OCCURRENCE OF NEUTRALIZING ANTIBODIES AGAINST BVDV-1 AND BVDV-2 IN CATTLE HERDS FROM MINAS GERAIS AND SÃO PAULO STATES, BRAZIL[1]

OCORRÊNCIA DE ANTICORPOS NEUTRALIZANTES CONTRA O BVDV-1 E O BVDV-2 EM REBANHOS BOVINOS DE MINAS GERAIS E SÃO PAULO, BRASIL

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SUMMARY

The occurrence of neutralizing antibodies against bovine viral diarrhea virus genotypes (BVDV-1 and BVDV-2) has been confirmed by virus neutralization test (VN) in samples of blood serum from 26 cattle herds which were not BVDV vaccinated, located in the states of Minas Gerais and São Paulo, Brazil. Ten blood samples were collected from each herd, five samples from 6 to 12-month-old calves and five samples from adult bovines. Of the total samples analyzed, 102 (39.2%) were reactive to BVDV, more specifically, 81 (31.1%) were reactive to BVDV-1 and BVDV-2, seven (2.7%) were reactive to BVDV-1 only and 14 (5.4%) were reactive to BVDV-2 only. Except for two herds, in all others at least one animal was detected reactive to BVDV, however, one of them was reactive to BVDV-2 only. In six herds, neutralizing antibodies were detected in blood serum from 6 to 12-month-old calves. Therefore, were indicative of recent BVDV infection and also suggested the likely presence of an infection source in the herd. The results showed the occurrence of neutralizing antibodies against BVDV genotypes in cattle herds located in the states analyzed, but these same results demonstrated the differences in the number of bovines reactive for BVDV-1 and BVDV-2, thus demonstrating the need to use strains from each genotype in VN tests for serological diagnosis of BVDV.

KEY-WORDS: Bovine viral diarrhea. BVDV-1. BVDV-2. Neutralizing antibodies. Virus neutralization.

RESUMO

A ocorrência de anticorpos neutralizantes contra os genótipos do vírus da diarréia viral bovina (BVDV-1 e BVDV-2) foi determinada pelo teste de virusneutralização (VN) em amostras de soro sangüíneo provenientes de 26 rebanhos bovinos não vacinados contra o BVDV, localizados nos Estados de Minas Gerais e São Paulo, Brasil. Foram analisadas 10 amostras por rebanho, sendo cinco de bovinos adultos e cinco de bovinos com idade entre 6 e 12 meses. Do total de 260 amostras analisadas, 102 (39,2%) reagiram ao BVDV, das quais 81 (31,1%) foram reagentes tanto ao BVDV-1 quanto ao BVDV-2, sete (2,7%) reagiram apenas ao BVDV-1 e 14 (5,4%) reagiram apenas ao BVDV-2. Com exceção de dois rebanhos, nos demais foram detectados pelo menos um animal reagente ao BVDV, entretanto, foram detectados animais reagentes apenas ao BVDV-2 em um deles. Em seis rebanhos foram detectados anticorpos neutralizantes nos bovinos da faixa etária de 6 a 12 meses, sendo, portanto, indicativos da infecção recente pelo vírus e também sugestivos da provável presença da fonte de infecção no rebanho. Os dados obtidos mostraram a ocorrência de anticorpos neutralizantes contra os genótipos do BVDV em rebanhos bovinos localizados nos Estados analisados, mas os resultados apresentaram diferenças no número de bovinos reagentes ao BVDV-1 e ao BVDV-2, ressaltando assim a necessidade da utilização de estirpes de cada genótipo nos testes de VN para o diagnóstico sorológico do BVDV.

PALAVRAS-CHAVE: Anticorpos neutralizantes. BVDV-1. BVDV-2. Diarréia viral bovina. Virusneutralização.

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INTRODUCTION

The bovine viral diarrhea (BVD) consists of a syndrome associated with clinical or asymptomatic infection with bovine viral diarrhea virus (BVDV) (BOLIN & RIDPATH, 1996). However, a significant percentage of infections (70 to 90%) is asymptomatic (BAKER, 1995). The disease is common in most countries and BVDV is a pathogen of cattle, particularly of the reproductive system (BROWNLIE, 2005).

