UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
CENTRO DE SAÚDE E TECNOLOGIA RURAL
UNIDADE ACADÊMICA DE MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA

Camila de Sousa Bezerra

Situação epidemiológica da infecção pelo vírus da estomatite vesicular em bovinos no estado da Paraíba

Patos/PB
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Dissertação submetida ao Programa de Pós-Graduação em Medicina Veterinária, da Universidade Federal de Campina Grande, como requisito parcial para obtenção do grau de Mestre em Medicina Veterinária.

Prof. Dr. Sérgio Santos de Azevedo

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SUMÁRIO

RESUMO

Os objetivos destes trabalhos foram estimar as prevalências em nível de rebanho e nível animal, identificar agrupamentos espaciais em nível de rebanho e fatores de risco associados à prevalência de rebanhos positivos para estomatite vesicular em bovinos no Estado da Paraíba, Nordeste do Brasil, bem como realizar uma revisão de literatura acerca da situação da doença no Brasil. O Estado foi dividido em três grupos amostrais: estrato amostral 1 (mesorregião do Sertão), estrato amostral 2 (mesorregião da Borborema) e estrato amostral 3 (mesorregiões da Zona da Mata e Agreste). Para cada estrato amostral, as prevalências de rebanhos positivos e de animais soropositivos foram estimadas por amostragem em dois estágios. No primeiro estágio, um número preestabelecido de rebanhos (unidades primárias de amostragem) foi selecionado aleatoriamente; no segundo estágio, um número pré-estabelecido de vacas com idade ≥ 24 meses (unidades secundárias de amostragem) foi selecionado aleatoriamente. No total, 2.279 animais foram amostrados de 468 propriedades. O diagnóstico sorológico foi realizado com o teste de soroneutralização viral. Um rebanho foi considerado positivo se incluiu pelo menos um animal positivo em rebanhos de até 10 fêmeas, dois animais positivos em rebanhos com 11 a 99 fêmeas e 3 animais positivos nos rebanhos com 100 fêmeas ou mais. A prevalência de rebanhos positivos no Estado da Paraíba foi de 38.5% (95% CI = 35.5-41.6%), 80.6% (95% CI = 73.6-86.2%) no Sertão, 7.0% (95% CI = 3.9-12.2%) na Borborema, e 2.6% (95% CI = 1.0-6.7%) no Agreste/Zona da Mata. A prevalência de animais soropositivos foi de 26.2% (95% CI = 20.6-32.8%) no Estado da Paraíba, 48.2% (95% CI = 41.5-54.9%) no Sertão, 6.3% (95% CI = 2.7-14%) na Borborema, e 1.9% (95% CI = 0.4-8.4%) no Agreste/Zona da Mata. Os fatores de risco identificados foram os seguintes: produção mista (OR = 3,86), tamanho do rebanho > 23 animais (OR = 3,40), presença de cervídeos (OR = 8,54), aluguel de pastagens (OR = 2,60) e compartilhamento de fontes de água (OR = 2,36). Foram detectados dois agrupamentos significativos de rebanhos positivos nas mesorregiões do Sertão e da Borborema. Os resultados obtidos indicam alta circulação do VSV na população bovina do estado da Paraíba, semiárido do Brasil, principalmente na mesorregião do Sertão, na qual foram observadas as maiores prevalências de propriedades e animais, bem como foram identificados aglomerados de propriedades positivas. Com base na análise de fatores de risco, sugere-se o desencorajamento das práticas de aluguel de pastagens e do compartilhamento de fontes de água devido à possibilidade do contato do VSIV presente no ambiente contaminado com animais suscetíveis.

PALAVRAS-CHAVE: Estomatite vesicular; Bovino; Epidemiologia; Análise de aglomerados espaciais; Controle; Nordeste Brasil.

ABSTRACT

This study focused on estimating the herd-level and animal-level prevalences, and identifying the risk factors associated with herd-level prevalence for vesicular stomatitis in bovines in the State of Paraíba, Northeastern Brazil, as well as to perform a literature review on the situation of the disease in Brazil. The state 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). For each sampling stratum, herd-level and animal-level prevalences were estimated by a two-stage sampling survey. In the first stage, a pre-established number of herds (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). Ten 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. In total, 2279 animals were sampled from 468 herds. Serological diagnosis was performed by virus neutralization. A herd was considered positive for VSV if it included at least one positive animal in herds of up to 10 females, two positive animals in herds of 11-99 females, and three positive in herds with more than 99 females. The herd level prevalence in the State of Paraíba was 38.5% (95% CI = 35.5-41.6%), 80.6% (95% CI = 73.6-86.2%) in the region of Sertão, 7.0% (95% CI = 3.9-12.2%) in Borborema, and 2.6% (95% CI = 1.0-6.7%) in Agreste/Zona da Mata. The animal-level prevalence was 26.2% (95% CI = 20.6-32.8%) in the State of Paraíba, 48.2% (95% CI = 41.5-54.9%) in Sertão, 6.3% (95% CI = 2.7-14%) in the region of Borborema, and 1.9% (95% CI = 0.4-8.4%) in Agreste/Zona da Mata. The risk factors identified were as follows: The risk factors identified were as follows: mixed production (milk/beef) (OR = 3.86), herd size > 23 animals (OR = 3.40), presence of cervids (OR = 8.54), rental of pastures (OR = 2.60) and sharing of water sources (OR = 2.36). Two significant groups of positive herds were detected in the Sertão and Borborema mesoregions. The results indicate high VSV circulation in the bovine population of the state of Paraíba, semi-arid region of Brazil, mainly in the Sertão mesoregion, in which the highest prevalences of properties and animals were observed, as well as agglomerates of positive properties were identified. Based on the analysis of risk factors, it is suggested the discouragement of pasture rental practices and the sharing of water sources due to the possibility of the presence of VSIV present in the environment contaminated with susceptible animals.

KEYWORDS: Vesicular stomatitis; Bovine; Epidemiology; Analysis of clusters; Control; Northeastern Brazil

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LISTA DE ABBREVIAÇÕES E SÍMBOLOS

%	Percentage
≡	Equal
<	Less than
>	Bigger than
≤	Less or equal
≥	Bigger or equal
°	Degree
°C	Degree Celsius
VS	Vesicular Stomatitis
VSV	Vesicular Stomatitis Virus
FMD	Foot and Mouth Disease
VN	Virus Neutralization
ELISA	Enzyme-Linked Immunosorbent Assay
OR	Odds Ratio
PCR	Polymerase Chain Reaction
SEDAP	Agricultural and Livestock Defense Service of the State of Paraíba
OD	Optical Densites
Se	Sensitivity
Sp	Specifity
CNPq	National Counsel of Technological and Scientific Development
CSTR/UFCG	Health Center and Rural Technology/Centro de Saúde e Tecnologia Rural/ Federal University of Campina Grande
Km	Kilometers
IC	Confidence Interval
UFSM	Federal University of Santa Maria
OR	Odds ratio
sp.	Species
spp.	Subspecie

INTRODUÇÃO GERAL

A estomatite vesicular (VS) é uma doença infecciosa, tendo como agente etiológico um vírus RNA negativo de fita simples, pertencente à ordem *Mononegavirales*, família *Rhabdoviridae,* gênero *Vesiculovirus*. A doença afeta animais ungulados e biungulados, sendo os equinos, bovinos e suínos os mais acometidos, além de animais silvestres e o homem. O vírus da estomatite vesicular (VSV) pode ser classificado em dois tipos segundo as suas características imunogênicas: New Jersey (VSNJV) e Indiana (VSIV), havendo subdivisão deste último em VSIV-1 (amostra clássica), VSIV-2 (Cocal e Argentina) e VSIV-3 (Alagoas) (De Stefano et al., 2002; Lichty et al., 2004; Brasil, 2012; Ferris et al., 2012).

Embora VS apresente baixa morbidade e mortalidade (Perez et al., 2010), a infecção tem um impacto direto na produção animal. Devido à semelhança clínica com a febre aftosa (FMD), existe uma restrição ao comércio e ao trânsito de animais em áreas com suspeitas até a confirmação do diagnóstico definitivo laboratorial, que geralmente é feito por ELISA e PCR (De Stefano et al., 2003, Arruda et al., 2015, De Stefano e Pituco, 2016). Sendo uma doença que requer um diagnóstico diferencial da FDM, é importante caracterizar as áreas de ocorrência da VSV, ajudando assim a elaborar medidas específicas de controle e prevenção direcionadas as áreas de importância epidemiológica e fatores de risco que favorecem a presença do vírus.

Esta dissertação consiste em dois capítulos. No Capítulo I, submetido ao periódico *Preventive Veterinary Medicine* (JCR 2.05, Qualis A2), foi determinada a prevalência de bovinos e de rebanhos soropositivos ao VSIV-3 no Estado da Paraíba, no Nordeste do Brasil, bem como os fatores de risco associados à ocorrência da infecção no rebanho e identificação de aglomerados espaciais de propriedades positivas. No Capítulo II foi elaborada uma revisão da literatura acerca da situação da VS no Brasil, e submetida ao periódico *Semina: Ciências Agrárias* (JCR 0.309, Qualis B1).

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CAPÍTULO I

Epidemiological situation of vesicular stomatitis virus infection in cattle in the state of Paraíba, semiarid region of Brazil

Artigo submetido ao periódico Preventive Veterinary Medicine
(JCR 2.05, Qualis A2)

Epidemiological situation of vesicular stomatitis virus infection in cattle in the state of Paraíba, semiarid region of Brazil

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ABSTRACT

The aim of this survey was to estimate the herd-level and animal-level seroprevalences, identifying risk factors and spatial clustering of vesicular stomatitis virus (VSV) positive herds in the state of Paraíba, semiarid of Brazil. The state was divided into three sampling strata: Sertão, Borborema and Zona da Mata/Agreste. For each sampling stratum, herd-level and animal-level prevalences were estimated by a two-stage sampling survey. First, a pre-established number of herds (primary sampling units) were randomly selected; second, within each herd, a pre-established number of cows aged ≥ 24 months were systematically selected (secondary sampling units). In total, 2279 animals were sampled from 468 herds. Serum samples were submitted to virus neutralization (VN) test for detection of antibodies to VSV using three viral strains: *VSIV-3 2013SaoBento/Paraiba E*, strain Indiana (VSIV-1) and VSNJV. A herd was considered positive for VSV if it included at least one positive animal in herds of up to 10 females, two positive animals in herds of 11-99 females, and three positive in herds with more than 99 females. The spatial clustering was assessed using the Cuzick–Edwards' k-nearest neighbor method and spatial scan statistics. The herd-level prevalence in the state of Paraíba was 38.5% (95% CI = 35.5-41.6%), 80.6% (95% CI = 73.6-86.2%) in the region of Sertão, 7.0% (95% CI = 3.9-12.2%) in Borborema, and 2.6% (95% CI = 1.0-6.7%) in Agreste/Zona da Mata. The animal-level prevalence was 26.2% (95% CI = 20.6-32.8%) in the state of Paraíba, 48.2% (95% CI = 41.5-54.9%) in Sertão, 6.3% (95% CI = 2.7-14%) in Borborema, and 3.2% 1.9% (95% CI = 0.4-8.4%) in Agreste/Zona da Mata. The risk factors identified were as follows: mixed production (milk/beef) (OR = 3.86), herd size > 23 animals (OR = 3.40), presence of cervids (OR = 8.54), rental of pastures (OR = 2.60) and sharing of water sources (OR = 2.36). Two significant clusters of positive herds were detected: the primary cluster covered the Sertão region and the secondary cluster covered part of the Sertão and Borborema regions. Our results suggest high VSV circulation in the bovine population of the state of Paraíba, semiarid region of Brazil, mainly in the Sertão mesoregion, and based on the risk factor analysis, the discouragement of pasture rental practices and sharing of water sources is recommended.

