BIA
BOLETIM DE INDÚSTRIA ANIMAL

ANIMAL SANITY

SEROLOGIC PROFILE OF BOVINE ALFAHERPESVIRUS TYPE1 (BOHV-1) AND PESTIVÍRUS A (BVDV-1) IN HERDS OF A FAMILY FARMING OF THE SAO PAULO STATE

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Received: 07/10/2020
Approved: 20/08/2021

Abstract

Serum samples from 719 dairy cattle of reproductive age, from 47 farms in a settlement in the municipality of Andradina, São Paulo, were analyzed for the presence of antibodies against the Nebraska strain of BoHV-1 and Singer type 1 of BVDV-1, using the virus neutralization technique (VN). Regarding BoHV-1, 72.0% of the samples analyzed (518/719) were positive, with a geometric mean of antibody titers of 318. For BVDV-1, 44.8% of the samples were reactive, only one farm had no reactive animals, and the majority of antibody titers found in the animals' sera were low. The results of this study revealed that BoHV-1 was present in all of the studied herds, with moderate titration in the majority of cases, with the presence of some high individual titers among the tested animals. Also, even with the reactivation or circulation of BoHV-1 in herds, the clinical disease was not observed, an epidemiological finding that also applied to BVDV-1. In view of the results obtained, the circulation of diseases in family farm herds in the studied region was evident, suggesting the need to study local risk factors and improve health education policies as a way to prevent or reduce the prevalence of these diseases.

Keywords cattle, reproductive diseases, health, rural settlement, serology

PERFIL SOROLÓGICO DO ALFAHERPESVIRUS BOVINOTIPO 1(BoHV-1) E PESTIVÍRUS A(BVDV-1) EM REBANHOS DA AGRICULTURA FAMILIAR NO ESTADO DE SÃO PAULO

Resumo

Coletou-se 719 amostras de soro sanguíneo de bovinos em idade reprodutiva, de 47 propriedades de um assentamento no município de Andradina-SP. As amostras foram analisadas para presença de anticorpos contra ambos os vírus estudados, pela técnica de virusneutralização, utilizando-se a estirpe Nebraska para o BoHV-1 e Singer tipo 1 para o BVDV-1. Em relação ao BoHV-1, 72,0% (518/719) das amostras foram positivas, sendo 318 a média geométrica dos títulos de anticorpos (GMT).Para o BVDV-1, 44,8% das amostras foram reagentes; apenas uma propriedade não teve animais reagentes, sendo a maioria dos títulos de anticorpos encontrados nos soros baixa. Observou-se que o BoHV-1 está presente em todos os rebanhos estudados com titulação moderada, com presença de alguns títulos individuais altos entre animais e com GMT alta em um dos rebanhos. Mesmo havendo a reativação ou mesmo a circulação do BoHV-1 nos rebanhos, não foi observado a doença clínica, fato epidemiológico que também ocorreu com o BVDV-1. Diante dos resultados obtidos, ficou evidente a circulação das doenças nos rebanhos da agricultura familiar na região estudada, sugerindo o estudo de fatores de risco local e melhorias de políticas de educação em saúde como forma de prevenir e ou reduzir a prevalência das enfermidades.

Palavras chave assentamento rural, bovinos, doenças reprodutivas, sanidade, sorologia

INTRODUCTION

According to official data, there are 9,431 registered rural settlements in Brazil, with 969,197 families living in these created or recognized settlements (CCIR, 2020). Of this number, in the state of São Paulo there are 275 regular settlements registered with the National Institute of Colonization and Agrarian Reform, encompassing 17,194 families engaged in farming and livestock breeding in an area of 348,729 hectares (INCRA, 2017). Within this scenario, there are 451 family farms in the municipality of Andradina, located near the border with the state of Mato Grosso do Sul, in the northwest region of São Paulo (INCRA, 2017), in an area of 12,591 hectares.

Aid programs for settled families are carried out by government agencies, to contribute to the development of agriculture and its sustainability. However, the full insertion of this segment in the market economy still depends on several factors, such as technological information, marketing routes, quality control, and regularity of products, among others.

Studies in settlements in the region have revealed that most of the families surveyed consume the products of animal and plant origin, and adopt different forms of marketing of the excess, such as direct sales to consumers and/or retailers, including fresh products and hand-processed products based on knowledge acquired within the family or the community and courses taught by institutions such as SENAR and Fundação ITESP. The limitations onsale are related to the production capacity and the difficulties of obtaining the health inspection seal (Santana et al., 2007).