The BVDV is an RNA virus that together with classical swine fever virus (CSFV) and the border disease virus (BDV) make up the genus *Pestivirus*, of the family *Flaviviridae* (NETTLETON & ENTRICAN, 1995). The BVDV is classified in two different species or genotypes BVDV-1 and BVDV-2, both of which can exist as two biotypes, cytopathogenic (CP) and non-cytopathogenic (NCP) (RIDPATH, 2010).

Theoretically, all cattle herds are infected or have suffered the infection at a given time (NETTLETON & ENTRICAN, 1995). BVD is widespread throughout the world, between 50 and 90% of the adult cattle herds has neutralizing antibodies against BVDV (HOUE & MEYLING, 1991; HOUE, 1999; KRAMPS et al., 1999). However, the occurrence of a recent infection or transmission of BVDV in the herd can be verified by detecting neutralizing antibodies in young animals (SMITH & GROTELUESCHEN, 2004).

BVDV infection is widespread in Brazilian herds and the virus strains isolated show remarkable antigenic variability and the phylogenetic analysis performed on these strains showed the presence of BVDV-1 and BVDV-2 (OLIVEIRA et al., 1996; CANAL et al., 1998; BOTTON et al., 1998; FLORES et al., 2000).

However, most studies conducted in Brazil researched only the antibodies against BVDV-1, which might result in failure to detect antibodies against BVDV-2 when they are present at low or moderate levels (FLORES et al., 2005). Thus, the aim of this study was to detect neutralizing antibodies against BVDV-1 and BVDV-2 in cattle herds from farms in Minas Gerais and São Paulo states, Brasil.

MATERIAL AND METHODS

Herds

During the period between January, 2005 and April, 2006, blood samples were drawn from 26 cattle herds from Minas Gerais and São Paulo. In Minas Gerais, the samples were collected from 6 herds located in the following municipalities: Machado, Poço Fundo and São Gonçalo do Sapucaí. In São Paulo, the samples were collected from 20 herds located in the municipalities of Jaboticabal, Monte Alto, Sertãozinho, São Carlos, Guariba, Pedregulho, São José do Rio Preto, Cristais Paulista, Buritizal and Rifaina.

These herds were not vaccinated against BVDV. Dairy and beef cattle were used in the study, and herds had between 20 and 480 animals. The herds were randomly sampled, regardless of management, production system, number of animals, breed, productivity and presence or not of reproductive problems.

Sampling

The number of samples per herd was based on the modified methodology adopted by Luzzago et al. (1999). Ten blood samples were obtained randomly from each herd, 5 from adult animals and 5 from animals aged between 6 and 12 months, totaling 260 samples.

Blood samples were drawn and stored in siliconized "vacutainer" BD® tubes, centrifuged at 1,080xg and divided into two 1.5-mL-serum aliquots for testing against BVDV-1 and BVDV-2. The serum aliquots were distributed into "eppendorf" tubes, identified and stored at -20ºC, until used. Blood serum samples were split in order to guarantee identical handling conditions before the serological tests against BVDV-1 and BVDV-2 were conducted.

Virus neutralization test – VN

All samples underwent VN test for antibodies against BVDV-1 and BVDV-2 (OIE, 2008). The VN test used epithelial cells of bovine kidney of the "Madin Darby bovine kidney" (MDBK) line, kept in Eagle MEM (Minimal Essential Medium) Gibco® medium, supplemented with 10% fetal bovine serum (SFB) Cultilab® free of *Pestivirus* and antibodies against BVDV**,** using the cytopathogenic strains (CP) of BVDV-1 (Singer) and of BVDV-2 (VS-253). Before testing, blood serum samples were previously inactivated at 56ºC, for 30 minutes. The virus neutralization tests were performed on disposable microtiter plates of 96 wells TPP®. To the maintenance medium Eagle-MEM Gibco® used for serum samples dilution, a 1% penicillin solution (10,000UI mL^{-1}) and streptomycin (10,000ug mL^{-1}) Gibco® was added.