Keywords: Vesicular Stomatitis; Bovine; Epidemiology; Control; Northeastern Brazil

1. Introduction

Vesicular stomatitis (VS) affects cattle, swine and horses, as well as other mammals, including humans (Rodríguez, 2002). The infection is characterized by the development of vesicular lesions in the mouth, tongue, roofs and coronary bands, with clinical course ranging from 2-3 weeks. The etiological agent of VS, vesicular stomatitis virus (VSV), belongs to the order *Mononegavirales*, family *Rhabdoviridae*, genus *Vesiculovirus* and is classified into two groups: New Jersey (VSNJV), considered exotic in Brazil; and Indiana (VSIV) described in Brazil, which is subdivided into VSIV-1 (classical), VSIV-2 (Cocal) and VSIV-3 (Alagoas) (Fauquet et al., 2005; De Stefano and Pituco, 2016).

VS is considered endemic in northern South America, and outbreaks in Brazil are mainly related to VSIV-3 (Panaftosa, 2017), but in some regions cases of the disease were associated with VSIV-2 (Alonso Fernández and Sondahl, 1985; Pauszek et al., 2011). The first isolation of VSV in Brazil was performed in 1964 (Andrade et al., 1980) in the state of Alagoas, from the oral epithelium of diseased horses and the isolate was classified as VSIV-3. Two years later, VSIV-2 was identified for the first time in Brazil in horses in the state of São Paulo. According to data from the Ministry of Agriculture, Livestock and Supply (MAPA) 169 VSV outbreaks caused mainly by VSIV-3 and sporadically by VSIV-2 were reported in several states (Bahia, Ceará, Goiás, Pernambuco, Maranhão, Mato Grosso, Minas Gerais, Pará, Paraíba, Piauí, Rio Grande do Norte, Rio de Janeiro, São Paulo and Tocantins) between 2005 and 2013 (De Stefano and Pituco, 2016).

The mechanisms of maintenance and transmission of VS are not fully understood, but it is known that the disease has a seasonal pattern with higher incidence in hot and humid seasons (Manson et al., 1978). Transmission may occur through direct contact of infected and healthy animals, fomites, and ingestion of infected vegetables. Due to its irregular spatial distribution in outbreak situations, there is the hypothesis of dissemination by wind, birds and mainly by insects (Tesh et al., 1969; Zimmer et al., 2013).

In Brazil, serological surveys for VS are scarce and based on unplanned sampling. Kotait (1990) conducted a survey of antibodies against VSIV-3 in 2181 bovine serum samples from Vale de Paraíba, state of São Paulo, and found 36 (1.64%) animals with positive serology. De Stefano et al. (2003) analyzed 1099 bovine serum samples from the region of Araçatuba, state of São Paulo, and found 28 (2.6%) seropositive animals for VSIV-1. Clementino et al. (2014) reported the first outbreak of VS in the state of Paraíba, in which of the 82 cattle from the outbreaks 43 (52.44%) presented clinical signs suggestive of SV, with

VSIV-3 identification. Cargnelutti et al. (2014) reported an outbreak of the disease in 14 horses and six cattle in the states of Paraíba and Rio Grande do Norte, with isolation of a virus related to VSIV-3. In Northeastern Brazil VSIV-3 infection is considered endemic (Panaftosa, 2017). Lunkes et al. (2016) investigated the presence of VSIV-3 antibodies in horses from the South, Central West and Northeast regions, with higher seropositivity in the Northeast region (87.3% in Ceará, 65.7% in Rio Grande do Norte and 45.4% in Paraíba states).

Although VS presents low morbidity and mortality (Perez et al., 2010), the infection has a direct impact on animal production. Due to the clinical similarity with foot-and-mouth disease (FMD) there is a restriction on the trade and transit of animals in areas with suspected VS until confirmation of the definitive laboratory diagnosis, which is usually made by ELISA and PCR (De Stefano et al., 2003; Arruda et al., 2015, De Stefano and Pituco, 2016). In the case of a disease that requires a differential diagnosis from foot-and-mouth disease, it is important to characterize its areas of occurrence, given the history of viral circulation in the region, thus helping to elaborate specific control and prevention measures directed to areas of epidemiological importance and risk factors that favor the presence of the virus. Thus, the aim of the present survey was to determine the epidemiological situation of VS in cattle of the state of Paraíba, semiarid region of Brazil, by determining the herd and animal-levels seroprevalence, and identification of risk factors and spatial clusters of positive herds.

2. Material and methods

2.1. 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 major 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 (Cabrera and Willink, 1973). Both Borborema and Sertão (the semiarid region) are typically within the Caatinga biome, which encompasses an area of 900,000 km² (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 (Andrade-Lima, 1981). 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 sometimes lasting for longer than one year is also a characteristic of the region (Batista et al., 2007).

2.2. 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 creating this stratification scheme, 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.

2.3. Sampling, target condition and herd-level case definition

The samples used in this study were obtained from a serological survey of bovine brucellosis in the state of Paraíba conducted by the National Program for Control and Eradication of Brucellosis and Tuberculosis (Clementino et al., 2016), and sampling design was adjusted for vesicular stomatitis. For each sampling stratum, the prevalence of herds infected with vesicular stomatitis and the prevalence of seropositive animals were estimated by a two-stage sampling survey. In the first stage, a pre-established number of herds (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). The allocation of the primary sampling units was random (random drawing), and was based on the records of farms of the SEDAP. If a selected herd could not be visited, the herd was replaced by another in the vicinity with the same production characteristics (management system and type of production).

The number of selected herds per sampling stratum was determined by using the formula for simple random samples proposed by Thrusfield (2007). The parameters adopted for the calculation were as follows: 95% confidence level, 2.5% estimated herd-level

prevalence (De Stefano et al., 2003), and 5% error. Further, the operational and financial capacity of the SEDAP 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. For this purpose, the concept of aggregate sensitivity and specificity was used (Dohoo et al., 2003). For the calculations, the following values were adopted: 98% e 95% (Allende and Germano, 1993) for the sensitivity and specificity, respectively, of the test protocol (virus neutralization) and 32.1% (De Stefano et al., 2003) for the intra-herd estimated prevalence. Herdacc version 3 software (Jordan, 1995) was used, 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, which involved selection of sampling units at equal intervals, the first animal being randomly allocated. For example, if one animal in every 100 were required, the first animal would be randomly allocated from the first 100. If this was animal 63, then the sample would comprise animals, 63, 163, 263, 363 and so on (Thrusfield, 2007). The target condition was a seropositive animal within an infected herd. 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 32.1% (De Stefano et al., 2003), and the sensitivity and specificity of the diagnostic test used (virus neutralization), with the goal of obtaining a herd sensitivity and specificity of ≥ 90%. A herd was considered positive for vesicular stomatitis antibodies if it included at least one positive animal in herds of up to 10 females, two positive animals in herds of 11-99 females, and three positive in herds with more than 99 females.

The field activities included blood collection, provision of an epidemiological questionnaire, and sending the samples to the laboratory. The veterinarians and agricultural and livestock technicians of 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 anticoagulant). 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. After draining, the serum was transferred to microtubes and was frozen at −20◦C until the serological analysis, approximately three years.

202 *2.4. Data collection*

203

204 A structured questionnaire including closed-ended questions was designed to
205 obtain information concerning (a) the identification and location of the herd; (b) management
206 practices; and (c) structure and composition of the herd. Questionnaires were applied to the
207 owner or person in charge of the herd either by the primary author or by a veterinarian from
208 the SEDAP at the same time of the visit to blood collection.

209

210 *2.5. Serological tests*

211

212 Serum samples were submitted to virus neutralization (VN) test for detection of
213 antibodies to VSV, according to the OIE (2015) protocol, using the isolate *VSIV-3 2013*
214 *SaoBento/ParaibaE* (Cargnelutti et al., 2014). After complement inactivation, serum samples
215 were diluted 1:40 and incubated with 400-500 $TCID_{50}$ of the isolate *VSIV-3 2013*
216 *SaoBento/ParaibaE* for 1h at 37°C, followed by addition of a suspension of Vero cells and
217 incubation at 37°C with 5% CO2. The cultures were monitored for citopathic effect (cpe) for
218 72h. Samples not presenting cpe were considered positive for VSV antibodies at the used
219 dilution. Then, positive samples were submitted to a quantitative VN test, in which a fixed
220 dose of virus (400-500 $TCID_{50}$) was incubated with serial 2-fold dilutions of sera, starting at
221 1:40. In this test, each sample was tested against three VSV strains/isolates: isolate *VSIV-3*
222 *2013SaoBento/Paraiba E*, strain Indiana (VSIV-1) and VSNJV. After 72h, the cell cultures
223 were monitored for cpe and the VN titers were considered as the reciprocal of the highest
224 serum dilution capable to prevent cpe.

225

226 *2.6. Prevalence calculations*

227

228 The calculation of the herd-level prevalence per sampling stratum employed the
229 sampling design of a simple random sample by using the following parameters: (a) number of
230 positive herds and (b) number of herds sampled in the stratum. EpiInfo 6.04 software was
231 used to calculate the apparent prevalence and respective confidence intervals (Dean et al.,
232 1996). Stratified random sampling was utilized to calculate the herd-level prevalence in the
233 State of Paraíba (Thrusfield, 2007). The required parameters were as follows: (a) condition of
234 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):

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

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):

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

2.7. Risk factor analysis

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: type of production (beef/milk/mixed), management system (intensive/semi-intensive/extensive), milking (no/yes), use of artificial insemination (no/yes), predominant breed (European dairy/Zebu, crossbreed), herd size (cut-off point: 3rd quartile), presence of goats/sheep (no/yes), presence of horses (no/yes), presence of swine (no/yes), presence of cervids (no/yes), animal purchasing (no/yes), location of animal slaughter (not slaughter/slaughterhouses/on the farm), rental of pastures (no/yes), sharing of pastures (no/yes), presence of flooded pastures (no/yes), use of maternity pens (no/yes), veterinary assistance (no/yes), sharing of water sources (no/yes), and type of farm (classic rural/Indian village, settlement, urban periphery). The variables were organized for presentation in ascending or descending order regarding scale of risk. When necessary, these variables were re-categorized. The lower-risk category was considered the basis 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 this cut-off 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). Confounding was assessed by monitoring the changes in the model parameters when adding new variables. If substantial changes (i.e., higher than 20%) were observed in the regression coefficients, this was considered as indicative of confounding. The calculations were performed by using SPSS software version 20.0.

2.8. Spatial analysis

Herd identification, geographical coordinates and results of serological tests were included in a database for spatial analysis. Firstly, the Cuzick–Edwards' k-nearest neighbor method (Cuzick and Edwards, 1990) 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). The existence of potential spatial clustering was analyzed 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 statistics by the SatScan software version 9.0 (Kulldorff and Nagarwalla, 1995) 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. The statistical significance level was set as 0.05 and the maps were constructed with the ArcGIS software.

