

# DETERMINATION OF THE SEROLOGICAL PREVALENCE OF EQUINE BABESIOSES BY IFA TEST IN THE STATE OF PARÁ, BRAZIL

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**SUMMARY:** The serological prevalence of equine babesiosis caused by *Babesia equi* and *Babesia caballi* was determined in three areas of Pará State, Brazil: Marajó Island, territory of Bragantina and in Paragominas county. The Immunofluorescence-Antibody-Test (IFAT) was realized for *B. caballi* in 336 serum samples of horses with a mean age of 5,22 years (7 months to 22 years) and for *B. equi* in 340 serum samples of horses with a mean age of 5,13 years (4 months to 22 years). On the Marajó Island, 37,50% of the samples were positive for *B. caballi* and 30,83% for *B. equi*. In the territory of Bragantina, 57,38% of the samples were positive for *B. caballi* and 85,48% for *B. equi*. In Paragominas county, 40,43% of the samples were positive for *B. caballi* and 69,79% for *B. equi*.

**KEY WORDS:** Equine babesioses, IFAT, prevalence, incidence.

## INTRODUCTION

The worldwide distribution of the two *Babesia* species in horses, *B. equi* (LAVERAN 1901) and *B. caballi* (NUTTALL and STRICKLAND 1910), corresponds with the distribution of its vectors. In almost all of the tropical and subtropical areas of the world occurs babesiosis (SCHEIN 1988). Little is known about the transmission of *B. equi* and *B. caballi* in South America. The only known vector of *B. caballi* in the New World is the tick *Anocentor* (= *Dermacentor*) *nitens* (ROBY & ANTHONY 1963, PFEIFER *et alii* 1992). The evolution cycle of *B. caballi* in his vector is transovarian and transstadial. Larvae, nymphs and adults are capable of transmitting it to the vertebrate host (SCHEIN 1988). For a long time, the vector of *B. equi* in the Americas was unknown. The ticks found to infest horses in Brazil are *A. nitens* and *Amblyomma cajennense*. They seem not to transmit *B. equi* (DENNIG 1988, PFEIFER BARBOSA 1993). On grassland, where horses are held together with bovines, the tick *Boophilus microplus* also infests these horses (FALCE *et alii* 1983). In the northeast of Pará State, in the region of Bragantina, *A. nitens*, *B. microplus* and *A. cajennense* were found to parasitize horses (PEREIRA *et alii* 1998). Experimentally, *B. microplus* was identified to transmit *B. equi* (STILLER *et alii* 1989).

The immune response of the animal depends on infection. After parasitemia the animal continues for a certain time immune.

A latent infection therefore, can be diagnosed only serologically (HOLBROOK *et alii* 1972) or by isotest (FRIEDHOFF 1982). Crossed immunity does not exist (DU TOIT 1919). In endemic areas, superinfections by permanent infestations with vector ticks cause stable immunity (KNOWLES *et alii* 1980). Newborns are protected during a certain period of time by maternal antibodies (PFEIFER BARBOSA 1993, HEUCHERT 1996) and infections are inapparent in areas with enzootic stability (KNOWLES *et alii* 1980). The importation of susceptible animals into these regions causes great losses (GAUTAM & DWIVEDI 1976).

In Brazil, in 1910, equine babesiosis was reported for the first time in São Paulo. DUPONT & BARREIROS TERRA (1952) diagnosed *B. equi* in Rio de Janeiro and in 1962 *B. caballi* was diagnosed by COSTA & MELLO. Serological studies showed high prevalences of both agents in horses in São Paulo and Rio de Janeiro (TENTER & FRIEDHOFF 1986, PFEIFER BARBOSA 1993, HEUCHERT *et alii* 1999).

## MATERIALS AND METHODS

### Animals and Sera

The sera samples were collected in 12 farms in three different regions of Pará State, the Marajó Island, the microregion of

Bragantina and the municipality of Paragominas. On all farms the horses are held extensively together with bovines. The samples were collected from various breeds (Marajoara, Mangalarga, Quarter-Horse, bastards, ponies, and others) and mules. The minimum age was five months for the detection of antibodies against *B. caballi* and four months for *B. equi*, in order to prevent false results caused by maternal antibodies (PFEIFER BARBOSA *et alii* 1995).

### IFA Test

The immunofluorescence antibody (IFA) test antigens were obtained from ponies belonging to the Veterinary Highschool of Hannover, Germany, experimentally infected with the USDA (US Department of Agriculture) strains. A commercial conjugate (Anti-Horse IgG (II+L) (rb) FITC Conjugated, Miles Laboratories Inc., n° 65-168) was used, and the test performed as described

by TENTER and FRIEDHOFF (1986). The sera were titrated from 1/20 to 1/160 in doubling dilutions and the degree of fluorescence was scored as 3+, 2+, 1+, trace and negative. The highest serum dilution showing a 1+ fluorescence was recorded as the IFA test titre. A titre of 1/40 or more was considered positive for *B. equi* and a titre of 1/80 or more was considered positive for *B. caballi* (TENTER & FRIEDHOFF 1986).

