Seroprevalence and risk factors associated with Bovine Herpervirus Type 1 (BoHV-1) in settlements in the state of São Paulo, Brazil

Soroprevalência e fatores de risco associados ao Herpesvírus Bovino Tipo 1 (BoHV-1) em assentamentos do estado de São Paulo, Brasil

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Article info

Abstract

Family farming is an agricultural activity that plays an important socioeconomic role in several Brazilian regions. In the Pontal do Paranapanema region of São Paulo, Brazil, dairy farming is one of the main agricultural activities generating income for many families. However, despite the region standing out for dairy farming, sanitary problems have been a primary cause of a reduction in production in the region. Among them are diseases caused by Bovine Herpes Virus Type 1 (BoHV-1) that result in respiratory and reproductive alterations in cattle and consequently generate economic losses. This study aimed to analyze the prevalence of BoHV-1 in dairy cattle in the settlements of Presidente Epitácio and Mirante do Paranapanema. In total, 168 blood serum samples were evaluated using a virus neutralization technique. The prevalence of BoHV-1 was 69.05% (116/168). This trend was observed in multiparous cows infected with the virus. The high seroprevalence suggests that BoHV-1 is disseminated in these settlements. Therefore, sanitary and control measures must be implemented to reduce the impact of these agents.

Resumo

A agricultura familiar é uma atividade agrícola que desempenha importante papel socioeconômico em diversas regiões brasileiras. Na região do Pontal do Paranapanema, no estado de São Paulo, Brasil, a pecuária leiteira é uma das principais atividades agrícolas geradoras de renda para muitas famílias. Porém, apesar da região se destacar pela pecuária leiteira, os problemas sanitários têm sido a principal causa da redução da produção na região. Entre elas estão as doenças causadas pelo Herpes Vírus Bovino Tipo 1 (BoHV-1) que resultam em alterações respiratórias e reprodutivas nos bovinos e consequentemente geram perdas econômicas. Este estudo teve como objetivo analisar a prevalência do BoHV-1 em bovinos leiteiros nos assentamentos Presidente Epitácio e Mirante do Paranapanema. Um total de 168 amostras de soro sanguíneo foram avaliadas por meio de técnica de vírus-soroneutralização. A prevalência do BoHV-1 foi de 69,05% (116/168). Houve uma tendência observada em vacas multíparas infectadas pelo vírus. A alta soroprevalência sugere que o BoHV-1 esteja disseminado nesses

assentamentos. Portanto, medidas sanitárias e de controle devem ser implementadas para reduzir o impacto desses agentes nos animais.

Pavaras-chave: clínica da reprodução; fertilidade; doenças respiratórias; clínica de ruminantes.

1 | Introduction

Family farming is an agricultural practice that holds significant social and economic importance in several regions of Brazil, contributing substantially to the domestic and global food supply (Bertolini et al., 2020). According to data from the Brazilian Institute of Geography and Statistics (IBGE), Brazil has approximately 5,073,324 agricultural establishments, of which 3,897,408 are classified as family farms, representing 76% of all establishments and occupying 23.0% of the total land dedicated to agricultural activities (IBGE, 2019). Family farming plays a vital role in the country's economy, contributing 10% of the national gross domestic product (GDP), and significantly impacting the economies of many municipalities (Beltrame and Pereira, 2017).

The state of São Paulo is known in the national economic scenario to have the best progress indicators in urban and rural areas, proving its importance to the Brazilian economy by representing 34% of the GDP (Firetti et al., 2012). The Pontal do Paranapanema region has been highlighted for dairy production, which is the most important livestock activity and serves as the primary source of income for families in the settlements (Barone et al., 2012; Urchei et al., 2013; Verges et al., 2014). However, despite its prominence in dairy farming, several health factors, including viral diseases caused by bovine alphaherpesvirus type 1 (BoHV-1), have reduced its productivity.

BoHV-1 belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae* (Blume et al., 2018), and the genus *Varicellovirus* (Queiroz-Castro et al., 2019). It is an enveloped double-stranded DNA virus with icosahedral symmetry and a diameter of 70–110nm (Costa et al., 2017). The capsid is covered by an amorphous protein coating called the tegument, the outer layer of which is a lipoprotein envelope with glycoprotein spikes on its surface that induce an immune response in the host (Fino et al., 2012).

