VETERINARY MICROBIOLOGY - SHORT COMMUNICATION

Molecular detection and phylogeny of bovine viral diarrhea virus 1 among cattle herds from Northeast, Southeast, and Midwest regions, Brazil

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Abstract

We examined the circulating BVDV species and genotypes among cattle herds from Northeast, Southeast, and Midwest regions in Brazil. A total of 77 animals tested positive through standard PCR. Phylogenetic analyses revealed the presence of BVDV-1a, highlighting the need for better surveillance strategies to prevent BVDV spread in the country.

Keywords BVDV · 5' UTR · Brazilian cattle · Economic burden · Veterinary pathogen

Bovine viral diarrhea virus 1 and 2, (BVDV-1 and BVDV-2), respectively, are two of the most important viral pathogens of cattle responsible for significant economic losses for cattle industry throughout the world [1–4]. BVDV-1 and 2 are associated with cases of gastroenteric disease and reproductive disorders such as temporary infertility, embryonic or fetal mortality, abortion or mummification, fetal malformations and birth of weak and unfeasible calves. Furthermore, other clinical outcomes include respiratory syndromes, hemorrhagic diseases, and a fatal form known as mucosal disease [5]. BVDV can also induce clinically inapparent infections as a consequence of fetal exposure, generating persistently infected (PI) calves. These animals are generally seronegative, clinically normal (able to survive for few years), and can disseminate the virus over their lifetimes. Therefore, the identification and elimination of PI calves is necessary for BVDV control [5–7].

The two species of BVDV (BVDV-1 and BVDV-2) belong to the *Pestivirus* genus in the *Flaviviridae* family. Their genomes consist of a single-stranded positive-sense RNA of approximately 12.3 Kb in length, flanked at both ends by untranslated regions (UTR). Different genomic regions have been used to study the viral genetic diversity, such as 5' UTR, and regions coding for glycoprotein E2, auto-protease Npro, and NS3 protease [8, 9]. To date, 21 BVDV-1 sub-genotypes (BVDV-1a to BVDV-1u) and 3 BVDV-2 sub-genotypes (BVDV-2a to BVDV- 2c) have been identified [10, 11]. A third species, referred as *Pestivirus H*, formerly known as HoBi-like virus or BVDV-3, is genetically and antigenically related to BVDV-1 and BVDV-2 [12].

The differences among the sub-genotypes are not related to the spectrum of clinical signs [13]. However, the viral sub-genotypes differences in cross-neutralization tests and monoclonal antibodies binding have been described [14]. Thus, the genetic diversity of BVDV isolates has not only scientific interest, but also practical implications for viral vaccine production, diagnosis, and epidemiology [15].

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Vaccination against BVDV is indicated for herds with positive diagnostic for BVDV, for animals with clinical/reproductive history compatible with BVDV clinical manifestations, or indicated for confined herds with constant turnover of animals [13]. Some studies also suggests that vaccination could be applied as a measure to reduce the damage caused by BVDV systematic testing and quarantine of purchased animals, as well as the use of vaccines composed of several BVDV sub-genotypes [16].

Studies focusing on BVDV worldwide have demonstrated a prevalence ranging from 18 to 93% [17–19]. In Brazil, many serological surveys have demonstrated the presence and broad distribution of BVDV infection in cattle herds with the frequency of positive animals ranging from 14% in Northeast region to 84% in Midwest region [20, 21]. However, serological data do not differentiate between BVDV sub-genotypes, leading to a gap in surveillance and better strategies of control. Studies using molecular tools are still scarce, and the prevalence of the genotypes circulating in Brazil is still poorly known [16, 22, 23]. Studies conducted in southern Brazil with animals with different clinical disease syndromes caused by BVDV demonstrated the presence of BVDV 2 in 45% of the analyzed samples, BVDV 1 in 40% and 15% of BVDV3 [24]. In the Southeast and central regions of Brazil, BVDV1a, BVDV1b, and BVDV2b sub-genotypes were detected [25].

Recent studies showed that genetic diversity of BVDV in a geographic area has been influenced by animal movement within countries and animal introduction from other countries [26–28]. Therefore, detection and molecular characterization of isolates is important to better understand the spatial distribution, as well as identification of other current genotypes circulating in the country, contributing to enhanced surveillance activities. In the present study, we focused on the molecular investigation and characterization of BVDV detected in cattle herds from different Brazilian states belonging to regions still under-researched for BVDV circulation.

