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Abstract - The aim of this study was to understand the dynamics of circulating antibodies against Borrelia burgdorferi in cows during the peripartum and in their calves during the first year of life, using an indirect enzyme-linked immunosorbent assay (iELISA). Sera from twenty cows and their calves were tested by an iELISA for detection of homologous antibodies against B. burgdorferi. The test showed 93.33% sensitivity and 86.66% specificity. At pre-partum, birth and post-partum, cows showed seropositivities of 90%, 30% and 86%, respectively. However, only 50% of the calves were seropositive after colostrum ingestion. The calves' seropositivity over their first year ranged from 0% in the second and third months to 64% at 12 months. Therefore this region should be considered an enzootic instability area.

Keywords : *spirochaetes, antibodies, bovines, elisa, borrelia.*

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Detection of Antibodies against *Borrelia Burgdorferi* in Periparturient Cows and Calves during the First Year Old by Indirect Enzyme-Linked Immunosorbent Assays (iELISA)

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Abstract - The aim of this study was to understand the dynamics of circulating antibodies against *Borrelia burgdorferi* in cows during the peripartum and in their calves during the first year of life, using an indirect enzyme-linked immunosorbent assay (iELISA). Sera from twenty cows and their calves were tested by an iELISA for detection of homologous antibodies against *B. burgdorferi*. The test showed 93.33% sensitivity and 86.66% specificity. At pre-partum, birth and post-partum, cows showed seropositivities of 90%, 30% and 86%, respectively. However, only 50% of the calves were seropositive after colostrum ingestion. The calves' seropositivity over their first year ranged from 0% in the second and third months to 64% at 12 months. Therefore this region should be considered an enzootic instability area.

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I. Introduction

Lyme borreliosis is an infectious disease caused by spirochaetes of the *Borrelia burgdorferi* sensu lato complex, which comprises at least 18 species worldwide (Margos et al. 2010). The spirochaetes can infect humans and animals (Fingerle et al. 2008), and lyme borreliosis is considered the most prevalent tick-borne disease in Europe and the United States of America (USA). In these countries, the infection is usually detected by identification of antibodies against specific antigens of *Borrelia* sp. in body fluids. However, in Latin America there are still no standardized techniques due to the difficulty of producing antigens of good quality on a large scale.

For the detection of antibodies against *Borrelia* sp. in immunoassays, such as enzyme-linked immunosorbent assay (ELISA) and enzyme-linked fluorescent assay (ELFA), antigens have been purified from long-term *in vitro* cultures. Establishing *in vitro* cultures from local strains to use as antigen sources is hampered primarily by the need for complex and costly culture media, as well as variation in growth rates and gene expression between strains (Yang et al., 2001).

In North America and Europe, most ruminants positive for Lyme disease are asymptomatic. Indirect enzyme-linked immunosorbent assays (iELISA) and Western blotting techniques are most suitable for serological diagnosis in these animals, but should be interpreted in conjunction with epidemiological data (Fonseca et al., 1996; Ishikawa et al., 1997).

In Brazil, seroepidemiological studies on domestic animals have previously detected antibodies against *B. burgdorferi* in dogs (Alves et al., 2004), horses (Gallo et al., 2009), cattle (Guedes Júnior et al., 2008) and buffaloes (Corrêa at al., 2012). Following on from this, the aim of this study was to use iELISA to determine the dynamics of circulating antibodies against *B. burgdorferi* in peripaturient cows, and in their calves during their first year of life.

II. Material and Methods

A longitudinal study was conducted between 2009 and 2011 in the dairy cow sector at the Seropédica Experimental Station, which belongs to the Agricultural and Livestock Research Company of the State of Rio de Janeiro (Empresa de Pesquisa Agropecuária do Estado do Rio de Janeiro; Pesagro-Rio). Monthly sera samples were taken from 20 cows during their last two months of pregnancy, month of birth and first two months of lactation. Their offspring were sampled monthly from birth through the first year of life.

