

**APPROACH ON RISK FACTORS OF BOVINE TRYPANOSOMOSIS DUE TO
Trypanosoma vivax IN THE BOLIVIAN AND BRAZILIAN PANTANALS**

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ABSTRACT

Trypanosoma vivax caused between 1995 and 1997 a devastating disease in Bolivian and Brazilian Pantanals. These regions are very important beef cattle breeding in Brazil and Bolivia. The principal clinical signs of bovine trypanosomosis in livestock were anemia, severe leucopenia, and abortion. The purpose of the present study was to analyze the risk factors of the natural bovine trypanosomosis in the Bolivian and Brazilian Pantanals. We concluded that the principal risk factors related to *T. vivax* transmission in the Bolivian and Brazilian Pantanals were the transportation by foot, because the conditions for acquisition or transmission of *T. vivax* were greatest at the numerous resting places along the route, the use of contaminated needles during vaccinations, and transmission by tabanids during the rainy season. The fauna contamination should be also considered.

Key-words: Bovine trypanosomosis, *Trypanosoma vivax*, risk factors, Bolivia, Brazil

**ABORDAGEM SOBRE OS FATORES DE RISCO DA TRIPANOSOMOSE BOVINA
DEVIDO AO *Trypanosoma vivax* NOS PANTANAIS BOLIVIANO E BRASILEIRO**

RESUMO

O *Trypanosoma vivax* causou entre 1995 e 1997 doença devastadora nos Pantanaís da Bolívia e Brasil. Estas regiões são importantes criatórios de bovinos de corte nos dois países. Os principais sinais clínicos da tripanosomose bovina foram anemia, leucopenia severa e aborto. O propósito do presente estudo foi analisar os fatores de risco da tripanosomose bovina natural nos Pantanaís da Bolívia e do Brasil. Concluímos que os principais fatores de risco relacionados à transmissão do *T. vivax* nos pantanaís boliviano e brasileiro foram o transporte a pé, porque as condições para a aquisição ou transmissão do *T. vivax* foram maiores nos numerosos locais de descanso do animais ao longo das estradas boiadeiras, o uso de agulhas contaminadas durante as vacinações e a transmissão pelos tabanídeos durante a estação chuvosa. A contaminação da fauna deveria ser considerada.

Palavras-chaves: Tripanosomoses bovina, *Trypanosoma vivax*, fatores de risco, Bolívia, Brasil.

**ABORDAJE SOBRE LOS FACTORES DE RIESGO DE LA TRIPANOSOMOSIS
BOVINA DEBIDO AL *Trypanosoma vivax* EN LOS PANTANALES BOLIVIANO Y
BRASILEÑO**

RESUMEN

Trypanosoma vivax ha causado entre 1995 y 1997 una enfermedad devastadora en los Pantanales bolivianos y brasileños. Los Pantanales son regiones muy importantes que crían ganados vacunos en el Brasil y Bolivia. Los principales síntomas de la tripanosomosis de los bóvidos fueron anemia, leucopenía severa y aborto. El propósito del actual estudio fue

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analizar los factores de riesgo de la tripanosomosis natural de los bóvidos en los Pantanales bolivianos y brasileños. Concluimos que los factores de riesgo principales se relacionaron con la transmisión del *T. vivax* fueron el transporte a pie, porque las condiciones para la adquisición o la transmisión del *T. vivax* eran mayores en los lugares de reclinación que son numerosos a lo largo de la ruta, del uso de agujas contaminadas durante vacunaciones, y de la transmisión por los tábanos durante la estación de lluvias. La contaminación de la fauna debe ser considerada.

Palabras-claves: Tripanosomosis de los bóvidos, *Trypanosoma vivax*, factores de riesgo, Boliva, Brasil.

