

STUDIES OF NATURAL INFECTION WITH *Anaplasma marginale* IN NELORE CATTLE IN THE UMUARAMA MUNICIPALITY, PARANÁ STATE, BRAZIL¹

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ABSTRACT.- YOSHIHARA E., VIDOTTO O., YAMAMURA M.H., MARANA E.R.M., PACHECO R., SILVEIRA A.P. **Studies of natural infection with *Anaplasma marginale* in Nelore cattle in the Umuarama municipality, Paraná State, Brazil.** [Estudos sobre a infecção natural com *Anaplasma marginale* em gado Nelore no Município de Umuarama, Estado do Paraná.] *Revista Brasileira de Parasitologia Veterinária*, v. 12, n. 1, p. 21-26, 2003. Fundação Faculdades “Luiz Meneguel” (FFALM), Departamento de Zootecnia, Bandeirantes, PR 86360-000, Brazil. Email: vidotto@uel.br

Anaplasma marginale antibodies were investigated in 226 Nelore cow (*Bos indicus*) sera from seven beef cattle herds, in the Umuarama Region, Northwest of Paraná State. A total of 172 (76,10%) animals were seropositive by Competitive Inhibition Enzyme-Linked Immunosorbent Assay (cELISA), indicating that a significant percentage of the animals could be unprotected and at risk of acquiring *A. marginale* infection and developing Anaplasmosis. Fourteen Nelore calves were monitored every thirty days from birth until six months old. Antibodies against *A. marginale* were detected in the calve sera, from birth until six months old by cELISA test. Twelve animals were seropositive since birth, showing the transference of colostral *A. marginale* antibodies. The serum antibody level of the calves dropped quickly. Thirty days after the birth only three calves were positive. At sixty days of age the serum antibody level rose again, increasing the number of seropositive animals, reaching the highest number at 180 days of age, when thirteen calves were seropositive, indicating an active production of *A. marginale* antibodies, due to infection after birth. In conclusion, the data obtained in this study suggest that Nelore breed cattle raised extensively in the region of Umuarama, PR, are partially immunized against *A. marginale* and susceptible to present outbreaks of anaplasmosis.

KEY-WORDS: *Anaplasma marginale*, cELISA, cattle, seropositivity, occurrence, *Bos indicus*.

RESUMO

Anticorpos contra *Anaplasma marginale* foram pesquisados em soros de 226 vacas de sete rebanhos de corte, constituídos exclusivamente por animais da raça Nelore, localizados

na região de Umuarama, Noroeste do Estado do Paraná. Do total de animais examinados, 172 (76,10%) mostraram-se reagentes ao teste cELISA, indicando que uma porcentagem significativa dos animais (23,90%) pode estar desprotegida, correndo o risco de adquirir a infecção pelo *A. marginale* e desenvolver a anaplasmoze. Quatorze bezerros da raça Nelore foram monitorados a cada trinta dias do nascimento até seis meses de idade. Anticorpos contra *A. marginale* foram detectados no soro de bezerros, do nascimento até seis meses de idade, pelo teste de cELISA. Doze animais eram soropositivos ao nascimento, demonstrando a transferência de anticorpos colostrais contra o *A. marginale*. Os níveis de anticorpos séricos caíram rapidamente, sendo que trinta dias após o nascimento apenas três bezerros eram positivos. A partir dos 60 dias de idade os títulos de anticorpos séricos voltaram a aumentar, elevando o número de animais positivos, atingindo o maior número com 180 dias de idade, quando treze bezerros foram soropositivos,

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indicando a produção ativa de anticorpos específicos contra o *A. marginale*, devido à infecção adquirida após o nascimento. Os resultados sorológicos deste trabalho sugerem que bovinos da raça Nelore criados extensivamente, na região de Umuarama, PR, estão parcialmente protegidos contra a infecção por *A. marginale*.

PALAVRAS-CHAVE: *Anaplasma marginale*, cELISA, soropositividade, ocorrência, *Bos indicus*.

INTRODUCTION

Anaplasmosis is a disease caused by the Anaplasmataceae *Anaplasma marginale* (DUMLER et al., 2001) widespread in the tropical, subtropical and temperate regions causing great losses to cattle raising worldwide (ALDERINK; DIETRICH, 1983; ZAUGG; KUTTLER, 1984, PALMER et al., 1986, 1989).