BoHV-1 was first isolated in Brazil in 1978, from pustules in cows (Alice, 1978; Costa et al., 2017). It is one of the main pathogens responsible for various clinical, reproductive, and respiratory diseases in cattle (Botton et al., 2022), including infectious bovine rhinotracheitis, pustular vulvovaginitis, pustular balanoposthitis, abortions, conjunctivitis, and meningoencephalitis (Blume et al., 2018; Gaeta et al., 2018; Silva et al., 2019).

BoHV-1 is found worldwide (Souza et al., 2018), except in some European countries that have managed to eradicate the infection (Bezerra et al., 2012a). Its high occurrence in small, medium, and large herds of beef and dairy cattle has been demonstrated in several Brazilian regions (Barbosa et al., 2019). Because of this, several seroepidemiological surveys have been conducted employing the virus neutralization techniques used to diagnose bovine herpesviruses.

A study conducted by Pasqualotto et al. (2015) using blood samples from cattle of reproductive age with a history of reproductive disorders, such as abortion and early embryonic mortality, found that 57.54% of the animals were seropositive for BoHV-1. Silva et al. (2019) evaluated 358 samples from cows aged 24 months or older, in the state of Pernambuco, that had not been vaccinated for infectious bovine rhinotracheitis (IBR) or bovine viral diarrhea (BVD); they detected anti-BoHV-1 antibodies in 52.8% (188/356) of the analyzed animals.

BoHV-1 is a virus with a high morbidity rate and low mortality; its main characteristic is its ability to remain in a latent state in its hosts for long periods, which could lead to viral reactivation (Oliveira et al., 2015) under stress conditions and/or immunosuppressive periods (Affonso et al., 2010), weaning, severe nutritional deficiencies, vaccinations, dehorning, transport, castration, and the introduction of new animals into herds (Blume et al., 2018; Botton et al., 2022).

Sensory ganglia that innervate the primary sites of infection, mainly the trigeminal and sacral ganglia (Viu et al., 2014), are the preferred tissues for establishing latency (Oliveira et al., 2015). Because of its latent nature, animals affected by the virus may show few clinical signs of the disease, making them potential virus carriers (Urzêda et al., 2018).

The main entry routes of BoHV-1 into the host are the respiratory mucosa, conjunctival epithelium, and genital organs (Costa et al., 2017). Transmission may also occur through aerosols and fomites (Viu et al., 2014), embryonic and fetal transplacental

infections through copulation and artificial insemination (Lopes et al., 2022), or through the use of contaminated semen obtained using cryopreservation techniques (Souza et al., 2018).

Owing to the lack of research on Brazilian settlements, the present study aimed to investigate the prevalence of antibodies against Bovine Herpesvirus type 1 (BoHV-1) in herds of cows raised in the settlements of Presidente Epitácio and Mirante de Paranapanema in the state of São Paulo. This study is the first to evaluate the presence of type 1 virus in this livestock category

2 | Materials and Method

2.1 | Study Design and Setting

This study was conducted in Pontal do Paranapanema, a region comprising 32 municipalities located far west of São Paulo. This region is bordered by the Paraná and Paranapanema rivers and boundaries with the states of Mato Grosso do Sul and Paraná (Verges et al., 2014).

According to the Köppen and Geiger classification, the climate in the Pontal do Paranapanema region can be categorized as AW or Cwa. An AW climate is characterized as humid tropical, with a rainy season during summer and a dry season in winter, and average temperatures ranging from 22–24°C, along with an average annual

precipitation of 1,500 mm. The Cwa climate, on the other hand, is the predominant type in the region and is defined as a mesothermal dry winter, with a rainy season during summer and a dry season in winter as well, but with an average annual temperature below 22°C (Ferreira et al., 2022).

2.2 | Study Design

A two-stage sampling design was implemented to determine the number of cows to be sampled. The first stage focused on determining the number of properties to be visited, whereas the second stage determined the number of animals to be sampled from each property using the EpiTool: Epidemiological Calculator (Sergeat, 2015). For sample size calculation, diagnostic tests with a sensitivity and specificity of at least 70%, were required. An expected prevalence of at least 47% was set for the animals and 10% for the properties, with a confidence level of 95%.

2.3 | Sampling

A total of 42 properties were visited. Blood samples were collected from 12 properties in Presidente Epitácio and 30 properties in Paranapanema, with four samples collected at each site, resulting in a total of 168 samples (Table 1).

Table 1. Distribution of properties visited and samples collected (serum) in Presidente Epitácio and Mirante do Paranapanema, São Paulo, Brazil, 2016

Municipality	No. of Properties Visited N (%)	No. of Samples N (%)
Presidente Epitácio	12 (28.57)	48 (28.57)
Mirante de Paranapanema	30 (71.43)	120 (71.43)
Total	**42 (100)**	**168 (100)**

Initially, a semi-structured epidemiological survey was applied to obtain information about the characteristics of the property and the general and reproductive health of the herd. Then, in order to assess the health status of the animals being studied, the methods described by Rosenberg (1993) for general clinical evaluation of the animals were used.

