



Sectional study of the occurrence of Bovine Viral Diarrhea Virus in pig farms in São Paulo State

Estudo seccional da ocorrência de vírus da diarreia viral bovina em fazendas de suínos no estado de São Paulo

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Abstract: Bovine Viral Diarrhea virus (BVDV) can infect pigs and lead to production losses. Data on the epidemiology of this virus in pigs are scarce. In order to obtain occurrence data of this disease, 600 blood serum samples of pigs from five farms in the northeast of the state of São Paulo were collected. Samples were divided into four categories: Weaning (W), Nursery (N), Finishing (F) and Sows (S), and were submitted to Virus neutralization (VN) test for BVDV-1 and BVDV-2. One (1/30) positive sample to BVDV-1 was detected in Property 2, showing 3.33% prevalence in (F) and a herd level prevalence of 0.84%. Regarding BVDV-2, in Property 2, 3.33% (1/30) of samples were seroreagent, obtained in (F) and 6.66% (2/30) in (S), with the herd prevalence of 2.44%. Properties 3 and 4 presented, 3.33% (1/30) of seroreagent samples in the (S), with 0.84% at herd level, respectively. In Property 5, we found 6.66% (2/30) of seroreagent samples, both in (S), with a final prevalence of 1.66%. No risk factor was significantly associated. Despite the low prevalence, evidence of infection was found in different categories within the farms, showing that inefficiency in biosecurity can lead to infection in swine.

Keywords: BVDV-1, BVDV-2, Epidemiology, Swine, Virus neutralization.

Resumo: O vírus da Diarreia Viral Bovina (BVDV) pode infectar os suínos e acarretar em perdas produtivas, no entanto, dados sobre epidemiologia e patogenia deste vírus em suínos são escassos. Visando obter informações sobre a ocorrência desta enfermidade em rebanhos suínos, foram coletadas 600 amostras de soro sanguíneo de suínos oriundos de cinco granjas do nordeste do estado de São Paulo. As amostras foram separadas em diferentes categorias, sendo elas maternidade (Mt), creche (C), terminação (T) e matrizes (M). As mesmas foram submetidas ao teste de Virusneutralização (VN) para a pesquisa de anticorpos contra BVDV-1 e BVDV-2. Das 600 amostras avaliadas, foi detectada uma (1/30) amostra sororreagente ao genótipo 1, obtendo-se 3,33% de positividade na (T), com prevalência no rebanho de 0,81% na Propriedade 2. Para o genótipo 2, na Propriedade 2 obteve-se 3,33% (1/30) de amostra sororreagente na (T) e 6,66% (2/33) para as (M), prevalência no rebanho de 2,44%. As Propriedades 3 e 4 apresentaram 3,33% (1/30) de amostras sororreagentes na (M), com 0,84% de prevalência no rebanho. Na Propriedade 5 encontrou-se 6,66% (2/30) de amostras sororreagentes, ambas nas (M), com prevalência final de 1,66%. Apesar da baixa prevalência, a infecção ocorreu em duas diferentes categorias dentro do sistema de produção, mostrando que a ineficiência na biosseguridade pode culminar na infecção dos suínos. Nenhum fator de risco teve associação significativa.

Palavras-chave: BVDV-1, BVDV-2, suídeos, vírus neutralização.

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Introduction

The genus Pestivirus comprises three recognized species that can infect swine, classical swine fever virus (CSFV), bovine viral diarrhea (BVDV-1 and BVDV-2), border disease (BDV) (BECHER et al., 2003) and more recently, an atypical Pestivirus responsible for causing congenital tremor in piglets has been identified (ARRUDA et al., 2016).

Bovine are described as the main hosts of BVDV, and the main source of infection for pigs and other wild ruminants (RIDPATH, 2010). Transmission may also occur through the use of milk and other derivatives from infected cattle in pig feed (TERPSTRA; WENSVOORT, 1988) as well as contaminated fomites (CARBREY et al., 1976). Contrary to what was previously believed, a model of experimental infection demonstrated that transmission also occurs among pigs, but to a limited extent; thus, the virus may disappear from a population if no new animal introductions occur (WIERINGA-JELSMA et al., 2006). Deng et al. (2012) affirm that the prevalence of BVDV in pig

herds is closely linked to the prevalence of the disease in bovine herds.

