

Seroprevalence of bovine alphaherpesvirus type 1 (BoHV-1) and risk factors associated with dairy properties of the municipality of Senador Guiomard, Acre, Brazil

Seroprevalência de alfa herpesvírus bovino tipo 1 (BoHV-1) e fatores de risco associados em propriedades leiteiras do município de Senador Guiomard, Acre, Brasil

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ABSTRACT: Bovine alphaherpesvirus type 1 (BoHV-1) is the etiological agent responsible for serious infections that compromise the respiratory and genital tracts of affected cattle. In order to estimate the seroprevalence of BoHV-1 and to identify the associated risk factors in dairy farms in the city of Senador Guiomard, Acre, Brazil, the present study was carried out through the analysis of 180 blood serum samples submitted to the screening of anti-BoHV-1 by the virus neutralization test (VN) and by means of the evaluation of the epidemiological questionnaire applied in the eight investigated properties. The prevalence of seropositivity for BoHV-1 was 61.1%, ranging from 43.3 to 86.2% among the analyzed properties. The variable absence of veterinary assistance showed statistically significant association (*odds ratio* — OR = 2.10; $p < 0.001$) with alphaherpesvirus infection. The results demonstrate that the frequency of BoHV-1 is high and needs to be controlled through prophylactic and health management measures.

KEYWORDS: livestock; epidemiological survey; virus neutralization.

RESUMO: O alfa herpesvírus bovino tipo 1 (BoHV-1) é o agente etiológico responsável por graves infecções que comprometem os tratos respiratório e genital dos bovinos acometidos. Com o objetivo de estimar a soroprevalência de BoHV-1 e identificar os fatores de risco associados em propriedades leiteiras do município de Senador Guiomard, Acre, foi realizado o presente estudo, por meio da análise de 180 amostras de soro sanguíneo submetidas à pesquisa de anticorpos anti-BoHV-1 pelo teste de vírus neutralização (VN) e por meio da avaliação do questionário epidemiológico aplicado nas oito propriedades investigadas. A prevalência de soropositividade para BoHV-1 foi de 61,1% variando de 43,3 a 86,2% entre as propriedades analisadas. A variável ausência de assistência veterinária apresentou associação estatisticamente significativa (*odds ratio* — OR = 2,10; $p < 0,001$) com a infecção pelo alfa herpesvírus. Os resultados demonstram que a frequência de BoHV-1 é alta e precisa ser controlada através de medidas profiláticas e de manejo sanitário.

PALAVRAS-CHAVE: pecuária; inquérito epidemiológico; vírus neutralização.

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INTRODUCTION

The bovine alphaherpesvirus type 1 (BoHV-1) belonging to the order Herpesvirales, family Herpesviridae, subfamily Alphaherpesvirinae and genus *Varicellovirus* is the etiological agent responsible for serious infections that compromise the respiratory and genital tracts of the affected animals. BoHV-1 can manifest clinically as bovine infectious rhinotracheitis (BIR) or infectious pustular vulvovaginitis / balanoposthitis (IPV / IPB), and infects mainly dairy and cutting cattle, but may also affect other domestic and wild species of ruminants (WORLD ORGANIZATION FOR ANIMAL HEALTH, 2010).

In the present study, BoHV-1 infection caused significant economic losses in livestock, as it is one of the main agents causing embryonic and fetal death, abortion, reduced fertility, decreased milk production and difficulty in gaining weight (VIU et al., 2014).

In Brazil, serological and etiological evidence indicates that BoHV-1 is widely distributed in dairy herds and has been isolated and identified in all regions of the country. However, the frequency of infected animals is indeterminate and varies considerably depending on the locality analyzed. Studies have identified the prevalence of 51.9% in Goiás (BARBOSA et al., 2005), 54.6% in Minas Gerais and São Paulo (ALEXANDRINO et al., 2011), 52.9% in Rio Grande do Sul (PIOVESAN et al., 2013), 14.0% in Amazonas (AGUIAR, 2014), 66.75% in Espírito Santo (SANTOS et al., 2014), 63.23% in Maranhão (FREITAS et al., 2014), and 57.54% in Santa Catarina (PASQUALOTTO et al., 2015).

In Acre state, the first report on the frequency of BoHV-1 was conducted by CAVALCANTE (1997), who examined 38 cattle from the experimental field of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) in Rio Branco and found prevalence of 44.73% positive animals. Another study, carried out by DINIZ et al. (2015) with 235 mestizo bovine females from 11 rural properties in six counties of Acre, confirmed the presence of anti-BoHV-1 antibodies in 43.82% of the evaluated females.

