UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
CENTRO DE SAÚDE E TECNOLOGIA RURAL
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA
CAMPUS DE PATOS – PB

EPIDEMIOLOGICAL AND SPATIAL CHARACTERIZATION OF BVDV AND BoHV-1 INFECTIONS IN BOVIDES FROM THE PARAÍBA STATE, NORTHEASTERN BRAZIL

LEÍSE GOMES FERNANDES

PATOS-PB
2017

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Tese apresentada ao Programa de Pós-Graduação em Medicina Veterinária do Centro de Saúde e Tecnologia Rural da Universidade Federal de Campina Grande, como parte dos requisitos para obtenção do título de Doutor em Medicina Veterinária.

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Doutoranda

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Orientador

PATOS-PB
2017

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECA DO CSTR

F363e Fernandes, Leíse Gomes

Epidemiological and spatial characterization of BVDV and BoHV-1 infections in bovides from the Paraíba State, Northeastern Brazil / Leíse Gomes Fernandes. – Patos, 2017.

63f. :il; Color.

Tese (Doutorado em Medicina Veterinária) - Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural, 2017.

“Orientação: Prof. Dr. Sérgio Santos de Azevedo”

Referências.

1. Epidemiologia. 2. Sorologia. 3. Análise de Cluster. I.Título.

CDU 616-036.22

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Tese aprovada pela Comissão Examinadora em: 23/02/2017

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"Um livro, uma caneta, uma criança e um professor podem mudar o mundo.
Não espere que outra pessoa venha e fale por você.
É você que pode mudar o mundo".

Malala Yousafzai

AGRADECIMENTOS

À Deus por minha vida e presença constante, pela saúde,sabedoria, paciência, forças e fé, que me permitiram concluir esta etapa.

À minha família por todo amor e dedicação durante todos os anos da minha vida e pelo apoio essencial que forneceram ao meu crescimento pessoal e profissional.

À todas as minhas amigas que estiveram sempre comigo, quer compartilhando risos, choros, alegrias e tristezas. Amo cada uma de vocês.

Ao meu namorado Rodrigo Monteiro, pelo amor, dedicação e motivação diária. Agradeço também a toda sua família, por todos os momentos juntos e o apoio durante toda esta etapa. Sou grata a Deus pela família que Ele me deu e pela a que escolhi me juntar.

Ao meu orientador, prof. Dr. Sérgio Santos de Azevedo, pela confiança e oportunidade em me orientar, pela amizade, atenção, paciência e principalmente, por todos os ensinamentos fundamentais para a conclusão desta etapa da vida.

Agradecer aos professores da pós-graduação e da unidade acadêmica, especialmente aos professores Dr. Clebert, Dr. Antonio Flávio e Dr. Silvano Higino, pelos ensinamentos, participações em bancas e na qualificação.

Aos professores colaboradores do projeto, Dra. Maristela Pituco, Dr. Søren Saxmose, Dr. Matt Denwood, pela cooperação durante a realização do trabalho, pelo apoio, boa recepção e contribuir de forma direta no trabalho.

Aos colegas do Laboratório de Doenças Transmissíveis pela valiosa contribuição na realização do experimento e pelos bons momentos de convivência.

À Jonas pela disponibilidade, auxílio e dedicação, que tornaram possíveis muitas viagens, experimentos e trabalhos..

Ao Cnpq e Ciencias sem Fronteiras pela oportunidade do Doutorado Sanduíche na Dinamarca, na qual obtive experiências profissional e pessoalmente enriquecedoras e inesquecíveis.

Ao Programa de Pós-Graduação em Medicina Veterinária e a Universidade Federal de Campina Grande (UFCG) pela obtenção do título de Doutora em Medicina Veterinária.

A CAPES pelo auxílio financeiro e pela concessão da bolsa de estudos.

E a todos que direta e indiretamente contribuíram para conclusão deste trabalho: *meus sinceros agradecimentos.*

ABSTRACT

This thesis is composed of three chapters. In Chapter I, a cross-sectional study based on a planned sampling was carried out to determine herd- and animal-level seroprevalences and to identify risk factors for Bovine Viral Diarrhea Virus (BVDV) infection in cattle from the State of Paraíba. The state was divided into three sampling strata, and for each stratum, the prevalence of herds infected with BVDV and the prevalence of seropositive animals was estimated by a two-stage sampling survey. In total, 2443 animals were sampled from 478 herds. In Chapter II, the study aimed to identify the risk factors associated with BVDV and Bovine Herpesvirus Type 1 (BoHV-1) infection in buffaloes in the State of Paraíba using 136 female buffaloes ≥ 24 months old from 14 herds. For the serological diagnosis of the BoHV-1 and BVDV infections, the virus-neutralization test was used and in each herd, an epidemiological questionnaire was applied in order to obtain data used in the risk factor analysis. In Chapter III, the study aimed to identify spatial clustering of positive herds by BVDV and BoHV-1 in cattle in the State of Paraíba, using the Cuzick-Edwards' *k*-nearest neighbor method and spatial scan statistics. The herd- and animal-level prevalences for BVDV in cattle in the state were 65.5% (95% CI =61.1–69.7%) and 39.1% (95% CI =33.1–45.6%), respectively, and the risk factors identified were: more than six calves aged ≤12 months (OR = 3.72; 95% CI=2.08–6.66), animal purchasing (OR = 1.66; 95% CI=1.08–2.55), pasture rental (OR = 2.15; 95% CI=1.35–3.55), and presence of veterinary assistance (OR = 2.04; 95% CI=1.10–3.79). In buffaloes, of the 136 animals 86 (63.2%) were positive for BoHV-1 and 12 (8.8%) for BVDV. The presence of watering points (OR = 17.68; 95% CI = 3.74–83.04) was identified as risk factor for BoHV-1, and animal purchasing was a risk factor for both BoHV-1 (OR = 68.64; 95% CI = 12.28–383.73) and BVDV (OR = 3.64; 95% CI = 1.04–12.76). Six significant clusters were detected for BVDV, a primary cluster (11 herds, 24.10 km, RR = 2,21; p <0.001) covering the eastern Sertão region, and five smaller significant clusters, involving one or two herds in Agreste/Zona da Mata region, near the border of the Borborema region. A significant clustering of BoHV-1 positive herds (p <0.001) was detected in Agreste/Zona da Mata region (103 cases, 77,17 km, RR=1.27). The findings suggest that the implementation of control and prevention measures should be adopted for cattle and buffaloes in the Paraíba state, with the aim of preventing dissemination of the agents in those herds.

Keywords: epidemiology, risk factors, serology, BoHV-1, BVDV, cluster analysis.

RESUMO

Esta tese é composta de três capítulos. No Capítulo I foi realizado um estudo transversal, com base em uma amostragem planejada, para a determinação de prevalências a nível de rebanhos e animais e a identificação de fatores de risco para infecção pelo Vírus da Diarreia Viral Bovina (BVDV) em bovinos do Estado da Paraíba. As prevalências de rebanhos e animais foram estimadas por um levantamento de amostragem em duas etapas. No total, 2443 animais foram amostrados de 478 rebanhos. No Capítulo II, objetivou-se identificar os fatores de risco associados às infecções por BVDV e Hepervírus Bovino do Tipo 1 (BoHV-1) em búfalos do Estado da Paraíba, utilizando-se 136 búfalos fêmeas ≥ 24 meses de 14 rebanhos. Para o diagnóstico sorológico de BoHV-1 e BVDV, foi utilizado o teste de virusneutralização e em cada rebanho foi aplicado um questionário epidemiológico para obtenção dos dados utilizados na análise do fator de risco. No Capítulo III, objetivou-se identificar o agrupamento espacial de rebanhos positivos por BVDV e BoHV-1 em bovinos no Estado da Paraíba, utilizando-se o método de Cuzick-Edwards e análise de varredura espacial. A prevalência de bovinos no estado foi de 65,5% (IC95% = 61,1-69,7%) e 39,1% (95% IC = 33,1-45,6%), respectivamente, e os fatores de risco identificados foram: mais de seis bezerros ≤ 12 meses (OR = 3,72, IC 95% = 2,08-6,66), compra de animais (OR = 1,66, IC 95% = 1,08-2,55), aluguel de pasto (OR = 2,15, IC 95% = 1,35-3,55) e presença de assistência veterinária (OR = 2,04; IC 95% = 1,10-3,79). Em búfalos, dos 136 animais, 86 (63,2%) foram positivos para BoHV-1 e 12 (8,8%) para BVDV. A presença de aguadas (OR = 17,68; IC95% = 3,74 - 83,04) foi identificada como fator de risco para BoHV-1 (OR = 68,64; IC95% = 12,28-383,73) e BVDV (OR = 3,64; CI = 1,04-12,76). Seis agrupamentos significativos foram detectados para BVDV, sendo um agrupamento primário (11 rebanhos, 24,10 km, RR = 2,21, p <0,001) cobrindo a região leste do Sertão, e cinco pequenos grupos, envolvendo um ou dois rebanhos na região de Agreste/Zona da Mata, próximos a fronteira da região de Borborema. Foi observado um agrupamento significativo de rebanhos positivos ao BoHV-1 (p <0,001) na região de Agreste/Zona da Mata (103 casos, 77,17 km, RR = 1,27). Os achados sugerem a adoção de medidas de controle e prevenção para rebanhos bovinos e bubalinos no estado da Paraíba, com o objetivo de prevenir a disseminação dos agentes nesses rebanhos.

Palavras-chave: epidemiologia, fatores de risco, sorologia, BoHV-1, BVDV, análise de cluster.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
≡	Equal
<	Less than
>	Bigger than
≤	Less or equal
≥	Bigger or equal
°C	Degree Celsius
BoHV-1	Bovine Herpesvirus type 1
BVDV	Bovine Viral Diarrhea Virus
CI	Confidence Interval
CNPq	National Counsel of Technological and Scientific Development
CP	Cytopathic effects
CSTR/UFCG	Health Center and Rural Technology/ Federal University of Campina Grande
GDP	Gross Domestic Product
GPS	Global positioning system
IBR	Infectious bovine rhinotracheitis
IPV	Infectious pustular vulvovaginitis
JCR	Journals Impact Factors
Km	Kilometers
NCP	Non-cytopathic effect
OR	Odds Ratio
PI	Persistently infected
RR	Relative Risk
SEDAP	Agricultural and Livestock Defense Service of the State of Paraíba
$TCID_{50}$	Tissue Culture Infective Dose

INTRODUTION

Paraíba has 1.3 million cattle distributed in 92 thousand livestock farms accouting for 2% of the state's GDP (Gross Domestic Product) (IBGE, 2009; IDEME, 2011), with predominance in family or subsistence agriculture and mixed farms (CLEMENTINO et al. 2015). Except for the Zona da Mata mesoregion, where sugarcane cultivation prevails, the cattle breeding is widespread in Agreste, Borborema and Sertão mesoregions, which 69% of the milk produced originates from family farms (IBGE, 2009). The buffalo exploitation has shown high growth in the state, mainly due to its significant adaptability to various environmental conditions and the dual ability to produce meat and milk (ALMEIDA et al., 2013).In this context, the study of infectious agents that cause economic losses in cattle and buffaloes, especially the reproductive viral agents, such as Bovine Viral Diarrhea Virus (BVDV) and Bovine Herpesvirus Type 1 (BoHV-1), assumes importance because they cause embryonic, fetal or neonatal mortalities, recurrence of heat, abortions, stillbirths and birth of debilitated animals (JUNQUEIRA; ALFIERI, 2006).

The BVDV is a member of the genus *Pestivirus*in the family *Flaviviridae*, and has two biotypes, cytopathogenic (CP) and non-cytopathogenic (NCP), being only the latter responsible for persistent infection. Persistently infected (PI) animals result from uterine exposure to NCP strains prior to fetal development (125 days of gestation).The animals are weak at birth or die before one year of age, but others may not present clinical signs of the disease and eliminate large amounts of virus, acting as constant sources of infection for nonimmune animals (RUFENACHT et al., 2001). BVDV infection may be responsible for economic losses related to reduced milk production, impaired reproductive performance, delayed growth, increased occurrence of other diseases, early slaughter and increased mortality among young animals (DAMMAN et al., 2015).