INTRODUCTION

Trypanosomosis is one of the most devastating diseases of animals and man in Sub-Saharan Africa and has a profound effect on rural development over vast areas (HOLMES, 1997). Non-tsetse transmitted trypanosomosis, caused by *Trypanosoma evansi* and *T. vivax*, occurs in various forms in South America, Africa and Asia (including China), and is a potential risk for 500 million cattle, 100 million buffalo and 12 million camels found in these regions (PEREGRINE, 1994).

In South America, four species of trypanosomes have social or economic importance. They are *T. cruzi*, the cause of Chagas' disease in humans, *T. equiperdum* causing a chronic sexually transmitted disease of horses, *T. evansi* causing acute disease in horses and other mammals, and *T. vivax* causing disease in bovines (WELLS et al., 1982, SILVA et al. 1995).

T. vivax is found throughout the tsetse belt in Africa. It has, however, spread to other parts of Africa, Central America, South America, the West Indies and Mauritius (LEVINE, 1973). *T. vivax* was reported in the New World for the first time in French Guyana and later to others parts of South America, Central America, and some Caribbean islands (LEGER & VIENNE, 1919, MELENDEZ et al., 1995). In Brazil, SHAW & LAINSON (1972) reported the first occurrence of *T. vivax*. The parasite was observed in a water buffalo (*Bubalis bubalis*) from the vicinity of Belém, Pará. Twenty-three years later, *T. vivax* was recorded in the north of Pantanal region (SILVA et al., 1995). In the following years, *T. vivax* outbreaks were reported in the Bolivian Pantanal (SILVA et al., 1998).

Pantanal is the name given to a large wetland located in the center of South America, which borders with Bolivia, between 16° and 21° S and 55° and 58° W, ranging in altitude from 80 to 130m above sea level. The Pantanal region is one of the most important beef cattle breeding regions of Brazil. At more than 138,000 km², Pantanal is known as the largest freshwater wetland in the world. Pantanal is home for at least 650 species of birds, 260 species of fish, 80 mammals species and 50 species of reptiles. The principal economic activities of Pantanal include fishing, mining, tourism, and cattle ranching. Extensive cattle ranching has been the dominant economic activity and land use in the region for the past two centuries. About 80 % (118,000 km²) of the lands are found on cattle ranches (SEIDL et al., 2001). The estimated present value of the existing herd of 3 million cattle is around US\$ 1 billion (SEIDL et al., 2001), not considering 4,966 buffaloes and 49,000 horses (SEIDL et al., 1998).

Each year, heavy tropical rains cause the main tributaries that supply water to Pantanal to overflow. During the dry season, floodwaters recede, resulting in a rich and complex mosaic of grasslands and forests, dotted with countless lagoons and marshes. This annual cycle makes the Pantanal one of the world's most productive wildlife habitats. Millions of waterfowl breed and feed along its rivers and lagoons, and dense populations of jaguars,

capybaras, marsh deer, giant anteaters, tapirs, hyacinth macaws, and others thrive in its forests and grasslands (ALHO et al., 1988).

The diversity of interacting habitat types and the direct connection with neighboring South American phytogeographic regions also produce a remarkable, albeit poorly known, plant diversity. The vegetation contains elements from the dry-savanna ("cerrado") of Central Brazil, the Bolivian "chaco" (semi-arid scrub forest), the Amazonian region to the north, and the Atlantic forest to the South and Southeast (ADAMOLI, 1986). Plant and animal life are strongly influenced by distinct seasonal flooding with water levels during the rainy season as much as 5 meters higher than during the dry season (JUNK & SILVA, 1995). The seasonal flooding regime supports a productive and diverse fauna (BROWN, 1986).