3. Results

A total of 2279 animals were sampled from 468 herds (range of herd sizes: 1–335). Herd-level and animal-level prevalence are presented in Tables 1 and 2, respectively. The geographic distribution of positive and negative herds is shown in Fig. 1. The herd-level prevalence was 38.5% (95% CI = 35.5-41.6%) and the animal-level prevalence was 26.2% (95% CI = 20.6-32.8%) in the state of Paraíba. The herd and animal-levels prevalence for sampling groups were, respectively, 80.6% (95% CI = 73.6-86.2%) and 48.2% (95% CI = 41.5-54.9%) in Sertão, 7.0% (95% CI = 3.9-12.2%) and 6.3% (95% CI = 2.7-14%) in

Borborema, and 2.6% (95% CI = 1.0-6.7%) and 1.9% (95% CI = 0.4-8.4%) in Zona da Mata/Agreste. Of the 491 samples positive for VSIV-3, 253 (51.5%) were positive for VSIV-1 with titers ranging from 40 to 1280 and 25 (5.1%) reacted to VSNJV, with titers ranging from 40 to 640, but almost all animals presented higher antibodies titles to VSIV-3 than VSIV-1 and VSNJV, indicating an immunology response to VSIV-3 infection whose antibodies cross-react with the other viruses/serotypes.

The results of the univariable analysis for the risk factors are presented in Table 3. The variables selected ($P \leq 0.2$) for the multiple analysis were as follows: type of production, management system, milking, predominant breed, herd size, presence of goats/sheep, presence of horses, presence of cervids, location of animal slaughter, rental of pastures, sharing of pastures, veterinary assistance, and sharing of water sources. In the final logistic regression model (Table 4), the risk factors identified were as follows: mixed production (OR = 3.86), herd size > 23 animals (OR = 3.40), presence of cervids (OR = 8.54), rental of pastures (OR = 2.60) and sharing of water sources (OR = 2.36).

The Cuzick–Edwards' test identified statistically significant (Simes $P = 0.01$) spatial global clustering of positive herds at all of the 10 neighborhood levels. Using Bernoulli model, two significant clusters were detected (Table 5, Fig. 2). There was no spatial overlap between clusters. In the primary cluster, that covered the Sertão region, the number of herds was 117, the radius of the cluster was 110.25 km, and the number of observed and expected cases (positive herds) were 102 and 35, respectively, where the risk for infection was 8.05 (relative risk = 8.05; $P < 0.0001$) times higher in herds located inside cluster than in those located elsewhere. The secondary cluster covered part of the Sertão and Borborema regions, and the number of herds was 32, the radius of the cluster was 42.48 km, and the number of observed and expected cases (positive herds) were 21 and 9.57, respectively, and the risk for infection was 2.40 (relative risk = 2.40; p = 0.033).

4. Discussion

This is the first seroprevalence survey for vesicular stomatitis virus (VSV) in cattle in Brazil using a planned sampling of herds and animals. Most reports of VSIV-3 infection in the country are related to sporadic outbreak situations or based on official reports. Prevalence of VSV-3 in herds (38.5%) and in animals (26.2%) confirmed the viral circulation in the state

of Paraíba described in previous reports in the same region (Cargnelutti et al., 2014; Clementino et al., 2014).

Serological surveys performed in two distinct regions of the state of São Paulo obtained low levels of seropositivity for VSV compared to the results of this reserach. In the Araçatuba region, 28 (2.6%) of the 1099 bovines sampled showed antibodies against VSIV-1 (De Stefano et al., 2003). Kotait (1990) conducted a survey of antibodies against VSIV-3 in sera from 2181 bovines from the Vale do Paraíba and found 36 (1.64%) animals with positive serology. The high seroprevalence of VSV-3 in the present survey may be related to viral activity in other animal species, such as horses. Lunkes et al. (2016) observed that among Southern, Center-Western and Northeastern Brazil, the last one presented the highest frequency of seropositive horses. The transmission of the virus between bovine and horses populations has already been proven and it is worth mentioning that the practice of consorted rearing is widely used in this region, besides the animal agglomeration in exhibition fairs, which provides direct and/or indirect contact of susceptible with infected animals, facilitating the maintenance of the agent. Another important factor that may explain the high prevalence of VSV-3 in the state of Paraíba is its border with the states of Ceará, Pernambuco and Rio Grande do Norte, where VSV-3 outbreaks have been described (Clementino et al., 2014 ; Cargnelutti et al., 2014).

The state of Paraíba is characterized by warm weather throughout the year, presenting favorable conditions to the proliferation and survival of insects. It is worth mentioning that the transmission of VSV by arthropods is probably an important route of virus propagation (Reis Jr et al., 2009). Thus, it is believed that the climate of the region favors the presence of the insect population and, consequently, greater opportunities for transmission, which may also justify the high seroprevalence of VSV infection in the state.

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 (Carpenter, 2001; Pfeiffer et al., 2008). In the present study, the Sertão mesoregion presented the highest herd-level (80.6%) and animal-level (48.2%) prevalences for VSIV-3, as well as the primary cluster identified covered almost the totality of this region and extended through the Rio Grande do Norte, Ceará and Pernambuco states. The Sertão is an area bordering these states, where there is an intense trade of animals without the

knowledge of their sanitary condition, which may justify the high prevalence found. It is worth noting that the state of Ceará is a high circulation area of VSIV-3 (López Inzaurralde et al., 1997; Lunkes et al., 2016). An important aspect is the family farm production in the Sertão, with low technification level of the properties, and without the support of important general sanitary measures for the control of infectious diseases, as quarantine of animals coming from other regions.

Out of the 491 samples positive for VSIV-3, 51.5% were also positive for VSIV-1, as well as 5% of the samples also reacted for VSNJV. These results are similar to those obtained by Lunkes et al. (2016) who examined 3626 samples of horses from six Brazilian states (Rio Grande do Sul, Goiás, Federal District, Pernambuco, Paraíba, Rio Grande do Norte and Ceará) and found 641 (17.7%) positive samples for VSIV-3, and 183 (28.5%) also reacted for VSIV-1 and seven (1.1%) were positive for VSNJV. According to Pauszek et al. (2011), a cross-reaction can be observed among VSIV-1, 2 and 3, despite their antigenic differences, which occur less frequently between VSIV and VSNJV (Cartwright and Brown, 1972; Lunkes et al., 2016). In addition, the positivity for VSNJV and VSIV-1 was probably due to a cross-reaction of VSIV-3 antibodies, since these serogroups are considered exotic in Brazil (Brasil, 2012; OIE, 2017; Panaftosa, 2017). In summary, results of VN tests indicated that the neutralizing antibodies detected were probably produced in response to infection by viruses antigenically related to VSIV-3 (VSIV-3 2013SaoBento/Paraiba*E*) (Lunkes et al., 2016), confirming the frequent circulation of this serotype in the Paraíba state.

By the risk factor analysis, it was possible to identify potentially important conditions for virus spread among/within herds. Mixed production was related to the occurrence of VS, corroborating the results obtained by Quincozes et al. (2007), in which farms with mixed production presented 1.73 and 12.98 times more chance to present positive animals to BVDV in relation to beef and milk properties, respectively, being justified by the lack or inefficiency of sanitary control measures. This variable may be related to other risk situations for any infection in herds, as observed by Silva et al. (2008), in which properties of this modality had a high rate of replacement of animals from other regions, favoring the spread of neosporosis.

Herd size is a classic risk factor for the occurrence of infectious diseases in animals. In the present study, the presence of more than 23 animals in the herds as a risk factor for VS can be justified by the closer contact of susceptible and infected animals in large herds, as well as the higher probability of vector transmission as a function of population density. Similarly, large herds are usually kept by animal purchasing, and if this practice is not

398 performed with the support of serological tests and quarantine, it can facilitate the entry of
399 infectious agents into susceptible populations. Seroepidemiological surveys for bovine
400 brucellosis conducted in Brazil also identified this variable as risk factor (Negreiros et al.,
401 2009, Ogata et al., 2009, Klein-Gunnewiek et al., 2009; t al., 2009, Dias et al., 2009b).

402 The presence of cervids was also identified as risk factor for VSIV-3 infection. A
403 research conducted in the Southeastern United States revealed high level of antibodies to
404 VSNJV in 60% of white-tailed deer (*Odocoileus virginianus*) (Karstad and Hanson, 1957). In
405 the same region of the USA, Jenney et al. (1970) identified antibodies against the VSNJV in
406 14 animals of the same species. According to the World Organization for Animal Health, the
407 white-tailed deer is considered the wild host of VSV (OIE, 2013). In Brazil, this cervidae has
408 a larger distribution in the Amazon region, the extreme north (Amapá and Roraima) and the
409 possibility of being found in Acre (Duarte, 1996; Eisenberg and Redford, 1999; Tiepolo et al.,
410 2009). However, there is no report of this species in Northeastern Brazil. Two cervidae
411 species have already been described in the state of Paraíba: the small red brocket (*Mazama*
412 *bororo*) (ICMBio, 2012) and the brown brocket (*Mazama gouazoupira*), according to
413 information from the administration of the Parque Zoobotânico Arruda Câmara in João
414 Pessoa, Paraíba. These species occur most commonly in the Sertão of the state, where the
415 highest herd and animal-level prevalences were observed, however, there is no information
416 about the presence of VSV in these species. Thus, further studies are needed to clarify the
417 potential role of these species in the epidemiology of VS.

418 The practice of pasture/grass rental is very frequent in the Northeastern Brazil,
419 especially during dry periods. This variable was identified as a risk factor for VS probably
420 because it allowed the indirect contact of susceptible animals with the agent in the
421 environment, which has already been observed in BVDV seroprevalence surveys (Fernandes
422 et al., 2015) and bovine brucellosis (Dias et al. 2009a; Klein-Gunnewiek et al., 2009). Some
423 authors suggest that VSV is a plant virus that undergoes a modification inside the insect when
424 it feeds on the infected plant (Johnson et al., 1969; Tesh et al., 1969) and that in endemic
425 areas the main transmission of VSV in susceptible animals is the contact of oral mucosa
426 lesions with contaminated pastures (Acha and Szyfres, 2003; Zimmer et al., 2013).

427 The sharing of water sources as a risk factor for VS can be justified by the indirect
428 contact between susceptible and infected animals through contaminated water, since the
429 possibility of VSV transmission by water has already been described (Zimmer et al., 2013). In
430 a study carried out in the Vale do Paraíba, São Paulo state, in 1986, the affected properties

were on the banks of the Paraíba river (Kotait, 1990), and there was no statistical association between the occurrence of the disease and other water sources available to the animals. In addition, VSV has high stability in suspension, which may facilitate the spread of virus in the herd, since infected animals release VSV through the lesions, leading to contamination of water sources (Zimmer et al., 2013).