BoHV-1 is an alphaherpesvirus, which belongs to *Varicellovirus* genus and may present clinically as infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), conjunctivitis and generalized infection in neonates, with reproductive problems, such as abortion, the most common manifestations (TAKIUCHI; ALFIERI; ALFIERI, 2001).This virus has the capacity to cause latent infections and its periodic reactivation in the animal is responsible for the perpetuation and dissemination in the bovine population, representing the main obstacle to the establishment of combat measures (OIE, 2010, FLORES et al., 2013).

In Paraíba, a study was carried out in six municipalities, from three mesoregions (Agreste, Cariri and Sertão Paraibano), using non-random sampling, where 2,343 bovines were tested for BVDV and BoHV-1 infections by the virusneutralization test. Of these, 22.2% (520) were seropositive for BVDV, and 46.6% (1,093) for BoHV-1, and there was at least one animal seropositive for BVDV and BoHV-1 in 88.9% and 100% of the herds, respectively (THOMPSON et al, 2006).

Considering the lack of epidemiological studies in the Paraíba state on BVDV and BoHV-1 infections that aim to determine epidemiological indicators (prevalences and risk factors) conducted on the basis of a planned sample in cattle and buffaloes, and to identify spatial groupings of positive herds, it was proposed to perform this thesis in order to carry out statistical treatment (prevalence calculation, risk factor analysis and spatial analysis), with a posterior analysis of the data obtained in the epidemiological study for BVDV and BoHV-1 in Paraíba; results that will be important in identifying areas of risk for BVDV and BoHV-1, monitoring positive herds and recommending and implementing measures to prevent and control infections.

The Thesis consists of three chapters consisting of original scientific articles. Chapter I refers to a research article published in Tropical Animal Health and Production - Qualis B1, and describes the prevalence and risk factors associated with BVDV infection in Paraíba state. Chapter II is composed of an article published in Semina: Ciências Agrárias - Qualis B1, in which the risk factors associated with BVDV and BoHV-1 infections in buffaloes in the state of Paraíba were identified. Chapter III comprises an article submitted to BMC Veterinary Research - Qualis B1, which described the spatial pattern of apparent prevalence estimates, as well as to identify spatial clustering of BVDV and BoHV-1 positive bovine herds in Paraíba, Northeast Brazil.

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CHAPTER I

Herd-level prevalence and risk factors for bovine viral diarrhea virus infection in cattle in the state of Paraíba, Northeastern Brazil

Published in Tropical Animal Health and Production, University of Edinburgh. Qualis B1; JCR 0.870.

Trop Anim Health Prod (2016) 48:157–165
DOI 10.1007/s11250-015-0937-x

REGULAR ARTICLES

Herd-level prevalence and risk factors for bovine viral diarrhea virus infection in cattle in the State of Paraíba, Northeastern Brazil

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Received: 12 March 2015 / Accepted: 16 October 2015 / Published online: 24 October 2015

Abstract Serological surveys based on a planned sampling on bovine viral diarrhea virus (BVDV) infection in Brazilian cattle herds are scarce. A cross-sectional study was carried out to determine herd- and animal-level seroprevalences and to identify risk factors associated with herd-level seroprevalence for BVDV infection in the State of Paraíba, Northeastern Brazil, from September 2012 to January 2013. The state was divided into three sampling strata, and for each stratum, the prevalence of herds infected with BVDV and the prevalence of seropositive animals was estimated by a two-stage sampling survey. In total, 2443 animals were sampled from 478 herds. A virus-neutralization test was used for BVDV antibody detection. A herd was considered positive when at least one seropositive animal was detected. The herd- and animal-level prevalences in the State of Paraíba were 65.5% (95% confidence interval (CI)=61.1–69.7%) and 39.1% (95% CI=33.1–45.6%), respectively. The frequency of seropositive animals per herd ranged from 10 to 100 % (median of 50%). The risk factors identified were as follows: more than six calves aged ≤12 months (odds ratio (OR)=3.72; 95% CI=2.08–6.66), animal purchasing (OR=1.66; 95% CI=1.08–2.55), pasture rental (OR= 2.15; 95% CI=1.35–3.55), and presence of veterinary assistance (OR=2.04; 95% CI=1.10–3.79). Our findings suggest that the implementation of control and prevention measures among farmers, with the aim of preventing dissemination of the agent in the herds, is necessary. Special attention should be given to addressing the identified risk factors, such as sanitary control prior to animal purchasing and to discourage the pasture rental, as well as to encourage the vaccination in the herds.

Keywords BVDV · Serology · Risk factors · Control · Northeastern Brazil

Introduction

With worldwide distribution and high prevalence, the bovine viral diarrhea virus (BVDV) is considered one of the most important infectious agents in the reproductive sphere in bovines due to its negative economic impacts on the reproduction (Gates et al. 2014), and it has been a target of many epidemiological surveys and control and/or eradication programs for decades (Greiser-Wilke et al. 2003; Presi et al. 2011; Graham et al. 2013).

BVDV is a *Pestivirus* of the Flaviviridae family and has a wide range of clinical manifestations, which include sub-clinical infections, gastroenteric, respiratory, reproductive and hemorrhagic diseases, temporary infertility, and a fatal form known as mucosal disease (Lanyon et al. 2014). The reproductive problems are considered the most important, mainly in pregnant females which, when infected in the initial stage of the gestation, may present death and embryonic or fetal reabsorption, fetal mummification, and abortion (Rodning et al. 2012), and when infected between 42 and 125 days of gestation, may generate persistently infected (PI) animals, which constitute the main sources of

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infection of the virus in the herds (Lindberg and Houe2005). The transmission may occur by direct and indirect contact or by iatrogenic transmission and contaminated semen (Flores 2003).

The conduction of serological surveys with determination of prevalence and risk factors permits to know the epidemiological situation of the infection and the conditions associated to its occurrence, allowing the elaboration of preventive and control measures and their application in a correct and efficient manner. In Brazil, many serological surveys have demonstrated the presence and broad distribution of BVDV infection in cattle herds with the frequency of seropositive animals and positive herds varying from 22.2 to 66.32% and 43 to 100%, respectively (Poletto et al. 2004; Samara et al. 2004; Thompson et al. 2006; Quincozes et al. 2007; Brito et al. 2010; Chaves et al. 2012; Almeida et al. 2013); however, the majority of these serological surveys were carried out in restricted geographical regions or were based on a limited number of samples without an adequate sampling design. In Brazil, there is not an official program for BVDV control and vaccination against BVDV infection is incipient and is performed irregularly in different regions and production systems, being held more frequently in dairy herds and intensive or semi-intensive beef herds in the Southeast and South of Brazil (Flores et al. 2005; Almeida et al. 2013). In Paraíba, Northeastern Brazil, the use of vaccines is not practiced.

During the last few decades, dairy cattle have become significantly important within animal husbandry in the State of Paraíba, Northeastern Brazil. In the mesoregions of Paraíba, except for the Zona da Mata region (where sugarcane crops prevail), small cattle-raising farms are widespread in the Agreste, Borborema, and Sertão regions. Whereas cultivated grasses (mostly *Brachiaria* spp.) are the basis for Agreste livestock, cattle are usually reared extensively on native Caatinga in most of the Borborema and Sertão farms. In Paraíba, most farms are family or subsistence farms and accounted for 69 % of milk production in the state (IBGE 2006; Clementino et al. 2015). In general, BVDV is an unknown disease for most of them. In this context, the performance of epidemiological studies to investigate infectious agents as well as BVDV infection is important. In the State of Paraíba, although there is a description of an epidemiological study for BVDV (Thompson et al. 2006), it was not performed based on planned sampling and the risk factors were not identified, not allowing the adequate survey of the epidemiological indicators of the infection. Thus, the aim of the present study was to determine the herd- and animal-level prevalences for BVDV in cattle in the State of Paraíba, as well as to identify the risk factors associated with herd-level prevalence.

Materials and methods

Characterization of the study area

The State of Paraíba, located in the Northeastern region of Brazil is characterized by warm weather throughout the year and geographically subdivided into the following four mesoregions: (i) Zona da Mata, (ii) Agreste, (iii) Borborema, and (iv) Sertão. The mesoregions are political/administrative subdivisions of the Brazilian states and based mostly on vegetation type and rainfall. The Zona da Mata and Agreste have relatively higher rainfall regimes, predominantly humid tropical climate, with autumn-winter rains, and dry season during the summer. On the coast, the mean annual rainfall is ≈1700 mm and temperatures averaging 24°C. Formed bythe Atlantic forest, the coastal vegetation as forests with the presence of evergreen tall trees, mangroves, and savannas (Cabrera and Willink 1973). Both Borborema and Sertão (the semiarid region) are typically within the Caatinga biome, which encompasses an area of 900,000 km^2 (11% of Brazilian territory) and is the only major biome that occurs exclusively in Brazil. Caatinga is xeric shrubland and thorn forest, which consists primarily of small, thorny trees that shed their leaves seasonally. Cacti, thick-stemmed plants, thorny brush, and arid-adapted grasses make up the ground layer; however, during the dry periods, there is no ground foliage or undergrowth. The weather is characterized by a hot and semiarid climate, with temperatures averaging 27°C, and the mean annual rainfall is typically ≈500 mm. There are typically two seasons: a rainy season from February to May, and a long drought period from June to January. However, occurrences of droughts some-times lasting for longer than 1 year are also a characteristic of the region (Andrade-Lima 1981).

Division of the State of Paraíba into stratified sampling groups

The State of Paraíba was divided into three sampling groups: sampling stratum 1 (mesoregion of Sertão), sampling stratum 2 (mesoregion of Borborema), and sampling stratum 3 (mesoregions of Zona da Mata and Agreste) (Fig. 1). When this stratification scheme was proposed, the operational capacity of the Agricultural and Livestock Defense Service of the State of Paraíba (SEDAP) was considered based on the areas of action of its regional units in order to ensure that the agency could conduct the fieldwork.

Sampling and blood collection

The samples used in this study were obtained from a study of bovine brucellosis in the state of Paraíba made by the National Program for Control and Eradication of

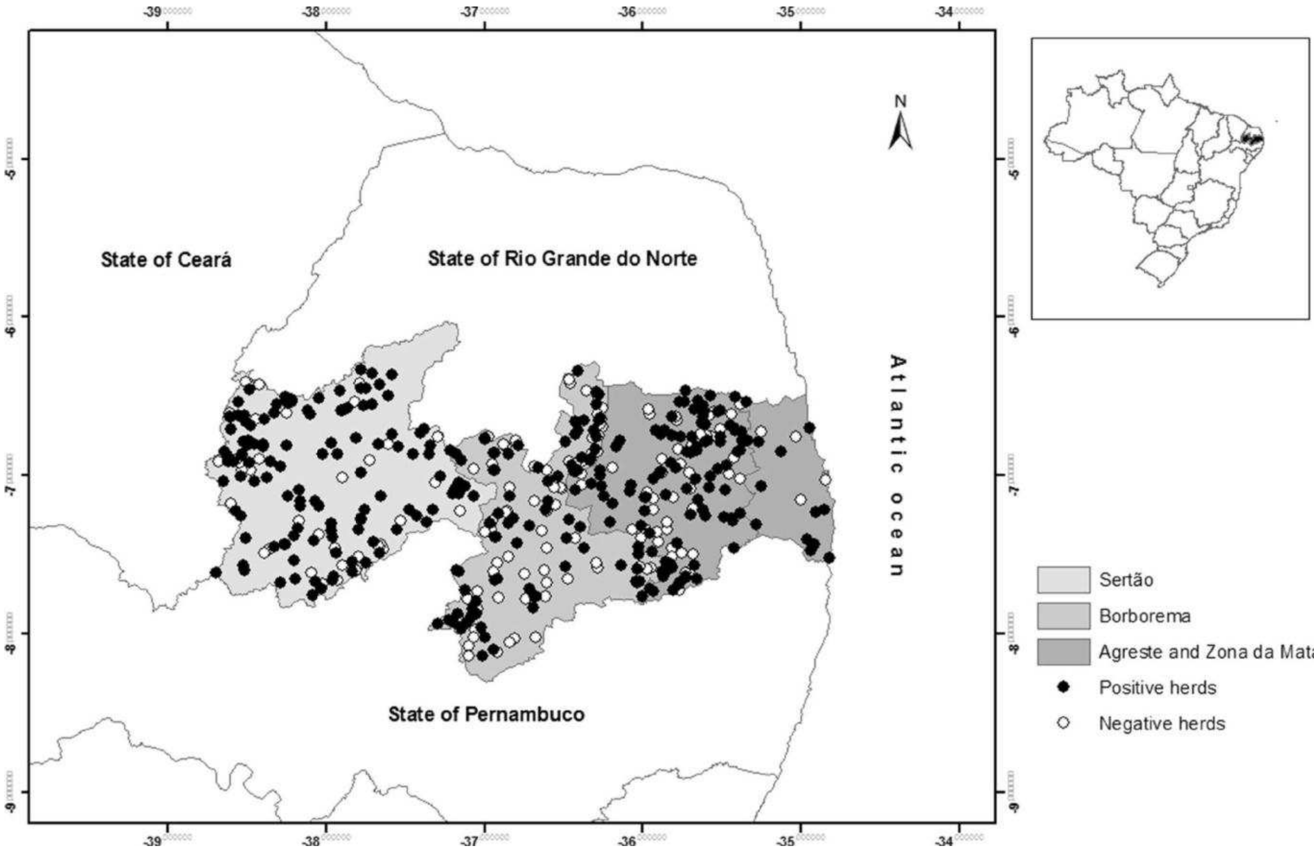

Fig. 1 Division of the State of Paraíba into three sampling groups, and geographical distribution of positive and negative herds. Detail shows the State of Paraíba within Brazil

