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EPIDEMIOLOGY OF ENZOOTIC BOVINE LEUKEMIA VIRUS (BLV) INFECTION

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ABSTRACT

The purpose of this study was to investigate the presence of anti-enzootic leukosis virus antibodies and also to identify the association between seropositivity and management variables for this infection in cattle in the State of Alagoas, Brazil. A total of 17 herds were examined, totaling 341 animals, distributed in eight counties. The antibody detection was performed by the technique of agar gel immunodiffusion (AGID), using the antigen constituted of lipids and proteins from bovine leukosis virus. Of the 341 samples analyzed, 95 (27.8%) were positive and the number of outbreaks was 12 (70.6%). Significant differences were observed for the variables: technical assistance ($p<0.000$), recent acquisition of animals ($p=0.003$), existence of maternity paddocks ($p<0.000$), and colostrum management ($p<0.000$). The enzootic bovine leukosis virus infection is present in the region studied and strict sanitary actions must be implemented to prevent the spread of the virus, which will avoid losses in the cattle production chain.

KEYWORDS: BLV; diagnosis; epidemiology.

EPIDEMIOLOGIA DA INFECÇÃO PELO VÍRUS DA LEUCOSE ENZOÓTICA BOVINA (LEB)

RESUMO

Objetivou-se com este trabalho investigar a presença de anticorpos do antivírus da leucose enzoótica além de identificar a associação entre variáveis de manejo e soropositividade para essa infecção em bovinos no Estado de Alagoas, Brasil. Foram examinados 17 rebanhos, perfazendo um total de 341 animais, distribuídos em oito municípios. A pesquisa de anticorpos foi efetuada pela técnica de Imunodifusão em Gel de Ágar (IDGA), utilizando-se o antígeno constituído por lipopolissacarídeos e proteínas do vírus da leucose bovina. Das 341 amostras analisadas, 95 (27,8%) foram positivas e o número de focos constatados foi de 12 (70,6%). Foram observadas diferenças significativas para as variáveis: assistência técnica ($p<0,001$), aquisição de gado recente ($p=0,003$), existência de piquete maternidade ($p<0,001$) e manejo de colostro ($p<0,001$). A infecção pelo vírus da leucose enzoótica bovina está presente na região estudada e medidas sanitárias rigorosas devem ser implementadas para controlar a disseminação do vírus, o que evitará perdas na cadeia produtiva da bovinocultura.

PALAVRAS-CHAVE: diagnóstico; epidemiologia; LEB.

INTRODUCTION

The Brazilian cattle herd grew 1.5% in 2009 compared with the previous year, and totaled 205.3 million head. Thus, Brazil has the second largest herd of cattle in the world, behind India. In 2008, after two years of decline, the number of cattle head increased 1.3% (IBGE, 2009). The census released by IBGE in 2009 identified an increase of 5.6% in milk production, totaling 29,112 billion liters per year in the country.

In Alagoas, data from the 2006 Agricultural Census revealed a cattle herd of 886,244 head (IBGE, 2006), reaching in 2009 a production of 238,229 million liters of milk and a yield of 1,486 liters / cow / year (IBGE, 2009).

The increase in cattle concentration per property, the introduction of imported genetic material, and changes in sanitary and reproductive management facilitated the spread of many pathogens of sanitary importance to dairy cattle production (POLETTO et al., 2004). Among these pathogens, the bovine leukemia retrovirus, responsible for the Enzootic Bovine Leukosis (EBL), stands out (OIE, 2008).

EBL is a disease of great economic importance due to several factors, such as losses of export markets that require animals free of infection, costs of diagnosis, and the treatment of complications of animals with lymphosarcomas, premature disposal or death of animals, particularly the ones with high genetic potential, and condemnation of carcasses in slaughterhouses with veterinary inspection service (DIGIACOMO, 1992).

Indiscriminate importation of cattle from the northern hemisphere, by elite cattle producers from the Southeast and South regions, is held responsible for the introduction of Enzootic Bovine Leukosis Virus (BLV) in Brazilian herds. Once the virus was established in these regions, it spread to the North and Northeast regions, favored by the heavy traffic of animals (ABREU et al., 1994) and, especially, the lack of sanitary policies to combat the occurrence of the disease in the country (GARCIA et al., 1991).

