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REGULAR ARTICLES

Description of risk factors associated with the detection of BVDV antibodies in Brazilian pig herds

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Abstract

Bovine viral diarrhea virus (BVDV) infects ruminants as primary hosts. However, other animals like pigs are susceptible. This study was conducted to investigate seroprevalence and risk factors associated with the detection of BVDV antibodies in pig herds. A total of 1.705 serum samples of 33 finisher herds, from seven Brazilian states, were collected in slaughterhouses. The samples were tested by virus neutralization (VN) test. In total, 5.35% (91/1.705) were positive and 64% (21/33) of the herds had positive animals. A significant association with "trucks are not cleaned and disinfected" and "visitors do not respect 72-h interval between visits to farms" ($P < 0.05$) was found in association with detection of BVDV-2 antibodies. This study suggests that important biosecurity gaps are present in Brazilian pig farms, as the presence of BVDV antibodies in pigs suggests (direct or indirect) contact with population(s) of ruminant species. Closing biosecurity gaps prevents spread of BVDV and other pathogens such as foot-and-mouth disease virus (FMDV) between pig and ruminant farms. This data should be taken in account by CSF surveillance programs, once cross-reaction in serologic tests between classical swine fever virus (CSFV) and BVDV antibodies has been shown to occur.

Keywords Interspecies infection · *Pestivirus* infection · Risk factors · Swine

Introduction

Challenging the health of livestock, there are several types of viral infections caused by pestiviruses. Pigs are susceptible to *Pestivirus* infection, including classical swine fever virus (CSFV), bovine viral diarrhea virus (BVDV-1), BVDV-2, and border disease virus (BDV) (Becher et al. 2003).

An epidemiological study has shown that bovine is the main host of BVDV and also the most important source of infection for other ruminants and pigs (Ridpath 2010). Interspecies transmission often occurs through the use of milk infected in the composition of pigs' feed (Terpstra and Wensvoort 1988) and through fomites (Carbrey et al. 1976). According to the model of experimental infection by Wieringa-Jelsma et al. (2006), BVDV transmission between pigs can happen, however, it is limited. Deng et al. (2012) found that the prevalence of BVDV in pig herds was closely linked to the prevalence of the disease in nearby cattle herds, suggesting area spread of the virus.

Risk factors have been associated with detection of BVDV antibodies in swine, including the presence of cattle in the farm, high small ruminant density within a 3-km radius, vaccines contaminated with BVDV, use of whey in feed, and age (Lenihan and Collery 1977; Vannier et al. 1988; Liess and Moennig 1990; Loeffen et al. 2009). BVDV infection in swine is typically subclinical; however, data on BVDV-associated clinical signs in growing pigs is not very consistent (Gatto et al. 2016).

It is important to understand the risk factors involved in the BVDV infection in pig herds in order to better plan surveillance in CSFV-free zones, as serologic cross-reaction between

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CSFV and BVDV antibodies was reported. Also, describing characteristics of swine herds infected with BVDV allows better understanding of biosecurity risks associated with contact of swine and ruminant operations. This study aimed to assess animal and herd level seroprevalence and associate potential risk factors with the presence of BVDV antibodies in Brazilian swine herds.

Materials and methods

Study design This is a cross-sectional study to assess the seroprevalence of BVDV in swine by testing serum of finisher pigs slaughtered in the state of São Paulo for BVDV-1 and BVDV-2 antibodies.

Two slaughterhouses were conveniently selected to collect samples for this study. Both were located in the northeastern region of the state of São Paulo and slaughtered animals from the major pig-producing regions in Brazil.

Eligibility criteria Study animals consisted of growing-finishing pigs from operations with at least 300 pigs (i.e., commercial pig production). Pigs originated from growing-finishing sites, where pigs were placed at 3 to 9 weeks of age and were fed with commercial pig diet until reaching slaughter age. All sampled animals had approximately 115–125 kg of live body weight and were of 18 to 21 weeks of age. The animal's origin data was collected from the mandatory Animal Transit Permit issued by the local official veterinary service office.

All pigs from the sampled farms were slaughtered between August 2013 and June 2014. During the sample collection period, the same sources (i.e., farms) of animals were not collected, avoiding duplication of the farm. To be eligible to participate, the farm owner had to fulfill a complete questionnaire to gather information for the risk factors analysis.

