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Frequency and risk factors associated with the bovine viral diarrhea virus in herds in the semiarid region of the states of Bahia and Pernambuco, Brazil

Frequência e fatores de risco associados a diarreia viral bovina em rebanhos na região semiárida dos estados da Bahia e Pernambuco

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ABSTRACT

Bovine viral diarrhea virus (BVDV) is an important viral pathogen in cattle. There are a few reports of this disease in northeastern Brazil, mainly in the semiarid region. The goal of this study was to determine both the frequency of seropositive animals, and the risk factors for BVDV in the municipalities of Bahia and Pernambuco. A non-probabilistic sampling was initially employed to determine the properties to be visited, whereas a probabilistic sampling was used to determine the number of animals per property. Serum samples were submitted to a seroneutralization test to determine the antibody titer for BVDV. An epidemiological questionnaire was used in the herds to evaluate the correlation and association between possible risk factors and BVDV infection in these herds. Of the 257 animals in the study, 144 were positive for infection BVDV, resulting in an overall frequency of 56.0%. The highest frequency was observed in Miguel Calmon (76.1%) and followed by Senhor do Bonfim (53.1%). The variables identified as risk factors for the BVDV infection were an extensive breeding system (OR = 2.58) and a mixed livestock purpose (beef and dairy) (OR = 3.32). The presence of BVDV-seropositive animals in the assessed municipalities suggests the circulation of the viral agent in these locations. Therefore, control and preventive measures must be implemented in order to minimize losses due to the disease, such as monitoring of the properties through laboratory tests, elimination of infected animals, and the use of vaccines in the indicated regions by the animal health department.

Palavras-chave:
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RESUMO

O vírus da Diarreia Viral Bovina (BVDV) é um dos principais patógenos virais de bovinos. Existe uma baixa prevalência dessa doença no nordeste brasileiro, principalmente na região semiárida. Objetivou-se determinar a frequência e os fatores de risco para BVDV em municípios da Bahia e Pernambuco. Uma amostragem não probabilística foi realizada para determinar o número de propriedades a serem visitadas, enquanto uma amostragem probabilística foi empregada para determinar o número de amostras a serem coletadas por propriedade. Amostras de soro foram obtidas e submetidas à técnica de soroneutralização para determinação do título de anticorpos para o BVDV. Para avaliação dos fatores de risco da infecção, aplicou-se um questionário investigativo nas propriedades. No total de 257 animais, 144 foram positivos para enfermidade, resultando uma frequência geral de 56%. A maior prevalência ocorreu em Miguel Calmon, 76,1% e Senhor do Bonfim, 53,15%. As variáveis identificadas como fator de risco foram sistema de criação extensivo (OR = 2.58) e exploração de animais para carne e leite (OR = 3.32). A presença de animais soropositivos para a doença nos municípios estudados sugere a circulação do agente viral nestas localizações. Assim, medidas de controle e prevenção devem ser colocadas em prática, visando minimizar as perdas, tais como monitoramento dos rebanhos por meio de

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exames periódicos, eliminação de animais positivos e o uso de vacinas nas regiões indicadas pelas agências de defesa agropecuária do país.

INTRODUCTION

Brazil has the second largest cattle herd worldwide, with approximately 215,199,488 heads, and the large areas used for cattle farming makes this an important activity. The tropical climate favors good development of livestock, with grasses and forage receiving high solar irradiance and appropriate rainfall in some regions (BRASIL, 2016). However, in the semiarid area of northeastern Brazil, cattle are prone to health problems, such as bovine viral diarrhea, which, according to Zheng et al. (2016), is responsible for productivity losses in the livestock industry.