This study was carried out from 2012 to 2014 in different regions of Brazil (Fig. 1 and Table 1), including Bahia State (Northeast), Espírito Santo and Minas Gerais States (Southeast), and Goiás State (Midwest). Two samplings were performed with cattle herds: The first sampling occurred from 2012 to 2013, where serum (blood fraction) and scab samples were collected from animals presenting clinical signs of ulcerative disease. The second sampling was performed from 2013 to 2014, in which peripheral blood mononuclear cells (PBMCs) from apparently healthy cattle were collected.

The blood cells were fractionated by density centrifugation through Ficoll Paque® (Life Science, USA). Total RNA was extracted from serum, scabs, and PBMCs by using Trizol® (Thermo Fisher, USA) according to manufacturer's instructions. The viral RNA was used for cDNA synthesis using 500 ng of random primers (Promega Corporation—USA)

and 500 ng of specific primers (12 reverse BVDV described by Ridpath et al., 1998). All samples were tested by PCR, targeting the 5' UTR region with primers and conditions previously described [29], resulting in amplicons of approximately 280 bp. Amplified cDNA fragments 5' UTR was directly sequenced by Sanger methodology on the ABI3130 platform (Life Technologies, Foster City, USA). Sequence quality was analyzed by the Applied Biosystems Sequence Scanner Software v1.0 (Applied Biosystems, 2012). A BLAST search was carried out to retrieve similar sequences that were aligned using the software MUSCLE. Selection of the best-fit nucleotide substitution model was performed using jModelTest. Phylogenetic tree reconstruction using maximum likelihood methods (ML) was performed using PhyML v.3.1 with 100 bootstrap replicates. The FigTree program v.1.4.0 was used for phylogenetic tree reconstruction. To identify significant association between captured variables with PCR results, a bivariate analysis was carried out using chi-square and Fisher's exact tests with a significance level of 5% by using EPI-INFO software version 7.2 (www.cdc.gov/epiinfo).

A total of 215 bovine samples were analyzed, in which 117 (54.0%) were from animals presenting clinical signs of ulcerative disease (94 serum samples and 23 scab samples) and 100 samples of PBMCs (46.0%) from apparently healthy animals. Of 117 samples collected from 2012 to 2013, 22 serum samples (23.4%) and 23 scabs (30.4%) tested positive for BVDV, and almost half of the PBMCs (49.0%) collected from 2013 to 2014 were also positive. Global distribution showed that most PCR positive samples were from Southeast region (23.5%), followed by Northeast and Midwest regions (8.3 and 3.7%, respectively) (Table 1).

Variables statistically associated with the molecular detection of BVDV-1 were location and year of sampling (Table 1). Animals from Minas Gerais State were almost four times more likely to be infected by BVDV-1 compared to animals from Bahia, Espírito Santo, and Goiás States (OR = 3.6; 95% CI = 1.9–7.0). Furthermore, animals sampled during the 2012–2013 period were almost three times more likely to be infected by BVDV-1 than those animals sampled during the 2013–2014 period (OR = 2.9; 95% CI = 1.6–5.2).

PCR products from three positive samples were sequenced and deposited in GenBank under accession numbers MF803824, MF803825, and MF803826. Sequenced samples were from Northeast region (named as Ba 1-2012 and Ba 2-2012) and from Midwest region (Go-2013) (Fig. 2). Phylogenetic analysis showed that our samples were grouped with BVDV genotype 1a, with identity values ranging from 98.9 to 100% among them (Table 2). Furthermore, we observed a 98–99% identity between our isolates and a sample detected in Chile in 2001 (AY671977) and a laboratory sample from the USA (AJ133738) (Table 2). By contrast, the lowest identity (88–90%) was observed with isolates from Santa Catarina (South Brazil) in 2012 (KP715123) (Table 2).