Brucellosis and Tuberculosis. For each sampling stratum, the prevalence of herds infected with BVDV and the prevalence of seropositive animals were estimated by a two-stage sampling survey. In the first stage, a pre-established number of herds with reproductive activity (primary sampling units) were randomly selected; in the second stage, a pre-established number of cows aged≥24 months were randomly selected (secondary sampling units).

In farms with more than one herd, the cattle herd of greater economic importance was chosen as the target of the study; the animals in the selected cattle herd were subjected to the same type of management system as the other herds, i.e., had the same risk factors as the other herds. The selection of the primary sampling units was random and was based on the records of farms of the SEDAP. If a herd that was selected could not be visited, the herd was replaced by another one in the vicinity with the same production characteristics. The number of selected herds per sampling stratum was deter-mined by using the formula for simple random samples proposed by Thrusfield (2007). The parameters adopted for the calculation were as follows: confidence level of 0.95, estimated prevalence of 50%, and standard error of 0.08. Further, the operational and financial capacity of the official veterinary service of the state was taken into consideration when determining the sample size of the sampling stratum.

For the secondary units, the minimum number of animals to be examined within each herd was estimated in order to allow its classification as positive herd (focus). For this purpose, the concept of aggregate sensitivity and specificity was used (Dohoo et al. 2003). For the calculations, the following values were adopted: sensitivity and specificity of the virus-neutralization test of at least 95 and 99.5% (Corbett et al. 2011), respectively, and 50% for the intraherd estimated prevalence. Herdacc version 3 (University of Guelph) software was used during this process, and the sample size was selected so that the herd sensitivity and specificity values would be ≥90%. Therefore, 10 animals were sampled in herds with up to 99 cows aged over 24 months; 15 animals were sampled in herds with 100 or more cows aged over 24 months; and all animals were sampled in those with up to 10 cows aged over 24 months. The selection of the cows within the herds was systematic, and only BVDV unvaccinated females were sampled. One herd was excluded from the survey because BVDV vaccination was performed prior to sampling. A herd was considered positive when at least one positive animal was detected.

The field activities included blood collection, provision of an epidemiological questionnaire, and sending the samples to the laboratory. The veterinarians and agricultural and live-stock technicians from the SEDAP were involved in the fieldwork. Blood samples (10-mL volume) were collected from September 2012 to January 2013, from cows aged ≥24 months by jugular vein puncture with a disposable needle and a 15-mL capacity vacuum tube (without anti-coagulant). An 11-digit code was used for identification of the tubes, of which the first nine digits referred to the herd code and the final two digits to the number sequence of the sampled cow. Serum samples were transferred and stored in plastic microtubes and were frozen.

BVDV serological diagnosis

For the serological diagnosis of the BVDV infection the virus-neutralization test was used (OIE-World Organization for Animal Health 2008). The cytopathic viral strain BVDV-1 (NADL) was used and supplied by the Virology Institute of the University of Veterinary Medicine of Hannover, Germany. The technique was used in two stages, screening and titration, and a sample was considered positive when it presented a titer ≥10. The neutralizing antibodies titers were considered the reciprocal of the higher serum dilutions capable of inhibiting the viral replication and the consequent production of cytopathic effect (CE) of BVDV. The infectious titer used was $10^{5.61}$ $TCID_{50}$/50μL, determined by the Reed and Müench method (Reed and Muench1938).

Prevalence calculations

A herd was deemed positive when at least one positive animal was detected. EpiInfo 6.04 software was used to calculate the apparent prevalences and respective confidence intervals (Dean et al. 1996). Stratified random sampling was used to calculate the herd-level prevalence in the State of Paraíba (Thrusfield 2007). The required parameters were as follows: (a) condition of the herd (positive or negative), (b) sampling stratum to which the herd belonged, and (c) statistical weight. The statistical weight was determined by applying the following formula (Dean et al. 1996):

$$\text{Weight} = \frac{\text{number of herds in the stratum}}{\text{number of herds sampled in the stratum}}$$

The calculation of the herd-prevalence per sampling stratum employed the sampling design of a simple random sample by using the following parameters: (a) number of positive herds and (b) number of herds sampled in the stratum.

The sampling design for calculating the animal-level prevalence in the State of Paraíba employed a two-stage stratified cluster sampling, and a two-stage cluster sampling in each stratum (Thrusfield 2007), where each herd was considered a cluster. The following parameters were used: (a) animal condition (seropositive or seronegative), (b) sampling stratum to which the animal belonged, (c) herd code (to identify each cluster), and (d) statistical weight. The statistical weight was calculated with the following formula (Dean et al. 1996):

$$\text{Weight} = \frac{\text{cows} \geq 24 \text{ months in the stratum}}{\text{cows} \geq 24 \text{ months in the sampled herds}} \times \frac{\text{cows} \geq 24 \text{ months in the herd}}{\text{cows} \geq 24 \text{ months sampled in the herd}}$$

In the above expression, the first term refers to the weight of each animal in the calculation of the animal-level prevalence within the stratum.

Risk factor analysis

A structured questionnaire including close-ended questions was designed to obtain information concerning (a) the identification and location of the herd, (b) management practices, (c) structure and composition of the herd, and (d) presence of other domestic and wildlife species in the farm. Questionnaires were administered to the owner or person in charge of the herd either by the primary author or by a veterinarian from the SEDAP at the same time of the visit to blood collection.

Data obtained with the epidemiological questionnaires were used in the analysis of risk factors associated with the herd-level prevalence. The analyzed variables and respective categories were as follows: sampling stratum (Sertão/ Borborema/Agreste and Zona da Mata), type of production (meat/milk/mixed), management system (confined/semi-confined/extensive), number of milking (not milking/1 time per day/2 or 3 times per day), number of lactating cows (cutoff point, 3rd quartile), daily milk production (cutoff point, 3rd quartile), use of artificial insemination (no/yes), predominant breed (Zebu/European dairy breed/crossbred/other breeds), number of cows aged ≥24 months (cutoff point, 3rd quartile), number of calves ≤12 months (cutoff point, 3rd quartile), herd size (cutoff point, 3rd quartile), presence of goats/sheep, horses, swine, wildlife, and cervids (no/yes), history of abortion (no/yes), abortion disposal method (left in the pasture lot/used to feed pigs or dogs/buried or burned), animal purchasing (no/ yes), pasture rental(no/yes), sharing of pastures (no/yes), flooded pastures (no/yes), maternity pens (no/yes), and veterinary assistance (no/yes).

The variables were organized for presentation in ascending or descending order regarding the risk scale. When necessary, these variables were re-categorized. The lower-risk category was considered the baseline for comparison for the other categories. An initial exploratory analysis of the data (univariable) was conducted for selection of variables with $P \leq 0.2$ by the chi-square test or Fisher's exact test; subsequently, the variables that passed

Table 1 Herd-level prevalence for BVDV in cattle in the State of Paraíba, Northeastern Brazil, according to sampling stratum

Sampling stratum	No. of herds			Prevalence (%)	95 % CI
	Total	Tested	Positive		
Sertão	24,356	159	118	74.2	66.8–80.4
Borborema	11,603	160	79	49.4	41.7–57.1
Agreste/Zona da Mata	18,398	159	102	64.2	56.4–71.3
State of Paraíba	54,357	478	299	65.5	61.1–69.7

below this cutoff were utilized for logistic regression (Hosmer and Lemeshow 2000). The fit of the final model was verified with the Hosmer and Lemeshow test, and collinearity between independent variables was verified by a correlation analysis; for those variables with a strong collinearity (correlation coefficient >0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (Dohoo et al. 1996). The calculations were performed by using SPSS software version 20.0.

Results

Prevalence

In total, 2443 animals were sampled from 478 herds. Herd- and animal-level prevalences are presented in Tables 1 and 2, respectively; further, the geographical distribution of positive and negative herds are shown in Fig. 1. The herd-level prevalence in the State of Paraíba was 65.5% (95% confidence interval (CI) =61.1–69.7%), 49.4% (95% CI =41.7–57.1%) in the region of Borborema, 64.2% (95 % CI =56.4–71.3 %) in Agreste/Zona da Mata, and 74.2% (95% CI=66.8–80.4%) in Sertão. The animal-level prevalence was 39.1% (95% CI = 33.1–45.6%) in the State of Paraíba, 31.9% (95% CI = 21.9–43.9%) in the region of Borborema, 42.1 % (95% CI = 33.5–51.2%) in Sertão, and 37.8% (95% CI = 27.6–49.1%) in Agreste/Zona da Mata. The frequency of seropositive animals per herd ranged from 10 to 100% (median of 50%).

Risk factors

The results of the univariable analysis for the risk factors are shown in Table 3. The variables selected ($P \leq 0.20$) for the multiple analysis were as follows: sampling stratum, management system, no. of milking, no. of lactating cows, daily milk production (liters), no. of cows aged ≥24 months, no. of calves aged ≤12 months, herd size, presence of wildlife, abortion disposal method, animal purchasing, pasture rental, maternity pens, and veterinary services. In the final logistic regression model (Table 4), the risk factors identified were as follows: more than six calves aged ≤12 months (odds ratio (OR) = 3.72), animal purchasing (OR = 1.66), pasture rental (OR = 2.15), and presence of veterinary assistance (OR = 2.04). Final model had a good fit (Hosmer and Lemeshow test: chi-square=3.66; P=0.454).

Discussion

Seroprevalence surveys at herd level for BVDV in Brazil are scarce, and this was the first study using planned sampling in the State of Paraíba and Northeastern Brazil. A considerable variation exists in the prevalence of positive herds, but a high prevalence were found in various Brazilian states, such as 48.8% in Rio Grande do Sul (Almeida et al. 2013), 78.2% in São Paulo (Silva 2014), and 88.3% in the State of Goiás (Brito et al. 2010). The herd-level prevalence found in this survey (65.5 %) is considered as being high; however, it was lower than that one obtained by Thompson et al. (2006), who found anti-BVDV antibodies in 88.9% of the sampled herds.