Each serum sample tested was duplicated by successive dilutions between 1:10 and 1:5,120. After adding the viral suspension containing 100 $TCID_{50}$ (50% tissue culture infective doses) of BVDV, the microplates were incubated in 5% CO_2 atmosphere at 37ºC. After 60 minutes, it was added to each cell well, a suspension of MDBK cells containing 300,000 cells mL^{-1} in Eagle-MEM Gibco® maintenance medium with 10% SFB Cultilab®. Subsequently, the plate was again incubated in an oven at 37ºC, in 5% CO_2 atmosphere for 96 hours. The serum samples that neutralized 100 $TCID_{50}$ of BVDV in 1:10 dilution were considered reactive. Samples reactive to 1:5,120 dilution were retested in duplicate to the dilution 1:20,480. The antibody titers were expressed as the reciprocal of the highest dilution where it was detected virus neutralization, and the final titer resulted from the geometric mean of the titers found in duplicates.

RESULTS

Blood serum samples were analyzed of 260 nonvaccinated animals from cattle herds of Minas Gerais and São Paulo, of which 102 (39.2%) were reactive to BVDV.

Table 1. Results of VN tests for BVDV-1 and BVDV-2 performed on blood serum samples of cattle not vaccinated against BVDV from herds located in Minas Gerais and São Paulo States.

		BVDV-1		
		Reactive	**Non-reactive**	**Total**
BVDV-2	**Reactive**	81 (31.1%)	14 (5.4%)	95 (36.5%)
	Non-reactive	7 (2.7%)	158 (60.8%)	165 (63.5%)
	Total	88 (33.8%)	172 (66.2%)	260 (100%)

Considering the age group analyzed, 73 (56.15%) of 130 samples from adult cattle and only 29 (23.3%) of 130 samples of animals between 6 and 12 months were reactive to BVDV.

Of the 102 samples reactive to BVDV, 81 (31.1%) were reactive to both BVDV-1 and BVDV-2, seven (2.7%) were reactive to BVDV-1 only and 14 (5.4%) were reactive to BVDV-2 only. A total of 88 (33.8%) samples were reactive to BVDV-1 and 95 (36.5%) to BVDV-2 (Table 1).

Regarding the VN tests for BVDV-1 and BVDV-2 performed in 60 blood serum samples from herds located in Minas Gerais, 36 (60%) were reactive to BVDV. Of these samples 30 (50%) were reactive to both BVDV-1 and BVDV-2, none was reactive to BVDV-1 only and six (10%) were reactive to BVDV-2 only. A total of 30 (40%) samples were reactive to BVDV-1 and 36 (60%) to BVDV-2. The VN tests performed for BVDV-1 and BVDV-2 in 200 serum samples from herds located in São Paulo detected 66 (33%) reactive to BVDV. Of these samples, 51 (25.5%) were reactive to both BVDV-1 and BVDV-2, seven (3.5%) were reactive to BVDV-1 and eight (4%) were reactive to BVDV-2 only. A total of 58 (29%) samples were reactive to BVDV-1 and 59 (29.5%) were reactive to BVDV-2.

The distribution of reactive samples per herd, variation of the respective mean antibody titer against BVDV-1 and BVDV-2, as well as the results for different age groups are shown in Table 2. From the total of 26 herds tested, it was found at least one animal reactive to BVDV-1 and to BVDV-2 in 23 and 24 herds, respectively. In the adult age group, animals reactive to BVDV-1 were found in 23 herds and, to BVDV-2, in 24 herds. For the calves, the 6 to 12 month age group, ten herds had animals reactive to BVDV-1 and ten herds to BVDV-2. The total number (adults and calves) of animals reactive to BVDV-1 per herd varied between 1 and 9, while antibody titers ranged from 10 to 2,560. For BVDV-2, the total number of reactive animals varied between 1 and 8 while antibody titers ranged from 10 to 3,620.

DISCUSSION

From the samples analyzed in this study, 39.2% of the animals were reactive to BVDV. However, half of the samples analyzed were from young animals aged between 6 and 12 months. When analyzing only the results of the VN tests of adult animals, the number of reactive animals of this age group (56.15%) was in the range proposed by Houe (1999), who considered that between 50 and 90% of the adult cattle population has antibodies against BVDV.

