

<https://doi.org/10.31533/pubvet.v15n02a759.1-5>

## Detection of hemoparasites in dairy cattle from the state of Espírito Santo

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**Abstract.** The objective of this study was to verify the occurrence of *Anaplasma marginale*, *Babesia bovis* and *Babesia bigemina* in dairy cattle from the central mesoregion of the State of Espírito Santo, through direct parasitological tests (blood smears) and serological tests (Enzyme Linked Immunosorbent Assay - ELISA). Blood samples were collected from 159 animals from 6 farms, concentrated in the central mesoregion of the state of Espírito Santo. The blood collected was stored in EDTA tubes, used to perform blood smears, and in tubes without anticoagulant, to obtain serum. The sera obtained were used for the detection of IgG antibodies to *A. marginale*, *B. bovis* and *B. bigemina*. Of the 159 blood smears analyzed, 64 (40.2%) and 11 (6.9%) were positive for *A. marginale* and *Babesia* sp., respectively. The ELISA of the 159 samples, 9 (5.7%), 59 (37.1%) and 47 (29.5%) were seropositive for *A. marginale*, *B. bovis* and *B. bigemina*, respectively. The results obtained characterize these farms of the central mesoregion of the state of Espírito Santo, as of enzootic instability for Bovine Parasitic Sadness (TPB) agents, presenting a high risk of occurrence of anaplasmosis and babesiosis outbreaks.

**Keywords:** Anaplasmosis, Babesiosis, ELISA, Bovine Parasitic Sadness

## Detecção de hemoparasitos em bovinos leiteiros do estado do Espírito Santo

**Resumo.** O presente trabalho teve como objetivo detectar a presença de *Anaplasma marginale*, *Babesia bovis* e *Babesia bigemina* em bovinos leiteiros da mesorregião central do estado do Espírito Santo, por exames parasitológicos diretos (esfregaço sanguíneo) e sorológicos (Ensaio Imunoabsorção Enzimática - ELISA). Foram colhidas amostras de sangue de 159 bovinos leiteiros provenientes de 6 propriedades rurais, concentradas na mesorregião central do estado do Espírito Santo. O sangue coletado foi armazenado em tubos de EDTA, utilizado para realizar os esfregaços sanguíneos, e em tubos sem anticoagulante, para obtenção do soro. Os soros obtidos foram utilizados para a detecção de anticorpos IgG para *A. marginale*, *B. bovis* e *B. bigemina*. Dos 159 esfregaços sanguíneos analisados, 64 (40,2%) e 11 (6,9%) foram positivos para *Anaplasma marginale* e *Babesia* sp., respectivamente. No ELISA das 159 amostras, 9 (5,7%), 59 (37,1%) e 47 (29,5%) foram soropositivas para *A. marginale*, *B. bovis* e *B. bigemina*, respectivamente. Os resultados obtidos, caracterizam as propriedades analisadas da mesorregião central do estado do Espírito Santo, como de instabilidade enzoótica para os agentes da Tristeza

Parasitária Bovina (TPB) possuindo alto risco da ocorrência de surtos de anaplasmoses e babesiose nestas propriedades.

**Palavras-chave:** Anaplasmoses, Babesiose, ELISA, Tristeza Parasitária Bovina

## Introduction

The Espírito Santo state has approximately 1.93 million livestock, milk production of 374 million, and gross production value of R\$ 426 million annually (Galeano et al., 2018). However, the presence of parasitic diseases in these animals is one of the limiting factors for the full development of this activity (Aguiar et al. 2017). In this sense, in the state of Espírito Santo and throughout Brazil, efforts are focused on the parasite, especially the *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*., which cause Bovine Parasitic Sadness complex (TPB) (Kessler, 2001; Kocan et al., 2003; Oliveira & Oliveira-Sequeira, 2004).

The diagnosis of TPB is based on epidemiological data, clinical signs, and especially on direct screening of infectious agents in stained blood smears, widely efficient in the acute phase of the disease. Serological, such as Enzyme Immunosorbent Assay (ELISA) and Indirect Immunofluorescence Reaction (RIFI), and molecular methods, such as Polymerase Chain Reaction (PCR), are used to identify chronically infected (Juliano et al., 2007; Kocan et al., 2010).

