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Trypanosomosis outbreaks due to *Trypanosoma evansi* in the Pantanal, Brazil. A preliminary approach on risk factors

R.A.M.S. Silva¹A.T.M. Barros²H.M. Herrera¹

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This study was carried out from January to July 1994 when several cases of trypanosomosis occurred in dogs and horses in the Pantanal. Blood samples collected from sick horses (n = 119) and dogs (n = 4) were examined by the microhematocrit centrifugation technique and mouse inoculation. All dogs and 116 horses (97 %) were infected with *Trypanosoma evansi*. The trypanosomosis outbreaks occurred during or just after the tabanid season in the region. The frequent transit of infected domestic animals through the properties could have contributed to the disease spreading by vectors. Other risk factors such as wild reservoirs and transmission by vampire bats are discussed.

Key words: Horse - Trypanosomosis - *Trypanosoma evansi* - *Tabanidae* - Centrifuging - Epidemiology - Brazil.

Introduction

The Pantanal is a seasonal floodplain of about 140,000 km² ranging in altitude from 80 to 120 m above sea level, located in the centre of South America, between 16° and 21° S and 55° and 58° W. (7). The Pantanal is divided into ten wetlands or *pantanaís*, differentia-

ted in terms of water courses, soil types, and the nature of historical occupation (figure 1). The most important one in terms of cattle raising is Nhecolândia (23,574 km²), located somewhat in the South (20). Extensive cattle ranches varying from 10,000 to 200,000 hectares occupy most of this wetland. It is populated by 3,996,000 cattle, 4,966 buffaloes and 139,760 horses (5).

The traditional cattle-raising system is based on calf and yearling production and its commercialisation involves taking the animals to market-places, river ports or railway stations. The most common way is, on average, for 906 animals to walk 230 km for eleven days (4). Usually, six cowboys herd the animals and each man has one extra horse. The Pantanal is one of Brazil's most important livestock regions and horses play a fundamental role in all steps of beef cattle production.

Equine trypanosomosis caused by *Trypanosoma evansi* is known in the Old World as surra and in the Pantanal and subtropical areas of Argentina as "Mal de Caderas" (14). According to Hoare (11) and Santos *et al.* (17), it was probably introduced in South America during the XVIth century by Spanish settlers. Apparently, the disease entered the Pantanal region in the 1850s but no effective drug treatment was available until the 1930s, and ranchers regularly had to import horses from other regions because of the high mortality rate caused by *T. evansi* (20). Since 1894, the disease has been reported in horses in the Nhecolândia subregion of Pantanal (2). The most serious epidemics followed extensive floodings and this disease became a significant limitation to the expansion of the cattle industry in the Pantanal (20). Franke *et al.* (8) reported the prevalence of the disease in horses as well as the prevalence of *T. evansi* infections in cattle, dogs and free-ranging capybaras in the Poconé subregion of Pantanal. In 0.3 % of the horses, 8.6 % of the dogs and 8 % of the capybaras, the infection was detected using standard parasitological methods. A seroprevalence of 4.1 % was found in horses, 2.3 % in cattle, 7.1 % in dogs and 22 % in capybaras, using an enzyme-linked immunosorbent assay for the detection of *T. evansi* antigen (ag-ELISA), whereas 9.6, 4.2, 18.6 and 14 %, respectively, of the animals investigated had *T. evansi* antibodies (Ab-ELISA). Despite the reduced incidence of the disease in horses and cattle since the 1950s due to the widespread application of drug treatment (20), the problem remains important. The aim of this study was to investigate some possible risk factors involved in trypan-

1. Laboratory of Ecopatolgy, EMBRAPA/Centro de Pesquisa Agropecuária do Pantanal, Rua 21 de Setembro 1880, CEP: 79.320-900, Corumbá, MS, Brazil. E-mail: ramss@sede.embrapa.br

2. Laboratory of Entomology, EMBRAPA/Centro de Pesquisa Agropecuária do Pantanal, Rua 21 de Setembro 1880, CEP: 79.320-900, Corumbá, MS, Brazil.