Bovine viral diarrhea is an infectious disease that negatively impacts the productivity and reproduction of the herd, notably through the reduction of milk yield, lower conception rates, respiratory disorders, and death due to acute infection. The causative agent of bovine viral diarrhea is bovine viral diarrhea virus, or BVDV (NEWCOMER; CHAMORRO; WALZ, 2017; PINIOR et al., 2017; RICHTER et al., 2017; SORAIRES SANTACRUZ et al., 2017; ZHAO et al., 2015), which is differentiated into two species (BVDV1 and BVDV2) of the family *Flaviviridae* and the genus *Pestivirus* (RIDPATH et al., 2015). Most of the acute infections caused by BVDV are subclinical and transitory (ALPAY; YESILBAG, 2015; PALOMARES et al., 2015).

A frequency of seropositive animals in Brazil has been reported in the northeast, especially in the states of Bahia and Pernambuco. Exposure to the virus is as widespread in Brazil as it is in the endemic regions of other countries. There are some studies in the scientific literature that relate the frequency and the main risk factors for the disease (CHAVES et al., 2010; 2012; ALMEIDA et al., 2013; SOUSA et al., 2013; MARQUES et al. 2016; TSCHOPP et al., 2017).

Some factors may influence directly the permanence and virulence of the BVDV, such as the type and size of the herd. Moreover, the impact of an infection depends on the type of strain and on the immunity of the herd, as well as due to animal and environment factors (BYRNE et al., 2017) such as animal density, herd ability (dairy or beef cattle), breeding system (intensive, semi-intensive or extensive systems), vaccination programs and management practices adopted by each farm. Therefore, the objective of this study was to determine the frequency and risk factors of BVDV seropositive animals in the municipalities of Miguel Calmon and Senhor do Bonfim in Bahia, as well as in Petrolina in Pernambuco.

MATERIAL AND METHODS

Location of experiment and sample collection

The study was approved under the protocol number 0006/180716 issued by the Committee on Ethics in the Use of Animals (CEUA). The blood sample collection was performed on herds with intensive, extensive, or semi-intensive breeding systems for dairy and/or beef cattle and on mixed-breed herds in the municipalities of Miguel Calmon and Senhor do Bonfim, in the state of Bahia, and in Petrolina, in the state of Pernambuco. A non-probabilistic sampling was initially used to determine the properties to be visited, whereas a probabilistic sampling was used to determine the number of animals per property. Four herds were chosen through non-probability sampling (convenience sampling) in each municipality, totaling 257 sampled animals (Table 1).

Table 1 – Number of sampled animals per municipality for the epidemiological study of the BVDV, 2017.

Municipality	Herd	Total
Petrolina	A	28
	B	16
	C	5
	D	5
Senhor do Bonfim	E	63
	F	29
	G	14
	H	5
Miguel Calmon	I	63
	J	14
	L	12
	M	3
Total	12	257

The software package Epi Info version 7 was used to calculate of the number of animals to be sampled on each property, with the following parameters: an expected disease frequency of 50% (value adopted to maximize the sample), a confidence interval of 95%, and an absolute error of 10%. The number of selected animals was determined for each individual herd to detect the presence of infection, using the following formula (THRUSFIELD, 2004):

$$n = [1 - (1-p)^{\frac{1}{d}}] \times (N-d/2) + 1$$

Where:

n = sample size;

p = probability of detection of at least one seropositive animal;

N = size of the herd;

d = number of seropositive animals in the herd.

Serum collection

To obtain serum, blood from 257 animals was collected on the herds. First, a complete asepsis of the site for the blood draw was carried out with 70% ethanol, and then the blood sample was drawn from the jugular vein. After collection, the blood was transported in isothermal boxes at 4 °C. Serum was later obtained through centrifugation (after transfer to sterile microtubes) and stored at -20 °C in the laboratory until analysis. The sampling was carried out during the coolest hours of the day during the early morning.

