Original Article

SURVEILLANCE OF NEUTRALIZING ANTIBODIES AGAINST *BOVINE HERPESVIRUS 1* IN CATTLE HERDS FROM DIFFERENT FARMING PROPERTY SYSTEMS

DISTRIBUIÇÃO DE ANTICORPOS NEUTRALIZANTES CONTRA O HERPESVIRUS BOVINO 1 EM PROPRIEDADES BOVINAS DE DIFERENTES SISTEMAS DE CRIAÇÃO

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ABSTRACT: The aim of this study was to investigate the presence of neutralizing antibodies against *bovine herpesvirus 1* (BoHV-1) in 722 non-vaccinated animals from eight properties that use different farming systems (extensive, semi-confinement and confinement). Serum samples were subjected to neutralization tests in order to search for anti-BoHV-1 antibodies. Serological results were categorized as negative, low titer, intermediate titer or high titer. One property showed no positive samples; while other properties presented frequency of positive samples ranging from 17.95% to 86.96%. For animals raised under confinement, the number of positive samples and neutralizing antibody titers were lower compared to others, possibly due to good sanitary practices adopted by this type of system. Altogether, our results can contribute towards the understanding of the endemic infection in Brazil.

KEYWORDS: Infectious bovine rhinotracheitis. Extensive. Semi-confinement. Confinement.

INTRODUCTION

Bovine herpesvirus 1 (BoHV-1) is classified as a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae* (ICTV, 2009), and it is known as one of the most important pathogens in cattle worldwide (MUYLKENS et al., 2007). BoHV-1 is capable to cause syndromes such as infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IPB). It can also cause abortion, infertility, conjunctivitis and encephalitis in cattle (NANDI et al., 2009). This herpesvirus causes latent infection in susceptible animals which will lead to the chronic stage (ACKERMANN; PETERHANS; WYLER, 1982). Major economic losses are associated with infection, mostly because of reproductive failure and intermittent viral excretion (TURIN; RUSSO, 2003).

Cattle farming systems can be divided into extensive, semi-confinement and confinement (ASSIS et al., 2005). The confinement systems are characterized by segregation of animals in specific areas with controlled food (PEIXOTO, 1988). The main characteristic of the semi-confinement system is the fact that the pasture is used for maintenance and energy source during part of the day, and after that, herd remains in places with controlled feed. Finally, in the extensive system the pasture area is the only energy source (ASSIS et al., 2005).

Cattle farming systems have a series of variables that can represent risk factors for BoHV-1 infection. Some of them are already known to increase the risk of infection; for instance, introduction of infected animals in the herd (PASTORET et al., 1982). Animals are exposed to each other in properties with extensive farming commonly because they share the same pasture; and that facilitates viral transmission by respiratory, ocular and genital secretions. Moreover, a potential infection can be considered via contact with another animal species which can act as carriers or vectors of the virus from one property to another (THORSEN et al., 1977). High density of animals sharing the same place can also increase the risk of infection (VAN WUIJCKHUISE et al., 1998). Furthermore, the frequent exposure to traders, farmers and veterinarians should be considered a risk factor for BoHV-1 infection (BOELAERT et al., 2005).

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BoHV-1 is present worldwide and varies in prevalence due to prevention and control methods. Particularly, in Brazil, antibodies against BoHV-1 have been extensively detected in herds (LOVATO et al., 1995; MELO; OLIVEIRA; FIGUEIREDO, 1997; MELO; AZEVEDO; ALFARO, 1999; MOLNÁR et al., 2001; ROCHA et al., 2001; DIAS et al., 2008; BEZERRA et al., 2012). Analysis of BoHV-1 infection in cattle herds raised in different systems has not been intensely investigated. Therefore, in the present work, our goal was to assess the presence of antibodies against BoHV-1 in non-vaccinated animals raised in extensive, semi-confinement and confinement systems.