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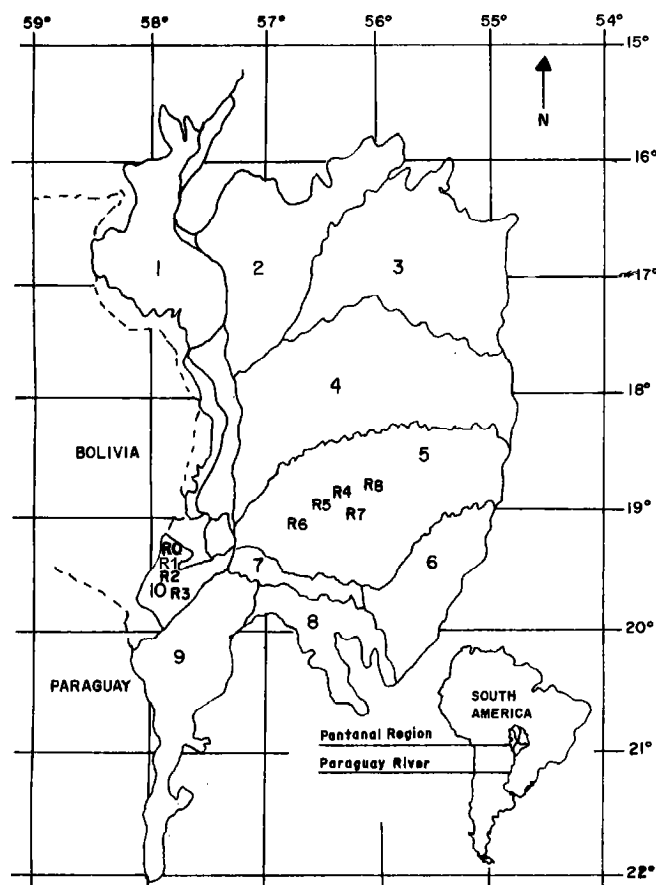


Figure 1: Location of the Pantanal and the *Trypanosoma evansi* outbreaks.

Pantanal Subregions

- | | |
|---------------------------------|---------------------------|
| 1- Pantanal of Caceres | 6- Pantanal of Aquidauana |
| 2- Pantanal of Pocone | 7- Pantanal of Abobral |
| 3- Pantanal of Barão de melgaço | 8- Pantanal of Miranda |
| 4- Pantanal of Paiguas | 9- Pantanal of Nabileque |
| 5- Pantanal of Nhecolândia | 10- Pantanal of Paraguay |

nosomosis outbreaks by *T. evansi* in dogs and horses that occurred in the Pantanal between January and July, 1994.

Material and Methods

The trypanosome survey was conducted from February to July, 1994, when cases of a disease with clinical signs comparable to those of trypanosomosis in horses and dogs were reported by ranchers in the Pantanal. After the first equine trypanosomosis outbreak in January in horses from R1 ranch, blood samples were collected from animals in neighbouring properties where trypano-

somosis-like symptoms or animal deaths were reported by the owners. A fly study carried out during this period allowed us to correlate trypanosomosis incidence and tabanid data.

Study area

The outbreaks occurred in two different regions. The first area, the Jacadigo Lake region located in the Pantanal of Paraguay River, is a plain region with abundant water bodies surrounded by mountains, mainly forested by species from the Paraguayan Chaco. This area includes ranches R0, R1, R2, and R3. The second area is located in the Pantanal of Nhecolândia and includes ranches R4, R5, R6, R7 and R8. It is a seasonal floodplain and its vegetation is characterized by a dominance of savanna species. Ranches R0, R1 and R2 are neighbours and R3 is located 20 km away. The other ranches (R4 to R8) are located about 150 km from the Jacadigo ranches (figure 1).

The climate is tropical with a 24.7 °C average temperature and a 72.7 % relative humidity. The annual rainfall is about 1,200 mm and the rainy season goes from September to April with more than 80 % of rainfall occurring from October to March (3).

