



RESEARCH ARTICLE

A Cross-Sectional and Exploratory Geospatial Study of Bovine Viral Diarrhea Virus (BVDV) Infections in Swines in the São Paulo State, Brazil

Henrique Meiroz de Souza Almeida*, Igor Renan Honorato Gatto, Anne Caroline Ramos dos Santos, Antônio Sérgio Ferraudo, Samir Issa Samara and Luís Guilherme de Oliveira

UNESP – University Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias (FCAV), Via de acesso Prof. Paulo Castellane, s/n, CEP 14884-900, Jaboticabal, São Paulo, Brazil

*Corresponding author: henri_almeida2003@yahoo.com.br

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ABSTRACT

Reports of the first isolation of a ruminant *Pestivirus* (BVDV) from swine were in 1973, and since then the occurrence of cross-infections has been reported in many countries and the serological cross-reaction between the Classical Swine Fever Virus (CSFV) and the Bovine Viral Diarrhea Virus (BVDV) antibodies can interfere in Classical Swine Fever (CSF) eradication. This study focused on establishing the prevalence of antibodies anti-BVDV in pigs of non-technified rearing farms, associating risk factors to the infection and using geospatial analysis tools to identify high risk of positive herd's areas and other epidemiological features. A set of 360 serum samples from 56 herds were collected and analyzed using the virus neutralization test (VN). In total, 4.72% (17) of the samples had antibodies and 26.79% (15) of the herds had at least one positive animal. The titers obtained ranged from 640 to 10 for BVDV-1 and 80 to 10 in BVDV-2. The Kernel's distribution map showed two high risk of infection areas, which were associated using a statistical multiple correspondence analysis (MCA) with the presence of bovine herds and median total swine herd size. The use of raw milk in the swine feed was associated with disease presence. In conclusion, BVDV antibodies were detected in swine serum and possible links between bovines and swine herds in transmission of BVDV from cattle to swine and the problems that might arise from serological cross-reaction in CSF tests due to the presence of anti-BVDV antibodies in swine serum are discussed.

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INTRODUCTION

The *Pestivirus* genus within the *Flaviviridae* family comprises viral pathogens of worldwide importance for animal production (Tao *et al.*, 2013). The Classical Swine Fever virus (CSFV), the Border Disease virus (BDV) and the Bovine Viral Diarrhea virus (BVDV) are the four-main species of this genus and despite being named after the species which these viruses preferably infect (Asfor *et al.*, 2014), scientific reports of cross-infections are common (Deng *et al.*, 2012; Tao *et al.*, 2013). Recently, a report of a novel porcine *Pestivirus* isolation from congenital tremor cases in piglets (Arruda *et al.*, 2016) and the experimental reproduction of the disease have brought up the discussion about pathogenicity of the genus in this species and consequently, the role cross-infections might play.

The first report of the isolation of a ruminant *Pestivirus* (BVDV) from swine happened in 1973 (Fernelius *et al.*, 1973) and since then, several scientific reports of this cross-infection were performed in different countries (Loeffen *et al.*, 2009; Deng *et al.*, 2012; Tao *et al.*, 2013). However, there is anecdotic information about the occurrence of BVDV infections in Brazilian swine.

BVDV infections in swine usually present mild clinical signs, despite some reproductive disorders such: birth of weak piglets, abortion, mummified fetuses, stillborn piglets, fever and diarrhea have been associated to these cross-infections (Tao *et al.*, 2013). Furthermore, reports of the birth of malformed piglets, fewer piglets per litter and even persistently infected piglets were related to vertical infection in pregnant sows exposed to the BVDV (Becher *et al.*, 2003).

Infected cattle are pointed out as the main infection source of BVDV to swine (Wieringa-Jelsma *et al.*, 2013). In addition, BVDV is widespread in Brazilian cattle herds, several studies show figures that range from 43% to 57.56%, and even reaching 66.32% of herd level prevalence in some regions (Samara *et al.*, 2004; Quincozes *et al.*, 2007; Almeida *et al.*, 2013). In the light of such high prevalence in cattle herds it is very likely that swine BVDV infection occur in Brazilian swine herds, even though there are few or almost no data about BVDV infections in swine as far as the authors know. The presence of antibodies anti-BVDV in swine serum can lead to misinterpretations or even false positive CSFV results in serological tests, hindering official CSF surveillance actions and causing troubles in official eradication programs (Loeffen *et al.*, 2009; Tao *et al.*, 2013). Since the main action in CSF outbreaks is the slaughter of positive animals or even the entire herd, a correct diagnosis is of utter importance to prevent unnecessary sanitary slaughter. In addition, false positive results bring the need of more specific confirmatory tests to differ real CSF outbreaks from putative BVDV infections, delaying the decision-making process and increasing surveillance programs expenses (De Smit *et al.*, 1999).

