding, these results would be predictable (Miike, 1987). Thus, the vector potential of horse flies for EIAV would be approximately 200 times greater than stable flies and 6,000 to 60,000

times greater than mosquitoes.

Regurgitation by stable flies during a second feeding has also been described using *in vitro* techniques (Butler et al, 1977), but results of EIAV and BLV transmission trials do not indicate a high rate of regurgitation. There have been a number of negative trials (five with over 100 stable flies) using EIAV donors with up to 10^6 IP/ml. Transmission was later demonstrated with groups of 224 - 400 stable flies and groups of 52 - 100 stable flies. Similar trials on BLV with groups of 50 - 75 flies and with donors with less than 10^5 bovine infectious doses (BID)/ml have been negative. Negative results of these transmission trials indicate that regurgitation by stable flies does not occur with high frequency under the reported conditions (Foil and Issel, 1991).

Once the amount of blood on the mouthparts of different insects is known, it is important to have an estimate of how often mixed blood meals are obtained. As discussed in the paper on vector control, the distance between animals has a significant impact on the percentage of mixed feeding. We conducted a study on tabanids and compared the relative feeding persistence of representative Louisiana tabanids. Horses were tethered in a 9m square design, and flies were marked, but not disturbed. *Tabanus lineola* was the most plentiful species present but only 2% of 599 marked *T. lineola* actually transferred to another host. Similarly, only 2.8% of *Chrysops spp.* transferred from one host to another, while of the larger tabanids, 7.1% of *T. sulcifrons* and 12.3% of *T. petiolatus* transferred to new host individuals. Although low numbers of other species were marked, it appeared that the larger the tabanid, the greater the potential for transfer between two host animals. Smaller tabanids may move between hosts less frequently than larger flies and individually transport less residual blood meal. However, differences in population density may change the relative importance of different-sized tabanid or other insect vectors.

CONCLUSION

Since there can be doubts about the relative importance of insects in the mechanical transmission of certain agents, it would be appropriate to establish criteria to qualify mechanical transmission by insects as important in the epidemiology of agents of livestock disease. The key elements to consider are:

- There should be some stage in the disease for which an appropriate titer naturally occurs that would indicate that mechanical transmission can occur.

- There should be reports of successful experimental transmission trials with insects transferred between natural hosts of the agent.

- The agent should be transmitted in areas in the absence of biological vectors.

- Contaminative, contagious or vertical transmission should not account for the major mode of transmission.

- Prevalence should be highest in areas with long insect seasons and high insect population.

- Studies should be conducted to establish temporal association of transmission with peaks of insects.

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BIOLOGY AND CONTROL OF TABANIDS AND STABLE FLIES

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ABSTRACT

Tabanids are among the most free-living adult flies that are livestock pests. A single blood meal is used as a source of energy for egg production (100 - 1,000 eggs per meal), and females of certain species can oviposit before a blood meal is obtained (autogeny). Therefore, only one out of fifty females is required to successfully oviposit for maintenance of annual populations and there are normally wild animal blood sources available to maintain annual tabanid population. The larval habitats are also independent of domestic livestock; thus use of repellents or partial repellents is the only chemical strategy to reduce the incidence of tabanids on livestock. Permanent traps and possibly treated silhouette traps can be employed to intercept flies. Selective grazing or confinement can also reduce the impact of tabanids. Stable fly adults are dependent upon vertebrate blood for survival and reproduction but the majority of the time is spent off the host. Stable fly larvae develop in manure, spilled feed, and decaying vegetation. Management of larval habitats by sanitation is the key to stable fly control. Treatment of animals with residual insecticides can aid in control; and thorough application to the lower body parts is important. Modified traps, either as treated targets or with solar powered electrocution grids, can be effective in reducing stable fly populations with proper use.

RESUMÉ

Les tabanides sont parmi les plus importants diptères adultes nuisibles au bétail. Un unique repas de sang est utilisé comme source d'énergie pour la production des œufs (100-1000 œufs produits par repas), et les femelles de certaines espèces peuvent pondre avant d'avoir pris un repas sanguin (espèces autogènes). En conséquence, seulement une femelle sur cinquante est nécessaire pour permettre le maintien d'une population donnée de tabanides. La présence générale d'animaux sauvages suffit le plus souvent à permettre ces repas sanguins et maintenir la population de taons. D'autre part les habitats larvaires sont souvent indépendants des élevages. En conséquence, l'utilisation de répulsifs ou de produits partiellement répulsifs est la seule stratégie chimique permettant de réduire l'impact des tabanides sur l'élevage. Des pièges permanents, et éventuellement des pièges écrans imprégnés peuvent être employés pour intercepter les insectes. Un pâturage sélectif, ou un confinement du bétail peuvent également réduire l'impact des tabanides. Les stomoxes adultes dépendent du sang d'hôtes vertébrés pour leur survie et leur reproduction, mais ils passent la majorité de leur temps éloignés des hôtes. Les larves de stomoxes se développent dans les bouses, les débris alimentaires et les végétaux en décomposition. L'aménagement des zones d'élevages par assainissement de l'habitat larvaire est la clef du contrôle des stomoxes. Le traitement des animaux à l'aide d'insecticides rémanents peut aider le contrôle; mais l'application des produits sur les parties basses est particulièrement importante. Des pièges modifiés, soit comme cible imprégnées soit à l'aide grilles d'électrocution solaires peuvent être utiles à la réduction des populations de stomoxes lorsqu'ils sont correctement utilisés.

RESUMEN

Los tábanos son entres las moscas adultas las que tienen mas libertad de vida y que son una peste para el ganado. Una sola oportunidad de alimententación con sangre es usada como fuente de energía para la producción de huevos (1 100-1,000 huevos por ración de alimento), y algunas hembras de ciertas especies pueden depositar sus huevos sin alimententarse antes (autogenia). Por lo tanto, solo se necesita una de cincuenta hembras para el éxito de la oviposición y así mantener la población anual. Normalmente hay disponibilidad de animales salvajes que sirven como fuente de sangre para mantener la población anual de tábanos. Los hábitats de las larvas también son independientes del ganado doméstico. De este modo, el uso de repelentes, total o parcial, es la única estrategia química para reducir la incidencia de tábanos en el ganado. Trampas permanentes y posiblemente trampas de tipo silueta previamente tratadas pueden se utilizadas para interceptar a las moscas. La estabulación selectiva o el confinamiento también pueden reducir el impacto de los tábanos. Una mosca adulta de establo depende de la sangre de los vertebrados para sobrevivir y su reproducción, pero la mayoría del tiempo se la pasa lejos del hospedador. Las larvas de moscas de establo se desarrollan en el estiércol, alimento derramado, y vegetación en descomposición. El manejo del hábitat de las larvas por medio del saneamiento es la clave para el control de la mosca de establo. El tratamiento en los animales con insecticidas residuales puede ayudar en el control; la aplicación de manera amplia y extensa en la parte inferior del cuerpo del animal es importante. Trampas modificadas, cualquiera que sea, objetivos

tratados o con trampas de rejillas de electroconducción por fuerza de energía solar, pueden ser efectivas en la reducción de la población de mosca de establo, utilizados apropiadamente.

INTRODUCTION

Control programs for arthropod pests of livestock are designed to increase economic benefits due to increased weight gains or animal fitness and to decrease transmission of agents of animal diseases. This paper will focus on potential methods for reducing the mechanical transmission of trypanosomes (*Trypanosoma vivax* and *T. evansi*) by insects, particularly stable flies, *Stomoxys calcitrans*, and tabanids. The integration of farm management practices and insect control measures are required to reduce transmission of disease agents.

Types of insecticides and application techniques are varied, and many of these are effective in controlling acarines and certain fly pests of livestock. However, sprays or dips using pyrethroid or organophosphate insecticides are the only elements of integrated programs effective in reducing mechanical transmission of disease agents. Application techniques such as self-treatment devices (dust bags or back rubbers), delivery of small quantities of insecticides by pour-on, injection, or impregnated ear tags, are not appropriate for tabanids or stable fly control (Foil and Hogsette, 1994). A review of the biology of these insects will be provided to support control recommendations. Surveillance techniques will also be discussed. Tabanids and stable flies both have a complete metamorphosis, that is egg, larva, pupa and adult. Only female tabanids blood feed while both male and female stable flies blood-feed. Stable flies and tabanids cause weight loss due to blood loss and annoyance as well as create feeding lesion sites which may promote contaminative transmission of agents or myiasis; thus, the recommended control methods would also supply economic benefit. Stable fly control may also reduce the incidence of transmission of cutaneous habronemosis or summer sore (Foil and Foil, 1988).

TABANIDS

Probably the most important factor that we should remember when talking about this group is that it is dangerous to generalize about a family of over 4,000 species and at least 137 genera. Flies of the family Tabanidae vary in colour, body markings, wing markings and size (9-33mm). Tabanids have three segmented antennae; the third segment is annulated and can have a tooth-like process at the base. The deer flies (*Chrysops spp.*) are relatively small (6-11mm in length), yellow-orange flies with dark body markings, that usually have pictured (distinct dark patterns) wings. Clegs (*Haematopota spp.*) are medium-sized flies with mottled wings. The term "horse fly" describes a much more diverse group of flies, and is essentially used to describe any tabanid other than deer flies and clegs.

Both male and female tabanids feed on carbohydrates for metabolic energy sources. Female flies obtain blood meals to support egg development. Females of most species must obtain a blood meal prior to the development of each batch of eggs; however, some species lay one batch of eggs before they seek an animal host (autogeny). Most species deposit their eggs in layered masses on vegetation above larval habitats. The period between the time a female obtains a blood meal, completes oogenesis and oviposition, and then begins to search for another blood meal is 3 - 4 days. Larvae feed on organic debris and/or small invertebrates in a variety of aquatic to semiaquatic habitats. The larvae mature and pupate in drier soil. Life cycles require from two months to two years depending upon the species and geographical location. In most regions, adults of most of the species occur for only about a month, but a succession of species often is seen. The result is that livestock may be attacked by one or more species of Tabanidae throughout all or most of the warm months of the year.

For surveillance, the Manitoba fly trap is effective. It consists of a tripod approximately two metres tall; the top half is covered with a clear plastic canopy with a collection chamber (normally a container with an inverted funnel) at the apex. A decoy, routinely a black sphere, is suspended under the canopy. Catts (1970) redesigned this trap as a pyramid with a clear top and a black bottom, the trap is erected using a centre pole and stakes at the end of ropes connected to the four corners of the pyramid. Recent updates of canopy trap designs are available (Hribar et al, 1989). The Manning trap incorporates some form of flight intercepting mesh panels normally green or gray plastic screen or nylon (Foil and Hogsette, 1989).

Worldwide, tabanids are considered to be the most challenging livestock pests to control and a multitude of factors

contribute to this fact. Females need spend only four minutes feeding on a host to generate eggs which develop into the next year's adults. Due to the complex life cycle, independent of livestock, integrated control strategies are required to reduce the impact of these pests. As with most other flies, control of the larval habitat is most effective if possible. Studies in both Africa and the United States demonstrated that widespread application of persistent insecticides on larval habitats can be very effective, however, potentially harmful environmental impact associated with such treatments, plus expense, has prevented adoption of these methods. Area wide control of adult tabanids has met with less success, and this has often been blamed on the mobility of adult tabanids. Ultra low volume application of pyrethroids has achieved some initial population reduction. Population levels often return to pretreatment levels with 1 - 3 days indicating a rapid influx of adult flies (Hansens, 1981). Removal of trees and other vegetation has been associated with a reduction in deer fly numbers. Vegetation control can also reduce oviposition if the oviposition substrate occurs along the margins of aquatic habitats. The use of vegetative barriers, water management, or spraying kerosene or other surfactant on the surface of small natural or artificial water pools where tabanids drink have also been mentioned as methods of control (Foil and Hogsette, 1994).

The effect of the use of insecticides and repellents applied to livestock on the tabanid burden has been studied to some degree. Early work establishing the economic impact of tabanids on cattle was conducted using synergized pyrethrins to provide protection from tabanid attack (Bruce and Decker, 1951). Similarly, cattle using self-treatment spray devices or alleyways are protected from mechanical transmission of agents.

Many of the new formulations for tabanid control contain one of several pyrethroids. We used the pyrethroid, fenvalerate, applied as a high pressure (100-200psi) spray as a model compound for determining the effects of pyrethroids on tabanid feeding success. We found that the feeding times for the horse fly species observed were reduced by 35%, and the amount of blood consumed was also significantly reduced by 30%. Mark-recapture and dissection (parity) studies indicated that at least 20% of the tabanids feeding on cattle contiguous to larval habitats had fed in that area at least four days previously. A 44% reduction in daily blood loss to tabanid feeding for cattle treated with fenvalerate would be predicted from the combination of reduced subsequent feeding and reduced blood meal size (Foil et al, 1989; Leprince et al, 1992).

As 20% of the population essentially can be controlled, pyrethroid ear tags, and sprays were evaluated against tabanids under field conditions in Louisiana. Overall mortality rates were 3% in control, 9% in permethrin tags, 15% in fenvalerate tags, 67% in fenvalerate spray at 0.01% and 79% in fenvalerate spray at 0.02%, and 100% in l-cyhalothrin tags (Leprince et al, 1992). There does appear to be some indication that the use of pyrethroid sprays will provide partial protection for livestock. However, effects of control programs on other arthropods should be considered. For example, partial protection is not warranted if such efforts increase pesticide resistance in other important livestock pests.

Animal management can influence the incidence of tabanids on livestock. Few tabanid species will enter barns or other structures; the species of horse flies that do enter structures are usually active during crepuscular or nocturnal periods. Tabanid attack can be reduced even when cattle are stanchioned beneath roofs supported by posts and with open sides. If given access to suitable structures, free-roaming livestock will seek shelter from tabanid attack. Fewer problems will be encountered on pastures located well away from wooded areas; therefore larger pastures should be used during peak tabanid activity.

The proximity of infected and susceptible hosts affects the probability of the mechanical transmission of agents, irrespective of the agent involved. The separation of infected and susceptible horses by at least 200m has been recommended to reduce the transmission of equine infectious anemia virus (EIAV). To test this recommendation we used groups of four horses, tethered in a square formation, each accompanied by a researcher. Tabanids that had initiated feeding, but were not visibly engorged, were marked and artificially repelled. When other horses were tethered 36.6m away, 87.5% of the tabanids returned to their original host and extrapolation from a linear regression indicated that up to 99% of the horse flies would be expected to return to the original host when separated as far as 49.3m from other hosts (Foil, 1983).

There are many natural infectious diseases, parasites, and predators of all life stages of tabanids. However, the diversity and dynamics of tabanid populations buffer this group from depletion by parasites and predators. For example, adult tabanid production from flooded hardwood forest floors in Louisiana averages 1 fly per sq. ft. of soil

surface, which is equivalent to 43,500 adult tabanids per acre (21,750 females). Our studies indicate that approximately 50% of these females successfully complete a blood meal and deposit around 200 eggs (or 2,150,000 eggs total). Thus, only 2% survival from egg to adult is required to maintain annual populations. In practice, the only form of biological control that provides protection for livestock occurs when predators intercept host-seeking tabanids around animals. Under some circumstances, sarcophagids and dragonflies can interrupt tabanid feeding. However, the most dramatic reduction in tabanid feeding relative to the presence of predators occurs when Bembecine wasps (Nyssonine) are active around livestock capturing tabanids as food for the larvae. Unfortunately, the larval habitats of these wasps are usually specific, and seasonal population peaks of the wasps are much shorter than tabanids.

STABLE FLIES

There are 18 species in the genus *Stomoxys*. The stable fly, *S. calcitrans*, is the most widely distributed and discussion regarding this species is generally applicable to other *Stomoxys* species. *S. calcitrans* resembles the house fly *Musca domestica* L., the sword-like proboscis projecting from beneath the head aids in differential identification. Adult stable flies are gray with four black stripes on the thorax and the abdomen tessellated (checkered) in black; the extreme width of the frons and the characteristic markings on the abdomen are usually sufficient for identification of these species. Size ranges from 4 - 7mm (Zumpt, 1973).

Stable flies hatch within 24 hours, and larval development takes approximately 12 - 13 days at 27°C. The pupal stage lasts approximately a week. Adults emerge, seek a blood meal and begin mating when 3 - 5 days old. Females begin laying eggs when 5 - 8 days old. Flies usually feed below the knees and hocks of their animal hosts, but they may move onto the sides and backs if populations are large. Female stable flies lay between 60 and 130 eggs per blood meal (Foil and Hogsette, 1994). Stable fly larvae develop in manure, spilled feed and decaying vegetation. In the midwestern United States, cattle manure on cattle feed lots is an important medium for stable fly larval development. Hay dropped by cattle while feeding from large bales of hay mixed with manure may constitute the greatest single stable fly breeding medium in the southern United States.

The most effective devices for surveillance or monitoring of adult stable fly populations are made of a reflective material such as fiberglass. The first of these, the Williams trap, consisted of two rectangular panels of Alsynite brand fiberglass notched halfway through the long sides and fitted together in a cross configuration. Corrugated fiberglass was formed into a cylinder trap (Broce, 1988), which was subsequently commercialized. The commercial cylinder trap is not coated with an adhesive, but is covered with a disposable clear plastic sleeve. Trap placement is paramount to success because numbers of captured flies will vary from place to place on any livestock facility. Counting the number of stable flies on the front legs of a predetermined number of animals is another convenient surveillance technique.

Larval habitats are normally impenetrable to insecticide application. Controlling larval habitats by other methods is critical, since adult control alone is unsatisfactory. A number of pesticides and repellents are available for management of adult stable fly populations on livestock. However, the efficacy of many of these is relatively short-lived (several hours). If treatments are applied to animals, care must be taken to thoroughly coat the lower extremities where stable flies normally feed. Orlon yarn impregnated with permethrin and wrapped in a continuous coil round the fiberglass panels of Williams traps can be used as a control method (Hogsette and Ruff, 1986).

Sanitation is the most important method to use for on-site reduction of stable fly populations. Stacking and burning active dry hay residues can result in a rapid decrease in adult stable fly populations. Wet hay residues can be stacked, covered with black polyethylene, and left to compost. Some more compatible materials can be composted by stacking in large piles. Internal materials, especially if wet, will compost and the resulting elevated temperatures will further limit fly development. If large hay bales being fed to stock are placed on wagons, residue accumulations can be minimized by moving the wagons a short distance once or twice a week. Routine removal or dispersal (drying) of rolled hay residues greatly reduces or eliminates stable fly populations.

Traps are considered useful for managing fly populations. The Williams trap has been used and traps employing electrocuting grids are very popular for fly control. By combining electrocuting grids with the reflective materials used in the pyramid traps, battery powered and then solar-powered electrocuting grid traps were developed for use in

the field. Several models of the solar-powered traps which attract both house flies and stable flies are being produced commercially in the US (Pickens, 1991).

Parasitic hymenoptera have been used for biological control of stable flies. Selecting the proper parasitoid for an area and supplementing with larval habitat management efforts increased the success of biological control efforts. Parasites purchased from commercial insectaries to be released in a particular geographic area must be naturally effective in that area if the parasites are to succeed. Andress and Campbell (1994) conducted studies on stable fly control with inundative releases of *Muscidifurax zoraptor* and *Spalangia nigroaena* in feed lots and dairy confinements. There was no control of stable flies in spite of weekly releases of high numbers of parasitoids.

Tremendous strides have been made toward development of environmentally friendly control methods for tsetse. These advances were made based on some logical observations (Vale, 1993). When a human was present, the catches from neither traps nor animals were representative (six ratio) of the natural populations. Electric grid technology was developed to study this difference and from that development have come descriptions of tsetse host-seeking behaviour. Exploitations of this behaviour (odors and visual stimuli) are used to encourage tsetse to light upon a surface treated with insecticide. Stimuli exploited in Zimbabwe are not even common to all species of tsetse, so direct application to tabanids or stable flies would not be possible. Significant differences exist between host-seeking behaviour of tsetse and many tabanid species. Stable fly biology is closer to tsetse although larval dynamics are different. The attraction of stable flies to the Williams trap is likely as a resting site rather than the mimicking of a host. Thus, developing host-like targets to augment resting traps may result in adequate adult stable fly control. For tabanids, developing target technology will likely take more innovative basic research, particularly since thousands of species have multiple host-seeking behaviours.

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CLINICAL FINDINGS IN *TRYPANOSOMA EVANSI* INFECTED GUINEA PIGS

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ABSTRACT

Trypanosoma evansi is the causative agent of a disease of horses called "derrengadera" or "surra". This hemoflagellate has been found in Africa, Asia and South America, where it infects horses, camels, dogs, cattle and buffaloes. Pathogenesis of *T. evansi* infection depends on its virulence, host susceptibility and epidemiological conditions in the region. In horses, the disease may be acute or chronic, with episodes of fever, anemia, edema, cachexia and hind end paralysis. These latter symptoms suggest nervous damage. We report the clinical and histological findings in guinea pigs experimentally infected with *T. evansi*. Three out of five infected guinea pigs showed neurological signs seven weeks after infection, mainly hind end paraplegia and both involuntary micturition and defecation. Histological changes were diagnosed as cellular infiltration by macrophages, both neuronal necrosis and phagocytosis, central chromatolysis and myelinic degeneration, compatible with an autoimmune condition. We suggest that it is necessary to conduct additional experiments on guinea pigs in order to comprehend whether *T. evansi* infection elicits an auto-antibody response which could recognize antigenic determinants in spinal cord tissues from uninfected animals and thus be responsible for damage in neuronal cells. Guinea pigs have proven to be an ideal experimental model for studying equine trypanosomiasis caused by *T. evansi*.

RESUMÉ

Trypanosoma evansi est responsable de la surra ou derrengadera chez les chevaux. Cet hémoflagellé a été trouvé en Afrique, en Asie et en Amérique du Sud, où il infecte les chevaux, chameaux, chiens, bovins et buffles. La pathogénicité des infections par *T. evansi* dépend de la virulence des souches, de la sensibilité des hôtes, et des conditions épizootiologiques régionales. Chez les chevaux, la maladie peut être aiguë ou chronique, avec des épisodes de fièvre, anémie, oedème, cachexie et paralysie postérieure. Ce dernier symptôme suggère l'existence de lésions nerveuses. Nous rapportons les observations cliniques et histologiques réalisées chez des cobayes expérimentalement infectés avec *T. evansi*. Sur cinq animaux, trois ont présenté des signes nerveux 7 semaines après l'infection; principalement une paralysie postérieure et des incontinences urinaires et fécales. Les modifications histologiques ont été interprété comme des infiltrations cellulaires par les macrophages, nécrose et phagocytose des cellules nerveuses, lyse de la chromatine et dégénérescence de la myéline, compatibles avec un syndrome auto-immun. Des études complémentaires sont nécessaires afin d'établir si l'infection par *T. evansi* chez le cobaye induit la synthèse d'auto-anticorps qui pourraient reconnaître des déterminants antigéniques des tissus de la moelle épinière, même chez des animaux non infectés, et pourrait être à l'origine des observations histologiques faites sur les cellules nerveuses. Le cobaye s'avère être un excellent modèle pour l'étude de la trypanosomose à *T. evansi* chez le cheval.

RESUMEN

El *Trypanosoma evansi* es el agente causal de una enfermedad de los equinos llamada "derrengadera" o "surra". Este hemoflagelado ha sido encontrado en Africa, Asia y Sur América, infectando a caballos, camellos, perros, bovinos y búfalos. La patogenia de la infección por *T. evansi* depende de su virulencia, la susceptibilidad de los hospedadores y las condiciones epizootiológicas de la región. En los caballos, la enfermedad puede ser de curso agudo o crónico, con episodios de fiebre, anemia, edema, caquexia y parálisis de las extremidades. Estos últimos síntomas sugieren un daño nervioso. Se reportan hallazgos clínicos e histológicos en cobayos infectados experimentalmente con *T. evansi*. Tres de cada cinco cobayos mostraron signos neurológicos a las siete semanas de infección, principalmente paraplejia de las extremidades e incontinencia (micción y defecación involuntarias). Los cambios histológicos observados consistieron en infiltración celular por macrófagos, necrosis neuronal y fagocitosis, degeneración cromatólisis central y degeneración mielínica, hallazgos compatibles con una condición autoimmune. Se sugiere la necesidad de ampliar estos experimentos en cobayos, de manera de saber si la infección por *T. evansi* provoca una respuesta de auto-anticuerpos que pudieran reconocer determinantes antigénicos de tejidos de la médula espinal en animales no-infectados y la cual podría ser responsable de la destrucción de la célula neuronal. Los cobayos han demostrado ser un modelo experimental ideal para el estudio de la trypanosomiasis equina causada por *T. evansi*.

INTRODUCTION

Trypanosoma evansi is the causative agent of a disease in horses called "derrengadera" or "surra". This hemoflagellate is widespread in Africa, Asia and South America (Luckins, 1988) and infects several domestic animals including horses, camels and dogs, which are severely affected by the infection. *T. evansi* has also been found in cattle, sheep, goats and pigs. The capybara (*Hydrochoerus hydrochoeris*) is a very important reservoir of this trypanosome (Arias, 1994). Transmission of the agent from one infected animal to another is accomplished through biting flies, particularly horseflies (Foil, 1989). In South America, the hematophagous bat *Desmodus rotundus* has been incriminated as a mechanical vector of this hemotropic agent.

The pathogenicity of *T. evansi* infection depends on strain virulence, host susceptibility and epizootiological conditions in the region (Hoare, 1972). In horses, the disease may be acute or chronic with episodes of fever, anemia, edema, cachexia and hind limb paralysis. Infected laboratory animals, such as mice, usually die within one week after infection, whereas guinea pigs develop a chronic disease with overt clinical signs within several weeks postinfection (Diaz-Ungria 1971). The symptoms suggest nervous damage and are similar to those observed in horses (Rangel 1905).

The objective of the present work was to study, by means of an experimental model, the histological changes in the lumbo-sacral spinal cord that could be related to the clinical signs observed in *T. evansi*-infected guinea pigs.

MATERIALS AND METHOD

Ten adult male guinea pigs, weighing an average of 500g were used. The experimental group (n=5) was infected with a *T. evansi* isolate, provided by the Venezuelan Institute of Veterinary Research at Maracay, Venezuela. Five uninfected guinea pigs were used as a control group. Animals were bled daily in order to perform thin blood smears and measure PCV values. Body temperature was recorded daily. The lumbo-sacral spinal cord from infected guinea pigs was used for the histological diagnosis.

RESULTS AND DISCUSSION

Average values of circulating trypanosomes, PCV and body temperature are shown in Fig. 1. Trypanosomes were seen for the first time on the fourth day post infection in thin smears stained with Giemsa. Packed cell volume displayed a decreasing pattern, reaching levels of 30% below preinfected values. Periodic absences of circulating haemoflagellates were seen during the chronic phase of the disease.

Three out of five *T. evansi*-infected guinea pigs showed neurological signs seven weeks after infection. These signs included hind limb paraplegia and involuntary micturition and defecation.

Perineuronal infiltrate of neuroglial cells is a feature of disseminated progressive paralysis. In the present work, the histopathological studies showed cellular infiltration by macrophages. Neuronal necrosis and phagocytosis, central chromatolysis and myelinic degeneration were found. These findings could explain the clinical signs observed in the guinea pigs experimentally infected with *T. evansi*.

Neuronal cell destruction in autoimmune diseases has been demonstrated in laboratory animals harbouring hemoflagellates (Avila, 1992). In fact, *T. cruzi* trypomastigote antigens share epitopes with sciatic nerve components (Gea et al., 1993). In addition, in the chronic phase of Chagas disease (McCormick and Rowland, 1993), autoimmune antibodies that recognize antigens of both heart and skeletal muscles from naïve mice have been demonstrated.

Muscles of *T. evansi*-infected horses have shown structural changes characterized by necrosis of muscle fibers and blood capillary necrosis. Cells were infiltrated mainly by macrophages which is a classical feature in autoimmune diseases (Quinones-Mateu et al., 1994).

In summary, the phagocytosis of neuronal cells observed in this study is compatible with an autoimmune condition. We suggest further experiments in guinea pigs, to determine if *T. evansi* infection elicits autoantibody responses

which could recognize antigenic determinants in spinal cord tissues from uninfected animals and be responsible for damages in neuronal cells. Guinea pigs have proven to be an ideal experimental model for studying equine trypanosomiasis caused by *T. evansi*.

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TRYPANOSOMA EVANSI IN HUNTING DOGS: A CASE STUDY

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ABSTRACT

Trypanosoma evansi was found in hunting dogs, and confirmed by the CIRAD-EMVT lab through the PCR test. Occurrence and clinical signs are described.

RESUMÉ

Trypanosoma evansi a été trouvé chez des chiens de chasse présentant des symptômes cliniques. Le diagnostic a été réalisé par examens parasitologiques et confirmé par PCR au CIRAD-EMVT-Guyane. L'importance et les signes cliniques sont décrits.

RESUMEN

El *Trypanosoma evansi* fue encontrado en perros de cacería, y confirmado por el laboratorio del CIRAD-EMVT a través de las pruebas de PCR. Su frecuencia y signos clínicos se describen.

Three out of five dogs belonging to a hunter fell ill with symptoms of loss of appetite, loss of weight, blindness and progressive wasting. The dogs were used for hunting purposes in Tibiti which is situated in Region III, District Para, Suriname. One died before anything was done, while another one was necropsied, upon which an extremely enlarged spleen and intraocular coagulation of blood was found. The three remaining dogs were bled and trypanosomes were found in the buffy coat, the direct (blood drop) observation and on the stained blood smears of two dogs. We came to the conclusion that the parasite most resembled *T. evansi*; and this view was supported by Dr. Desquesnes at the CIRAD-EMVT laboratory.