Approximately 8mL of blood were collected from each animal by venipuncture of the external jugular vein, using a vacuum system (BD vacutainer® dry). After collection, the tubes were centrifuged at 600xg for 15 minutes to obtain serum, which was

placed in microtubes and frozen for later processing at the Laboratório de Viroses de Bovídeos (LVB) located at the Biological Institute of the State of São Paulo.

2.4 | Laboratory testing

For the virus neutralization reaction, 96-well flat-bottom microtiter plates (TPP, Techno Products AG, Switzerland) were used for the following purposes: reaction, serum control, dose control, and

back titration. In the plates where the reaction occurred, 100µL of MEM (Minimum essential medium, Cultilab, Campinas, Brazil) were added. After the addition of 40µL of each sample, 50µL from each well in row A were transferred to row B, and the procedure was repeated up to row H. Forty microliters of the corresponding viral doses of 1.56 TCIDs (tissue culture infectious dose), 3.125 DICTs, 6.25 DICTs, 12.5 DICTs, 25 DICTs, 50 DICTs, 100 DICTs, 200 DICTs, 400 DICTs, and 500 DICTs, respectively. All plates were incubated in an incubator with 5% CO_2 at 37°C for 1h. Then, all plates received 50µL of MDBK cells at a concentration of $2\times2\times10^5$cells/mL, except for the serum control. Finally, the plates were incubated again at 37°C in an incubator with 5% CO_2 for four days. Samples that did not present the CPE were considered reactive. The presence of CPE indicates the absence of antigen-antibody binding; therefore, the sample was considered non-reactive.

3 | Results and Discussion

Of the 168 blood serum samples analyzed and obtained from farms in Presidente Epitácio and Mirante de Paranapanema, 116 tested positive for BoHV-1. The serological prevalence of BoHV-1 was 69.05%, which confirmed the infection of the herds with bovine herpes virus type 1.

The serological tests performed in this study identified considerable seropositivity, which demonstrated the wide dissemination of the agent in the herds of the analyzed farms. The prevalence of BoHV-1 (69.05%) in the studied region was similar to those reported by Freitas et al. (2014) in Maranhão (63.23%), Oliveira et al. (2015) in Pernambuco (52.3%), Silva et al. (2015) in Garanhuns (79.5%), Duarte and Santana (2018) in Alegrete-RS (40.90%), Arruda et al. (2019) in Acre (61.1%), and Silva et al. (2019) in Pernambuco (52.8%). However, Haas et al. (2020) found a considerably higher seroprevalence (95.7%) in northern Minas Gerais.

The variations in the prevalence of BoHV-1 demonstrated in the aforementioned studies can be explained by differences in the production systems adopted by the farms, animal management, age, number of animals sampled, sampling techniques, diagnostic methods, and the regional characteristics of each study, which can cause differences in the results (Affonso et al., 2010; Freitas et al., 2014; Silva et al., 2015; Arruda et al., 2019).

Regarding the prevalence of antibodies against BoHV-1 by studied region, settlements located in Mirante de Paranapanema exhibited a higher prevalence than those in Presidente Epitácio. The variations in prevalence between the two regions in this study can be explained by the different types of management and degrees of technification of each sampled farm.

In this study, a trend was observed between the presence of antibodies against BoHV-1 and the number of births (P=0.0556). However, no significant differences were observed in body condition, pregnancy, date of last birth, postpartum alterations, retained placenta, or vaginal alterations, as shown in Table 2.

Table 2. Evaluation of reproductive parameters between positive and negative animals for BiHV-1

Variable	P-Value (BoHV-1)
Number of births	0.0556
Body condition score	0.8605
Pregnancy	0.8620
Date of last calving	0.4901
Reproductive alterations*	0.1653
Vaginal alterations**	0.6766

*Reproductive alterations (abortion, stillbirth, malformations, and dystocia); **Vaginal alterations (edema, discharge, trauma, vesicles, nodules, and pustules).

Although BoHV-1 is reportedly closely related to reproductive disorders in cows, such as embryonic death, repeated estrus, irregular calving intervals, and abortions (Barbosa et al., 2019), these variables were not significant in the present study, possibly because these characteristics were not reported at many of the properties. A study carried out on the prevalence and risk factors associated with BoHV-1 infection by Bezerra et al. (2012a) detected a higher prevalence of the agent on properties with a history of reproductive alterations. In this study, the calving interval tended to increase, indicating that the presence of herpes virus in the herd caused settler losses.