Blood samples

Blood samples were taken from four horses at ranch R1, ninety-five at R2, four at R3, one at R5 and five each at R6, R7 and R8. Sick dog samples were obtained, one each from R3 and R4 and two from R5. Horses were bled from the jugular vein and dogs from the radial vein using a vacuum system (Vacuum II, Labnew, Campinas, Brazil). The diagnosis was made using the microhematocrit centrifuge test (MHCT), according to Woo (21). From each blood sample and buffy coat thin smears were prepared.

Mouse inoculation

Blood samples (0.2 ml) were injected intraperitoneally into two outbred white mice. After inoculation, Giemsa-stained thin smears of tail blood from each mouse were examined daily for two months. Seventeen blood samples (17.9 %) from the R2 horses were tested by mouse inoculation. Parasite identification was based on morphological characteristics and biometrical data, obtained from working with an eye piece micrometer as described by Hoare (12).

Fly collecting

A tabanid survey using a horse bait was done monthly from June 1992 to May 1993 in the Nhecolândia subregion. Each sampling day lasted from before sunrise

till after sunset. The flies were caught by two collectors with hand-nets (30 cm diameter). All flies that landed and/or fed on horses were captured and immediately killed in ethyl-acetate bottles. These flies were brought to the Laboratory of Entomology for identification and counting.

Results

The outbreaks began in January when seven horses from R0 and, two weeks later, four horses from R1 became sick. The clinical signs observed were fever, anemia, conjunctivitis, edema of legs and lower parts of the body, progressive weakness, a gradual paresis of hind quarters, loss of condition and inappetence (figure 2). After trypanosomosis had been diagnosed, all horses on the property were immediately treated with Naganol® (Bayer) and the symptoms disappeared.

In February, new cases occurred in a neighbouring ranch (R2) where some horses became sick. All animals in this ranch (n = 95) were sampled and 100 % of the horses proved infected with *T. evansi*. In this ranch, 48 horse deaths (50.5 %) and an abortion were recorded before treatment. One week later, 10 horses (20 %) died in R3. Before death, blood samples from four horses were collected and found to be infected with *T. evansi*. After treatment no other horses died or showed any of the symptoms. In February, four dogs, one from R3, one from R4 and two from R5, and a horse from R5, became sick. All these animals were diagnosed and treated for trypanosomosis.

Between March and June no case was reported by the ranchers. However, in July 12 horse deaths occurred in three other ranches (R6, R7 and R8) located close to R4



Figure 2: Horse presenting signs of Mal de Caderas due to *Trypanosoma evansi*. The signs include edema of the legs and lower parts of the body and loss of condition.

and R5. A horse in R6 died with the same previously described symptoms. Another horse from R6 passed by R7 and R8 where it died with symptoms of "Mal de Caderas". Later on, five (6.3 %) horses died in R7 and five (12.5 %) in R8. Before death, all of these horses showed symptoms similar to those of trypanosomosis but no laboratory diagnosis was possible. After the deaths in R8, MHCT was used to test five animals and trypanosomes were found in all of them.

Seasonal fluctuation of tabanids in the region and seasonableness of the most abundant species are shown in figure 3. The tabanids reached a population peak from September to November, during the first half of the rainy season. However, population levels of these vectors still remained high until March, end of the rainy season. *Tabanus importunus* was the most abundant and important species throughout the rainy season and reached a population peak from October to January.

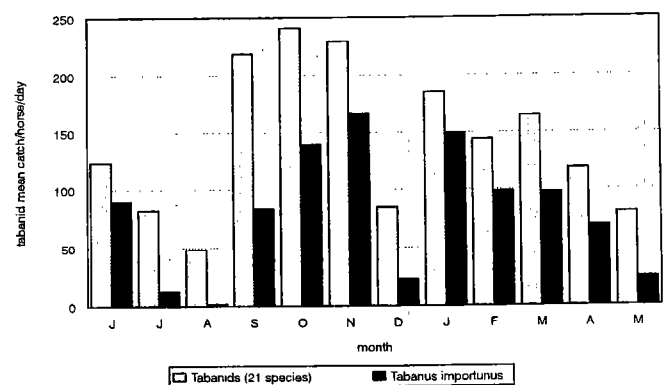


Figure 3: Seasonal distribution of tabanids captured on horses in the Pantanal region, Brazil, from June 1992 to May 1993.