To determine the prevalence of this infection among Brazilian cattle, several studies were conducted in different regions of the country. In Rio Grande do Sul State, FLORES et al. (1990) and MORAES et al. (1996) found 20.7% and 9.2% of positive animals, respectively. In the state of Paraná, BARROS FILHO et al. (2010) studied dairy cattle and analyzed 268 samples from animals reared in the metropolitan region of Curitiba and determined a prevalence of 56.3% of positive animals. In the Northeast, in Bahia State, TÁVORA & BIRGEL (1991) found 16.1% positive animals and MATOS et al. (2005) found 41.0% animals with positive sera. In Pernambuco State, MENDES et al. (2011) observed a prevalence of 23.1% in dairy herds. In Alagoas State, BIRGEL JUNIOR et al. (1999) determined a prevalence of 9.6% in bovines raised in the dairy region. This report was the first record of infection in the state. Therefore, this new study was designed to examine the current situation of this infection, by determining the seroprevalence of anti-virus of enzootic leucosis in cattle in Alagoas State, Brazil, and to identify the association between management variables and seropositivity for this infection.

MATERIAL AND METHODS

The State of Alagoas is located at the East-Central portion of the Brazilian Northeast, between parallels 8°48'12" and 10º30'12" of south latitude and meridians 35º09'36" and 38º13'54" of west longitude. For planning purposes, the state was divided into three Mesoregions: East, Agreste, and Hinterland (Figure 1); and in 13 Microregions: Serrana do Sertão Alagoana, Alagoana do Sertão do São Francisco, Santana do Ipanema, Batalha, Palmeira dos Índios, Arapiraca, Traipu, Serrana dos Quilombos, Mata Alagoana, Litoral Norte Alagoano, Maceió, São Miguel dos Campos and Penedo, where the 102 municipalities that make up this Unit of the Federation, whose capital city is Maceio, are distributed (UFAL, 1999; ASSIS, 2007).

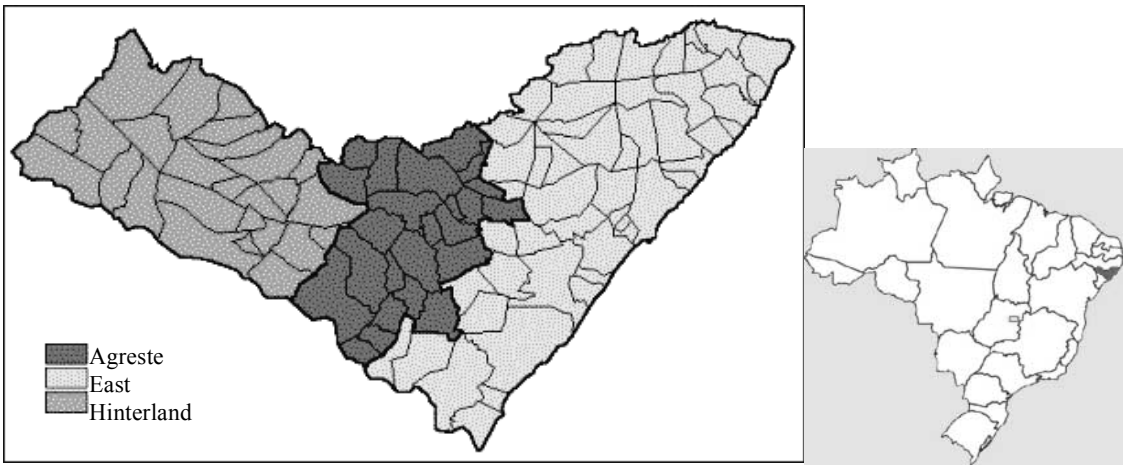

Figure 1 - Mesoregions the State of Alagoas
Source: http://www.zonasuldemaceio.com.br/alagoas/imagens/250px-Alagoas_MesoMicroMunicip.svg.png

The study was developed in the microregion of Batalha, meso hinterland of Alagoas, which has a total of 96,034 cattle distributed among the municipalities of Batalha (17,280), Belo Monte (10,000), Jacaré dos Homens (10,924), Jaramataia (8012), Major Izidoro (27,547), Monteirópolis (6035), Olho D'Água das Flores (9536) and Olivença (6,700) (IBGE, 2006). We decided to work at this micro-region because it is considered the dairy industry of the state of Alagoas.