Despite the notorious health and economic importance of BoHV-1 infection, there is still a limitation of studies investigating its frequency and associated risk factors in Amazon cattle herds, especially in Acre, mainly due to the difficulties of access and the scarcity of human resources, material technology, finances and diagnostics.

Given the above, the present study aimed to estimate the BoHV-1 seroprevalence and to identify possible associated risk factors in dairy farms in the municipality of Senador Guimard, Acre, Brazil.

MATERIALS AND METHODS

The present work deals with a cross-sectional study carried out in the rural area of Senador Guimard (10°09'08" S;

67°44'21" W). This municipality belongs to the dairy basin of the Rio Branco Microregion. The total bovine herd of Senador Guimard identified in the 2010 by Demographic Census was 213,369 animals, of which 7,996 were identified with milk aptitude (IBGE, 2010).

For the calculation of the sample, the size of the dairy herd of the study area, the estimated prevalence of 50%, based on the results obtained in studies carried out in the state (CAVALCANTE, 1997; DINIZ et al., 2015), the confidence interval of 95% (CI95%) and the margin of error of 5%, which determined a minimum sampling of 180 animals, were considered.

Between June and November 2016, 180 serological samples of non-vaccinated animals for the BoHV-1 were randomly collected, being 176 of female bovine and four males, with ages ranging from 2 to 8 years old. All were apparently healthy. These animals came from eight properties, distributed in the municipality that constitutes the study area, which were selected for non-probabilistic convenience.

Blood was collected through jugular venipuncture with a sterile disposable needle and vacuum tubes, previously identified with the animal number. After collection, the samples were centrifuged at 5,000 G for 10 min to obtain the serum. The serum aliquots were then stored in plastic microtubes and kept at -20°C until the serological test (BEZERRA et al., 2012a).

For each of the eight properties, an epidemiological questionnaire composed of objective questions was used to obtain information about the type of exploration and management practices used to characterize the risk factors associated with BoHV-1 infection.

The analysis of the samples was carried out through the neutralization virus test (NV) at the Laboratório de Virologia Animal of the Universidade Federal Rural de Pernambuco, using the standards and procedures defined by the World Organization for Animal Health (2010).

For the test, Madin-Darby bovine kidney (MDBK) and type culture collection (CCL-22) cells were used. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), with penicillin (40 mg / L) and streptomycin (200 mg / L) supplemented with 4% fetal bovine serum (Sigma®) (SANTOS et al., 2014).

In the test, 96 polystyrene plates were used, with constant dilution of the serum (1:2), *versus* 100 infective doses to 50% of the cell cultures / well (Infecting Dose for 50% of Cell Cultures — DICC50) for the BoHV-1 isolate. Antibody detection was performed by incubating dilutions in the base 2 (1:2 and 1:4) of the serum against constant virus doses in duplicate.

After incubation of the virus serum for 24 hours at 37°C and 5% CO₂, a suspension of MDBK cells was added (400,000 / mL), followed by incubation in CO₂ oven at 37°C. The reading was performed after 72 hours of incubation, through cytopathic effect (CPE) research. It was considered a

positive sample when in the absence of CPE, indicating viral neutralization, and negative in the presence of CPE (SILVA et al., 2015).

In the statistical analysis, the frequency measures of BoHV-1 infection were calculated, and the strength of the association between the risk factors (independent variables) and the diagnostic test result (dependent variable) was evaluated through *odds ratio* (OR), with their respective CI95%.

For this, Fisher's exact test was used, and p value less than 0.05 was considered statistically significant, according to the program Statistical Package for the Social Sciences (SPSS, IBM, New York, United States), in the version 21.0.

The study was approved by the Committee on Ethics in Animal of the Universidade Federal do Acre, as determined by process No. 23107.010820/2015-10 and protocol No. 64/2015, being in accordance with the ethical principles in animal experimentation established by the National Council of the Control of Animal Experimentation and Federal Council of Veterinary Medicine.

RESULTS AND DISCUSSION

The overall prevalence of seropositive animals for BoHV-1 was 61.1% (Table 1). This result resembles the values found by DIAS et al. (2008) in Paraná (64.41%) and by SOUSA et al. (2013) in Maranhão (67.3%). However, it differs from the prevalence obtained by HOLZ et al. (2009) in Rio Grande do Sul (29.2%), AGUIAR (2014) in Amazonas (14.0%) and OLIVEIRA et al. (2015) in Paraná (14.25%) — they were lower frequencies. On the other hand, it is also distinguished from the seroprevalences recorded by OKUDA et al. (2006) in Rondônia (86.2%), GUARINO et al. (2008) in Uruguay (99%) and AFFONSO et al. (2010) in Goiás (84.5%), for having verified higher values of seropositivity for BoHV-1.