MATERIAL AND METHODS

Sampling and property

This study was performed using blood samples from 772 animals (age approximately 24 months) from eight herds not vaccinated against BoHV-1, coming from Bom Despacho, Cajuri and Viçosa cities (Minas Gerais State), and Dores do Rio Preto (Espirito Santo State). From a total of 772 samples, 579 originated from dairy herds and 194 came from one herd of beef cattle exploration. Animals used in dairy farming were derived from two properties that uses the extensive system (E-P1: 98 animals, E-P2: 69 animals), two farms that uses the semi-confined system (SC-P4: 114 animals, SC-P5: 100 animals), and two properties that uses the confined farming system (C-P6: 115 animals, C-P7: 39 animals, C-P8: 43 animals). Beef cattle were originated from an extensive system production farm (E-P3:194 animals). All properties presented restrictions on animal's entry, except property EP-3. Samples were centrifuged and sera were stored at -20°C.

Cells and viral stock

MDBK (Madin-Darby bovine kidney) cells were cultured in Minimum Essential Media (MEM) (Cultilab), and supplemented with 5% fetal bovine serum (FBS) (Cultilab) at 37°C and 5% CO_2. For production of BoHV-1 stock, we used BoHV-1 LA strain (BoHV-1 Los Angles). Viral titer was obtained by $TCID_{50}$ and calculated according to Reed and Muench (1938).

Survey of neutralizing antibodies

Tests for neutralizing antibodies against BoHV-1 were performed using serum neutralization (SN) technique as described by House and Baker (1971), with minor modifications. Briefly, serum samples were thawed and placed in the water bath at 56°C for 30 minutes, and 200 DICC50/50µL of BoHV-1 LA was added to serum dilutions from each animal. Serum-virus mixture was incubated for 60 minutes at 37°C, and then, 50µL of MDBK cells suspension (300,000 cells/mL) were added. Tests were read after 72 hours of incubation by monitoring the cytopathic effect (EPC). Positive and negative control sera were also used.

Statistical analysis

Serological results were classified according to the titer obtained. Titers less than 2 were classified as negative, titers 2 and 4 as low, and titers 8, 16 and 32 as intermediate, and 64 and 128 as high. The chi-square test was used to verify differences between frequencies of positive and negative evidences against the system of creation. The non-parametric Kruskal-Wallis test was used to verify differences between titers of neutralizing antibodies and variables. Geometric mean values were expressed as mean $\log_4$ of the titer obtained. To discriminate differences within a group we used the Dunn test. Tests were performed by GraphPad Prism version 5.00 for Windows (San Diego, USA), and significance was based on $p<0.05$.

RESULTS

Seven out of eight properties selected for this study were positive. The positivity of farms varied from 17.95 to 86.96% (table 1). Our results demonstrate that properties C-P7 and C-P8 presented the greatest numbers of negative samples. In fact, the property C-P8 was the only one that did not have positive animals. On the other hand, property E-P2 showed high frequency of intermediate and high titers of antibodies against BoHV-1.

Table 1. Evaluation of the presence of neutralizing antibodies to BoHV-1 in different properties.

Farm	Negative samples (%)	Positive samples (%)			Total positive samples (%)
		Low	Medium	High	
E-P1	42.8	15.3	33.6	8.2	57.2
E-P2	13.0	18.8	40.6	27.5	87.0

E-P3	39.2	19.1	32.0	9.8	60.8
SC-P4	43.9	10.5	36.8	8.8	56.1
SC-P5	42.0	19.0	29.0	10.0	58.0
C-P6	47.0	36.5	15.7	0.9	53.0
C-P7	82.1	2.6	15.4	0	17.9
C-P8	100.0	0	0	0	0

As showed in Figure 1, significant differences were observed between the systems adopted. Animals raised in the extensive and semi-confinement systems showed a higher titer frequency (12.7% and 9.3%, respectively) compared to the confinement system (0.6%). Therefore, for the confinement system a higher frequency of negative samples (65.5%) was statistically significant compared to the extensive (35.2%) and semi-confinement (43%) systems. Between extensive and semi-confinement systems, no substantial difference was observed.