Average annual rainfall is slightly greater than 2000 mm at the northern boundary of the Paraguay basin, and falls to 800 mm with distance south and west. Maximum rainfall occurs in January in the south of the basin, and in February-March farther north (COLLISCHONN et al., 2001). The land declivity, almost null, is only 6 to 12 cm/km in the East-West direction and from 1 to 2 cm/km in the North-South direction, favoring flooding which propagates from North to South and from East to West along the Paraguay River which is the only natural drainage way of the Pantanal. The Pantanal is divided in 11 sub-regions. The Pantanal soils in their majority are constituted of poor sand soils with small spots of some rich clay and lime soils. The climate is hot during the summer with an average temperature around 32°C, but it is cold and dry during the winter season, presenting an average temperature around 21°C. Frost can occasionally take place during the months of July and August. The Pantanal region contains large extensive livestock farms which, associated to environmental factors, have consolidated a rural structure constituted of large properties (56% of the area with farms greater than 10,000 ha). The milk production is insignificant economically and it is usually consumed by low income people (SEIDL et al., 1998, SEIDL et al., 2001).

Bolivia is a sub-tropical country located in the center of South America. It is divided into nine departments (Chuquisaca, Cochabamba, El Beni, La Paz, Oruro, Pando, Potosí, Santa Cruz, and Tarija). Santa Cruz is the largest and most important one economically, and its production includes petroleum, natural gas, sugar cane, cotton, timber, soy bean, rice, wheat, corn and cattle (HALL et al., 1993). Santa Cruz has approximately 371,000 km² and 1,364,389 habitants and produces 34.5% of the national agricultural gross domestic product (GDP). Livestock contributes to 21.6% of the agricultural GDP and utilizes 29% of the total area (SOLANO et al., 2000).

Santa Cruz is considered to be one of the most important livestock regions of Bolivia maintaining a population of 1,598,957 cattle. The department of Santa Cruz has 15 provinces (Andrés Ibáñez, Angel Sandoval, Chiquitos, Cordillera, Florida, Germán Busch, Guarayos, Ichilo, Ignacio Warnes, José Miguel de Velasco, Manuel María Caballero, Ñuflo de Chávez, Obispo Santistevan, Sara, and Vallegrande). The greatest number of cattle is found in extensive lowland provinces of Ñuflo de Chavez, Guayros, Velasco, Chiquitos, Angel Sandoval and Cordillera (HALL et al., 1993). Within Santa Cruz, the cattle movement is generally centripetal. The western valleys and specially dry subtropical zone have more breeding stock, and the excess of animals of about 1-year-old are exported for fattening to better pasture of the subtropical humid zone. The final destination of most of this produce is the city of Santa Cruz de la Sierra, but the production is also exported to other Departments of Bolivia (CARRIQUE-MAS, et al., 2000). Annual rainfall varies from 1,200 to 1,800 mm, while temperature fluctuates between 25 and 35°C (SOLANO et al., 2000).

The Beni Department is a northeastern department of Bolivia in the lowlands region of the country. It is the second largest department in Bolivia. Beni has 8 provinces (Cercado, Iténez, José Ballivián, Mamoré, Marbán, Moxos, Vaca Díez, and Yacuma). It has a tropical

and humid climate, with an average annual rain precipitation that varies between 1,000 mm and 4,000 mm. in all its territory. Beni has a population of around 5,000,000 cattle. Most of the information of the Pantanal region can be applied to the Pantanals of Santa Cruz and Beni. The purpose of the present study was to analyze the risk factors of the natural bovine trypanosomosis by *T. vivax* in the Bolivian and Brazilian Pantanals.

MATERIALS AND METHODS

Blood sampling in Brazil.

All bovines sampled in Brazil were bled from their jugular vein using a vacuum system (Vacuum II, Labnew, Campinas, Brazil).

Pantanal of Poconé

In the Pantanal of Poconé were sampled 29 zebu purebred and crossbred (*Bos taurus taurus* x *Bos taurus indicus*), with ages from 1-9 years old.

Pantanal of Paiaguás

In the Pantanal of Paiaguás were sampled 2 beef cattle (all zebu breed) of an outbreak involving hundreds of animals with sudden death of 20 bovines.