The variation in the prevalence of BoHV-1 found in these studies can be explained by the type of breeding, management of animals, age range of animals, sampling techniques, diagnostic methods, and regional characteristics of each study.

The prevalence of BoHV-1 infection in the dairy herd of the municipality of Senador Guiomard is considered high, which shows that the virus is present and widely disseminated in the region. However, this value may be even higher, since the seropositivity found does not accurately reflect the number of infected animals and previous studies have demonstrated the existence of latently infected animals seronegative to the diagnostic tests.

According to TAKIUCHI et al. (2001), among the main reasons for the high frequency of infected animals in a given area are the lack of knowledge about the existence and transmission of the virus, its probable respiratory and reproductive consequences, as well as the main forms of prophylaxis and

viral control. Already for DEL FAVA et al. (2002), the high prevalence rates of BoHV-1 can be justified by age, sex, type of breeding, management and exploration of the animals that constitute the risk factors for infection.

Regarding sex, 61.4% of the females and 50.0% of the males were seroreactive in the population evaluated (Table 1). Because they came from dairy herds, most of the positive animals was expected to be female, similar to the study by SILVA et al. (2015), in which the majority of BoHV-1 seropositive animals was found to be female. As for the breeders, half of the analyzed ones also presented seropositivity to the alphaherpesvirus, which demonstrates and emphasizes the relevance of the male in the spread of the infection. According to OLIVEIRA et al. (2011), infected bulls can transmit BoHV-1 directly by natural or indirect mating through contaminated semen during artificial insemination, thus contributing to the increase of low rates of return to service, shortening of the estrous cycle and low rate of fertilization in the infected females and favoring the maintenance of the virus in the establishment.

All of the eight investigated properties presented positive animals, and the prevalence distribution by property ranged from 43.3 to 86.2% (Table 1). Similar results were verified by BEZERRA et al. (2012a, 2012b) in Maranhão; both studies similarly found seropositive animals for BoHV-1 in all analyzed properties. According to ACKERMANN; ENGELS (2006), once the animal is infected by the alphaherpesvirus,

Table 1. Prevalence of bovine alphaherpesvirus type 1 infection by sex, age group and dairy properties in the municipality of Senador Guiomard, Acre, Brazil.

Variable	Viral neutralization			
	Positive		Negative	
	n	%	n	%
Sex				
Female	108	61.4	68	38.6
Male	2	50.0	2	50.0
Age Group				
2 to 4 years old	38	60.31	25	39.69
> 4 years old	72	61.53	45	38.47
Properties				
1	11	73.4	4	26.6
2	8	80.0	2	20.0
3	17	63.0	10	37.0
4	11	47.8	12	52.2
5	13	43.3	17	56.7
6	25	86.2	4	13.8
7	13	44.8	16	55.2
8	12	70.5	5	29.5
Total	110	61.1	70	38.9

it becomes a carrier and source of infection, which endangers the health of other susceptible animals.

Regarding the risk factors, the statistical analysis revealed significant association ($p < 0.001$) between the rate of infection by BoHV-1 and the absence of veterinary care, and the lack of veterinary medical services identified in this study was a risk factor to seropositivity to alphaherpesviruses (OR = 2.10), as shown in Table 2.

The finding of this study corroborates that one obtained by BEZERRA et al. (2012a), that when investigating dairy cattle in the Amazon region of Maranhão the presence of veterinary assistance as a protection factor was identified (OR = 0.37) for the occurrence of viral infection, with significant statistical association ($p < 0.05$). Similarly, SOUSA et al. (2013) also verified the absence of veterinary assistance as a risk factor (OR = 7.714) for increased exposure to the agent ($p < 0.05$). For BEZERRA et al. (2012b), the lack of veterinary medical assistance can contribute to the delay of diagnosis and the absence of implantation of programs of prophylaxis and infection control.

Regarding the age group, 61.53% of seropositivity was detected in animals aged four years old or more. ALEXANDRINO et al. (2011) report that the older the animals are, the more likely they are to contract BoHV-1, due to the longer exposure to the virus and the risk factors for infection. In addition, intensive management in older animals can lead to stress and intensify the reactivation of the latent virus in the environment, ensuring that the infection remains in the herd which favors viral dissemination to the rest of the flock.

