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A genetic profile of bovine pestiviruses circulating in Brazil (1998–2018)

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Review

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Abstract

The pestiviruses bovine viral diarrhea virus 1 (BVDV-1), 2 (BVDV-2), and HoBi-like (HoBiPeV) are endemic among Brazilian cattle, the world's largest commercial bovine herd. In the last two decades (1998–2018) over 300 bovine pestiviruses have been partially or fully sequenced in Brazil, including viruses from different regions, different epidemiological backgrounds, and associated with diverse clinical presentations. Phylogenetic analysis of these viruses demonstrated a predominance of BVDV-1 (54.4%), with subgenotypes −1a (33.9% of total) and −1b (16.3%) being more frequent and subgenotypes −1d, −1e, and −1i at very low frequencies. The overall BVDV-2 frequency was 25.7% but it varied largely by region, reaching up to 48% in Southern states. BVDV-2b was the predominant subgenotype (84.8% of BVDV-2), followed by BVDV-2a (8.86%). HoBiPeV accounted for 19.9% (61/307) of the genotyped viruses and were detected at high frequency in cattle from Northeastern states. These findings demonstrate a unique mix of pestivirus species and subgenotypes, unlike that seen in Europe or North America. The design of effective diagnostic tools, vaccines, and control programs for limiting bovine pestivirus infections in Brazil must take into consideration this unique mix of viruses. This article provides a critical review of two decades of genetic identification of pestiviruses in Brazil.

Introduction

The genus *Pestivirus*, family *Flaviviridae*, comprises important animal viruses, including the cattle pathogens bovine viral diarrhea virus 1 (BVDV-1) and 2 (BVDV-2), and the recently classified HoBi-like pestivirus (HoBiPeV) (ICTV, 2017; Simmonds *et al.*, 2017). Pestiviruses are small (40–50 nm), enveloped viruses, containing a single-stranded, positive sense RNA genome of approximately 12.3 kb in length. The viral genome contains a long open reading frame (ORF) flanked by two untranslated regions (5′ and 3′ UTRs), respectively. The ORF encodes a long polyprotein which is co- and post-translationally cleaved by viral and host proteases in 11–12 mature viral polypeptides: N^{pro}, C, E0/E^{rns}, E1, E2, p7, NS23 (NS2-3), NS4A, NS4B, NS5A, and NS5B (Tautz *et al.*, 2015).

Historically, pestivirus species were defined by several criteria including the origin host species, comparisons of the complete coding nucleotide sequences, and cross-neutralization titers (Becher *et al.*, 2003; Simmonds *et al.*, 2017). Nucleotide sequencing and comparison of the highly conserved 5′ UTR, in addition to N^{pro} and E2 sequences – or even the complete coding sequence – have been employed for pestivirus phylogeny and genotyping (Pellerin *et al.*, 1994; Ridpath *et al.*, 1994; König *et al.*, 1997; Becher *et al.*, 2003; Liu *et al.*, 2009*a*, 2009*b*). According to these criteria, BVDV-1 isolates have been allocated into at least 21 subgenotypes (named −1a to −1u), whereas four BVDV-2 subgenotypes (−2a to −2d) have been described to date (Giangaspero *et al.*, 2008; Yeşilbağ *et al.*, 2017). Based on a limited number of isolates/genomes already genotyped, the newly classified HoBiPeV may be allocated into, at least, four subgenotypes or subgroups (Giammarioli *et al.*, 2015).