Detection of anti-BVDV antibodies through a seroneutralization test

Antibodies against BVDV were detected using a method described by Eschaumer et al. (2014). All neutralization tests were performed in triplicate on 96-well tissue culture plates. The sera were diluted in cell culture medium (Dulbecco's modified eagle medium, DMEM) and divided into two parts: (1) 12 wells with virus + DMEM + fetal horse serum + Madin Darby Bovine Kidney, MDBK cells; (2) 12 wells with MDBK cells + DMEM + fetal horse serum (in this case presenting some modifications to the reported method). Initially, the standard cell culture was obtained. For cultivation, fetal bovine serum (5% FBS) was added to cultures maintained in a CO_2 incubator with humidity control at 37 °C. DMEM (50 µL), serum, and virus were added to 100 TCID50 in all wells. After 1 h, the cell culture was added to all the wells of the plate. After this, the plates were incubated at 37 °C for 96 h, thus determining the presence of seroneutralization. The serologic results were classified as low, medium, or high antibody titer according to Santos et al. (2014), where samples with titers lower than 2 were classified as negative, those with titers between 2 and 4 were classified as low, those with titers of 8, 16, or 32 were classified as intermediate, and those with titers of 64 or 128 were classified as high.

Evaluation of risk factors associated with infection with BVDV

The risk factor assessment was carried out according to the method described by Almeida et al. (2013). A structured questionnaire was created in order to evaluate the risk factors regarding the BVDV infection. A cross-sectional study was performed based on information about the owner and the general features of the farm, such as the farming system (intensive, semi-intensive, or extensive), livestock purpose (beef, dairy, or both), availability of veterinary care, and health examinations. The questionnaire also contained questions about applied farm management and commercialization of the herd.

Statistical analysis

The analysis of risk factors was carried out in two stages, including both univariate analysis and multivariate analysis. For the univariate analysis, each independent variable was crossed with a dependent variable. The variables with a value of $P < 0.2$ for the Chi-square test or the Fisher Exact test were selected and used for the multivariate analysis, using multiple logistic regression (HOSMER; LEMESHOW 2000). The collinearity between the predictive variables was verified by means of a correlation analysis and, for the variables with strong collinearity (correlation coefficient ≥ 0.9), one of the two was excluded from the multiple analyses according to biological plausibility. The adopted significance level for the multiple analyses was 5%. Herd A in Senhor do Bonfim, Bahia was excluded from the analysis due to vaccination against BVDV. All analyses were performed with SPSS 13.0 for Windows. For assessment of the influence exerted by environmental variables, Spearman's correlation was used for intra-herd frequency, and for the variables temperature and rainfall.

RESULTS AND DISCUSSION

Frequency of animals seropositive for bovine viral diarrhea in the states of Bahia and Pernambuco

Of the 257 samples included in this study, 144 were found to be positive for bovine viral diarrhea, resulting in an overall frequency of 56% of the evaluated animals, and 91.6% of the herds. The municipalities of Miguel Calmon and Senhor do Bonfim had the highest frequencies of seropositive animals, with 76.1% and 53.15%, respectively, as shown in Table 2. There were variations in frequency between the assessed herds in each municipality that are due to epidemiological factors regarding the transmission of the disease, environmental factors, and different rearing methods.

The frequency discrepancy observed in the states of Pernambuco and Bahia might be associated with the different sampling regions and the overall number of evaluated samples. Seasonal changes in frequency for bovine viral diarrhea may be observed and are generally associated with the close contact between animals.

Likewise, small herds tend to show a greater tendency toward the self-elimination of the disease (DAMMAN et al., 2015). The results of the present study confirmed that smaller herds presented low seropositivity, in accordance with data described by Talafha et al. (2009), who verified that the frequency of the BVDV in medium- and large-sized herds was higher than that in small-sized herds.

Table 2 – Evaluation of frequency for bovine viral diarrhea in the municipalities in Bahia and Pernambuco, 2017.