Pantanal of Nabileque

In the Pantanal of Nabileque were sampled 21 beef cattle (all zebu breed) and 40 crossbred cows (Holstein Friesian x White Nelore cattle). All bovines were between 1-10 years old.

Blood sampling in Bolivia

The study in Bolivia was carried out in several provinces of Santa Cruz (Velasco, Nuflo de Chaves, Guayaros, Chiquitos and German Bush).

Province of Velasco, Nuflo de Chaves, Guayaros and Chiquitos

Between January and May of 1996, thin blood smears stained with May-Gruewald-Giemsa from Province of Velasco, Nuflo de Chavez, Guayaros and Chiquitos were submitted by veterinaries to the Laboratorio de Diagnostico e Investigaciones Veterinárias (LIDIVET) in Santa Cruz de la Sierra, Bolivia. In September of the same year, blood samples were collected from the caudal vein of 29 bovines in the Laguna Concepción (Province of Chiquitos). Samples were taken using the same vacuum system used in the province of German Bush. The sampled animals, all Bolivian criollo-zebu crossbreeds, were between 1 and 10 years old (average 4 years old).

Province of German Bush

In February of 1997, blood samples were collected from the caudal vein of 87 bovines belonging to 4 ranches of province of German Bush, Bolivia were bled from their jugular vein using a vacuum system. The sampled animals, all nelore purebred and crossbreeds (*Bos taurus taurus* x *Bos taurus indicus*) between 1 and 9 years old (average 7 years old).

DIAGNOSIS OF TRYPANOSOMOSIS

The diagnosis of trypanosomosis in studies developed in Brazilian and Bolivian Pantanals were done using hematocrite centrifuge technique and Giemsa-stained smears. Blood from each sample and the concentrated parasites in the buffy coat of micro-hematocrite

tubes were used to prepare thin smears. The trypanosomes were identified based on morphological and biometrical data according to Hoare (1972). *T. vivax*, subgenus Duttonella, were distinguishable by their mensural characteristics, comprising total length, including flagellum, distance from posterior end of body to kinetoplast and to nucleus, distance from kinetoplast to nucleus, from latter to anterior end of the body and length of free flagellum. *T. vivax* as seen in the blood of beef cattle was also essentially monomorphic. The kinetoplast was large and terminal or almost so. The nucleus was centrally placed, but the bulk of the cytoplasm was found in the posterior part of the body as this is somewhat swollen. The posterior extremity was swollen and blunt.

RESULTS

Parasitological and clinical studies in Brazil

Pantanal of Poconé

In January of 1995, outbreaks of ophthalmitis, abortion, dysentery, and death occurred in ranches located in the Poconé sub-region of Pantanal. Information that a drug (diminazene diacetate) was being used with success, to cure sick animals of one ranch led us to investigate the possibility that trypanosome infection was responsible. Twenty nine bovines were sampled. During the clinical examination the principal signs observed were fever, lethargy, loss of appetite, weakness, lacrimation, dysentery and abortion. Some animals had substantial loss of weight in a short period of time. Other animals had a more chronic form of the disease for four or six months with emaciation and severe cachexia. Ten of 29 (34.48%) sampled bovines were infected by *T. vivax*.

Pantanal of Paiaguás

In the beginning of November 1996 several cases of fever, intense anemia, progressive weakness, loss of condition, loss of appetite, lethargy, substantial weight loss over short period of time and death were observed. Because of a sudden death of 20 bovines, 2 blood samples were sent to the EMBRAPA-Pantanal Research Center located in Corumbá, MS. One of two samples were positive to *T. vivax*.

Pantanal of Nabileque

Beef cattle: Clinical signs similar to bovines from the Pantanal of Paiaguás were observed. Death of bovines were observed. Twenty one animals were sampled, all zebu breed, with ages between 1 to 10 years. *T. vivax* was diagnosed in 80.95% (17/21). During the outbreak, death of 5 marsh deer (*Blastocerus dichotomus*) was recorded, but without diagnosis.