As for all farmers, the health and welfare of the animals is of great relevance to the sustainability of these producers. Good sanitary practices, no matter how small the property or the type of production, are essential to guarantee profitability. Health is one of the main factors that directly influence the productive and reproductive indexes of a herd, and diseases can cause serious losses to producers.

Among other diseases of economic importance, bovine infectious rhinotracheitis (IBR) is responsible for low productivity of Brazilian livestock, affecting mainly the reproductive capacity of animals. IBR, caused by bovine herpesvirus type 1 (BoHV-1), is a virus belonging to the order Herpesvirales, family Herpesviridae, subfamily Alphaherpesvirinae and genus Varicellovirus; in this subfamily there is also the species BoHV-5, responsible for causing encephalopathy in cattle (ICTV, 2021). The

subtypes BoHV-1.2, related to episodes of respiratory symptoms, infertility and stillbirths; BoHV-1.2a, associated with a variety clinical manifestations, such as stillbirths and other reproductive disorders, including diseases of the genital tract and respiratory problems; and BoHV-1.2b, causing mild respiratory disease and problems of the genital tract (Fino et al., 2012).

The main cause of IBR is the establishment of latent infections for BoHV-1 virus (Lemaire et al., 1994) that maintain the viral genome in ganglion cells. Therefore, every infected animal should be considered a carrier and a potential recurrent virus transmitter (Alfieri and Alfieri 2017). The infection has been described in cattle herds around the world, but has been eradicated in several European countries (OIE, 2017).

The bovine viral diarrhea virus (BVDV) is classified as a pathogen in the family Flaviviridae, genus Pestivirus (Dezen et al., 2013), currently, with the new nomenclature, sub-divided into three species: Pestivirus A (BVDV-1), Pestivirus B (BVDV-2) and, Pestivirus H (Hobi-like virus) (ICTV, 2021). The pathogenesis of the agent depends on a combination of factors that can influence the consequences of infection, such as: immunotolerance, immunocompetence, animal age, gestation and gestational age of the fetus, emergence of fetal immunocompetence, immune status of the animals and presence stressors (Silva et al., 2011).

The main characteristic of epidemiological importance of BVD is probability of birth of animals persistently infected (PI) by the virus, which excrete it through secretions and excretions, playing a fundamental role in the occurrence of the disease (Alfieri and Alfieri, 2017). PI animals result in fetal infection via the transplacental route in the first trimester of pregnancy and the virus is eliminated by nasal discharge, milk, urine, and saliva (Silva et al., 2011). Infection of the non-immunocompetent fetus between 40 and 120 days of gestation with BVDV-1 non-cytogenic strains (NCP) can result in a PI calf (Dezen et al., 2013).

Clinically, the infection can be manifested as mild acute gastroenteric and respiratory diseases, severe acute gastroenteric, respiratory and hemorrhagic diseases, mucosal disease, and chronic BVD. However, the main problems involve reproduction, since in pregnant cows the infection can lead to embryonic absorption with the return of estrus, stillbirth, fetal mummification, birth of weak calves, as well as delayed growth and the possibility of PI animals if infected in the first three months of pregnancy (Piovesan et al., 2013). In Brazil, seroepidemiological surveys have revealed

that the infection is widespread (Alexandrino et al., 2011, Pasqualotto et al., 2015), including in small herds kept by family farmers (Rego et al., 2016; Gaeta et al., 2018).

The main activity in the surveyed settlement is dairy farming. Investments have been directed towards the nutritional improvement of the herd, in addition to the genetic selection of the sires and cows. However, it is not part of the farmers' routine to check the health status of these animals. In view of the above and the absence of studies on these diseases in the region analyzed, we investigated the occurrence of antibodies against BoHV-1 and BVDV-1in cattle from these properties, so as to provide, in addition to scientific data, information on preventive health measures needed by the target community.

MATERIAL AND METHODS

The study was carried out in a settlement in the northwest region of the state of São Paulo. The research design was approved by the Research Ethics Committee of the School of Veterinary Medicine of Araçatuba (Process 004153/09).

According to the Land Institute of the State of São Paulo (ITESP/Andradina office), the settlement was created in 2002, with 74 lots/families, covering an area of 1,534.48 hectares. For data analysis, 47 properties were considered that have as main economic activity the rearing of dairy cattle, with an average of 31 head per family, for a total population of approximately 2,014 animals.