5. Conclusion

The results indicate high VSV circulation in the bovine population of the state of Paraíba, semiarid of Brazil, mainly in the Sertão mesoregion, which borders the states of Ceará, Rio Grande do Norte and Pernambuco, where the highest prevalence of properties and animals, as well as clusters of positive herds were identified. Based on the risk factor analysis, pasture rental practices and sharing of water sources are highly discouraged due to the possibility of contact with VSIV present in the contaminated environment.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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695 **Figure caption**

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

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700 **Fig. 2.** Significant clusters of positive herds for stomatitis vesicular virus antibodies in cattle
701 in Paraíba state, Northeastern Brazil. Primary cluster: circular red line; secondary cluster:
702 circular dark line. Detail shows Paraíba state within Brazil.

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728 **Table 1** Herd-level seroprevalence for VS in cattle in the state of Paraíba, Northeastern
729 Brazil, according to sampling stratum.

	No. of herds				
Sampling stratum	Total	Tested	Positive	Prevalence (%)	95% CI
Sertão	24,356	155	125	80.6	[73.6-86.2]
Borborema	11,603	157	11	7	[3.9-12.2]
Agreste/Zona da Mata	18,398	156	4	2.6	[1-6.7]
State of Paraíba	54,357	468	140	38.5	[35.5-41.6]

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Table 2 Animal-level prevalence for VS antibodies in cattle in the state of Paraíba, Northeastern Brazil, according to sampling stratum.

	Animals				
Sampling stratum	Total	Tested	Positive	Prevalence (%)	95% CI
Sertão	288,764	908	452	48.2	[41.5 – 54.9]
Borborema	83,428	701	26	6.3	[2.7 – 14]
Agreste/Zona da Mata	192,320	670	13	1.9	[0.4 – 8.4]
State of Paraíba	564,512	2279	491	26.2	[20.6 – 32.8]

782 **Table 3** Univariable analysis for risk factors associated with the herd-level seroprevalence of
783 VS in cattle in the state of Paraíba, Northeastern Brazil.

Variables	Categories	No. of herds sampled	No. Of positive herds (%)	*P*
Type of production	Beef	58	8 (13.8)	< 0.001*
	Milk	134	28 (20.9)	
	Mixed	276	104 (37.7)	
Management system	Intensive	28	5 (17.9)	< 0.001*
	Semi-intensive	263	62 (23.6)	
	Extensive	177	73 (41.2)	
Milking	No	114	22 (19.3)	0.006*
	Yes	354	118 (33.3)	
Use of artificial insemination	No	465	140 (30.1)	0.558
	Yes	3	0	
Predominant breed	European dairy / Zebu	71	7 (9.9)	< 0.001*
	Crossbreed	397	133 (33.5)	
Herd size	1 – 23	352	85 (24.1)	< 0.001*
	> 23	116	55 (47.4)	
Presence of goats/sheep	No	286	94 (32.9)	0.100*
	Yes	182	46 (25.3)	
Presence of horses	No	212	52 (24.5)	0.027*
	Yes	256	88 (34.4)	
Presence of swine	No	315	94 (29.8)	1.000
	Yes	153	46 (30.1)	
Presence of cervids	No	461	135 (29.3)	0.027*

	Yes	7	5 (71.4)	
Animal purchasing	No	310	97 (31.3)	0.422
	Yes	158	43 (27.2)	
Location of animal slaughter	Not slaughter	210	38 (18.1)	< 0.001*
	Slaughterhouses	255	101 (39.6)	
	On the farm	3	1 (33.3)	
Rental of pastures	No	359	87 (24.2)	< 0.001*
	Yes	109	53 (48.6)	
Sharing of pastures	No	391	111 (28.4)	0.137*
	Yes	77	29 (37.7)	
Presence of flooded pastures	No	298	88 (29.5)	0.892
	Yes	170	52 (30.6)	
Use of maternity pens	No	348	104 (29.9)	1.000
	Yes	120	36 (30)	
Veterinary assistance	No	394	107 (27.2)	0.004*
	Yes	74	33 (44.6)	
Sharing of water sources	No	395	103 (26.1)	< 0.001*
	Yes	73	37 (50.7)	
Type of farm	Classic rural	430	132 (30.7)	0.313
	Indian village	3	1 (33.3)	
	Settlement	19	2 (10.5)	
	Urban periphery	16	5 (31.2)	

784 * Variables selected and used in the multiple analysis ($P \leq 0.2$)

Table 4 Risk factors associated with herd-level seroprevalence of VS in cattle in the state of Paraíba, Northeastern Brazil.

Risk factors	Logistic regression coefficient	Standard error	Wald	Degrees of freedom	Odds ratio (OR)	95% CI	*P*
Mixed production	1.35	0.453	8.887	1	3.86	1.56 – 9.37	0.003
Herd size > 23 animals	1.224	0.258	22.539	1	3.40	2.05 – 5.63	<0.001
Presence of cervids	2.145	0.915	5.496	1	8.54	1.42 – 51.36	0.019
Rental of pastures	0.957	0.258	13.77	1	2.60	1.57 – 4.31	<0.001
Sharing of water sources	0.859	0.294	8.511	1	2.36	1.33 – 4.20	0.004
Intercept	-3.813	0.571	44.542	1	0.022	...	<0.001

Hosmer and Lemeshow chi-square = 6.259; degrees of freedom = 6; $P = 0.395$.

Table 5 Statistically significant clusters of herds seropositive for vesicular stomatitis in cattle in the state of Paraíba, Northeastern Brazil.

Radius (km)	No. of herds in cluster	No. of positive herds in cluster		RR[a]	*P*
		Observed	Expected		
110.25[b]	117	102	35	8.05	< 0.0001
42.48	32	21	9.57	2.40	0.033

[a] Relative risk

[b] Primary cluster

HIGHLIGHTS

- The herd-level seroprevalence of vesicular stomatitis (VSV) in cattle from the State of Paraíba, semiarid region of Brazil, was 38.5% (95% CI = 35.5-41.6%).
- Spatial clusters of positive herds were identified in a region that borders other Brazilian states, where there is an intense trade of animals without the knowledge of their sanitary condition.
- Pasture rental practices and sharing of water sources are highly discouraged due to the possibility of contact with VSV present in the contaminated environment.

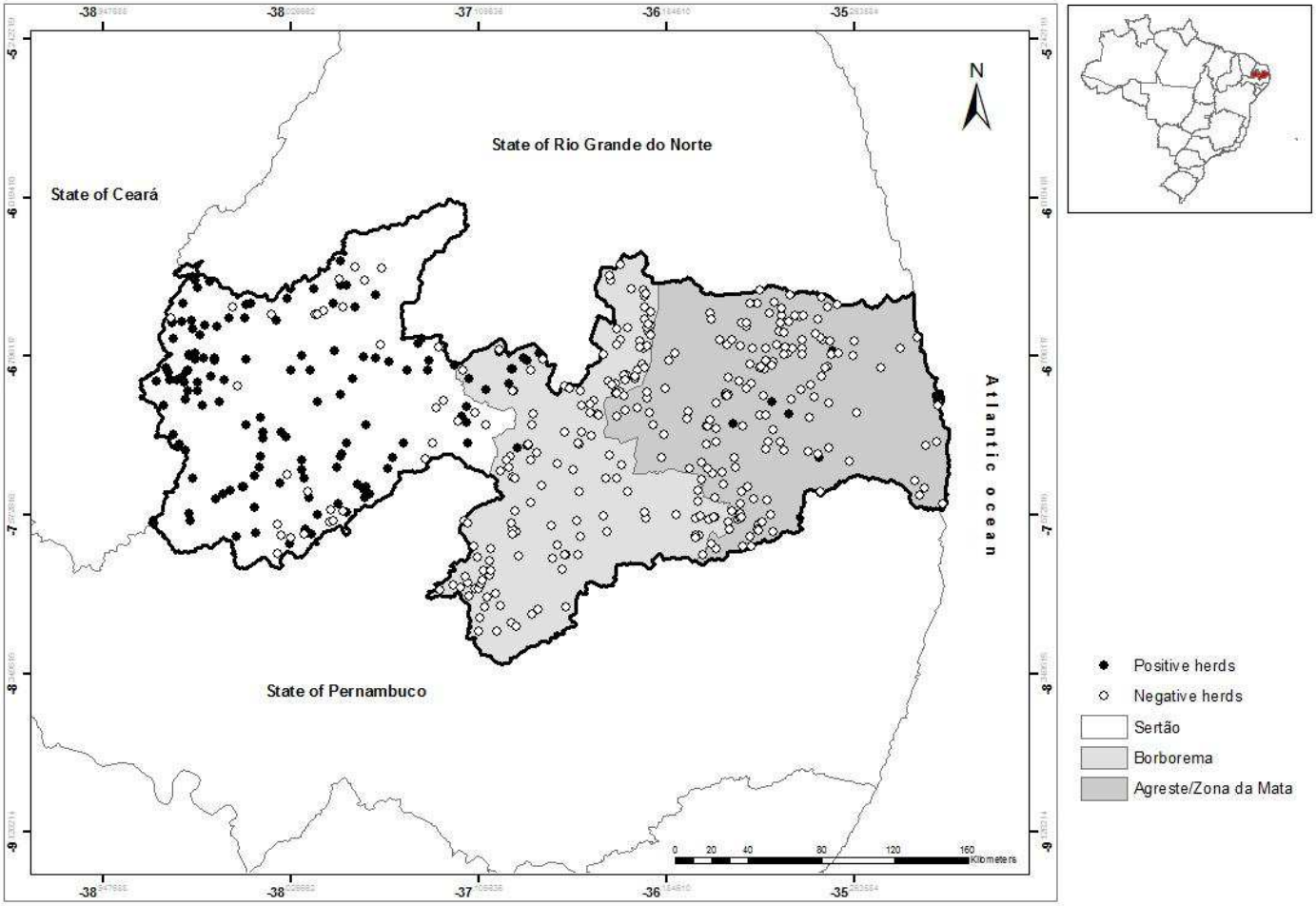
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Fig. 1