Table 2 Animal-level prevalence for BVDV in cattle in the State of Paraíba, Northeastern Brazil, according to sampling stratum

Sampling stratum	Animals			Prevalence (%)	95 % CI
	Total	Tested	Positive		
Sertão	288,764	990	337	42.1	33.5–51.2
Borborema	83,428	729	180	31.9	21.9–43.9
Agreste/Zona da Mata	192,320	724	271	37.8	27.6–49.1
State of Paraíba	564,512	2443	788	39.1	33.1–45.6

Table 3 Univariable analysis for risk factors associated with the herd-level prevalence of BVDV in cattle in the State of Paraíba, Northeastern Brazil

Variables	Categories	No. of herds sampled	No. of positive herds (%)	P
Sampling stratum	Sertão	159	118 (74.2)	<0.001*
	Borborema	160	79 (49.4)	
	Zona da Mata	159	102 (64.2)	
Type of production	Meat	60	43 (71.7)	0.282
	Milk	138	86 (62.3)	
	Mixed	280	170 (60.7)	
Management system	Confined	28	15 (53.6)	0.054*
	Semi-confined	271	160 (59.0)	
	Extensive	179	124 (69.3)	
No. of milking	Not milking	124	70 (56.5)	0.152*
	1 times/day	305	194 (63.6)	
	2or 3 times/day	49	35 (71.4)	
No. of lactating cows	0–4	382	220 (57.6)	<0.001*
	>4	96	76 (82.3)	
Daily milk production (liters)	0–15	368	212 (57.6)	<0.001*
	>15	110	87 (79.1)	
Use of artificial in semination	No	475	298 (62.7)	0.559
	Yes	3	1 (33.3)	
Predominant breed	Zebu	25	18 (72.0)	0.246
	European dairy breed	43	27 (62.8)	
	Crossbred	405	249 (61.5)	
	Other breeds	5	5 (100)	
No. of cows aged ≥24 months	1–9	368	205 (55.7)	<0.001*
	>9	110	94 (85.5)	
No. of calves ≤12 months	1–6	370	207 (55.9)	<0.001*
	>6	108	92 (85.2)	
Herd size	1–23	364	200 (54.9)	<0.001*
	>23	114	99 (86.8)	
Presence of goats/sheep	No	296	192 (64.9)	0.217
	Yes	182	107 (58.8)	
Presence of horses	No	219	130 (59.4)	0.218
	Yes	259	169 (65.3)	
Presence of swine	No	324	198 (61.1)	0.399
	Yes	154	101 (65.6)	
Presence of wildlife	No	302	198 (65.6)	0.092*
	Yes	176	101 (57.4)	
Presence of cervids	No	471	293 (62.2)	0.265
	Yes	7	6 (85.7)	
History of abortions	No	447	281 (62.9)	0.732
	Yes	31	18 (58.1)	
Abortion disposal method	Left in the pasture lot	379	228 (60.2)	0.078*
	Used to feed pigs or dogs	7	6 (85.7)	
	Buried or burned	92	65 (70.7)	
Animal purchasing	No	316	182 (57.6)	0.002*
	Yes	162	117 (72.2)	
Pasture rental	No	366	214 (58.5)	0.001*
	Yes	112	85 (75.9)	
Sharing of pastures	No	398	245 (61.6)	0.381
	Yes	80	54 (67.5)	
Flooded pastures	No	304	184 (60.5)	0.266
	Yes	174	115 (66.1)	
Maternity pens	No	356	209 (58.7)	0.004*
	Yes	122	90 (73.8)	

Table 3 (continued)

Variables	Categories	No. of herds sampled	No. of positive herds (%)	*P*
Veterinary assistance	No	404	241 (59.7)	0.003*
	Yes	74	58 (78.4)	

*$P \leq 0.2$, variables selected and used in the multiple analysis

These results are concerning, as the herds of the State of Paraíba do not vaccinate cattle against BVDV, and it may be affirmed that these antibodies found may be originated from the exposure of the animals to the virus, suggesting its dissemination in the cattle herds (Sousa et al. 2013).

The animal-level prevalence found in this study (39.1%) is considered low when compared with some works carried out in the country: 64% in the State of Goiás (Brito et al. 2010), 65.66% in Maranhão (Chaves et al. 2012), 66.32 % in Rio Grande do Sul (Quincozes et al. 2007), and 78.21% in São Paulo (Silva 2014). However, it was higher than that one obtained by Thompson et al. (2006); they reported a frequency of anti-BVDV antibodies of 22.2%, demonstrating that the virus is disseminated. According to Almeida et al. (2013), the low prevalence in the present study may be related to the low density of animals in the herds when compared with other regions, as regional demographic factors. However, Flores et al. (2005) reported that high prevalences must be interpreted with caution, as the majority of seroepidemiological surveys carried out in Brazil do not use planned sampling, with a small number of samples and generally herds with reproductive problems, not considering the possibility of interference of vaccination on serological results. In the present survey, a planned sample of herds and animals was used, as well as the vaccination against BVDV is not a common practice in the State of Paraíba, suggesting that the influence of vaccine antibodies, if there was, was low. Considering the frequency of seropositive animals per herd, which ranged from 10 to 100%, it is suggested that the virus is widespread in the herds from the State of Paraíba (Chaves et al. 2012; Sousa et al. 2013).

The Sertão mesoregion presented the highest herd- (74.2%) and animal-level (42.1%) prevalences. Such result may be associated to the fact that the Sertão is one of the main dairy regions in the State of Paraíba, and according to Flores (2003), several serological surveys revealed prevalence higher than 70% in dairy herds. Furthermore, the Sertão mesoregion borders the States of Rio Grande do Norte, Ceará and Pernambuco, where there is an intense animal trade, many times without the knowledge of the sanitary condition of the animals. It is worth highlighting that recently the BVDV was isolated in a herd in the county of Pombal (Weber et al. 2014), located in the Sertão mesoregion.

By the risk factor analysis, it was possible to identify the conditions which possibly are playing a role in the dissemination of the infection in the herds. The occurrence of more than six calves aged ≤12 months was identified as a risk factor. The seroprevalence among calves <6 months was similar to the seroprevalence among mature animals in the State of Paraíba in 2000 (Thompson et al. 2006). These authors suggest that the influence of passive antibodies in young animals is high in this infection. In addition, pregnant females, when infected between 40 and 120 days of gestation, may generate PI animals, which are immune-tolerant calves, generally seronegative, which continuously shed virus in large amounts through secretions and excretions (Lanyon et al. 2014). In this context, it is suggested that the higher the number of calves aged ≤12 months greater is the chance of occurrence of PI animals. Variables related to herd size are not liable of correction, and in this case, the identification of PI animals with their posterior removal from the herd assumes an important role as a control measure.

Despite the low prevalence of PI animals (Dias et al. 2010), these are the main source of infection in a herd. Houe (1992) demonstrated that herds with PI animals present higher prevalence than herds without PI animals. The same study reported that herds without PI animals often have an age limit above which all animals were seropositive. This is due to the fact that antibodies most

Table 4 Risk factors associated with herd-level prevalence of BVDV in cattle in the State of Paraíba, Northeastern Brazil

Risk factors	*b*	SE	Odds ratio (OR)	95 % CI	*P*
More than 6 calves aged ≤12 months	1.314	0.297	3.72	2.08–6.66	<0.001
Animal purchasing	0.505	0.219	1.66	1.08–2.55	0.021
Pasture rental	0.767	0.255	2.15	1.31–3.55	0.003
Presence of veterinary assistance	0.715	0.315	2.04	1.10–3.79	0.023

often are lifelong, and this age limit is a likely indicator of how long ago the herd was exposed to PI animals. According to Thompson et al. (2006), young animals beyond 6 months old are more likely to be seronegative and older animals are more likely to be seropositive to BVDV. This complies with other studies which have reported that seroprevalence of BVDV increases with age (Brito et al. 2010; Chaves et al. 2012). In the present study, in which only females over 24 months were sampled, non-use of young animals in the sampling would not cause impact in the inference of seroprevalence of BVDV in the state of Paraíba; however, the detection of BVDV antibodies in young animals would indicate the degree of persistence of the sources of infection and its within-herd spread (Thompson et al. 2006).

Animal purchasing was identified as a risk factor and corroborates the results of other epidemiological surveys (Talafha et al. 2009; Saa et al. 2012; Gates et al. 2013). Indeed, the animal purchasing is a classic risk factor for the occurrence of infectious diseases, and in the case of the BVDV, the possibility of introduction of PI animals and pregnant females gestating PI fetuses assumes importance, being the control of the introduction of infected animals in the herd an important prevention strategy (Gates et al. 2014; Graham et al. 2015).

The pasture rental was also considered a risk factor, which suggests indirect contact between herds. Silva (2014) also identified this risk factor in a survey with planned sampling in the State of São Paulo. The practice of pasture rental may favor the contact of the animals with previously contaminated environments, and in the dependence of environmental temperature, the BVDV may survive in feces of infected animals for up to 3 h at 35 °C (Niskanen and Lindberg 2003). In this way, it is suggested that the pasture rental is a practice that must be discouraged aiming to avoid not only the dissemination of the BVDV but of other infectious agents.

In some surveys carried out in Brazil, the herds which did not have veterinary assistance were more likely to be BVDV positive when compared with the herds which had veterinary assistance, as the lack of veterinary assistance may be reflected especially in the diagnosis and absence of implementation of BVDV control programs (Quincozes et al. 2007; Chaves et al.2012). However, the presence of veterinary assistance was considered a risk factor for BVDV infection in the present study. According to Silva et al. (2009), due to the existence of the habit of some farmers of only hiring veterinary services after finding reproductive disorders in the animals, this should not be regarded as a cause for the occurrence of the disease, but a consequence of the infection.

Although the prevalence of BVDV in cattle is relatively low in the State of Paraíba compared with other regions, the majority of farmers are not aware of the impact of the infection and the economic losses that it can cause. Thus, emphasizing correction of the risk factors among these farmers is crucial in order to avoid the dissemination of BVDV in the herds. Notably, the majority of farms in the state are family farming systems and due to their characteristics, raising the awareness of the farmers regarding the adoption of changes in the management conditions of their farms and the adoption of biosafety practices will be a difficult task, and this is a future challenge.

Conclusion

We conclude that BVDV infection occurs in cattle herds in the State of Paraíba, Northeastern Brazil. Our findings suggest that the implementation of control and prevention measures among farmers, with the aim of preventing dissemination of the agent in the herds, is necessary. Special attention should be given to addressing the identified risk factors, such as sanitary control prior to animal purchasing and to discourage the pasture rental, as well as to encourage the vaccination in the herds.

Acknowledgments This study was financed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), under the codes 471571/2011-3, 302131/2012-4, and 476596/2013-0. The authors would like to thank the SEDAP for help during the sample collection.

Compliance with ethical standards The study was approved by the Ethics Committee of the Federal University of Campina Grande, Brazil—protocol number 48-2012.

Conflict of interest The authors declare that they have no conflict of interest.

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CHAPTER II

Risk factors associated with BoHV-1 and BVDV seropositivity in buffaloes (*Bubalus bubalis*) from the state of Paraíba, Northeastern Brazil

Published in Semina: Ciências Agrárias, Londrina - PR. Qualis B1; JCR 0.229.

DOI: 10.5433/1679-0359.2016v37n4p1929

Risk factors associated with BoHV-1 and BVDV seropositivity in buffaloes (*Bubalus bubalis*) from the State of Paraíba, Northeastern Brazil

Fatores de risco associados com as soropositividades para BoHV-1 e BVDV em búfalos (*Bubalus bubalis*) no Estado da Paraíba, Nordeste do Brasil

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Abstract

This study aimed to identify the risk factors associated with Bovine Herpesvirus Type 1 (BoHV-1) and Bovine Viral Diarrhea Virus (BVDV) infections in buffaloes in the State of Paraíba, Northeastern Brazil, using 136 female buffaloes ≥ 24 months old from 14 herds. For the serological diagnosis of the BoHV-1 and BVDV infections, the virus-neutralization test (VN) was used and in each herd, an epidemiological questionnaire was applied in order to obtain data to be used in the risk factor analysis. Of the 136 animals 86 (63.2%) were positive for BoHV-1 and 12 (8.8%) for BVDV. The presence of watering points (odds ratio = 17.68; 95% CI = 3.74 – 83.04) was identified as risk factor for BoHv-1, and animal purchasing was a risk factor for both BoHV-1 (odds ratio = 68.64; 95% CI = 12.28 – 383.73) and BVDV (odds ratio = 3.64; 95% CI = 1.04 – 12.76). The results from the present study showed an evidence of the presence of BoHV-1 and BVDV infections in buffaloes from the State of Paraíba, Northeastern Brazil. It is suggested that control and prevention measures should be adopted, such as the use of diagnostic tests prior to animal purchasing and the use of vaccines to avoid the introduction of infected animals into the herds and the consequent dissemination of the infections, minimizing economic losses.