Serological surveys in Brazil have indicated two distinct epidemiological situations, represented by areas of enzootic stability, where *A. marginale* prevalence has been high (RIBEIRO; REIS, 1981; RIBEIRO et al. 1984; ARTILES et al. 1995; DALAGNOL et al. 1995) and areas of enzootic instability, especially in Northeast (OLIVEIRA et al. 1992) and South (ARTILES et al. 1995) regions, where the climatic conditions are unfavorable at certain periods of the year for the development of the main vectors, such as ticks and hematophagous flies.

A competitive immunosorbent assay (cELISA) test detects specific antibodies against the main surface protein 5 (MSP5) of *A. marginale* (TORIONI DE ECHAIDE et al. 1998; MARANA et al., 1998), which is highly conserved among different *A. marginale* strains of *A. ovis* and *A. centrale* (VISSER et al. 1992). VIDOTTO et al. (1998) analyzed the seroprevalence of Anaplasmosis in dairy herds in Londrina county, Paraná State, using this cELISA test, and demonstrated that the epitope on MSP5 defined by the monoclonal antibody (mAb) ANAF16C1 is conserved within *A. marginale* in this region. KANO et al. (2002) analyzed, by Western Blotting, *A. marginale* strains from different regions of Brazil and showed that MSP5 was present in all strains investigated.

ANDRADE et al. (2001) studied the seroprevalence in herds and the dynamics of colostral and post-natal *A. marginale* acquired antibodies in dairy cows, heifers and calves from Londrina region. There were no rate differences of seropositive animals among cows and heifers categories (98,3% and 96,6% respectively), but the calves showed significantly low seropositive rate (81,2%). The antibody levels in a group of calves, monitored from the birth until one year old, decreased about the 3rd and 4th months of age. In the subsequent months, following period, several calves presented clinical signs of anaplasmosis with visible rickettsemia. In a parallel manner, the tick burden counting increased approximately in the 4th and 5th months after birth.

Bos taurus and *B. indicus* susceptibility to *A. marginale* is controversial. Parker et al. (1985) reported that *B. taurus* is

more susceptible to *A. marginale* than *B. indicus*. Other researchers did not detect significant difference in the susceptibility among *B. indicus*, *B. Taurus* or in the crosses of them under natural or experimental conditions (PATARROYO et al. 1978; OTIM et al. 1980; BOCK et al. 1999).

The present study aimed to use the cELISA test to assess the percentage of seropositivity of *A. marginale* antibodies in Nelore (*B. indicus*) herds and serological profile of calves from birth to weaning, adding more epidemiological informations to anaplasmosis in Paraná State.

MATERIAL AND METHODS

Animal and serum sample selection

From August to October 1999, 226 samples of serum of cows, raised extensively, belonging to seven beef herds, consisting exclusively of Nelore breed animals from Umuarama region, Paraná State, were examined by cELISA, for the presence of antibodies against *A. marginale*. The EPI info program 6.04b version (Center for Disease Control and Prevention, CDC, USA) was used to calculate the total number of samples. Umuarama is located in the Northwest of Paraná State (23°44'S and 53°17'W) at 480 meters of altitude. The mean minimum temperature was 18.7°C, the mean maximum temperature was 29.5°C, the mean rainfall was 92.17mm and relative humidity was 60.8% in the region during the experiment (IAPAR, 1999).

After the serological results, one of the seven herds that presented high percentage of seropositivity, was selected to investigate the *A. marginale* natural infection. For this study, carried out from November 1999 to May 2000, were utilized 14 pregnant cows and its newborn calves. The calves stayed with their dams until weaning, when they were seven months old.