The pasture system consisted of rotation in fields of *Brachiaria decumbens* and *Panicum maximum,* with stocking density of three animals per hectare. During the study ticks were observed on animals and pastures. The animals were dewormed (levamisole phosphate, 1ml/50kg, BLLVER) every three months and treated against ticks (Fluazuron, 1ml/10kg, Ouro Fino) monthly.

Blood serum samples were tested by iELISA using the technique described by Machado et al. (1997) for *Babesia bovis,* adapted to *B.burgdorferi*. The protein

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concentration of antigen, from *B. burgdorferi* sensu stricto G 39/40 of North American origen, was adjusted to 5 μg/mL. The plates (Costar 3590, Corning Co.) were sealed and incubated overnight at 4 ° C. The test samples were diluted 1:400 in PBS-Tween with 5% rabbit serum, and 100μl were added to ELISA plate. After an incubation at 37°C in humid chamber for 90 minutes, 100 μl of the alkaline phosphatase anti-bovine IgG (Sigma Chemical Co.) at 1:30000 according to manufacturer's recommendations were added to each well. The plates were incubated in the same conditions before. The substrate p-nitrophenyl phosphate (PNPP, Sigma Chemical Co.) was added at 5mg per plate and the plates were incubated at room temperature. After 50 min, the absorbance was read at 405 nm in a micro-ELISA reader (Labsystems iEMS Reader MF).

The immunological response of each serum was estimated at each dilution by determining the positive serum ratio (S/P), calculated from positive and negative reference sera (Machado et al. 1997) using the following equation:

$$S/P = \frac{(\text{mean sample absorbance}) - (\text{mean absorbance of negative reference serum})}{(\text{mean absorbance of positive serum reference}) - (\text{mean absorbance of negative reference serum})}$$

Optical density values were grouped into ELISA levels (EL), which ranged from 0 (lowest level) to 9 (highest level). The maximum amplitude from the lowest level was determined by the mean absorbance values of the negative group plus two standard deviations, as established by Machado et al. (1997). The subsequent levels were determined by increments of 35%. Cutoff was calculated according to Frey et al. (1998), with 12 negative sera for each plate.

The chi-square test or Fisher's exact test was used to determine significant differences in the percentages of samples testing. P values <0.05 were considered statistically significant. Analysis was performed by the statistical software R Foundation for Statistical Computing, version 2.12.2 (2011).

III. Results

The serological results for the presence of homologous antibodies against *B. burgdorferi* in periparturient cows and their calves during the first year of life are shown in Table 1 and Figure 1.

Three weeks before birth, 90% of cows were seropositive, a week before the birth this dropped to 30%. The same cows two months after birth showed 86% seropositivity for *B. burgdorferi*. Despite the high percentage of seropositive cows and a significant migration of antibodies to colostrum, only 50% of the calves were observed to be seropositive after ingestion of colostrum. In addition, there was a significant variation in the prevalence of homologous antibodies against *B. burgdorferi* in calves during the first year of life.

All the calves were seronegative for *B. burgdorferi* during their second and third months. Subsequently, between the fourth and tenth months, the percentage of seropositive animals ranged from 20% (4/20) to 40% (8/20). In the 11th and 12th months, the calves showed a significant increase in the prevalence of antibodies to *B. burgdorferi,* reaching a maximum of 64% seropositivity in 11th month (Figures 2 and 3).

Only four calves were consistently seropositive from the first infection (four months old) until the twelfth month, while five animals were seronegative throughout the study. After the loss of maternal antibodies, 20% of the calves became positive for the first time at four months, 5% at five months, 10% at six months, 10% at nine months, 20 % at eleven months and 10% at twelve months.