Key words: BoHV-1. BVDV. Buffaloes. Northeastern Brazil. Risk factors. Serology.

Resumo

Este estudo teve como objetivo identificar os fatores de risco associados à infecções pelo Herpesvírus Bovino Tipo 1 (BoHV-1) e Vírus da Diarreia Viral Bovina (BVDV) em búfalos no Estado da Paraíba, Nordeste do Brasil, utilizando 136 búfalas com idade ≥ 24 meses procedentes de 14 rebanhos. Para o diagnóstico sorológico das infecções por BoHV-1 e BVDV, empregou-se o teste de virusneutralização (VN) e em cada rebanho foi aplicado questionário epidemiológico para obtenção de dados a serem utilizados na análise de fatores de risco. Dos 136 animais 86 (63,2%) foram positivos para BoHV-1 e 12 (8,8%) para BVDV. Presença de aguadas (*odds ratio* = 17,68; IC 95% = 3,74-83,04) foi identificada como fator de risco para o BoHV-1, e compra de animais foi um fator de risco para BoHV-1 (*odds ratio* = 68,64; IC 95% = 12,28-383,73) e BVDV (*odds ratio* = 3,64; IC 95% = 1,04-12,76). Os resultados do presente estudo evidenciaram a presença das infecções por BoHV-1 e BVDV em búfalos do Estado da Paraíba, Nordeste do Brasil. Sugere-se que medidas de controle e prevenção sejam adotadas, como o

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Recebido para publicação 21/03/15Aprovado em 13/04/16

uso de testes de diagnóstico antes da compra de animais e a utilização de vacinas para evitar a introdução de animais infectados nos rebanhos e consequente disseminação das infecções, minimizando perdas econômicas.

Palavras-chave: BoHV-1. BVDV. Búfalos. Nordeste do Brasil. Fatores de risco. Sorologia.

Introduction

Bubaline presents as distinctive features their rusticity and adaptability to climatic and topographic factors and poor soils, added to the double aptitude for the production of both meat and milk, which make it a good alternative for the production of animal protein, mainly in tropical countries such as Brazil. Currently, Brazil has a prominent position in buffalo rearing and has a headcount of more than a million animals, of which the Northeastern region has about 122 thousand animals (IBGE, 2012). In the State of Paraíba, the bubaline headcount has a total of approximately 933 animals (IBGE, 2012).

Over the past years, with the expansion of the buffalo farming in Brazil, the interest on the sanitary state of these animals has increased, not only due to its importance but also because of the possibility of these animals acting as sources of infection of diseases for bovine and other species (SCHEFFER, 2013). Some infectious diseases can determine reproductive failures and may compromise simultaneously many animals of different categories, causing several clinical signs such as oestrus recurrence, abortions, perinatal mortality, birth of weak animals and infertility, which result in great economic damage (JUNQUEIRA; ALFIERI, 2006). In this context, the infections by the Bovine Herpesvirus Type 1 (BoHV-1) and the Bovine Viral Diarrhea Virus (BVDV) are of importance, and are responsible for the Infectious Bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis (IBR/IPV) and Bovine Viral Diarrhea/Mucosal Disease (BVD/MD), respectively.

BoHV-1 is an important bovine pathogen, causing significant economic losses related to failures in the reproduction and an increase of the mortality in the herds (CORTEZ et al., 2001). BoHV-1 has been reported to adapt itself to other species (THIRY et al., 2006) and the buffaloes' susceptibility has been demonstrated both in isolation and in viral identification, as in serological studies (SCICLUNA et al., 2010; MONTAGNARO et al., 2014). BVDV has a worldwide distribution and is endemic in the majority of the bovine populations, causing economic losses mainly of reproductive origin (GIRALDO et al., 2013). One of the most important characteristics of this virus is its high mutation frequency and the tendency to recombine, leading to various clinical manifestations and the difficulty to control the disease (VARGAS et al., 2009).

Considering the lack of epidemiological studies on BoHV-1 and BVDV in buffaloes in the State of Paraíba, Northeastern Brazil, this study aimed to identify the risk factors associated with seropositivity for BoHV-1 and BVDV in buffaloes.

Materials and Methods

The study was carried out in 14 herds with buffalo farming in the counties of Alagoa Nova, Areia, Campina Grande, Guarabira, Juripiranga, Santa Helena, Sapé, Rio Tinto, Santana dos Garrotes, Itatuba, Solânea and Cacimbas (Figure 1). The studied population was formed of bubaline females with aptitude for meat and milk, mixed-bred and of the Murrah breed, with age ≥ 24 months. For the calculation of the number of animals to be sampled, the formula for simple random sampling was used:

$$n = \frac{Z^2 P\,(1 - P)}{d^2}$$

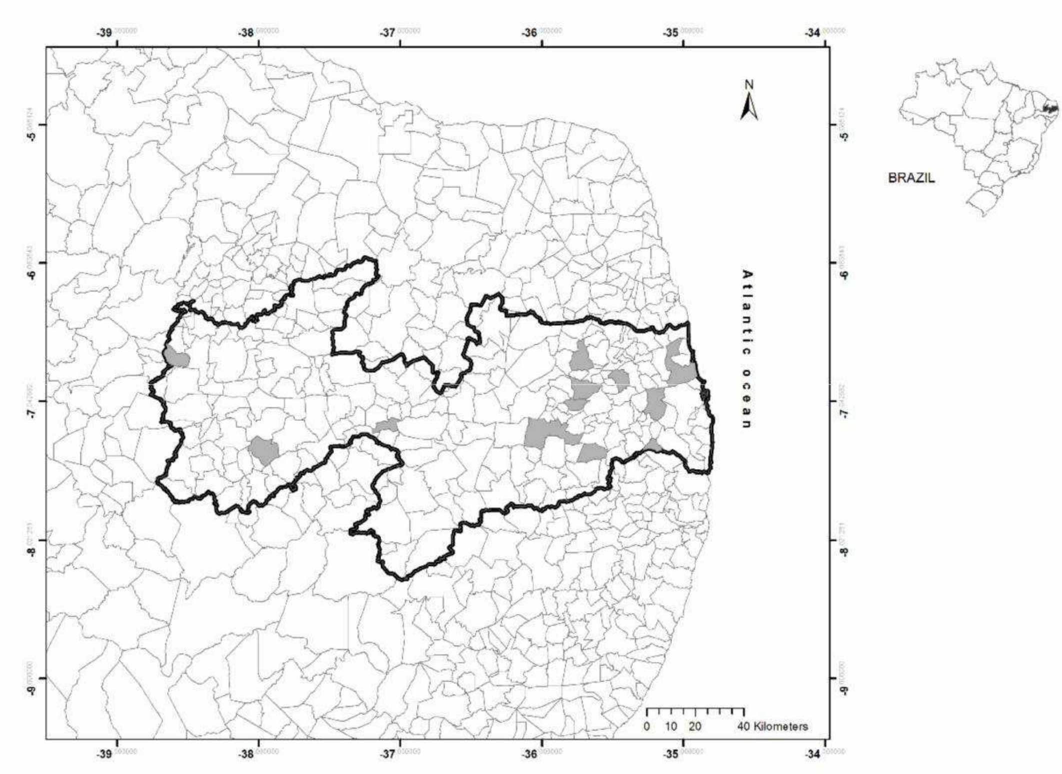
n = number of animals to be sampled

Z = value of normal distribution for the 95% confidence level

P = expected prevalence of 50% (for the maximization of the sample)

d = error of 10%

Figure 1. Geographical distribution of the counties used in the State of Paraíba, Northeastern Brazil. Detail shows the State of Paraíba into Brazil.



In total, 136 bubaline females with age ≥ 24 months were selected from 14 herds. Blood samples were collected in the period from November 2012 to July 2013, by venipuncture of the jugular with disposable needle and a tube with vacuum (without anti-coagulant) with capacity for 15ml. During blood collection, an epidemiological questionnaire was applied in order to obtain data to be used in the risk factor analysis. The variables and respective categories used were: management system (intensive, semi-intensive, extensive), type of exploration (meat, milk or mixed), type of milking (manual, mechanical), number of milking per day (non-milking, once a day, twice a day), presence of other animal species (bovine, equine, goats/sheep, swine, poultry, dog, cat), presence of wildlife (no, yes), occurrence of miscarriages during the last 12 months (no, yes), presence of rodents (no, yes), rodent control (no, yes), feeding on native pasture (no, yes), water source (drinking troughs, watering points), animal purchasing (no, yes), pasture rental (no, yes), presence of flooded areas (no, yes), presence of maternity pens (no, yes), separation of young from adults animals (no, yes), and presence of veterinary assistance (no, yes).

For the serological diagnosis of BoHV-1 and BVDV infections the virus-neutralization technique was used (OIE, 2008, 2010). The cytopathic viral strains BoHV-1 e BVDV-1 (NADL), supplied by the Virology Institute of the Veterinary Medicine College of Hanover, Germany were used. The technique was used in two stages, screening and titration, and a sample was considered to be positive when it presented a titer ≥ 2 for BoHV-1 and ≥ 10 for BVDV. The neutralizing antibody titers were considered as being the reciprocal of the higher serum dilutions that inhibited viral replication and the consequent production o cytopathic effect of BVDV and BoHV-1.

Risk factor analysis was performed in two steps: univariable and multivariable analysis. Univariable analysis was performed using the chi-square test or Fisher's exact test (ZAR, 1999), and those variables that presented $P \leq 0.20$ were used for multivariable logistic regression. The multivariable analysis was then performed, using the stepwise forward method (HOSMER; LEMESHOW, 2000). The significance level in multivariable analysis was 5%. Collinearity among independent variables was assessed using correlation analysis, and when two variables were highly collinear (correlation coefficient > 0.90), only one variable was likely to enter the multivariable analysis, and therefore the selection of which collinear variable to enter the model was guided by biological plausibility (DOHOO et al., 1996). The tests were performed using the SPSS for Windows software package, version 13.0.

Results and Discussion

Of the 136 buffaloes used 86 (63.2%) and 12 (8.8%) were seropositive for BoHV-1 and BVDV, respectively. Results of the univariable analysis of risk factors for BoHV-1 and BVDV with the most associated variables ($P \leq 0.20$) are shown in Table 1, and in Table 2 are shown the final models of logistic regression analysis for BoHV-1 and BVDV risk factors. Presence of watering points (odds ratio = 17.68; 95% CI = 3.77 – 83.04) was identified as risk factor for BoHV-1, and animal purchasing was considered a risk factor for both BoHV-1 (odds ratio = 68.64; 95% CI =12.28 – 383.73) and BVDV (odds ratio = 3.64; 95% CI = 1.04 – 12.76).

The high frequency of seropositivity for BoHV-1 found in this study corroborates the results found in recent studies carried out in the Brazilian states of Pará and Amapá (FERREIRA et al., 2009), and in Rio Grande do Sul (SCHEFFER, 2013), with prevalence rates of 82.4% and 44%, respectively. However, these results indicate that BoHV-1 is broadly disseminated in the region, which reinforces the possibility of these animals acting as sources of infection, mainly when dealing with animals with latent infection, which may disseminate the virus throughout their lives (BEZERRA et al., 2012). For BVDV, the positivity rate found was 8.8%, similar to the results found by Martins et al. (2012) in the State of São Paulo (12.9%) and Scheffer (2013) in Rio Grande do Sul (10.8%). However, these results suggest the circulation of BVDV and BoHV-1 within herds in Paraíba and due to the fact that it is common in the region to associate the farming of bovine and bubaline, the bovine-bubaline transmission is possibly being important (SCHEFFER, 2013).