BVDV-1 and BVDV-2 have the broadest global distribution and have been associated with a variety of clinical manifestations in cattle (Houe, 2003) and a wide range of ruminant species, both domestic and free ranging. HoBiPeV were initially identified as contaminants of fetal bovine serum (FBS) of Brazilian origin (Schirrmeier *et al.*, 2004) and subsequently associated with a variety of clinical manifestations in cattle in South America (Cortez *et al.*, 2006; Decaro *et al.*, 2011; Bauermann *et al.*, 2013; Marques *et al.*, 2016; Weber *et al.*, 2016*a*, 2016*b*; Mósena *et al.*, 2017*a*, 2017*b*; Silveira *et al.*, 2017), Italy (Decaro *et al.*, 2011), Thailand (Liu *et al.*, 2009*a*, 2009*b*), India (Mishra *et al.*, 2014), and Bangladesh (Haider *et al.*, 2014). It has also been isolated from water buffalo. The origin, distribution, and relevance of this novel bovine pestivirus is still unclear (Bauermann *et al.*, 2013).

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Brazil has the world's largest commercial bovine herd with estimates exceeding 211 million cattle (BRASIL, 2014). Several studies have demonstrated a widespread distribution of BVDV among Brazilian cattle (Canal *et al.*, 1998; Gil *et al.*, 1998; Flores *et al.*, 2002; Cortez *et al.*, 2006; Lunardi *et al.*, 2008; Bianchi *et al.*, 2011, 2017; Otonel *et al.*, 2014; Weber *et al.*, 2014; Mósena *et al.*, 2017*a*, 2017*b*; Silveira *et al.*, 2017; Monteiro *et al.*, 2018*a*, 2018*b*). A few inactivated vaccines are available in Brazil, most containing both BVDV-1 and BVDV-2, but some containing only BVDV-1 (Anziliero *et al.*, 2015). Recently, an attenuated vaccine containing genetically modified BVDV-1 and BVDV-2 strains was licensed in the country. Regardless, vaccination against BVDV-associated diseases is not a widespread practice in Brazil and only 5 million doses of BVDV-containing vaccines were sold in 2016. HoBiPeV antigens have not been introduced in commercial vaccines yet in spite of a crescendo identification of these viruses in Brazilian cattle (Monteiro *et al.*, 2018*a*, 2018*b*).

In the last two decades (1998–2018), selected BVDV-1 and BVDV-2 subgenotypes have been shown to circulate among Brazilian cattle and other related species (Canal *et al.*, 1998; Gil *et al.*, 1998; Flores *et al.*, 2002; Cortez *et al.*, 2006; Lunardi *et al.*, 2008; Bianchi *et al.*, 2011, 2017; Otonel *et al.*, 2014; Weber *et al.*, 2014; Mósena *et al.*, 2017*a*, 2017*b*; Silveira *et al.*, 2017; Monteiro *et al.*, 2018*a*, 2018*b*). Likewise, atypical pestiviruses (subsequently identified as HoBiPeV) have been isolated from animals since the early 2000s and a number of studies indicate that these viruses are endemic in Brazil (Cortez *et al.*, 2006; Bianchi *et al.*, 2011; Marques *et al.*, 2016; Weber *et al.*, 2016*a*, 2016*b*; Mósena *et al.*, 2017*a*, 2017*b*; Silveira *et al.*, 2017, 2018; Monteiro *et al.*, 2018*a*, 2018*b*). This article provides a critical review of the published work concerning the genetic identification and genotyping of bovine pestiviruses, with the goal of generating an approximate genetic profile of Brazilian bovine pestiviruses. A chronological history of the genetic identification, clinicpathological, and geographical origin of these viruses is presented in Table 1. The Supplementary file contains more details and sequence information on these viruses.

Early studies

The earliest article of genetic identification of bovine pestiviruses in Brazil dates from the late 1990s and reports the genotypic identification and phylogeny of two non-cytopathic BVDV isolates (Soldan and BR275) based on a 247 nt sequence within the 5′UTR (Canal *et al.*, 1998). The Soldan strain – identified as BVDV-2 – had been isolated from an animal with mucosal-like disease (MD) six years earlier (1992) in Rio Grande do Sul (RS), the southernmost Brazilian state. Thus, the isolation of Soldan strain preceded the first reports of the identification of BVDV-2 in North America (Pellerin *et al.*, 1994; Ridpath *et al.*, 1994). Strain BR275 was isolated from the serum of an antibody-negative heifer in RS and identified as BVDV-1a. The study by Canal *et al.* paved the way for a number of reports of genetic identification of bovine pestiviruses in Brazil (Table 1).