Region	Animals (+)	Total	Seropositive animals (%)
Petrolina	15	54	27.8
Senhor Do Bonfim	59	111	53.1
Miguel Calmon	70	92	76.1
Total	144	257	56.0

Table 3 shows the frequency of animals positive for BVDV in comparison to the total number of animals on each herd in the municipality of Petrolina, Pernambuco. There was no vaccination against the disease, and the frequency of seropositive animals varied, with recorded percentages of 3.6%, 81.2%, 20.0%, and 0.0% for the assessed herds.

Table 3 – Frequency for bovine viral diarrhea on herds in the municipality of Petrolina, Pernambuco.

Herd	Vaccine	Animals (+)	Total	Seropositive animals (%)
A	No	1	28	3.6
B	No	13	16	81.2
C	No	1	5	20.0
D	No	0	5	0.0
TOTAL		15	54	27.8

Table 4 shows the frequency of animals positive for BVDV in comparison to the total number of animals on each herd in the municipality of Miguel Calmon, Bahia. There was no vaccination against the disease, and the recorded percentages of seropositive animals were 87.3%, 42.9%, 58.3%, and 66.6% for the assessed herds.

Table 4 – Frequency for bovine viral diarrhea on herds in the municipality of Miguel Calmon, Bahia.

Herd	Vaccine	Animals (+)	Total	Seropositive animals (%)
E	No	55	63	87.3
F	No	6	14	42.9
G	No	7	12	58.3
H	No	2	3	66.6
TOTAL		70	92	76.1

Table 5 shows the frequency of animals positive for BVDV in comparison to the total number of animals on each herd in the municipality of Senhor do Bonfim, Bahia. Vaccination was not performed against the disease, with the exception of herd A. The recorded percentages of seropositive animals were 68.2%, 31%, 21.4%, and 80.0% for the assessed herds. The presence of vaccinated animals on herd A indicates the frequency results obtained from the vaccination practice. However, there may have been vaccine failure, as some animals in the herd did not show seroconversion. In addition to low and medium titers observed in a study by Vogel et al. (2002), crossed neutralization tests revealed significant variation in the neutralizing activity against antigenically different field isolates. These results show that the vaccination did not induce a serologic response of appropriate magnitude and duration in most of the animals, mainly due to the great antigenic diversity of the samples of BVDV.

The present study is the first to broadly investigate the frequency of bovine viral diarrhea in this region in northeastern Brazil. Studies carried out by Mineo et al. (2006) and Fernandes et al. (2016) found a frequency of 62.0% and 65.5% for BVDV in the states of Minas Gerais and Paraíba, respectively. A study in the state of Rio Grande do Sul reported a frequency of 48.8% in dairy cattle herds (ALMEIDA et al., 2013). As for the Northeast region, a higher prevalence of seroreactive animals using iELISA was described by Chaves et al. (2010) (61.5%, 246/400) and Sousa et al. (2013) (67.30%, 105/156) in dairy herds unvaccinated against BVDV in the state of Maranhão. These results suggest a lower prevalence of infection in beef cattle, probably due to the shorter period of stay of the animals within the properties and,

consequently, to a decreased direct contact of susceptible animals with infected animals (VIANA et al., 2017).

Table 5 – Frequency for bovine viral diarrhea on herds in the municipality of Senhor do Bonfim, Bahia.

Herd	Vaccine	Animals (+)	Total	Seropositive animals (%)
I	Yes	43	63	68.2
J	No	9	29	31.0
L	No	3	14	21.4
M	No	4	5	80.0
TOTAL		59	111	53.2

A study conducted by Byrne et al. (2017) in Ireland found that 663 out of 4,828 samples (13.73%) were identified as positive for the viral disease (BVDV), whereas in Australia, Hay et al. (2016) found a positivity of 0.24% for the BVDV-1.

Evaluation of risk factors associated to infection with BVDV

No positive correlation was observed in the correlation analysis performed between intra-herd frequency and the environmental variables of annual mean temperature and rainfall in the municipalities. Risk factor analyses are crucial for the comprehension of disease epidemiology and the proposal of control measures. According to Gates; Humphry; Gunn (2013), livestock movement records, routine serologic tests, and questionnaires can provide epidemiological data.