The dogs were treated with Penta Carinat (Penta Medina) and recovered. After one month the animals were still negative for trypanosomes. Within a few months, another dog showed the same clinical signs. The diagnosis was confirmed by Capillary Centrifuge Test (CCT). Though parasitemia was not very high, some samples were kept in sealed capillary tubes in liquid nitrogen in the hope of finding laboratory mice in which to multiply the parasites. Unfortunately, in Suriname, laboratory mice are in very short supply. The owner of the dog did not want the dog treated due to the expense of the prescribed drug, therefore the dog was euthanised.

ACKNOWLEDGMENTS

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SENSITIVITY OF THE WOO TEST FOR DETECTION OF *TRYPANOSOMA VIVAX*

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ABSTRACT
The Woo test, or Haematocrit Centrifuge Technique (HCT), is the technique most commonly used for diagnosis of animal trypanosomiasis, but its sensitivity is not well defined. According to the authors, the sensitivity of the Murray technique (dark ground buffy coat method, or DG/BCM) is higher, equal or lower than that of the Woo test for the detection of *Trypanosoma vivax*. Most authors have compared particular techniques relative to other techniques, but not relative to a fIxed reference. The relative sensitivity of a particular test appears to vary between technicians. The objective of the present study was to measure the sensitivity of the Woo test for *T. vivax* detection, using blood samples with pre-determined levels of parasitemia, ranging from 1 to 1,767 parasites/ml, that were prepared by mixing infected ovine blood with non-infected ovine blood. A simple technique is described for the enumeration of parasites in the blood. The mean positivity level of the Woo test was about 200 +/- 100 *T. vivax* /ml. The sensitivity of the test was 100% above 700 parasites/ml; 80% between 300 and 700; 50% between 60 and 300, and negligible below 60 parasites/ml. Factors are provided to quantify parasitemia relative to the number of parasites observed between microscope slide and cover slip (parasitemia > 2000) or in the capillary tube (parasitemia < 2000). Sensitivity of the techniques for the detection of active infection could be evaluated with reference to fixed values such as known parasitemias, or parasitemias created artificially, as described.

RESUMÉ
Le test de Woo, ou technique de centrifugation hématocrite (HCT), est la technique la plus employée pour le diagnostic des trypanosomoses du bétail, mais sa sensibilité est mal définie. Selon les auteurs, la sensibilité de la technique de Murray (buffy coat dark ground method ou DG/BCM) est supérieure, égale ou inférieure à celle du test de Woo pour la détection des infections par *Trypanosoma vivax*. La plupart des auteurs ont comparé les techniques entre-elles, et non par rapport à une référence fixe. La sensibilité relative d'un test d'observation parasitologique dépend étroitement du technicien qui le réalise. L'objectif de la présente étude est de mesurer la sensibilité du test de Woo pour la détection de *T. vivax*, en utilisant des échantillons sanguins de parasitémies pré-déterminées, allant de 1 à 1767 trypanosomes/ml, préparés par mélange de sang de mouton infecté dans du sang de mouton non infecté. Une méthode simple est décrite pour le dénombrement des parasites dans le sang. Le seuil moyen de positivité du test de Woo a été de 200 +/-100 *T. vivax*/ml. La sensibilité du test était de 100% au-delà de 700 parasites/ml ; 80% entre 300 et 700 ; 50% entre 60 et 300, et négligeable en-dessous de 60 parasites/ml. Des facteurs de conversion sont fournis pour la quantification de la parasitémie selon le nombre de parasites observés au microscope entre lame et lamelle (parasitémie > 2000/ml) ou dans le tube capillaire (parasitémie < 2000/ml). La sensibilité des techniques de détection des infections actives par les trypanosomes circulants pourrait être évaluée par rapport à des valeurs connues de parasitémies, ainsi qu'il a été décrit.

RESUMEN
La prueba de Woo, o Técnica del microhematocrito (HCT), es la mas comúnmente usada para el diagnóstico de la trypanosomiasis en animales, pero su sensibilidad no está bien definida. De acuerdo a los autores, la sensibilidad de la técnica de Murray (método de la capa blanca a fondo oscuro, o DG/BCM) es mas alta, igual o menor que la de las pruebas de Woo en la detección del *Trypanosoma vivax*. La mayoría de los autores han comparado técnicas en particular con otras, pero no relacionándolas a una referencia fija. Esta sensibilidad relacionada a una prueba en particular aparentemente varía de una técnica a otra. El objetivo del presente estudio fue medir la sensibilidad de la prueba de Woo para la detección del *T. vivax*, usando muestras de sangre con niveles pre-determinados de parasitemia oscilando de 1 a 1767 parásitos/ml, que fueran preparadas mezclando sangre de ovinos infectados con sangre de ovinos no infectados. Se describe una técnica simple para la cuantificación de los parásitos en las muestras de sangre. El promedio de los niveles de positividad de la prueba de Woo fueron de 200 +/- 100 *T. vivax*/ml. La sensibilidad de la prueba fue de un 100% cuando habían 700 parásitos/ml; de 80% entre 300 y 700; 50% entre 60 y 300, y negativo por debajo de 60 parásitos/ml. Se proveeyó de factores para cuantificar la parasitemia relacionada al número de parásitos observados al microscopio en láminas preparadas de las muestras

(parasitemia > 2000) o en tubos capilares (parasitemia < 2000). La sensibilidad de las técnicas para la detección de infecciones activas podrían ser evaluadas relacionándolas a valores fijos, tales como los conocidos de parasitemia creados artificialmente, como se describió en el presente trabajo.

INTRODUCTION

The Hematocrit Centrifuge Technique (HCT), or Woo test (Woo, 1969 and 1970), is the technique most commonly used for diagnosis of animal trypanosomiasis, but its sensitivity is not well defined. According to the authors, the sensitivity of the dark ground buffy coat method, or DG/BMC is higher (Murray et al., 1977; Parris et al., 1982), equal (Camus, 1983), or lower (Kalu et al., 1986; Betancourt et al., 1979; Van Vlaenderen, 1996; Desquesnes, 1996) than that of the Woo test for the detection of *Trypanosoma vivax*. Most authors have compared particular techniques relative to other techniques, but not relative to a fixed reference. The relative sensitivity of particular tests appears to vary between technicians. The objective of the present study was to measure sensitivity of the Woo test for *T. vivax* detection, and to determine factors to quantify parasitemia in relation to the number of parasites observed between microscope slide and cover slip or in the capillary tubes.

MATERIAL AND METHODS

Five ml of infected blood was collected from a sheep experimentally infected with an isolate of *T. vivax* from French Guiana (TVFG2, isolated by Desquesnes and Demarty, unpublished), 140ml of blood was collected from a non-infected sheep (both sheep were originally from a non-infected farm (36 sheep all negative for indirect-ELISA). Estimation of the parasitemia was done as follows: 10µl of infected blood were deposited on a microscope slide with a micropipette and covered with a cover slip of 22x22mm; air bubbles were carefully avoided and the blood was equally distributed between slide and cover slip. The microscope slide was rejected if these parameters were not achieved. The natural cell constituted has a depth of 0.0207mm (h). At a magnification of x400 the volume of blood examined in one microscope field is $V = \pi R^2 . h = 3.707 \times 10^{-3}$ mm^3 (R = 0.239mm). Twenty-five fields were observed from the right extremity of the cover slide to the left, and another 25 fields were read from the top to the bottom. The total number of parasites observed was recorded. An average of one trypanosome/field corresponds to a parasitemia of about 270,000/ml. Nine counts were done on nine different preparations, by two technicians, to determine the parasitemia of this diluted sample with minimal error, and the mean was established as indicated by Schwartz (1963):

$$m = m_0 +/- \frac{tS}{\sqrt{n}}, \qquad \text{[with n = 9, ddl = 8 and t = 2,3.]}$$

RESULTS

On the day of the experiment, the parasitemia was about 10^6/ml. A pre-dilution of the infected blood yielded a parasitemia of about 10^5/ml. The nine counts were processed on the pre-diluted blood.

The results of the count are presented in Table 1.

m = 107,000 +/- 14,000 parasites/ml or 107,000 +/- 13%

Table 1: Results of the count and estimation of the parasitemia in the pre-dilution sample:

Count number	Number of trypanosomes observed/50 fields	Mean number of trypanosomes per field	Corresponding parasitemia (trypanosomes/ml)
1	17	0,34	91702
2	16	0,32	86308
3	17	0,34	91702
4	25	0,50	134856
5	25	0,50	134856
6	23	0,46	124067
7	17	0,34	91702
8	19	0,38	102490
9	20	0,40	107884
MEAN:	19,89	0,40	$m_0 = 107.285$
S:	3 38	0,07	$S = 18.238$
		Confidence interval of the mean number of parasites/ml in the pre-dilution:	$M = 107.000$ +/- 14.000

$$m = m_0 +/- t \frac{S}{\sqrt{n}}$$

m = 107,000 +/- 14,000 parasites/ml, or 107,000 +/- 13%

Serial dilutions of the infected blood into the non-infected blood were then performed to prepare samples with parasitemia ranging from 1 to 1,767 parasites/ml. The error on the dilution was considered as negligible, so the dilutions are affected with the same uncertainty as the initial pre-dilution, e.g. +/- 13%.

Six capillary tubes were filled with 70μl of blood for each dilution, centrifuged as indicated by Woo (1970); and observed at a magnification of x100 (microscope Leitz Biomed Laboratoire®); tubes were blindly observed; three tubes were read by one technician, three by the other. Readings were done under four different positions, by three rotations of a quarter of a circle of the capillary tube in its holder. The total number of parasites observed was recorded.

Results of the readings are presented in Table 2; and grouped by probability categories as indicated in Table 3.

Table 2: Number of trypanosomes observed in each capillary tube, and probability of a positive result in relation to the parasitemia

Parasitemia in trypanosomes/ml*	Series of capillary tubes						Probability of a positive response
	1	2	3	4	5	6	
1767	20	15	14	8	15	10	1
1325	9	10	10	8	6	12	1
883	5	3	7	4	4	7	1
707	4	4	3	3	2	4	1
530	1	0	1	4	3	2	0,83
442	2	0	2	4	2	4	0,83
353	3	0	4	0	3	1	0,67
265	1	0	1	1	2	2	0,83
177	1	1	0	0	1	0	0,50
88	0	0	0	1	2	2	0,50
71	0	0	0	0	1	1	0,33
57	1	0	0	1	2	0	0,50
44	0	0	0	0	0	0	0,00
35	0	0	0	1	0	0	0,17
26	0	0	1	0	0	0	0,17
18	0	0	0	0	0	0	0,00
13	0	0	0	0	0	0	0,00
9	0	0	0	0	0	0	0,00
7	0	0	0	0	0	0	0,00
4	0	0	0	0	0	0	0,00
2	0	0	0	·	0	0	0,00
1	0	0	0	0	0	0	0,00

* parasitemia indicated subject to uncertainty of 13%

Table 3: Sensitivity of the Woo test in relation to the parasitemia

Parasitemia (number of T. vivax / ml)	Number of positive samples/ total number of samples	Sensitivity
> 700 +/- 90	24/24	100%
from 265 +/- 35 to 700 +/- 90	19/24	79%
from 57 +/- 7 to 265 +/- 35	11/24	46%
< 57 +/- 7	2/36	negligible

In order to establish a comparison between the number of parasites observed in a capillary tube and the parasitemia, the results of the reading were regrouped by the number "n" of trypanosomes observed (Table 4 and Table 5).

Table 4: Frequency of the capillary tubes presenting a number "n" of trypanosomes at a given parasitemia, and calculation of the mean corresponding parasitemia

Parasitemias (number of trypanosomes/ml) x_i	Number "n" of trypanosomes observed per capillar tube (n_i):					
	1	2	3	4	5 to 9	10 to 20
1767	0	0	0	0	1	5
1325	0	0	0	0	3	3
883	0	0	1	2	3	0
707	0	1	2	3	0	0
530	2	1	1	1	0	0
442	0	3	0	2	0	0
353	1	0	2	1	0	0
265	3	2	0	0	0	0
177	3	0	0	0	0	0
88	1	2	0	0	0	0
71	2	0	0	0	0	0
57	2	1	0	0	0	0
44	0	0	0	0	0	0
35	1	0	0	0	0	0
26	1	0	0	0	0	0
18	0	0	0	0	0	0
13	0	0	0	0	0	0
9	0	0	0	0	0	0
7	0	0	0	0	0	0
4	0	0	0	0	0	0
2	0	0	0	0	0	0
1	0	0	0	0	0	0
$\sum n_i$	16	10	6	9	7	8
$\sum n_i x_i$	3144	3326	3533	5654	8391	12810
mean parasitemia $\sum n_i x_i / \sum n_i$ (Trypanosomes/ml)	197	333	589	628	1199	1601
and uncertainty*	+/- 87	+/-154	+/-225	+/-150	+/-309	+/-191

* the uncertainty indicated is the one of $n_i x_i$ distribution, the total uncertainty must also include the uncertainty of the pre-dilution (+/- 13%), as indicated in Table 5.

Table 5: Estimation of the parasitemia from the number of trypanosomes observed in a capillary tube

Number of *T.vivax* observed in a capillary tube	Approximate corresponding parasitemia in parasites/ml	
1	200	+/- 112
2	330	+/- 197
3	590	+/- 301
4	630	+/- 231
5 à 9	1200	+/- 465
10 à 20	1600	+/- 399

CONCLUSION

A simple technique was described for the direct enumeration of parasites in the blood. Factors are provided to quantify parasitemia in relation to the number of parasites observed between slip and cover slip (parasitemia > 2000) or in the capillary tube (parasitemia < 2000).

The mean positivity level of the Woo test was about 200 +/-100 *T. vivax* /ml.

Sensitivity of the techniques for detection of active infection could be evaluated with reference to fixed values such as known or artificially created parasitemias as described.

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IMPROVED ELISAs FOR TICK-BORNE PATHOGENS

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ABSTRACT

Tick-borne diseases (TBDs) are responsible for causing enormous economic losses in domestic ruminants in large areas of the tropics and subtropics. Currently available diagnostic methods that utilize light microscopy for direct detection of parasites and serological methods utilizing crude antigens are inadequate because of their lack of sensitivity and specificity. Since 1992 ILRI has been involved in developing improved serological tests for four important tick-borne pathogens (TBPs) namely, *Theileria parva*, *Theileria mutans*, *Babesia begemina* and *Anaplasma marginale*. Enzyme linked immuno-assays (ELISAs), based on well defined, parasite-specific conserved antigens produced in recombinant forms, were selected as the basis for developing the serological tests as these have proven to eliminate most of the problems associated with other serological tests. This paper briefly describes the approaches used to develop, standardize and validate antibody detection ELISAs for *T. parva*, *T. mutans*, *B. bigemina* and *A. marginale*.

RESUMÉ

Les maladies transmises par les tiques provoquent d'énormes pertes économiques chez les ruminants domestiques dans les zones tropicales et sub-tropicales. Les méthodes courantes de diagnostic ayant recours à l'utilisation du microscope optique pour la détection directe des parasites et les méthodes sérologiques utilisant des antigènes parasitaires bruts sont peu performantes du fait de leurs faibles sensibilité et spécificité. Depuis 1992, l'ILRI s'est investi dans la recherche pour développer de meilleures techniques de diagnostic sérologique pour quatre agents pathogènes véhiculés par les tiques: *Theileria parva*, *Theileria mutans*, *Babesia bigemina* et *Anaplasma marginale*. Des techniques immuno-enzymatiques (ELISAs) basée sur des antigènes recombinants bien définis, spécifiques des parasites, ont été choisies pour développer des tests sérologiques, car elles permettent d'éliminer tous les problèmes rencontrées avec les techniques sérologiques classiques. Cette communication décrit brièvement les approches utilisées pour développer, standardiser et valider les techniques de détection des anticorps par ELISAs pour *T. parva*, *T. mutans*, *B. bigemina* et *A. marginale*.

RESUMEN

Las enfermedades transmitidas por garrapata son responsables de enormes pérdidas económicas en rumiantes domésticos en grandes regiones de los trópicos y subtrópicos. Los métodos para diagnosticar disponibles actualmente que utilizan la microscopia de luz para la detección directa de parásitos y los métodos que utilizan antígenos no refinados son inadecuados por falta de sensibilidad y especificidad. Desde 1992 ILRI ha estado involucrado en el desarrollo de pruebas serológicas mejoradas para cuatro importantes patógenos transmitidos por garrapata (TBP), es decir, *Theileria parva*, *Theileria mutans*, *Babesia bigemina* y *Anaplasma marginale*. Las inmuno-pruebas ligadas a las enzimas (ELISAs), basadas en antígenos bien definidos y conservados Parásitos-específicos producidos en formas recombinantes, fueron seleccionados como base para desarrollar las pruebas serológicas dado que se ha comprobado que estos eliminan la mayoría de los problemas asociados con otras pruebas serológicas. Esta ponencia describe brevemente los enfoques utilizados para desarrollar, estandarizar y validar la detección de anticuerpos ELISAs para *T.parva*, *T. mutans*, *B. bigemina* y *A. marginale*.

AN APPROACH TO DEVELOPING IMPROVED ELISAs

A stepwise approach to the development and testing of improved ELISAs for the selected organisms was adopted. Broadly, this involved four steps:

1) Identification and selection of parasite-specific candidate antigens;

2) Cloning, sequencing and expression of genes for the selected antigens;

3) Standardization and laboratory validation of the selected antigens; and

4) Field validation of the improved ELISAs.

1.   Identification and selection of antigens

In developing ELISAs with high specificity and sensitivity, the most important consideration was the selection of appropriate candidate antigens. Parasite specific antigens that are conserved for the species, immunodominant, recognized very early in the infection, and which remain in the host as long as possible following recovery from the disease, provide the highest sensitivity and specificity. In order to select such antigens we adopted an approach that involved collection of sera from cattle exposed to various TBPs by polyacrylamide gel electrophoresis. This method of testing enabled us to identify parasite-specific antigens that were recognized by the cattle in the field following natural exposure to the disease. The identified antigens were then electroeluted from the gels and used to generate murine monoclonal antibodies (MAb) and bovine monospecific sera. The MAb were then used in ELISA's to trap parasite specific antigens and tested against a selection of positive and negative control sera, and compared with the existing indirect fluorescent antibody or capillary agglutination tests for specificity and sensitivity. Those candidate antigens that proved to be equally good or better than the crude antigens used in the existing tests were selected for further analysis and investigation. The major parasite specific conserved antigens that were identified as diagnostic antigens using bovine immune sera in immunoblots were as follows:-

T. parva. The polymorphic immunodominant molecule (PIM) of *T. parva* is predominantly found on the surface of the schizont stage. It is also present at low levels on the surface of sporozoites. The molecular weight varies between 69 kD to 110 kD depending on the parasite stock examined.

T. mutans. The only conserved antigen that was identified by bovine sera in immunoblots was the P32 molecule. This antigen is located on the surface of trophozoites and merozoites of erythrocytic stages of the parasite. It is not known if the antigen is present on the schizont stage. The molecular weight is 32 kD and it does not vary in size between different parasite stocks.

B. bigemina. The 200 kD antigen, found to be present on the surface of the trophozoite and merozoite stages of the parasite, was identified as the candidate antigen. The size of the molecule is conserved.

A. marginale. The 19kD molecule found to be the major surface protein of the initial bodies found in erythrocytes was selected as the candidate diagnostic antigen. This molecule was identified by researchers at Washington State University and was obtained from them through our collaboration.

2.   Cloning, sequencing and expression of genes for putative diagnostic antigens

Various genomic and cDNA libraries were prepared for use in cloning genes for antigens of interest as identified above. The MAbs and monospecific sera were then used to immunoscreen these libraries. The positive plaques were selected, further characterized, cloned and subcloned into the pGEX plasmid vector for expression of the appropriate protein as a fusion product with glutathione-s-transferase (GST). With the pGEX vector the expressed product could be easily produced in large quantities and purified using commercially available sepharose-GST columns or beads. The recombinant antigens were then characterized in ELISA formats for sensitivity and specificity for the detection of appropriate antibodies. Only the antigens that proved superior to the existing ones were further characterized.

3. Standardization and laboratory validation of improved ELISAs

The procedures described for international standardization and validation of ELISA protocols and reagents by IOE/FAO/WHO/IAEA were followed. Standardization involved the determination of methods for data expression for comparison of results between laboratories, consensus on primary reference standards and quality assurance.

A major aspect of the validation exercise involved the establishment of a bank of standard bovine reference sera. Two sets of sera were considered to be important for this purpose. These were:-

    i)   serial serum samples collected over a period of 250 days from cattle experimentally infected with defined parasites

    ii)   field sera obtained from different geographical regions representing different epidemiological environments for TBDs. The field sera that were used were from Kenya, Zimbabwe, South Africa, Mali, Sudan, Australia, UK and Holland.

These reagents were used to compare the diagnostic performance of the improved ELISAs using candidate antigens with the tests in current use.

Two ELISA formats were used. The first involved direct coating of the purified recombinant antigen onto the microtitre plate before introduction of test sera. This was followed by a second antibody, anti-bovine immunoglobulins conjugated to horse radish peroxidase. The reaction was revealed by addition of the substrate/chromogen (hydrogen peroxide/2,2-azinobis 3-ethyl-benzylthiazoline 6-sulphonic acid, ammonium salt). The second approach involved the use of a MAb bound onto the microtitre plate to trap the recombinant antigen from a bacterial lysate. The immobilized antigen was then used to detect antibodies in the test sera following the procedure described above.

Initially, both approaches were used when the candidate antigens were first evaluated against the standard set of sera generated in the laboratory by experimental infections. This study showed that the first approach of direct coating was better than the second approach. Following this exercise, the putatively selected antigens were only used by direct coating of ELISA plates against the field sera and the specificity and sensitivity of each antigen determined in improved ELISA. The results are summarized below:

Antibody detection ELISA for *Theileria parva*. The polymorphic immunodominant molecule (PIM) showed the highest sensitivity when compared with other *T. parva* antigens and the IFA test. The antigen has been tested in this format against a range of sera obtained from cattle experimentally infected with different cattle-derived stocks (Muguga, Marikebuni, Uganda, Boleni) and one buffalo-derived stock (7014). Additionally, the antigen has been evaluated using field sera obtained from East Coast fever endemic areas of Kenya and Uganda and non-endemic areas of Kenya, West Africa, Great Britain, Sudan and Australia. The results of these studies have shown that the PIM-based antibody detection ELISA for *T. parva* has greater than 95% sensitivity and specificity. The test has been validated in the field.

Antibody detection ELISA for *Theileria mutans*. The recombinant 32 kD antigen (P32), identified as a candidate antigen for use in a *T. mutans* antibody detection ELISA, has been characterized and a number of alleles of the gene encoding the antigen have been identified. Two of these alleles, clone21 and clone23, have been expressed as fusion proteins with GST and compared for sensitivity and specificity in an antibody detection ELISA. The GST/clone23 has been found to show higher sensitivity and specificity that the GST/clone21. The recombinant protein expressed by the allele GST/clone23, representing the full length P32, has been evaluated using sera from tick-transmitted *T. mutans* endemic areas of Uganda and Kenya and non-endemic areas. The tests show over 99% sensitivity and 95% specificity. Studies on epitope mapping using experimental sera have shown that GST/clones 21 and 23 express epitope polymorphisms. Therefore studies are in progress to map epitopes using field sera. The results may open up the possibility of using defined epitopes rather than the whole protein for antibody detection in the ELISA. The P32-based ELISA is awaiting field validation.

Antibody detection ELISA for *Anaplasma marginale*. The gene for the 19 kD protein, identified as a candidate

antigen for use in an *A. marginale* antibody detection ELISA, has been obtained through collaboration with Washington State University and expressed in *E. coli* as a fusion protein with GST using the pGEX vector. The recombinant protein has been used in the antibody detection ELISA system, similar to that used for *T. parva* and *T. mutans*, and evaluated for specificity. The recombinant antigen has been found to detect *A. marginale*-specific antibodies from experimentally infected cattle and does not cross-react with sera raised against *Cowdria ruminantium*. The antigen has not been tested for specificity against sera from cattle experimentally infected with *Ehrlichia* species. Tick-transmission experiments in cattle are in progress to collect sequential sera for evaluating the specificity of the test.

Antibody detection ELISA for *Babesia bigemina*. A 200 kD antigen of *B. bigemina* has been identified as a candidate antigen for use in the antibody detection ELISA system. A 3.8kb of the coding sequence has been expressed in *E. coli* as a fusion protein with GST and is being evaluated using a range of sera. The antigen reacts with sera from cattle following infection with a sporozoite stabilate of *B. bigemina*. In addition, 21% of 200 sera collected from cattle in Tasmania, where *Babesia spp.* do not occur, show cross-reactivity with the recombinant antigen. In an attempt to remove the cross-reacting epitopes, epitope libraries from the DNAse-digested fragments of the gene expressing the 200 kD protein were constructed in *lambada* gt11. The libraries containing DNA fragments between 100-500 bp were immunoscreened with hyperimmune sera from cattle and one strongly reacting clone of 190 pb was isolated, recloned in the pGEX vector and expressed as a fusion protein. The fusion protein has been evaluated with a limited number of sera in western blots and antibody detection ELISA. The smaller recombinant protein has the same specificity as the protein expressed by the 3.8kb coding sequence and it cross-reacts with only 7% of the 200 sera from Tasmania. Studies are in progress to test the small fusion protein derived from the 200 kD antigen against a number of other reference sera to determine its specificity.

Although the test has shown over 95% sensitivity and specificity with a range of sera from various parts of the world, the "false positive" results with the Tasmanian sera are causes for concern. However, the field validation will go ahead with the 200 kD based test further research efforts will be made to determine the basis of the reactivity of the Tasmanian sera to this antigen.

4.   Field validation of the improved ELISAs

The main objective of the field validation exercise was to compare the performance of the improved ELISA's developed at ILRI with a selected number of diagnostic laboratories in different parts of the world. The validation exercise was planned in three phases. The first phase was the identification of laboratories within the TBDs region with adequate facilities for performing ELISAs. The second phase was the selection and training of technicians from these designated laboratories and the third phase was the comparison of the performance of the newly developed tests in these designated laboratories and ILRI.

This exercise has been carried out with Burkina Faso, Mali, Kenya, Zimbabwe and India. In this study, good concordance has been found between the performance of tests in the field laboratories and ILRI. The evaluation still needs to be completed in Latin America.

CONCLUSIONS

Studies were initiated to develop antibody detection ELISAs for *T. parva, T. mutans, B. bigemina* and *A. marginale*. Using a rational, step-wise approach, candidate diagnostic antigens were identified for all the parasites. Molecular biological techniques were used to produce recombinant forms of these antigens in large quantities. A standard laboratory protocol has been developed for purification of these antigens. These antigens have been tested in an ELISA system against a range of standard sera from serum bank established for this purpose, and have been found to be superior to the existing serological tests in terms of sensitivity, specificity and the ease of the performance of the assay.

FUTURE PLANS

The improved ELISAs for *A. marginale* and *B. bigemina* are currently undergoing field validation. It is expected that after the completion of this exercise the tests will be transferred to various laboratories for wider evaluation.

The reagents that have been generated during the development of improved ELISAs will be exploited to develop a second generation of diagnostic tools. Currently the sequences of the genes cloned for the parasite specific diagnostic antigens are being exploited to synthesize DNA primers that can be used in PCR-based assays to detect and differentiate minute quantities of *T. theileria* parasites. It is expected that such assays will be ready for field testing within 12 - 18 months. These assays will be extremely useful as research tools for many national laboratories and international agencies involved in control of TBDs.

SENSITIVITY AND SPECIFICITY OF THE ANTIGEN-ELISA TESTS FOR *TRYPANOSOMA* SPECIES

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ABSTRACT

Three species-specific monoclonal antibody-based antigen-ELISA tests (AG-ELISA) have been developed for diagnosis of the main livestock trypanosomes, by Nantulya and Lindqvist (1989). They are AG-ELISA *T. vivax*; AG-ELISA *T. brucei*, which also recognizes *T. evansi* antigens; and AG-ELISA *T. congolense*. According to the authors, species-specificity and sensitivity of the AG-ELISA were very satisfactory. Used for the first time in South America in 1991 during an epidemiological survey in cattle in French Guiana, the AG-ELISA gave surprising results which justified a re-evaluation of sensitivity and specificity. The evaluation was carried out at CIRAD-EMVT French Guiana and at ILRI, Kenya. Blind tests were performed to ensure standardization of the results between the two laboratories; no significant difference was found. The tests were evaluated with 2,953 samples from cattle eventually infected by *T. vivax* only, 130 samples from non-infected cattle imported from Metropolitan France, 160 samples from four calves and 338 samples from two sheep experimentally infected with an isolate of *T. vivax* from French Guiana, and 70 samples from two sheep experimentally infected with an Asian clone of *T. evansi*. Results indicated that the sensitivity of the AG-ELISA *T. vivax* was very low; 2-4% in experimentally infected cattle or sheep. The sensitivity of the AG-ELISA *T. brucei* was approximately 60% in sheep experimentally infected with *T. evansi*, slightly below the parasitological technique (78%). Specificity of the tests was also very low: 6-10% false positive results were obtained with the three tests in cattle from France; 4-22% false positive results were obtained with AG-ELISA *T. congolense* in cattle and sheep naturally or experimentally infected with *T.vivax*; and 20-26% false positive results were obtained with AG-ELISA *T. brucei*, in cattle and sheep experimentally infected in *T. vivax*. Tests developed using these monoclonal antibodies or with monoclonal antibodies derived from these will not allow the development of sensitive and specific diagnosis. The use of these reagents should be discontinued. For a sensitive and specific diagnosis based on AG-ELISA, there is need to develop new monoclonal antibodies; such a project should be based on collaborative research by several institutions.