Next, Gil *et al.* (1998) performed the genetic analysis of 16 BVDV isolates from various sources, including ten viruses obtained from fetuses in a slaughterhouse in RS (1997–1998), two isolated from animals with BVD signs (Santa Catarina state, SC, southern Brazil) and four isolated from the sera of calves from herds with reproductive problems in Sao Paulo state (SP), southeastern country (Table 1, Supplementary file). Genetic analysis based on the 5′ UTR sequences identified 11 BVDV-1 (three BVDV-1a and nine BVDV-1b) and four BVDV-2 (no genetic subtyping of BVDV-2 was conducted at that time). Two of these BVDV-2s had been isolated from clinical cases resembling the acute BVD described in North America a few years earlier (Pellerin *et al.*, 1994; Ridpath *et al.*, 1994).

A subsequent study by Flores *et al.* (2002) provided the first evidence of the existence of subgenotypes within the BVDV-2 species. Comparison of BVDV-2 sequences available to that date demonstrated the existence of two genetically distinct BVDV-2 subgroups. Subgenotype BVDV-2a included most North American, European, and Asian isolates while subgenotype BVDV-2b comprised South American isolates. Currently, comprehensive genetic analysis including a high number of isolates from different locations based on the 5′UTR, N^{pro}, and E2 indicates the existence of four BVDV-2 subgenotypes (−2a to −2d) (Giangaspero *et al.*, 2008; Yeşilbağ *et al.*, 2017). It should be noted that only one isolate was identified as BVDV2d in the analysis by Giangaspero *et al.* (2008).

A new player comes into play

An article by Schirrmeier *et al.* (2004) represented a hallmark on the recent history of bovine pestiviruses. A quality control routine of FBS conducted in Germany led to the identification of a novel pestivirus – named D32/00_'HoBi' – in a batch of Brazilian serum. Sequence analysis of the entire N^{pro} and E2-coding sequences, complemented by cross-neutralization and monoclonal antibody-binding assays indicated that the isolate D32/00_'HoBi' was distinctly different from the known pestiviruses (Schirrmeier *et al.*, 2004; Bauermann *et al.*, 2012). Thus, isolate D32/00_'HoBi' was proposed as the prototype of a novel pestivirus genetic group, variously referred to as HoBi-*like*, atypical pestiviruses or, simply, BVDV-3 (Liu *et al.*, 2009*a*, 2009*b*; Decaro *et al.*, 2011; Bauermann *et al.*, 2013). Viruses belonging to this genetic group were subsequently found in FBS from different geographical origins and in clinical specimens obtained from cattle with a variety of clinical manifestations, in South America (Cortez *et al.*, 2006; Marques *et al.*, 2016; Weber *et al.*, 2016*a*, 2016*b*; Mósena *et al.*, 2017*a*; Monteiro *et al.*, 2018*a*; Silveira *et al.*, 2018), Italy (Decaro *et al.*, 2011), Thailand (Liu *et al.*, 2009*a*, 2009*b*), India (Mishra *et al.*, 2014), and Bangladesh (Haider *et al.*, 2014). Recently, this group of viruses has been recognized as a new pestivirus species, named *Pestivirus H* (ICTV, 2017) (proposed abbreviation, HoBiPeV). The origin, distribution, and relevance of HoBiPeV are still unclear, yet a number of studies demonstrated that these viruses are endemic in Brazil (Cortez *et al.*, 2006; Weber *et al.*, 2016*a*, 2016*b*; Silveira *et al.*, 2017, 2018; Monteiro *et al.*, 2018*a*, 2018*b*).