In this sense, the present study aimed to detect the occurrence of *Babesia bovis*, *Babesia bigemina*, and *Anaplasma marginale* in dairy cattle from the central mesoregion of the state of Espírito Santo, through direct parasitological (stained blood smear) and serological (ELISA) tests.

## Material and methods

Samples were collected from six dairy herds, concentrated in the central mesoregion of the state of Espírito Santo. A total of 159 animals were sampled, 80 cows (between 3 and 9 years) and 79 calves (between 3 and 6 months). Blood samples were collected from the jugular, mammary, or coccygeal vein by Vacutainer® system (B.D. – Juiz de Fora – MG). Ten milliliters of whole blood was collected into an anticoagulant-free vacutainer tube (B.D. – Juiz de Fora – MG), for serum collection. Serum samples were split into duplicates and stored at -20 °C until required for analyses. Blood smears were performed by tail punctured and then stained with Diff-Quick (Newprov – Pinhais – PR). Blood smears were analyzed at a light microscope under 1000x magnification in order to identify erythrocytes parasitized by *A. marginale* and *Babesia* sp.

The ELISA was carried out as described by Machado et al. (1997), with minor modifications, for IgG anti-*B. bovis* and anti-*B. bigemina*, and as described by Andrade et al. (2004) for IgG anti-*A. marginale*. Total antigen produced at the FCAV/UNESP Immunoparasitology Laboratory, Campus de Jaboticabal, was used. All samples were tested in duplicate, and positive and negative controls were tested in quadruplicate. The reaction was read by a microplate reader (Microplate Reader, Model 680, Bio-Rad) at 405 nm. The blank well did not contain serum. The cut-off point was calculated as described by Machado et al. (1997).

Tick infestation was also evaluated as proposed by Fraga et al. (2003), with minor modifications. A scale ranging from 0 to 3 was used, where 0 = no parasites (teleogines and/or larvae); 1 = low infestation; 2 = medium infestation; and 3 = high infestation.

## Results and discussion

Among the 159 blood smears analyzed 64 (40.2%) and 11 (6.9%) were positive for *A. marginale* and *Babesia* sp., respectively. However, by ELISA, 9 (5.7%), 59 (37.1%), and 47 (29.5%) were seropositive for *A. marginale*, *B. bovis*, and *B. bigemina*, respectively (Table 1). The parasitological technique detected more *A. marginale*, while the ELISA detected more seropositivity for *Babesia* sp.. The blood smear identifies animals mainly during the parasitemic phase, being observed the corpuscle of *A. marginale* and the merozoites of *Babesia* sp. in the erythrocytes and animal may present clinical signs. In the other hand, ELISA detects chronically infected animals, where antibodies remain present (Santos et al., 2017).

**Table 1.** Blood smears and ELISA results for *A. marginale*, *B. bovis* and *B. bigemina*, from 6 farms localized in the central mesoregion of the state of Espírito Santo.

Farms	Samples		Blood smears				ELISA				
			<i>A. marginale</i>		<i>Babesia</i> sp.		<i>A. marginale</i>		<i>B. bovis</i>		<i>B. bigemina</i>
	N	%	N	%	N	%	N	%	N	%	N
A	31	19.5	13	41.9	2	6.4	5	16.1	15	48.4	14
B	30	18.9	13	43.3	4	13.3	1	3.3	11	36.7	3
C	26	16.4	16	61.5	4	15.4	1	3.8	5	19.2	1
D	31	19.5	9	29	1	3.2	0	0	3	9.7	5
E	22	13.8	8	36.4	0	0	2	9.1	12	54.5	12
F	19	11.9	5	26.3	0	0	0	0	12	63.1	12
Total	159	100	64	40.2	11	6.9	9	5.7	59	37.1	47
											29.5

When only adults were analyzed ([Table 2](#)), it was identified that 23 (28.7%) and 1 (1.2%) were positive for *A. marginale* and *Babesia* sp., respectively, in the blood smear. By ELISA, the number of seropositive animals was 6 (7.5%), 30 (37.5%), and 17 (21.2%) for *A. marginale*, *B. bovis*, and *B. bigemina*, respectively. These results differ from Franque ([2010](#)) in dairy cattle from the southern region of the state of Espírito Santo, where the prevalence was over 75% for the three hemoparasites, being considered as an area of enzootic stability based on this category. The area of the present work is considered enzootic instability, with a predisposition to the occurrence of outbreaks ([Mahoney & Ross, 1972](#)).