RESUMÉ

Trois tests de détection des antigènes par ELISA immunocapture (Ag-ELISA), basés sur l'utilisation d'anticorps monoclonaux spécifiques d'espèce, ont été développés pour le diagnostic des trypanosomoses du bétail par Nantulya et Lindqvist (1989). Ag-ELISA *T. vivax*, Ag-ELISA *T. brucei* qui reconnaît également les antigènes de *T. evansi*, et Ag-ELISA *T. congolense*. Selon les auteurs du test, la spécificité d'espèce et la sensibilité des tests sont très satisfaisantes. Utilisés pour la première fois en Amérique du Sud lors d'une enquête épidémiologique chez les bovins en Guyane Française, les tests ont donné des résultats surprenants qui justifiaient une ré-évaluation de leur sensibilité et spécificité. L'évaluation a été menée au CIRAD-EMVT-Guyane et à l'ILRI (Nairobi, Kenya). Des tests en aveugle ont été réalisés afin de s'assurer de la reproductibilité et de la standardisation de la technique entre les laboratoires; aucune différence significative n'a été trouvée dans l'échantillonnage sélectionné. Les tests ont été évalués à partir de 2953 échantillons de sérum de bovins éventuellement infectés par *T. vivax* (bovins de Guyane Française), 130 échantillons de bovins indemnes (importés de France métropolitaine), 160 échantillons provenant de 4 veaux et 338 échantillons provenant de 2 moutons expérimentalement infectés avec une souche guyanaise de *T. vivax* (témoins positifs), et 70 échantillons de moutons expérimentalement infectés avec une souche asiatique de *T. evansi*. Les résultats indiquent que la sensibilité de l'Ag-ELISA *T. vivax* est très faible, de 2 à 4% chez les bovins et ovins expérimentalement infectés; la sensibilité de l'Ag-ELISA *T. brucei* a été de 60% chez les moutons expérimentalement infectés par *T. evansi*, inférieure à la sensibilité de la technique de Woo (78% de positifs). La spécificité des tests a également été très faible : 6 à 10% de faux positifs sont obtenus avec les trois tests sur les échantillons d'animaux indemnes; 4 à 22% de faux positifs sont obtenus avec les Ag-ELISA *T. congolense* chez les animaux infectés naturellement ou expérimentalement par *T. vivax*, et 20 à 26% de faux positifs sont obtenus chez les avec l'Ag-ELISA *T. brucei* chez les bovins et ovins expérimentalement infectés avec *T. vivax*. Les tests développés à l'aide de ces anticorps monoclonaux ou de dérivés de ces anticorps monoclonaux ne permettront pas de mettre au point des techniques de diagnostic sensibles et spécifiques; l'utilisation de ces réactifs n'est pas recommandée. Pour un diagnostic sensible et spécifique basé sur l'Ag-ELISA, il est nécessaire de développer de nouveaux anticorps monoclonaux, et, étant donné l'ambition d'un tel projet, il est suggéré que cette entreprise résulte d'une collaboration entre divers instituts de recherche.

RESUMEN

Basados en la técnica de ELISA para antígenos (Ag-ELISA) desarrollada por Nantuyla y Lindqvist (1989) se produjeron tres anticuerpos monoclonales especie-específicos para el diagnóstico del principal trypanosoma del ganado. Ellos fueron, los Ag-ELISA-*T. vivax*, Ag-ELISA-*T. brucei* (los cuales también reconocen el antígeno del *T. evansi*) y el Ag-ELISA-*T. congolense*. De acuerdo a sus autores, la especie-especificidad y la sensibilidad del Ag-ELISA son muy satisfactorias. Usado por primera vez en Sur América en 1991 durante una investigación epidemiológica realizada en bovinos en la Guyana Francesa, el Ag-ELISA dió sorprendentes resultados los cuales justificaron la re-evaluacion de su sensibilidad y la especificidad. La evaluación se llevó a cabo en el CIRAD-EMVT en la Guyana Francesa y en el ILRI, Kenya. Se realizaron ensayos ciegos para asegurar la estandarizacion de los resultados entre los dos laboratorios; no encontrándose diferencias significativas. Las pruebas fueron evaluadas con 2953 muestras de bovinos eventualmente infectados por *T. vivax* solamente, 130 muestras de bovinos no infectados importados de la Francia Metropolitana, 160 muestras de cuatro becerros y 338 muestras de dos ovejas experimentalmente infectadas con un aislado de *T. vivax* de la Guyana Francesa, y 70 muestras de dos ovejas experimentalmente infectadas con un clon asiático de *T. evansi*. Los resultados indicaron que la sensibilidad del Ag-ELISA-*T. vivax* fue muy bajo, es decir, 2-4% en bovinos y ovinos experimentalmente infectados; la sensibilidad del Ag-ELISA-*T. brucei* fue aproximadamente de un 60% en los ovinos experimentalmente infectados con *T. evansi*, ligeramente por debajo de la observada por la técnica parasitológica (78%). La especificidad de la prueba también fue muy baja, se obtuvo: 6-10% de falsos positivos en tres pruebas en bovinos de Francia; 4-22% de falsos positivos con el Ag-ELISA-*T. congolense* en bovinos y ovinos natural o experimentalmente infectados con *T. vivax*; y 20-26% falsos positivos con el Ag-ELISA-*T. brucei*, con bovinos y ovinos experimentalmente infectados con *T. vivax*. Las pruebas desarrolladas con el uso de estos anticuerpos monoclonales o con anticuerpos monoclonales derivados de ellos, son el preludio para el futuro establecimiento de un nuevo método de diagnóstico de sensibilidad y especificidad aseguradas. El uso de estos reactivos (de origen africano) debe ser descontinuado. Para los diagnósticos de sensibilidad y especificidad basados en los Ag-ELISA, persiste la necesidad de desarrollar nuevos anticuerpos monoclonales; y se sugiere que tal proyecto debe ser en colaboración entre varias instituciones.

Three species-specific antigen-ELISA tests (Ag-ELISA), based on monoclonal antibodies, have been developed for diagnosis of the main livestock trypanosomes, by Nantulya and Lindqvist (1989). These are: Ag-ELISA *T.vivax*, AG-ELISA *T. brucei* which also recognizes *T. evansi* antigens, and Ag-ELISA *T. congolense*. According to the authors, species-specificity and sensitivity of the Ag-ELISA were very satisfactory.

Specificity was first evaluated with lysates of parasites and considered to be 100%. This finding was confirmed by the absence of false positives in 100 steers grazing in Kapiti, close to Nairobi, in a tsetse-free area (Nantulya, 1990), and in well controlled medical conditions (Stevenson, unpublished). Under experimental conditions Nantulya and Lindqvist indicated that there was no cross-reaction but their article did not mention whether all sera were tested in heterologous systems. Sensitivity was first evaluated in cattle experimentally infected with clones of the parasites and found to be much higher than the parasitological examinations. During the first 51 days of infections, sensitivity of the tests in homologous infections were *T. vivax* Ag-ELISA: 71%; *T. brucei* Ag-ELISA: 54%; *T. congolense* Ag-ELISA: 70% (Nantulya and Lindqvist, 1989). Higher sensitivity of the Ag-ELISA was confirmed in the field by the fact that some positive results of Ag-ELISA were negative on parasitological techniques. These animals were not found to be infected, but were declared infected because of positive results on the tests.

A positive result on the Ag-ELISA became synonymous with infection, as Nantulya said "....a significant number (52.6%) of the animals.....which had been missed by parasite detection techniques were found to be infected as shown by the test for antigen...". Ag-ELISA's were then widely used in Africa with a very high level of confidence, either for epidemiological studies, or trypanocide resistance studies, and even as an indirect tool to evaluate the results of tsetse control and eradication programmes (Nantulya et al., 1989; Ries and Connor, 1993; Doku, 1993; Diall et al., 1993; Okuna et al., 1993; Nantulya, 1994; Singh et al., 1994; Mansion et al., 1995). Some authors realized later that the epidemiological data obtained from AG-ELISA were sometimes too surprising to be credible, but in Africa, one can never be certain that a trypanosome is totally absent from a particular area. Re-evaluation in the field was very difficult.

Ag-ELISA tests were first used in South America in 1991, during the epidemiological survey carried out in French Guiana, by Desquesnes and Gardiner (1993). Surprising results (Table 1) led to a re-evaluation of the Ag-ELISA

under natural and experimental conditions. This report is a summary of this evaluation, carried out with 2,953 sera from cattle from French Guiana, 130 sera from metropolitan France, 160 samples from cattle and 338 samples from sheep experimentally infected with *T. vivax*, and 70 samples from sheep experimentally infected with *T. evansi*.

Table 1: Origin and number of samples tested, positive results to the 3 Ag-ELISA, and meaning

	number of samples tested	Ag-ELISA *T. vivax*	Ag-ELISA *T. brucei**	Ag-ELISA *T. congolense*
Non-infected cattle *(imported from metropolitan France)*	130	8% false positive	6% false positive	10% false positive
Cattle eventually infected by *T. vivax* *(cattle from French Guiana)*	2953	12% positive	˙ 20% false positive	18% false positive
Cattle infected with *T. vivax* IL4007 *(experimental infections)*	160	3.8% true positive	4.4% false positive	4.1% false positive
Sheep infected with *T. vivax* IL4007 *(experimental infections)*	338	2% true positive	26% false positive	22% false positive
Sheep infected with *T. evansi* *(experimental infection with an Asian clone RoTat 1.2)*	70	4.3% false positive	60% true positive	7.1% false positive

* used for the detection of *T. evansi* antigens

I   STANDARDIZATION OF AG-ELISA RESULTS

Half of the samples from cattle from French Guiana, and all samples from cattle experimentally infected were processed at ILRI; the other samples were processed at CIRAD-EMVT-Guyane. Results of the tests processed in French Guiana were verified in blind tests of 80 samples at CIRDES, Burkina Faso, and ILRI, Kenya. There was no significant difference between the results of the three laboratories (Desquesnes and La Rocque, 1995).

Parasites
The evaluation was carried out with a stock of *T. vivax* isolated by Lancelot (1988) in French Guiana. This isolate was used at ILRI under the name IL4007, having been shown to be a pure *T. vivax* on the basis of:

1. Morphology

2. Morphometry and motility of the parasites

3. Inability to grow in mice

4. Positive PCR reactions with *T. vivax* oligonucleotides (TVW1 and 2) but negative reactions with *T. brucei* oligonucleotides (TBR1 and 2, Masiga et al. 1992)

5. Positive reaction with the oligonucleotides and DNA hybridation developed by Masake et al. (unpublished)

The clone RoTat 1.2 of an Asian *T. evansi* was used (Bajyana-Songa and Hamers, 1988).

## II   SENSITIVITY OF THE AG-ELISA *T. VIVAX*

Four Boran calves at ILRI (Desquesnes, 1996 b) and two Creole x Blackbelly sheep at CIRAD-EMVT-Guyane (Desquesnes and La Rocques, 1995) were experimentally infected with IL4007 and bled almost daily for parasitological and serological examinations. In calves, 160 samples were collected during the first 51 days of infection (40 samples per calf); HCT test yielded 68% positive results (109/160) and Ag-ELISA *T. vivax* 3.8%

(6/160). In sheep 338 samples were collected during the first 130 and 270 days of infection; HCT gave 52% positive results and AG-ELISA 2%. The sensitivity of the test was negligible with this isolate.

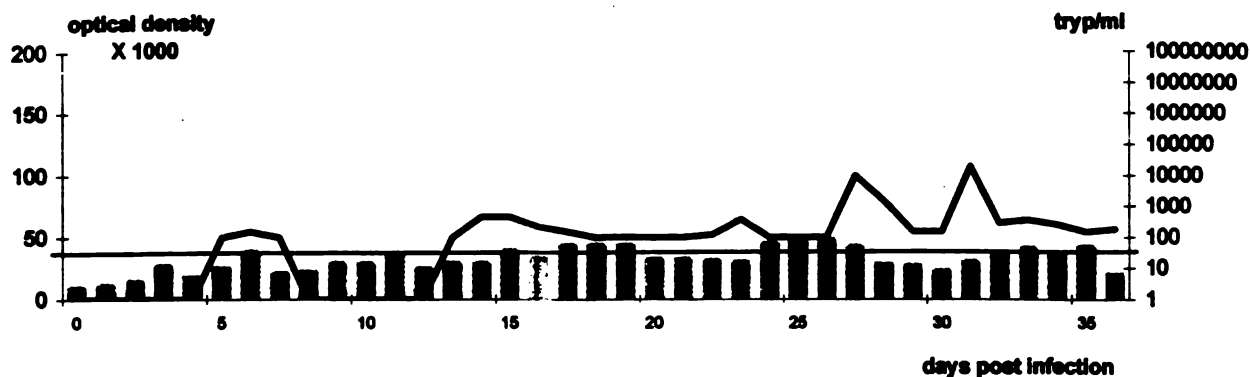## III   SENSITIVITY OF THE AG-ELISA *T. BRUCEI* FOR DETECTION OF *T. EVANSI* ANTIGENS

Two sheep were infected with RoTat 1.2 and bled daily for parasitological and serological examination. Seventy samples were collected during the first 36 days of infection; HCT yielded 78% positive results and Ag-ELISA *T. brucei*: 60%. Figures 1 and 2 indicate parasitemia and optical density of the samples with *T. brucei* Ag-ELISA. Sensitivity of the *T. brucei* Ag-ELISA was lower than that of HCT, but both tests found 84% of positive samples. Addition of AG-ELISA to HCT could be proposed to improve the general sensitivity of the diagnosis, but further results on specificity of the AG-ELISA *T. brucei* led to discontinuation of the use of the test. Suratest® is another test based on derived monoclonal antibodies, developed by Nantulya (1994).

Figure 1:   Sheep 1, experimentally infected with *T. evansi*: parasitaemia and optical densities (X1000) with, from top, *T. brucei* Ag-ELISA, *T. congolense* Ag-ELISA and *T. vivax* Ag-ELISA (from top to bottom)
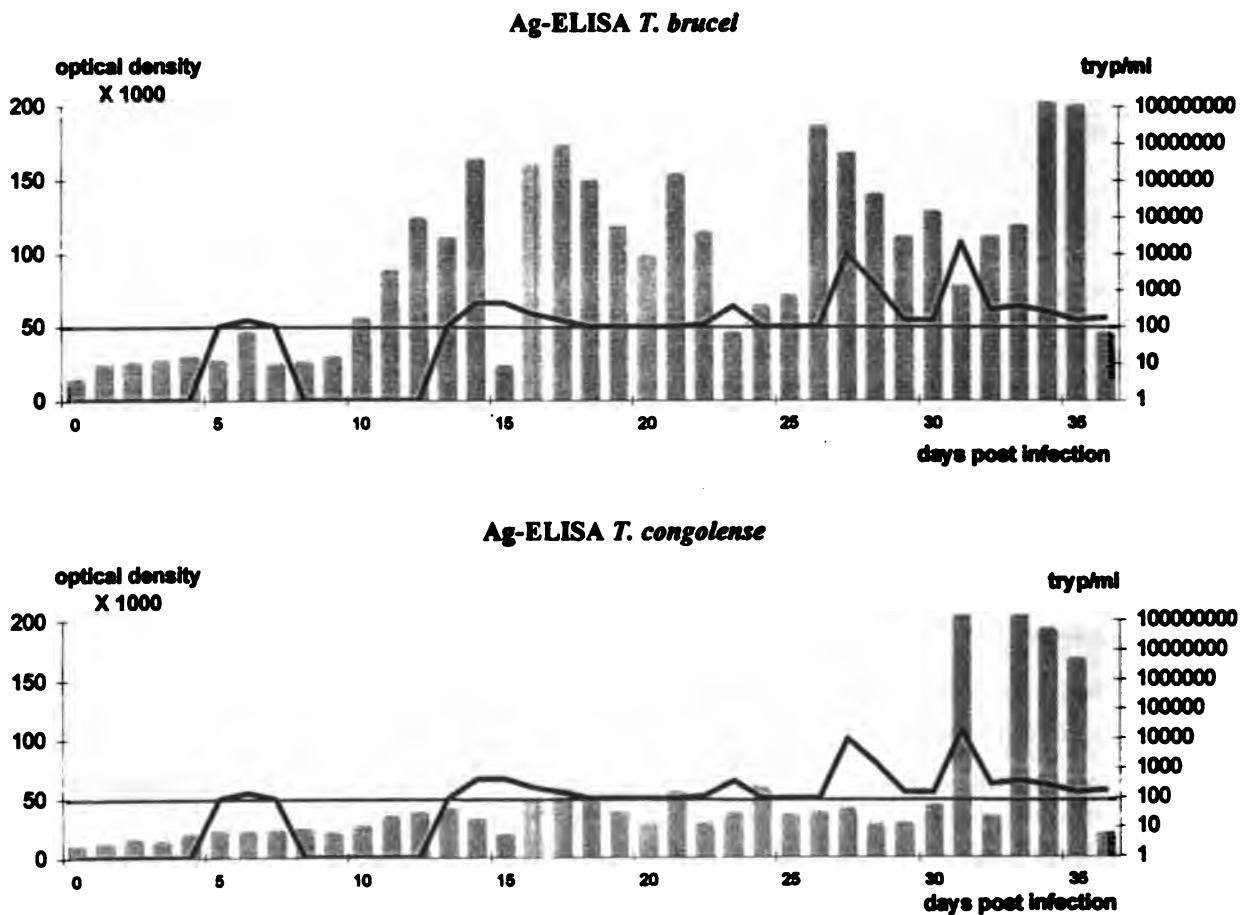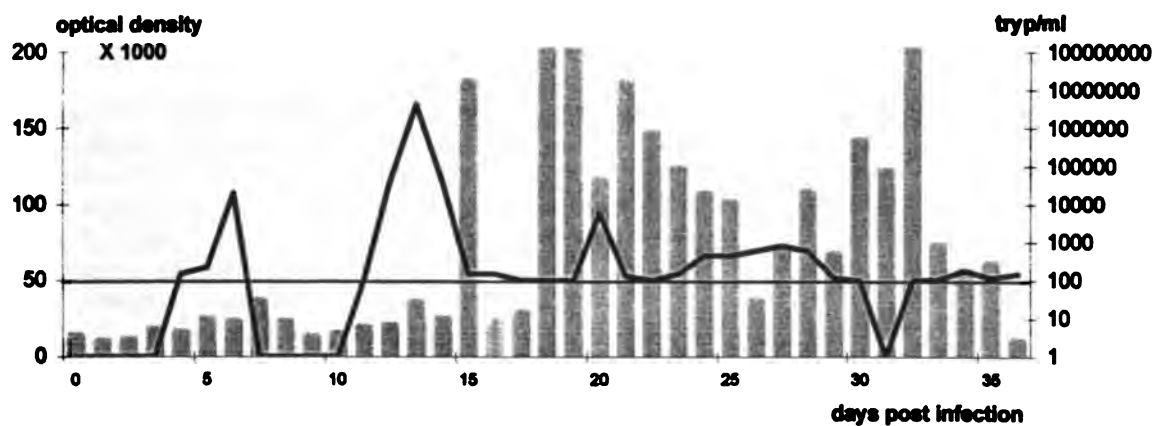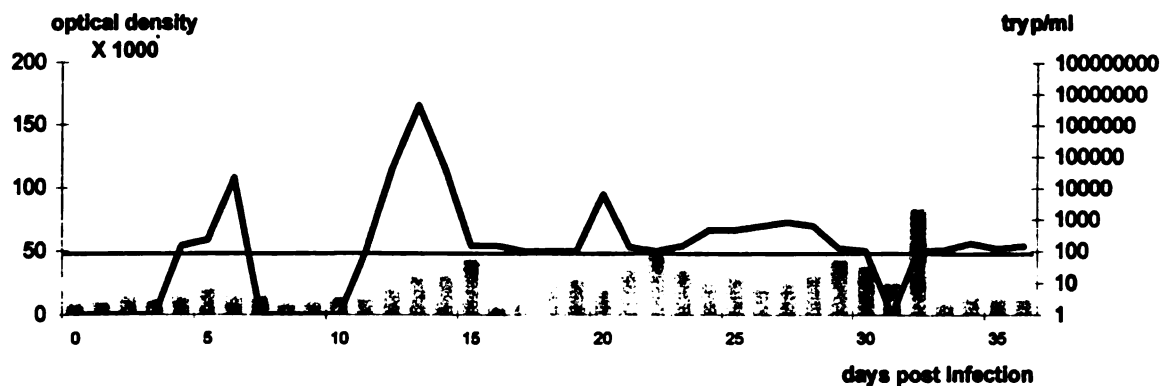

Ag-ELISA *T. brucei*

Ag-ELISA *T. congolense*

Ag-ELISA *T. vivax*

Legend :

curve	:	parasitemia
bars	:	optical density X 1000
horizontal line	:	cut off line of the ELISA tests

Figure 2: Sheep 2, experimentally infected with *T. evansi*: parasitaemia and optical densities (X1000) with *T. brucei* Ag-ELISA, *T. congolense* Ag-ELISA and *T. vivax* Ag-ELISA (from top to bottom)
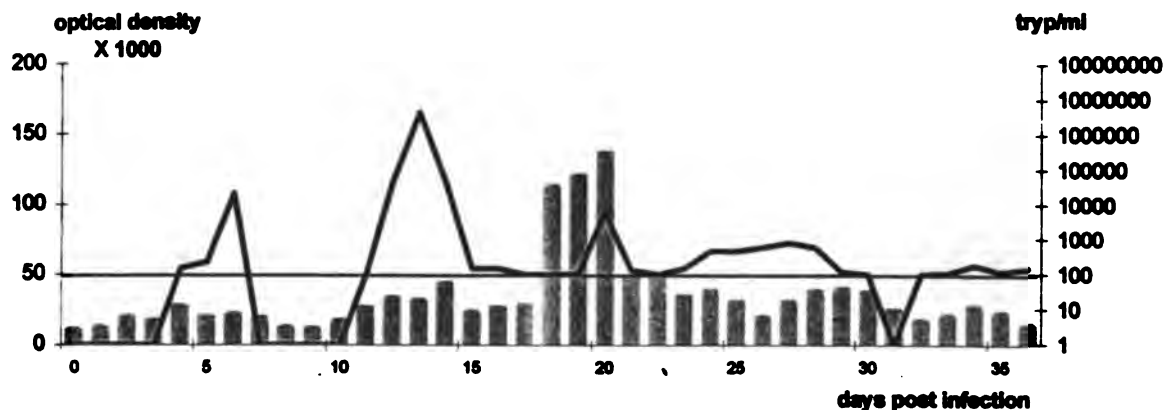
Ag-ELISA *T. brucei*

Ag-ELISA *T. congolense*

91

Ag-ELISA *T. vivax*

Legend :

      curve : parasitemia
       bars : optical density X 1000
horizontal line : cut off line of the ELISA tests

IV   CONCLUSION

The sensitivity of *T. congolense* Ag-ELISA was not evaluated because this parasite is not present in Latin America, but its specificity was shown to be poor; the test being more sensitive to detecting *T. vivax* antigens than the Ag-ELISA *T. vivax* itself. Cross-reactions with *T. vivax* and *T. evansi* were observed.

Sensitivity and/or specificity of the three Ag-ELISA are extremely unsatisfactory, and researchers working with these reagents in Africa, America and Asia should be aware of the significant bias of these tests. The utilization of these reagents is not advisable; however, antigen detection would be an ideal tool for the study of trypanosomiasis, providing the test is highly specific and sensitive. There is a need for a collaborative project of several researchers to develop new reagents, and, for the New World, taking into consideration the small four: *T. vivax, T. evansi, T. equiperdum* and *T. cruzi*, keeping in mind possible interference of *Megatrypanum* species. An alternative to the antigen detection tests could be species-specific antibody detection tests although attempts to develop *T. brucei* antibody detection tests were made by Ijagbone et al. (1989), such tests are not yet available.

V   DOT-ELISA

Recent publications by Bosompem et al. (1996 a and b) report on the development of new dot-ELISA tests based on monoclonal antibody for diagnosis of infection in biting insects. According to the authors, these reagents are 100% specific for *T. vivax, T. brucei, T. congolense* or *T. simiae,* and highly sensitive, and the ideal concentration to detect trypanosomes with a high sensitivity is 10^4 trypanosomes/μl/dot (Bosompem et al., 1996 a); sensitivity of the dot-ELISA is low below this concentration.

In usual terms, 10^4 trypanosomes/μl/dot is 10^7 parasites/ml. At this concentration, microscopic observation is highly sensitive. Monoclonal antibody technology is highly sophisticated but is not required for such low performances. The conclusion of the laboratory evaluation is that the dot-ELISA is less sensitive than a parasitological examination.

However, in the field, Bosompem et al. (1996 b) mentioned that sensitivity of the dot-ELISA is much higher than that of the parasitological examinations. This paradox does not seem to surprise the authors. The most probable explanation for this test presenting a higher sensitivity in the field than in the laboratory would be due to false positive results.

Validation of this dot-ELISA was carried out in a similar manner as previously described for AG-ELISA:

- capability of the test to recognize specifically parasitic lysates was used to demonstrate the high specificity of the tests;

- in the field, negative results with parasitological examination and positive results with dot-ELISA were considered proof of the higher sensitivity of the new test.

As previously in cattle with the AG-ELISA, it has never been demonstrated that these flies were infected. Moreover, in the present case, discordance between sensitivity in the laboratory and in the field is not explained.

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CHARACTERISTICS AND INTERPRETATION OF INDIRECT-ELISA FOR T. VIVAX; PROPOSAL FOR THE STANDARDIZATION OF RESULTS

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ABSTRACT

Indirect-ELISA for *T. vivax* as described by Ferenc et al. (1990) is the most sensitive technique for antibody detection of *T. vivax* infections. A group of experts from IAEA, FAO, WHO and OIE has given recommendations for standardization of the indirect-ELISA (Wright et al., 1993). Based on these recommendations, we have added some proposals for standardization and presentation of the results. The example of the indirect-ELISA for *T. vivax* in cattle is presented. Characteristics of the indirect-ELISA have been studied under experimental conditions in sheep, and in natural infections in cattle in French Guiana. The results are presented in relative percentage positivity (RPP): percentage of positivity relative to negative and positive reference samples; to avoid any variation from plate to plate, or dependent upon the activity of the reagents. Alternatively, instead of an arbitrary choice, reference samples were chosen to represent infected and non-infected populations. Three hundred samples from each population were tested, mean response to the test was recorded and the reference samples selected for a similar response. Such presentation of the results allows the determination of sensitivity and specificity of the test when fixing the cut-off line either on a distribution graph of the responses in infected and non-infected populations, or, directly with the Gaussian distributions. More accurate determination of the cut-off line can be applied if the seroprevalence in the investigated population is known. Characteristics of the test in 13 sheep experimentally infected with *T. vivax* indicated that the RPP of the samples was below 5% before infection; reached 50% in 12-30 days post infection (p.i.); remained above 30% throughout the infection; and decreased below 30% within 2-3 months after a sterilizing treatment. RPP should be used for the presentation of the results in ELISA tests in order to ensure better standardization and allow comparison of the results between laboratories.

RESUMÉ

L'ELISA-indirecte *Trypanosoma vivax* décrite par Ferenc *et al.* (1990) est la technique la plus sensible pour la détection des infections par *T. vivax*. Un groupe d'experts de l'AIEA, la FAO, l'OMS et l'OIE a donné des recommandations pour la standardisation des ELISA-indirectes (Wright et al., 1993). Sur la base de ces recommandations, et en ajoutant des propositions pour la standardisation régionale et l'expression des résultats, l'exemple de la standardisation de l'ELISA-indirecte *T. vivax* est présenté. Les caractéristiques de l'ELISA-indirecte *T. vivax* ont été étudiées dans des conditions expérimentales chez les moutons, et dans des conditions naturelles chez les bovins de Guyane Française. Il est proposé de présenter les résultats en pourcentage de positivité relatif (PPR), en fonctions des résultats obtenus avec des échantillons de référence positifs et négatifs afin d'éviter les variations entre plaques, dépendant de l'activité des réactifs. Une alternative à la sélection arbitraire des échantillons de référence est proposée; ceux ci sont choisi au sein de la population étudiée, pour leur réactivité représentative de la réactivité moyenne des échantillons provenant d'animaux indemnes ou infectés de la zone étudiée. 300 échantillons de chaque population sont testés, la réponse moyenne est enregistrée, et les échantillons de référence sont sélectionné selon cette valeur. Une telle représentation des résultats permet de déterminer la sensibilité et la spécificité du test selon le seuil de positivité déterminé soit sur la représentation graphique de la distribution des populations indemne et infectée, soit à l'aide de la gaussienne de distribution de ces échantillons. Une détermination plus précise du seuil de positivité peut être effectuée si la prévalence des infections dans le secteur étudié est connue ou estimée à la suite d'une pré-enquête. Les caractéristiques du test chez 13 moutons expérimentalement infectés par *T. vivax* indique que le PPR des échantillons est inférieur à 5% avant l'infection, qu'il atteint 50% en 12 à 30 jours post infection (p.i.), reste supérieur à 30% pendant toute la durée de l'infection, et décroît jusqu'à une valeur inférieure à 30% dans les 2 à 3 mois qui suivent un traitement stérilisant. Il est suggéré d'utiliser le PPR pour l'expression des résultats des tests ELISA, pour une meilleure standardisation et une comparaison des résultats entre laboratoires.

