Natural infection of calves by *Anaplasma marginale* in dairy herds of the Metalúrgica Region, Minas Gerais


The dynamic of natural infections by *Anaplasma marginale* in calves was evaluated during a period of one year on two farms located in the Metalúrgica Region, State of Minas Gerais, Brazil. Blood samples were collected weekly for rickettsemia and packed cell volume (PCV) determination. The animals born from March to July suffered the infection in October and November, independently of their age, whilst calves born from September to December acquired the infection during the first days of life. These animals presented patent rickettsemia from 30 days of life. During the patent period PCV decreased after one week of infection, ranging from 20 to 23%. It was concluded, that in the region studied, the transmission of *A. marginale* is influenced by climatic conditions, and that calves born during the dry season are more likely to acquire the infection when they are exposed to high transmission levels during the subsequent raining season.

**INDEX TERMS:** *Anaplasma marginale*, anaplasmosis, cattle, epidemiology.
and another called AW with temperatures above 18°C in Winter (Antunes 1986).

Climate, soil and cattle biotypes modulate the capacity of the land to support Boophilus microplus (Guglielmone 1995) and hematophagous diptera population, considered the main vectors for bovine anaplasmosis (Yeruham & Braverman 1981).

Despite the high prevalence of antibodies against A. marginale previously reported in the State of Minas Gerais and other States (Ribeiro & Reis 1981b, Madruga et al. 1985), clinical anaplasmosis has occurred, suggesting situations of enzootic instability. Although the importance of A. marginale is recognized, the existing epidemiological data have been limited to certain areas and cannot be applied to the whole State. This is due to the existence of different ecological conditions, and also to the different management systems adopted (Ribeiro & Reis 1981a,b).

The purpose of the present study was to determine the dynamic of A. marginale natural infections in a CW climatic area, during a period of one year, aiming to understand the epidemiology of this disease, providing knowledge for the proposal of effective prevention and control measures for this area.

**MATERIALS AND METHODS**

The study was carried out from March/1997 to June/1998 in two dairy farms (A and B), located at 766 m altitude, 19°27'S latitude and 44°14'W longitude, with a mean temperature of 21.5°C, in the Metalúrgica Region of the Minas Gerais State (CW climatic area, Fig. 1).

On Farm A, the newborn female Holstein calves were kept with their respective mothers for two days after birth and then they were transferred to individual pens where they were kept until 30 days of age. The calves received four liters of milk daily and balanced ration ad libitum. After this period, they were transferred to collective pens with restricted access to pasture (twice a week), where they were kept until 90 days of age. During this period they received balanced ration, hay and water ad libitum. The calves were then maintained on star grass pastures, receiving corn silage and concentrate from May to October or pasture plus concentrate from November to April.

On this farm, 55 females calves were divided into two groups, according to the period of their birth:

- Group 1 was composed of 30 animals born from March to July 1997;
- Group 2 was composed of 25 animals born from September to December 1997.

On Farm B, Holstein and Brown Swiss female calves were kept with their respective mothers for two days and were transferred to calf hutch, where they stayed up to 30 days of age, and then they were put on Brachiaria pasture. The management was similar to that described for Farm A. In Farm B, 33 female calves born from March to July 1997 were monitored.

Blood samples were collected weekly over a period of one year. Giemsa stained thin blood smears were prepared and examined microscopically to calculate the rickettsemia. The packed cell volume (PCV) was determined using the microhematocrit technique (Schalm et al. 1975). Serum samples were tested monthly by the indirect fluorescent antibody test (IFAT) in order to measure anti-A. marginale antibody levels (IICA 1987).

Data were analyzed by the Chi-square test (Sampaio 1998).

**RESULTS**

During the experimental period, the pluvial precipitation was concentrated from November to March, and a defined dry period occurred from May to August.

From April to August, the mean temperature was below 20°C and pluvial precipitation was less than 50 mm, conditions considered unfavorable to the development of the biological cycle of ticks and biting flies. From September on, there was an increase in temperature and rainfall values, generating more favorable conditions for the development of Anaplasma vectors (Fig. 1).