Sampling and sample design The number of samples was calculated to estimate the prevalence of BVDV in Brazilian pigs herds with reasonable accuracy using the methodology proposed by Thrusfield (2010) shown as:

$$n = \left(\frac{1.96}{d}\right)^2 \times \frac{[(S \times p) + (1-E) \times (1-p)] \times [(1-S \times p) - (1-E) \times (1-p)]}{(S + E - 1)^2}$$

where $P = 0.05$ (disease's expected prevalence) (Gatto et al. 2016), $d = 0.02$ (maximum permissible error), $N = 13{,}500$ (regional swine population), $S = 0.8$ (animal level sensitivity), and $E = 1$ (animal level specificity) (Sturza et al. 2011; Corbett et al. 2011).

Then, the result was adjusted according to the regional population size through the formula (Thrusfield 2010):

$$n_c = \frac{(N \times n)}{(N + n)}$$

Regarding the number of herds, it was sampled the highest possible number of different herds during the study period (convenience sample). The minimum number of samples needed to be collected was 553 in each slaughterhouse. For a higher power, 1.705 serum samples from finishing pigs at the slaughterhouse were collected, comprising 33 different herds from seven Brazilian states (Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Goiás, Mato Grosso do Sul, and Mato Grosso) during the years of 2013 and 2014. All collected batches were from commercial farms and approximately 91% (30/33) were vaccinated. At all sampling moments, all the animals of the slaughtered batch had blood samples collected.

Virus neutralization assay Samples were tested between 7 and 15 days post collection by virus neutralization (VN) test for the detection of BVDV antibodies, as recommended by the "*Manual of Diagnostics Tests and Vaccines for Terrestrial Animals*" (World Organization for Animal Health 2015). Bovine kidney "Madin-Darby bovine kidney (MDBK)" cell line and cytopathic strains of BVDV-1 (Singer) and BVDV-2 (VS-253) were used, with an incubation time of 96 h, and positive and negative controls further from the internal controls were used. All seropositive samples were tested twice to estimate the geometric mean of titer (GMT) values. A sample was considered positive when the total neutralization of 100 $TCID_{50}$ occurred in the serum and no cytopathic effect (CPE) was observed in the cell layer in serum dilutions higher than 1:10 (World Organization for Animal Health 2015). Positive herds to BVDV infection were those with at least one positive animal. All seropositive samples to BVDV were submitted to test for specific antibodies for CSFV by enzyme-linked immunosorbent assay—ELISA Ab.[1]

Questionnaire The animals' owners answered an epidemiological questionnaire (composed of 35 questions) to obtain data about the herd, mostly related to adoption of classical biosecurity measures. The authors use as basis Loeffen et al. (2009) and the possible answers were only "yes" or "no." The variable "herd size" was included in the model.

Data analysis To verify if the variables were associated with the occurrence of positive animals for BVDV, Fisher's exact test, odds ratio estimation, and confidence intervals were used. Thus, the analysis of risk factors was performed at the herd level, considering that all animals within the herd were

[1] See PrioCHECK® (CSFV Antibody 2.0 ELISA Kit, strip, Zurique, Suiça)

exposed to the same investigated variables of infection occurring the same for all animals. The herd sensitivity and specificity were calculated based on the average number of animals per herd using software R, package epiR.

For the inclusion and analysis of the variable "sample size," the herds were divided into four categories, based on the quartiles (20 to 29, 30 to 49, 50 to 99, and ≥ 100 animals). The variables with $P < 0.2$ in the univariate analysis and univariate logistic regression were submitted to multivariate logistic regression. In order for the variable to be maintained in the final model, a significance of $P < 0.05$ was adopted. No adjustment of P values was made to account for multiple testing. In the comparison between the models by the likelihood ratio test, we also adopted a significance level of 0.05. The influence of the state of origin on the proportion of infected herds was checked. The calculations were performed using the software Epi Info 7.

Results

The farms were contacted, and the questionnaire response rate was 100%. Out of 1.705 tested samples, 5.35% (91; CI 95% 4.27–6.40) were positive. Out of 33 pig herds involved in this study, 63.63% (21; 95% CI 40.22–80.04) had at least one seropositive animal to BVDV, regardless the genotype tested (Table 1), resulting in a positive herd status. The distribution of within-herd prevalence is represented in the histogram (Fig. 1). Considering an expected prevalence, average number of animals per herd and an infected herd ≥ 1 positive animal was detected that herd level sensitivity = 0.87 and herd level specificity = 1.

The variables "presence of dairy cattle in same farm," "cattle purchase in the last 6 months," and "disinfection program" were identified as possible risk factors for BVDV-1 infection in the univariate analysis (Table 2). However, in the multivariate logistic regression analysis, no risk factors remained associated with BVDV-1 antibodies.