Fig. 1 A general overview of the studied area. On the left, a map of South America targeting Brazil and some neighbor countries. On the right, a box highlighting Brazil and the four states where bovine herd were samples, which include Bahia, Espirito Santo, Minas Gerais, and Goiás

Bovine viral diarrhea virus has a global distribution and impacts animal health and reproductive performance, resulting in significant financial losses even though vaccination programs are available [1–4]. In the present study, we detected BVDV in 24% (29/117) of animals with clinical signs of ulcerative disease. The occurrences of ulcerative disease affecting livestock have been largely described in different regions of Brazil [24, 25, 30–33]. Monitoring ulcerative disease is of high importance for livestock due to the economic burden associated especially with cattle industry [34] as well as the possibility their clinical signs may be confused with foot-and-mouth disease, a high-morbidity disease, which has

Table 1 Variables associated with BVDV-1 detection in Brazilian cattle herd, 2012–2014

Variables	Total cattle population $n = 215$ (%)	Positive animals $n = 77$ (%)	Negative animals $n = 138$ (%)	OR (95% CI)	*p* value
Location					
Bahia	86 (40.0)	18 (23.4)	68 (49.3)		Reference
Minas Gerais	98 (45.6)	48 (62.3)	50 (36.2)	*3.6 (1.9–7.0)*	*< 0.0001*
Espírito Santo	12 (5.6)	3 (3.9)	9 (6.5)		0.99
Goiás	19 (8.8)	8 (10.4)	11 (8.0)		0.1
Year of sampling					
2012–2013	117 (54.4)	29 (37.7)	88 (63.7)		
2013–2014	98 (45.6)	48 (62.3)	50 (36.3)	*2.9 (1.6–5.2)*	*0.0004*