IV. Discussion

The use of enzyme linked immunosorbent assays with recombinant antigens produced from cell culture systems has improved sensitivity and specificity for the detection of antibodies against *B. burgdorferi* in humans, canines, and horses (Schulte-Spechtel et al. 2003). For studies in cattle, *B. burgdorferi* antigens produced from cell cultures are used in ELFA and iELISA (Wells et al. 1993).

The occurrence of probable Lyme disease in Brazil, known there as Baggio-Yoshinari (Mantovani et al., 2007), and the close phylogenetic association found in affected cattle from Brazil from PCR for *B. burgdorferi* using *flgE* primer by Mantovani et al. (2012), suggested the possibility of serological cross-reactivity between spirochetes of the genus *Borrelia* (Rogers et al., 1999), justifying the seroepidemiological study in cattle. The rate of seropositive cattle indicates the presence of some agent of tick-borne spirochetosis in the herd assessed, as previously observed by Guedes Júnior et al. (2008), who found 54.9% seropositivity to *B. burgdorferi* in dairy cows in the state of Pará, Brazil.

The age of the animal demonstrated a positive relationship with seropositivity, in which cows had higher percentage of seropositivity than calves. Stefancikova et al. (2002) found a similar higher seroprevalence in older cows. Lyme borreliosis has often been found in first-birth heifers, presenting as a problem affecting particular herds (Parker; White, 1992).

The fall in antibody titers in cows during the pre-partum demonstrated efficiency in passive transport of

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antibodies against *B. burgdorferi* via colostrum. However, the small number of seropositive calves after colostrum ingestion shows failure in some stage of lactation (Sasaki et al., 1976).

The increase in the percentage of seropositive calves during the first year of life demonstrates the existence of some environmental transmission, since most of the agents of the complex *Borrelia burgdorferi* sensu lato are transmitted by ticks of the family Ixodidae (Bennett, 1995; Barbour et al., 1996).

V. Conclusions

Although an organism of the genus *Borrelia* has not been isolated from cattle in Brazil, the high seroprevalence observed in this study is a strong indication that this pathogen exists. Molecular studies should be undertaken in order to confirm the agent in these animals.

The decrease in antibody titers in cows during the pre-partum demonstrated efficiency in passive transport of antibodies against *B. burgdorferi* via colostrum. Furthermore, the increase in the percentage of seropositive animals during the first year of life, suggests the existence of some environment transmission, however, studies of possible vectors, such as *Rhipicephalus microplus* and wild hosts, are necessary.

Acknowledgements

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Legends for Illustrations and table

Table 1 : Values of positive serum ratio for ELISA levels 0-9, during the periparturient cows and their calves in the first year of life

EL	S/P	Calves		Cows		
		<6 months	>6 months	Pre-partum	Birth	Post-partum
0	0 – 0.173	15.0%	0.0%	0.0%	20.0%	0.0%
1	0.174 – 0.235	22.0 %	5.0%	8.0%	30.0%	15.0%
2	0.236 – 0.318	21.0%	12.0%	5.0%	20.0%	10.0%
3	0.319 – 0.430	18.0%	30.0%	20.6%	20.0%	15.0%
4	0.431 – 0.582	17.0%	30.0%	40.0%	10.0%	20.0%
5	0.583 – 0.787	7.0%	14.0%	10.0%	5.0%	15.0%
6	0.788 – 1.0.63	0.0%	4.0%	2.0%	0.0%	10.0%
7	1.064 – 1.436	0.0%	3.0%	10.0%	0.0%	10.0%
8	1.437 – 1.940	0.0%	2.0%	5.0%	0.0%	5.0%
9	> 1.941	0.0%	0.0%	0.0%	0.0%	0.0%

EL – ELISA levels; S/P - positive serum ratio

Figure 1 : Absorbance values of sera from cows naturally infected with *Borrelia burgdorferi* during the peripartum