Some risk factors that may be associated with the occurrence of BVDV in pigs, such as the presence of cattle on the same property, high density of small ruminants close to the swine herd, BVDV contaminated vaccines, and the age of the animals (LOEFFEN et al., 2009).

This study aimed to investigate the occurrence of antibodies against BVDV in pigs of different age groups and to try to determine associated risk factors.

Material and methods

Experimental design

For this study, five pig farms located in the region of Jaboticabal - SP were selected, presenting a commercial character with intensive cycle system. To obtain representative samples of all the properties, the animals were randomly selected, and 30 blood samples were collected at each breeding stage, which was classified as: Weaning (W), Nursery (N), Finishing (F) and Sows (S). All herds underwent clinical assessments and general facility inspection.

In addition to the serological sampling, a structured questionnaire was applied to the owners, aiming to obtain epidemiological information about the herd. The questionnaire lists the main risk factors potentially related to the possibility of BVDV infection in swine herds, such as the presence of ruminants on the farm; dairy cattle breeding; cattle, goats, sheep or pigs acquisition in the last 6 months; presence of goats, sheep and cattle within a 3 km radius of the herd; occurrence of reproductive problems (abortion, birth of weak piglets, stillbirths, mummification, return to estrus) in the pig herd in the last 6 months; occurrence of reproductive problems in cattle, goats and sheep of the property; occurrence of reproductive problems in pigs and ruminants of neighboring properties; use of ruminant milk or derived in pig feeding; contact of the staff with different species of animals; use of community boar; vaccination of pigs and supply of treated water.

Sample collection

The blood samples were collected with sterile disposable syringe, free of anticoagulants, and obtained from the puncture of the jugular vein, centrifuged at 9000 x g for 10 minutes to obtain blood serum, which was packed in duplicates in microtubes graduated of 2, 0 mL, identified

and stored in freezer -20°C until the time of the serological tests.

Virus neutralization test (VN)

Serum samples were submitted to Virus neutralization test. The sera to be tested were collected in duplicates and subjected to successive dilutions starting at 1:10 up to 1:5,120, considering positives the samples that showed total neutralization of the 100 TCID₅₀ at a dilution above 1:10, as recommended by the "Manual of Diagnostic Tests and Vaccines of Terrestrial Animals" (OIE, 2012). For the test were used bovine kidney epithelial cell lineage "Madine-Darby Bovine Kidney" (MDBK) and as standard virus the BVDV-1 (Singer strain) cytopathic (CP). The antibody titer considered for positive samples was equivalent to the reciprocal of the highest dilution in which total neutralization of the 100 TCID₅₀ occurred, evidenced by the absence of cytopathic effect in the cell culture.

No differential diagnosis was made for PSC since the region selected for the study is demonstrably free of the infection and the Ministry of Agriculture, Livestock, and Supply (MAPA) did not allow the test to be made outside the official laboratories.

Data analysis

The results obtained in the

serological test were considered essentially positive or negative, and the samples diagnosed as suspect were considered positive. For the correlation between the serological data and the presence of the investigated risk factor, the OR (Odds Ratio) and its confidence interval according to Thrusfield (2010) were calculated. The data were then submitted to univariate statistical analysis using Fisher's exact test.

Results

A total of 600 serum samples were collected, being 120 samples per property. With regard to BVDV-1, only Property 2 was positive, and a seroreagent sample was detected at the Finishing (F), which prevalence was 0.84% (1/120) (CI:

95, 0.14-4.46%). Regarding the prevalence in the class, we obtained 3.33% of positivity (1/30) (CI 95: 0.59-16.67%).

Property 2 was also the only one in which reproductive disorders occurred in ruminants and pigs in the last six months, and although other risk factors such as presence of ruminants on the same property, presence of cattle in neighboring properties and acquisition of pigs in the last 6 months were observed, there was no significant association between the data. Therefore, out of the 600 samples collected, only one animal was seropositive to BVDV-1. The results obtained for BVDV-1 in Property 2 are shown in Table I.

Table I. Data on the prevalence of BVDV-1 in Property 2.