Fig. 2 Phylogenetic trees based on the nucleotide sequences of the BVDV 5' UTR region. Phylogenetics trees constructed based on nucleotide sequences of 222 bp 5' UTR region. **a** Phylogenetic tree showing the genotype grouping of BVDV-1a. The samples sequenced in the present study are indicated with black dots: GO-2013 (MF803826), BA1-2012 (MF803824), and BA2-2012 (MF803825). Phylogenetic tree were constructed using the maximum likelihood method with 100 bootstrap of replicates by using the Akaike information criterion (AIC). Based on the AIC, the TIM2 with gamma correction (TIM2 + G) and Kimura 80 model with estimated invariable site and gamma correction (K80 + I + G) were the best-fit model. **b** Phylogenetic tree showing a general overview of BVDV genotypes from 1a to 2c
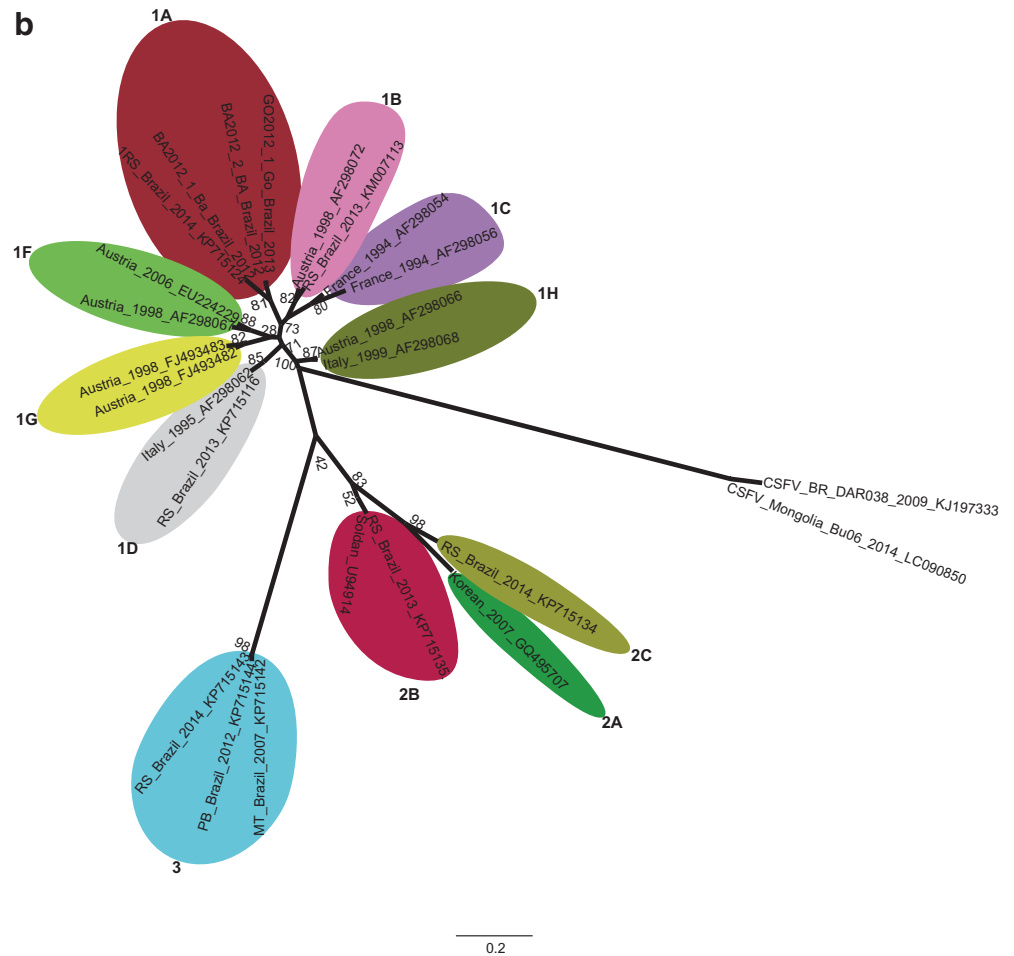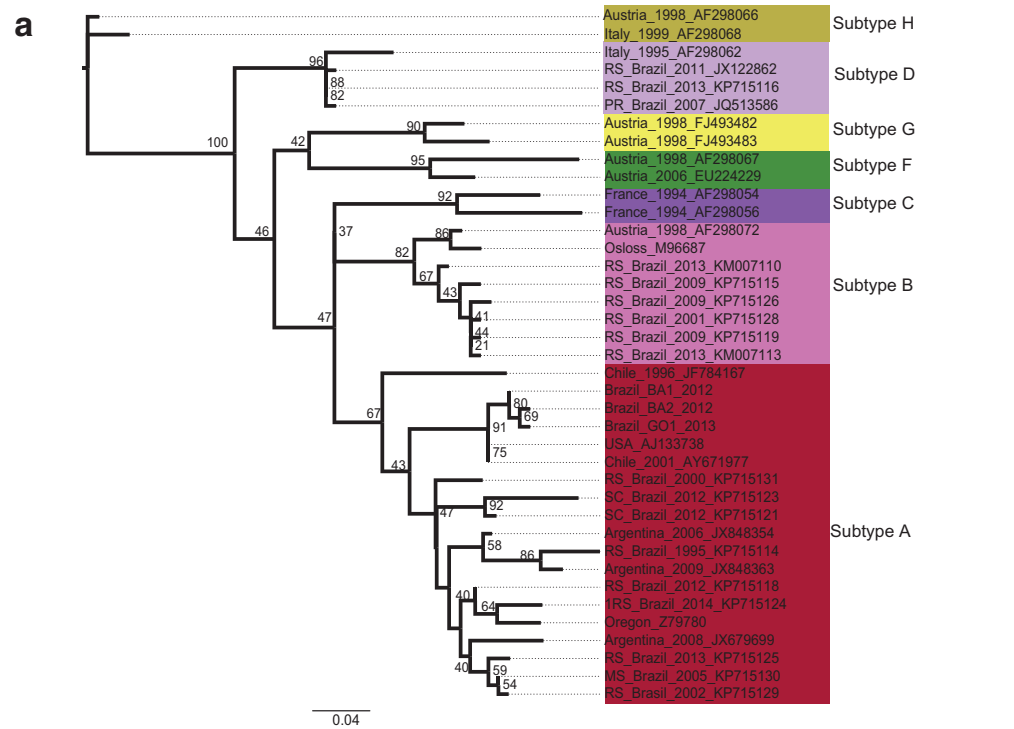
Table 2 Nucleotide percentage of identities among sequences of BVDV strains