The variables associated with BVDV-2 infection in the risk factor analysis ($P < 0.2$) were cattle purchase in the last 6 months, "background of biosecurity measures," "trucks are not cleaned and disinfected," and "visitors do not respect 72-h interval between visits to farms" (Table 3) and were submitted to a univariate regression analysis. Only three variables remained significant in the univariate logistic regression since background of biosecurity measures presented a $P > 0.05$ and was excluded (Table 4). The variables cattle purchase in the last 6 months, trucks are not cleaned and disinfected, and visitors do not respect 72-h interval between visits to farms had $P < 0.2$ and were submitted to a multivariate regression analysis. Only trucks are not cleaned and disinfected ($P = 0.01$, 95% CI 2.47–386.33) and visitors do not respect 72-h interval between visits to farms ($P = 0.05$, 95% CI 0.01–0.99) remained significant ($P \leq 0.05$) and were associated with occurrence of BVDV-2 antibodies in pigs.

Another logistic regression was performed with the remaining two variables to check if after excluding the cattle purchase in the last 6 months of the model they would still be significant. Both variables had $P < 0.05$ (Table 5) and therefore were considered as risk factors. The two independent variables are not associated with each other, and there was no interaction between these two variables ($P > 0.05$).

The variable sample size was not associated with the trucks are not cleaned and disinfected or visitors do not respect 72-h interval between visits to farms ($P > 0.05$). The sample size was shown to be associated with the frequency of BVDV-2 seropositive herds ($P = 0.02$). In the comparison between the models with and without variable sample size, the inclusion of the variable significantly influenced ($P < 0.05$) in the model, although it showed a $P = 0.06$ in the Wald test. There is no significant difference between states in the proportion of infected herds ($P = 0.27$). None of BVDV positive samples had antibodies against CSFV.

Discussion

This study investigated risk factors associated with seroprevalence of BVDV in finishing pigs. Infection of pigs with BVDV suggests direct or indirect contact of pigs with ruminants, which may be a risk for transmission of other pathogens. Understanding risk factors associated with BVDV infection in pig herds allows improvements in biosecurity, reducing risk of pathogen introduction.

Regarding the variables analyzed, there was a significant association in the logistic regression analysis for trucks are not cleaned and disinfected ($P = 0.004$) and visitors do not respect 72-h interval between visits to farms ($P = 0.02$) by BVDV-2 infection in pigs. The associations found can be attributed to gaps in the biosecurity program.

When including the number of samples collected per herd as a variable in the logistic regression analysis, a significant influence was noted ($P < 0.05$) and the sample size presented a significant association with the frequency of BVDV-2-positive herds ($P < 0.05$). Therefore, the authors acknowledge that the BVDV-2 prevalence might be super estimated.

Gaps in biosecurity contribute significantly to the spread of pathogens in several ways. The outbreak of porcine epidemic diarrhea virus (PEDV) in the USA in 2013 revealed failures in biosecurity in pig industry. Some risk factors have been associated with the spread

Table 1 Within-herd prevalence of antibodies for BVDV-1 and BVDV-2 from 33 different herds