The results presented in this study are very similar to other studies conducted in Brazil for adult animals reactive to BVDV (PELLEGRIN et al., 1997; PITUCO et al., 1998; GUIMARÃES et al., 2001; DIAS & SAMARA, 2003; NORONHA et al., 2003; ALFAIA et al., 2004).

In Brazil, the first serological survey for BVDV was conducted by Wizigmann et. al (1972). According to Flores et al. (2005), several studies were also conducted in other Brazilian states, but from the epidemiological point of view, the number of reactive cattle detected should be interpreted with caution, since some herds had reproductive problems and the majority did not take into account that part of the reactive samples could be the result of vaccination. However, in this study the herds were not vaccinated against BVDV and the herds were randomly chosen independent of management, production system, quantity of animals, breed, productivity and the presence or not of reproductive problems.

The number of animals reactive to BVDV from the cattle herds located in Minas Gerais was proportionally greater than those from São Paulo. This difference could be related to the fact that most of the herds analyzed in Minas Gerais were more densely populated and consequently, the more intensive management facilitated the spreading of the virus (HOUE & MEYLING, 1991; HOUE, 1995; HOUE, 1999; MOCKELIUNIENE et al., 2004; SMITH & GROTELUESCHEN, 2004).

The results of the VN tests of the 102 samples reactive to BVDV, for both virus genotypes showed that 81 (31.1%) were reactive to both BVDV-1 and BVDV-2, while seven (2.7%) and 14 (5.4%) were

Table 2. Number of reactive animals according to age bracket and variation of antibody titers in the VN tests against BVDV-1 and BVDV-2, from herds located in Minas Gerais and São Paulo States.

Herd	City	State	BVDV-1				BVDV-2			
			Adults		Calves		Adults		Calves	
			Reactive animals/sample	Variation of antibody titers	Reactive animals/sample	Variation of antibody titers	Reactive animals/ sample	Variation of antibody titers	Reactive animals/ sample	Variation of antibody titers
1	Machado	MG	4/5	80 a 2.560	4/5	80 a 160	4/5	160 a 3.620	4/5	20 a 160
2	S. G. do Sapucaí	MG	3/5	160 a 640	4/5	40 a 320	3/5	20 a 640	5/5	40 a 3.620
3	S. G. do Sapucaí	MG	1/5	20	0/5	0	1/5	453	1/5	14
4	Poço Fundo	MG	2/5	20 a 2.560	3/5	640 a 1.280	2/5	160 a 2.560	5/5	160 a 2.560
5	Poço Fundo	MG	4/5	10 a 320	1/5	20	5/5	20 a 1.810	2/5	20 a 40
6	Machado	MG	4/5	80 a 2.560	0/5	0	4/5	14 a 640	0/5	0
7	S. J. do Rio Preto	SP	2/5	10 a 320	1/5	10	4/5	10 a 120	0/5	0
8	Jaboticabal	SP	0/5	0	0/5	0	0/5	0	0/5	0
9	Sertãozinho	SP	1/5	1.280	0/5	0	1/5	320	0/5	0
10	São Carlos	SP	2/5	10 a 20	0/5	0	3/5	10 a 80	0/5	0
11	São Carlos	SP	0/5	0	0/5	0	0/5	0	0/5	0
12	Jaboticabal	SP	2/5	40 a 80	0/5	0	1/5	10	0/5	0
13	Jaboticabal	SP	2/5	10 a 20	0/5	0	2/5	10 a 20	0/5	0
14	Guariba	SP	3/5	160	0/5	0	3/5	80 a 160	0/5	0
15	Guariba	SP	2/5	80 a 640	0/5	0	2/5	20 a 240	0/5	0
16	Guariba	SP	4/5	40 a 2.560	1/5	10	3/5	80 a 905	0/5	0
17	Pedregulho	SP	5/5	80 a 320	4/5	10 a 1.280	5/5	56 a 120	3/5	10 a 80
18	Pedregulho	SP	1/5	20	0/5	0	1/5	40	0/5	0
19	Cristais Paulista	SP	2/5	320	0/5	0	2/5	160 a 196	0/5	0
20	Buritizal	SP	5/5	226 a 905	1/5	640	5/5	10 a 905	2/5	40 a 905
21	Pedregulho	SP	4/5	80 a 320	2/5	10 a 320	4/5	28 a 80	1/5	40
22	Buritizal	SP	2/5	40 a 320	0/5	0	2/5	80 a 226	1/5	10
23	Buritizal	SP	3/5	40 a 80	0/5	0	3/5	452	0/5	0
24	Cristais Paulista	SP	0/5	0	0/5	0	2/5	10 a 20	0/5	0
25	Monte Alto	SP	4/5	10 a 640	0/5	0	4/5	10 a 320	0/5	0
26	Rifaina	SP	4/5	40 a 1.280	1/5	10	4/5	10 a 320	1/5	28