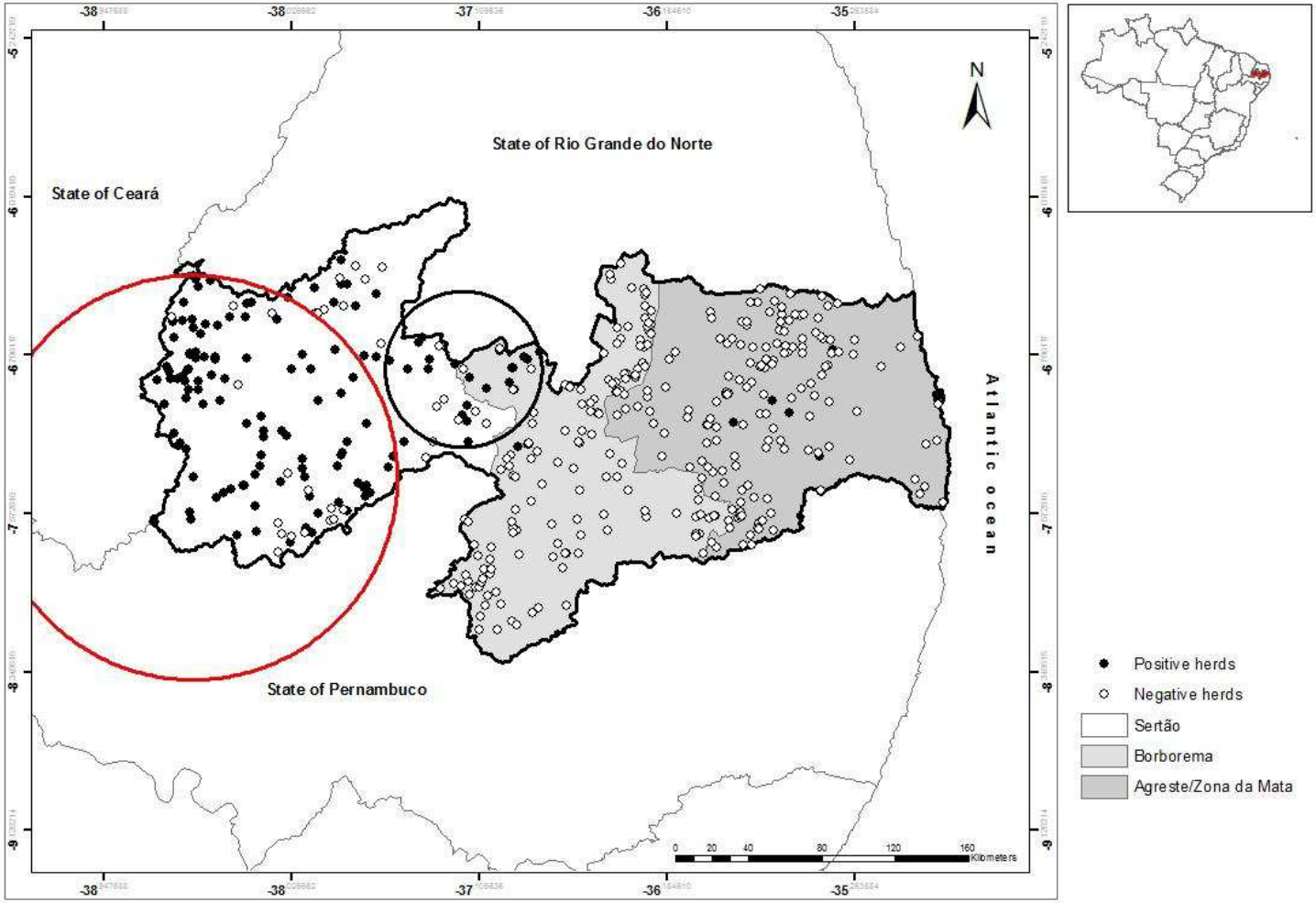
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Fig. 2

CAPÍTULO II

REVISÃO DE LITERARURA:

UMA ATUALIZAÇÃO SOBRE A ESTOMATITE VESICULAR NO BRASIL

Artigo submetido ao periódico Semina: Ciências Agrárias
(JCR 0.309, Qualis B1)

REVISÃO DE LITERATURA:
UMA ATUALIZAÇÃO SOBRE A ESTOMATITE VESICULAR NO BRASIL

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RESUMO

A estomatite vesicular (VS) é uma doença infecciosa viral que afeta animais ungulados e biungulados, sendo os equinos, bovinos e suínos os mais acometidos. Devido à semelhança clínica com a Febre Aftosa (FMD), além da diminuição na produção de leite e carne causada pela doença, a mesma possui impacto socioeconômico significativo. Este trabalho tem como objetivo fornecer informações sobre a VS, principalmente no que diz respeito à situação da infecção no Brasil.

Palavras-chave: Estomatite vesicular, revisão.

ABSTRACT

Vesicular stomatitis (VS) is an infectious viral disease that affects ungulate and biungulate animals, with horses, cattle and pigs being the most affected. Due to the clinical similarity with foot-and-mouth disease (FMD), in addition to the decrease in milk and meat production caused by the disease, it has a significant socioeconomic impact. This work aims to provide information about VS, especially regarding the infection situation in Brazil.

Keywords: Vesicular stomatitis, review.

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1. Introdução

A estomatite vesicular (VS) é uma doença infecciosa que afeta animais ungulados e biungulados, sendo os equinos, bovinos e suínos os mais acometidos, além de animais silvestres e o homem (Rodríguez, 2002). Embora apresente baixos níveis de morbidade e mortalidade, a ocorrência da VS tem impacto econômico direto na produção animal, devido à semelhança clínica com a Febre Aftosa (FMD), além da diminuição na produção de leite e carne (Perez et al., 2010). A VS foi anteriormente categorizada como uma doença pertencente à lista "A" da World Organisation for Animal Health, com requisitos obrigatórios de relatórios internacionais e restrições comerciais severas, porém em função da morbidade e mortalidade não serem significativas foi retirada da lista de doenças da OIE (Brasil 2014).

A manutenção e o modo de transmissão do vírus da estomatite vesicular (VSV) não estão totalmente esclarecidos, porém alguns estudos mostraram maior distribuição dos surtos após o período das chuvas em locais de vegetação exuberante, o que sugere maior adaptação do vírus em climas de maior umidade (López Inzaurralde et al., 1997; Brasil, 2012).

Os principais achados clínicos da doença são lesões vesiculares e ulcerações nos lábios, língua, mucosa oral, narinas, tetos e bordas coronárias dos cascos, levando à redução do consumo de alimentos e de água pelos animais, o que acarreta perda de peso e diminuição na produtividade. As lesões dos tetos são observadas em aproximadamente 2-10% dos animais afetados e podem ocasionar mastite com perda total ou parcial da função mamária (Reis Jr et al., 2009).

Devido à semelhança clínica com a FMD, o comércio e o trânsito de animais são restringidos em áreas com suspeita de VS até que ocorra a confirmação do diagnóstico laboratorial definitivo, que pode ser realizado por ELISA e PCR (De Stefano et al. 2003, Fernández et al. 2008, Perez et al. 2010).

Este trabalho tem como objetivo fornecer informações sobre a VS, provendo uma atualização da situação da infecção no Brasil de acordo com relatos de doença, bem como inquéritos sorológicos conduzidos em várias regiões do país.

2. Etiologia

O vírus da estomatite vesicular (VSV) pertence à ordem *Mononegavirales,* família *Rhabdoviridae,* gênero *Vesiculovirus*. Possui como genoma uma molécula de RNA linear de sentido negativo com pelo menos cinco genes na ordem '3-N-P-M-G-L-5'. Semelhante a outros rhabdovírus, o VSV possui o formato de projétil, variando entre 100 a 430 nm de extensão por 40 a 100 nm de diâmetro (Rodríguez et al., 2017). O nucleocapsídeo possui simetria helicoidal e é circundado por uma camada lipoproteica de onde partem projeções de 5 a 10 nm que constituem a glicoproteína viral (Fauquet e Fargette, 2005). Através desta região o vírus interage com as células susceptíveis, estando envolvida na neutralização viral e diferenciação de sorotipos do VSV (De Stefano et al., 2002). São reconhecidos dois sorotipos imunologicamente distintos do VSV: New Jersey (VSNJV) e Indiana (VSIV). O VSIV por sua vez possui três subtipos, com base nas relações sorológicas: VSIV-1, também conhecido como vírus IND clássico; VSIV-2 ou vírus Cocal (COCV), originalmente isolado a partir de ácaros de roedores em Trindade em 1961 (Jonkers et al., 1964); e VSIV-3 ou vírus de Alagoas (VSAV), com o primeiro isolamento no estado de Alagoas, Brasil (Andrade et al., 1980). De acordo com o International Committee on Taxomony of Viruses (ICTV, 2014), há pelo menos 20 sorotipos adicionais a serem caracterizados.

3. Aspectos Epidemiológicos

A primeira descrição do agente etiológico da VS ocorreu em 1926, no estado de Indiana, EUA, recebendo o nome de vírus da estomatite vesicular de Indiana (VSIV) (Oltsky et al., 1926). Um ano depois, um agente sorologicamente relacionado ao VSIV foi isolado de bovinos em Nova Jersey, sendo denominado vírus da estomatite vesicular de Nova Jersey (VSNJV) (Cotton, 1927).

A doença é limitada às Américas, no entanto, já foram descritos casos de doenças vesiculares na França (1915 e 1917) e na África do Sul (1886 e 1897) (Hanson, 1952). A infecção pelo VSV é endêmica no Norte da América do Sul (Peru, Colômbia, Equador, Venezuela), América Central e Sul do México, havendo casos esporádicos na Bolívia, Norte do México e Sudoeste dos Estados Unidos, onde 80% dos casos estão relacionados ao VSNJV e

esporadicamente ao VSIV-1 (Federer et al., 1967; Alonso Fernandez e Sondahl, 1985; Rodríguez, 2002). No Brasil, esses dois vírus não foram detectados, sendo os surtos causados por vírus relacionados sorologicamente ao VSIV-2 e VSIV-3 (Pauzesk et al., 2011; Panaftosa, 2017; Rodríguez et al., 2017).

A infecção pelo VSV pode afetar bovinos, equídeos, suínos, além de insetos, pássaros e mamíferos silvestres. Os ovinos e caprinos são mais resistentes à infecção pelo VSV, sendo raramente afetados (Rodríguez, 2002). Pesquisas sorológicas demostraram a presença de anticorpos contra o vírus em animais silvestres como morcegos, veados-da-cauda-branca, suínos selvagens, roedores, porco-espinho e várias espécies de primatas não humanos (Hanson et al., 1968; Tesh et al., 1969; Yuill, 1981; Stallknecht et al., 1985; Stallknecht e Ericson, 1986; Hayek et al., 1998).

O modo pelo qual o vírus é mantido no ambiente durante os surtos e a forma de transmissão não está totalmente esclarecido, no entanto sabe-se que, assim como em outros arbovírus, os insetos fazem parte do ciclo de vida do VSV, havendo relatos do isolamento viral em artrópodes, tanto em condições naturais quanto experimentais (Reis Jr. et al., 2009).

Shelokov et al. (1967) relataram o primeiro isolamento do VSIV a partir de mosquitos-palha (Diptera: *Psychodidae*) em áreas de ocorrência da infecção em animais domésticos no Panamá. Durante um surto da VS em bovinos e equinos no México, em 1965, o VSIV foi isolado pela primeira vez em mosquitos *Aedes sp.*, surgindo a hipótese dos insetos como vetores da VS (Sudia et al., 1967).

Em condições experimentais, Mead et al., (1997; 1999; 2004) descreveram o isolamento do VSNJV em moscas-pretas (Diptera: *Simuliidea*), observando o desenvolvimento da doença clínica em suínos e roedores, quando picados por moscas contaminadas. Os insetos não hematófagos podem participar do ciclo de vida do VSV, uma vez que há relatos do seu isolamento em moscas domésticas, durante surtos da doença no Colorado, em 1982 (Walton et al., 1987).

O ciclo de vida dos arbovírus está relacionado à presença de animais reservatórios capazes de manter níveis de viremia, de forma que quando os vetores se alimentam desses animais virêmicos, tornam-se infectados, completando o ciclo de vida dos arbovírus (Beaty et al., 1996), no entanto, essa manutenção dos níveis de viremia nos animais infectados pelo VSV não é observada (Howerth et al., 1997). A transmissão do VSV durante os surtos, na ausência de hospedeiros mamíferos virêmicos, pode ser justificado pelos resultados obtidos por Mead et

al. (2000), que demonstraram a transmissão do VSV entre moscas-pretas infectadas e não infectadas ao se alimentarem em um mesmo animal não-virêmico.