Two blood samples were collected, via jugular vein using vacutainer tubes, one containing anticoagulant (EDTA) for blood smear and Packed Cell Volume (PCV) and, another without anticoagulant, to obtain serum used in the cELISA test. From the cows in the herds and, from the calves and their dams in the maximum 18 hours after the birth and afterward, only from the calves, every thirty days until they were six months old. Samples of serum were obtained and stored at -20 °C until to be tested.

cELISA

The serum samples were tested by the cELISA technique essentially as described by Torioni De Echaide et al. (1998). The test used the purified *A. marginale* recombinant MSP-5 (rMSP-5), fused to the maltose binding protein (MBP) as antigen and its respective mAb ANAF16C1 labeled with peroxidase enzyme. The sera used as negative controls were obtained from animals negative to *A. marginale* by the cELISA and IFI serological tests. Sera obtained from two calves inoculated experimentally with *A. marginale* and kept on isolation at the Veterinary Hospital at Londrina State

University were used as positive controls. The control sera and the tested samples were adsorbed (v/v) with 120 mL of MBP (1,3mg/mL), to neutralize antibodies against this protein present in the *E. coli* wall of the cattle microbiota. After adsorption 100mL of serum were incubated with 1,0 mg per well of the rMSP-5 at room temperature and, in the next step 100mL of peroxidase-labeled ANAF16Cl was added per well. The reaction was developed by the addition of substrate consisting of hydrogen peroxide and OPD (O-phenylenediamin dihydrochloride) in citrate buffer and stopped with 25 mL of 2N NH₂SO₄.

Optical Density at 492 nm (OD₄₉₂) was determined with a MAXline Microplate Readers (Molecular devices Corporation Sunnyvale, Calif.) reader. The percentage of inhibition (PI) for the test sera was calculate relative to the negative control serum using the formula: $PI = 100 - (100 \times \text{test serum absorbance} / \text{negative control absorbance})$. The cutoff point selected to discriminate between negative and positive sera was 25% inhibition.

Packed Cell Volume (PCV) and Blood smears

The PVC of each calf was analyzed in every blood sample collected from birth until 180 days of age. The technique was performed using a 75.0 x 1.0 mm capillary tube and centrifuge at 10,000 rpm for 5 minutes, analyzing the result on a reading card for PCV. Thin blood smears were made from the blood of each calf, on the stipulated collection days, stained with Giemsa, to detect *A. marginale* and to determine the parasitemias of the animal (IICA, 1984).

RESULTS AND DISCUSSION

The seropositivity of *A. marginale* in Nelore cattle, determined by cELISA, is shown in Table 1. One hundred and seventy-two of the 226 (76.10%) sera tested were positive. The number of positive animals, although high, was smaller than those found by other authors, indicating that a significant percentage of the animals could be unprotected and in risk of acquiring *A. marginale* infection and developing Anaplasmosis. Figure 1 shows the distribution of cELISA

Tabela 1. Percentage of seropositivity of *Anaplasma marginale* in Nelore cows (*Bos indicus*) of the Umuarama Region, Paraná State, Brazil, by cELISA test.

Herds	Samples	Positives	Negatives
01	25	22 (88,00%)	3 (12,00%)
02	46	33 (71,74%)	13 (28,26%)
03	30	21 (70,00%)	9 (30,00%)
04	39	38 (97,44%)	1 (2,56%)
05	35	23 (65,71%)	12 (34,29%)
06	30	15 (50,00%)	15 (50,00%)
07	21	20 (95,24%)	1 (4,76%)
TOTAL	226	172 (76,10%)	54 (23,90)

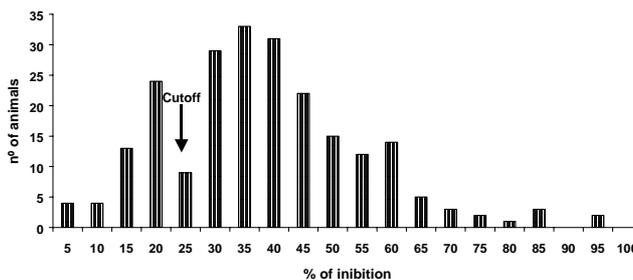


Figure 1. Frequency distribution of the cELISA percent inhibition values for 226 sera collected in Nelore breed cows herds, endemic for *Anaplasma marginale*, from the Umuarama Region, Paraná State, Brazil.

percent inhibition values for the herd cows. These values show that although positive by the cut-off point of the reaction, many of the animals presented relatively low inhibition percentages, indicating low levels of antibodies against *A. marginale*. These data suggest enzootic instability in the region studied according to the criteria established by Mahoney and Ross (1972) to determine areas of enzootic stability or instability. Different authors have found *A. marginale* prevalence rates varying from 0.89 to 99%. This variation may be due to geographic location. El-Metenawy (2000) found a prevalence rate of 0.89% in the arid and mountainous region of Saudi Arabia, while in semiarid regions in Idaho (USA) prevalence was about 31% to 37% (MAAS et al. 1986). The prevalence rates are much higher in the tropical regions, from 80% to 99% (PATARROYO et al. 1978; NICHOLLS et al. 1980; KNOWLES et al. 1982; PAYNE; OSÓRIO, 1990; COSSIO-BAYÚGAR et al. 1997).