**Fig. 1. Temperature and rainfall in the Metalúrgica Region of Minas Gerais, 1997/98.**

**Fig. 2. Anaplasma marginale infection in calves born from March to June (n=30) in Farm A, Metalúrgica Region of Minas Gerais, 1997.**
Among the animals born from March to July (Group 1), 70.0% (21/30) acquired the infection when they were five months old or older (Table 1). The animals had higher levels of infection from October to December, independently of their ages (Fig. 2). The mean rickettsemia ranged from 0.4 to 3.8%, being higher in animals with ages between 7 and 8 months (p< 0.05), during November and December.

Table 2 shows the frequency of positive sera by IFAT among calves in Group 1. The number of positive animals decreased to 13.6 % during the period of 90 to 150 days. Thereafter, the number of positive animals increased gradually, and the highest titers were recorded.

### Farm A
Among the animals born from March to July (Group 1), 70.0% (21/30) acquired the infection when they were five months old or older (Table 1). The animals had higher levels of infection from October to December, independently of their ages (Fig. 2). The mean rickettsemia ranged from 0.4 to 3.8%, being higher in animals with ages between 7 and 8 months (p< 0.05), during November and December.

Table 2 shows the frequency of positive sera by IFAT among calves in Group 1. The number of positive animals decreased to 13.6 % during the period of 90 to 150 days. Thereafter, the number of positive animals increased gradually, and the highest titers were recorded.

### Farm B
On farm B, the majority of animals (57.6%) was infected when they were 5 to 8 months old. The higher infection rates occurred from September to December, when 69.7% acquired the infection (Fig. 4). The mean rickettsemias ranged between 0.9% and 2.9%, being higher (p<0.05) in animals over 5 months of age (Table 1). In this group the dynamic of anti-\textit{A. marginale} antibodies was similar to that described for Farm A (Table 2).

Recrudescence was observed in 8 (24.0%), out of 33 animals. The rickettsemias ranged from 0.3% to 1.5% and started 20 days after the primary infection.

Before the patent rickettsemia of \textit{A. marginale}, the mean PCV was 32.0%. After rickettsemias, this value decreased to 24.0% during the first week of infection and was significantly different (p<0.05) from those observed before infection. The mean PCV value during recrudescence was 25.0%, but no difference (p<0.05) was observed between PCV values during the first infection and recrudescence.

### Table 1. Natural infection of calves by \textit{Anaplasma marginale} in two dairy herds, in the Metalúrgica Region of Minas Gerais, 1997/98

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Farm A Infection (%)</th>
<th>Rickettsemia (%)</th>
<th>Farm B Infection (%)</th>
<th>Rickettsemia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2</td>
<td>26.7</td>
<td>1.8</td>
<td>32.0</td>
<td>0.6</td>
</tr>
<tr>
<td>3-4</td>
<td>3.3</td>
<td>0.4</td>
<td>36.0</td>
<td>0.7</td>
</tr>
<tr>
<td>5-6</td>
<td>56.7</td>
<td>1.3</td>
<td>28.0</td>
<td>1.4</td>
</tr>
<tr>
<td>7-8</td>
<td>13.3</td>
<td>3.8</td>
<td>4.0</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15.1</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27.3</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21.2</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36.4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

The mean PCV, registered before the patent rickettsemia was 33.0% in both groups. After infection this value decreased to 25.0 % in Group 1, and to 27.0% in Group 2, and were statistically different (p< 0.05) from the values observed before infection. On the other hand, no difference (p<0.05) was observed in PCV during recrudescence.

### Table 2. Frequency of calves with anti-\textit{Anaplasma marginale} antibodies detected by IFAT on two dairy farms in the Metalúrgica Region of Minas Gerais, 1997/98

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Farm A</th>
<th>Farm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>90.9</td>
<td>81.8</td>
</tr>
<tr>
<td>31 - 60</td>
<td>68.2</td>
<td>63.6</td>
</tr>
<tr>
<td>61 - 90</td>
<td>27.3</td>
<td>45.5</td>
</tr>
<tr>
<td>91 - 120</td>
<td>13.6</td>
<td>30.3</td>
</tr>
<tr>
<td>121 - 150</td>
<td>13.6</td>
<td>45.5</td>
</tr>
<tr>
<td>151 - 180</td>
<td>81.8</td>
<td>63.6</td>
</tr>
<tr>
<td>181 - 211</td>
<td>96.0</td>
<td>66.7</td>
</tr>
<tr>
<td>212 - 240</td>
<td>98.0</td>
<td>81.8</td>
</tr>
</tbody>
</table>

*Serological tests were carried out only in Group 1.

