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Communication

Antibodies against *Bovine herpesvirus 1* in dairy herds in the state of Espírito Santo, Brasil¹

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ABSTRACT

Bovine herpesvirus 1 (BoHV-1) causes major losses in worldwide livestock, affecting the respiratory and reproductive tracts of bovine. In the past decades, the number of cases in Brazil has been gradually increasing. Therefore, it is important to assess the distribution of infection in different regions of the country. In the state of Espírito Santo (ES) the BoHV 1 infection rate in dairy cattle herds is unknown. Thus, the aim of this study was to detect neutralizing antibodies against BoHV-1 in serum samples from 1,161 non-vaccinated cows from 59 dairy cattle herds in 23 municipalities of the Metropolitan, North, Northwest and South macro-regions. The identification of seropositive cows was evaluated by the virus neutralization test. The results showed that of all serum samples evaluated 775 (66.75%) had neutralizing antibodies against BoHV-1. Moreover, all herds were found positive; however, the percentage of positive cows varied among regions; 49.06%, 62.15%, 67.21% and 80.04% for the Metropolitan, South, North and Northwest macro-regions, respectively. In this study, the results clearly indicate the dissemination of the viral agent in dairy cattle in the ES state, requiring the monitoring and control of diseases related to BoHV-1 infection.

Key words: Infectious bovine rhinotracheitis (IBR), virus neutralization, dairy cows, BoHV-1, Espírito Santo.

RESUMO

Anticorpos contra herpesvírus bovino 1 em rebanhos leiteiros do Estado do Espírito Santo, Brasil

O herpesvírus bovino 1 (*Bovine herpesvirus 1* - BoHV-1) causa grandes prejuízos econômicos à pecuária bovina mundial, por comprometer os sistemas respiratório e reprodutivo de bovinos de corte e leiteiros. Nas últimas décadas, os registros de casos clínicos ocasionados pelo BoHV-1, no Brasil, mostram que têm aumentado gradualmente, o que torna importante o delineamento de estudos que mostrem a distribuição da infecção nas diversas

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regiões do país. O objetivo deste estudo foi investigar a frequência de anticorpos anti-BoHV-1 em rebanhos bovinos leiteiros do Estado do Espírito Santo (ES), região ainda carente de informações sobre a distribuição desse patógeno. Para o estudo, foram utilizadas amostras de soro de bovinos leiteiros, não vacinados contra o BoHV-1, de 1.161 animais, provenientes de 59 rebanhos, oriundos de 23 municípios pertencentes às macrorregiões metropolitana, norte, noroeste e sul do ES. Para a identificação de animais soropositivos, foi aplicada a técnica de vírus-neutralização em microplacas. Das amostras de soros avaliadas, 775 (66,75%) apresentaram anticorpos neutralizantes contra BoHV-1, sendo todos os rebanhos considerados positivos. As percentagens das frequências de animais positivos foram bastante variadas, apresentando 49,06, 62,15, 67,21 e 80,04%, para as macrorregiões metropolitana, sul, norte e noroeste, respectivamente. Neste estudo, os resultados claramente indicam a circulação do agente viral no rebanho bovino leiteiro do ES, sendo necessário o controle e monitoramento das enfermidades relacionadas com o BoHV-1.

Palavras-chave: Rinotraqueíte infecciosa bovina (IBR), vírus-neutralização, vacas leiteiras, BoHV-1, Espírito Santo.

INTRODUCTION

Brazil has the second largest commercial cattle herd in the world with 205,292 million head in 2009 (IBGE, 2010). However, it still maintains low production rates, resulting from several factors, especially diseases of the reproductive cycle (Takiuchi *et al.*, 2005). In this context, infections caused by bovine herpesvirus 1 (*Bovine herpesvirus 1* - BoHV-1) (ICTV, 2012) are considered the main causes of reduced reproductive efficiency and beef production in Brazil and worldwide (OIE, 2008).

BoHV-1 is the etiological agent of clinical syndromes that include infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB) and reproductive disorders characterized by embryonic, fetal, and neonatal death. The BoHV-1 belongs to the family Herpesviridae, subfamily Alphaherpesvirinae, and has the ability to develop latent infection, mainly in the ganglion cells. This condition makes the animal chronically infected, favoring viral spread to susceptible animals (Winkler *et al.*, 2000).

Several risk factors are associated with the spread of BoHV-1. Studies show that high density herds are associated with greater likelihood of infection by the agent (Miller, 1991; McDermott *et al.*, 1997; Dias *et al.*, 2008). The association between herd size and seropositivity to BoHV-1 can be directly attributed to the often purchase of animals, participation in agricultural events, professional visits and the presence of regular and temporary workers in the properties (Van Schaik *et al.*, 2002).

A number of studies have been conducted to determine the prevalence of BoHV-1 in Brazilian herds (Ravazzolo *et al.*, 1989; Lovato *et al.*, 1995; Medici *et al.*, 2000; Dias *et al.*, 2008). However, regional variations in rates of prevalence (Kampa *et al.*, 2009) may occur. This

demonstrates the need for studies to characterize and quantify the BoHV-1 circulation in regions not yet been evaluated, as the State of Espírito Santo, Brazil. For these reasons, this study aimed to perform a serologic survey of BoHV-1 in dairy cattle herds from the different macro-regions of the state.

MATERIALS AND METHODS

A total of 1,161 serum samples were collected from cows of 59 dairy herds, in 23 municipalities of the four macro regions (Metropolitan, North, Northwest and South) of the state of Espírito Santo (ES), between 2007 and 2008. The samples of bovine sera were distributed as follows: Metropolitan (n = 269, 6 municipalities), South (n = 214, 7 municipalities), North (n = 274, 4 municipalities) and Northwest (n = 404, 6 municipalities). The sampled herds were selected on the basis of easy access and availability by the owners. The samples were taken on non-probability convenience sampling basis, because in addition to not keeping a list of specific elements of the population, there was not a defined sampling design (Medronho *et al.*, 2009). The objective of the study was to use the sampling to show the main characteristics of a specific study group, in this case, the dairy cattle herds of the ES macro-regions.