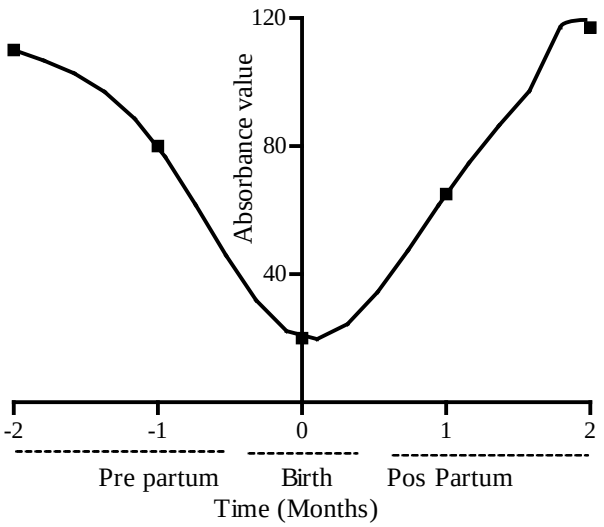

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Figure 2 : Absorbance values of sera from calves naturally infected with *Borrelia burgdorferi* during the first year of life

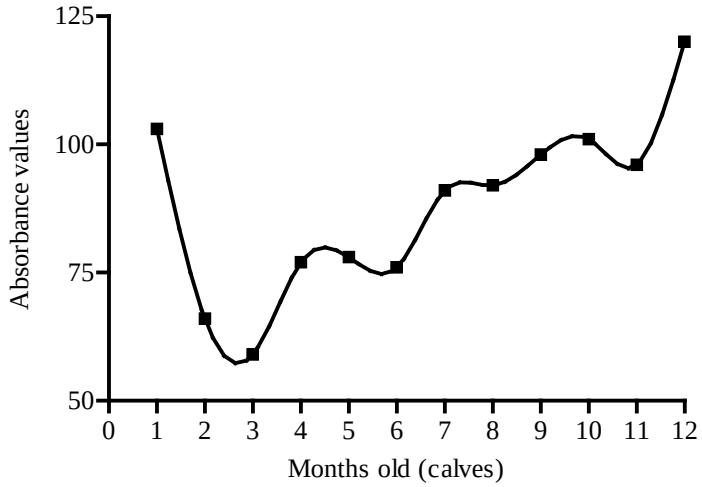
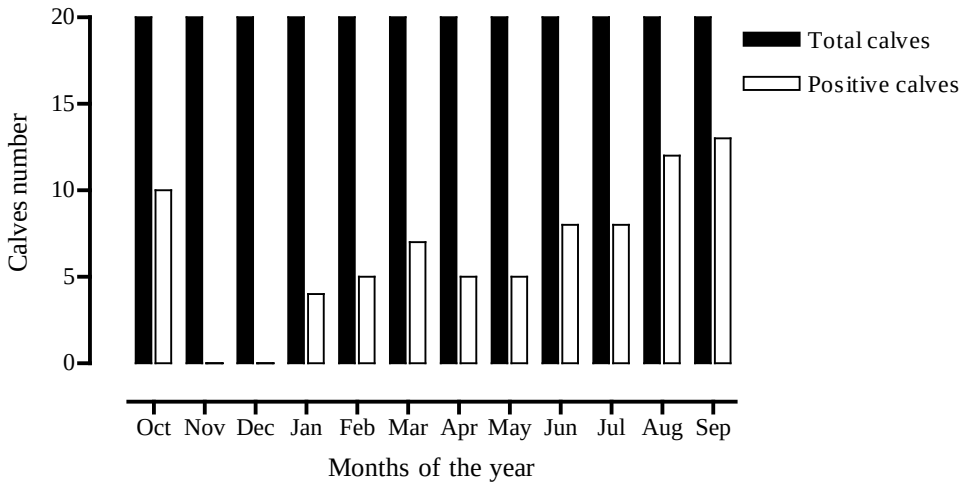


Figure 3 : Calves number naturally infected with *Borrelia burgdorferi* during the first year of life



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