Property	Age group	Positive samples	Total of samples	Prevalence within group (%)	CI 95% (%)	Prevalence within the herd
Property 2	Weaning	0	30	0%	0 - 11.35	
	Nursery	0	30	0%	0 - 11.35	
	Finishing	1	30	3.33%	0.59 - 16.67	0.84%
	Sows	0	30	0%	0 - 10.43	
Total		1	120		0.14 - 4.46	

Regarding BVDV-2, four properties showed seropositive animals in different classes. Only Property 1 did not

present, in any class, seroreagent sample for the referred agent.

Property 2 presented 3 seropositive animals (3/120) for the agent, being one finishing animal (F) and two sows (S), which represents 2.44% of positivity in the herd (CI: 95, 0.83-6.93%). Concerning the classes, we observed the

prevalence of 3.33% (CI: 95, 0.59-16.67%) in Finishing (F) and 6.66% (CI: 95, 1.68-19.61%) in Sows (S). Table II.

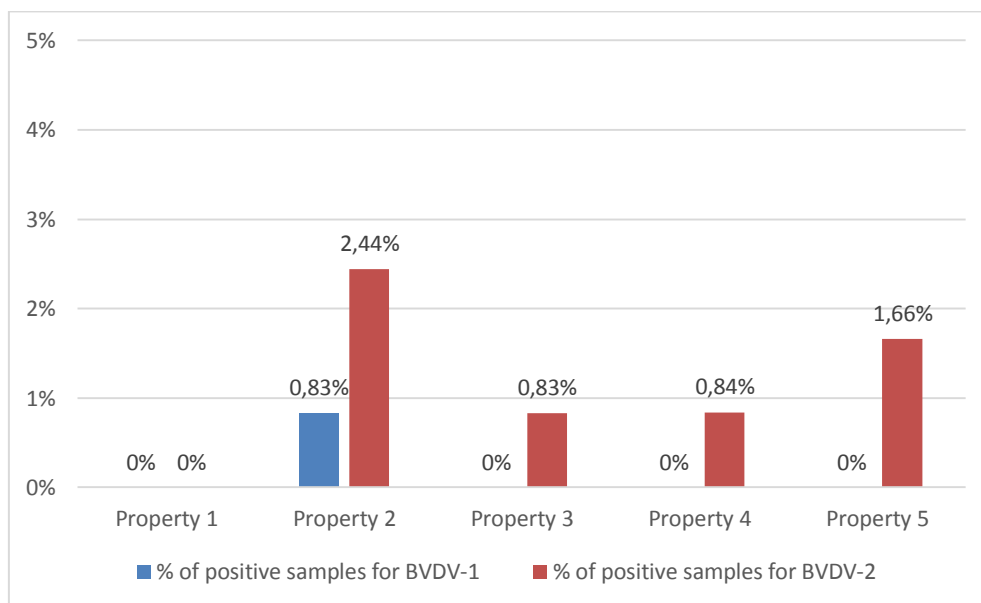
Table II. Prevalence data of Properties considered positive for BVDV-2 with their respective seroreagent classes.

Property	Age group	Positive samples	Total of samples	Prevalence within group (%)	CI 95% (%)	Prevalence within the herd
Property 2	Finishing	1	30	3.33%	0.59 - 16.67	2.44%
	Sows	2	30	6.66%	1.68 - 19.61	
Property 3	Sows	1	30	3.33%	0.59 - 16.67	0.84%
Property 4	Sows	1	30	3.33%	0.59 - 16.67	0.84%
Property 5	Sows	2	30	6.66%	1.85 - 21.32	1.66%
Total of samples		7	600		0.56 - 2.36	1.16%

Properties 3 and 4, presented, each one, a seroreagent sample, being in Property 3 the category (S), obtaining 0.84% (CI: 95, 0.15-4.57%) of positivity in the herd, and prevalence of 3.33% (CI: 95, 0.59-16.67%) for the category; and in Property 4 the class (F), representing a prevalence of 0,84% in the herd (CI: 35, 0.15-4.61%), and 3.33% (CI: 95, 0.59-16.67%) for the category.