Animais silvestres frequentemente possuem anticorpos neutralizantes contra o VSV em áreas endêmicas, porém o papel desses animais no ciclo natural do vírus ainda não foi esclarecido. Tesh et al. (1970) estudaram os efeitos da inoculação do VSV em vertebrados silvestres, observando que o vírus rapidamente desaparecia da circulação sanguínea, sugerindo que a fonte natural de VSV poderia não ser hospedeiros vertebrados e sim as plantas. A disseminação do VSV, por sua vez, ocorreria através do contato do vírus no ambiente com lesões da mucosa oral dos animais susceptíveis; os insetos atuariam na disseminação do vírus para outras plantas e animais (De Stefano et al., 2002). Esta hipótese justificaria a distribuição espacial irregular da infecção pelo VSV, pois frequentemente não são observados casos adjacentes às propriedades afetadas (De Stefano et al., 2002; Acha e Szyfres, 2003).

Outras formas de transmissão em condições experimentais, como por via intranasal, intradérmica, intravenosa, escarificação da pele ou mucosa, contato direto entre animais, além da transmissão mecânica e biológica por insetos, têm apresentando resultados positivos (Howerth et al., 1997; Stallknecht et al., 2001; Mead et al., 2004; Scherer et al., 2007).

Em áreas endêmicas a infecção pelo VSV ocorre com intervalos entre os surtos inferiores a um ano, frequentemente associados as transições dos períodos chuvosos e secos (Hanson, 1981). Nas áreas não-endêmicas os surtos da doença ocorrem em ciclos de um a dois anos com intervalos de oito a dez anos (Rodríguez et al., 2017).

Embora a VS se apresente com baixos níveis de morbidade e mortalidade, a infecção tem impacto econômico direto na produção animal. Devido à semelhança clínica coma Febre Aftosa (FMD), o comércio e trânsito de animais são restringidos em áreas com suspeita de VS, até que haja confirmação do diagnóstico laboratorial definitivo, que é feito por ELISA e PCR (De Stefano et al. 2003, Fernández et al. 2008, Perez et al. 2010).

4. Patogenia e sinais clínicos

Uma importante característica do VSV é o tempo de sobrevivência relativamente alto fora dos hospedeiros, tanto em suspensão como em superfícies secas. Zimmer et al. (2013) avaliaram a estabilidade e inativação do VSV, observando que o vírus é sensível a altas temperaturas, permanecendo mais de 28 dias à 4°C em condições de laboratório; o VSV possui

alta estabilidade em suspensão, podendo facilitar a sua disseminação no rebanho através da água contaminada (Thurmond et al., 1987). Vale salientar que a transmissão por águas já foi comprovada e que o compartilhamento de fontes de água pode ser um fator de risco à infecção pelo VSV (Kotait, 1990; Zimmer et al., 2013). Uma pesquisa sorológica de anticorpos neutralizantes contra o VSIV-3 em bovinos no estado da Paraíba identificou o compartilhamento de fontes de água como fator de risco à presença de animais soropositivos (Bezerra, 2018).

O período de incubação do VSV nos bovinos, equinos e suínos varia de dois a quatro dias e a infecção é caracterizada por lesões vesiculares na boca (língua, lábios, gengivas), tetos e epitélio da banda coronária dos cascos. Outros sinais clínicos como depressão, febre, laminite e salivação excessiva são frequentemente observadas antes da formação das vesículas. Estudos epidemiológicos têm demonstrado que o estado fisiológico (ex. gestação, lactação, idade) pode influenciar o desenvolvimento de sinais clínicos. Na maioria dos casos, a doença é autolimitante e o seu curso clínico dura cerca de duas a três semanas (Reis Jr et al., 2009).

Os bovinos e equinos raramente apresentam lesões em mais de um local, enquanto os suínos frequentemente desenvolvem vesículas em vários sítios (Rodríguez et al., 2017). Em um surto ocorrido no Colorado, EUA, dos 2400 bovinos estudados, 378 apresentaram sinais clínicos da infecção pelo VSV, com presença de lesões somente na região oral em 69.3% dos casos; lesões somente nos tetos em 23% dos animais; lesões orais e nos tetos em 5,8% dos bovinos e 1,9% dos animais avaliados apresentaram lesões apenas nos cascos (Alderink, 1984). Geralmente 10-15 % dos animais apresentam sinais clínicos, ocorrendo principalmente em animais adultos (Francy et al., 1988; Hayek etal., 1998; OIE, 2015).

Trabalhos com o VSNJV demostraram a variação dos sinais clínicos de acordo com a via de inoculação, onde as lesões vesiculares foram observadas nas aplicações intradérmicas na banda coronária dos cascos, na cavidade oral e nasal (Howerth et al., 1997; Clarke et al., 1996; Howerth et al., 2006; Scherer et al., 2007), no entanto a transmissão por escarificação nasal e de pele, picadas de insetos, via intravenosa e intradérmica auricular, não resultaram em formação de vesiculas, ocorrendo apenas a soroconversão dos animais (Howerth et al., 1997; Perez e Tabachnick, 2006; Scherer et al., 2007).

As glândulas salivares dos insetos contêm substâncias que regulam negativamente a resposta imune do hospedeiro, além de potencializar a multiplicação viral em cultivos celulares e em camundongos inoculados (Osorio et al., 1996; Edwards et al., 1998; Limesand et al.,

2000; 2003; Schneider et al., 2006; Schneider e Higgs, 2008). Em um experimento realizado por Reis Jr et al. (2008), foi avaliado as alterações histopatológicas causadas pelo VSNJ através da escarificação e picada de moscas-pretas, ambos localizados nas bandas coronárias dos cascos, observando maior número de células positivas no sítio de inoculação dos animais picados por insetos.

5. A estomatite vesicular no homem

A VS no homem é por vezes despercebida devido à sintomatologia semelhante à gripe. O período de incubação é de 48 horas e os principais sintomas são dores musculares, especialmente nas pernas e globo ocular, dores de cabeça, náuseas, vômitos e faringite (Chaverri, 1970, Quiroz et al., 1988).

A ocorrência natural da infecção nos humanos é mais observada em áreas endêmicas, onde há a proliferação de insetos (Shelokov e Peralta, 1967), havendo também relatos de casos por exposição ao vírus em laboratório. Três pesquisadores da Universidade de Winscosin, Estados Unidos, que apresentavam febre e dores musculares, foram soropositivos ao VSNJV, no entanto não houve o isolamento do vírus (Hanson et al., 1950). Alguns anos depois, em Greenport, EUA, o VSNJV foi isolado pela primeira vez a partir de uma amostra de sangue de um pesquisador, sendo este o primeiro relato de viremia de VSV no homem (Fellowes et al., 1995). No diagnóstico sorológico realizado em Beltsville, EUA, observou-se que dos 54 casos soropositivos ao VSV, 31 (57.4%) apresentaram sinais clínicos e em 16 (29.6%) não foram observados sintomas característicos da doença (Patterson et al., 1958).

Em um surto da VS no Colorado, Estados Unidos, as amostras de soro colhidas de veterinários responsáveis pelo controle da infecção, apresentaram 12,8% de soropositividade na população exposta e de 5,8% nos não expostos (Reif et al., 1987).

Quiroz et al. (1988) descreveram no Panamá um caso de um menino de três anos de idade que apresentava febre, tremores, vômitos e um ataque clônico-tônico generalizado, com duração de 3-5 minutos. Foi isolado o VSIV a partir do raspado da garganta, além da detecção de anticorpos neutralizantes, sendo este o primeiro caso de encefalite associado com infecção pelo VSIV em humanos.

6. Prevenção e controle

Nas áreas de ocorrência da VSV as medidas profiláticas incluem o controle de insetos, limpeza e desinfecção dos recipientes de alimentos e água, equipamentos de ordenha e utensílios que podem veicular o vírus entre os animais. Como a escarificação da pele parece ter influência na penetração do vírus, pastagens altas e feno grosseiro devem ser evitados (Rodríguez et al., 2017).

Uma vez que o compartilhamento de fontes de água e o aluguel de pastagens podem atuar como fator de risco a ocorrência da VS no rebanho (Bezerra, 2018), é necessário desestimular essas práticas, uma vez que elas permitem o contato indireto de animais infectados com animais susceptíveis, além de facilitar a entrada do VSV em rebanhos livres da infecção.

Vacinas inativadas contendo os sorotipos VSNJV e VSIV-1 têm sido utilizadas na América Central e do Sul. Apesar da eficácia dessas vacinas não ter sido avaliada, as vacinas bivalentes, contendo adjuvante oleoso, aplicadas a cada seis meses, têm reduzido significativamente a incidência da doença (Rodríguez et al., 2017)

7. Diagnóstico

Por fazer parte do complexo das doenças de diagnóstico diferencial da febre aftosa, o diagnóstico da estomatite vesicular deve ser feito imediatamente à notificação. Os métodos de diagnóstico utilizados incluem o isolamento viral, a detecção de antígenos por ELISA, fixação de complemento, RT-PCR (transcrição reversa e reação da polimerase em cadeia) e RT-PCR em tempo real. Além desses, a detecção de anticorpos por soroneutralização (SN) e determinação de IgM por ELISA são também utilizados. Amostras de epitélio e fluido vesicular são as indicadas para o diagnóstico. Alternativamente, quando as lesões vesiculares estão ulceradas ou erosivas, pode-se coletar suabes. O meio de transporte deve conter pH neutro, enviando-se as amostras em gelo, evitando-se congelá-las (OIE, 2015; Rodríguez et al., 2017).

O ensaio imunoenzimático sanduíche indireto (IS-ELISA) é atualmente o método de diagnóstico de escolha para a identificação de sorotipos virais da VS e outras doenças vesiculares. O IS-ELISA é capaz de diferenciar todas as cepas do VSIV e o sorotipo do VSNJV, conforme a adequação da técnica. Por possuir maior sensibilidade, o IS-ELISA é

preferível quando comparado à Fixação de Complemento (FC), no entanto quando os reagentes não estão disponíveis, a FC pode ser realizada (Alonso et al., 1991).

A RT-PCR pode ser utilizada para amplificar pequenas áreas genômicas do VSV (Wilson et al., 2009). Esta técnica detecta a presença de RNA do vírus em amostras de tecido vesicular e cultura celular, mas não pode determinar se o vírus é infeccioso. Em geral, as técnicas de PCR não são rotineiramente utilizadas para triagem de casos de diagnóstico do VS (OIE, 2015), uma vez que envolve técnicas laboriosas, tonando-se inviável a sua realização em grande número de amostras.

A detecção de anticorpos é o meio de diagnóstico de escolha para realização da triagem dos casos de VS. Os anticorpos geralmente podem ser detectados entre cinco e oito dias após a infecção (Katz et al., 1997), podendo persistir por oito anos, com flutuação de até mil vezes dentro de um mês (De Stefano et al., 2002).

O ensaio imunoenzimático de bloqueio de fase líquida (LP-ELISA) pode ser utilizado para detecção e quantificação de anticorpos de diferentes sorogrupos do VSV e possui maior especificidade quando comparada a vírus neutralização (VN), que tem como princípio a detecção de anticorpos neutralizantes contra o sorogrupo do VSV testado (Allende et al., 1992).