RESUMEN

El ELISA-indirecto para el *T. vivax* como lo describieron Ferenc *et al* (1990) es la técnica mas sensible para la detección de anticuerpos en las infecciones por *T. vivax*. Un grupo de expertos de la IAEA (Agencia Internacional de Energía Atómica), FAO, WHO (Organización Mundial de la Salud) y la OIE (Organización Internacional de Endemias) han aportado su recomendaciones para la estandarizacion del ELISA-indirecto (Wright *et al.*, 1993). Basándose en estas recomendaciones hemos añadido algunas propuestas para la estandarizacion y presentación de los resultados; presentamos el ejemplo del ELISA-indirecto para el *T. vivax* de los bovinos. Las características del ELISA-indirecto fueron estudiadas bajo condiciones experimentales en ovinos y en bovinos de la Guyana Francesa infectados naturalmente. Se propuso presentar los resultados en porcentajes de positividad relativa (RPP): porcentajes de positividad relacionándolos a las muestras de referencia negativas y positivas para evitar cualquier variación de lámina a lámina, o dependiente de la actividad de los reactivos. Alternativamente, en vez de una elección arbitraria, se escogieron las muestras de referencia para representar la población infectada y la no infectada. Se registró la media de la respuesta a la prueba, examinando y probando 300 muestras de cada población y las muestras de referencias seleccionadas para una respuesta similar. Tal presentación de los resultados permite la determinación de la sensibilidad y la especificidad de la prueba cuando se fijan los puntos de corte por medio de un gráfico de distribución de las respuestas en la población infectada y en la no infectada, o directamente con la distribución de Gauss. Una determinación mas exacta del punto de corte se pudiera aplicar si la seroprevalencia en la población investigada es conocida. Las características de la prueba en 13 ovinos experimentalmente infectados con *T. vivax* indicaron que el RPP de la muestras estuvo por debajo del 5% antes de la infección, alcanzando el 50% en 12-30 días post infección (pi.), permaneciendo por encima del 30% a lo largo de la infección, y decreciendo por debajo del 30% dentro de los 2-3 meses después del tratamiento de esterilización. Se sugiere el uso del RPP para la presentación de los resultados de las pruebas de ELISA, para la mejor estandarizacion y comparación de los resultados entre los laboratorios.

INTRODUCTION

The Indirect-ELISA technique is commonly used for antibody detection, but the technique requires a high level of standardization between laboratories. A group of experts from IAEA, FAO, WHO and OIE have recently given recommendations for standardization of the indirect-ELISA (Wright et al., 1993).

Indirect-ELISA for *T. vivax* as described by Ferenc et al., (1990) is the most sensitive technique for antibody detection of *T. vivax* infections. Genus specificity of the test is high, but sub-genus specificity is poor. On the basis of this indirect-ELISA *T. vivax*, we have added to the recommendations of Wright et al., (1993) some proposals for standardization and presentation of the results. Characteristics of the indirect-ELISA *T. vivax* under experimental conditions in sheep, and in natural infections in cattle in French Guiana are identified.

STANDARDIZATION OF THE ELISA TECHNIQUE

Production of natural antigens is biologically variable and this characteristic will not be further discussed.

A rodent adapted strain of *T. vivax* (IL3568) was cultivated in irradiated rats, and when parasitemia reached 10^8, the animals were anesthetized and bled to death. The blood was collected on glucose/saline buffer (PSG) and centrifuged. Buffy coats were deposited on a column of DEAE as described by Lanham and Godfrey (1970) to separate parasites from blood cells. Three cycles of sonication, freezing/thawing were done. The lysate was then centrifuged at high speed, to eliminate insoluble particles. The soluble antigens were used at 15µg/ml to coat the ELISA plates.

The protocol of the ELISA reaction was optimized to obtain the highest possible rate of positive samples/negative samples. Recommendations of Boquentin and Duvallet (1990) and personal recommendations of Katende (ILRI) were very useful for the establishment of the protocol and the optimization of the reaction.

PROPOSAL FOR STANDARDISATION OF THE INDIRECT-ELISA FOR *T. VIVAX*

In the first standardisation technique in order to fix a cut-off line, a value of the optical density (OD) was chosen

either arbitrarily, or as a multiple of a negative reference sample (for example, 2.5 times the optical density of the negative reference sample).

In their recommendations, Wright et al., (1993) indicated that results should be expressed as percentage of positivity of the samples compared to a strongly positive reference sample. The cut-off line was then chosen as 30% or more, depending on the general reaction of known infected or non-infected samples.

To avoid variation of the whole chromogenic reaction, due to individual quality of the plates, temperature, or intrinsic activity of the reagents (enzyme, substrate, chromogene) etc., the result was expressed not only relative to a positive sample, but to both positive and negative reference samples. This parameter was designated 'Relative Percentage of Positivity' or 'RPP'.

RPP of a sample $\quad = \quad \dfrac{\text{OD of the sample - OD negative reference sample}}{\text{OD of positive reference sample - OD of negative reference sample}}$

In such conditions, with fixed reference samples, variations of the whole reaction from one plate to another would be negligible. Once the reference sera have been selected, they must be allocated and kept at a negative temperature (-20°C or -80°C).

CHOICE OF REFERENCE SAMPLES

Weakly reactive negative samples and highly reactive positive samples can be arbitrarily chosen when the reactivity of the infected and non-infected population are unknown.

Instead of arbitrarily choosing the reference sera, we propose to study the infected population (IP) and non-infected population (NIP), and select reference sera representative of the mean reactivity of the IP and the NIP.

Figure 1a represents the OD obtained with sera from 300 cattle found naturally infected with *T. vivax* and 300 sera from non-infected cattle.

Figure 1a: Optical densities in indirect-ELISA *T. vivax* observed in 300 infected and 300 non infected cattle
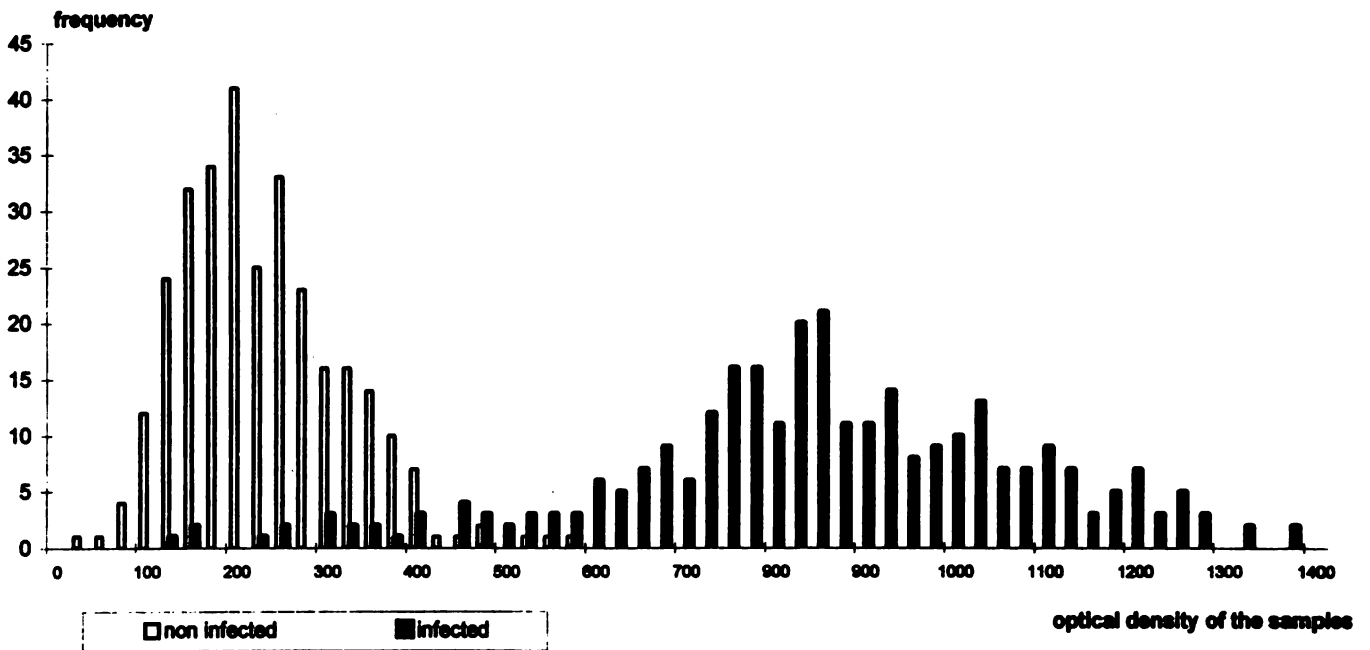
Mean OD of negative is $m_N = 229$, with a standard deviation of $\sigma_N = 89$;
Mean OD of positive is $m_p = 845$, with a standard deviation of $\sigma_p = 234$. The Equation of the Gaussian distribution is recalled hereafter:

$$y = \left(\frac{1}{\sigma\sqrt{2\pi}} \right) e^{-\left(\frac{(x-\mu)^2}{2\sigma^2} \right)}$$

Figure 1b represents the Gaussians of distribution NIP ($m_N = 229$, $\sigma_N = 89$) and IP ($m_p = 845$, $\sigma_p = 234$).

Figure 1b: Theoretical distribution of optical densities in indirect-ELISA for *T. vivax* infected and non infected cattle
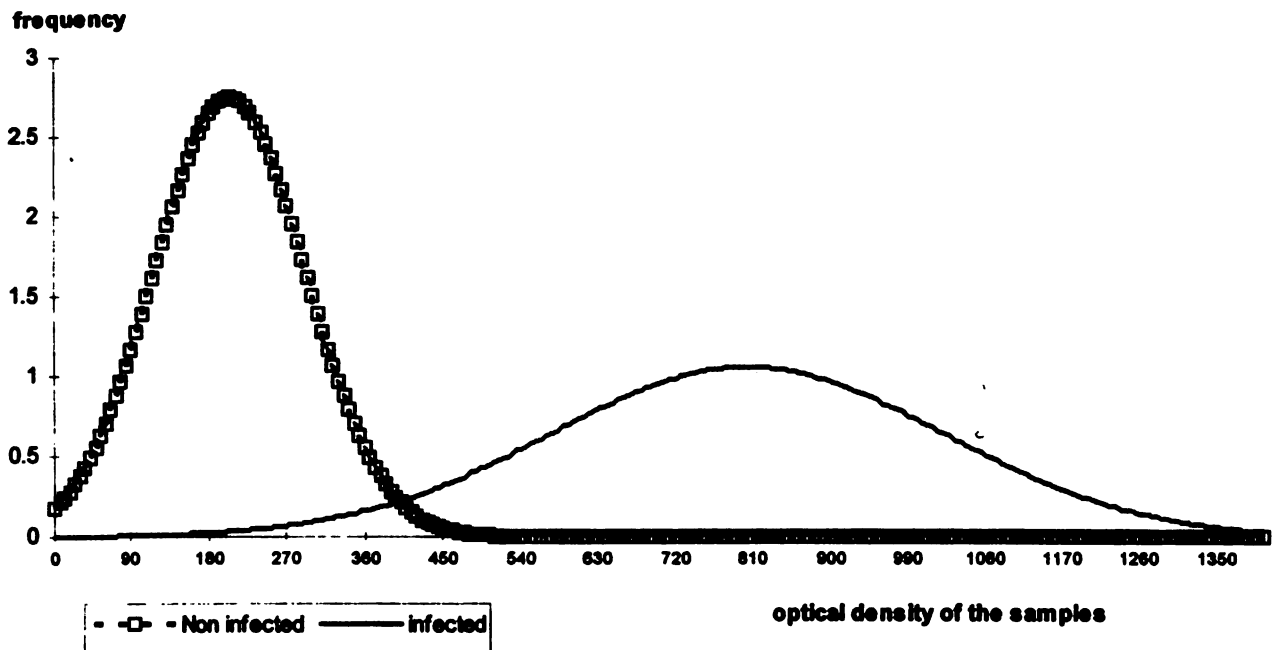

Reference sera were chosen for their reactivity close to the means, e.g. 229 and 845. Figure 2a represents the RPP of the infected and non-infected populations.

The advantage of such reference samples is that the cut-off line can be determined on the basis of chosen values for sensitivity and specificity which are read directly on the figure, or deduced from the equations of the Gaussian distribution.

In the case of *T. vivax* in cattle, with a sensitivity of 98%, the specificity was 93%; and with a specificity of 99%, the sensitivity was 95%. When prevalence is known, other criteria can be used, like very high positive or negative predictive values, a minimum risk of error (number of false positives = number of false negatives) etc. On a regular basis, in French Guiana, with a prevalence of 22% (Desquesnes and Gardiner, 1993), the cut-off line was established at RPP 50%, for a maximum positive predictive value (99.9%). The distribution of the population with such prevalence is illustrated in Figure 2b. Comparison of Figures 2a and 2b clearly indicates that the choice of cut-off line depends on the prevalence of infection in the population under investigation. For reliable results, the cut-off line must be increased when prevalence is below 50%, and decreased if prevalence is above 50%.

Figure 2a: RPP in indirect-ELISA *T.vivax*: Theoretical seroprevalence of 50%
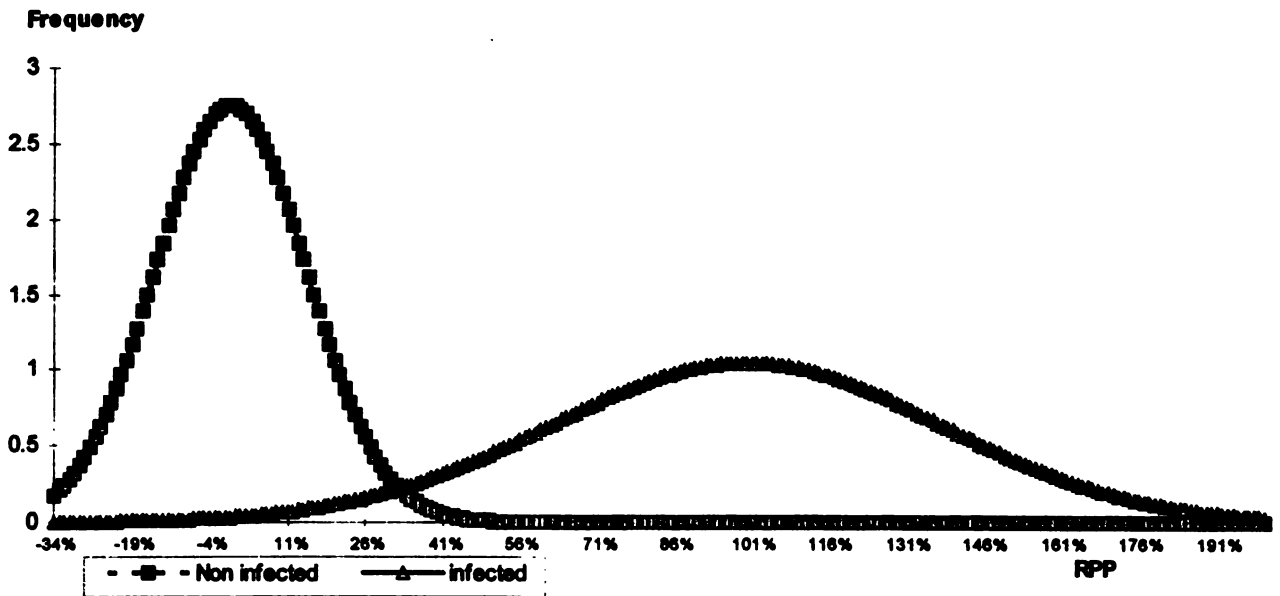
Figure 2b: RPP in indirect-ELISA *T.vivax*: Theoretical distribution with an observed seroprevalence of 22%
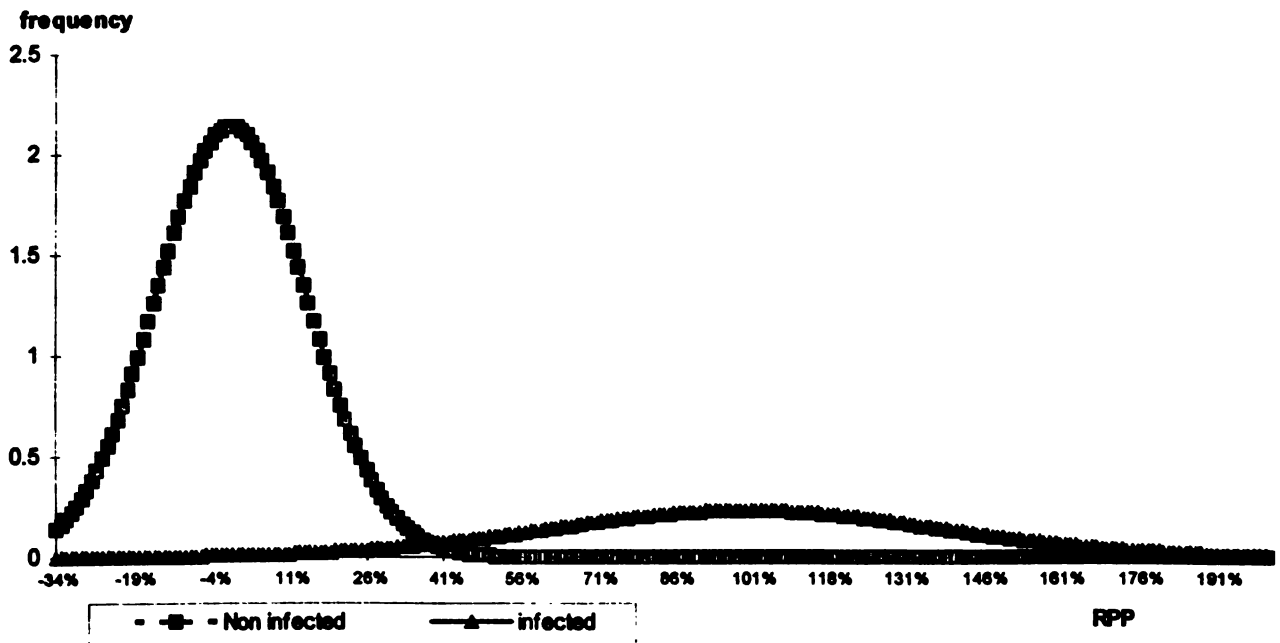
It must be emphasised that the specificity of the test decreases when the prevalence decreases; to avoid the increase of false positive results, a higher cut-off line must then be chosen, according to the real or estimated prevalence of the infection.

100

CHARACTERISTICS OF THE TEST IN NATURALLY INFECTED CATTLE

A herd of 23 cattle imported from metropolitan France was introduced into French Guiana in December 1995 and mixed with a herd of 37 local cattle previously infected by *T. vivax* in the epidemic of 1994-95. In June 1996, clinical signs including fever, anemia, and significant weight loss alerted the farmer, and *T. vivax* was found in up to 20% of the imported cattle and 5% of the local cattle. Natural evolution of the infection was observed during 10 days, then the entire herd was treated with isometamidium chloride at a dose of 1mg/kg. Serodiagnosis through indirect-ELISA yielded the following data in imported cattle:

⇒ the animals were all negative upon their entry into French Guiana. RPP were homogeneous and low;

⇒ the total sero-incidence reached 70% within one month;

⇒ seroprevalence remained around 60% during three months post treatment (PT), and then decreased rapidly down to 10% at three and a half months PT;

⇒ values of the RPP were highly heterogeneous during the infection period, revealing presence of infected and non-infected animals. Later on they became homogeneous and high;

⇒ the mean RPP of the herd (mRPP) reached 83% and decreased regularly down to 26% by three and a half months PT; the herd was considered as negative three months PT (mRPP < 50%);

⇒ the mean RPP of positive samples (mRPPP) was approximately 100% during active infection, with individual scores of 200%, and then decreased down to 70% three and a half months PT, confirming the efficacy of the treatment on all the animals;

⇒ the mean RPP of the negative samples (mRPPN) was 9.5% at the beginning of the survey, increased to 30% at three months PT, indicating the slow decrease of the antibody titers after a sterilising treatment.

Figure 3 represents seroprevalence, mRPP, mRPPP and mRPPN of the imported herd, before and after sterilizing treatment against *T. vivax*.

Figure 3: Imported herd infected with *T.vivax* and treated with isometamidium chloride: seroprevalence, mean RPP of the herd (mRPP), mean RPP of the positive samples (mRPPP) and mean RPP of the negative samples (mRPPN)
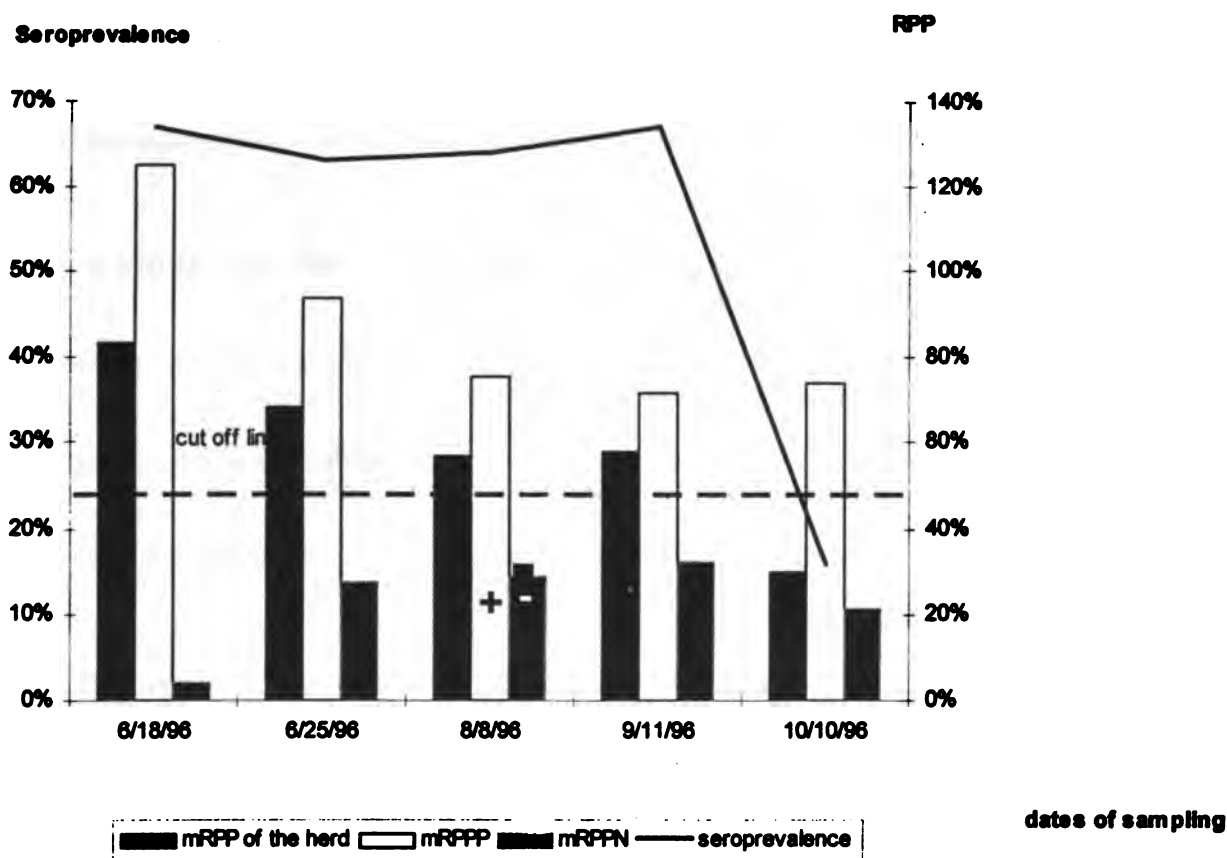

CHARACTERISTICS OF THE TEST IN EXPERIMENTALLY INFECTED SHEEP

Field observations in sheep did not allow the establishment of reference samples representative of infected or non-infected sheep. Reference samples were arbitrarily taken from one of the first sheep infected (with IL4007), negative samples before infection and positive samples at day 30 post infection (PI). The cut-off line of the test was fixed at 50% RPP, according to the observations made during experimental and natural infections.

The indirect-ELISA *T. vivax* was carried out on a daily basis on 13 sheep experimentally infected with isolates of *T. vivax* from French Guiana or Venezuela. The animals were sampled during 70 to 350 days post infection. Before inoculation, all the samples were below 5% of RPP. Whichever isolate was used, RPP reached 30% within 7 - 15 days post infection (PI), and 50% within 12 - 30 days PI. Samples were almost always above 50%. Figure 3 indicates RPP and parasitemia of three sheep experimentally infected with *T. vivax*. In seven sheep bled almost daily during seven months for a total of 1226 samples, the mean negative results were 9.8%, indicating a sensitivity of about 90%. Just after non-sterilizing treatments, the RPP either increased slightly and then decreased, or remained stable, or decreased, but always increased again later on, when parasites reappeared in the circulating blood. After a sterilizing treatment, RPP decreased below 30% two to three months PT (Figure 4a, 4b, 4c).

Figure 4a: Indirect-ELISA in sheep experimentally infected with *T. vivax:* TVVG1 (Venezuela)
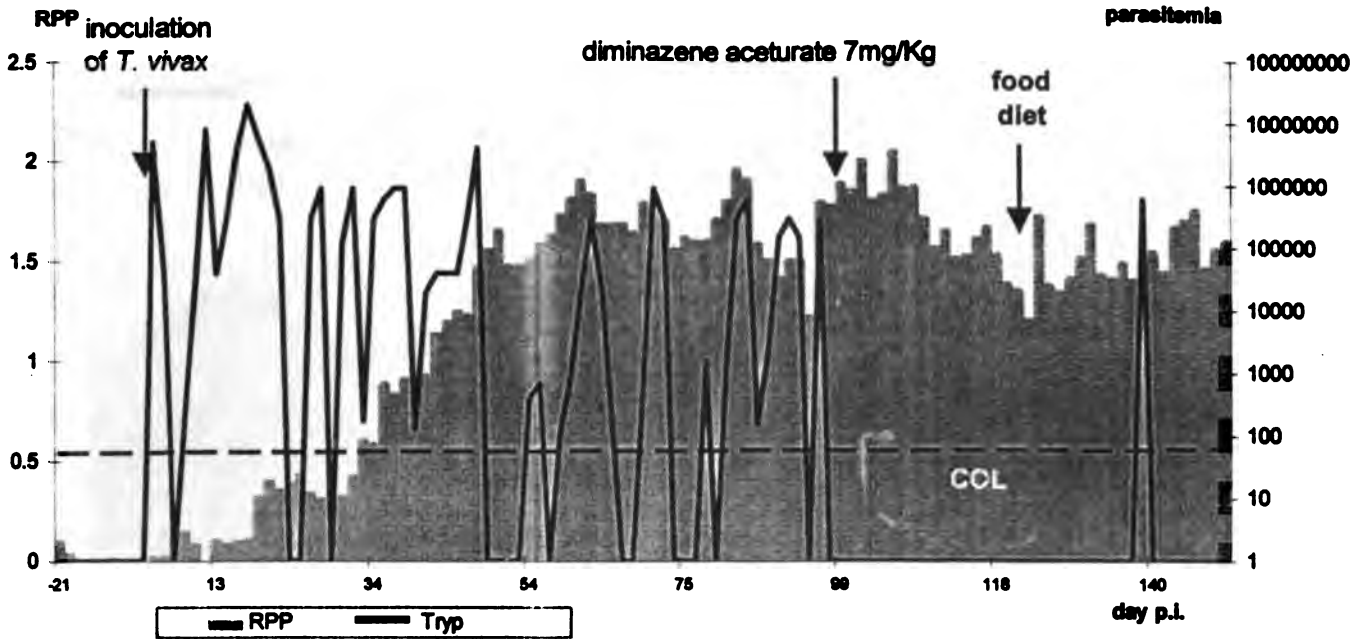
Figure 4b: Indirect-ELISA in sheep experimentally infected with *T. vivax:* TVFG3 (French Guiana 1996)
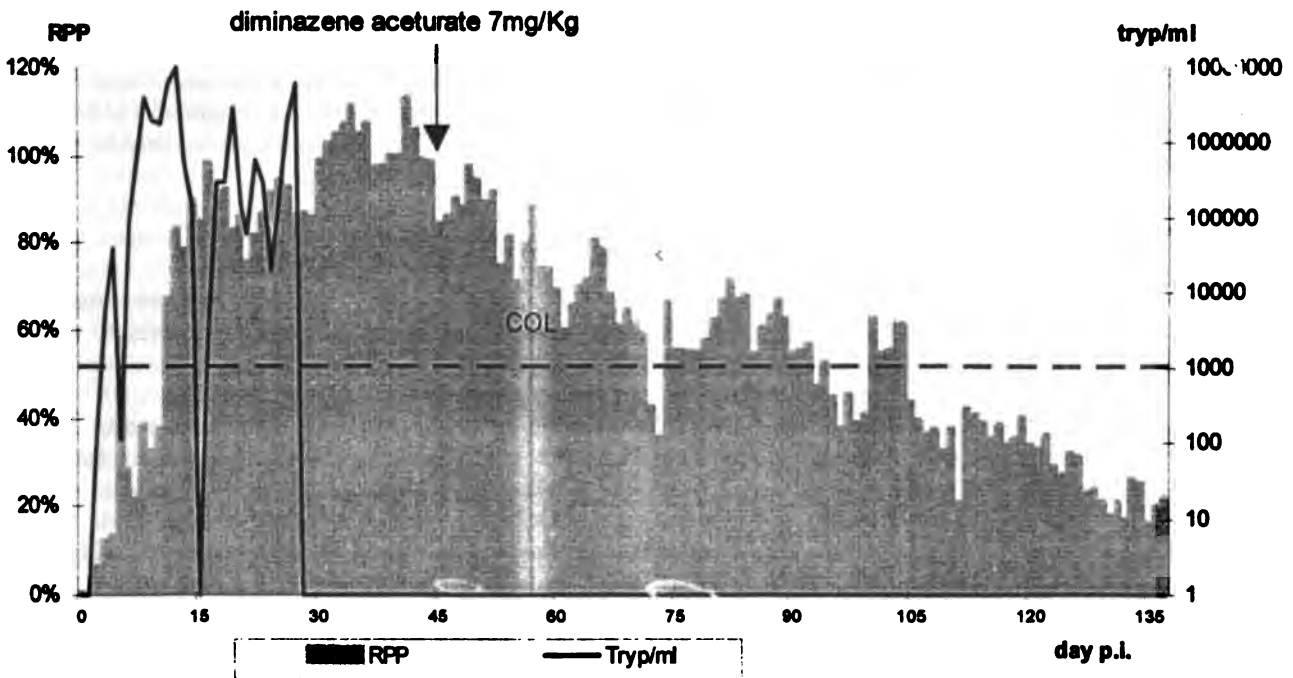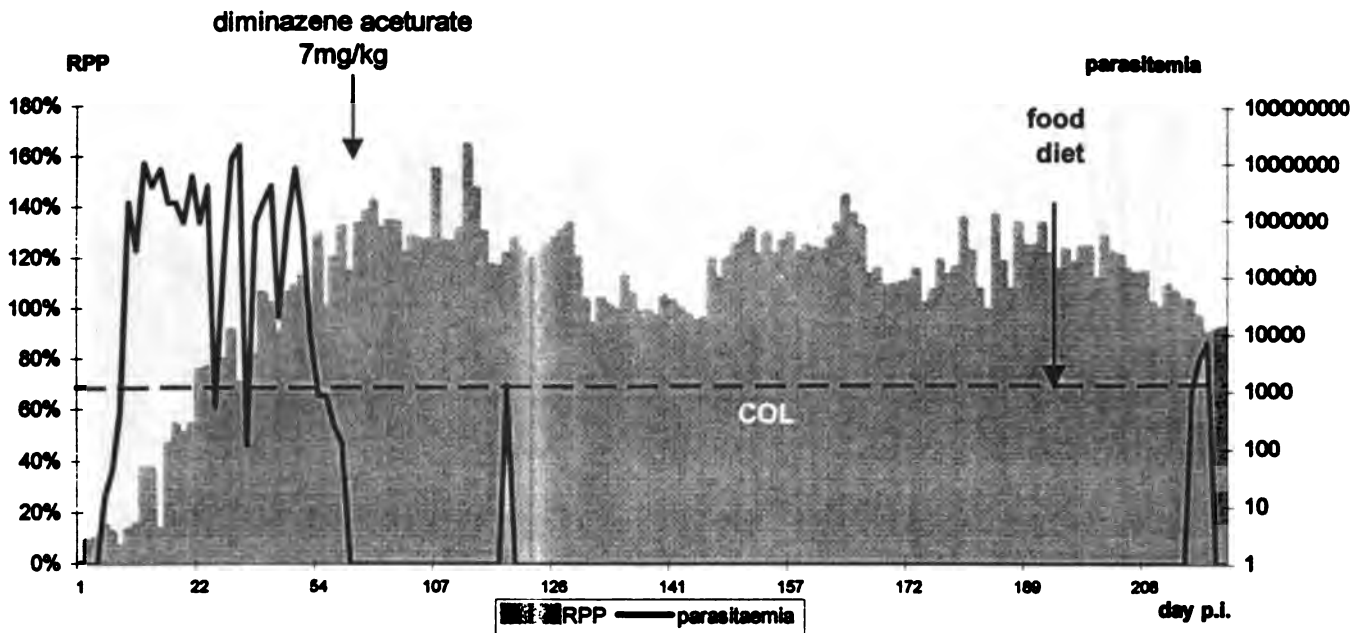
Figure 4c: Indirect-ELISA in sheep experimentally infected with *T. vivax:* TVFG2 (French Guiana 1994)