The virus-neutralization technique can be impracticable as a diagnostic method in herds which adopt the vaccination against BoHV-1 and BVDV, since the differentiation of the vaccine antibodies from those produced as a result of the infection is not possible (TAKIUCHI et al., 2001). However, in the State of Paraíba, the vaccination of bubaline against BoHV-1 e BVDV is not a disseminated practice, accordingly this a fact that excludes the possible interference of the serology results. On the other hand, the practice of not vaccinating the animals could justify the high seropositivity rates found.

Animal purchasing was identified as a risk factor for BoHV-1 and BVDV, a similar result found by Dias et al. (2008) in bovine in the State of Paraná, Brazil. Animal purchasing is a classical risk factor associated with infectious diseases, and it suggests the importance of carrying out a sanitary control when purchasing animals by serological diagnosis at the origin and the destiny of the animals, as a way of avoiding the introduction of infected animals into the herds. It is important to highlight that in the State of Paraíba the serological testing for BoHV-1 and BVDV is not a common practice among rural producers.

Table 1. Results of the univariable analysis of the risk factors for BoHV-1 and BVDV seropositivities in buffaloes in the State of Paraíba, Northeastern Brazil, with the most associated variables ($P \leq 0.20$).

Variable	Category	Total no. of animals	No. of positive animals (%)	*P*
BoHV-1				
Presence of swine	No	121	72 (59.5)	
	Yes	15	14 (93.3)	0.023
Rodent control	No	48	42 (87.5)	
	Yes	88	44 (50)	0.001
Presence of watering points	No	58	28 (48.3)	
	Yes	78	58 (74.4)	0.003
Animal purchasing	No	99	55 (55.6)	
	Yes	37	31 (83.8)	0.005
Presence of flooded areas	No	6	0 (0)	
	Yes	130	86 (66.2)	0.002
Separation of young from adult animals	No	87	45 (51.7)	
	Yes	49	41 (83.7)	<0.001
BVDV				
Presence of equine	No	15	3 (20)	
	Yes	121	9 (7.4)	0.130
Animal purchasing	No	84	4 (4.8)	
	Yes	52	8 (15.4)	0.058

Table 2. Risk factors for BoHV-1 and BVDV infections in buffaloes in the State of Paraíba, Northeastern Brazil, identified by multiple logistic regression.

Variable	Odds ratio	95% CI	*P*
BoHV-1			
Presence of watering points	17.68	[3.77 – 83.04]	<0.001
Animal purchasing	68.64	[12.28 – 383.73]	<0.001
BVDV			
Animal purchasing	3.64	[1.04 – 12.76]	0.044

The presence of watering points was a risk factor for BoHV-1, which points to the possibility of indirect transmission by the ingestion of contaminated water, once that these animals have the habit of bathing in small mud puddles or watering points and also to urinate and defecate whilst ingesting water in these places. Scicluna et al. (2010) detected the presence of active viral particles in feces of buffaloes positive for BoHV-1, which may represent an elimination route of the agent and a source of environmental contamination.

Conclusions

The results from the present study showed an evidence of BoHV-1 and BVDV seropositivity in buffaloes from the State of Paraíba, Northeastern Brazil. It is suggested that control and prevention measures should be adopted, such as the use of diagnostic tests prior to animal purchasing and the use of vaccines to avoid the introduction of infected animals into the herds and the consequent dissemination of the infections, minimizing economic losses.

Acknowledgements

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for finantial support (code 302131/2012-4).

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CAPÍTULO III

Spatial analysis for Bovine Viral Diarrhea Virus and Bovine Herpesvirus type 1 infections in the state of Paraíba, Northeastern Brazil

Article submitted to BMC Veterinary Research. Qualis B1; JCR 1.643.

Spatial analysis for Bovine Viral Diarrhea Virus and Bovine Herpesvirus type 1 infections in the state of Paraíba, Northeastern Brazil

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Abstract

Background: Bovine Viral Diarrhea Virus (BVDV) and Bovine Herpesvirus type 1 (BoHV-1) cause reproductive problems in cattle and restrictions on international trade in animals wideworld. Both infections were detected in cattle herds in the Paraíba state, however, the spatial distribution and geographic identification of positive herds for these viruses has never been examined. Therefore, the aim of this study was to describe the spatial pattern of apparent prevalence estimate and to identify spatial clustering of positive herds of BVDV and BoHV-1 infections in cattle herds from the state of Paraíba, Northeastern Brazil.

Results: The herd-level prevalence for BVDV and BoHV-1 infections in Paraíba were, respectively, 65.5% (95% CI: 61.1–69.7) and 87.8% (95% CI: 84.5–90.5). The average apparent within-herd prevalence of BVDV was 31.8% and of BoHV-1 was 62.4%. The predicted prevalence was highest (0.42 – 0.75) for BVDV in the west, north and eastern part of Sertão and in the central and eastern part of Agreste/Zona da Mata. For BoHV-1, the highest predited prevalence (0.74 – 0.97) was in some local areas across Sertão and thoughout the eastern part of Agreste/Zona da Mata. Six significant clusters were detected for BVDV, a primary cluster covering the eastern Sertão region, with 11 herds, radius of 24.10 km and risk relative (RR) of 2.21 (P<0.001) and five smaller significant clusters, involving one or two herds in Agreste/Zona da Mata region with a high RR. A significant clustering of BoHV-1 positive herds (P<0.001) was detected in Agreste/Zona da Mata region with a radius of 77.17 km and a RR of 1.27, with 103 cases. Consistency was found between kriging and SatScan results for identification of risk areas for BVDV and BoHV-1 infections.

Conclusions: The clusters detected contemplated different areas of the state, with BVDV cluster located in the Sertão and BoHV-1 in Agreste/Zona da Mata stratum. Through the risk mapping, it was possible to identify the areas in which the risk is significantly elevated, coincided with areas where there are borders with other states and in which there is a high movement of animals.

Keywords: cattle, epidemiology, cluster analysis, BVDV, BoHV-1, kriging estimate.

Background

The reproductive infectious diseases are those that cause more damage in productivity in cattle herds, assuming importance viral agents, especially Bovine Viral Diarrhea Virus

(BVDV) and Bovine Herpesvirus type 1 (BoHV-1), which cause reproductive problems in both beef and milk cattle, and restrictions on international trade in animals and animal products [1, 2, 3].

BVDV is a member of the genus *Pestivirus,* which belongs to the *Flaviviridae* family, and although capable of manifesting in different clinical presentations and acute, sub acute or chronic character, the reproductive consequences of this virus and its persistent infection has greater economic and epidemiological importance [4, 5]. The persistently infected (PI) animals result from uterine exposure to noncytopathic strains of BVDV before development of fetal immunocompetence (generally before 125 days of gestation) [6]. PI calves are often weak at birth or die before one year of age, but others may not show clinical signs, being epidemiologically important due to shed large amounts of virus, acting as constant sources of infection for susceptible animals [7]. The detection and removal of PI animals and vaccination of susceptible animals are key strategies for the control of herds from BVDV [8, 9, 10].

The BoHV-1 is a alphaherpesvirus belonging to the genus *Varicellovirus*, which infects cattle and presents clinical manifestations such as pustular vulvovaginitis or balanoposthitis, abortion, rhinotracheitis and meningoencephalitis, and causes great economic losses to the livestock industry [11, 12]. BoHV-1 has the ability to cause latent infections, and periodically, BoHV-1 may be reactivated, and then sheded and transmitted, which guarantees its perpetuation and spread in herds, thus being the main obstacle to the establishment of control measures [13, 14].

The seroprevalence of BVDV and BoHV-1 have numerous references available all over the world, with variable values, but generally higher, depending on the control measures practiced [15]. In Brazil, regional data obtained from serological surveys reveal significant spread of the virus in beef and dairy herds [16, 17, 18]. In the state of Paraíba, Thompson et al. [19] found anti-BVDV antibodies in 22.2% of animals and 88.9% of herds and anti-BoHV-1 antibodies in 46.6% of animals and 100% of herds. These authors concluded that the within-herd spread of BVDV and BoHV-1 were relatively slow and its ubiquitous nature makes it difficult to generalize the rate of among-herds spread.

According to Carpenter [20], whether it is an outbreak investigation or epidemiological research, more emphasis should be placed on the spatial and temporal components of health events in order to identify unusual occurrences of events that happen close together in either time and/or space. Spatial analysis of infectious diseases allows for the detection of disease clusters, which can occur due to common risk factors among herds or the transmission between neighbors herds, being a useful tool in epidemiological surveillance providing better visualization and hypothesis survey for the occurrence of clusters, facilitating the elaboration of control strategies [21].

BVDV and BoHV-1 infections occur in cattle herds in the state of Paraíba, however, the spatial distribution and geographic identification of positive herds for these viruses has never been examined. Hence, the present study aimed to describe the spatial pattern of apparent prevalence estimate of BVDV and BoHV-1 infections in cattle herds from the state of Paraíba, Northeastern Brazil, including identification of areas with increased risk of occurrence of these viruses.

Methods

Characterization of the study area

The state of Paraíba, located in the Northeastern region of Brazil, is characterized by warm weather throughout the year. The state is geographically subdivided into the following four regions, based mostly on vegetation type and rainfall: (i) Zona da Mata (Atlantic forest), (ii) Agreste, (iii) Borborema, and (iv) Sertão. The Zona da Mata and Agreste have relatively higher rainfall regimes [22]. Borborema and Sertão (semi-arid region) are typically within the Caatinga biome, a xeric forest and a forest of thorns composed by cacti, thick plants, spiny brush and adapted arid grasses [23]. The climate is characterized by a warm and semi-arid climate, with average temperatures of 27 °C, and the average annual rainfall is typically ≈500 mm. There are typically two seasons: a rainy season from February to May, and a long drought period from June to January. However, occurrences of droughts lasting more than one year are also characteristic of the region [24].

The animal husbandry has an increasingly important in the Agreste, Borborema and Sertão regions, where small cattle-raising farms and family farms are widespread. The cattle are usually reared extensively on native Caatinga in most of the Borborema and Sertão farms. Following the Brazilian scenario of milk production, in the state of Paraíba around 69% of milk was produced in small cattle-raising farms [25].

Data source

The data used in this study were obtained from a survey of bovine brucellosis in the state of Paraíba made by the National Program for Control and Eradication of Brucellosis and

Tuberculosis, in which samples were collected from September 2012 to January 2013. In total, 2,443 cows aged ≥ 24 months were sampled from 478 herds.

Study design

The state of Paraíba was divided into three sampling groups: sampling stratum 1 (mesoregion of Sertão), sampling stratum 2 (mesoregion of Borborema), and sampling stratum 3 (mesoregions of Zona da Mata and Agreste) (Fig. 1). When this stratification scheme was proposed, the operational capacity of the Agricultural and Livestock Defense Service of the State of Paraíba (SEDAP) was considered based on the areas of action of its regional units in order to ensure that the veterinarians and agricultural and livestock technicians from the SEDAP could conduct the fieldwork. For each sampling stratum, a pre-established number of herds with reproductive activity (primary sampling units) were randomly selected and then, a pre-established number of unvaccinated cows aged ≥ 24 months were randomly and systematic selected (secondary sampling units), using the following criteria: 10 animals were sampled in herds with up to 99 cows aged over 24 months; 15 animals were sampled in herds with 100 or more cows aged over 24 months; and all animals were sampled in those with up to 10 cows aged over 24 months.

The field activities included blood collection and sending the samples to the laboratory. The coordinates in each herd were identified using a global positioning system device (GPS).

BoHV-1 and BVDV serological diagnosis

For the serological diagnosis of BVDV and BoHV-1 infections the virus-neutralization test was used [26, 27]. The cytopathic viral strains BVDV-1 (NADL) and BoHV-1 supplied by the Virology Institute of the Veterinary Medicine College of Hanover, Germany were used. The technique was performed in two stages, screening and titration, and a sample was considered positive when it presented a titer ≥ 10 for BVDV and ≥ 2 for BoHV-1. The neutralizing antibodies titers were considered the reciprocal of the higher serum dilutions capable of inhibiting the viral replication and the consequent production of cytopathic effect of BVDV and BoHV-1. The infectious titer used was $10^{5.61}$TCID$_{50}$/50 µL for BVDV and $10^{6.25}$ TCID$_{50}$/50 µL for BoHV-1, determined by the Reed and Müench method with adaptations [28].