Dairy cattle: In August 1997, an outbreak of bovine trypanosomosis occurred in a dairy-cattle ranch. The animals showed fever, intense anemia, progressive weakness, loss of condition, loss of appetite, downfall in the milk production, leading to 3 dead cows, 5 abortions and 7 placenta retentions. The prevalence for *T. vivax* was 42.5% (17/40). All bovines of the herd were sampled.

Parasitological and clinical studies in Bolivia

Province of Velasco, Nuflo de Chaves, Guayaros and Chiquitos

In early 1996, several cases of intense anemia, abortion and death were reported in cattle from the wetland provinces of Santa Cruz. *T. vivax* was diagnosed in thin blood smears from 159 cases: from Province of Velasco (57), Nuflo de Chavez (20), Guayaros (30) and Chiquitos (52) and in 86.20% of 29 beef cattle from Laguna Concepcion. Similar clinical signs were observed in Brazil, and some veterinarians reported the occurrence of edema and signs of central nervous involvement such as hyper-reactivity to stimuli, tremors, lack of coordination. The increase in the lymph node size on the neck was reported. In Laguna Concepcion some animals presented hematocrite as low as 17%. The average hematocrite was 26%.

Province of German Bush

In February of 1997, the first recorded cases of bovine trypanosomosis-like diseases, were discovered in a ranch of German Bush province. The mortality rate was 5% (50/1000). The clinical signs observed were fever, anemia, abortion, progressive weakness, substantial weight loss in relatively short time, and progressive emaciation and lymph node increase. Some animals registered a hematocrite as low as 17%. *T. vivax* was identified in 47.82% of examined cattle (39/87) by micro-hematocrite test.

DISCUSSION

In October of 1995, *T. vivax* was reported by the first time in the north of the Pantanal region. In the following years this parasite was reported in the Bolivian Pantanal. After the report of *T. vivax* outbreaks in Santa Cruz in 1996, other Bolivian departments were affected. In 1997 according to local press, *T. vivax* caused the death of 150,000 bovines in Beni and affected 5 % of 5 million cattle herd (PRESENCIA DEL PAIS, 1997). In Beni, the bovine trypanosomosis was recorded in the provinces of Iténez, Mamoré, Ballivián, Moxos, and Cercado. Beef-cattle production is the predominant form of land-use in most Bolivian and Brazilian wetland areas, with low human population density. The traditional cattle-raising system in the Pantanal, Brazil, is based on calf and yearling production. Its commerce involves animal transportation to market places, river ports and railway stations. The most common method of transportation is by foot. Herds, which average 906 animals, takes a mean of 11 days to cover 230 km (CADAVID-GARCIA, 1985).

Similar cattle-raising systems are used in the Pantanal of Santa Cruz. Conditions for the acquisition or transmission of *T. vivax* are greatest at the numerous resting places along the route, when the interaction among animals from different properties and host proximity provides an excellent opportunity for disease transmission by vectors. Similarly to Gardiner (GARDINER, 1989), a temporal association between the rainy season when biting flies, particularly *Tabanidae*, are abundant and an increased prevalence of *T. vivax* infections in cattle has been noted. Horse flies and deer flies have been shown to mechanically transmit more than 35 agents that cause disease of animals (BARROS & FOIL, 1999). In Colombia, Otte and Abuabara (OTTE & ABUABARA, 1991) found the transmission of *T. vivax* by *Tabanus nebulosus*.