CONCLUSION

The test is robust since it always gives positive results from 20 days PI. During 2 - 3 months post-sterilization, it gives false positive results, which are expected in an antibody-based test. Knowledge of the epidemiological situation of the herd allows interpretation of the results of the test. When parasitological results and indirect-ELISA *T. vivax* were interpreted together, the percentage of false negative results decreased to 2.8% in the seven sheep bled daily during seven months.

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UTILIZATION OF *T. EVANSI* ANTIGENS IN INDIRECT-ELISA FOR THE DIAGNOSIS OF *TRYPANOSOMA* SP. IN LIVESTOCK

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ABSTRACT
The only non-commercial reagent necessary to process the indirect-ELISA Trypanosoma species is the trypanosome antigen. Trypanosomes for indirect-ELISA are generally produced in rodents. Rare *T. vivax* strains have been adapted to rodents, and irradiation of the rats is often necessary to produce high parasitemia in order to prepare antigens for indirect-ELISA. As a result, production and utilization of *T. vivax* antigens is a limiting factor for most laboratories. *T. evansi* grows easily in mice, with very high spontaneous parasitemic levels, and is known to have antigenic similarities with other salivarian trypanosomes. The utilization of *T. evansi* antigens in the diagnosis of *T. vivax* infections could be an interesting alternative for laboratories which cannot produce *T. vivax* antigens. Qualitative studies in western blotting, and quantitative studies in indirect-ELISA *T. vivax* and indirect-ELISA *T. evansi* have been conducted with sera from sheep experimentally infected with *T. vivax* or with *T. evansi*. Qualitative studies showed that some common antigens are present in both species, but most of them are inconsistently recognized. The quantitative study in indirect-ELISA with the antigens of *T. vivax* showed strong responses in homologous systems (with sera from sheep infected with *T. evansi*). On the contrary, with *T. evansi* antigens, the heterologous and homologous systems both give strong responses. It is therefore suggested that *T. evansi* antigens could be used in indirect-ELISA to detect both infections, and that positive samples to that test be tested with indirect-ELISA *T. vivax* to allow differentiation of the infection from another parasite. In such a system mixed infections would not be detected. However, indirect-ELISA *T. evansi* could be used for detection of both parasites, especially for laboratories which do not have the facilities to produce *T. vivax* antigens.

RESUMÉ
Le seul réactif qui n'est pas disponible dans le commerce, pour la réalisation des ELISA-indirectes *Trypanosoma* spp. est l'antigène de trypanosome. La production des antigènes de trypanosomes est généralement réalisée par culture des parasites sur rongeurs de laboratoire. De rares souche de *T. vivax* ont été adaptées aux rongeurs, mais nécessitent toutefois une irradiation préalable des animaux afin d'obtenir une parasitémie suffisamment élevée pour la préparation des antigènes. La production de tels antigènes est un facteur limitant pour la plupart des laboratoires de diagnostic. *Trypanosoma evansi* cultive facilement chez les rongeurs avec des parasitémies spontanées très élevées, et possède de fortes communautés antigéniques avec les autres trypanosomes de la section des *Salivaria*. Pour ces raisons, l'utilisation des antigènes de *T. evansi* pour le diagnostic des infections du bétail par *T. vivax* est une alternative pour les laboratoires qui ne sont pas en mesure de produire les antigènes de *T. vivax*. Des études qualitatives en western-blotting, et des études quantitatives en ELISA-indirecte *T. evansi* et ELISA-indirecte *T. vivax* ont été menées avec des sérums de moutons infectés par *T. vivax* ou *T. evansi*; les études qualitatives indiquent qu'une grande partie des antigènes sont communs aux deux espèces, mais que ceux-ci sont reconnus de manière inconstante. Les études quantitatives en ELISA-indirecte *T. vivax* indiquent des réponses fortes en système homologue (animaux infecté par *T. vivax*) mais faibles en système hétérologue (animaux infectés par *T. evansi*). En revanche, avec l'ELISA-indirecte *T. evansi* les réponses hétérologues et homologues sont fortes. Il est en conséquence suggéré que les antigènes de *T. evansi* soient utilisés en ELISA-indirecte pour détecter les deux infections, et que seuls les échantillons positifs soient testés en ELISA-indirecte *T. vivax* pour une éventuelle différenciation des infections. Quoiqu'il en soit, l'ELISA-indirecte *T. evansi* peut être utilisée pour la détection de ces deux parasites, en particulier pour les laboratoires qui ne possède pas l'équipement ni le matériel biologique nécessaires à la préparation des antigènes de *T. vivax*.

RESUMEN

El único reactivo no comercial necesario para procesar el ELISA-indirecto para las especies de *Trypanosoma* es el antígeno-trypanosoma. La producción de trypanosomas para el ELISA-indirecto se realiza generalmente en roedores. Una cepa rara de *T. vivax* ha sido adaptada a los roedores, y además, la irradiación de las ratas es a menudo necesaria para producir alta parasitemia de manera de preparar el antígeno para el ELISA-indirecto. La producción de tales antígenos es un factor limitante para la mayoría de los laboratorios. El *T. evansi* crece fácilmente en ratones y se logran niveles altos de parasitemia y se sabe que tiene similitudes antigénicas con otras trypanosomas salivariarios. Por estas razones, la utilización de los antígenos del *T. evansi* en el diagnóstico de las infecciones de *T. vivax* podrían ser una alternativa interesante para los laboratorios que no puedan producir antígenos de *T. vivax*. Se han realizado estudios cualitativos con Western Blotting, y estudios cuantitativos con el ELISA-indirecto-*T. vivax* y ELISA-indirecto-*T. evansi*. con sueros de ovinos infectados experimentalmente con *T. vivax* o con *T. evansi*. Los estudios cualitativos demostraron que algunos antígenos comunes están presentes en ambas especies, pero la mayoría de ellos no se les reconoce consistentemente. Los estudios cualitativos con el ELISA-indirecto con antígenos de *T. vivax* mostraron una fuerte respuesta en los sistemas homólogos (con suero de ovinos infectados con *T. vivax*), pero con una baja respuesta en los sistemas heterólogos (con suero de ovinos infectados con *T. evansi*). Por el contrario, con los antígenos del *T. evansi*, en ambos sistemas: homólogos y heterólogos se presentó una fuerte respuesta. Por ello, se sugiere que los antígenos del *T. evansi* podrían ser usados en el ELISA-indirecto-*T. evansi* para detectar ambas infecciones, y las muestras positivas a esta prueba sean luego examinadas con el ELISA-indirecto- *T. vivax* para permitir la diferenciación de la infección de un parásito y del otro. En cada sistema, las infecciones mixtas pudieran no ser detectadas. Sin embargo, el ELISA-indirecto-*T. evansi* podría ser usado para la detección de ambos parásitos, especialmente en los laboratorios que no tengan facilidad de producir el antígeno de *T. vivax*.

INTRODUCTION

The only non-commercial reagent necessary to process the indirect-ELISA for the *Trypanosoma* species is the trypanosome antigen. The trypanosomes needed for the preparation of soluble antigens for indirect-ELISA are usually produced in rodents. Rare *T. vivax* strains have been adapted to rodents, but even with these isolates, irradiation of the rats is often necessary to produce high parasitemia. Production of such antigens is a limiting factor for most laboratories. Indeed, we have prepared our stock of *T. vivax* antigens at ILRI, under such conditions.

T. evansi grows easily, with very high spontaneous parasitemic levels in mice, and is known to have antigenic similarities to other salivarian trypanosomes. Utilisation of *T. evansi* antigens in the diagnosis of *T. vivax* infections could be an interesting alternative for laboratories which cannot produce *T. vivax* antigens. Antigenic similarities between these sub-genus of the genus *Trypanosoma* have been suspected for a long time, but little investigation has been done on their potential application to heterologous diagnosis. Qualitative studies of the reaction of sera from *T. vivax* infected animals with *T. evansi* antigens have been carried out recently by Rossi et al.(1993 and 1995). The authors found that *T. evansi* polypeptides from 25 to 117KDa were recognised by these sera. Only one of these polypeptides was recognised by almost all the *T. vivax* infected animals (96KDa), but none appeared to be fully satisfactory for a diagnosis based on a single polypeptide.

Qualitative and quantitative studies of these cross reactions were initiated at CIRAD-EMVT-Guyane.

I   QUALITATIVE STUDY

The qualitative study was carried out by western blottings.

When *T. evansi* antigens were used, sera from sheep infected with *T. evansi* or *T. vivax* recognised numerous common polypeptides, whose molecular weights were estimated at 160, 140, 70, 72, 56, from 53 to 48.5, and 30Kda. Some other bands were inconsistently recognized. The maximum reaction was observed from 48.5-53 Kda. The band of Kda described by Rossi et al. was not found.

With *T. vivax* antigens, sera from sheep infected by *T. evansi* or *T. vivax* recognised numerous common polypeptides, whose molecular weights were estimated at 70, 68, 64-56 and 40 Kda. Numerous other bands were

inconsistently recognized. Two bands of 22 and 26 Kda were recognized only by *T. vivax* infected animals, but not by all of them. Two bands of 180 and 190Kda were recognised by all *T. vivax* infected animals and did not react with sera from *T. evansi* infected animals.

These first results require confirmation to determine the frequency of recognition of these polypeptides in *T. vivax* infected animals. Since these antigens do not appear in high quantities on electrophoresis gels, such studies would require substantial amounts of *T. vivax* antigens and could only be performed in specialized laboratories.

II   QUANTITATIVE STUDY

In four sheep experimentally infected with *T. vivax*, and four sheep experimentally infected with *T. evansi*, the indirect-ELISA for IgG were processed with *T. vivax* antigens and *T. evansi* antigens. When the antigen used is the same as the parasitic species diagnosed we refer to a homologous system. If the antigen used differs from the parasitic species diagnosed, we refer to a heterologous system. Results of the indirect-ELISA are presented in RPP (relative percentage of positivity) as described by Desquesnes (1996).

Extracts of the results are illustrated in Figure 1 (animals infected with *T. vivax*), and Figure 2 (animals infected with *T. evansi*). In homologous systems RPP are represented with a continuous curve, and in heterologous systems, with a discontinuous curve.

Figure 1: Parasitemia, and RPP in indirect-ELISA *T. vivax* (homologous system) and indirect-ELISA *T. evansi* (heterologous system) of two sheep experimentally infected with *T. vivax* (Venezuelan isolate: TVVG1)

sheep 12: infected with TVVG1

sheep 13: infected with TVVG1

Parasitemia   RPP vivax   RPP evansi
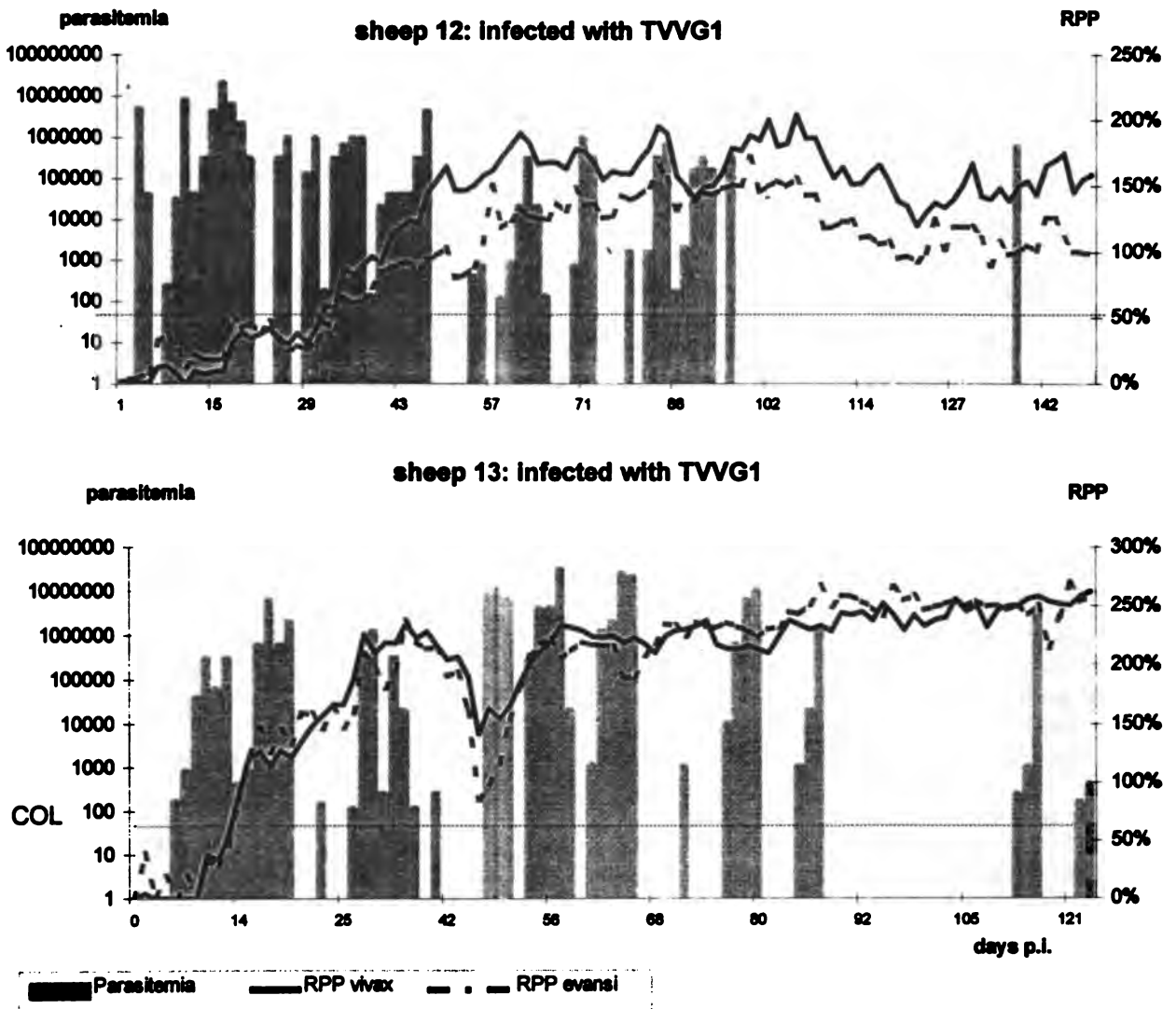
Figure 2: Parasitemia, and RPP in indirect-ELISA *T. evansi* (homologous system) and indirect-ELISA *T. vivax* (heterologous system) of two sheep (n° 14 and 15) experimentally infected with *T. evansi* (Venezuelan isolate: TEVA1)
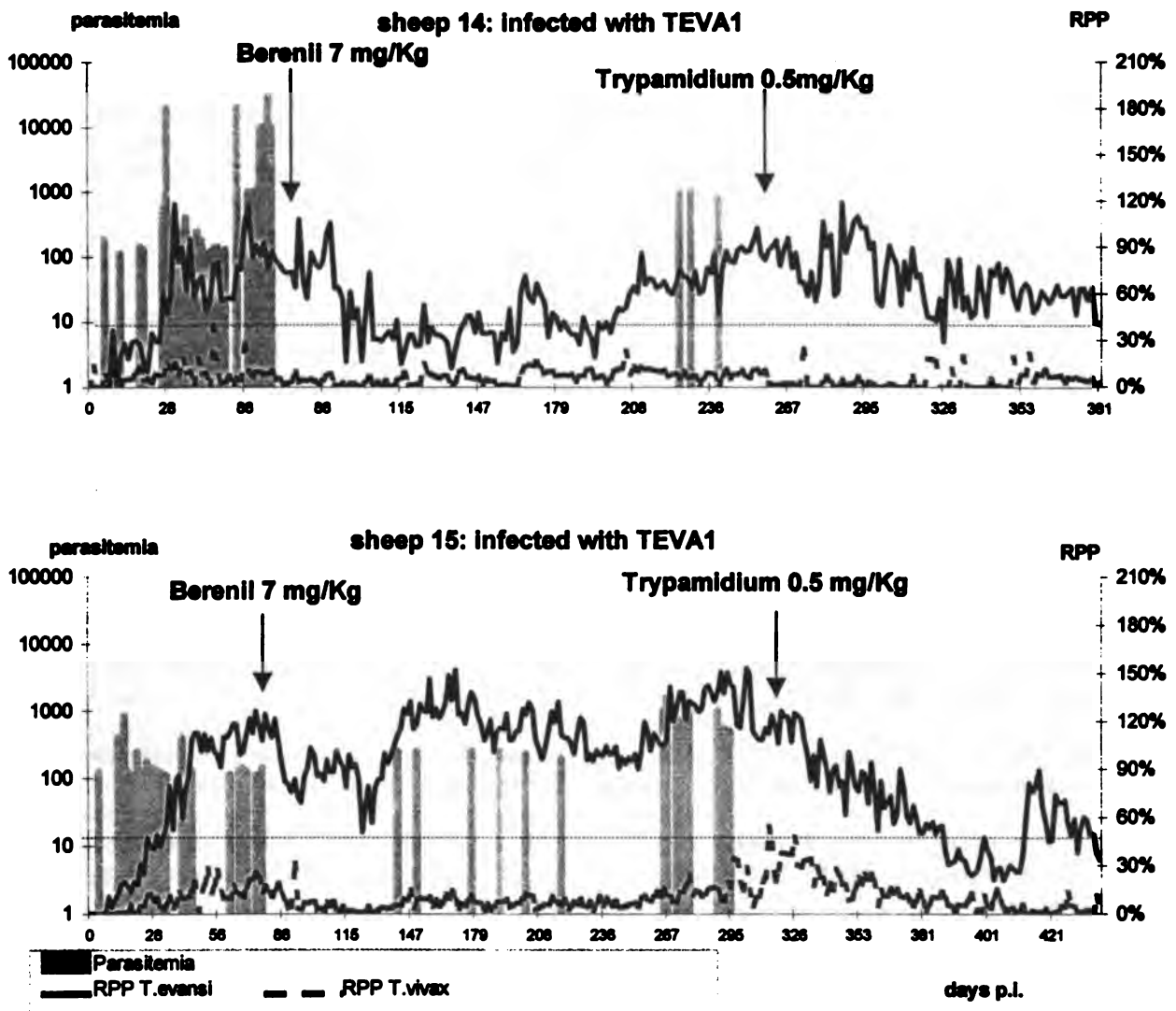
This study revealed that the RPPs obtained from animals infected with *T. vivax* are high and very similar in both homologous and heterologous systems (on *T. evansi* antigens) but generally low or nil in the heterologous system. Only one of the four sheep infected by *T. evansi* reached 50% of RPP during the course of the infection.

These observations confirmed that:

> *T. evansi* infections can interfere with the diagnosis of *T. vivax* infections, but, from this study, apparently at a low level. A study on a larger number of animals is necessary to confirm these first observations.

T. vivax infections not only interfere, but are fully detected by indirect-ELISA *T. evansi*; this may not be an important problem in epidemiology since *T. evansi* is generally studied in horses more than in ruminants (*T. vivax* hosts). On the other hand, in ruminants, *T. evansi* antigens could be used for the diagnosis of *T. vivax* infections.

Diagnosis in host species potentially infected by both parasites could be done as follows:

A primary analysis through indirect-ELISA *T. evansi* could be processed for all samples, and, for the positive samples, a secondary analysis through indirect-ELISA *T. vivax* could be processed. Samples positive on the first and negative on the second test would most probably reveal an infection by *T.* evansi. Positive results on both would reveal infection by at least *T. vivax*. Mixed infections would reveal infection by at least *T. vivax*, but the infections could not be identified. Field evaluation of this method is necessary, and will be carried out during the seroepidemiological survey in the Guianas.

III   CONCLUSION

T. evansi antigens appear to be convenient and useful for diagnosis of *Trypanosoma* species infections in livestock. However, apart from *T. vivax* which was under study, cross reactions with the other *Trypanosoma* species found in livestock, *T. cruzi* in all host species, and *T. equiperdum* in horses, must also be considered when using *T. evansi* antigens.

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UTILIZATION OF *T. EVANSI* ANTIGENS IN INDIRECT-ELISA FOR DIAGNOSIS OF CHAGAS' DISEASE IN HUMANS

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ABSTRACT
Because the incidence of Chagas' disease in French Guiana is very low (an estimated 5-10 cases a year) tests are not locally available, and samples must be sent by courier to Metropolitan France. Two cases of Chagas' disease with cardiac symptoms were suspected by a cardiologist in Cayenne Hospital and serum samples were submitted to CIRAD-EMVT Guyane. Antigenic similarities between salivarian and stercorarian trypanosomes have seldom been investigated, so the potential use of *T. evansi* antigens for the diagnosis of *T. cruzi* infection was evaluated. Western blottings on *T. evansi* antigens showed reactions of the two sera, while four negative samples did not react at all. On indirect-ELISA for *T. evansi* the reactions of the two sera were very strong. Fifty sera from the serum bank of the Pasteur Institute were used as negative controls. The rates of optical density of suspected sera/negative control were 10 to 40. Infection of these two sera was later confirmed in homologous systems by the Pasteur Institute in Paris. Evaluation of the indirect-ELISA *T. evansi* for the diagnosis of Chagas' disease in humans was conducted in collaboration with F. Breniere, from Orstom Bolivia; and 211 reference sera were tested blindly at CIRAD-EMVT-Guyane. The cut off line of the indirect-ELISA *T. evansi* was later determined on the basis of the results of several other tests specific for *T. cruzi* including PCR, Buffy Coat, ELISA IgG and IgM, hemagglutination and immunofluorescence. The sensitivity and specificity of the new test was not significantly different from the other serological tests based on *T. cruzi* antigens, showing the potential use of the indirect-ELISA *T. evansi* for the diagnosis of Chagas' disease in humans.

RESUMÉ
La maladie de Chagas a une très faible incidence en Guyane Française, estimée à environ 5 à 10 cas cliniques par an. Pour cette raison, les tests de dépistage ne sont pas disponible localement, et les échantillons suspects doivent être adressés par expédition rapide vers la France métropolitaine. Cette année, deux cas de maladie de Chagas cruzi avec signes cardiaques ont été suspecté par un cardiologiste de l'Hopital de Cayenne; des échantillons de sérum ont été adressés en France, mais également au CIRAD-EMVT-Guyane, à Cayenne. Les communautés antigéniques entre les trypanosomes de la section des *Salivaria* et ceux de la section des *Stercoraria* ont été très peu étudiées, aussi, l'utilisation des antigènes de *T. evansi* pour la détection des anticorps dirigés contre *T. cruzi* a-t-elle été évaluée. Des western-blottings sur antigènes de *T. evansi* ont été réalisés, et ont révélé des réactions prononcées avec les deux sérums suspects alors que 4 sérums témoins négatifs ne réagissaient pas du tout. En ELISA-indirecte *T. evansi* les sérums suspects ont également fourni de fortes réponses alors que 50 échantillons issus de la banque de sérum de l'Institut Pasteur de Cayenne, utilisés comme contrôles négatifs, ne réagissaient pas. Le rapport des densités optiques des sérums suspects/sérums négatifs était de 10 à 40. L'infection de ces deux patients a par la suite été confirmée en système homologue (ELISA-indirecte *T. cruzi*) par l'Institut Pasteur de Paris. L'évaluation de l'ELISA-indirecte *T. evansi* pour le diagnostic de la maladie de Chagas chez l'homme a été conduite en collaboration avec F. Brenière, de l'ORSTOM Bolivie; 211 échantillons de référence ont été testés en aveugle au CIRAD-EMVT-Guyane. Le seuil de positivité de l'ELISA-indirecte *T. evansi* a été déterminé ultérieurement sur la base des résultats obtenu avec plusieurs autre tests, spécifiques de *T. cruzi*, parrmi lesquels la PCR, le test de Murray, les ELISA IgG et IgM, et les tests d'hémagglutination et d'immunofluorescence. La sensibilité et la spécificité du nouveau test n'était pas sensibliment différentes de celles des autres techniques sérologiques basées sur les antigènes spécifiques de *T.*; l'intérêt de l'ELISA-indirecte *T. evansi* pour le diagnostic de la maladie de Chagas chez l'homme a ainsi été confirmé.

RESUMEN
La enfermedad del mal de Chagas tiene muy baja incidencia en Guyana Francesa, se estima como en un 5-10 casos por año. Por esta razón, pruebas locales de diagnóstico no están disponibles, y las muestras deben ser enviadas por valija a la Francia Metropolitana. Dos casos de las enfermedad de Chagas con síntomas cardiacos fueron sospechados por los cardiólogos en el Hospital de Cayenne y las muestras de suero fueron entregados al CIRAD-

EMVT Guyane. Las similitudes de antígenos entre trypanosomas salivarios y estercolarios han sido investigadas muy poco, así que se evaluó el uso potencial de los antígenos de *T. evansi* para el diagnóstico de las infecciones por *T. cruzi*. El Western blotting de los antígenos del *T. evansi* mostró reacciones en dos de los sueros, mientras que cuatro muestras resultaron negativas. En el ELISA-indirecto con el *T. evansi* las respuestas de los dos sueros fueron muy fuertes. 50 sueros del banco de suero del Instituto Pasteur fueron usados como controles negativos. Las tasas de densidad óptica de los sueros sospechosos/control negativo fueron de 10 a 40. La infección de estos dos sueros fue luego confirmada en sistemas homólogos por el Instituto Pasteur en París. La evaluación del ELISA-indirecto-*T. evansi* para el diagnóstico de la enfermedad del Chagas en humanos fue dirigida en colaboración con el Sr. F. Brenierer de Örstom Bolivia; 211 sueros de referencia fueron puestos a prueba en un ensayo ciego en el CIRAD-EMVT-Guyana. Los puntos de corte del ELISA-indirecto-*T. evansi* fueron luego determinadas en base a los resultados a otras pruebas específicas para el *T. cruzi*, incluyendo el PCR, frotis de capa blanca, Ac-ELISA (IgG e IgM), hemaglutinacion e immunofluorescencia. La sensitividad y la especificidad de las nuevas pruebas no fueron significadamente diferentes de otras pruebas serológicas basadas en el antígeno del *T. cruzi*. Se demostró el uso potencial del uso ELISA-indirecto-*T. evansi* para el diagnóstiço del mal de Chagas en los humanos.