All samples were taken from dairy cows and not vaccinated against BoHV-1. The serum samples were stored in microcentrifuge tubes and stored at -20 °C until the serological test.

Madin-Darby bovine kidney cells (MDBK) were cultured for the virus neutralization test (VN). The cells were grown in minimal essential medium (Cultilab®) containing 1.6 mgL⁻¹ of penicillin (Cultilab®) and 0.4 mgL⁻¹ of streptomycin (Cultilab®), filtered through Millipore 0.22 µm filter, pH 7, added of 0.2% sodium bicarbonate

and 5% pestivirus-free fetal bovine serum (FBS) (Cultilab®) and antibodies against bovine viral diarrhea virus (BVDV). For the production of BoHV-1, the sample BoHV-1 Los Angeles (BHV-1 LA ATCC 864), kindly provided by the Laboratory of Animal Virology, Federal University of Santa Maria - RS, was amplified in cell culture with MEM supplemented with 5% fetal bovine serum. The viral titer in the suspension was obtained by the Reed and Muench method and expressed as 50% cell culture infective dose (CCID₅₀).

The serological diagnosis used the VN test, according to the method proposed by OIE (2008).

RESULTS AND DISCUSSION

The virus neutralization test (VN) indicated that 775 (66.75%) of the 1,161 serum samples were positive for BoHV-1, while 386 (33.25%) were negative.

The macro-regions of the state showed differences for the frequency of cows infected with BoHV-1. The highest frequency (80.04%) of seropositive cows for BoHV-1 was identified in the northwest regions and the lowest (49.06%) in the metropolitan region (Table 1). The samples representative of the macro-regions showed high frequency variation of low, medium and high anti-BoHV-1 antibody titers. The different results found among the macro-regions may be due to different factors such as cattle raising practices, health management and climate of each region included in the study.

The high frequency of positive sera in this work may be related to several factors that contribute to the introduction and maintenance of BoHV-1 in the herds, since 100% of the farms were found positive. The trade of animals without health requirements necessary to prevent infection, the lack of mandatory virological control of semen sold in Brazil, the virus ability to remain in a latent state, the lack of information on this disease for farmers and authorities contributes to the viral spread intra- and inter-herds (Moreira *et al.*, 2001; Van Schaik *et al.*, 2002).

Several studies were performed to determine the BoHV-1 infection rates in Brazilian herds. A study in

the State of Goiás, Barbosa *et al.*, (2005) reported 51.9% of infection rate. In the state of Paraná, in a study including farms with a history of reproductive problems, 41.9% of the animals and 90.5% of dairy herds were positive for BoHV-1 (Medici *et al.*, 2000). Bezerra *et al.* (2012) found prevalence of 71.3% in dairy cattle in a study in the State of Maranhão. In the state of Rio Grande do Sul, Holz *et al.* (2009) reported prevalence of 29.2% of seropositive animals for BoHV-1 and BoHV-5. Dias *et al.* (2008) found prevalence of 64.41% in Western Paraná State.

The virus neutralization test showed that BoHV-1 was disseminated throughout the sampled municipalities. High frequency of positive samples, over 75%, were found in the municipalities of Cariacica (84.09%), Pinheiros do Norte (82.43%), Montanha (76.36%), São Gabriel da Palha (79.59%), Boa Esperança (83.58%), Colatina (75.92%), Ecoporanga (79.42%), Baixo Guandu (91.66%), Presidente Kenedy (81.81%) and Alegre (90.9%).

BoHV-1 and BoHV-5 are antigenically related (Varela *et al.*, 2009; Mahony, 2010). Therefore, they show antigenic cross-reactivity in the diagnosis test used (VN), not being possible to differentiate between antibodies and viral species. Thus, some animals seropositive to BoHV-1 may alternatively be positive for BoHV-5. Even so, the diagnostic test used in this study is considered standard for the BoHV-1 diagnosis (Rocha *et al.*, 2001) and many authors have used this method to estimate the prevalence of BoHV-1 in different states of Brazil (Medici *et al.*, 2000; Barbosa *et al.*, 2005). The results of this study confirm the presence of BoHV-1 in dairy cattle herds of all studied macro-regions.

Some considerations regarding the sampling method should be made. Because the samples of this study are non-probability samples, they cannot be considered statistically and epidemiologically representative of the origin population. The state of Espírito Santo has 30,935 cattle herds (IBGE, 2006). However, the sampling involved 23 of the 78 municipalities of the state, in which the samples were taken at random and in a dispersed manner, thus covering most of the state's territory.

Table 1. Sampling description considering the number of municipalities, farms, cows and virus neutralization test for identification of anti-BoHV-1 antibodies according to each macro-region of the state of Espírito Santo, Brazil

Macro-region	Number:			(%)
	Municipalities	Farms	Cows	Positive samples
Metropolitan	6	13	269	49.06
North	4	12	274	67.21
Northwest	6	19	404	80.04
South	7	15	214	62.15
Total	23	59	1161	66.75*

*Percentage of positive animals in the total sampling

CONCLUSION

This study is the first to report the dissemination of BoHV-1 in dairy cattle in the state of Espírito Santo. The results show the occurrence of endemic viral infection in the tested samples. Given the economic and social importance of cattle farming in Brazil and the economic losses related to infection with BoHV-1, it is important to adopt measures for control and prevention of infection, aiming at reducing its impact on the herd health profile, particularly those related to the reproductive and respiratory systems.

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