Supported by a credit policy that finances the construction of housing and the maintenance of families in the first year, all the producers who participated in this study live in masonry houses with a structured sanitation system. The water comes from a well and the sewage is discharged septic tanks (elaborated by Embrapa and adopted by the CATI/SAA Microbasin Program).

The production system relies on crossbred cattle, living on the same property with animals of other species such as goats, pigs, horses, dogs, and cats. The cattle are kept in pasture, but some of the farmers supplement their animals with some type of feed. The animals' drinking water comes from streams or wells.

Reproduction is carried out exclusively by natural means, and farmers often share their pastures with neighbors. The animals are purchased from livestock traders or from neighboring farmers, most of the time without any health certificate. All farmers have technical assistance from a veterinarian and an ITESP technician,

including vaccination against foot-and-mouth disease and brucellosis. There is no record of vaccination against IBR and BVD in this settlement.

719 cattle (approximately 50% of the herd on each property) were included in the study, of both sexes. The animals selected to check for the occurrence of antibodies against BoHV-1 and BVDV-1were adults of reproductive age, that is, over 24 months old, among those with the greatest economic value.

Blood collections were performed by venipuncture, using sterile disposable needles and vacutainers, and then were kept at room temperature for one hour for serum separation. The samples were sent to the Araçatuba Regional Laboratory of the Biological Institute for complete separation of blood serum and transferred to 2 ml tubes (duplicate), duly identified, and kept in a freezer at a temperature of negative 20 ^{o}C until serological testing.

The samples were analyzed at the Virus Reproduction Laboratory of the Department of Preventive Veterinary Medicine and Animal Reproduction, São Paulo State University (UNESP), Jaboticabal Campus, using the virus neutralization technique as recommended by the World Organization for Animal Health (OIE, 2013), using the Nebraska and Singer strains for the detection of antibodies against BoHV-1 and BVDV-1, respectively. Animals were considered positive with titer greater than or equal to 2 was found for BoHV-1 and greater than or equal to 10 for BVDV-1. Antibody titers were transformed into mean titer geometric (GMT) (ANZILIERO et al., 2011).

RESULTS AND DISCUSSION

During visits to collect material, no animals were found with clinical signs of the diseases investigated. However, 32% (15/47) of the farmers reported the occurrence of stillbirth at some point in their herds.

For BoHV-1, the occurrence of contact with the virus in the studied properties was 100%, since all herds had at least one reactive animal. Of the 719 serum samples analyzed, 518 (72.0%) were reactive to BoHV-1, with geometric mean antibody titers (GMT)of positive animals having a value of 114,4.

Of the animals tested, 8.6% (62/719) had low titers (≤8), with a GMT of 4.2; 18% (130/719) had medium titles (between 16 and 64) with a GMT of 39.3 and most of them, 45.3% (326/719) had high titles (≥ 128), with a GMT of 329 (Table 1). The classification as low, medium and high was according to Becker et al. (2015).

Table 1. Geometric mean of antibody titers against BoHV-1 in family farming herds in the northwest of the state of São Paulo

Antibody titers	Absolute number of animals (%)	Titers
< 2	201 (28)	Negatives
2	16 (2.2)	Low **GMT 4.2**
4	25 (3.5)	
8	21 (2.9)	
Subtotal	**62 (8.6)**	
16	28 (3.9)	Intermediate **GMT 39.3**
32	37 (5.2)	
64	65 (9.0)	
Subtotal	**130 (18.0)**	
128	78 (10.8)	High **GMT 329**
256	110 (15.3)	
512	80 (11.1)	
≥ 1024	58 (8.0)	
Subtotal	**326 (45.3)**	
Total	**518 (72.0)**	**GMT 114.6**

In the state of São Paulo, a high prevalence of animals reactive to BoHV-1 has also been recorded, demonstrating the rapid spread of bovine herpesvirus in herds. Silva (2011) observed 70.36% prevalence and Santos et al. (2014) noted 61.4%. In a more recent study, also in settlements in São Paulo, there was 31.7% prevalence in calves (Gaeta et al., 2018), showing the presence of the infection in this category as well. In the present study, animals under two years of age were not analyzed.

According to Fino et al. (2012), in a review of several studies, found average of seropositivity for Brazilian herds of 76% in the Northeast, 67.45% in the Midwest, 50.75% in the Southeast, and 41.6% in the South of the country, varying with the type of breeding analyzed and the diagnostic method used.