In view of the lack of data about BVDV infection in swine, altogether with the fact that some features of this cross-infection remain quite unclear and the importance of serological cross-reaction between BVDV and CSFV antibodies in serological tests, more studies about this type of infection are required. This research focused on assessing the prevalence of anti-BVDV antibodies in swine herds, associating risk factors to the infection and using geospatial analysis tools to identify potential geospatial feature involved in the disease distribution.

MATERIALS AND METHODS

Sample design: Serum samples of 360 swine from 56 different herds were collected during the years of 2014 and 2015, in different 12 municipalities (Jaboticabal, Taiúva, Taiaçu, Guariba, Pradópolis, Ibatinga, Borborema, Itápolis, Motuca, Monte Alto, Taquaritinga and Santa Ernestina) of the northeastern region of the state of São Paulo. The choice of the sampled herds was by convenience (not random) and the only criteria used in the selection were the low adoption of techniques and biosecurity measures in the production site, since the animals of such farms were our target population. All samples came from a CSF-free zone recognized by OIE (OIE, 2016), so consequently all sampled animals were free of CSFV infection.

If the herd had five or less animals, all had samples collected, however in larger herds, 10% of the animals were randomly chosen and sampled. In order to achieve a representative sample size, we used the following equation:

$$n = \frac{z^2 \cdot p \cdot q}{d^2} \text{ (Thrusfield, 2010)}$$

In which: n = sample size, Z = normal standard deviation, p = disease's expected prevalence, q = 1 - p; and d =

maximum admitted error value. An expected prevalence of 6% value according to a 5.34% prevalence found in industrial finisher animals (Gatto *et al.*, 2015). The obtained value (n) was adjusted (na) to the regional population size (N), by the equation:

$$na = \frac{n \times N}{n + N} \text{ (Thrusfield, 2010)}$$

The N value used was 4,100 animals, according to the regional swine population published in the São Paulo state animal census (São Paulo, 2008). Only animals with more than two months old were included in this study. The blood samples collected were centrifuged and the serum was spared and stored at -20°C in micro tubes until usage.

Risk factor association: For the risk factor analysis, we used epidemiological information regarding the 56 sampled herds (each herd was a single unit for this analysis), gathered in an interview with the herd owner before the sample collection. The questionnaire approached questions related to variables that might be involved with the presence of infection (Table 2).

Virus neutralization test: The anti-BVDV antibodies presence was assessed through the VN assay described in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE, 2015) using MDBK (*Madine Darby Bovine Kidney*) tissue culture cells and both cytopathic strains Singer (BVDV-1) and VS253 (BVDV-2) in a 100 TCID₅₀ (50% Tissue culture infective dose) concentration. The positive samples were those in which occurred total neutralization of the 100 TCID₅₀ in a dilution higher than 1:10. The antibody titer considered was the reciprocal of the highest dilution in which there were total neutralization of the viral dose, and the final titer obtained by the geometric mean of the four results.

Geospatial analysis: The geographical coordinates of the sampled herds was obtained in the South American Geodesic system (SAD 69) format, using GPS device model GPSMAP® 60Cx. After the VN results, herds with at least one positive animal had, the geographical coordinates subjected to the Kernel intensity estimator tool, in the software Terraview® version 4.2.2.

Data analysis: All prevalence values found had the respective confidence interval (CI 95%) calculated using the methodology described by Thrusfield (2010). In order to detect association between the variables investigated and BVDV infections, it was used the Fisher's exact test (95%), and further on the Odds Ratio (OR) values and the CI 95% were calculated.

Continuous variables such: bovine herd size, swine herd size, total number of sows, total number of piglets, total number of boars, goat herd size and sheep herd size, were all categorized in small, medium and large herd based on the means values for each category. Afterwards, the herd size and BVDV presence data were subjected to a multiple correspondence analysis (MCA) to detect associations or similarity between the variables using the Statistica® version 7 software.

This research was evaluated and approved by the institution's ethics committee and was registered under the certificate n°07998/14 on 8th of May of 2014.