8. Estomatite Vesicular no Brasil

8.1 Diagnóstico de surtos

No Brasil, o primeiro caso de VS foi registrado em equinos no estado de Alagoas em 1964, sendo esta amostra classificada como VSIV-3, devido a diferenças nos sorogrupos VSIV-1 e VSIV-2 (Andrade et al., 1980). Dois anos depois, também no estado de Alagoas, o serviço veterinário brasileiro registrou um surto da doença em muares com isolamento do VSIV-3 (Brasil, 1988). Até o momento não há relato do isolamento do VSIV-1 e VSNJV no Brasil, sendo estes considerados exóticos (Brasil, 2012; OIE, 2017; Panaftosa, 2017).

Pustiglione Netto et al. (1967) descreveram o isolamento do VSIV-2 no Brasil, a partir de amostras de epitélio de equinos doentes, no município de Rancharia, São Paulo. Em 1979, no município de Ribeirão Preto, São Paulo, Arita e Arita (1983), isolaram o mesmo subtipo do VSV em equinos. Em Minas Gerais, Rocha Araújo et al. (1977) relataram o primeiro isolamento do VSIV-3 em bovinos. Em 1984 no estado de Sergipe, Alonso Fernandez

e Sondahl (1985) isolaram o VSIV-3 de equinos e no mesmo ano Arita et al. (1985) descreveram o isolamento do VSIV-3 em bovinos doentes. Pituco et al. (1989), após a ocorrência de um surto de VS em bovinos e equinos, isolaram o VSIV-3 em bovinos, na região do Vale do Paraíba, São Paulo. Clementino et al. (2014) relataram o primeiro surto de VS no estado da Paraíba, onde dos 82 bovinos provenientes das propriedades focos, 43 (52.44%) apresentavam sinais clínicos sugestivos à VS, com identificação do VSIV-3. No mesmo ano Cargnelutti et al. (2014) descreveram um surto da doença em 14 equinos e seis bovinos nos estados da Paraíba e Rio Grande do Norte, com isolamento do vírus relacionado ao VSIV-3.

De acordo com o trabalho realizado por López Inzaurralde et al. (1997), o qual avaliou os resultados laboratoriais para VS realizados pelo Centro Panamericano de Febre Aftosa, entre os anos de 1964-1996, os subtipos VSIV-2 e VSIV-3, apresentam importância epidemiológica no Brasil, com identificação do VSIV-2 apenas nos estados de São Paulo e Rio Grande do Sul em dois episódios com intervalo de 10 anos entre eles. Já o VSIV-3 apresentou circulação ativa, com isolamento nos estados de Minas Gerais, São Paulo, Alagoas, Ceará, Goiás e Rio de Janeiro, sendo identificadas duas áreas onde a infecção assume caráter endêmico: em seis mesorregiões do Estado do Ceará e na mesorregião Norte do estado de Minas Gerais.

Segundo dados do serviço veterinário brasileiro no período de 1997-2011, 164 focos de VS estavam relacionados ao VSIV-3 e 219 focos ao VSIV-2, esse últimos limitados aos anos de 1998 e 1999, nos estados do Paraná e Santa Catarina (Brasil, 2012). O VSIV-3 manteve a sua ocorrência endêmica no Brasil ao longo dos anos e, considerando o período de 2007-2011, apresentou maior número de casos na Bahia, Minas Gerais, Ceará e Rio Grande do Norte. A tendência de maior ocorrência dos surtos do VSV compreendeu o período de maio a junho, onde 67.7% envolviam apenas bovinos, 14% apenas equídeos e 1.8% envolviam bovinos e equídeos (Brasil, 2012).

8.2 Inquéritos sorológicos

No Brasil, sorologia positiva para o VSV foi detectada em vários estados em diferentes espécies de animais. Allende e Germano (1993) ao comparar dois testes sorológicos para detecção de anticorpos contra o VSIV-3 analisaram 305 soros de bovinos, equinos e suínos, detectando 300 (98,40%) amostras positivas na técnica de soroneutralização viral (SN).

Na região de Araçatuba, 28 (2.6%) dos 1.099 bovinos amostrados apresentaram anticorpos contra o VSIV-1 (De Stefano et al., 2003). Kotait (1990) realizou uma pesquisa de anticorpos contra o VSIV-3 em amostras de soros de 2.181 bovinos do Vale da Paraíba, encontrando 36 (1.64%) com sorologia positiva.

Inquéritos sorológicos realizados na população equina apresentaram altas prevalências de anticorpos contra o VSIV-3 na região Nordeste do Brasil. Dados da investigação conduzida pelo Ministério da Agricultura, Pecuária e Abastecimento (MAPA), utilizando 6.517 amostras de equinos provenientes de oito estados da região Nordeste, além do estado do Espírito Santo e Norte do estado de Minas Gerais, apresentaram maiores prevalências de equinos soropositivos nos estados do Piauí (86.2%), Pernambuco (51.7%), Rio Grande do Norte (50.4%), Paraíba (42.4%) e Maranhão (42.2%) (Brasil, 2012). Resultados semelhantes foram observados no inquérito sorológico do VSIV-3 em equinos das regiões Sul, Centro-oeste e Nordeste, observando-se maior soropositividade na Região Nordeste: 87,3% no Ceará, 65,7% no Rio Grande do Norte e 45,4% na Paraíba (Lunkes et al., 2016).

No estado da Paraíba foi realizado um inquérito sorológico de anticorpos contra o VSIV-3 em bovinos, onde dos 2.279 animais testados, 491 (26.2%) foram soropositivos, sendo a mesorregião do Sertão a que apresentou maior prevalência de rebanhos (80.6%) e de animais (48.2%) positivos (Bezerra, 2018).

9. Considerações finais

Por ser diagnóstico diferencial da febre aftosa, a estomatite vesicular gera grandes percas na comercialização de animais e seus subprodutos, no entanto existem aspectos epidemiológicos da infecção que ainda não foram elucidados, sendo necessário mais pesquisas voltadas a caracterizar a epidemiologia da VS. No Brasil o VSIV-3 possui circulação endêmica, em várias regiões com maiores casos localizados na região Nordeste. Até o presente momento não foi encontrado casos relacionados ao VSIV-1 e VSNJV, sendo estes considerados exóticos no país.

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CONCLUSÃO GERAL

Devido à semelhança da estomatite vesicular com a febre aftosa, é importante a condução de estudos epidemiológicos para verificar a circulação do VSV na população bovina e com base em amostragem planejada. De fato, este trabalho é o primeiro no Brasil a determinar a situação epidemiológica da estomatite vesicular, em nível estadual, utilizando amostragem planejada de propriedades rurais e de animais, e os resultados obtidos indicam alta circulação do VSV na população bovina do estado da Paraíba, semiárido do Brasil, principalmente na mesorregião do Sertão, que faz fronteira com os estados do Ceará, Rio Grande do Norte e Pernambuco, na qual foram observadas as maiores prevalências de propriedades e de animais, bem como foram identificados aglomerados de propriedades positivas. Com base na análise de fatores de risco, sugere-se o desencorajamento das práticas de aluguel de pastagens e do compartilhamento de fontes de água devido à possibilidade do contato do VSIV presente no ambiente contaminado com animais suscetíveis.

ANEXO I

Preventive Medicine Veterinary – Diretrizes

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Preventive Veterinary Medicine's Editors and reviewers use several published guidelines for reporting standards; the websites are listed in the Appendix to this Guide for Authors. Conformation to these reporting standards allows our Editors and reviewers to judge the quality and originality of your work; conformation also offers readers sufficient information to judge the relevance of your study to the readers' own situations. Omission of substantive items from relevant guidelines for reporting standards is sufficient reason to reject your manuscript.

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• Provide captions to illustrations separately.
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A detailed guide on electronic artworkis available.
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Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):EPS (or PDF): Vector drawings, embed all used fonts.
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Please do not:
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• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.
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Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.
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Please submit tables as editable text and not as images. Tables can be placed either next to there levant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

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Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or' Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

In the text refer to the author's name (without initial) and year of publication, followed – if necessary– by a short reference to appropriate pages. Examples: "Since Peterson (1988) has shown that..."

"This is in agreement with results obtained later (Kramer, 1989, pp.12–16)".

If reference is made in the text to a publication written by more than two authors the name of the first author should be used followed by "et al.". This indication, however, should never be used in the list of references. In this list names of first author and co-authors should be mentioned. References cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically on authors' names, and chronologically per author. If an author's name in the list is also mentioned with co-authors the following order should be used: publications of the single author, arranged according publication dates – publications of the same author with one co-author – publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as1974a, 1974b, etc.

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Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

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One author has been designated as the corresponding author with contact details:
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• Full postal address
• Phone numbers
All necessary contents of the manuscript text have been uploaded, and contain:
• Keywords

• All figure captions
• All tables (including title, description, footnotes)
Further considerations
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Appendix
Authors: These minimum items of information are needed by our referees and Editors to evaluate your manuscript. Additional information may be appropriate, depending on your study design and objectives.
Excellent guidelines for standardizing and strengthening the reporting of biomedical research are available from the CONSORT, MOOSE, PRISMA, REFLECT, STARD, and STROBE statements. Westrongly urge you to consult these guidelines before submitting papers to Preventive Veterinary Medicine. The guidelines are freely available (with considerable elaborations and explanations) at the following websites:
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http://jama.ama-assn.org/cgi/reprint/283/15/2008 (for MOOSE: Meta-analysis of Observational Studies in Epidemiology: A Proposal for Reporting, Donna F. Stroup et al.; published in J Am Med Assoc 2000; 283:2008-2012)
http://prisma-statement.org (for meta-analyses and systematic reviews)
http://reflect-statement.org (for clinical trials in livestock)
http://www.stard-statement.org (for evaluations of diagnostic tests)
http://www.strobe-statement.org (for observational studies; there is an elaboration for studies of genetic associations)

1. For **ALL descriptive and comparative studies:**
a. **Source** of subjects
b. **Eligibility** criteria
c. **Sample-size justification** appropriate for the study design and primary hypothesis. This should include details of adjustment for clustering (including the levels of **clustering**, the assumed cluster size, and either the **design effect** or the **intra cluster correlation**) if clustering was present.
d. Methods by which the data were acquired
e. Diagnostic **sensitivity and specificity** of any tests used. (Analytic sensitivity and reproducibility might be appropriate alternatives for some studies.) Correction to the **true prevalence** is expected for e.g., seroprevalence studies.

f. Descriptions of the observed data (including measures of subject-level variation), stratified on the outcome implied by the primary hypothesis. These descriptions should include time, place, "demographics," and relevant management and health information.
g. Declaration of the **experimental unit**
h. Descriptions of the **formal random mechanism** (e.g., lottery or table of random numbers) and the list frame (enumerating every eligible subject and/or cluster) used at any step claimed to be "random"
i. Descriptions of the **pilot, repeatability, and validation testing of any questionnaire** used to acquire data for the study. Also needed are: the language of the survey instrument, the time it took to complete, how it was administered, the types of questions (e.g., closed, semi-closed, open), and the training of any persons administering the survey. Making a copy available to the review process is desirable (in English as well as the language of administration).