Regarding the calving variables, multiparous cows were found to have a higher seropositivity than primiparous cows. This result was similar to that described by Tadeg et al. (2021) who evaluated the seroprevalence of IBR and Brucellosis and their effects on dairy cattle. They observed a higher prevalence of the disease in multiparous cows than in primiparous cows, demonstrating a strong association between BoVH-1 and the number of

births. It is assumed that the higher prevalence of BoHV-1 in cows with more calves is because these animals are more exposed to the risk factors for this disease (Bezerra et al., 2012b). Regarding the body condition score (BCS), this study found that most animals that tested positive for BoHV-1 had scores between 2.75 and 3.5, which are typical in dairy production. This indicates that infection by the virus can occur regardless of body condition. Consistent with this finding, in a seroprevalence study, Dilma et al., (2024) demonstrated that animals with good body condition scores were considerably more seroprevalent than those with low body condition scores.

Several factors contribute to the occurrence of BoHV-1 in cattle herds worldwide, including production systems, which are strongly correlated with the occurrence of the virus in the herd. In this study, variable production systems (extensive and semi-intensive) did not show statistical significance (P=0.7317) for the prevalence of BoHV-1 in the evaluated herds. The results of this study do not support the findings of Silva et al. (2015), who demonstrated a higher prevalence of the virus on properties that used a semi-intensive system.

The presence of wetlands on the studied farms was not statistically significant (P=0.2509) for BoHV-1 prevalence. This is in contrast to the findings of Fernandes et al. (2016) who analyzed the risk factors associated with BoHV-1 and BVDV in buffalo in the state of Paraíba; they found that the presence of wetland areas was an important risk factor for BoHV-1 (P=0.002).

Although herd size, number of animals, total number of cows, and total number of calves did not show any significance for BoHV-1 (P=0.4332, 0.1869, 0.1404, and 0.8605, respectively) in the current study, some previous studies have demonstrated that herd size and high animal density are strongly associated with BoHV-1 seropositivity because animal-to-animal transmission is more frequent, increasing the risk of transmission of infectious disease (Woodbine et al., 2009; Fernandes et al., 2019).

Adult animals are frequently affected by viruses because of increased stress from management practices and greater exposure to the virus, particularly when new seropositive animals are introduced into the herd and shed the virus through secretions (Bezerra et al., 2012b).

Barbosa et al. (2019) observed that a higher number of animals in a herd is an important risk factor

for BoHV-1 infection, and that high seroprevalence is shown under high density conditions. Another study indicated that older cows are more susceptible to BoHV-1 infection than younger animals (Dilma et al., 2024).

When the feeding system was analyzed as a risk factor for BoHV-1 infection, no significant difference was observed for this variable (P=0.7317). However, Silva et al. (2015) emphasized that the feeding system can be a potential risk factor for BoHV-1 infection, as contact with nasal secretions from infected animals, inhalation of aerosols, and consumption of contaminated water or food are the main transmission routes of the virus. In the present study, because most animals were raised in a semi-intensive system, this risk factor could not be evaluated.

Although the statistical data were insignificant (P=0.4243) for the reproductive management variable, Barbosa et al. (2019), when evaluating the risk factors associated with BoHV-1 in dairy and crossbred herds with reproductive problems, it demonstrated that the non-use of artificial insemination constitutes an important risk factor for viral infection.

The presence of other domestic animals analyzed in this study, as well as other variables, did not show any statistical significance (P=0.5263). Although this variable does not appear to be a risk factor for infection, Silva et al. (2015) reported that cattle and other domestic species, even if they do not play a significant role in the spread of the virus, can still act as reservoirs and mechanical transmitters when moving from one place to another.

4 | Conclusion

The results of this study revealed a high prevalence of BoHV-1 seropositivity in dairy farms in Paranapanema, suggesting that the virus is widespread in the region. The adoption of effective prophylactic and sanitary measures, such as vaccination and good management practices, should be integrated into farms to control infection in herds and consequently avoid economic losses caused by the infectious agent. Periodic seroepidemiological surveys in these regions are necessary to assess their sanitary status for the prevalence of BoHV-1 infections in herds.

5 | Conflict of Interest Statement

The authors declare no conflict of interest.

6 | Ethics Committee

This study was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/VET) under protocol number CEUA 7447020215.

7 | Acknowledgements

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