**Table 2.** Blood smears and ELISA results for *A. marginale*, *B. bovis* and *B. bigemina*, of 80 cows from 6 farms localized in the central mesoregion of the state of Espírito Santo.

Farms	Samples		Blood smears				ELISA				
			Samples		<i>Babesia</i> sp.		<i>A. marginale</i>		<i>B. bovis</i>		<i>B. bigemina</i>
	N	%	N	%	N	%	N	%	N	%	N
A	15	18.8	2	13.3	0	0	4	26.7	9	60	6
B	15	18.8	3	20	1	6.7	1	6.7	6	40	2
C	15	18.8	10	66.7	0	0	1	6.7	3	20	0
D	13	16.1	0	0	0	0	0	0	3	23.1	1
E	15	18.8	6	40	0	0	0	0	5	33.3	6
F	7	8.7	2	28.5	0	0	0	0	4	57.1	2
Total	80	100	23	28.7	1	1.2	6	7.5	30	37.5	17
											21.2

When only calves were analyzed ([Table 3](#)), positivity on blood smears, for *A. marginale* and *Babesia* sp., was 41 (51.9%) and 10 (12.6%), respectively.

**Table 3.** Blood smears and ELISA results for *A. marginale*, *B. bovis* and *B. bigemina*, of 79 calves from 6 farms localized in the central mesoregion of the state of Espírito Santo.

Farms	Samples		Blood smears				ELISA				
			Samples		<i>Babesia</i> sp.		<i>A. marginale</i>		<i>B. bovis</i>		<i>B. bigemina</i>
	N	%	N	%	N	%	N	%	N	%	N
A	16	20.3	11	68.7	2	12.5	1	6.2	7	43.7	8
B	15	19	10	66.7	3	20	0	0	5	33.3	1
C	11	13.9	6	54.5	4	36.4	0	0	2	18.2	1
D	18	22.8	9	50	1	5.5	0	0	0	0	4
E	7	8.8	2	28.6	0	0	2	28.6	7	100	6
F	12	15.2	3	25	0	0	0	0	8	66.7	10
Total	79	100	41	51.9	10	12.6	3	3.8	29	36.7	30
											37.9

On ELISA, seropositive for *A. marginale*, *B. bovis*, and *B. bigemina* was 3 (3.8%), 29 (36.7%), and 30 (37.9%), respectively. When compared to Franque ([2010](#)) study results were similar to the prevalence of *A. marginale*, being smaller than 75% of seropositivity. In contrast, the prevalence for *B. bovis* and *B. bigemina* were different, where this author found seropositivity over 75%.

Individual analysis of the farms showed that farms E and F presented enzootic stability for *B. bovis* (E-100%) and *B. bigemina* (E-85.7% and F-83.3%) when calves were analyzed. Comparison between

farms (all categories together) showed that farms D and E presented the biggest difference between the percentage of seropositive for *B. bovis* and *B. bigemina*. Despite the study conducted by Guimarães et al. (2011) demonstrate that the breeding system did not interfere with the seropositivity of animals, in our research this factor may have interfered, once cows and calves from farm D were housed in the free stall and suspended individual stalls, while in farm E animals were housed on pasture, being more exposed to *Rhipicephalus microplus*. This is evident once from the 31 animals sampled from farm D only eight presented ticks, while in all 22 animals from farm E ticks were detected (Table 4).

**Table 4.** Tick score of 159 bovines from 6 farms localized in the central mesoregion of the state of Espírito Santo.

Farms	Samples		Score			
	N	%	0	1	2	3
A	31	19.5	3	18	9	1
B	30	18.9	0	19	11	0
C	26	16.4	4	13	7	2
D	31	19.5	23	7	1	0
E	22	13.8	0	7	11	4
F	19	11.9	4	12	3	0
<b>TOTAL</b>	<b>159</b>	<b>100</b>	<b>34</b>	<b>76</b>	<b>42</b>	<b>7</b>

## Conclusion

Thus, the present study characterizes these farms from the central mesoregion of the state of Espírito Santo as enzootic instability for TPB agents, showing a high risk for anaplasmosis and babesiosis outbreaks.

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