However, in the present study, the age group did not present significant association, according to Table 2, differing from the results obtained by MAGAÑA-URBINA et al. (2005), in Mexico, where cows aged 4 years old or more had increased risk of BoHV-1 when compared to the younger ones (OR = 2.36), and by CARBONERO et al. (2011), in Ecuador, where the cows older than 4 years old were identified as a risk factor associated with infection (OR = 1.44).

However, in this study, animals younger than two years old were not analyzed, which may explain the lack of statistical

Table 2. Risk factors associated with bovine alphaherpesvirus type 1 infection in dairy farms in the municipality of Senador Guimard, Acre, Brazil.

Variable	n	Positive VNT (%)	OR (95%CI)	p-value
Age Group				
2 to 4 years old	63	38 (60.31)	1.05 (0.56–1.97)	0.847
> 4 years old	117	72 (61.53)		
Replenishment of animals				
To recreate	25	19 (76.0)	1.11 (0.83 – 1.34)	0.123
Purchase	155	91 (58.70)		
Type of creation				
Semiconfining	46	25 (54.34)	1.45 (0.73 – 3.19)	0.297
Extensive	134	85 (63.43)		
Sharing the grass				
No	51	37 (75.54)	1.49 (0.80 – 1.93)	0.062
Yes	129	73 (56.58)		
Conditions of the Facilities				
Suitable	17	12 (70.58)	1.62 (0.92 – 1.80)	0.709
Inadequate	163	98 (60.12)		
Veterinary assistance				
Yes	138	79 (57.24)	2.10 (1.18 – 2.99)	< 0.001*
No	42	31 (73.80)		
Reproductive management				
Natural mount	163	98 (60.12)	1.59 (0.53 – 4.73)	0.501
Reproductive biotechnology	17	12 (70.58)		
History of abortion				
No	36	27 (75.24)	1.45 (0.94 – 1.66)	0.059
Yes	144	83 (57.63)		

VNT: virus neutralization test; OR: *odds ratio*; 95%CI: confidence interval of 95%.

significance, since younger animals have less contact with adult animals, thus are less exposed to alphaherpesvirus.

The variables type of breeding and reproductive management also had no significant association (Table 2), but may be contributing to the infection, even to a lesser extent, since they presented representative OR and were already identified by SILVA et al. (2015) as potential risk factors in cattle herds in the state of Pernambuco, but without statistical significance.

As for the form of animal replacement, BARBOSA et al. (2005) report that the purchase of cattle represents a risk factor for alphaherpesvirus infection. The reason for this can be explained by the greater likelihood that animals purchased without the health marketing criteria may be infected. However, this variable had no significant association with BoHV-1 infection in this study (Table 2).

With regard to grass sharing, ACKERMANN; ENGELS (2006) and DIAS et al. (2008) emphasize that the use of pastures in common with other properties contributes to the contact between susceptible and infected animals, favoring the spread of the virus through ocular and nasal secretions, inhalation of aerosols and by direct contact with infected fetal attachments, but in this research there was no statistically significant association between this variable and seropositivity for the alphaherpesvirus (Table 2).

Regarding the history of abortion, FRANDOLOSO et al. (2008) say that BoHV-1 infections can cause infertility, embryonic death, fetal death and abortion, especially between

the fifth and eighth month of gestation, and this factor was already associated with DIAS et al. (2008) to positivity of BoHV-1 in creations in the state of Paraná. However, in this study, previous history of abortion did not present statistical significance, according to Table 2. This finding corroborates the results of BARBOSA et al. (2005), SOUSA et al. (2013) and BEZERRA et al. (2012b), who also did not verify the relationship between the history of abortions and the prevalence of BoHV-1 in the cattle herds investigated.

CONCLUSIONS

The results obtained in this study demonstrated high prevalence of seropositivity for BoHV-1 in dairy farms in the municipality of Senador Guimard, indicating that the virus is widely distributed in the region. The variable lack of veterinary care showed statistically significant association and was identified as a risk factor for seropositivity to alphaherpesvirus. Thus, it is essential that the infection of the herds is controlled by prophylactic measures and sanitary management by the owners in conjunction with the animal health services through periodic serological tests, vaccination campaigns and gradual substitution of infected animals for healthy ones. In addition, it is also necessary to develop new studies in order to describe and understand the epidemiological profile of alphaherpesvirus infection in Acre cattle.

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