ID[a]	State/municipalities	Within-herd prevalence % BVDV-1 (P/N)[a]	CI 95 (%)[b]	Within-herd prevalence % BVDV-2 (P/N)	CI 95 (%)
	Goiás	–	–	–	–
1	Rio Verde	3.28 (2/61)	0.90 a 11.19	1.64 (1/61)	0.29–8.72
2	Rio Verde	3.33 (1/30)	0.59 a 16.67	0.00 (0/30)	0–11.35
	Mato Grosso do Sul	–	–	–	–
3	Campo Grande	3.33 (1/30)	0.59 a 16.67	3.33 (1/30)	0.59–16.67
4	Dourados	3.33 (1/30)	0.59 a 16.67	6.67 (2/30)	1.85–21.32
5	Ponta Porã	0.00 (0/30)	0 a 11.35	3.33 (1/30)	0.59–16.67
6	Vicentina	0.00 (0/50)	0 a 7.13	4.00 (2/50)	1.10–13.46
	Mato Grosso	–	–	–	–
7	Itiquira	0.00 (0/20)	0 a 16.11	0.00 (0/20)	0–16.11
8	Primavera do Leste	16.67 (5/30)	7.34 a 33.56	10.00 (3/30)	3.46–25.62
	Paraná	–	–	–	–
9	Espigão Alto do Iguaçu	0.00 (0/29)	0 a 11.70	0.00 (0/29)	0–11.70
10	Laranjeiras do Sul	13.33 (4/30)	1.17 a 25.50	6.67 (2/30)	1.85–21.32
11	Siqueira Campos	1.18 (3/255)	0.40 a 3.40	2.75 (7/255)	1.34–5.56
12	Siqueira Campos	1.92 (2/104)	0.53 a 6.74	0.96 (1/104)	0.17–5.25
	Rio Grande do Sul	–	–	–	–
13	Rodeio Bonito	9.18 (9/98)	4.91 a 16.54	2.04 9 (2/98)	0.56–7.14
	Santa Catarina	–	–	–	–
14	Águas Frias	3.33 (1/30)	0.59 a 16.67	3.33 (1/30)	0.59–16.67
15	Águas Frias	3.33 (1/30)	0.59 a 16.67	6.67 (2/30)	1.85–21.32
16	Águas Frias	0.00 (0/30)	0 a 11.35	0.00 (0/30)	0–11.35
17	Concórdia	1.92 (2/104)	0.53 a 6.74	0.96 (1/104)	0.17–5.25
18	Chapecó	0.00 (0/30)	0 a 11.35	0.00 (0/30)	0–11.35
19	Iomerê	3.33 (1/30)	0.59 a 16.67	3.33 (1/30)	0.59–16.67
20	Nova Erechim	6.67 (2/30)	1.85 a 21.32	0.00 (0/30)	0–11.35
21	Pinhalzinho	0.00 (0/30)	0 a 11.35	6.67 (2/30)	1.85–21.32
22	Pinhalzinho	7.41 (2/27)	2.06 a 23.37	7.41 (2/27)	2.06–23.37
23	Salto Veloso	0.00 (0/30)	0 a 11.35	10.00 (3/30)	3.46–25.62
24	Saudades	3.91 (5/128)	1.68 a 8.82	0.78 (1/128)	0.14–4.29
25	Saudades	6.90 (2/29)	1.91 a 21.96	0.00 (0/29)	0–11.70
26	União do Oeste	3.33 (1/30)	0.59 a 16.67	3.33 (1/30)	0.59–16.67
	São Paulo	–	–	–	–
27	Colina	1.67 (2/120)	0.46 a 5.87	1.67 (2/120)	0.46–5.87
28	Cristais Paulista	0.00 (0/27)	0 a 12.46	0.00 (0/27)	0–12.46
29	Guariba	2.04 (1/49)	0.36 a 10.69	0.00 (0/49)	0–7.27
30	Ipuã	0.00 (0/31)	0 a 11.03	6.45 (2/31)	1.79–20.72
31	Jaboticabal	4.69 (3/64)	1.61 a 12.90	0.00 (0/64)	0–5.66
32	Jaboticabal	0.00 (0/31)	0 a 11.03	0.00 (0/31)	0–11.03
33	Monte Alto	0.00 (0/28)	0 a 12.06	0.00 (0/28)	0–12.06
Total	–	3.00 (51/1705)	2.28–3.91	2.35 (40/1705)	1.73–3.18

Equal numbers indicate the same home city, but a different farm

ID farm identification

[a] The fraction in brackets correspond to total of animals tested positive (P) for BVDV in the virus neutralization (VN) test divided by total of animals tested (N) in the batch

[b] Confidence interval of 95%

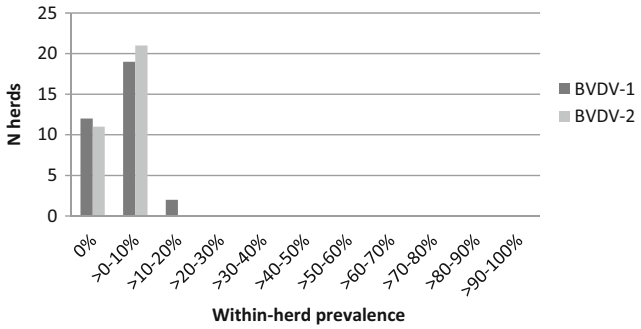

Fig. 1 Distribution of within-herd prevalence for bovine viral diarrhea virus antibodies

of PEDV as: contaminated trucks in loading and/or unloading pig area, or the contact by the driver in this area with different farms (Lowe et al. 2014).

Similar to what has been reported as risk factor for PEDV infection, the lack of truck disinfection was associated with the presence of BVDV-2 antibodies in swine, showing that trucks with poor decontamination practices could have been responsible for introducing BVDV into swine herds. Proper disinfection of vehicles is a pivotal biosecurity measure that when not applied could contribute to further occurrence of infections.