A study developed by Raymond (1990) in French Guiana found that *T. vivax* was transmitted between zebu bull calves by blood meal interruption and immediate resumption of female *Tabanus importunus*. In the Pantanal, tabanids are the assumed vectors of *T. evansi* and the Equine Infectious Anemia Virus (BARROS & FOIL, 1999). A two years (1992-1994) study developed by Barros & Foil (1999) reported the predominant tabanid species from Nhecolandia sub-region of the Pantanal, as well as their relative abundance and seasonal occurrence. In the grassland, a total of 1,458 (51.5%) tabanids from 20 species was caught,

while 1,374 (48.5%) tabanids from 19 species were collected from the savannah. A seasonal change of tabanid abundance related to the habitat was observed throughout the year. In general, population peaks occurred around the beginning of the rainy season. After this period, the tabanid population decreased gradually, reaching the lowest levels from February to July. The number of flies increased as the temperature and relative humidity increased in the period preceding the rainy season each year. The most abundant species were *Tabanus importunus* (29.5%), *Tabanus occidentalis* (25.4%), *Tabanus claripennis* (12.3%), and *Lepiselaga crassipes* (7.5%).

In Santa Cruz, studies on zoning of the province into three regions of estimated tabanid fly challenge by Hall et al. (1993) showed that the provinces of Velasco, Nuflo de Chavez, Guayaros and Chiquitos are in an area of high fly challenge. In the Pantanal, studies showed that the population peak of tabanids occurs in the first half of rainy season, from September/October to December/January. However, the tabanids remain in high numbers until the end of the rainy season. This season represents the period of major risk for trypanosome transmission by these insects due to their abundance and population peak, most notably *Tabanus importunus* (SILVA et al., 1996).

Since *T. vivax* transmission out of Africa is mechanical, some common practices in the region, such as the use of one needle for several animals during vaccination and medicine applications, could be an important way for infection. The sampled bovines were generally in poor conditions. The clinical findings were very similar in all regions studied. The abortions occurred in beef and dairy cattle, but only among the dairy cattle it was possible to determine the correct number of cases.

The treatment of affected cattle with diminazene aceturate at 3.5 mg/kg (Brazil) and isometamidium 0.5-1.0 mg/kg (Bolivia) usually resulted in recovery of all animals. In some cases, despite a trypanocidal treatment used, animals died and the weight loss of the others was not compensated three or four months later. The anemia and leukopenia were the major signs of bovine trypanosomosis in the Brazilian Pantanal and Bolivian Pantanal and could be the principal cause of mortality of trypanosome-infected animals.

Leukopenia has been reported in trypanosomosis and it is attributed to a reduced myelopoiesis. The severe leukopenia could cause immunosuppression (ILEMOBADE et al., 1982). The study of cattle groups infected with *T. vivax* or *T. congolense* and, with a combination of *T. vivax* and *T. congolense*, and vaccinated against contagious bovine pleuropneumonia (CBPP) suggested that the protective immunity to CBPP engendered by vaccination is impaired during infection with African trypanosomes. The severe leukopenia observed in our studies (SILVA et al., 1999) demonstrated that the importance of trypanosomosis control in ensuring success of vaccination campaigns in Brazil and Bolivia should be considered.

CONCLUSIONS

We conclude that the principal risk factors related to *T. vivax* transmission in the Bolivian and Brazilian Pantanal were the transportation by foot, because the conditions for acquisition or transmission of *T. vivax* were greatest in the numerous resting places along the route, increased movement of horses and cattle through the properties of both countries, and transmission by the tabanids during the rainy season. According to Gardiner (1989), *T. vivax* also infects various species of antelopes, for which it is non-pathogenic. In Brazilian and Bolivian Pantanal the ungulate fauna is very rich and co-exists with domestic livestock. In the Pantanal, surveys found an abundance of about 44000 marsh deer (MOURAO et al., 2000). The same survey indicated that there were at least 60000 pampas deer inhabiting the Pantanal. These animals could serve as an important reservoir of infection. The wildlife

infection should be considered. Current and recent historical evidence indicate that bovine trypanosomosis in the Brazilian and Bolivian Pantanals has the potential to become an important animal disease. However more studies are necessary to determine the epizootology of *T. vivax* and the impact of the disease on the economy of the region.

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