### RESULTS

The IFA test was realized in 336 serum samples for *B. caballi* in horses with a mean age of 5,22 years (between 5 months and 22 years). Of this total, 153 (45,54%) were positive ( $\geq 1:80$ ), 56 (16,67%) showed a doubtful reaction (1:40) and 127 (37,80%) were negative ( $\leq 1:20$ ). 340 serum samples were examined for *B. equi* originating from horses with a mean age of 5,13 years

Table 1 - IFA test results as prevalence values for *B. equi* and *B. caballi* in serum samples from horses in the State of Pará, Brazil.

	<i>Babesia caballi</i>	<i>Babesia equi</i>
Paragominas	n = 94 negative 40% positive 41% doubtful 19%	n = 96 negative 30% positive 70%
Marajó Island	n = 120 negative 49% positive 38% doubtful 13%	n = 120 negative 69% positive 31%
Bragantina	n = 122 negative 25% positive 57% doubtful 18%	n = 124 negative 15% positive 85%
Total	n = 336 negative 38% positive 45% doubtful 17%	n = 340 negative 38% positive 62%

(between 4 months and 22 years). Of these, 210 (61,77%) were positive ( $\geq 1:40$ ) and 130 (38,24%) showed a negative reaction ( $\leq 1:20$ ) (Table 1).

On the Marajó Island, 120 serum samples were examined, of which 45 (37,50%) were positive for *B. caballi*, 16 (13,30%) had a doubtful reaction and 59 (49,20%) were negative. For *B. equi*, 37 (30,83%) samples were positive and 83 (69,17%) negative (Table 1).

In the microregion of Bragantina 122 samples were examined for *B. caballi* of which 70 (57,38%) were positive, 22 (18,03%) were doubtful and 30 (24,59%) were negative. 124 samples were examined for *B. equi*, of which 106 (85,48%) showed a positive and 18 (14,52%) a negative reaction (Table 1).

In the municipality of Paragominas, 94 samples were examined for *B. caballi*, of which 38 (40,43%) showed a positive, 18 (19,15%) a doubtful and 38 (40,43%) a negative reaction. For *B. equi*, in 96 serum samples examined 67 (69,79%) were positive and 29 (30,21%) were negative (Table 1).

An infection with *B. equi* induces a permanent immunity for the rest of the animal's life. Therefore, the rate for the annual incidence can be calculated by the mean age of the examined horses (FRIEDHOFF, personal communication). For the total of 340 animals examined we calculated an annual incidence of 12%. On the Marajó Island this incidence was 5,04%, in the microregion of Bragantina it was 17,34% and in the municipality of Paragominas 16,70% (Table 2).

## DISCUSSION

In epidemiological studies it is indispensable using immunoserology for the detection of latent infections. Nevertheless, the interpretation of the serological tests is sometimes difficult, because of persisting titres after spontaneous elimination or chemical therapy of *Babesia* spp., the existence of doubtful titres, and the absence of antibodies during the prepatent phase of infection. The first test developed for the detection of *Babesia*-antibodies in horses was the complement fixation test (CFT) by HIRATO *et alii* (1945). Since 1969 this test is the official test for the importation of horses in some countries. The CFT is very specific, but has a low sensibility (28,8% according to BOESE & PEYMANN 1994). The titres turn negative after some months of infection and therefore give false negative results (TENTER & FRIEDHOFF 1986, YOUNG 1988). That is the reason why the IFA test that detects antibodies soon after infection and during a prolonged period was chosen to realize this investigation.

Table 2 - Annual incidence of *B. equi* in Pará State, Brazil

Region	Mean Age (years)	Incidence (%)
Marajó Island	6,12	5,04
Microregion Bragantina	4,93	17,34
Paragominas	4,18	16,70
Total	5,13	12,00

The presence of *B. equi* and *B. caballi* in the North of Brazil is not surprising, because it is a region with tropical climate where the ticks that transmit *Babesia* spp. find ideal temperatures for their development.

Nevertheless, exists a regional difference. The Marajó Island stands out for having a lower prevalence and incidence than the other two regions. This is, because it is an island with two defined seasons, the rainy (December-June) and the dry (July-November) season. These radical climatical changes inhibit the evolution of the ticks, mainly of the transmitter tick *Boophilus microplus* which was identified transmitting *B. equi* (STILLER *et alii* 1989). The distribution of these ticks in Central and South America is limited by drought and tropical forests and others (YOUNG 1988).

## SUMÁRIO

A prevalência sorológica da Babesiose equina causada por *Babesia equi* e *Babesia caballi* foi determinada em três regiões do Estado do Pará: Ilha do Marajó, Microrregião Bragantina e no Município de Paragominas. Através do teste de imunofluorescência indireta (IFI) foram examinados 336 amostras de soros para *B. caballi* de cavalos com a idade média de 5,22 anos (entre 7 meses e 22 anos) e 340 amostras de soros para *B. equi* provenientes de cavalos com a idade média de 5,13 anos (entre 4 meses e 22 anos). Na região da Ilha do Marajó 37,50% das amostras foram positivas para *B. caballi* e 30,83% para *B. equi*. Na microrregião Bragantina 57,38% das amostras foram positivas para *B. caballi* e 85,48% para *B. equi*. No Município de Paragominas 40,43% das amostras tiveram uma reação positiva para *B. caballi* e 69,79% para *B. equi*.

PALAVRAS-CHAVE: Babesiose equina, sorocpidemiologia, prevalência, incidência.

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