Herd-level case definition

The herd-level case definition was based on the size of the population (cows aged ≥24 months), number of females sampled, an intra-herd apparent prevalence of 50%, and the sensitivity and specificity of the virus-neutralization test of 95 and 99.5% for BVDV [29] and 94.4% and 93.2% for BoHV-1 [30], respectively, with the goal of obtaining a herd sensitivity and specificity of ≥90%. After simulations using Herdacc software, a herd was deemed positive for BVDV when at least one positive animal was detected, and for BoHV-1 infection, a herd was considered positive if it included at least one positive animal in herds of up to seven females; two positive animals in herds of 8-99 females; and three positive animals in herds with more than 99 females.

Apparent prevalence calculations

EpiInfo 6.04 software was used to calculate the apparent prevalences and respective confidence intervals [31]. Stratified random sampling was used to calculate the herd-level prevalence in the state of Paraíba [32]. The required parameters were as follows: (a) condition of the herd (positive or negative), (b) sampling stratum to which the herd belonged, and (c) statistical weight. The statistical weight was determined by applying the following formula [31]:

$$Weight = \frac{\text{number of herds in the stratum}}{\text{number of herds sampled in the stratum}}$$

The calculation of the herd-prevalence per sampling stratum employed the sampling design of a simple random sample by using the following parameters: (a) number of positive herds and (b) number of herds sampled in the stratum.

Spatial analysis

Identification of the herd, geographical coordinates and results of serological tests were included in a database for spatial analysis. Firstly, the Cuzick–Edwards' k-nearest neighbor method [33] was used to detect the possibility of spatial clustering at herd level using the ClusterSeer 2.5.1 software (BioMedware, Ann Arbor, MI, United States). Existence of potential spatial clustering was analysed at each of the first 10 neighborhood levels, and the overall p-value was adjusted for multiple comparisons with the Simes approach. In a second step, scan statisticis by the SatScan software version 9.0 [34] was used to identify local clusters of positive herds. A Bernoulli model was applied, the scanning window was circular, and the spatial size of scan window was limited to 25% of the total population. Because of the large proportion of positive herds, analysis was not run on herd-level, and then considering within-herd prevalence. The statistical significance level was set as 0.05 and the maps were constructed with the ArcGIS software.

The kriging method was used to estimate the spatial pattern of apparent within-herd prevalence of BoHV-1 and BVDV. This method has the advantage that along with a smooth surface of predicted prevalence a map of prediction variance is also produced. The method assumes stationarity, but it has been shown [35] to also work well on non-stationary rates. Kriging is based on the spatial correlation between measurements which is modelled by the semivariogram. An exponential model given by

$$\gamma(d) = c_0 + c(1 - \exp(-\frac{d}{a}))$$

was fitted to the sample semivariogram. The parameters in the model (nugget c_o, partial sill c, and range a) were estimated by minimizing $\sum wj(\gamma(d_j) - \hat{y}(dj))^2$, where the weights w_j were chosen to be the number of observation pairs at distance d_j. The practical range of influence in this model is $a' = 3 \times a$. Directional semivariograms in four directions (north, north-east, east, south-east) were also fitted to test for anisotropy.

Results

Figure 1 shows the spatial location of the herds in the state of Paraíba. The herd-level prevalence for BVDV and BoHV-1 infections in Paraíba were, respectively, 65.5% (95% CI: 61.1–69.7) and 87.8% (95% CI: 84.5–90.5) (Tab. 1). Two proprieties were excluded because they showed errors in their geographic coordinates, totaling 476 herds used in the spatial analysis. Figure 2 shows the spatial localization of positive and negative herds for BVDV (Fig. 2a) and BoHV-1 (Fig. 2b). The average apparent within-herd prevalence of BVDV was 31.8%, ranging from 0% to 100%, and for BoHV-1 it was 62.4% ranging from 0% to 100%. Figures 3 and 4 show the kriging estimate of the apparent within-herd prevalences for BVDV and BoHV-1, respectively. The predicted prevalence was highest (0.42 – 0.75) for BVDV (Fig. 3a) in the west, north and eastern part of Sertão and in the central and eastern part of Agreste/Zona da Mata. For BoHV-1 (Fig. 4a), the highest predited prevalence (0.74 – 0.97) was in some local areas across Sertão and thoughout the eastern part of Agreste/Zona da Mata. The prediction variance reflects the location of tested herds: low values in the western part of Sertão and north, central and southern part of Agreste/ Zona da Mata for BVDV (Fig. 3b) and north and central part of Agreste/Zona da Mata for BoHV-1 (Fig. 4b), where herd density was high.

The Cuzick–Edwards' test identified statistically significant (P <0.05) spatial clustering of BVDV and BoHV-1 cases at k = 18 and k = 7 neighborhood levels, respectively. Using Bernoulli model, six significant clusters were detected for BVDV and one significant cluster for BoHV-1 (Tab. 2, Fig. 5). There was no spatial overlap between the clusters. For BVDV, a primary cluster covered the eastern Sertão region, with 11 herds, radius of 24.10 km and the risk for infection was 2.21 (relative risk (RR) = 2.21; P <0.001) times higher in herds located inside cluster than in those located elsewhere. The others significant BVDV clusters were smaller, involving one or two herds in Agreste/Zona da Mata region, near the border of the Borborema mesoregion, however, with a high relative risk. The BoHV-1 cluster covered the Agreste/Zona da Mata region with a radius of 77.17 km and a relative risk of 1.27 (P <0.001), with 103 cases.

Discussion

In Brazil, prevalence rates of BVDV range from 22.2% to 85.4% [18, 36, 37], and seropositivity frequencies of BoHV-1 between 18% and 90% were frequently observed in non-vaccinated herds throughout the different geographical areas of Brazil [38, 39, 40]. In the Northeastern Brazil, seroprevalences for BVDV infection of 72.6% [41] and 51.1% in family farms [42] in Pernambuco state, and for BoHV-1 infection of 79.5% [43] also in Pernambuco state, 62.67% [44] in Sertão of Paraíba and 63.23% [45] in Maranhão state have been reported. The herd-level seroprevalences for BVDV (65.5%) and BoHV-1 (87.8%) estimated for Paraíba state in this survey indicate that these viruses are present in most herds of the state.

In this work, six significant clusters were detected for BVDV infection and one significant cluster for BoHV-1. Consistency was found between kriging and SatScan results for identification of risk areas (location of areas with high apparent within-herd prevalence) for BVDV and BoHV-1 infections. Both methods identified the same local areas: the western part of Sertão for BVDV and the western part of Agreste/Zona da Mata for BoHV-1.

The primary cluster for BVDV was detected in the Sertão region, with a radius of 24.10 km, and covering its eastern region. The Sertão was the stratum of higher prevalence for BVDV and is bordered on the north by Rio Grande do Norte state, west by Ceará state, and south by Pernambuco state. Border regions are always associated with large circulations of animals due to intense trade and often without the knowledge of the health status of animals [46, 47, 48]. Furthermore, recently BVDV was isolated in a herd in Pombal, a municipality of the Sertão mesoregion [49]. Knowing that PI animals are the main sources of infection of BVDV, it is important to consider that the cluster for BVDV may have PI herds. Ersbøll et al. [50] in a study on the dynamics of BVDV infection among neighboring herds in Denmark found that the presence of PI-herds increased the risk of a herd becoming infected (PI-herd), showing *odds ratios* (OR) of 1.37, 1.40 and 1.70 for 1, 2, and $\geq$ 3 PI-herds in the neighborhood, respectively, as well as the risk of becoming a PI-herd was negatively associated with the mean distance from the neighboring herds (OR = 0.7 for an increase of 1 km), concluding that the occurrence of PI-herd in a certain area has influence on the risk of a neighbor herd becoming infected.

In Brazil, Hein et al. [51] found two BVDV clusters of dairy herds in the Arroio do Meio region, Rio Grande do Sul state, a primary (P = 0.391) and a secondary (P = 0.773) by scanning analysis, but both without statistical significance. According to the author, the occurrence of no association may have been due to the scan test limitation or because transmission of the virus via aerosols or other vehicles is not an effective way to disseminate. Kirchgessner et al. [52] in an exploratory cluster analysis identified clusters in different locations for domestic livestock and white-tailed deer in New York state, United States, suggesting that BVDV is maintained independently in domestic livestock herds in the western part of the state and in the white-tailed deer population in the northwestern part. According to the authors, the spatial point pattern analyses provide information necessary for the epidemiological risk assessment that should precede the development of any regional BVDV management plan.

The significant BoHV-1 cluster (P <0.001) showed a radius of 77.17 km with 103 herds whose 359 animals were positive when it was expected 297 positive animals, and involved a large part of the Agreste/Zona da Mata stratum, which has a high herd-level prevalence (87.4%). Despite the seroprevalence is high throughout the state, the presence of a significant BoHV-1 cluster requires epidemiological interpretation of its spatial distribution and, consequently, the decisions based on that evidence [53]. The mesoregions of Agreste and Zona da Mata had the highest rainfall in the state and a bovine livestock characterized by dairy farms and predominant confined rearing [54]. Miranda et al. [55] detected BoHV-1 clusters in southeastern Brazil and observed that disease clusters may occur either because herds share common risk factors or via transmission between herds through the movement of infected animals. The high prevalence of BoHV-1 in the Agreste/Zona da Mata stratum and the presence of cluster suggest that the relationship between the cases and the spread of the virus is due to the proximity of the animals in the herd [20]. BoHV-1 clusters were detected in the state of Rio Grande do Sul, in which the most significant (P = 0.00027) had a radius of 122.33 km, with 173 properties, 109 positive animals, and RR of 1.31, showing a moderate presence of BoHV-1 infection in the southern herds [20].

In the risk mapping described in this study there is a significant relation between the spatial effect and the development of the diseases, indicating that there are some important predisposing factors in the occurrence of infections studied as well as there may be other variables not considered in this work that can determine the standard spatial distribution for both BVDV and BoHV-1 infections in a particular area. These variables influence a greater risk of disease in areas of higher spatial effect and, consequently, a highest relative risk space.

Conclusions

The spatial analysis enabled the identification of spatial clustering of risk for BVDV and BoHV-1 in the state of Paraíba. The clusters detected contemplated different areas of the state, with BVDV cluster located in the Sertão stratum and BoHV-1 in Agreste/Zona da Mata stratum. Through the risk mapping, it was possible to identify the areas in which the risk is significantly elevated, coincided with areas where there are borders with other states and in which there is a high movement of animals. Clusters detection analysis enabled a better view of the occurrence of the BoHV-1 and BVDV and its result indicates the highest risk areas in the state of Paraíba, facilitating control strategies for specific actions and directed to those areas.

List of Abbreviations

BoHV-1: Bovine Herpesvirus type 1; BVDV: Bovine Viral Diarrhea Virus; PI: persistently infected; RR: Risk Relative; SEDAP: Agricultural and Livestock Defense Service of the State of Paraíba; GPS: global positioning system; $TCID_{50}$: 50% Tissue culture Infective Dose; OR: odds ratio.

Declarations

Ethics approval

The study was approved by the Ethics Committee of the Federal University of Campina Grande, Brazil — protocol number 48-2012.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

This study was financed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), under the codes 471571/2011-3, 302131/2012-4, and 476596/2013-0.The authors would like to thank the SEDAP for help during the sample collection.

Authors ' contributions

LF conducted the serological diagnosic tests and drafted the manuscript. IC conduted the field activities and study design. EP conceived the laboratory for the serological diagnosis. AM was involved in the preparation of the methods. SA conceived the study, participated in its design and coordination of the study and performed the spatial analysis. All authors read and approved the final manuscript.

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Table 1. Census data of the cattle population in the State of Paraíba, Northeastern Brazil, according to sampling stratum, and herd-level prevalence for BVDV and BoHV-1.