The results indicate that the BoHV-1 virus was circulating in the properties of the settlement, since there was no history of vaccination of the animals in the 47 farms studied. In all of them, the presence of animals that were reactive for the presence of antibodies against BoHV-1 was detected, and the main characteristic was the variation of the titers found, with values from low to high. In most of the properties (44/47, 93.6%), the animals presented high titers.

Presence of antibodies in unvaccinated animals indicates that the virus is present since the disease causes latency and several factors can interfere with the animal's immune system, which can lead to a re-excretion of the virus. In relation to

BVDV-1, 44.8% (322/719) of the analyzed samples were positive, presenting GMT of 41.31 for the reactive animals. Rego et al. (2016) also found 51.1% prevalence in family farming, also in animals over 24 months old, and Gaeta et al. (2018) reported 24.4% in calves. Of the animals tested, 9% (65/719) had low titers (≤10), with GMT of 10; 19.9% (143/719) had medium titers (between 20 and 40) with GMT of 27.1; and 15.9% (114/719) had high titers (≥80), with GMT of 157.1 (Table 2).

Table 2. Geometric mean of antibody titers against BVDV-1 in family farming herds in the northwest of the state of São Paulo

Antibody titers	Absolute number of animals (%)	Titers
< 10	397 (55.2)	Negatives
10	65 (9.0)	Low GMT 10
Subtotal	**65 (9.0)**	
20	80 (11.1)	Intermediate GMT 27.1
40	63 (8.7)	
Subtotal	**143 (19.9)**	
80	53 (7.3)	High GMT 157.1
160	31 (4.3)	
320	15 (2.0)	
640	11 (1.5)	
1280	3 (0.4)	
≥ 2560	1 (0.1)	
Subtotal	**114 (15.9)**	
Total	**322 (44.7)**	**GMT 41.3**

The result indicates that there is low virus circulation, because to suggest viral circulation, animals from the same property must have different titers or high titers in virus neutralization; and that most of the animals 55.2% (397/719) are susceptible, as they did not show antibodies against BVDV-1.

Some characteristics of family farming can be important risk factors for the transmission of diseases such as BoHV-1 and BVDV-1. The purchase of animals without a health certificate and the habit of sharing pastures and breeding animals is very common among the farmers. Semen is one of the main routes for elimination of the virus, and natural mounting is one of the main risk factors for infection by BoHV-1 (Barbosa et al., 2019).

The use of common pastures among farms contributes to the contact between susceptible and infected animals, favoring the spread of the virus by ocular and nasal secretions, aerosol inhalation, and direct contact with infected fetal remains (Barbosa et

al., 2019). Among some factors that contribute to the immunosuppression of animals, due to the stress caused, which could facilitate the transmission of the virus in the herd and between herds, we can mention malnutrition, stressful management, pregnancy, and postpartum. This disease is related to disorders of the reproductive system, in addition to affecting the respiratory, digestive, circulatory, immune, lymphatic and other systems; so BoHV-1can manifest clinically as bovine infectious rhinotracheitis or infectious pustular vulvovaginitis/balanoposthitis. However, its symptoms are difficult to perceive, due to the few clinical signs that are presented. However, in the farms participating, these findings were not reported, and few farmers mentioned the occurrence of abortion at some point in the past.

The rates of infections by IBR and BVDV-1 can be highly variable, so investigation of the epidemiological profile of infections in different herds and in different categories of animals of the same herd is of paramount importance, as well as the analysis of risk factors and mitigation measures (Alfieri and Alfieri, 2017). The elaboration of a nutritional program that meets the requirements of the animals, control of the purchase and introduction of new animals, control of the quality of semen used and the artificial insemination procedure, adoption of regular vaccination, periodic serodiagnosis of animals and disposal of animals considered problematic are strategies that can eliminate the causative agents of diseases (Pasqualotto et al., 2015).

The need to promote health education in the settlement was also evident, in order for farmers to make use of good health practices, allowing improvement in the production and reproduction of animals, consequently assuring sustainability.

CONCLUSIONS

BoHV-1 was present in all herds studied, with moderate titers in the majority of animals. Even with the reactivation or circulation of BoHV-1 in herds, clinical disease was not observed, an epidemiological fact that also occurred with BVDV-1. The circulation pattern of diseases in the herds of the studied settlement suggests the need to study local risk factors and to improve health education, to prevent or reduce the prevalence of diseases.

ACKNOWLEDGEMENTS

Foundation for Research Support of the State of São Paulo (FAPESP).

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