reactive only to BVDV-1 and BVDV-2, respectively. Similar results were found in the study by Flores et al. (2000), who reported 2.5% of samples reactive to BVDV-1 only and 3.3% to BVDV-2. A total of 88 (33.8%) were reactive to BVDV-1 and 95 (36.5%) to BVDV-2.

These results showed that the use of only one genotype strain in the VN tests resulted in false-negative diagnosis for the other genotype according to the observations made by Flores et al. (2000), Fulton et al. (2000), Fulton et al. (2002), Chase et al. (2003) and Flores et al. (2005).

The use of BVDV-2 strains in the VN test would also be required in the case of herd individual analysis, since herd 24 from São Paulo (Table 2), would be diagnosed as false-negative because it had only two animals reactive to BVDV-2 and none reactive to BVDV-1. Another aspect to be reported is the higher antibody levels of VN tests (3,620) found in animals reactive to BVDV-2, specifically in herds 1 and 2 (Table 2).

All herds from Minas Gerais had at least one animal reactive to BVDV. In São Paulo, with the exception of herds 8 and 11 (Table 2), every other herd had at least one reactive animal, the same was reported by Richtzeinhain et al. (1999) and Dias & Samara (2003), which shows that at some point, these herds have experienced infection with BVDV (NETTLETON & ENTRICAN, 1995).

The number of reactive adult animals was greater than the number of young animals infected. According to Mockliuniene et al. (2004), the number of reactive animals increases with age, thus suggesting the occurrence of old infections, after all the titers of antibodies against BVDV are long lasting after natural infection (LINDBERG & HOUE, 2005) and they decrease only a few years after the occurrence of infection (FREDRIKSEN et al., 1999).

Many of the cattle herds analyzed had high titers of antibodies, but this is not enough to predict the presence of BVDV in the herd. The fact that BVDV antibodies are long lasting after natural infection occurs, poses a challenge when trying to detect the true infection stage of the herd (HOUE, 1999; LINDBERG & HOUE, 2005).

In some of the cattle herds with reactive animals and high antibody titers, a number of the animals were between 6 and 12 months. This was seen in herds 1, 2, 4, 17, 20 and 21 (Table 2). In this case, the detection of neutralizing antibodies in this age bracket, as well as the high titers indicated recent infection by BVDV and the likely presence of a source of infection in the herd (SMITH & GROTELUESCHEN, 2004).

The pathogenesis of BVDV infections has peculiar characteristics, which is reflected in the epidemiology and is not observed in other diseases. In epidemiological studies of BVDV, the main challenges are to detect the stage of infection, the way the BVDV is spreading within the herd and the source of infection and finally, the challenge of quantifying the economic losses (HOUE, 2003; SMITH & GROTELUESCHEN, 2004).

CONCLUSIONS

The results confirm the occurrence of cattle with neutralizing antibodies against BVDV-1 and BVDV-2 in cattle herds from Minas Gerais and São Paulo, and in most analyzed herds it was found at least one reactive animal. The use of only one genotype strain of BVDV in VN tests resulted in false-negative diagnosis for the other genotype, which indicated the need to use strains of each genotype in the VN test to diagnose BVDV accurately. The neutralizing antibodies detected in young animals suggested the presence of BVDV in some of the analyzed herds.

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