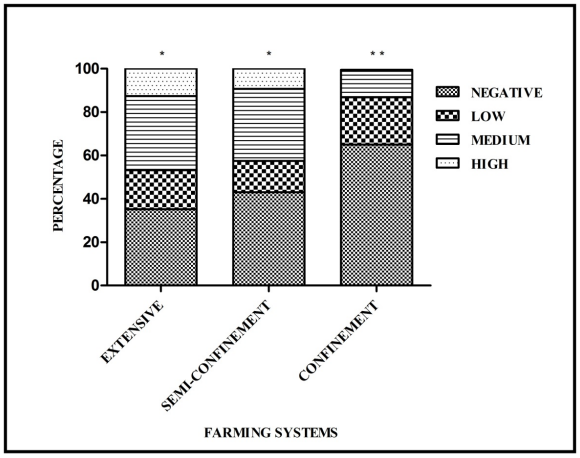

Figure 1. Frequency of samples containing low, intermediate, and high antibody titers. Negative samples in the different farming systems studied. **: frequencies of negative, intermediate and high antibody titers in the confinement system showed significant differences compared to other systems. ($p < 0.05$).

The confinement system also had a lower geometric mean for neutralizing antibodies (0.38 ± 0.655) when compared to the extensive (1.24 ± 1.16) and semi-confined (1.06 ± 1.127) systems evaluated (Figure 2).

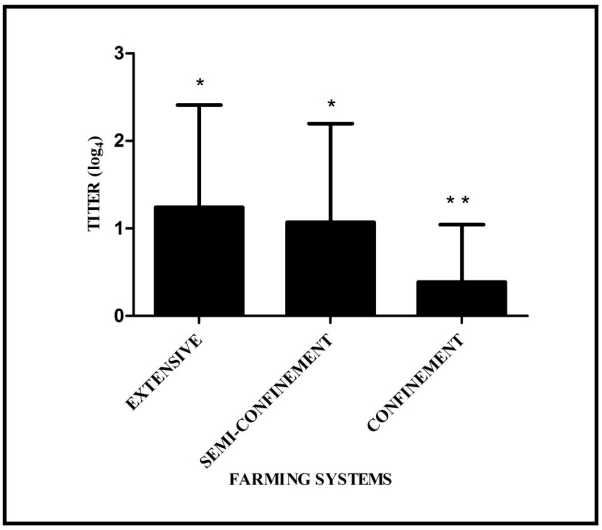

Figure 2. Mean of antibody titers converted by log4 at the different farming systems, **: significant difference between the confinement system compared to other systems

DISCUSSION

The main cattle systems used in Brazil are both extensive and semi-confined. However, the confinement system is increasing significantly throughout the country (BRAGA, 2010). Consequently, understanding the dynamics of BoHV-1 in these systems is important to improve animal management. Studies about viral circulation in animals naturally infected with BoHV-1 and its association with creation system remain scarce. Here, the present study aimed to present information on the distribution of infection in different farming systems.

The frequency of positive animals showed high variation among the sampled properties, which corroborates with results shown in serological studies for BoHV-1 performed in various regions of Brazil (ROCHA; GOUVEIA; LEITE, 1995; MELO; OLIVEIRA; FIGUEIREDO, 1997; BEZERRA et al., 2012). Furthermore, our results demonstrated a lower frequency of seropositive animals in the breeding confinement system. That can be explained by the sanitary management which is best controlled at the feedlot. Moreover, typically in this system there are no restrictions on the contact between herd animals and animals from neighboring properties (FRASER, 1980).

Another advantage of the containment system is the control of external factors such as contact with other species that could be infected by BoHV-1. Infection of cattle transmitted by other species may occur under natural conditions. Indeed, this type of infection has been described (AGUIRRE et al., 1995) and experimented (THORSEN et al., 1977) in wild cervids. For our study this possibility was ruled out since the properties sampled are located in regions between the Cerrado and Atlantic Forest which are inhabited by cervids.