INTRODUCTION

The incidence of Chagas' disease in French Guiana is low, estimated at about 5 - 10 cases a year, so tests are not locally available and samples must be sent by courier to Metropolitan France. There has been little investigation into antigenic similarities between salivarian and stercorarian trypanosomes. The only study that we could find in the literature is by Monzon and Colman (1988), in horses in Argentina, in which cross reactions and/or mixed infections with *T. evansi* and *T. cruzi* were suspected.

I.   Preliminary study on western blotting and indirect-ELISA

Two cases of Chagas disease with cardiac symptoms were suspected by Dr. Beaudet, cardiologist at Cayenne Hospital, and serum samples were submitted to CIRAD-EMVT Guyane. Western blottings on *T. evansi* antigens showed reactions of the two sera especially with a large band from 50 to 56KDa, and a small band around 78KDa. Two other bands from 60 to 70 and at 36KDa were recognized in one of the samples. Four negative samples did not react at all on western blot.

On indirect-ELISA *T. evansi*, the reactions of both sera were very strong. Fifty sera from the serum bank of Cayenne's Pasteur Institute were used as negative controls. The optical density rates of suspected sera versus negative control were 10 to 40. On IgG detection the mean optical density (OD) of negative controls was 83 (OD = optical density of the ELISA reader X 1000), and the mean OD of positive samples was 821. On IgM detection of the mean, OD of negative samples was 31, and the mean OD of positive samples was 1350.

Diagnosis was confirmed in a homologous system by the Pasteur Institute in Paris.

II   Evaluation of the test with reference sera

F. Breniere (Orstom, La Paz, Bolivia) was kind enough to participate in the evaluation of the test, and send a batch of reference samples to our laboratory.

One of the previously diagnosed positive samples was used as a positive control and one of the negative samples was selected as a control. The results of the test are presented in relative percentage of positivity (RPP) as follows:

$$RPP = \frac{OD\ sample - OD\ negative\ ref.}{OD\ positive\ ref. - OD\ negative\ ref.}$$

The ELISA reagents and protocol are indicated in Annex 1. The cut-off line of the test was fixed using the indications of F. Breniere, on the basis of the results obtained, with 22 samples negative with four other serological tests, namely:

IF IgG IBBA®
ELISA IgG IBBA®
ELISA IgG Pasteur®
ELISA IgM Pasteur®

ANNEX I: Preparation of the antigens

An isolate of *T. evansi* from Venezuela (TEVA1) was inoculated intraperitoneally into 30 mice (C3H) at day 0. Parasitemia was evaluated from day 3 and the animals were bled when parasitemia reached 10^8 (day 4-6). Blood was collected with citrate/glucose buffer and parasites were separated on a DEAE column as described by Lanham and Godfrey (1970). Parasites were washed 3 times by 10 minutes centrifugation at 4.500rpm in phospate saline glucose (PSG); pellet of trypanosomes were resuspended in PBS and exposed to 5 cyles of 5 minutes freezing/ 5 minutes defreezing in liquid nitrogen and in an incubator at 25°C and then sonicated on ice for 3 X 2 minutes at PW60. The lysate was centrifuged at 4500 rpm for 10 minutes at 4°C, and the pellet was discarded ; the supernatant was centrifuged at 13000 rpm for 20 minutes at 4°C. The protein concentration of the supernatant was estimated by UV readings with a spectrophotometer at 260nm and 280nm, according to the technique of Adams (Normograph) (based on Warburg and Christian, 1942). In our experiment the protein concentration was 2.5mg/ml under a volume of 5 ml. The soluble antigen was aliquoted and stored at -20°C or -80°C.

ELISA Protocol:
Materials:
Polysorp Nunc microtiter plates®;
phospate buffered saline pH 7.4 (PBS) ;
washing buffer is PBS 0.1% tween 20® (WB 0.1%) or 0.2% tween 20® (WB 0.2%);
blocking buffer is PBS with 5% skimmed milk (BB);
diluting buffer is BB with 0.1% tween 20 (DB);
anti-IgG human SIGMA®;
ABTS 2% ;
H_2O_2 1%;
substrate buffer is a citrate buffer pH4 (SB).

Method:
Coating: 100µl/well at a protein concentration of 15µg/ml in PBS, overnight at 4°C.
Rincing with PBS twice.
Blocking with 200µl of BB/well, overnight at 4°C.
Sera are diluted1/50 in DB and transferred under a volume of 100µl.
Incubation : 15 min. at 37°C, permanent shaking.
Rincing X5 with PBS.
Washing X 8 with WB 0.1%.
Conjugate 1:1000 in WB 0.2% ;
incubation 15min. at 37°C, permanent shaking.
Rincing X5 with PBS.
Washing X 8 with WB 0.1%.
Substrate: 10 ml of SB with 80µl of H_2O_2 1% and 80µl of ABTS 2% ; 100µl/well.
Incubation in a dark room with permanent shaking for about 20 minutes, and
Reading of the plate when the mean of positive controls reachs 1 OD.

Results are presented in relative percentage of positivity « RPP » as follows:

$$RPP = \frac{OD\ sample - OD\ negative\ ref.}{OD\ positive\ ref. - OD\ negative\ ref.}$$

With the indirect-ELISA *T. evansi* the mean RPP of the 22 samples was 2% with a standard deviation of 4%. The

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cut-off line was determined as the value of the mean +/- 2 standard deviations: COL = 2 + 2 x 4 = 10%.

Results of the evaluation with reference sera are indicated in Table 1.

IgG detection in indirect-ELISA *T. evansi* was very sensitive to detect *T. cruzi* infections in adults, and very specific in this epidemiological context since there were no false positives in adults and children. The sensitivity was lower in infected children, probably due to a low level of specific immungloblins in early infections.

Cross reactions were observed with sera from people infected by *Leishmania* sp. These cross reactions were also observed with some of the other tests performed. Since the samples come from a low endemic area of *T. cruzi*, the positive reaction could be due to *T. cruzi* infection or to a cross reaction with *Leishmania* sp.

Table 1: **Results of the evaluation of the indirect-ELISA IgG *T. evansi* for the diagnosis of Chagas disease in humans**

Reference samples	Number of samples tested	Number of positives at a COL 10%	Number of false positives	Number of false negatives
Infected with cardiac symptoms	10	9	0	1
Infected with digestive symptoms	10	8	0	2
Infected without symptoms	2	2	0	0
Leishmaniasis	7	3	3	0
Leishmaniasis positive at 1/80	9	0	0	0
Leishmaniasis positive at 1/320	4	4	4	0
Non infected children in endemic area	10	0	0	0
Infected children in endemic area	30	25	0	5
Infected adults in endemic area	30	30	0	0
Non infected adults in endemic area	10	10	0	0
TOTAL	122	91	7	8

CONCLUSION

It appears that *T. evansi* antigens could be useful for the diagnosis of Chagas' disease in humans by IgG detection through indirect-ELISA. Production of the antigen is simple and the cost of the test very low (around US$ 0.50 per sample). This technique could be used in seroepidemiological surveys and screening of blood donors.

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DETECTION OF PARASITE DNA IN HEMATOPHAGOUS FLIES

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ABSTRACT

A major problem in studying mechanically transmitted parasites is detecting the minute amounts of parasite material present in the infected vector. The use of DNA-based probes could overcome this problem and contribute significantly to efforts aimed at monitoring and controlling trypanosomiasis by helping to evaluate the performance of different control strategies. Improved methods, simplified DNA preparation from animal blood and fly extracts allow probe methods to be used in less sophisticated laboratories. A wide range of DNA-based techniques have been used for trypanosome detection including polymerase chain reaction (PCR), random amplification of polymorphic DNA (RAPD), kDNA minicircle analysis and hybridisation using repetitive DNA sequences. Published work suggests that PCR is the most sensitive of these techniques. PCR amplification of a repetitive *T. evansi* specific DNA sequence detected the DNA equivalent of a single trypanosome. The same probe in a southern blot detection system detected 1000 *T. evansi* parasites in a 10ml blood sample. We have evaluated the probes reported as *T. evansi* specific for the ability to detect parasite DNA in artificially fed *Stomoxys calcitrans*. The most sensitive probe was used in an Indonesian field-trial. We present the preliminary results of this work.

RESUMÉ

Un des problèmes majeurs dans l'étude des parasites transmis mécaniquement est de détecter des quantités infimes de matériel parasitaire présent chez les vecteurs infectés. L'utilisation de sondes d'ADN permet de résoudre ce problème et contribue de manière significative à l'amélioration de la connaissance et du contrôle des trypanosomoses en aidant à évaluer les performances de différentes stratégies de contrôle. L'amélioration des méthodes, simplification de la préparation de l'ADN à partir de l'insectes ou du sang des animaux infectés, permet de vulgariser l'utilisation des techniques de sondes d'ADN dans des laboratoires moyennement équipés. Une large game de techniques basées sur la détection de l'ADN des trypanosomes, incluant la PCR, l'utilisation d'amorces aléatoires (RAPD), l'analyse des minicercles d'ADN kinétoplastique et l'hybridation à l'aide de séquences d'ADN répétitives a été utilisée. Les publications suggèrent que la PCR est la technique la plus sensible. L'amplification d'une séquence répétitive spécifique de l'ADN de *Trypanosoma evansi* permet de détecter l'ADN d'un unique trypanosome. La même séquence ne permet que de détecter 1000 parasites dans un échantillon de 10 ml de sang par Southern blot. Les sondes spécifiques de *T. evansi* ont été évaluées pour la détection de l'ADN parasitaire chez des *Stomoxys calcitrans* nourris artificiellement. La sonde la plus sensible a été utilisée sur le terrain en Indonésie. Les résultats préliminaires de ce travail sont présentés.

RESUMEN

Un problema grave en el estudio de parásitos transmitidos mecánicamente es la detección de cantidades minúsculas de materia parásita presente en el vector infectado. El uso de sondas basadas en el ADN podría resolver este problema y contribuir significativamente a los esfuerzos dirigidos a monitorear y controlar la Tripanosomiasis ayudando a evaluar la actuación de diferentes estrategias de control. Los métodos mejorados, las preparaciones de ADN simplificadas de sangre de animales y extractos de moscas permiten la utilización del método de sonda en laboratorios menos sofisticados. Una amplia gama de técnicas basadas en ADN han sido utilizadas para la detección de Tripanosoma incluyendo la reacción en cadena polimerasi (PCR), la ampliación al azar de ADN polimórfico (RAPD), el análisis minicírculo kADN y la hibridización utilizando secuencias repetitivas de ADN. Los estudios publicados sugieren que la PCR es la más sensible de estas técnicas. La ampliación PCR de una secuencia repetitiva de ADN específica *T. evansi* ha detectado ADN equivalente a una sola Tripanosoma. La misma sonda en el sistema de detección de la mancha austral detectó 1000 parásitos de *T. evansi* en una muestra de sangre de 10 ml. Hemos evaluado las sondas que se han reportado específicamente a *T. evansi* por su capacidad en detectar el ADN parasítico en *Stomoxys calcitrans* alimentado artificialmente. La sonda más sensible fue utilizada en una prueba de campo en Indonesia. A continuación se presentan los resultados preliminares de este trabajo.

Disease caused by *Trypanosoma evansi* infection results in economic losses in livestock in many tropical countries. Up to now, control has been based on the treatment of infected animals with an ever decreasing stock of expensive trypanocidal drugs. At present the introduction of alternative control regimes, especially those based on vector reduction, cannot be developed as little is known about the factors influencing transmission of *T. evansi*. The aim of our work is to develop assays that can be used to provide qualitative data on the vector efficiency of a variety of diptera for *T. evansi*, combining both laboratory and field-based studies. The information gained from this study will be used to assess the feasibility of vector reduction measures as a means of controlling *T. evansi* infection.

The ability to maintain hematophagous diptera as laboratory-bred colonies, along with improvements in artificial feeding techniques, provides the means to investigate the interaction of parasites such as *T. evansi* with potential vectors under controlled conditions. A wide range of blood-sucking diptera has been implicated as vectors for *T. evansi* (Nieschulz, 1926, Foil 1993). Of these, *Stomoxys* sp. has a number of advantages over other fly species for developing transmission models for *T. evansi*, notably that large numbers of *S. calcitrans* can be easily maintained, and that *S. calcitrans* also feeds readily either through membranes or open capillary systems which have been used to study feeding behavior and transmission of various pathogens. As well as serving as a general model for studies of transmission of *T. evansi*, *S. calcitrans* has been implicated as a vector for *T. evansi* (Nieschulz, 1926). A small scale survey carried out by Foil (1993) identified *Chrysops* sp., *Tabanid* sp. and *Stomoxys* sp. as potential vectors for *T. evansi* in East Java and highlighted the need to establish the vector status of different flies.

The major problem in studying mechanically transmitted parasites such as *T. evansi* is the need to develop assays capable of detecting the minute amounts of parasite material likely to be present in the infected vector e.g. a 5 µl blood meal from a parasitemia of 10^6 trypanosomes /ml of blood would contain five trypanosomes – even less as residual blood on the mouthparts. The high sensitivity and specificity of DNA-based assays could overcome the problem of low trypanosome concentrations and contribute significantly to efforts aimed at monitoring and controlling trypanosomiasis by helping to evaluate the performance of different control strategies. Improved enzyme-based detection systems now enable DNA-probe methods to be used in less sophisticated laboratories (Majiwa et al. 1993). A wide range of DNA-based techniques have been used for trypanosome detection, principally polymerase chain reaction (PCR), random amplification of polymorphic DNA (RAPD), kDNA minicircle analysis, and hybridisation using repetitive DNA sequences. PCR and hybridisation both offer high specificity and sensitivity and are applicable to the large-scale analysis of trypanosome samples (Hide and Tait, 1991). However, there are considerable differences in the sensitivity claimed for these assays in the literature, dependent on reaction condition e.g. PCR amplification of *T. evansi* minicircle DNA is reported to be capable of detecting the DNA equivalent of five trypanosomes (Ol Diall et al., 1992; Artama et al., 1991) while amplification of a repetitive *T. evansi* specific DNA sequences is reported to detect the DNA equivalent of a single trypanosome (Panyim et al., 1992; Viseshakul and Panyim, 1990).

DNA probes have been used to detect and differentiate between trypanosome species including *T. brucei* (Hide et al., 1990; Mathieu-Daude et al., 1994;) *T. evansi* (Masiga and Gibson, 1990), *T. equiperdum* and *T. congolense* (Moser et al., 1989). Such probes have also been used to detect *T. brucei*, *T. congolense*, *T. simiae* and *T. vivax* parasite DNA in tsetse flies (Masiga et al., 1992; Kukla et al., 1987; McNamara and Snow, 1991; Gibson et al, 1988; Majiwa and Oteni, 1990).

As an initial step in our work we tested 10 sets of PCR primers reported in the literature as capable of detecting *T. evansi* DNA. We tested these primers using the conditions recommended by the authors as well as using different concentrations of primers, increased number of cycles and Taq DNA polymerase sources. All these assays were carried out using purified genomic DNA of *T. evansi*.

Master mixes excluding primers were prepared with per 50µl reaction:-

1 x PCR buffer; 2mM MgC12; 0.2mM dNTP's; 1.25 units Taq DNA polymrase. Primers at 2µM were added and the PCR carried out using a standard cycling protocol:- 1 min @ 94°C; 2 min @ 55°C; 2 min @ 72°C for 30 cycles then 5 min @ 72°C. The PCR products were visualized on 2% agarose stained with ethidium bromide.

Results from initial studies suggested that the TBR1/TBR2 primer set with PCR products with sizes of 177, 354 and 531 was capable of detecting 0.01 picogram of DNA (equivalent to ten times the amount of DNA expected in a single trypanosome).

We then addressed the problems of detecting *T. evansi* DNA in *T. evansi*-infected donkey blood: principally the inhibitory effects of hemoglobin in Taq. We examined this by spotting donkey blood containing different concentrations of *T. evansi* onto nylon membrane, nitrocellulose membrane and Whitman filter paper. After air-drying, the blood spots were eluted with distilled water for thirty minutes. The water was removed by centrifugation and replaced with a second aliquot. The samples were then heated to 100°C to elute the *T. evansi* DNA. Eluted samples were analyzed by the PCR using TBR1/TBR2 primers and the PCR products visualized by ethidium bromide staining after agarose gel electrophoresis. We then assessed this process for its ability to detect *T. evansi* DNA in *S. calcitrans* fed on *T. evansi*-infected donkey blood. In this study *S. calcitrans* were fed for two minutes on *T. evansi* -infected blood containing 10^6 trypanosomes/ml donkey blood. They were then dissected into mouthparts, crop and midgut which were homogenized in distilled water, spotted onto filter paper and double eluted and then analyzed by PCR for detecting *T. evansi* DNA. The method was confirmed to be capable of detecting the equivalent of a single trypanosome in an infected blood meal. The cost of each PCR reaction was estimated at $0.90.

Species or sub-group		Primer sequence	PCR product (bp)
T. brucei	TBR1	CGA ATG AAT ATT AAA CAA TAC GCA GT	177
	TBR2	AGA ACC ATT TAT TAG CTT TGT TGC	
	MG1	GAATATTAAACAATGCGCAG	164
	MG2	CCATTTATTAGCTTTGTTGC	
	GM1	ACA TAT CAA CAA CGA CAA AG	162
	GM2	CCC TAG TAT CTC CAA TGA AT	
T. evansi	ARTAM A1	CAA CGA CAA AGA GTC AGT	373
	ARTAM A2	ACG TGT TTT GTG TAT GGT	
T. evansi	DAUDE1	CGC CCA TAA GAT TTC CGG TT	930
	DAUDE2	GGT GTA ATA CTC ACC CGG TT	
T. evansi	PANYIM 1	AGT CAC ATG CAT TGG TGG CA	122
	PANYIM 2	GAG AAG GCG TTA CCC AAT CA	
T. evansi	SMILEY 1	CTC CTA GAA GCT TCG GTG TCC T	227
	SMILEY 2	TGC AGA CGA CCT GAC GCT ACT	
T. evansi	GEN046	CCG GCG TAT C	227
	IL0525	CGG ACG TCG C	287*
T. evansi	PRO1	CAC AAT GGC ACC TCG TTC CC	300-400
	PRO2	TTA GAA TGC GGC AAC GAG A	
T. vivax	TVW1	CTG AGT GCT CCA TAT GCC AC	150
	TVW2	CCA CCA GAA CAC CAA CCT GA	

We are currently applying this technique to field caught fly samples in Central Java.

District Species	Village	Fly	Samples for PCR		
			Collected	Tested	Positive
Batang	Wonosegoro	T. rubidus	9	9	0
		T. striatus	2	1	0
		S. exiujua	19	14	8
		M. domestica	1	1	0
Batang	Kedung Segog	T. rubidus	248	51	1
		T. striatus	9	9	0
		M. domestica	29	20	0
Pemalang	Surajija	T. rubidus	4	2	0
		T. striatus	2	1	0
		S. exigua	16	7	0
		M. domestica	27	16	0'
Pemalang	Kejambon	T. rubidus	67	24	7
		T. striatus	30	15	4
		S. exigua	55	14	4
		M. domestica	149	26	5
Pemalang	Banjarmulya	T. rubidus	56	40	15
		T. striatus	124	18	8
		S. exigua	31	15	0
		M. domestica	27	18	0
		Chrysops sp.	1	1	1

The work is scheduled to continue for two years in collaboration with RIVS, Bogor, Java.

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PCR FOR THE DIAGNOSIS OF TRYPANOSOMA SPECIES IN LIVESTOCK; SAMPLE PREPARATION TO INCREASE SENSITIVITY

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ABSTRACT

As the antigen-ELISA developed by Nantulya and Lindqvist (1989) was shown to have poor sensitivity and specificity, *T. vivax* DNA detection through polymerase chain reaction (PCR) using the oligonucleotides developed by Masiga et al. (1992) appeared to be an alternative for a species-specific diagnosis of *T. vivax* active infections in livestock. PCR is highly sensitive when processed on purified DNA from DNA extraction with ethanol/chloroform, but this technique is too expensive and time-consuming to be applied for diagnosis. First evaluation of the PCR directly applied on serum for the diagnosis of *T. vivax* in cattle indicated a low sensitivity of about 10^3 parasites/ml. Improvement in sample preparation should improve performance. Sensitivities of several low cost techniques were evaluated with pre-determined parasitemia. Twenty-two blood samples with known numbers of *T. vivax* /ml, ranging from one to 1,767 were prepared by dilution of infected sheep blood with blood from a non-infected sheep. Sensitivity of the PCR was evaluated in the following blood sample preparations: heparinized blood, plasma, lysed blood, buffy coat from hematocrit capillary tubes, pellet from plasma centrifugation, and DNA purified with a commercial ion exchange resin. Crude heparinized blood usually inhibited the PCR. Sensitivity of PCR with plasma and lysed blood was low, around 450 parasites/ml. PCR on buffy coat was more sensitive, but PCR products were sometimes hardly visible. Pellet of plasma centrifugation is an original, fast and economical preparation, which presented a high sensitivity: 100% of the samples were positive when the parasitemia was over 13 parasites/ml. DNA purification is slightly more time-consuming and expensive, since it proceeds from several manipulations and the use of a commercial kit, but it appeared to be the most sensitive technique among those investigated: 100% of the samples were positive when the parasitemia was above two *T. vivax*/ml. However PCR products are sometimes hardly interpreted because of a high background of fluorescence. Plasma pellet is recommended for the diagnosis of *T. vivax* by PCR with the appropriate oligonucleotides. These techniques should be evaluated for the other pathogenic trypanosomes of livestock.

RESUMÉ

Puisque les antigène-ELISA développés par Nantulya et Lindqvist (1990) se sont avéré peu sensibles et spécifiques, la détection de l'ADN de *T. vivax* par PCR à l'aide des oligonucléotides développés par Masiga *et al.* (1992) apparaît comme une alternative pour la détection des infections actives du bétail par *T. vivax*. La technique de PCR est très sensible quand elle est menée sur de l'ADN purifié par la méthode classique d'extraction éthanol/chloroforme, mais cette technique de préparation est longue et coûteuse, et son utilisation pour le diagnostic de routine n'est pas envisageable. Une première évaluation de la technique de PCR appliquée directement sur sérum a montré une sensibilité très faible, de l'ordre de 10^3 trypanosomes /ml. Une modification de la technique de préparation des échantillons devait permettre d'améliorer ces performances. La sensibilité de la PCR évaluée avec plusieurs techniques de préparation des échantillons sanguins, possédant un faible coût, a été réalisée sur 22 échantillons de sang, de parasitémie pré-déterminée, allant de 1 à 1767 trypanosomes/ml, préparés par dilution de sang de mouton infecté dans du sang de mouton non infecté. La sensibilité de la PCR a été évaluée avec les types d'échantillons suivants: Sang total hépariné, plasma, sang lysé à la saponine, buffy coat extrait de tubes à hématocrite, culot de centrifugation de plasma et ADN purifié sur résine échangeuse d'ion. Le sang total inhibe la réaction de PCR. La sensibilité de la PCR sur plasma et sang lysé est faible, environ 450 parasites/ml. La PCR sur buffy coat est plus sensible, mais le produit de l'amplification est parfois peu visible. Le culot de plasma est une préparation originale, rapide et économique qui présente une bonne sensibilité puisque 100% des échantillons sont positifs quand la parasitémie est supérieure à 13 *T. vivax*/ml. La purification de l'ADN est plus longure et coûteuse, puisqu'elle résulte de plusieurs manipulations et de l'utilisation d'une résine commerciale, mais c'est la technique la plus sensible: 100% des échantillons sont positifs quand la parasitémie est supérieure à 2 *T. vivax*/ml ; toutefois, les produits d'amplification sont parfois difficiles à interpréter du fait de la présence de nombreuses bandes de poids non spécifique et d'une bruit de fond élevé. Le culot de centrifugation de plasma est recommandé pour le diagnostic de

T. vivax par PCR avec les amorces TVW 1 et TVW2. Cette technique devra être évaluée avec d'autres espèce de trypanosomes pathogènes et d'autres oligonucléotides.

RESUMEN

Desde que la técnica del antígeno-ELISA (Ag-ELISA) fuera desarrollada por Nantulya y Lindqvist (1989) mostró tener una pobre sensibilidad y especifidad, La detección del ADN del *T. vivax* a través de la prueba de reacción en cadena de las polimerasas (PCR) usando los oligonucleótidos desarrollada por Masiga *et al.* (1992) mostró ser una alternativa para los diagnósticos especie-específicos del *T. vivax* en las infecciones activas en el ganado. El PCR es altamente sensible cuando se procesa con ADN purificado de la extracción de ADN con etanol/cloroformo, pero esta técnica es muy cara y toma mucho tiempo para ser utilizada para el diagnóstico. La primera evaluación del PCR directamente aplicado al suero para el diagnóstico del *T. vivax* en los bovinos indicó una baja sensibilidad de cerca 10^3 parásitos/ml. Mejoras en la preparación de las muestras deben dar mejor resultados. Fue evaluada la sensibilidad de varias técnicas de menor costo teniendo cada una de ellas una parasitemia pre-determinada. 22 muestras de sangre con un número conocido de *T. vivax*/ml, oscilando entre 1 a 1767, se prepararon por dilución de muestras de sangre de ovinos infectados mezcladas con muestras de sangre de ovinos no infectados. La sensibilidad del PCR se evalúo en las siguientes muestras de sangre preparadas: sangre heparinizada, plasma, sangre lisada, capa blanca proveniente de los tubos capilares para determinación de microhematocrits, pellets de plasma centrifugado, y ADN purificado con una resina de intercambio iónico comercial. La sangre completa heparinizada usualmente inhibía al PCR. La sensibilidad del PCR con plasma y sangre lisada fue baja, alrededor de 450 parásitos/ml. El PCR con la capa blanca fue mas sensitiva, pero los productos del PCR algunas veces eran difícilmente visibles. Los pellets de plasma centrifugado es una preparación original, rápida y económica, y presentó una alta sensibilidad: 100% de las muestras fueron positivas cuando la parasitemia estaba por encima de 13 parásitos/ml. La purificación del ADN es ligeramente mas costosa y toma mas tiempo, ya que su procedimiento incluye varias manipulaciones y el uso de un kit comercial, pero aparentemente es el mas sensible dentro de las técnicas que se han investigado: 100% de las muestras fueron positivas cuando la parasitemia estaba por encima de 2 *T. vivax*/ml. Sin embargo los productos del PCR algunas veces son difíciles de interpretar debido a la alta fluorescencia de fondo. Los pelletss de plasma se recomiendan para el diagnóstico del *T. vivax* por el PCR con los oligonucleótidos apropiados. Estas técnicas deben ser evaluadas con otros tyrpanosomas patógenos del ganado.

INTRODUCTION

Polymerase chain reaction (PCR) techniques for diagnosis of *Trypanosoma* species have recently been developed, particularly since several oligonucleotides specifically amplifying *Trypanosoma* species DNA sequences were described. Some are available for the parasites present in South America, *T. vivax, T. evansi* and *T. cruzi.* These techniques can be used for diagnosis in both host and vector.

Theoretically, PCR is able to detect a single DNA strand, then a single parasite (Panyim et al., 1993, with *T. evansi),* or even less when the sequence involved is a repetitive sequence, as it is generally used. The sensitivity of the PCR for diagnosis is potentially very high, but when using biological samples, DNA purification is required previous to the reaction for a satisfactory sensitivity, due to inhibition of the reaction by the host or vector components, and poor availability of DNA in biological samples. PCR is highly sensitive when processed on purified DNA from DNA extraction with ethanol/chloroform, but this technique is too expensive and time-consuming to be applied for diagnosis. Preparation of the samples for PCR should then be improved. On the other hand, clearance of the parasite DNA from the host blood needs to be evaluated to establish whether a positive PCR means active infection.

The sub-genus-specificity of the reagents published by Masiga et al., (1992), based on Moser (1989a) for *T. vivax* (TVW1 and 2) and *T. brucei* (TBR1 and 2), and those published by Moser et al. (1989b) for *T. cruzi* (TCZ1 and 2) has so far been 100%. PCR was thus considered a potentially very useful technique for the diagnosis of trypanosomiasis in mammals and for epidemiological studies in vectors of the parasites.

I   GENERALITIES ON PCR

PCR allows for the detection of a totally or partially known DNA sequence. The technique is based on the use of Taq DNA polymerase, extracted from *Thermophilus aquaticus,* a thermostable enzyme which allows polymerization of desoxyribonucleic phosphates acids (dNTP) by duplication of the DNA matrix.

Three steps are processed successively:-

i)   Denaturation of the DNA matrix, at a high temperature (94°C): the double DNA strand is opened and two strands become available for the next step;

ii) Annealing of the oligonucleotides and the matrical strands at 50-60°C: the double DNA strand is opened and two strands become available for the next step;

iii) Extension at the optimal polymerization temperature, around 72°C.