The *A. marginale* seroprevalence rate in Brazil, according to studies in different regions, varies from 81.1 to 96.9% (RI-BEIRO et al., 1984; ARAUJO et al. 1998; VIDOTTO et al. 1998; MADRUGA et al. 2000; ANDRADE et al., 2001), indicating that most area of the country can be considered in enzootic stability.

In Paraná State, Vidotto et al. (1998) also used the cELISA to analyze sera from Holstein (*B. taurus*) and Cross-breeding cows and found a higher rate (87.56%) of seropositive animals than in the present study (76.88%), studying Nelore breed animals (*B. indicus*). *B. taurus* and *B. indicus* susceptibility to *A. marginale* is controversial. Parker et al. (1985), under experimental conditions, reported that animals in the 1-2 year old group of *B. taurus* breed were more susceptible than the animals of the *B. indicus* breed to *A. marginale*, considering the percentage of parasitemia, globular volume and antibody titers, while other authors did not find significant difference between the breeds (PATARROYO et al. 1978; OTIM et al. 1980; BOCK et al. 1999).

A. marginale antibodies were detected in calves sera from birth to six months old by the cELISA test. Twelve of the 14 calves studied were seropositive at the birth (Figure 2), showing the transference of colostral *A. marginale* antibodies. Most of the calves' dams showed high inhibition percentages in the

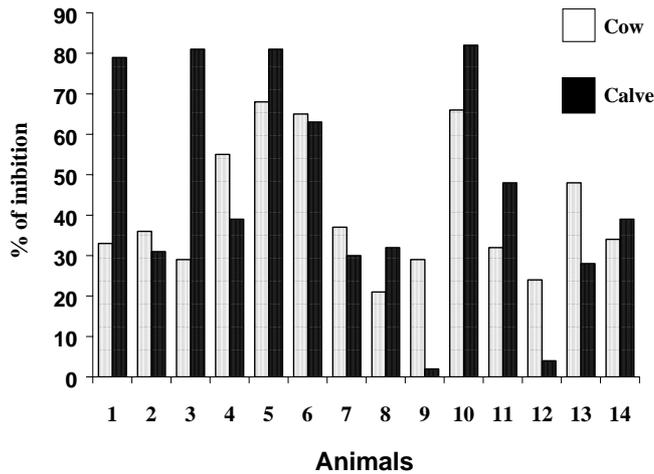


Figure 2. Frequency distribution of the cELISA percent inhibition values for Nelore breed dams and calves sera collected in a herd, endemic for *Anaplasma marginale*, at the birth day, from the Umuarama Region, Paraná State, Brazil.

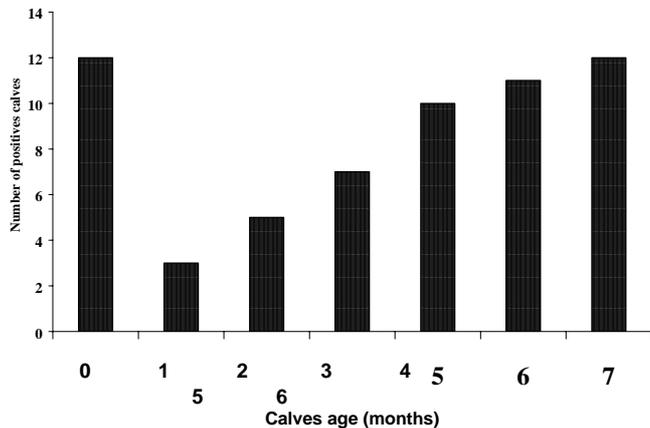


Figure 3. *Anaplasma marginale* serological variation on the number of positive Nelore breed calves from the birth until six months old, in Umuarama Region, Paraná State, Brazil.