Tables 6 and 7 show the results for the risk factor analyses on the assessed herds. In Table 5, the univariate analysis points to a relationship between the evaluated variables and the disease ($P \leq 0.20$).

Table 6 – Univariate analysis of management variables associated with bovine viral diarrhea in the northeastern semiarid region of Brazil.

Variable	N° of animals	N° of positives (%)	P
Farming system			
Intensive	33	2 (6.1)	
Semi-intensive	52	27 (51.9)	
Extensive	108	63 (58.3)	< 0.001*
Rearing method			
(Calves born on-farm only)	63	18 (28.6)	
(Calves born on-farm + purchased weaned calves and yearlings)	115	68 (59.1)	
(Purchased weaned calves and yearlings + animals purchased for fattening)	14	5 (35.7)	0.001*
Livestock use			
Beef	14	5 (35.7)	
Dairy	111	39 (35.1)	
Mixed purpose	68	48 (70.6)	< 0.001*
Quarantine/examinations			
Both	28	1 (3.6)	
Only quarantine	16	13 (81.2)	
Only examinations	14	5 (35.7)	
None	135	73 (54.1)	< 0.001*
Reproductive Management			
Natural	96	56 (58.3)	
Controlled	5	1 (20.0)	
Artificial Insemination	92	35 (38.0)	0.009*
Technical Assistance			
No	55	17 (30.9)	
Yes	99	68 (68.7)	< 0.001*

Table 7 – Environmental risk factors associated with seropositivity for bovine viral diarrhea in the northeastern semiarid region of Brazil.

Risk Factor	Regression coefficient	Standard Error	Odds ratio	IC 95%	P
Extensive Breeding System	0.943	0.376	2.58	1.24 – 5.38	0.010
Mixed-Use	1.199	0.386	3.32	1.55 – 7.07	0.001

Through univariate analysis, an association was observed between seropositivity and breeding type, with the highest frequency being verified in farms that acquired and reared animals (59.1%). A high prevalence was also observed in properties that did not perform any type of laboratory tests (54.1%). With regard to reproductive management, those properties that presented natural breeding as reproductive management also presented the highest seropositivity (58.0%). Properties that adopt a more rigorous reproductive management, with periodic tests for detection of important diseases tend to present a lower frequency of the disease.

The multivariate analysis allowed to identify the extensive breeding system (OR=2.58) and the mixed-use (OR=3.32) variables as risk factors for bovine viral diarrhea. In the assessed region, it was common for herds that adopted an extensive farming system to have a deficient health management program, favoring the emergence of important health issues. Along with health management, extensive systems can also influence the occurrence of the infection (QUINCOZES et al., 2007). Likewise, herds that produced beef and milk concomitantly did not have appropriate disease prevention programs. Fernandes et al. (2016) observed that the purpose of the livestock did not constitute a risk factor for bovine viral diarrhea, but that introduction of animals into the herd was considered an important risk factor for this disease. Gates et al. (2014) stated that on-site transmission of the virus was higher in dairy herds than in beef cattle herds, especially when there had been recent livestock movement. Similar data were obtained by Byrne et al. (2017) for dairy herds (20.95% positive animals) (10.42%, OR: 3.03, $P < 0.001$).

Biosecurity measures aiming to reduce the introduction of the virus into herds are essential in order to reduce reproductive losses associated with bovine viral diarrhea. It is similarly of utmost importance to minimize the contact of pregnant females with possible sources of infection during the first 125 days of gestation, given the probability of persistently infected calves.

CONCLUSION

The presence of BVDV-seropositive animals in the assessed municipalities suggests circulation of the viral agent in these locations. Therefore, control and preventive measures must be implemented in order to minimize losses due to the disease, such as the monitoring of the properties through laboratory tests, elimination of infected animals, and the use of vaccines in the indicated regions.

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