Thirty cycles are generally required to synthesize a substantial amount of a specific molecular weight product which can then be detected on an electrophoresis gel f agarose, with the addition of ethidium bromide for fluorescent observation under UV lamp. The molecular weight of the PCR product is generally sufficient to affirm that a specific reaction occurred; however, confirmation of the specificity of the amplified sequence can be achieved by total or partial DNA probing.

Since both reactions make the technique more complicated, it is not adapted to large scale diagnosis. The classical technique of DNA purification through ethanolchloroform extraction is also technically complicated and time-consuming, therefore it is rarely used for diagnosis, and especially for large scale diagnosis in epidemiological surveys.

Although potentially highly sensitive and specific, the PCR technique required simplification of the procedure for routine diagnosis or for application to large scale surveys. PCR was successfully applied by Majiwa et al. (1994) to detect the presence of trypanosomes in tsetse flies and in the buffy coat of hematocrit tubes from centrifuged cattle blood. However, sensitivity has never been evaluated under experimental conditions.

II   PRELIMINARY STUDIES ON CATTLE AND SHEEP SERA

Detection of *T. vivax* with TVW1 and TVW2 in cattle
Four Boran calves were experimentally infected with an isolate of *T. vivax* from French Guiana (IL4007) and bled almost daily for 51 days. HCT (Woo, 1970), BCM (Murray, 1979), Ag-ELISA (Nantulya and Lindqvist, 1989) and PCR (Masiga et al., 1989) were processed. PCR analysis was processed as described by Masiga et al., 1989) except that the reactional volume was 20µl and the serum sample volume was 1µl. PCR was positive when more than 5 - 10 parasites could be observed in the capillary tube, indicating a sensitivity of about 500-1200 parasites/ml (Desquesnes and Tresse, 1996). Two calves were treated with diminazene aceturate to evaluate the persistence of DNA in the circulating blood. In one case the PCR was negative the day after the treatment; in the other it remained positive 24 hours after treatment. It can therefore be considered that a positive result to PCR is synonymous with active infection.

Detection of *T. evansi* in sheep with TBA1 & TBR2, and oligonucleotides for *T. evansi* described by Diall (1993)
A similar study was carried out in two sheep experimentally infected with a Venezuelan isolate of *T. evansi* (TEVA1). PCR analysis was conducted as described by the authors. TBR oligonucleotides give positive results when parasitemia was above 10^3 parasites/ml. The specific oligonucleotides for *T. evansi* gave positive responses when parasitemia was above 10^4-10^5, indicating that TBR oligonucleotides would probably be more sensitive. In Latin America, the use of TBR oligonucleotides is recommended, but, in areas where *T. equiperdum* could also be present, specific oligonucleotides should be used to differentiate *T. evansi* from *T. equiperdum* infections.

Note: Diagnosis can also be performed from the material collected on a blood smear (even stained); it was processed to confirm an infection by T. evansi in a dog from Suriname (Bansse, 1996).

III   PREPARATION OF THE SAMPLES FOR IMPROVEMENT OF THE SENSITIVITY

Materials and methods
To accurately evaluate the sensitivity of the PCR for detection of *T. vivax* in sheep blood samples, blood from an

experimentally infected sheep was diluted with the blood of a non-infected sheep. Twenty-two samples were prepared, ranging from 1,767 to 1 parasite/ml. The enumeration of the parasites in blood and the further dilutions were carried out as previously described for the evaluation of the sensitivity of the Woo test (Desquesnes and Tresse, 1996).

Six types of preparations were evaluated in PCR, under the same reactive conditions.

i)   crude heparinized blood;

ii) plasma from low speed blood centrifugation (1200 rpm; 304g for 5 minutes);

iii) lysed blood using the technique described by Diall (1993) (saponin lysis);

iv) buffy coat from hematocrit capillary tubes of 70µl (Woo, 1970; Majiwa *et al.*, 1994);

v)   pellet from high speed centrifugation (14,000 rpm; 17,530g for 10 minutes) of 0.5ml of plasma from low speed centrifugation (1,200 rpm; 304g for 5 minutes);

vi) DNA purified from 0.8ml of heparinized blood, with a commercial ion exchange resin ("ReadyAmp Genomic DNA Purification System", Promega®), as described by Penchenier *et al.* (1996, in press).

Results and discussion:
Results of the PCR are indicated in Table 1. As expected (Higuchi, 1989), crude heparinized blood usually inhibited the PCR. Sensitivity of PCR with plasma and lysed blood was low, around 450 parasites/ml. PCR on buffy coat was more sensitive, but PCR products were sometimes hardly visible. Plasma pellets demonstrated a high sensitivity: 100% of the samples were positive when the parasitemia was over 13 parasites/ml. The PCR product was always clearly visible, and there was a total absence of fluorescent background. DNA purification was slightly more time-consuming and expensive, as it resulted from several manipulations and the use of a commercial kit, but it appeared to be the most sensitive technique of those investigated. All of the samples were positive when the parasitemia was over two *T. vivax* /ml; however, PCR products were sometimes difficult to interpret because of a highly fluorescent background. Costs and durations of the six preparations under study are indicated in Table 2.

Table 1: PCR Results with several types of blood preparations and rates of parasitemia

Parasitemia trypanosomes/ml*	Type of preparation of the blood samples					
	In Plasma	Plasma pellet	Lysed blood	Heparinized blood	Buffy coat	Purified DNA
1767	3	3	3	0	3	3
1325	2	3	3	0	3	3
883	3	3	3	0	3	3
707	3	3	3	0	3	3
530	3	3	3	0	3	3
442	3	3	3	0	3	3
353	0	3	0	0	3	3
265	1	3	2	0	3	3
177	1	3	0	1	3	3
88	2	3	1	0	3	3
71	1	3	2	2	3	3
57	2	3	1	2	3	3
44	1	3	2	0	3	3
35	2	3	1	0	2	3
26	0	2	0	0	1	3
18	1	3	0	0	2	3
13	1	3	0	0	0	3
9	2	3	0	0	2	3
7	0	2	0	0	0	3
4	0	1	0	0	0	3
2	0	1	0	0	0	3
1	0	0	0	0	0	0
Total positives	31	57	27	5	46	63
Percentage of positives	47%	86%	41%	8%	70%	95%

*Numbers of trypanosomes/ml are indicated with an uncertainty of 13%

Table 2: Sensitivity of the PCR with several types of blood preparation, cost (in US$) and time of preparation

Sensitivity level:		Plasma	Plasma pellet	Lysed blood	Buffy coat	Purified DNA
442	+/- 60	94%	100%	100%	100%	100%
44	+/- 6	64%	100%	67%	100%	100%
9	+/- 1,3	57%	98%	50%	85%	100%
2	+/- 0,3	49%	90%	43%	73%	100%
Cost of the preparation		0.15	0.18	0.37	0.35	1.27
Cost of the PCR		0.6	0.6	0.6	0.6	0.6
Total cost of the diagnosis		0.78	0.78	0.97	0.95	1.87
Preparation time		1 min.	3 min.	6 min.	3 min.	4 min.
Total incubation time:		10 min.	25 min.	75 min.	5 min.	44 min.

Results with the DNA purification method were very similar to those of Penchenier et al. 1996, in press) who found PCR to be highly sensitive to detection of *T. brucei* in humans at levels down to one parasite/ml of blood.

IV   CONCLUSION

Plasma pellets are recommended for sensitive diagnosis of active infections of livestock with *T. vivax* through PCR with the appropriate oligonucleotides. This technique should be evaluated for the other pathogenic trypanosomes of livestock. It is speculated that the plasma pellet would be adapted to diagnosis of *T. evansi* infections, but not *T. congolense* infections due to the small size of the parasite and the absence of an undulating membrane which will not cause a satisfactory separation of blood cells and parasites during the first step of low speed centrifugation.

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RESISTANCE TO DIMINAZENE ACETURATE AND ISOMETAMIDIUM CHLORIDE IN SOME SOUTH AMERICAN T. VIVAX AND T. EVANSI; CONSEQUENCES OF TREATMENT AND CHEMOPROPHYLAXIS

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ABSTRACT

Little information is available on the sensitivity to trypanocides of Latin American isolates of livestock trypanosomes. Most of the information is derived from field observations. The trypanocides most commonly used in Latin America are diminazene aceturate and isometamidium chloride; although homidium has also been used, its use should be avoided because of its carcinogenic and mutagenic activity. Resistance to diminazine aceturate and isometamidium chloride was evaluated in sheep and calves for three isolates of T. vivax from French Guiana, one from Venezuela, and one isolate of T. evansi from Venezuela. Naïve animals were selected and infected intravenously with 10^4-10^5 parasites, kept under fly-proof conditions, and bled daily for parasitological (Woo test) and serological (indirect-ELISA) examinations. Treatments were applied intramuscularly when parasitemia reached 10^7 for T. vivax infections, and 10^4 for T. evansi infections. Whichever treatment was applied, parasites were no longer detectable the day after the treatment. All the isolates were resistant to diminazene at the dose of 7mg/kg except one isolate from French Guiana; relapses were recorded 12 to150 days after treatment, either spontaneously, or after transport or nutritional stress. Sheep were shown to be potentially healthy carriers of T. vivax and T. evansi. Resistance was not recorded for the treatment with 0.5 mg/kg isometamidium chloride in T. vivax infected sheep; duration of the protection was evaluated in some of the animals by re-inoculation of the same isolates after every 15 days; the animals were protected for more than 4.5 months. Although T. evansi infection could not be ascertained either by direct examination, or by mouse inoculation, the antibody level as measured by indirect ELISA T. evansi had not decreased four months after the treatment; suggesting that this isolate of T. evansi is resistant to isometamidium chloride. Utilization of these drugs in relation to medical or epidemiological strategy is discussed. The use of diminazene aceturate and isometamidium chloride in enzootic and epizootic, monospecies or multispecies situations is discussed, and is contrary to the strategy generally applied in Africa. The role of ruminants and dogs as reservoirs of T. evansi, and of horses for T. vivax is reviewed.

RESUMÉ

Peu d'informations objectives sont disponibles sur la sensibilité aux trypanocides des souches sud-américaines de trypanosomes du bétail. La plupart des informations reposent sur des observations de terrain. Les trypanocides les plus utilisés en Amérique du Sud sont l'acéturate de diminazène et le chlorure d'isométamidium; bien que l'homidium soit également été utilisé, il serait préférable d'éviter sont usage étant donné ses propriétés mutagènes et cancérogène. La résistance à l'acéturate de diminazène et au chlorure d'isométamidium de trois isolats de T. vivax de Guyane Française, d'un isolat de T. vivax du Venezuela et d'un isolat de T. evansi du Venezuela a été évaluée chez des moutons et des veaux expérimentalement infectés. Les animaux indemnes de trypanosomose ont été infectés par voie intraveineuse avec 10^4-10^5 parasites, élevés en étable imperméable aux insectes, et prélevés quotidiennement pour les examens parasitologiques (test de Woo) et sérologiques (ELISA-indirecte). Les traitement ont été appliqués par voie intramusculaire quand la parasitémie atteignait 10^7 pour les infections à T. vivax et 10^4 pour les infections à T. evansi. Quel que soit le traitement appliqué les parasites ont disparu de la circulation le lendemain des traitements. Tous les isolats testés (T. vivax et T. evansi) ont été résistants à l'acéturate de diminazène à la dose de 7mg/kg, à l'exception d'un isolat de T. vivax de Guyane Française; la résurgence des parasites ont été enregistrée entre 12 et 150 jours après les traitements, soit spontanément, soit à la suite de stress de transport ou de restrictions alimentaires. Les moutons se sont avérés capables d'être infectés par T. vivax et T. evansi en l'absence de symptômes. Aucune résistance au chlorure d'isométamidium n'a été enregistrée chez les moutons infectés par T. vivax; chez certains animaux la durée de protection a été évaluée par réinoculation des parasites tous les 15 jours à la suite du traitement; elle a toujours été supérieure à 4,5 mois. Chez les moutons infectés par T. evansi, bien que la persistance de l'infection n'ait pu être démontrée, par examen direct et inoculation aux souris, la persistance des anticorps plus de quatre mois après le traitement suggère que cette souche de T. evansi est résistante au chlorure d'isométamidium. L'utilisation de ces trypanocides est présentée selon les stratégies médicale et épidémiologique.

L'utilisation de l'acéturate de diminazène et du chlorure d'isométamidium dans les secteurs enzootiques et épizootiques, et selon que les infections sont monospécifiques ou multispécifiques est discutée, et opposée aux stratégies généralement recommandées en Afrique. Les rôles de réservoir des ruminants et des chiens pour *T. evansi*, et celui éventuel du cheval pour *T. vivax* sont rappelés.

RESUMEN

Se dispone de poca información sobre la sensibilidad a las tripanocidas en los aislamientos de tripanosomas en ganado de América Latina. La mayor parte de la información se deriva de observaciones de campo. Las tripanocidas más comúnmente usadas en América Latina son aceturate de diminazene y cloruro de isometamidium; a pesar de que el homidium ha sido usado también, su uso debería evitarse debido a su actividad carcinogénica y mutógena. La resistencia al aceturate de diminazene y cloruro de isometamidium fue evaluada en ovejas y terneras en tres aislamientos de *T. vivax* en la Guyana Francesa, una en Venezuela; y otro aislamiento de *T. evansi* en Venezuela. Se seleccionaron unos animales que fueron infectados intravenosamente con 10^4 - 10^5 parásitos, guardados bajo condiciones a prueba de moscas, sangrados diariamente para análisis parasitológico (la prueba Woo) y serológico (ELISA indirecto). Los tratamientos se aplicaron intramuscularmente cuando la parasitemia alcanzaba 10^7 para infecciones de *T. vivax*, y 10^4 para infecciones de *T. evansi*. Independientemente del tratamiento aplicado no se detectaron parásitos el día después del tratamiento. Todos los aislamientos eran resistentes al Diminazene en una dosis de 7 mg/kg excepto un aislamiento en la Guyana Francesa; las recaídas se observaron de 12 a 150 días después de los tratamientos, o espontáneamente o después del agotamiento del transporte o dieta. Las ovejas se mostraron potencialmente portadoras sanas de *T. vivax* y *T. evansi*. No se observó la resistencia para el tratamiento con 0.5 mg/kg de cloruro de isometamidium en ovejas infectadas de *T. vivax*; se evaluó la duración de la protección en algunos de los animales a través de la vacunación de los mismos aislamientos después de cada quince días; los animales quedaron protegidos por más de 4.5 meses. Aunque no se puede establecer la presencia de las infecciones *T. evansi* por observación directa, o por vacunación de ratones, el nivel de anticuerpos encontrados en ELISA indirecto *T. evansi* no había disminuido cuatro veces después del tratamiento; sugiriendo que este aislamiento de *T. evansi* es resistente al cloruro de isometadmidium. Se discute la utilización de estos medicamentos en relación a la estrategia médico o epidemiológica. El uso de aceturate de diminazene y cloruro de isometamidium en situaciones enzóticas y esotéricas, monoespecíficas o multiespecíficas se analiza y se contrapone a la estrategia generalmente adoptada en Africa. El papel de rumiantes y perros como depósitos de *T. evansi* y de caballos para *T. vivax* se hace notar.

INTRODUCTION

Little information is available on the sensitivity to trypanocides of Latin American isolates of livestock trypanosomes. Most of the information is derived from field observations. However, resistance of *T. vivax* to diminazene aceturate in cattle, at a dose of 3.5mg/kg has been recorded in Colombia by Hull (1971) and Betancourt (1978). The authors suggested that a dose of 7 mg/kg could control the parasite but evaluation under laboratory conditions was not performed. Resistance of *T. evansi* to diminazene aceturate in horses was described in Argentina by Mancebo and Monzon (1986).

The trypanocides most commonly used in Latin America are diminazene aceturate and isometamidium chloride. Although homidium has also been used, its use should be avoided because of its carcinogenic and mutagenic activity (MacGregor and Johnson, 1977; Probst et al. 1981).

This paper describes the efficacy of diminazene aceturate and isometamidium chloride in sheep and/or calves experimentally infected with South American isolates of *T. evansi* and *T. vivax*.

MATERIALS

The study was carried out with four isolates of *T. vivax* and one of *T. evansi*. The isolates were:-

    a)   TVFG1, isolated in French Guiana from a Zebu by Lancelot (1988) during the epizootic of 1988-1989;

    b)   TVFG2, isolated in French Guiana from a Zebu by Desquesnes and Demarty (unpublished) during the

epizootic of 1994-1995;

c) TVFG3, isolated in French Guiana by Desquesnes, Tresse and Garrain (unpublished) during a post-epizootic period in 1996;

d) TVVG1, isolated from a Zebu in Venezuela, in the State of Guarica, and transferred by courier under dry ice protection to our laboratory by Dr. E. Espinoza, from the Universidad Nacional Experimental Simon Rodriguez, in Caracas, Venezuela;

e) TEVA1, isolated from a horse in Venezuela, in the State of Apure, and transferred to our laboratory by Dr. E. Espinoza.

Sheep and calves naïve to *Trypanosoma* species were used and kept under fly-proof conditions.

METHODS

For each isolate, two sheep were experimentally infected intravenously with 10^4-10^5 parasites, and bled almost daily for parasitological and serological examination. Two Boran calves were also inoculated with TVFG1 (IL4007). When parasitemia reached about 10^7 for *T. vivax* infections, and about 10^4 for *T. evansi* infections, animals were treated with either diminazene aceturate (Beronal®), given intramuscularly, at doses of 3.5mg/kg, 7mg/kg or 10.5mg/kg, or, later during the course of the infection, with isometamidium chloride (Trypamidium® in sheep and Samorin® in cattle), given intramuscularly, at a dose of 0.5mg/kg.

Animals were bled every day for two to four months post treatment. Parasitological examination through the HCT and serological examination through indirect-ELISA *T. vivax* or indirect-ELISA *T. evansi* were carried out to monitor either a relapse of parasitemia, or to measure the decreasing level of antibodies, in the case of curative treatment.

In some cases treated with isometamidium chloride, if parasites were not observed after one month, sheep were re-inoculated with the same isolates every 15 days to evaluate the duration of the protection.

RESULTS

All *T. evansi* and *T. vivax* isolates except TVFG3 (Figure 4) were resistant to diminazene aceturate at the dose of 7 mg/kg (Figures 1-3 and 5-9). With *T. vivax* TVFG3, IgG level decreased below the cut-off line three months after treatment. TVFG3 has been recently isolated and only one animal was injected; further experiments are necessary to confirm the sensitivity of this isolate to diminazene aceturate.

The TVFG1 was tested at a higher dose of 10.5/kg, and was shown to be resistant (Figure 1). In all cases, following treatment, trypanosomes disappeared from the circulating blood the day after the treatment, indicating most of the parasites were killed, but, in the case of resistance, parasites re-appeared between 12 and 150 days later. When the animals remained aparasitemic for a long period, relapses of the parasitemia could be induced by transport stress (Figure 1) or nutritional stress (Figures 2 and 6). Specific antibody levels remained high in cases of resistance (Figures 1-2, 5-6 and 8-9).

All the *T. vivax* isolates were sensitive to isometamidium chloride (Figures 3 and 7), in sheep and calves. The duration of protection was evaluated in sheep, and was greater than four and a half months for TVFG1 (Figure 7) and TVVG1, and greater than five months for TVFG2. In animals that were not re-inoculated, the decrease of specific IgG was observed by indirect-ELISA, and results fell below the cut-off line between two and three and a half months after treatment (Figure 3).

T. evansi was resistant to diminazene aceturate at 7 mg/kg, but the parasite could not be observed after treatment with isometamidium chloride, even after a nutritional stress period of 60 days, but the level of specific IgG remained high for more than three and a half months, when the experiment had to be discontinued for practical reasons (Figures 8-9). However, prior to the end of the experiment, intraperitoneal inoculation of 0.3ml of blood from each sheep into two mice did not confirm that the animals were still infected. It is therefore suspected that this isolate of

T. evansi is resistant to isometamidium chloride in sheep, but it could not be established whether the animals would ever be infected again.

Results of these studies are summarized in Table 1.
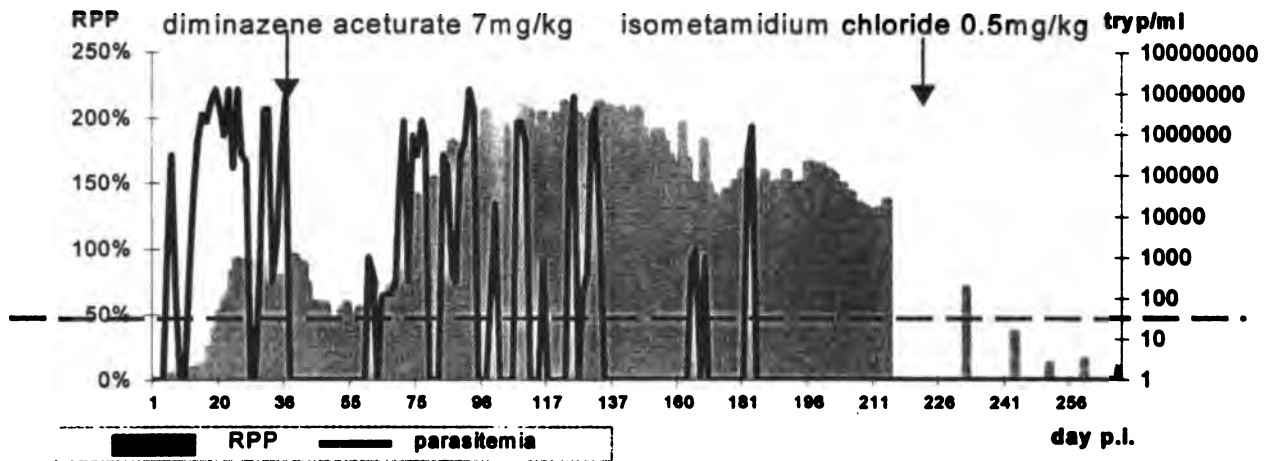
Figure 1:   Sheep 1 infected with TVFG1, treatments and transport stress, parasitemia and RPP in indirect-ELISA *T. vivax*
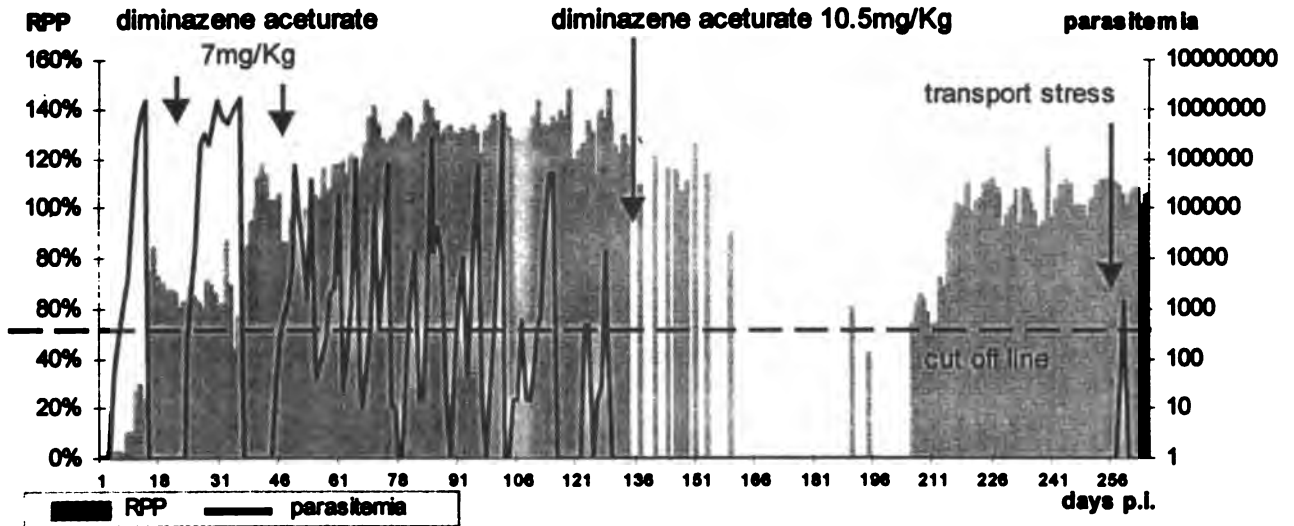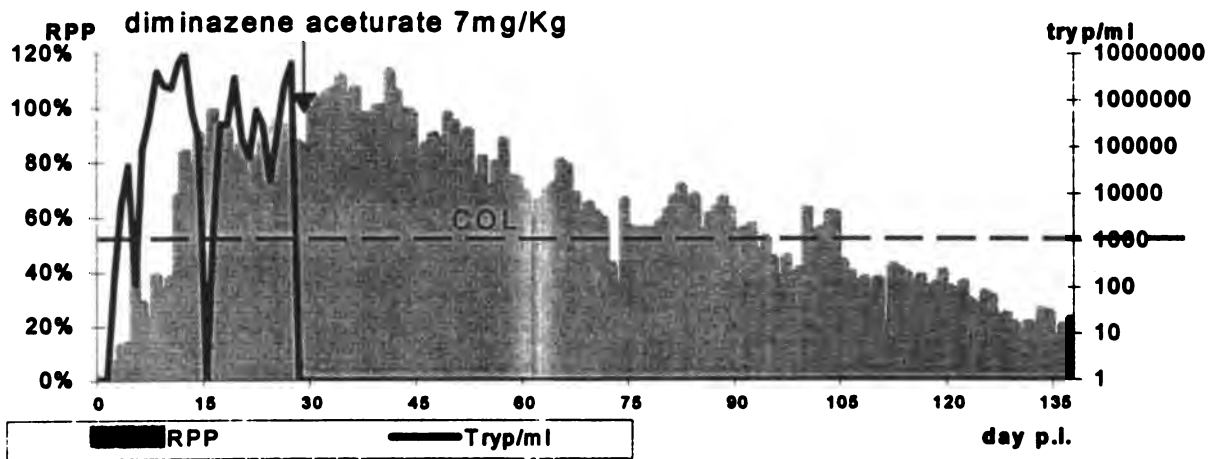

Figure 2:   Sheep 2 infected with TVFG2, treatment and diet, parasitemia and RPP in indirect-ELISA T. vivax
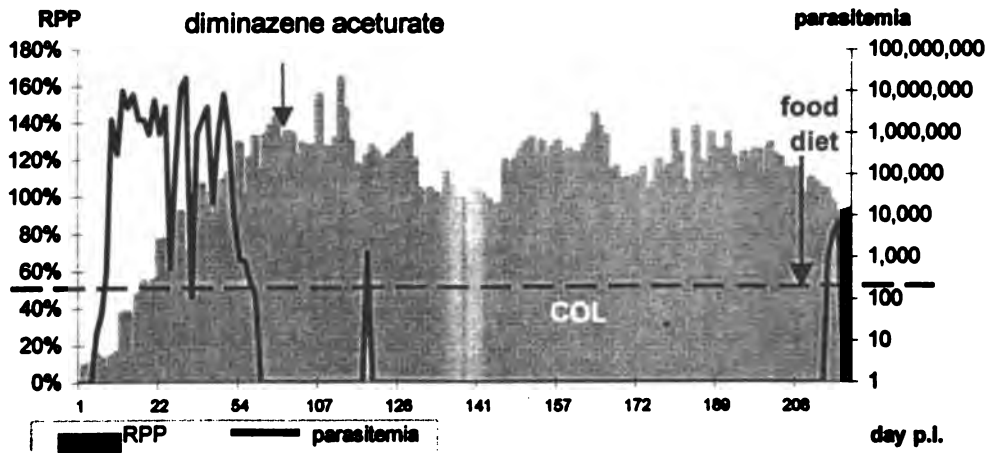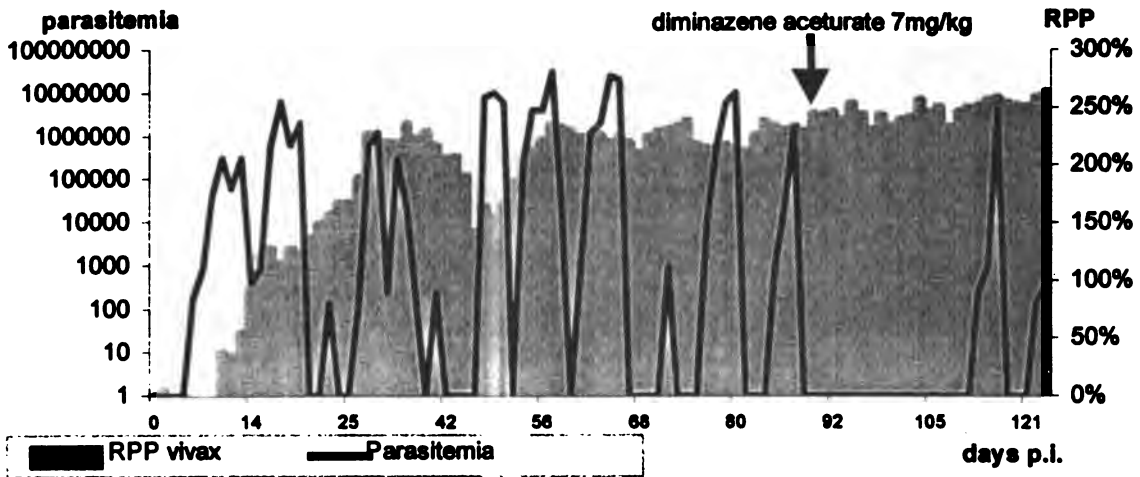
Figure 3: Sheep 3 infected with TVFG2, treatments, parasitemia and RPP in indirect-ELISA T. vivax
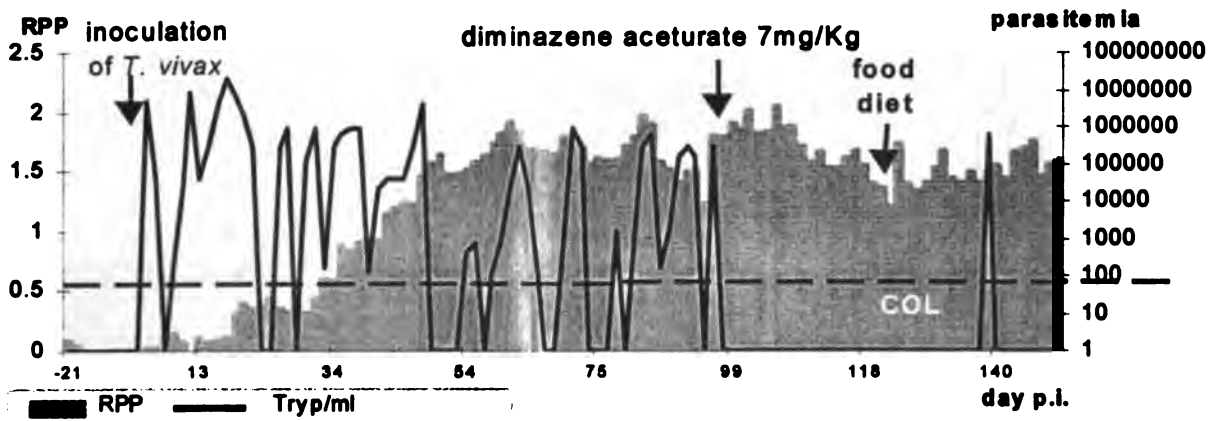