cELISA test (Figure 3) that indicates the presence of seric antibodies which were transferred to the calves. However, the antibody titers transferred to the calves decreased rapidly (Figure 4). Only three animals were positive by the cELISA test one month after birth (Figure 2). When the calves were two months old the level of seric antibody increased again, reaching higher percentage at six months of age (Figure 4) indicating active specific *A. marginale* antibody production possibly due to infection acquired after birth. Swift and Thomas (1983) had already shown experimentally passive colostrum antibodies transmission to the calves in heifers experimentally infected with *A. marginale* during pregnancy. Madruga et al. (1987) monitored crossed Nelore breed calves from birth until 210 days of age and also detected a fall in colostrum antibodies against *A. marginale* at the mean age of 47 days, indicating the disappearance of the passively acquired humoral immunity or its presence at very low levels. In another study Madruga et al.

Figure 4. Mean frequency distribution of the cELISA percent inhibition values for Nelore breed calves sera collected in a herd, endemic for *Anaplasma marginale*, from the birth until six months old, in Umuarama Region, Paraná State, Brazil.

(1985) examined 50 Nelore, Ibagé and crossed Nelore breed calves and reported that the number of serologically negative animals increased at 30 days and reached a maximum at 60 days, suggesting that at around 60 days of age there is a calf population in potential risk of contracting the infection and presenting clinical signs of the disease.

All the 14 calves at 30 days of age were infested by low level of *Boophilus microplus* and this parasitism continued until the end of the study when the animals were six months old. Although other mechanisms of transmission for *A. marginale* are mentioned, such as mechanical transmission by hematophagous insects (VANZINI; RAMIRES, 1994; EWING et al. 1997), it seems to have a very direct relationship between the tick presence and the active production of specific *A. marginale* antibodies by the calves, indicating a possible horizontal transmission of this rickettsia by the *B. microplus*. Andrade et al. (2001) carried out a similar study with Holstein breed calves from birth until one year of age and verified that there was a later decreasing in the seric antibody levels, with a greater number of negative animals around three months of age. These authors also showed that the antibodies raised again at five months of age and remained high until the end of the observations. Holstein calves similarly to the Nelore breed calves, it seemed to have a direct relationship between the presence of ticks and the increase in the seric antibody levels.

The PCV values of the calves remained between 36% and 49% during the controlled period, which are within the normal parameters for cattle (FELDMAN et al., 2000). There was no detectable *A. marginale* parasitemia by the Giemsa stained blood smears and any animal was observed with clinical signs of anaplasmosis. In contrast to these data, Madruga et al. (1985) reported the presence of *A. marginale* in blood smears in Nelore, Ibagé and crossed Nelore breed calves with 30 days old. Furthermore, Andrade et al. (2001) detected rickettsemia, varying from 0.1% to 3.8% associated with clinical signs of anaplasmosis, in Holstein breed calves heavily infested by *B. microplus*.

The increase in the seric antibody levels in calves exposed to natural *B. microplus* infection, the detection of the parasite in blood smears and the arising of the clinical signs of anaplasmosis, coinciding with the presence and increase in the tick population indicate the involvement of this Ixodidae in the *A. marginale* transmission to calves. How transmission takes place still is unclear. Stich et al. (1989) proved transestadial transmission but could not prove transovarian transmission in *Dermacentor variabilis*. Smith et al. (1993) mentioned several studies where transovarian transmission was not produced, while the transestadial and intraestadial transmission were successfully obtained in different genus of ticks. Vanzini and Ramirez (1994) reported few authors that had successful in the *A. marginale* transovarian transmission by ticks of the *Dermacentor* genus. On the other hand, these same authors mentioned several studies that report that transovarian transmission did not take place but intraestadial and transestadial transmissions did.

Several aspects of *A. marginale* epidemiology are still not clear, and require more detailed studies on the transmission mechanisms of this rickettsia by *B. microplus*. The serological data obtained in this study suggest that Nelore breed cows and calves raised extensively in the region of Umuarama, PR, are apparently suitable to present outbreaks of anaplasmosis.

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