Discussion

At least two different outbreaks could be identified with apparently no connection between them, involving at least 116 infected horses of which 70 died before treatment. Also, four dogs became infected.

A trypanosome survey carried out on domestic and wild animals in the Pantanal by Nunes and Oshiro (16) showed the presence of *T. evansi* in dogs, coatis (*Nasua nasua*) and capybaras (*Hydrochaeris hydrochaeris*). Stevens *et al.* (19) observed a 27 % *T. evansi* infection rate in wild capybaras and a 58 % rate in semi-captive animals in Pantanal. These authors also isolated *T. evansi* from sick dogs in the same region. According to Morales

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et al. (15), healthy capybaras can harbour *T. evansi* and can constitute a wild reservoir for horses and dogs in Colombia. Other wild mammals, such as the coati (16), the ocelot (*Felis pardalis*) (18) and the common vampire bat (*Desmodus rotundus*) (11), could harbour *T. evansi*, but their roles as reservoirs in the Pantanal is still unknown.

The vampire bat appears to play an important role in initiating outbreaks of "Mal de Caderas" in horses and, once initiated, the infection can be spread by tabanid flies (6). Greenhal *et al.* (9) described both sequential and simultaneous feeding of the common vampire bat from a single wound. This feeding behaviour, i.e. returning to the same host, does not favour the spread of the infection.

Many tabanid species have been reported as trypanosome vectors throughout the world. Krinsky (13) reviewed the role of horse flies as vectors of many trypanosome species and cited about 50 species of tabanids incriminated in the transmission of *T. evansi*. Gruvel and Balis (10) observed a seasonal correlation between the incidence of trypanosomosis in camels and the abundance of tabanids in Chad.

In the Pantanal, studies showed that the vector season coincides with the first half of the rainy season, from September/October to December/January (figure 3). However, the tabanids still 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 peaks of species with high vectorial capacity, notably *Tabanus importunus*. Lutz, in 1908, cited by Barreto, (1) concluded that trypanosomosis was transmitted mechanically by tabanids, mainly *T. importunus* and *T. trilineatus*, during an outbreak in the Marajo Island (North of Brazil). Epidemiologic observations in Venezuela revealed a high possibility of mechanical transmissions of *T. evansi* by *T. importunus* (13).

The increase in cattle trading in the Pantanal over the last four years has caused an increase in the movement of horses, cattle and dogs through various properties. This could have contributed to the spread of the disease. The optimal conditions for the animals to acquire or transmit *T. evansi* are at resting places during the trips, mainly near market places, where the number and the proximity of animals from different properties provide an excellent opportunity for disease transmission by the vectors.

Conclusion

There is strong circumstantial evidence that "Mal de Caderas" outbreaks are governed by factors such as the presence of domestic carriers (cattle, horses and dogs), wildlife reservoirs (mainly capybaras and coatis), abun-

dance of vector populations and local husbandry practices such as intense livestock trading. Whilst vampire bats can play an important role in initiating the outbreaks, tabanids are fundamental for the spread of the infection. We believe that seasonal occurrence of these factors determines the regional epidemiological situation. Finally, knowledge of risk factors is essential to control trypanosomosis in the Pantanal.

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Cette étude a été effectuée de janvier à juillet 1994 lorsque plusieurs cas de trypanosomose sont apparus chez des chiens et des chevaux du Pantanal. Des échantillons de sang obtenus de 119 chevaux malades et de 4 chiens ont été examinés par les techniques de centrifugation en tubes microhématocrites et d'inoculation à la souris. Tous les chiens et 116 chevaux (97 p. 100) étaient infectés avec *Trypanosoma evansi*. Les foyers trypanosomiens sont apparus durant ou aussitôt après le pic de prévalence des tabanides dans la région. Le passage fréquent dans les propriétés d'animaux domestiques infectés peut avoir contribué à la propagation de la maladie par les vecteurs. D'autres facteurs de risque tels que les réservoirs sauvages et la transmission par des chauves-souris sont discutés.

Mots-clés : Cheval - Trypanosomose - *Trypanosoma evansi* - *Tabanidae* - Centrifugation - Epidémiologie - Brésil.