In Property 5, two positive samples were detected in (S), which characterizes a prevalence of 0.84% (CI: 95, 0.46-5.87%) within the herd, reaching 6.66% of positivity in the class (CI: 95, 1.85-21.32%).

All the information regarding BVDV-2 positive samples within each herd is outlined in Table II. The relative and absolute prevalence of each property are shown in Graph 1.



Graph I. Prevalence of BVDV-1 and BVDV-2 by property.

Discussion

In this study, it was observed that the only animal categories that presented seroreagent samples were (F) and (S). Loeffen et al. (2009), O’Sullivan et al. (2011) and Lipowski (2014) attributed the low prevalence of BVDV in swine herds to the high specialization of swine production, in which interspecies contact was reduced with the option of a single zootechnical breeding in the property.

Loken et al. (1991) determined BVDV-1 seroprevalence (NADL strain) of 2.2%, which is the closest approximation to the prevalence found in this study of 3.33% (F), as well as two only brief reports of BVDV in pigs in Brazil, in the state of Rio Grande do Norte (4.13%) and in the state of São Paulo (2.32%), (GATTO et al., 2014a, b), different from Loeffler et al. (2009) which estimated a BVDV-1

seroprevalence of 0.42% in finishing pigs, almost eight times lower than the value found for that same category.

In Properties 2 and 5, prevalences of 6.66% were found, a value similar to that found by Holm-Jensen (1985), which showed a seroprevalence in domestic pigs of 6.4% for BVDV-1 (strain Ug59), Denmark. However, Loeffen et al. (2009) showed lower values for the category of sows at reproductive age, 2.5% prevalence for BVDV-1. This difference can be justified by the conditions of sanitary management, biosafety and types of exploitation adopted in different countries.

In the finishing animals, seroreagent samples were found in properties 2, 3 and 4, both with a prevalence of 3.33%. Prevalence data supporting this study were carried out in Norway, demonstrating seroprevalence to

BVDV-1 (NADL strain) of 2.2% (LOKEN et al., 1991) and in France (4.4%), (PLATEAU et al., 1978).

Considering the prevalence per herd, Properties 3, 4 and 5 presented percentages of 0.84, 0.84 and 1.67% respectively, values almost equal to that found by Lipowski (2014), 1.04% for BVDV-1, in a serological survey carried out in Poland. In Property 2, a prevalence of 2.44% in animals of (F) and (S) was determined, a value close to that already demonstrated by a brief report by Gatto et al. (2014b), with 2.32% for genotype 1.

Only 20% (1/5) of the studied properties presented a seroreagent sample, four of the properties did not present any seroreagent samples, corroborating with O'Sullivan et al. (2011) who did not identify any seropositive animal for BVDV in the state of Ontario, Canada.

No serological surveys were found for BVDV-2 in pigs; this lack of information prejudiced the comparison of the data found and the discussion of the results.

It is noted that Property 2, where the highest prevalence occurred for genotype 2, also presented a BVDV-1 reactive animal. It was the only property that reported reproductive problems in pigs and ruminants in the last 6 months. In addition,

other possible risk factors were observed, such as presence of ruminants on the same property, presence of cattle in neighboring properties, acquisition of pigs in the last 6 months.

Although no risk factors were observed and described in property 5, the presence of cattle/sheep/goats in neighboring properties could probably be the closest contact with the source of infection that determined the cause of 2 reactive animals, this information comes with the description of Loeffen et al., (2009), which identified a high density of sheep and/or goats within a three-kilometer radius as potential risk factors associated with BVDV infection in pigs.

All risk factors were used in a descriptive way, no possible risk factor was significantly associated with the statistical analysis and thus were described as trends for the occurrence of the infection.

Due to the fact that animals were held in intensive breeding with biosecurity measures, the low prevalence of antibodies to BVDV was expected, but genotype 2 was more present in the herd than genotype 1. It was observed that the seropositive animals were in older age categories, such as finishing pigs and sows. Statistically, there was no significant association between the risk factors and prevalences

found for both genotypes.

Conclusion

This study found BVDV seroreagent samples from pigs from intensified farms, leading to the belief that this agent circulates in herds of technical properties. The data found to serve as a warning to the health authorities because of the possibility of false-positive results in tests for PSC.

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