To accomplish this epidemiological study and establish the prevalence of serum anti-BLV antibodies, we used the formula proposed by THRUSFIELD (2004). We considered an estimated prevalence in the country of 23.7 (FERNANDES et al., 2009), with a confidence level of 95%, and a statistical error of 20.0%, which resulted in a minimum sample size of 310. However, we chose to work with 341 samples, as a safety margin, which were collected in 17 properties distributed in eight municipalities. The properties were chosen due to their convenience. For the calculation of samples per property, we used the computer program WinEpiscope 2.0. The herds consisted of animals of various breeds, ages, at different stages of lactation, reared under intensive or semi-intensive system and submitted to mechanical and / or manual milking.

Blood samples were obtained from bovines at reproductive age, by jugular vein puncture with a vacuum collection system, into siliconized tubes with capacity for 10 mL. The collected blood was kept at room temperature until the blood clot retraction, and then transported, under refrigeration, to the laboratory where they were centrifuged for 10 minutes at 1000g. Serum was transferred to polypropylene tubes and stored in a freezer at -20°C until the moment of the serological tests. The collection period was from January to April 2008.

Detection of serum anti-BLV was made by Agar Gel Immunodiffusion (AGID) test, using an antigen for virus diagnosis produced by the Technological Institute of Paraná (TECPAR), according to the methodology recommended by the manufacturer.

For analysis of the results, we considered dispersion of absolute and relative frequencies. To identify the association between management variables and seropositivity for the infection, questionnaires with objective questions about the hygiene-sanitary management were applied. The characterization of significance between the differences observed in the frequency of positive animals by sex, technical assistance, last purchase of livestock, maternity paddocks and colostrum management was determined by chi-square test or Fisher exact test. The level of significance was 5% (ZAR, 1999). We considered the properties that presented at least one animal reactive to the serology test as focus.

RESULTS AND DISCUSSION

Of the 341 analyzed samples, 95 (27.8%) were positive and 246 (72.2%) negative (Table 1). The prevalence of positive animals in herds ranged from 5.0% to 65.2%, with a mean prevalence of 22.3%. Regarding the municipalities and the corresponding BLV prevalence in positive animals, we obtained rates ranging from 8.3% (1/12) to 50% (63/126). The results obtained in this study were higher than those reported by BIRGEL et al. (1999), who found 9.6% positivity in the city of Batalha in Alagoas State, and close to those reported by MENDES et al. (2011), who observed a prevalence of 23.1% in dairy herds in Pernambuco State. The differences found in these studies may be associated with different forms of

management, breeds, technologies (BIRGEL JUNIOR et al., 1995), and the type of sampling performed.

Regarding the municipalities, we found that 100.0% presented properties with positive animals and the number of foci was 70.6% (12/17). MOLNÁR et al. (1999) carried out a similar study in the state of Pará in 14 extensive herds, and reported that all the herds had positive animals, thus identifying, 100.0% of outbreaks. Likewise, CARNEIRO et al. (2003) carried out a study in the state of Amazonas and identified 8.9% serum-reactive bovines in all herds; these results differ from those found in this study. Despite the difference we found, the number of outbreaks in the studied area can be considered high, indicating that the agent is widespread in rural properties in Alagoas. Although there are no studies reporting clinical cases of BLV in cattle in this state, it is important that veterinarians and producers are attentive to this disease, since the number of foci was high.

In assessing the significance of the analyzed variables, we observed a significant association for technical assistance ($p < 0.000$), recent cattle purchase ($p = 0.003$), colostrum management ($p < 0.000$) and the existence of maternity paddock ($p < 0.000$) (Table 2).

Table 1. Serological results for enzootic bovine leukosis in the microregion of Batalha in the State of Alagoas, in the period from January to May 2008

Serology (AGID)	N	R.F. (%)	Propriedades	R.F. (%)	Municipalities	R.F. (%)
Negative	246	72.2	5/17	29.4	-	-
Positive	95	27.8	12/17	70.6	8	100.0
Total	341	100.0	17	100.0	8	100.0

N = Number of animals; RF = Relative Frequency.