Sampling stratum	No. of herds	Tested	BVDV			BoHV-1[b]		
			Positive	Prevalence	95% CI	Positive	Prevalence	95% CI
Sertão	24,356	159	118	74.2	[66.8 – 80.4]	145	91.2	[85.6 – 94.7]
Borborema	11,603	160	79	49.4	[41.7 – 57.1]	130	81.3	[74.4 – 86.6]
Agreste/Zona da Mata	18,398	159	102	64.2	[56.4 – 71.3]	139	87.4	[81.3 – 91.8]
Stateof Paraíba	54,357	478	299	65.5	[61.1 – 69.7]	414	87.8	[84.5 – 90.5]

Table 2. Statistically significant clusters of herds with a high within-herd prevalence of BVDV and BoHV-1 in the state of Paraíba, Northeastern Brazil.

Radius (km)	No. of herds in cluster	No. of cases in cluster		RR[a]	p-value
		Observed	Expected		
BVDV					
24.10[b]	11	55	25.87	2.21	< 0.001
2.11	2	8	2.59	3.11	0.045
0	1	13	4.53	2.90	0.004
0	1	10	3.23	3.12	0.005
0	1	10	3.23	3.12	0.005
0	1	10	3.23	3.12	0.005
BoHV-1					
77.17	103	359	297.39	1.27	< 0.001

[a] Relative risk

[b] Primary cluster

Fig. 1 Spatial localization of cattle herds in the State of Paraíba, Northeastern Brazil, according to sampling stratum. Detail shows Paraíba state within Brazil.

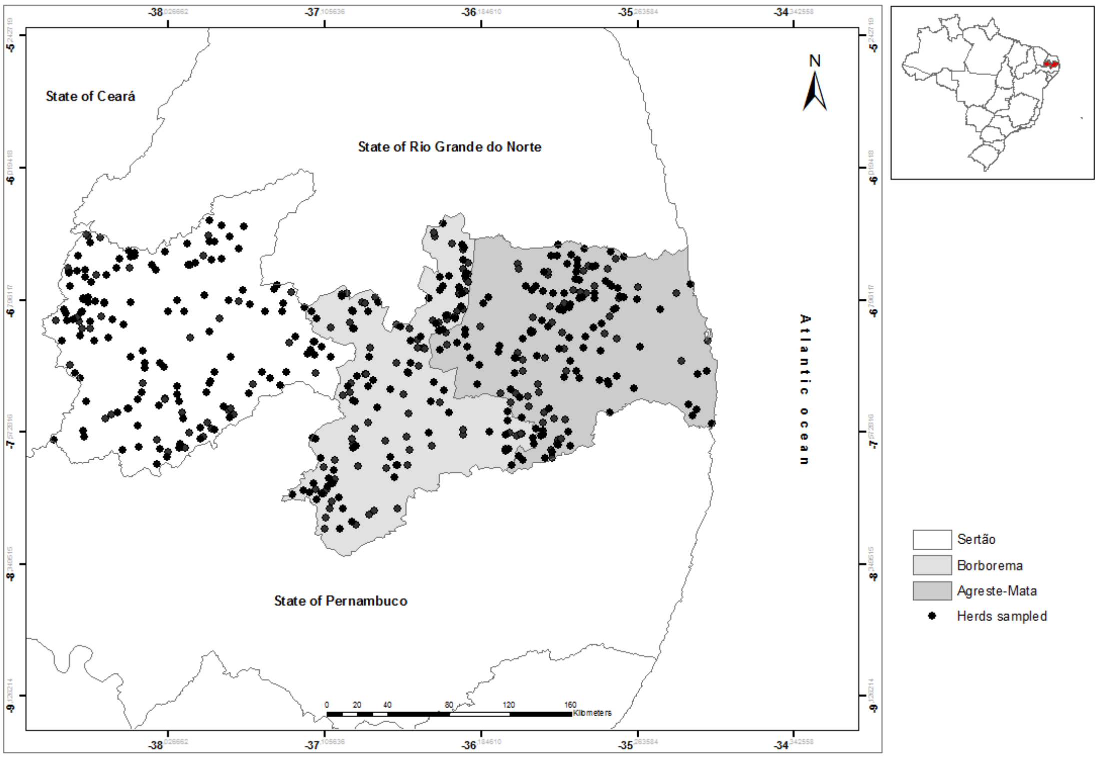


Fig. 2 Spatial localization of positive and negative herds in the State of Paraíba, Northeastern Brazil: (**a**) Herd status of BVDV; (**b**) Herd status of BoHV-1.

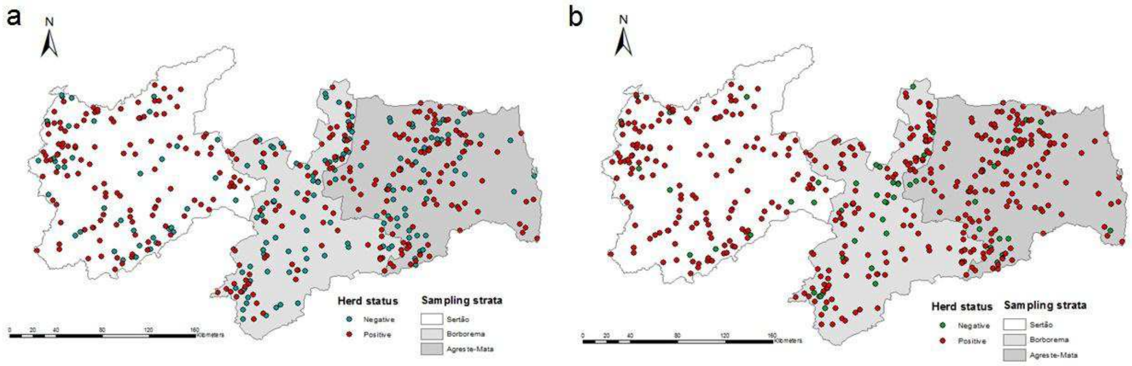


Fig. 3 Apparent within-herd prevalences for BVDV in the State of Paraíba, Northeastern Brazil: (**a**) Kriging surface of predicted apparent prevalence and (**b**) the variance of kriging estimates of predicted apparent prevalence.

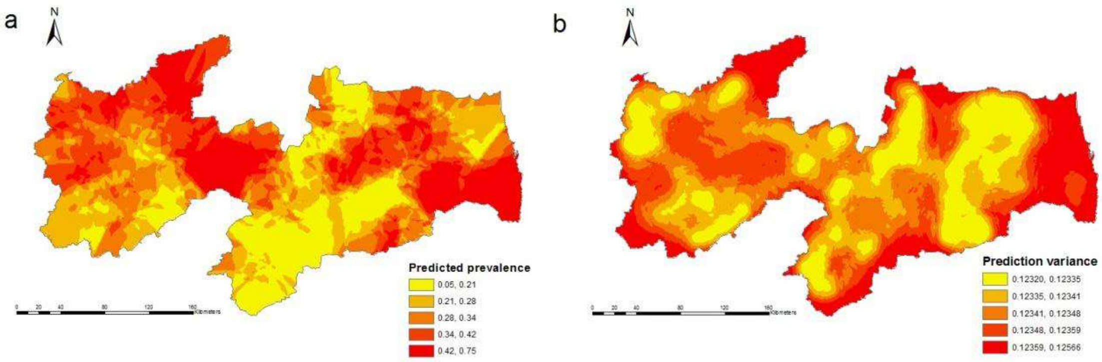


Fig. 4 Apparent within-herd prevalences for BoHV-1 in the State of Paraíba, Northeastern Brazil: (**a**) Kriging surface of predicted apparent prevalence and (**b**) the variance of kriging estimates of predicted apparent prevalence.

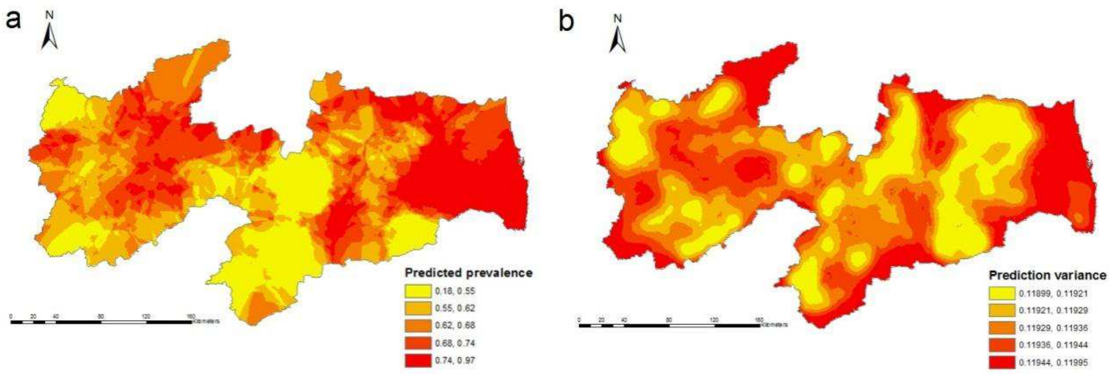
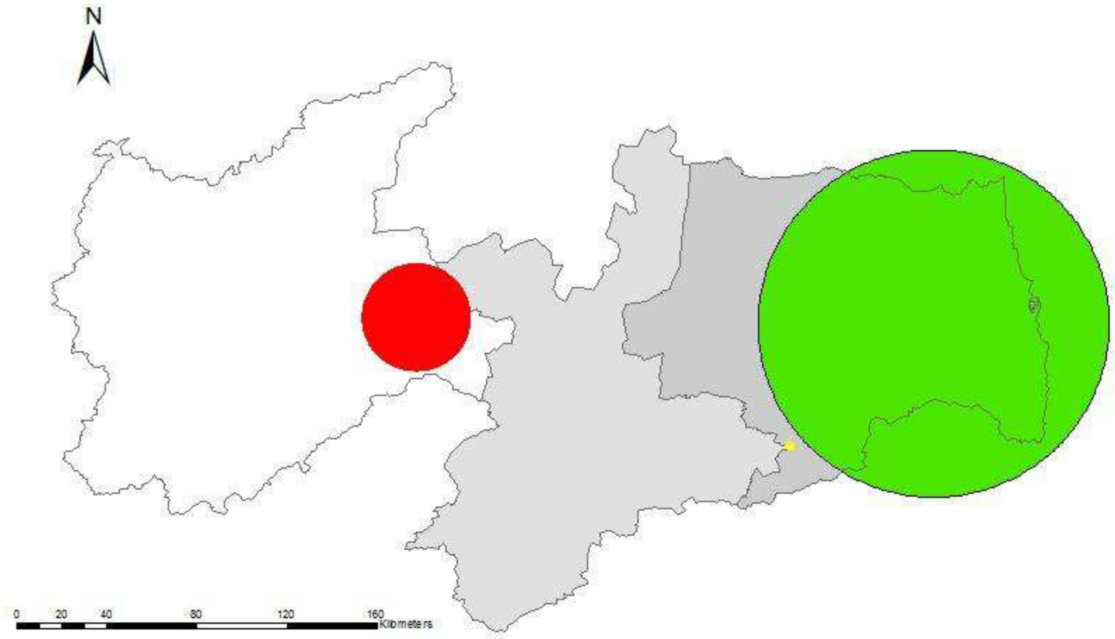


Fig. 5 Significant clusters of herds with a high apparent within-herd prevalence of BVDV (primary cluster: red, secondary clusters: yellow) and BoHV-1 (green).



CONCLUSIONS

Briefly, the three articles that compose this thesis led to the following conclusions:

- Antibodies to BVDV infection in bovine and BVDV and BoHV-1 infections in buffaloes were detected from the State of Paraíba Northeast of Brazil.
- Based on the analysis of risk factors, it is suggested to implement control and prevention measures among farmers, with the aim of preventing dissemination of the agent in herds, such as sanitary control before to animal purchasing, to discourage the pasture rental, to encourage vaccination in herds, as well as the use of diagnostic tests prior to animal purchasing and the use of vaccines to avoid the introduction of infected animals into the herds and the consequent dissemination of the infections, minimizing economic losses.
- The spatial analysis enabled the identification of spatial clustering of risk for BVDV and BoHV-1 in cattle in the Paraíba state, allowing a better view of the occurrence of these viruses and its result indicates the highest risk areas in the state, facilitating control strategies for specific actions and directed to those areas.

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