RESULTS

Prevalence results and risk factor analysis: Out of 360 swine serum samples, 4.72% (17/360; IC 95%: 2.97-7.43%) were positive in the VN. Table 1 presents all the prevalence values found at animal level for both genotypes. The BVDV-1 strain Singer sample's GMT ranged from 10 to 640, while in BVDV-2 strain VS253, the sample's GMT ranged from 10 to 80. In both cases, the titers obtained were predominantly low. Table 2 shows the antibody titers obtained for each sample and viral strain used. Regarding to herd level prevalence 26.79% (15/56, CI 95%: 15.19-38.38%) had at least one positive animal.

Concerning to the risk factor analysis performed, almost all variables investigated in the epidemiological questionnaire had $P > 0.2$ when analyzed using the Fisher's exact test, being non-significant. Consequently, no potential associations between the variables investigated and disease distribution could be established by this study. The only variable that had association with the disease occurrence was the use of raw bovine milk in the feed given to swine (OR: 2.82, 95%CI 0.82-9.76; $P < 0.13$), indicating a possible association with disease cases. More detailed data of this analysis can be check in Table 2 which presents the OR and p value of all variables.

Geospatial analysis results: The visual assessment of the map generated by the Kernel intensity estimator (Fig. 1) pointed the existence of two major areas with a higher risk of BVDV infection.

Area 1 – Upper left side (Fig. 1) embracing the cities of Taiúva, Taiacu, Jaboticabal, Monte Alto and Taquaritinga.

Area 2 – Lower right side, comprising the cities of Motuca, Guariba and Pradópolis.

As an attempt to understand the presence of those areas in the map, the herds had the size and presence of infection data subjected to an exploratory MCA. The value of the general chi-squared of the test was 1463.57 ($P < 0.01$) pointing out the existence of associations between the variables.

Analyzing the residual values, it was possible to identify: a strong association ($P < 0.05$) between BVDV-2 infection in swine and the presence of bovine herds (with more than 16 animals) within the same farm (residual value: 2.00). In addition, it was identified a moderate association ($P < 0.15$) between BVDV-1 infection in swine and the presence of a medium swine herd size (25 to 50 animals; residual value: 1.86).

Afterwards, distribution maps of the farms with bovine herds larger than 16 animals and medium swine herd size were generated by the Kernel intensity estimator tool (Fig. 2), in an attempt to explain the areas shown in Fig. 1.

When comparing the maps, it was possible to notice a matching of areas 1 with high-risk of infection (Fig. 1) and the distribution of bovine herds with 16 animals or more (Fig. 2). Another similarity observed was also between area 1 (Figure 1) and the distribution of medium swine herd (Fig. 3).

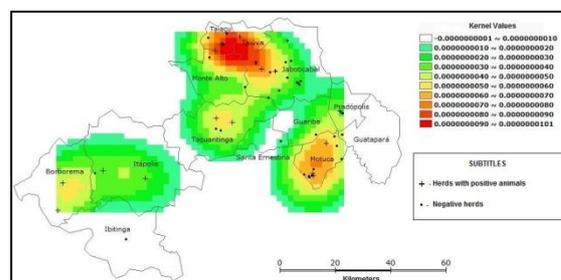


Fig. 1: Map of the sampled region presenting two main high-risk of infection areas and herds with positive cases shown.

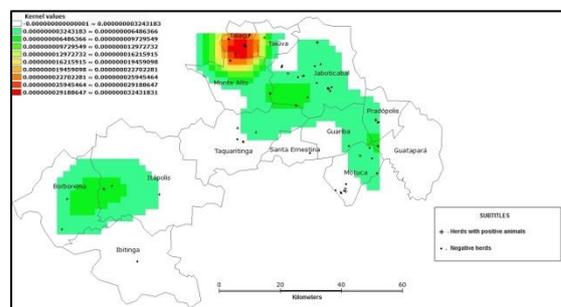


Fig. 2: Map of the sampled area in the northeastern regions of São Paulo state in the year of 2015 presenting the distribution of farms with bovine herds within (more than 16 animals) generated using the Kernel intensity estimator tool.

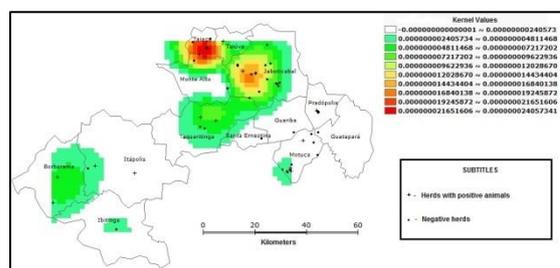


Fig. 3: Map of the sampled area in the northeastern regions of São Paulo state in the year of 2015 presenting the distribution of farms with medium swine herd size (25 to 50 animals) generated using the Kernel intensity estimator tool.