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1- Brazil BA1 2012 MF803824																	
2- Brazil BA2 2012 MF803825	99%																
3- Brazil GO1 2013 MF803826	99%	99%															
4- 1a MS Brazil 2005 KP715130	91%	90%	91%														
5- 1a RS Brasil 2002 KP715129	91%	90%	91%	99%													
6- 1a RS Brazil 1995 KP715114	93%	92%	93%	93%	93%												
7- 1a RS Brazil 2000 KP715131	93%	92%	92%	96%	96%	91%											
8- 1a RS Brazil 2012 KP715118	93%	92%	93%	97%	97%	93%	97%										
9- 1a RS Brazil 2013 KP715125	91%	90%	90%	98%	98%	93%	96%	97%									
10- 1a RS Brazil 2014 KP715124	92%	91%	91%	94%	93%	91%	96%	97%	93%								
11- 1a SC Brazil 2012 KP715121	92%	91%	92%	95%	95%	93%	94%	96%	93%	95%							
12- 1a SC Brazil 2012 KP715123	89%	89%	89%	91%	91%	89%	90%	92%	89%	92%	95%						
13- 1a Strain Draper L32880	87%	86%	87%	92%	92%	86%	90%	90%	90%	92%	92%	89%					
14- 1a strain Oregon Z79780	90%	88%	89%	94%	93%	89%	94%	97%	93%	93%	92%	88%	86%				
15- 1a USA AJ133738	99%	98%	98%	93%	93%	93%	94%	94%	92%	93%	93%	90%	88%	91%			
16- 1a Chile 2001 AY671977.1	99%	98%	98%	93%	93%	93%	94%	94%	92%	93%	93%	90%	88%	91%	100%		
17- 1a Argentina 2008 JX679699.1	90%	88%	89%	95%	96%	90%	94%	96%	94%	94%	93%	89%	90%	92%	91%	91%	
18- 1a Argentina 2009 JX848363.1	94%	93%	94%	94%	94%	97%	93%	94%	94%	92%	94%	90%	90%	91%	94%	94%	91%

Identities were calculated as the percentage of nucleotide substitution on two by two comparisons of strain sequences. Sequences alignments and the percentage of identities were calculated by the software MEGA. Nucleotide sequence identities are shown on the down left side of the table. The calculations were based on 222 bp of the 5' UTR region

a major factor for livestock trade when considering its economic impact [1–4].

Although several studies have focused on viral pathogens implicated with ulcerative disease affecting cattle such as *Pseudocowpoxvirus*, *Bovine popular stomatitis virus*, Bovine herpesviruses, and Orthopoxviruses, few are the studies reporting BVDV characterization and specific genotypes circulating in Brazilian cattle herds [13, 22, 24, 25, 35–38]. BVDV-1a seems to be the most prevalent genotype circulating in Brazil, followed by BVDV-2b [23]. BVDV-1a also circulates in neighboring countries, such as Argentina and Uruguay [39, 40]. In this study, sequenced samples showed a greater identity with a sample detected in Chile in 2001 (Table 2). The same profile was observed for the sample that was circulating in Brazil in 1995 (KP715114), which showed a higher identity with samples from Argentina in 2009 (JX848363.1) than with Brazilian samples from the nearest years (Table 2). This may be due to the high viral mutational rate and/or a possible viral circulation between Brazil and neighboring countries.

Livestock movements and trade have been implicated in the spread of infectious diseases worldwide [41–43]. Hence, the detection of BVDV genotypes similar to those detected in neighbor countries could be attributed to associated with animal movement and trade at domestic and international levels, being fundamental to help alert sanitary authorities to the spread of BVDV strains and also improve surveillance and control strategies.

We were also able to detect BVDV-1a in clinically inapparent infected cattle. A high BVDV seroprevalence (up to 50%) has been reported in Brazilian cattle herd through seroepidemiological studies [38, 44, 45]. Our samples do not represent all the country and did not have complete coverage of all farms in the sampled regions due to labor and budget limitations. However, our findings add important information on BVDV molecular epidemiology in Brazilian cattle herds. To better understand the molecular profile of BVDV circulation in Brazil, a nationally representative study of the cattle herds is needed.

In conclusion, our findings provide relevant information to the epidemiological surveillance of BVDV in Brazilian cattle herds, thus allowing a better understanding of its geographical distribution in the country. These data also support the need for improving sanitary politics in the veterinary sector in order to suggest preventive programs that could potentially decrease the economic burden to the cattle industry.

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