2. For **comparative studies** (including **both observational and intervention** studies):
a. Numerical descriptions of **all tested risk factors** or pre-intervention characteristics of the subjects, **stratified** on the primary hypothesis/outcome of the study
b. Descriptions of how **blindness** was accomplished for all subjective evaluations

3. For **randomized controlled trials and other intervention studies:**
a. **Approval** by your institution's **animal-welfare committee** and description of measures taken for rescue analgesia or rescue euthanasia.
b. Methods by which the owners of the animals gave **informed consent** for their animals to be in the trial
c. Methods used for **allocation concealment** after the animals were determined to be eligible for random assignment to the various experimental or control groups
d. **Description and justification of the "control" group's "treatment"** (e.g., standard therapy, placebo to mimic the delivery system in the absence of a standard therapy, or "do nothing" to mimic both the treatment and its delivery)
e. Methods used for **active monitoring for adverse effects** ("harms")

4. For **simulation studies and risk assessments:**
a. Distinction between deterministic and stochastic processes
b. Descriptions of (and justifications for) all choices of **distributions and their parameter Values**
c. Description of numbers, training, experience, and representativeness of any **"experts"** used to provide opinions
d. Declaration of the **stakeholders** for any risk assessment
e. Distinction between assumptions, input data, calculations from intermediate steps in the modeling process, and model predictions
f. Descriptions of the assumed chance variation and assumed knowledge uncertainty in the inputs, and methods used to deal with those sources of total uncertainty
g. **Sensitivity analyses** of key assumptions and of the input variables that had the greatest uncertainty
h. Descriptions of the **variability in the "outputs"** from stochastic models

5. For **statistical-hypothesis tests:**
a. Declarations of the unit of statistical analysis and of the dependent ("outcome") variable

b. **Alpha** and **tails**, and any methods used to adjust for multiple comparisons (to protect experiment wise alpha from the problem of **multiplicity**)
c. **Methods used to adjust for clustering within the data**
d. Methods used to determine that the **statistical assumptions were met** (e.g., that the data were
Gaussian or that the odds ratio or hazards ratio was constant across the observed range of the risk factor)
e. Methods used to look for **collinearities** or other interrelationships among the risk factors being tested
f. Methods used to select or to retain risk factors within multivariable models (including the **test criterion**)
g. Clear declaration of any variables "forced into" the model (not allowed to drop out; this implies a need to account for that factor) or offered to the model on a priori grounds despite any screening results (this implies that the factor was part of a major hypothesis)
h. Description of the **goodness-of-fit** of any models
i. How **missing data** were handled

AFTER ACCEPTANCE

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Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor.
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ANEXO II

Semina: ciências agrárias – Diretrizes

Normas editoriais para publicação na Semina: ciências agrárias

A revista Semina: Ciências Agrárias, com periodicidade trimestral, é uma publicação de divulgação científica do Centro de Ciências Agrárias da Universidade Estadual de Londrina. Tem como objetivo publicar artigos, comunicações, relatos de casos e revisões relacionados às Ciências Agronômicas, Ciência e Tecnologia de Alimentos, Medicina Veterinária, Zootecnia e áreas afins**.**

Categorias dos Trabalhos

a) Artigos científicos: no máximo 25 páginas incluindo figuras, tabelas e referências bibliográficas;
b) Comunicações científicas: no máximo 12 páginas, com referências bibliográficas limitadas a 16 citações e no máximo duas tabelas ou duas figuras ou uma tabela e uma figura;
b) Relatos de casos: No máximo 10 páginas, com referências bibliográficas limitadas a 12 citações e no máximo duas tabelas ou duas figuras ou uma tabela e uma figura;
c) Artigos de revisão: no máximo 35 páginas incluindo figuras, tabelas e referências bibliográficas.

Apresentação dos Trabalhos

Os originais completos dos artigos, comunicações, relatos de casos e revisões podem ser escritos em português, inglês ou espanhol e devem ser enviados em três cópias impressas em papel A4, com espaçamento duplo, elaborado no editor de texto Word for Windows, fonte Times New Roman, tamanho 12 normal, com margens esquerda e direita de 2,5 cm e superior e inferior de 2 cm, respeitando-se o número de páginas, devidamente numeradas, de acordo com a categoria do trabalho. Figuras (desenhos, gráficos e fotografias) e tabelas serão numeradas em algarismos arábicos e devem estar separadas no final do trabalho. As figuras e tabelas deverão ser apresentadas nas larguras de 8 ou 16 cm com altura máxima de 22 cm, lembrando que se houver a necessidade de dimensões maiores, no processo de editoração haverá redução para as referidas dimensões. As legendas das figuras deverão ser colocadas em folha separada obedecendo à ordem numérica de citação no texto. Fotografias devem ser identificadas no verso e desenhos e gráfico na parte frontal inferior pelos seus respectivos números do texto e nome do primeiro autor. Quando necessário deve ser indicado qual é a parte superior da figura para o seu correto posicionamento no texto.

Preparação dos manuscritos

Artigo científico:

Deve relatar resultados de pesquisa original das áreas afins, com a seguinte organização dos tópicos: Título; Título em inglês; Resumo com Palavras-chave (no máximo seis palavras); Abstract com Key-words (no máximo seis palavras); Introdução; Material e Métodos; Resultados e Discussão com as conclusões no final ou Resultados, Discussão e Conclusões separadamente; Agradecimentos; Fornecedores, quando houver e Referências Bibliográficas. Os tópicos devem ser escritos em letras maiúsculas e minúsculas e destacados em negrito, sem numeração. Quando houver a necessidade de subitens dentro dos tópicos, os mesmos devem

receber números arábicos. O trabalho submetido não pode ter sido publicado em outra revista com o mesmo conteúdo, exceto na forma de resumo de congresso, nota prévia ou formato reduzido.

Na primeira página do manuscrito devem constar as seguintes informações:
1. Título do trabalho: O título, acompanhado de sua tradução para o inglês, deve ser breve e suficientemente específico e descritivo, contendo palavras que permitam ao leitor ter uma idéia do conteúdo do artigo.
2. Nomes dos autores: Deverão ser escritos por extenso, separados por ponto e vírgula, logo abaixo do título do trabalho. A instituição, os órgãos de fomento e a identificação dos autores deverão ser feitos por inserção numérica de notas de rodapé ao final do título e dos nomes. O autor para correspondência com endereço completo, telefone, fax e E-mail deverá ser destacado com um asterisco sobrescrito junto ao seu número de identificação.
A partir da segunda página do manuscrito a apresentação do trabalho deve obedecer à seguinte ordem:
1. Título do trabalho, acompanhado de sua tradução para o inglês.
2. Resumo e Palavras-chave: Deve ser incluído um resumo informativo com um mínimo de 150 e um máximo de 300 palavras, na mesma língua que o artigo foi escrito, acompanhado de sua tradução para o inglês (Abstract e Key words).
3. Introdução: Deverá ser concisa e conter revisão estritamente necessária à introdução do tema e suporte para a metodologia e discussão.
4. Material e Métodos: Poderá ser apresentado de forma descritiva contínua ou com subitens, de forma a permitir ao leitor a compreensão e reprodução da metodologia citada com auxílio ou não de citações bibliográficas.
5. Resultados e discussão com conclusões ou Resultados, Discussão e Conclusões: De acordo com o formato escolhido, estas partes devem ser apresentadas de forma clara, com auxílio de tabelas, gráficos e figuras, de modo a não deixar dúvidas ao leitor, quanto à autenticidade dos resultados, pontos de vistas discutidos e conclusões sugeridas.
6. Agradecimentos: As pessoas, instituições e empresas que contribuíram na realização do trabalho deverão ser mencionadas no final do texto, antes do item Referências Bibliográficas.

Observações:
Quando for o caso, antes das referências, deve ser informado que o artigo foi aprovado pela comissão de bioética e foi realizado de acordo com as normas técnicas de biosegurança e ética.
Notas: Notas referentes ao corpo do artigo devem ser indicadas com um símbolo sobrescrito, imediatamente depois da frase a que diz respeito, como notas de rodapé no final da página.
Figuras: Quando indispensáveis figuras poderão ser aceitas e deverão ser assinaladas no texto pelo seu número de ordem em algarismos arábicos. Se as ilustrações enviadas já foram publicadas, mencionar a fonte e a permissão para reprodução.
Tabelas: As tabelas deverão ser acompanhadas de cabeçalho que permita compreender o significado dos dados reunidos, sem necessidade de referência ao texto.
Grandezas, unidades e símbolos: Deverá obedecer às normas nacionais correspondentes (ABNT).
7. Citações dos autores no texto: Deverá seguir o sistema de chamada alfabética escrita com letras maiúsculas seguidas do ano de publicação de acordo com os seguintes exemplos:
Os resultados de DUBEY (2001) confirmam que o.....
De acordo com SANTOS et al. (1999), o efeito do nitrogênio.....

Beloti et al. (1999b) avaliaram a qualidade microbiológica.....
.....e inibir o teste de formação de sincício (BRUCK et al., 1992).
.....comprometendo a qualidade de seus derivados (AFONSO; VIANNI, 1995).
8. Referências Bibliográficas: As referências bibliográficas, redigidas segundo a norma NBR 6023, ago. 2000, da ABNT, deverão ser listadas na ordem alfabética no final do artigo. Todos os autores participantes dos trabalhos deverão ser relacionados, independentemente do número de participantes (única exceção à norma – item 8.1.1.2). A exatidão e adequação das referências a trabalhos que tenham sido consultados e mencionados no texto do artigo, bem como opiniões, conceitos e afirmações são da inteira responsabilidade dos autores.
As outras categorias de trabalhos (Comunicação científica, Relato de caso e Revisão) deverão seguir as mesmas normas acima citadas, porem, com as seguintes orientações adicionais para cada caso:

Comunicação científica
Uma forma concisa, mas com descrição completa de uma pesquisa pontual ou em andamento (nota prévia), com documentação bibliográfica e metodologia completas, como um artigo científico regular. Deverá conter os seguintes tópicos: Título (português e inglês); Resumo com Palavras-chave; Abstract com Key-words; Corpo do trabalho sem divisão de tópicos, porém seguindo a seqüência – introdução, metodologia, resultados (podem ser incluídas tabelas e figuras), discussão, conclusão e referências bibliográficas.

Relato de caso
Descrição sucinta de casos clínicos e patológicos, achados inéditos, descrição de novas espécies e estudos de ocorrência ou incidência de pragas, microrganismos ou parasitas de interesse agronômico, zootécnico ou veterinário. Deverá conter os seguintes tópicos: Título (português e inglês); Resumo com Palavras-chave; Abstract com Key-words; Introdução com revisão da literatura; Relato do (s) caso (s), incluindo resultados, discussão e conclusão; Referências Bibliográficas.

Artigo de revisão bibliográfica
Deve envolver temas relevantes dentro do escopo da revista. O número de artigos de revisão por fascículo é limitado e os colaboradores poderão ser convidados a apresentar artigos de interesse da revista. No caso de envio espontâneo do autor (es), é necessária a inclusão de resultados próprios ou do grupo envolvido no artigo, com referências bibliográficas, demonstrando experiência e conhecimento sobre o tema.
O artigo de revisão deverá conter os seguintes tópicos: Título (português e inglês); Resumo com Palavras-chave; Abstract com Key-words; Desenvolvimento do tema proposto (com subdivisões em tópicos ou não); Conclusão; Agradecimentos (se for o caso) e Referências Bibliográficas.

Outras informações importantes
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2. A publicação dos trabalhos depende de pareceres favoráveis da assessoria científica "Ad hoc" e da aprovação do Comitê Editorial da Semina Ciências Agrárias, UEL.
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