DISCUSSION

The occurrence of BVDV infections in swine has been reported in countries worldwide, such as United States, Netherlands and China (Fernelius *et al.*, 1973; Loeffen *et al.*, 2009, Deng *et al.*, 2012) and as far as the authors know this might be one of the first reports of BVDV infections in Brazilian swine.

The prevalence values found in this study, 4.72% at animal level and 26.79% at herd level, differ from the values found by Loeffen *et al.* (2009), who found 2.5% prevalence in sows, 0.42% in finisher pigs and 11% of sow herds and by O'Sullivan *et al.* (2011) did not detect positive swine in the state of Ontario Canada.

An important feature of BVDV infections is that bovines are the main host of BVDV (Ridpath, 2010; Tao *et al.*, 2013) and the direct contact with swine is the main transmission pathway of BVDV between these two species (Terpstra and Wensvoort, 1988; Jelsma *et al.*, 2013). Consequently, the prevalence of BVD in bovine herds is pointed as directly related to presence of infections in swine (Loeffen *et al.*, 2009; O'Sullivan *et al.*, 2011; Deng *et al.*, 2012).

Table 1: The obtained prevalence values and respective CI 95% of BVDV infection at animal and herd levels

	Genotype	Positive/ Tested	Prevalence	Confidence Interval (95%)
Animal level	BVDV-1 Singer	7/360	1.94%	0.95%-3.96%
	BVDV-2 VS253	11/360	3.06%	1.71%-5.39%
	Total	17/360*	4.72%	2.97%-7.43%
Herd level		15/56	26.79%	15.19%-38.38%

* - One samples were positive for both genotypes of the virus; however, it was counted only once in the total prevalence.

Table 2: Antibody titers and Geometric Mean Titer (GMT) obtained by all four VN assays using BVDV-1 strain Singer and BVDV-2 strain VS253

Virus	Sample ID	1° test	2° test	3° test	4° test	GMT
BVDV-1	1	20	10	10	10	11.89
	2	20	40	80	80	47.57
	3	40	40	20	80	40
	4	10	10	10	20	11.89
	5	10	10	10	10	10
	6	10	10	10	10	10
	7*	640	640	1280	320	640
BVDV-2	1	20	10	10	10	11.89
	2	10	10	10	10	10
	3	10	10	10	10	10
	4	10	10	10	10	10
	5	10	10	10	10	10
	6*	160	80	80	40	80
	7	10	10	10	10	10
	8	10	10	10	10	10
	9	10	10	10	10	10
	10	10	10	10	10	10
	11	10	10	10	10	10

* - The same sample was reagent to both species.

In Brazil, BVDV is widespread among cattle herds (Quincozes *et al.*, 2007, Almeida *et al.*, 2013), and the prevalence data of the same region in which samples were collected for this study showed a 56.49% BVD prevalence in cattle (Samara *et al.*, 2004). The high prevalence value among cattle could be responsible for more prevalent BVDV infection found in this study than the ones found by Loeffen *et al.* (2009) in the Netherlands, where there is an effective BVD eradication program since 1997 (Mars and Maanen, 2005). On the other hand, the absence of BVD infection in swine from the state of Ontario, Canada is counter intuitive since BVD still prevalent in Canadian cattle herds (Taylor *et al.*, 1995; O'Sullivan *et al.*, 2011). This fact could be related to the adoption of biosecurity measures by most of the farms in Ontario reducing close contact between swine and cattle (O'Sullivan *et al.*, 2011).

All sampled farms in this study had poor or no adoption of biosecurity practices. Thus, in several farms,

swine and ruminants were in close contact resulting in higher prevalence values found and highlighting the critical role of biosecurity measures in avoiding interspecies transmission of pathogens.

Regarding the risk factor analysis, the only variable that had a significant association with the presence of the disease ($P < 0.2$) was adding bovine raw milk in the swine feed (Table 3). This practice is known to be another transmission pathway of BVDV from cattle to swine (Terpstra and Wensvoort, 1988), due to the large number of viral particles present in the milk of infected cattle (Lindberg, 2003; Mars and Maanen, 2005; Fulton *et al.*, 2013). However, since the $P < 0.2$ this association must be interpreted with caution.

As for the other variables analyzed, a limitation of the study could explain the lack of risk factor association, since all selected herds came from small farms, what implied in very similar rearing conditions among all the sampled herds, what could have had a negative effect in the risk factor association.