Furthermore, considering the raising system as a risk factor, a study of dairy herds in northeastern China showed that animals reared in extensive system had higher rates of infection by *Neospora canimum* when compared to animals raised in the confinement system (WANG et al., 2010). In fact, another study in Italy by Rinaldi et al. (2007) also demonstrated that *N. caninum* infection increases the risk of BoHV1 infection in cattle. However, in our study we did not investigate whether animals raised in the extensive system were also co-infected with *N. caninum*.

Taking into consideration that the sanitary practices in the extensive, and semi-confined systems are less controlled than the confinement system (FRASER, 1980), it is possible to assume that animals raised under poor health management practices have potentially higher rate of infection by different pathogens. Additionally, some studies have shown that animals infected by other pathogens are also the most susceptible to BoHV-1 infection (MENSIK et al., 1976; MSOLLA et al., 1983). But in a another study, Dias et al. (2008) evaluated different risk factors for BoHV-1 between extensive and semi-confined/confined systems and found no difference in the percentage of positive animals. In this case, this result could be explained because analyses of confined and semi-confined systems were placed in the same group. Moreover, antibody titers against BoHV-1 were not assessed during this study, which is important when evaluating results.

It is also relevant to mention the ability of BoHV-1 in establishing latent infection. This characteristic makes them indefinitely attached to their hosts, and viruses can circulate between them during the primary infection or by reactivation from latency (MUYLKENS et al., 2007). This type of infection is characterized by the absence of viral expression and, therefore, inexistent production of antigenic molecules that stimulate the immune response (NANDI et al., 2009; JONES; SILVA; SINANI, 2011). At this point, antibody titers against BoHV-1 are lower but they tend to increase when viral infection is reactivated (MUYLKENS et al., 2007). Although it is difficult to determine the latent infection *in vivo*, our results demonstrated that neutralizing antibody titers were higher in animals from the extensive and semi-confined systems than those from the confined system, which suggests either a recent viral reactivation or primary infection.

Altogether, our study showed that extensive and semi-confined management systems seem to favor BoHV-1 infection in cattle because of the higher frequency of seropositivity. Therefore, our data indicates that cattle raised extensively are more susceptible to infection by BoHV-1. Since in Brazil extensive and semi-confined systems prevail over confined, our results suggest that the extensive system can be a risk factor for the occurrence of BoHV-1infection.

CONCLUSION

Although the present work did not aim to study the prevalence of BoHV-1 in Brazil, our results can help to understand the risk factors associated with the handling of animals. Our data indicate that the confinement system contributes to a

lower risk of BoHV-1 infection when compared to extensive and semi-confinement systems.

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RESUMO: Este trabalho teve por objetivo avaliar a distribuição de anticorpos neutralizantes contra o *herpesvirus bovino 1* (BoHV-1) em 722 animais não vacinados pertencentes a oito propriedades que utilizam diferentes sistemas de criação (extensivo, semi-confinamento e confinamento). As amostras sorológicas foram submetidas ao teste de soroneutralização para pesquisa de anticorpos anti-BoHV-1. Os resultados foram classificados em títulos negativo, baixo, médio e alto. Uma propriedade não apresentou amostras positivas, enquanto que nas demais propriedades a frequência de amostras positivas variou de 17,95% a 86,96%. Os animais do sistema de criação por confinamento apresentaram menor número de amostras positivas quando comparado aos animais do outros sistemas de criação, possivelmente devido às boas práticas sanitárias adotadas por esse sistema. Dessa forma, nossos resultados podem contribuir para o entendimento dessa infecção endêmica no Brasil.

PALAVRAS-CHAVE: Rinotraqueíte infecciosa bovina. Extensivo. Semi-confinamento. Confinamento

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