Table 2. Distribution of samples submitted to the Agar Gel Immunodiffusion test (AGID), according to the variables associated with the presence of infection for Enzootic Bovine Leukosis (EBL) in cattle herds of the region of Batalha in the State of Alagoas, in the period from January to May 2008

Variable	Leukosis				Total		P value
	Reactive		Non-reactive				
	N	%	N	%	N	%	
Sex							
Male	3	33.3	6	66.7	9	100.0	$p^b = 0.713$
Female	92	27.7	240	72.3	332	100.0	
Technical assistance							
Yes	49	19.9	197	80.1	246	100.0	
No	1	3.8	25	96.2	26	100.0	$p^a < 0.000$*
Sporadic	45	65.2	24	34.8	69	100.0	
Recent cattle purchase							
Yes	6	11.3	47	88.7	53	100.0	$p^a = 0.003$*
No	89	30.9	199	69.1	288	100.0	
Maternity paddock							
Yes	80	36.5	139	63.5	219	100.0	$p^a < 0.000$*
No	15	12.3	107	87.7	122	100.0	
Colostrum management							
Yes	31	19.0	132	81.0	178	100.0	$p^a < 0.000$*
No	64	36,0	114	64,0	163	100,0	

[a]By the chi-square test; [b]By Fisher exact test; [c]Significant association at 5.0%.

When analyzing the variable sex, we observed that males are just as susceptible to the infection as females (p = 0.713). Regarding this variable, BIRGEL JUNIOR et al. (1995) stated that the difference must be attributed to the influence of the production system instead of to sex because, in dairy herds, both male breeders or steers produced for sale are kept isolated, which makes the virus horizontal transmission harder.

Risk factors associated with BLV infection were analyzed in the State of Tocantins by FERNANDES et al. (2009), who concluded that only the type of milking practiced in the properties showed a significant association ($p < 0.05$) and OR of 2.7 for manual milking. However, these authors emphasized that despite the variable technical assistance did not show significant association, it was high (40.1%) in properties that have veterinary assistance compared to the ones that do not.

We found in this study a greater number of positive animals on those properties where there were technical assistance and recently purchased cattle. It is known that one of the main forms of BLV transmission is horizontal, through contact with contaminated materials (rectal palpation gloves, earring applicators), and the interference of the veterinarian, without proper hygienic care, may be a contributing factor to the occurrence of infection in many of the properties. FERNANDES et al. (2009) reported that the negligent introduction of genetically qualified and more productive breeders and / or matrices from European breeds, mainly Holstein, without the implementation of strict sanitary criteria, creates favorable conditions for the occurrence of VLB infection (FERNANDES et al. 2009).

According to FLORES et al. (1988), some factors positively affect the spread of the agent: the presence of several infected animals, large rotation (purchase and sale) of animals, use of semi-intensive production system and routine use of practices such as vaccinations, deworming and other therapy measures, minor surgeries, dehorning, earring application and rectal palpation for pregnancy diagnosis.

The properties structured with maternity paddocks and that did not carry out colostrum-origin control showed higher positivity levels. These results are important from an epidemiological point of view, because preventive measures can be implemented in the properties to reduce the number of foci of infected animals. The largest number of infected animals in the properties that had maternity paddocks must be due to the contact with contaminated pasture and water during parturition. The use of BLV-free colostrum is an important control measure, preventing transmission (LEUZZI JUNIOR, 2001); also, pasteurization is one way to inactivate the virus (BAUMGARTENER et al. 1976).

Considering the high number of foci found in the studied region, we recommend the carry-out of a strict control in the properties by hygienic-sanitary measures, serological routine, euthanasia of positive animal, and, in the properties with low positive rates, segregation of the herd according to the serologic status. According to FLORES et al. (1988b), infection control in the properties with one or two positive animals could be done simply by replacing them with negative descendants or seronegative animals from other property. The maintenance of seropositive animals in the herd endangers other animals, which, with the coexistence, will certainly become infected, making control more difficult.

The detection and elimination of seropositive animals are the key points in controlling the infection by BLV. In this sense, the mandatory serological tests in herds, the requirement for seronegative animals for fairs and exhibitions, artificial insemination centers, blood donors for premonition and animals to be imported are necessary in an attempt to control the infection (FLORES et al., 1988b). This control is necessary, since the infection is considered both a health and an economic problem, because the presence of the virus imposes serious restrictions on the exportation and importation of cattle with high genetic potential, in addition to causing mortality and decreasing the productivity of infected bovines (BRAGA et al. 1997).

CONCLUSION

The infection by Enzootic Bovine Leukosis virus is present in cattle in the studied region and strict sanitary measures should be implemented to control the spread of the virus, which will prevent losses in cattle production chain.

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