Geospatial mapping tools have an increasing importance in epidemiology investigations due to its potential to assess geographical links between disease's outbreaks and cases distribution. The Kernel's intensity maps generated in this study showed an overlapping between the cattle herds presence and the areas of high-risk of BVDV positive swine herds (Fig. 1 & 2).

This fact is reinforced by a strong association ($P < 0.05$) found in MCA between BVDV-2 positive swine herds and the presence of cattle within the same farm and by the moderate association ($P < 0.15$) between medium swineherds and BVDV-1 infections. Both findings allowed the authors to point that the proximity of cattle and swine herds in this study could have been involved in the occurrence and prevalence of BVDV infections in swine.

In addition, the area 2 shown in the Kernel map is a settlement of small farms most of them with poor adoption of biosecurity measures. In such farms, keeping cattle and swine in the same pen, barn or pasture is a common practice. As it was earlier mentioned in this section this kind of mixed farming practices are associated with the presence of BVDV infections in swine (O'Sullivan *et al.*, 2011; Tao *et al.*, 2013) while, the species specialization of farms reduces the odds of this type of infections (Loeffen *et al.*, 2009).

Table 3: Univariate statistical analysis for the association between the investigated variables and the presence of BVDV-1 infections. The odds ratio (OR) at 95% CI and the p values obtained by the two-tailed Fisher's exact ($P < 0.05$) are presented

Investigated variables	Exposed herds (%)	Exposed herds with positive cases	OR (CI 95%)	P value (two-tailed Fisher)
Presence of ruminants within the same farm	44 (78.57%)	12	1.125 (0.26-4.87)	1.00
Presence of dairy cattle in the farm	23 (41.07%)	5	0.64 (0.19-2.20)	0.5510
Introduction of new bovines in the farm during the last 6 months	13 (23.21%)	3	0.775 (0.18-3.31)	1.00
Introduction of new goats and ewes in the farm during the last 6 months	5 (8.93%)	1	0.66 (0.07-6.43)	1.00
Introduction of new swine in the herd during the last 6 months	22 (39.29%)	6	1.04 (0.31-3.49)	1.00
Presence of cattle in the surrounding farms	47 (83.93%)	14	3.39 (0.39-29.75)	0.4184
Presence of goats and ewes herds in the surrounding farms	29 (51.79%)	7	0.76 (0.23-2.47)	0.7655
Occurrence of reproductive disorders in the swine herd	15 (26.78%)	2	0.33 (0.07-1.69)	0.3064
Occurrence of reproductive disorders in ruminants	8 (14.28%)	1	0.35 (0.04-3.09)	0.4276
Occurrence of reproductive disorders in ruminants or swine in the surrounding herds	4 (7.14%)	1	0.90 (0.09-9.44)	1.00
Addition of raw bovine milk in the swine feed	27 (48.21%)	10	2.82 (0.82-9.76)	0.1334
The same person dealing with ruminants and swine	44 (78.57%)	13	2.10 (0.40-10.92)	0.4809
Use of BVD vaccines in cattle	5 (8.93%)	0	0.00	0.3093

The presence of antibodies anti-BVDV in swine serum, detected in this study, could cross-react in CSF serological diagnostic tests leading to false positive results and hindering CSF surveillance or eradication programs (Loeffen *et al.*, 2009; Tao *et al.*, 2013). Half of the main Elisa commercial kits used for CSF diagnostics were not able to differentiate CSFV from BVDV antibodies (Schroeder *et al.*, 2012). Consequently, in outbreaks it would demand more time and financial resources for an accurate diagnostic and since the detection of such disease implies in the sacrifice of the entire herd leading to great economic losses (De Smitt *et al.*, 1999; Brazil, 2004) correctly diagnosing CSF is of utter importance.

Conclusions: This study detected anti-BVDV antibodies in 4.72% of the swine from non-technified herds in the northeastern regions of São Paulo state, as far as we know, this the first report of BVDV infections occurring in such rearing sites in Brazil which could interfere with CSF diagnosis, surveillance and eradication programs. Thus, areas of high risk of infection seemed to be related to the proximity between cattle and swine herds, reinforcing the role that mixed farming practices may have in epidemiology of swine infections.

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Authors contribution: HMSA authored the manuscript, designed the research collected, processed samples and analyzed the data. IRHG and ACRS participated in samples collection and processing. ASF participated in the geospatial data analysis. SIS and LGO helped with the design of the study, data analysis and the writing process.

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