In Group 2 (animals born from September to December), 68.0% (17/25) were infected during the first 4 months of life, while the remaining calves were infected when 5 to 8 months old. Maximum infection levels occurred from January to March, when 72.0% of calves showed rickettsemia (Fig. 3). In this group, the rickettsemia was significantly lower (p< 0.05) than in Group 1 (Table 1).

On Farm A, recrudescence was observed in 22 (40.0 %) among the 55 studied animals, being 8 (15.0%) in calves from Group 1 and 14 (25.0 %) in calves from Group 2. The first recrudescence appeared 30 days after the primary infection, with rickettsemias ranging from 0.03% to 5.1%. A second recrudescence was observed in 24.0% of the calves, between 20 and 40 days after the first recrudescence.

**Fig. 3. Anaplasma marginale infection in calves born from September to December (n=25) on Farm A, Metalúrgica Region of Minas Gerais, 1997/98**

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DISCUSSION

The epidemiology of anaplasmosis is difficult to understand because the transmission of *Anaplasma marginale* under natural conditions is not well defined. *A. marginale* can be transmitted by ticks, as well as by biting flies in tropical and subtropical areas (Wanduragala & Ristic 1993). Among the species of ticks mentioned as vectors of anaplasmosis, *Boophilus microplus* has been considered the main transmitter of this rickettsia (Rogers et al. 1978, Hermans et al. 1994). Nevertheless, the transovarian transmission of *A. marginale* in *B. microplus* is still controversial, since several studies could not demonstrate it, and its occurrence appears to be at low frequency in nature (Potgieter 1979, Ribeiro et al. 1996). The transestadial transmission of anaplasmosis assumes real importance, particularly considering that suckling calves can be infested with ticks from their mothers. The transestadial and intraestadial transmissions are likely to be more frequent in intensive exploitation systems, where a close physical contact among the animals is common, allowing ticks to be transferred (Mason & Norval 1981, Aguirre et al. 1994).

In the region studied, under natural conditions, *B. microplus* ticks have four generations during the year. Tick infestation is observed throughout the year, with the highest incidence occurring from November to January, when the mean temperature is higher than 20°C (Magalhães 1989). Transmission of *A. marginale* by hematophagous diptera is known to be of importance under field conditions, but no data are available for South America regarding their relevance under local conditions (Guglielmone 1995). As far as flotation of biting fly populations in this area is concerned, there is a seasonal occurrence with two peaks of high incidence, one in October (Spring) and another in January (Summer), when the climatic conditions are favorable for the development of these vectors (Rodriguez 1998).

The region studied is characterized by two well defined seasonal periods, suggesting a direct relationship between climatic conditions and transmission of *A. marginale*.

From March to September (Autumn–Winter) there is a reduction of the population of vectors, caused by less favorable climatic conditions (decrease in temperature and rainfall), which affect their biological life cycle. On both farms, the majority of animals born in this period acquired the *A. marginale* infection at the beginning of October and November, independently of their age. This period coincides with an increase in temperature and the beginning of the rainy season, which results in an increased number of anaplasmosis vectors. At this time, most animals were 6 to 7 months old and were vulnerable to have clinical disease. It is known that susceptibility to anaplasmosis increases with age and with decline of colostral antibodies (Roby et al. 1961).

In contrast, animals born from September to December (Group 2) acquired the primary infection during their first days of life. Patent rickettsemias were detected from day 30 and were concentrated in January. These results coincide with previous studies in endemic areas (Corrier & Guzman 1977, Ribeiro & Reis 1981a, Madruga et al. 1985).

Comparing the two management systems, it is noticed that on Farm B calves are exposed to the vectors at an early age, as they have access to grass since their first days of life. Under this circumstance the primary infection occurs earlier than that occurring in calves kept in calf hutch.

The results from this study suggest that, in the Metalúrgica Region of the Minas Gerais State, there is a need to adopt preventive measures to protect calves born between April and August from having clinical infections at the beginning of the rainy season. This would help to control the disease, avoiding severe economical losses due to mortality and treatment of a high number of animals.

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