Figure 4: Sheep 15 infected with TVFG3, treatments, parasitemia and RPP in indirect-ELISA *T. vivax*
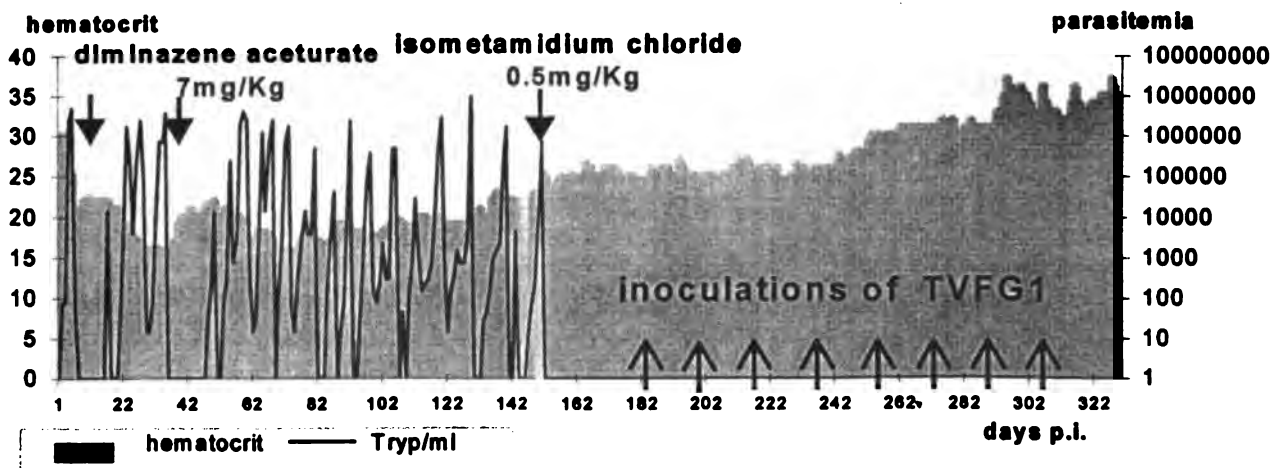
Figure 5: Sheep 12 infected with TVVG1, treatment, parasitemia and RPP in indirect-ELISA *T. vivax*
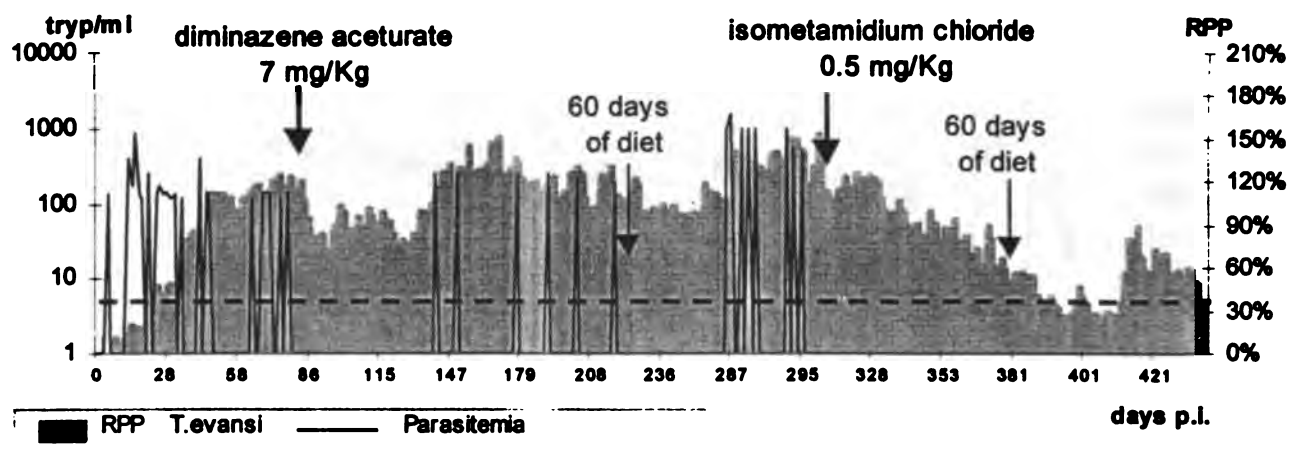

Figure 6: Sheep 13 infected with TVVG1, treatment, parasitemia and RPP in indirect-ELISA *T. vivax*
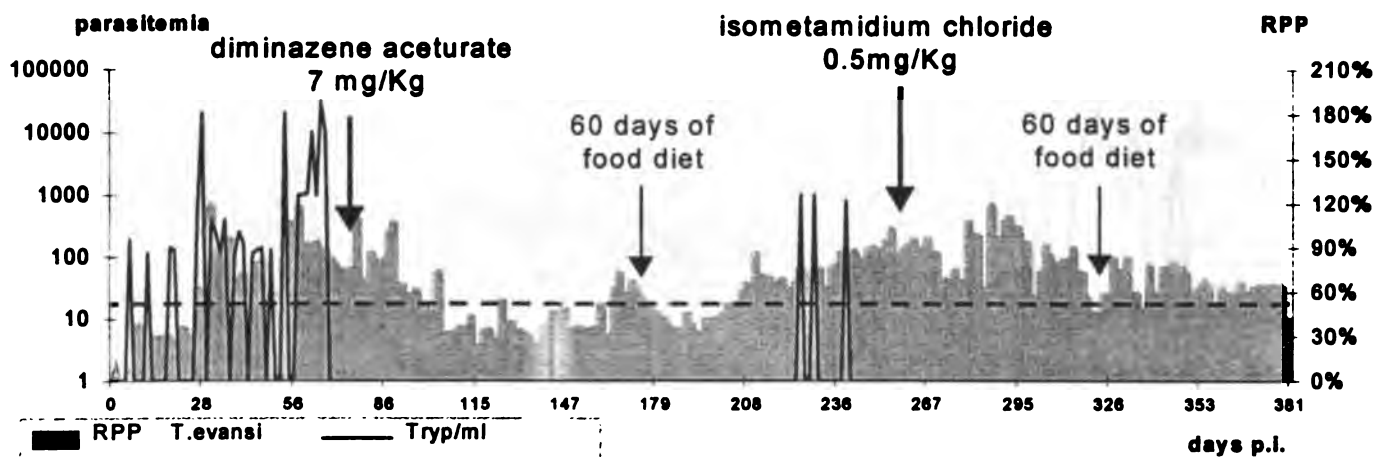

Figure 7: Resistance to diminazene aceturate and duration of the protection with isometamidium chloride against TVFG1: haematocrit and parasitemia

Figure 8: Sheep infected with TEVA1, treatment, parasitemia and RPP in indirect-ELISA *T. evansi*

Figure 9: Sheep infected with TEVA1, treatment, parasitemia and RPP in indirect-ELISA *T. evansi*

Table 1: Resistance of several isolates of *T.* vivax and *T.* evansi from South America to treatments with diminazene aceturate and isometamidium chloride

Name of the isolate (host species)	diminazene aceturate 3.5mg/Kg	diminazene aceturate 7mg/Kg	diminazene aceturate 10.5mg/Kg	isometamidium chloride 0.5mg/Kg	duration of protection
TVFG1 (sheep)	resistant	resistant	resistant	*sensitive*	> 4.5 months
TVFG1 (calves)	not tested	resistant	not tested	*sensitive*	not determined
TVFG2 (sheep)	not tested	resistant	not tested	*sensitive*	> 5 months
TVFG3 (sheep)	not tested	*sensitive*	not tested	not tested	not determined
TVVG1 (sheep)	not tested	resistant	not tested	*sensitive*	> 4.5 months
TEVA1 (sheep)	not tested	resistant	not tested	resistant ?	not determined

DISCUSSION

In French Guiana, according to the farmers, and in the laboratory with the isolates tested, the treatment is effective,

in the sense that it abrogates clinical signs. However an asymptomatic carrier state follows treatment, and could be the cause of a relapse several months or even several years later. This cannot be appreciated by the farmers, and could hardly be demonstrated in the field; in such cases, laboratory evaluation is very helpful.

The mechanism which induces apparent resistance of the parasites to diminazene aceturate was not investigated. A number of hypotheses may explain this apparent resistance:

- the existence of extra-vascular foci of the parasites which could escape the effect of the drug, for example in the nervous system, as was shown in buffaloes with *T. evansi*, and in goats with *T. vivax* (Whitelaw et al. 1988); indeed, bioavailability of diminazene aceturate is only 23% of the plasma rate in the cerebrospinal fluid and 32% in the lymph (Peregrine et al. 1992);

- cross resistance induced by the use of imidocarb, a babesiocide, which is not frequently used in most countries, including French Guiana, due to its very high price;

- innate resistance (Desquesnes *et al.* 1995)

Whatever the mechanism of resistance, it is a fact that treatments with diminazene aceturate do not sterilize the animals; the treatment could even produce the asymptomatic carriers since it enhances the immune system of the host, but does not kill all the parasites. Under laboratory conditions, the use of diminazene aceturate has always or almost always led to aparasitemic and asymptomatic infection. This finding suggests that such mechanisms could occur in the field. Diminazene aceturate could then be recommended in enzootic areas when the aim is to enhance the immune system of the domestic hosts. However, if the aim is eradication of the parasite from the area, the use of isometamidium chloride is recommended.

With respect to *T. evansi*, none of the trypanocides tested could apparently sterilize the infected sheep; suggesting that ruminants may act as a reservoir for *T. evansi*.

Finally, it is important to emphasize the fact that studies on the effect of a trypanocide must be carried out for at least three to four months before drawing any conclusions, since the aparasitemic carrier state can last for long periods; moreover, observations should always associate HCT and indirect-ELISA.

CONSEQUENCES OF TREATMENT AND CHEMOPROPHYLAXIS

In the control of trypanosomiasis, therapeutic and epidemiological strategies are different. Therapeutic strategy aims to control or prevent pathogenic effects of parasites in a sensitive host, and epidemiological strategy aims to eradicate the parasite population, and/or to avoid the creation of a reservoir.

THERAPEUTIC STRATEGY

Due to the high cost, the use of trypanocides requires therapeutic and/or economical justification, and is only applied in associations of host/parasite with highly pathogenic effects. On the other hand, the choice of a trypanocide is also determined by tolerance of the drug in the infected animals. For these reasons, isometamidium chloride is most commonly employed in cattle for the control of *T. vivax*, and suramin in horses for the control of *T. evansi*.

Control of *T. vivax* in Cattle

Enzootic areas: In some areas of Africa, the vector pressure of tsetse flies is very high throughout the year. In addition, there is wide genetic and antigenic diversity of the parasites linked to the cyclical development in tsetse flies, and to a wide and multi-host species reservoir in domestic and wild hosts. In enzootic areas, livestock are exposed to numerous and varied inoculums. These multiple species infections prevent the cattle from controlling infection by the immune system. The consequence is that, in enzootic areas, chemoprophylaxis is required and must be applied continuously to protect the animals from the disease. In Africa, enzootic stability and permanent chemoprophylaxis are therefore associated. Isometamidium chloride is the most suitable chemical for such a

situation. Unfortunately, in some areas, the use of this chemical leads to high levels of resistance, and the duration of the protection decreased to one month at 1 mg/kg (Morzaria, personal communication).

The situation is very different in Latin America. In enzootic areas, vector pressure can be seasonal, or even permanently very high, but mechanical transmission of the parasites does not create genetic and antigenic variations. Moreover, except for some deer found infected, wild reservoirs of *T. vivax* have not been clearly identified. The genetic and antigenic diversity of the Latin American parasite is thus most probably very limited due to mechanical transmission between cattle and sheep. Hosts are then exposed to multiple infections, although infections are usually monospecific. In such conditions, immunization of the hosts seems to become established naturally and gradually. The immunity can be enhanced by the use of trypanocide treatments, especially if treatment does not result in sterilization. Later, complete control of the parasites can be acquired for long periods. Relapses are due to stress or insufficient water or food supply.

In Latin America enzootic stability and occasional treatments with a non-sterilizing drug should therefore be associated. Diminazene aceturate (DA) is the most suitable chemical for such a situation in *T. vivax* DA-resistant areas.

Sporadic or Epizootic Areas: In Africa, when trypanosomiasis is sporadic or epizootic, it is generally due to a temporary presence of the cyclical vector which has brought the parasite into a non-infected area. Chemotherapy is usually recommended to get rid of these seasonal infections, which mostly occur during the wet season, therefore in Africa, sporadic and epizootic situations are usually associated with chemotherapy. DA is the most suitable chemical for such a situation in trypanosomes DA-sensitive areas.

In Latin America, when trypanosomiasis is sporadic or epizootic, it is generally due to the introduction of infected cattle into a non-infected area or herd. The infected animals then constitute a potentially permanent source of infection for biting insects and consequently, for the rest of the herd. The only way to prevent the spread of the infection is to sterilize the infected animals, which involves treating all the animals exposed; and, because it is practically impossible to treat all the animals in a particular area in one day, it is preferable to put the rest of the population under protection in the form of chemoprophylaxis. In Latin America, sporadic and epizootic situations should therefore be associated with the use of chemoprophylactic drugs to prevent the spread of the disease. Isometamidium chloride is the most suitable chemical for such a situation.

Control of *T. evansi* in Horses

The epidemiology of *T. evansi* in Africa and Latin America is not very different, featuring mechanical transmission and very broad domestic and wild reservoirs. In the New World, *T. evansi* is transmitted by biting insects as well as vampire bats; however, the disease mostly appears as epizootic. Hoare (1972) suggested that vampire bats are a reservoir causing outbreaks further spread by tabanids. For practical reasons, it is impossible to treat all the animals exposed to the infection, so chemoprophylactic drugs are more suitable to avoid the spread of the infection. Since isometamidium chloride does not seem to be effective, other chemicals should be evaluated with the local strains of *T. evansi* before any chemical is recommended. Suramin, quinapyramine and melarsamine could also be evaluated.

EPIDEMIOLOGICAL STRATEGY

For the control of a parasite population, control measures must take into consideration not only with respect to the individual protection of a herd or an area, but also the eventual creation of a reservoir in domestic and/or wild population, especially when the objective is eradication.

Eradication of *T. vivax*

Whether T. *vivax* can infect horses (and dogs), and whether these animals could consequently act as a reservoir have never been determined in Latin America. Detailed studies are hampered by lack of specific and sensitive diagnostic tools. To date, except for deer, which are not abundant everywhere, a significant wild reservoir of *T. vivax* has not been identified in Latin America.

Since mechanical vectors can only transmit the parasites for short distances (200-300m), the strategy for *T. vivax* control can almost be defined for each herd, provided there is limited space. This strategy does not apply to very large extensive breeding areas, but in confined or delimited breeding areas, where eradication of *T. vivax* by systematic treatment of all ruminants with isometamidium chloride is feasible. This treatment must also be applied to all animals prior to their introduction on the farm.

In areas enzootic for *T. vivax* and *T. evansi*, the chemicals used to control of *T. vivax* in ruminants are not effective against *T. evansi*, and ruminants can therefore become reservoirs of *T. evansi*. Since *T. evansi* possesses a large wildlife reservoir, the domestic reservoir constituted by ruminants may not be significant, but when the management system allows for close contact between cattle and horses, the role of cattle in the epidemiology of *T. evansi* may be important. When an outbreak of *T. evansi* occurs in horses, the associated ruminants should be treated with a drug that sterilizes infection. Such a drug should be identified.

Dogs and even cats may also act as reservoirs of *T. evansi*; curative treatments should also be applied to these species.

Eradication of *T. evansi*

In areas where *T. evansi* is enzootic, eradication of the parasite is not feasible due to its very large wildlife reservoir. In these areas, only therapeutic strategies can be applied. In epizootic areas, chemoprophylaxis of horses, ruminants and dogs is recommended.

CONCLUSION

In practical terms, economical and medical constraints have priority over epidemiological constraints, therefore, treatments are only applied if economically justified. Moreover, there is no ideal treatment which allows control of both *T. vivax* and *T. evansi* in horses, ruminants and dogs. Here again, the lack of species-specific diagnostic tools limits the seroepidemiological studies and therefore the planning of strategic chemotherapy and chemoprophylaxis. In addition, the lack of new efficient drugs against *Trypanosoma* species continues to be a problem.

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A COMPARISON BETWEEN SURAMIN-SENSITIVE AND SURAMIN-RESISTANT *TRYPANOSOMA EVANSI* USING DIFFERENTIAL DISPLAY ANALYSIS

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ABSTRACT

Control of surra, a disease caused by *T. evansi*, relies heavily on the trypanocide suramin which has led to the development of suramin-resistant strains of the parasite. A test for identifying drug resistant strains of *T. evansi* would help in the control of surra and increase the efficacy of the available trypanocides. We have used Differential Display analysis to compare suramin-sensitive and suramin-resistant stock of *T. evansi*. To accomplish this, a stock of *T. evansi* susceptible to suramin at a dose rate of 0.7μg/ml was made resistant to suramin at a dose rate of 700μg/ml. The total RNA from the two stocks was extracted and Differential Display analysis was carried out using a combination of upstream and downstream primers. Fifty-five differentially expressed cDNA bands were obtained from the suramin-resistant and suramin-susceptible populations. These bands were screened by affinity capture using a northern blot technique and then cloned prior to sequencing. Four of the cDNA bands differentiate between genomic DNA extracted from the homologous suramin-sensitive and suramin-resistant stocks of *T. evansi.*

RESUMÉ

Le contrôle du "surra", maladie causée par Trypanosoma evansi, repose principalement sur l'utilisation d'un trypanocide, la suramine, qui a rapidement conduit à l'apparition de souche suramino-résistantes. Un test permettant de diagnostiquer la résistance des souches de T. evansi à la suramine serait particulièrement utile, et permettrait d'accroître l'efficacité des autres trypanocides. Nous avons utilisé une technique de caractérisation par analyse d'ADNc pour comparer des souches de T. evansi sensibles et résistantes à la suramine. Pour ce faire, une souche de T. evansi sensible à la suramine, à la dose de 0,7μg/ml a été rendue résistante à la dose de 700μg/ml. L'ARN total des deux souches a été extrait, et une analyse de l'ADNc a été menée l'aide d'une combinaison d'amorces oligonucléotidiques situés en amont et en aval. 55 bandes différentes d'ADNc ont ainsi été obtenues à partir des deux souches suramine-sensible et suramine-résistante. Ces bandes ont été étudiées par capture d'affinité à l'aide d'une technique de Northern-blot, puis clonées avant d'être séquencées. Quatre d'entre elles permettent de différencier l'ADN génomique extrait des souches sensible et résistante de T. evansi.

RESUMEN

El control de la "surra", enfermedad causada por el *T. evansi*, ha dependido fuertemente de la droga trypanocida denominada suramina y ha conllevado al desarrollo de una cepa de parásito suramina-resistente. Una prueba para identificar las cepas resistentes a las drogas de *T. evansi* podría ayudar al control de la "surra" e incrementar la eficacia de los trypanocidas disponibles. Hemos usado el analisis diferencial para comparar stocks de *T. evansi* suramina-sensitivos y suramina-resistentes. Se logró que un stock de *T. evansi* susceptible a suramina a una dosis de 0.7ug/ml se hiciera resistente a la suramina a una tasa de dosificación de 700pg/ml. Se extrajo el ARN total de los dos stocks y se realizó un análisis diferencial usando una combinación de alta y baja tensión de corriente eléctrica. Se obtuvieron expresiones de cincuenta y cinco bandas diferenciables de c-ADN de las poblaciones de parásitos suramina-resistentes y suramina-susceptibles. Estas bandas fueron clasificadas por afinidad con la técnica Northern-blot y luego clonadas antes de la secuenciación. Cuatro de las bandas de c-ADN diferenciaron entre el ADN genómico extraído de los stocks homólogos de *T. evansi* suramina-sensitivos y suramina-resistentes.

INTRODUCTION

Trypanosomiasis caused by *T. evansi* (surra) affects livestock in both the Old and New Worlds. In camels, buffalo, cattle and horses the disease processes induced by *T. evansi* cause abortion, infertility, low productivity and, if untreated, death. Agricultural development is often impaired in endemic areas due to restrictions on draught animal power, and low productivity in livestock breeding programmes also occurs due to the pathogenic effects of the disease.

With little prospect in the near future for immunological control, chemotherapy will remain the most widespread and effective means of controlling trypanosomiasis for the foreseeable future. Since the disease is probably restricted to domestic livestock and no stage of development is found in the insect vector(s), any successful control programme is likely to have permanent effects.

Chemotherapy of surra relies on suramin, quinapyramine and cymelarsan in camels, and diminazene, homidium and isometamidium in cattle. All these drugs except cymelarsan have been heavily used over the past 40 years. This has resulted in an increase in the number of reports of resistance to these trypanocides. The situation will not improve in the near future because the major pharmaceutical companies are reducing their expenditure directed at producing new trypanocides (current estimate for developing a new drug is £80 million) and there are no new trypanocides under development. It is therefore of crucial importance that we utilize our existing drugs as effectively as possible in order to prevent the possibility of *T. evansi* developing resistance to all the existing trypanocides. Parasite resistance to trypanocidal drugs is a problem in many parts of Africa and is an important constraint to the control of trypanosomiasis. *In vitro* methods for assaying drug resistance have appeared in recent literature but are not widely used as they require specialized equipment and skilled personnel. A simple method of detecting drug resistance in the field would therefore be a valuable tool in the chemical control of disease caused by *T. evansi*.

DIFFERENTIAL DISPLAY ANALYSIS

One technique for analyzing the differences between suramin-sensitive and suramin-resistant stocks of *T. evansi* is Differential Display analysis (Lian and Pardee 1992). This technique allows the identification of genes differentially expressed at the messenger RNA (mRNA) level on a systematic basis and therefore offers the possibility of identifying new markers for diagnosis of diseases. The technique allows simultaneous display of both up- and down-related genes and permits side-by side comparisons of mRNA from the different sources. More important, these differences can be used to identify and isolate the corresponding genes.

We have used Differential Display to compare a *T. evansi* stock resistant to suramin at a dose-rate of $0.7\mu g/ml$, to the same stock which had been induced to become resistant to suramin at a dose rate of $700\mu g/ml$ under in-vitro culture. After comparing some 50% of the gene products, a total of 6,366 cDNA bands, 49 bands were identified that clearly differentiated between the suramin-sensitive and suramin-resistant *T. evansi* stocks. After re-screening, the number of bands that differentiated between suramin-sensitive and suramin-resistant stocks were reduced to 15 and this number of bands was reduced to only four after affinity northern blotting. The four cDNAs were specific for suramin-resistant *T. evansi* and this was confirmed by hybridizing ^{32}P labeled probes prepared from the cDNAs with genomic DNA extracted from the sensitive and resistant *T. evansi* stocks. The labeled probes hybridized only with the suramin-resistant DNA. The cDNAs have been cloned, amplified and sequenced. The sequences have been deposited in the EMBL nucleotide database and the accession numbers are given in a table after the description of the Differential Display technique.

Technique for Differential Display analysis

The first step in the Differential Display analysis is the extraction of high quality total RNA, totally free of contaminating DNA. The isolation of the RNA from *T. evansi* was a lengthy process.

The second step in the Differential Display technique divided the suramin-sensitive RNA and the suramin-resistant RNA each into nine identical 300ng aliquots. The RNA aliquots were then reverse transcribed using nine anchored downstream primers into cDNA fractions.

The third step involves PCR amplification of cNDAs from each fraction using a set of 24 arbitrary primers. The amplification utilizes α ^{33}P-dATP to label the products which are subsequently separated by non-denaturing electrophoresis on 0.2mm thin polyacrylamide gels. The gels are dried onto thick filter paper and exposed overnight to X-ray film. Comparison of the corresponding gel tracks reveals differences in gene products between the suramin-sensitive and suramin-resistant *T. evansi* populations.

The first differential display analysis run with amplified cDNAs from the suramin-sensitive and suramin-resistant

T. evansi involved nine primers each with 48 individual PCR runs and produced many differences. These differences were seen either as differences in the cDNA fragment intensities on the autoradiogram or more excitingly as cDNA fragments present only in the suramin-resistant *T. evansi* stock and not in the suramin-sensitive stock. Further analysis was carried out on gene products generated using downstream primers 1, 4 and 9. From the respective gels 49 bands were cut out from the dried polyacrylamide gel using the autoradiogram as a template. Each band was eluded from the gel slice and then reamplified using the original primer combination.

The whole technique up to this point was then repeated with a second aliquot of the original RNA preparations from the suramin-sensitive and suramin-resistant *T. evansi* populations. This confirmed that the differences were not arbitrary. The whole technique was then repeated twice more with new preparations of RNA from the suramin-sensitive and suramin-resistant *T. evansi* populations. Downstream primers 1, 4 and 9 gave rise to a total of 49 differences in bands between the suramin-resistant and suramin-sensitive *T. evansi* populations.

The fourth step was to purify differentially displayed cDNAs by affinity capture using the total RNA used to prepare them originally. Each cDNA was labeled using $_\alpha$-^{32}P-dATP and used in a northern blotting experiment to probe total RNA from the suramin-sensitive and suramin-resistant *T. evansi* populations covalently bound to Hybond N membranes. All northern blots were washed to high stringency (0.1 x SSX/0.1%SDS at 65°C) before exposure to X-ray film for three hours. Positive bands were eluted from the Hybond N membrane and then reamplified using the original primer combination. From the original 49 bands four were affinity purified to this step in the technique.

The penultimate step in Differential Display analysis was to clone the affinity purified cDNAs into a suitable vector and then sequence the DNA insert. This proved to be very difficult and took a number of trials with different vectors. The four inserts were finally ligated and transformed into Amersham's pMOS vector. Colonies containing inserts of the appropriate size were selected, these were: W463; W472; W315 and W361. For the work to identify and characterize molecular markers for the detection of stocks of *T. evansi*, the inserts have been sequenced and the data submitted to the EMBL nucleotide database. Accession numbers for the sequences and information on the plasmids containing the isolated suramin-resistant markers are in the following table:-

Colony I.D.	Insert Size	EMBL Accession No.
W463	~ 500bp	Z82041
W472	~ 550bp	Z82042
W315	~ 394bp	Z82039
W361	~ 344bp	Z82040

Plasmid DNA preparations have been prepared from each of the four inserts (clones W315, W363, W472 and W463). The DNA was then reamplified and purified by preparative agrose gel electrophoresis using Geneclean®. The purified inserts were then labeled with a $_\alpha$-^{32}P-dATP and probed against the homologous suramin-sensitive *T. evansi* and suramin-resistant *T. evansi*. The labeled probes recognized only the suramin-resistant *T. evansi* DNA down to a concentration of 1µg. (Boid *et al.* In preparation).

cDNA previously generated from the Suramin-sensitive and Suramin-resistant RNA pools using one of the 9 Downstream (DS) primers was amplified by PCR using the 24 Upstream (US) primers and a ^{33}P-dATP radiolabel.

Electrophoretic separation of the radiolabeled cDNA products was carried out using a non-denaturing gel separation procedure.

The gel was dried under vacuum and exposed to X-ray film overnight. The bands of interest were cut out from the dried gel using the autoradiogram for localisation. Eluted bands were subsequently reamplified and used for further

studies.

The photograph shows a typical autoradiogram produced using the protocol described.

The samples are organised as below from Track 1 to Track 24.

Track 1: Upstream primer 1 /Downstream primer 1
Left lane: Suramin-resistant cDNA's
Right lane: Suramin-sensitive cDNA's

Track 12: Upstream primer 1 /Downstream primer 12
Left lane: Suramin-resistant cDNA's
Right lane: Suramin-sensitive cDNA's

Track 20: Upstream primer 1 /Downstream primer 20
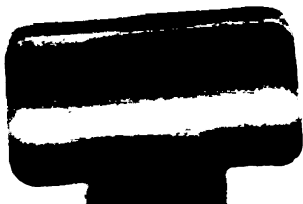Left lane: Suramin-resistant cDNA's
Right lane: Suramin-sensitive cDNA's

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