Considering the two risk factors that were associated with BVDV infection in this study, it is plausible to speculate that there are similarities in the procedures adopted in farms, mainly in those from southern Brazil, where approximately 49.25% of the Brazilian swine herd is (Instituto Brasileiro de Geografia e Estatística 2012). The distance between herds and the herd size can influence directly on the adoption of biosecurity measures in animal rearing, mainly in the transport of pigs, if the truck goes to several farms on the same day, enhancing the spread of pathogens. Importantly, it is common to have dairy cattle reared in swine, for subsistence purposes, a practice that increases the chances of BVDV infection in pigs.

Our study's findings were not inconsistent with those of Ridpath (2010) who hypothesized that infected cattle

Table 2 Univariable analyses of risk factors for the presence of positive pigs for BVDV-1

Risk factor	OR	CI OR (95%)	*P***
Presence of dairy cattle in the same farm	20.0	1.99 to 200.53	0.01*
Cattle purchase in the last 6 months	5.5	0.96 to 31.43	0.07*
Disinfection program	–	–	0.03*

OR odds ratio, *CI* confidence interval

*Variables considered for logistic regression analyses; ***P* Fisher's exact test, no adjustment is made for multiple testing

Table 3 Univariable analyses of risk factors for the presence of positive pigs for BVDV-2

Risk factor	OR	CI OR (95%)	*P***
Cattle purchase in the last 6 months	5.5	0.96 to 31.43	0.07*
Background of biosecurity measures	–	–	0.01*
Trucks are not clean and disinfected	13.3	2.08 to 84.99	0.01*
Visitors do not respect 72-h interval between visits to farms	1.87	0.44 to 7.85	0.11*

OR odds ratio, *CI* confidence interval

*Variables considered for logistic regression analyses; ** *P* Fisher's exact test, no adjustment is made for multiple testing

was the main host of BVDV and the main source of infection for pigs, as well as Carbrey et al. (1976) who showed the importance of between-herd transmission of BVDV through fomites. Importantly, the risk factors presence of dairy cattle in same farm, cattle purchase in the last 6 months, and disinfection program show as having an association with BVDV infection in pigs by univariate analysis. Despite Loeffen et al. (2009), Ridpath (2010) and Deng et al. (2012) have asserted that cattle is the main source of BVDV infection in pigs; the data raised by this study was not accurate enough to confirm it.

It is interesting to understand the risk factors involved in the BVDV infection in pigs in order to support the monitoring of CSFV-free zones as serologic tests cross react between CSFV and BVDV antibodies. However, it is important to conduct other studies in larger pig populations to identify new risk factors or more detailed of those surveyed in this study. Also, understanding aspects associated with BVDV infection of pig operations from ruminant species may help target control measures to mitigate the spread of eventual introduction of other pathogens such as FMDV.

To the best of our knowledge, this is the first study in Brazil describing seroprevalence and risk factors associated with

Table 4 Univariable logistic regression analyses obtained with model including the variables associated with infection ($P < 0.2$ in the univariable analyses) for the presence of positive pigs to BVDV-2

Risk factor	OR	CI OR (95%)	*P*
Cattle purchase in the last 6 months	5.50	0.96 to 31.42	0.05
Background of biosecurity measures	4522915.39	0.00 to > 1.0E12	0.97
Trucks are not clean and disinfected	13.30	2.08 to 84.99	0.01
Visitors do not respect 72-h interval between visits to farms	0.23	0.049 to 1.13	0.07

OR odds ratio, *CI* confidence interval

Table 5 Multivariable logistic regression analyses obtained with model including the variables associated with infection ($P < 0.2$ in the univariable logistic regression analyses) for the presence of positive pigs to BVDV-2

Risk factor	OR	CI OR (95%)	*P*
Trucks are not cleaned and disinfected	40.77	3.20 to 520.33	0.004
Visitors do not respect 72-h interval between visits to farms	0.06	0.01 to 0.69	0.02

OR odds ratio, *CI* confidence interval

BVDV antibodies in finishing pig herds. All risk factors were closely related to major gaps in biosecurity programs, such as trucks were not cleaned and disinfected and visitors did not respect 72-h interval between visits to farms.

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Compliance with ethical standards

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

Ethics committee approval The institution's ethics committee approved this study, and the certificate registered under the protocol no. 07998/14 on 8 May 2014.

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