Subsequently, Cortez *et al.* (2006) performed the phylogenetic analysis of 18 BVDV isolates obtained from laboratories in five Brazilian states, between 1997 and 2004, and isolated from cattle with varied clinical backgrounds, including persistently infected (PI) animals and aborted fetuses. Based on comparison of the 5′UTR, 10 were identified as BVDV-1 (seven as BVDV-1a and three as BVDV-1b) and six were identified as BVDV-2 (two as BVDV-2a and four as BVDV-2b). Two viruses grouped with the prototype D32/00_ HoBi (Schirrmeier *et al.*, 2004) and were classified as 'atypical pestiviruses' at the moment. The clinical history of these two viruses dates back to 2000 and 2002, respectively, when they were isolated from aborted fetuses in SP state (Table 1). This was apparently the first published isolation of 'atypical

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Table 1. Identification, typing, and subgenotyping of bovine pestiviruses from Brazil (1998–2018)

Year isolation/ detection	State	*n*	History	Target	Genotypes/ subgenotypes	Reference
1992, NI[a]	RS (2)	2	Animal with MD signs (Soldan, BVDV-2b); healthy animal of a herd with reproductive problems.	5′UTR	BVDV-1a (1) BVDV-2b (1)	Canal *et al.* (1998)
1995–1997	RS (11) SC (1) SP (3) PB (1)	16	Fetal serum collected in a slaughterhouse (9), animals with BVD signs (3), healthy calves of herds with reproductive problems (4)	5′UTR	BVDV-1a (3) BVDV-1b (9) BVDV-2b (4)	Gil *et al.* (1998)
1995–2014	RS MS MT SC PB PR	41* (89)	Collection of isolates from animals (25), cell culture (9), unknown origin (4), FBS (3) *Many isolates have been described in other articles or have no origin/description	5′UTR, N^{pro}, E2	BVDV-1a (18) BVDV-1b (5) BVDV-1d (4) BVDV-1e (1) BVDV-2b (5) BVDV-2c (2) HoBiPeV (6)	Silveira *et al.* (2017)
1997–2004	RS (8) MG (1) MT (1) PR (5) SP (3)	19* (18)	Respiratory and/or gastroenteric disease, abortions, PI animals *One isolate previously genotyped	5′UTR	BVDV-1a (7) BVDV-1b (3) BVDV-2a (2) BVDV-2b (4) HoBiPeV (2)	Cortez *et al.* (2006)
2000–2010	RS (19)	20* (19)	Gastroenteric, respiratory disease, PI animals, abortions, MD-like disease, retarded growth, semen *One isolate previously genotyped in other study	5′UTR	BVDV-1a (6) BVDV-1b (1) BVDV-2a (3) BVDV-2b (6) HoBiPeV (3)	Bianchi *et al.* (2011)
2004	*RS (5)*	10* (5)	Fetal bovine sera and serum of PI animals *Five viruses were previously genotyped by Gil *et al.* (1998)	5′UTR, N^{pro}	BVDV-1a (3) BVDV-1b (1) BVDV-1d (1)	Vilcek *et al.* (2004)
2004	BR	1	Lot of FBS produced in Brazil	5′UTR, N^{pro}, E2, NS3, 3′UTR	HoBiPeV	Schirrmeier *et al.* (2004)
2004	PR (1)	1	Acute BVD in a beef cattle herd – six steers affected	5′UTR	BVDV-1b (1)	Lunardi *et al.* (2008)
2006	MG (2)	2	Healthy PI animals	5′UTR	BVDV-1b (2)	Dias *et al.* (2017)
2006–2014	BR (13)* SP/MS (31)	44	Lots of pooled fetal bovine serum (31) and packed/labeled FBS produced in Brazil (13) *No state of origin available	5′UTR	BVDV-1a (23) BVDV-1b (8) BVDV-1d (3) BVDV-2* (1) BVDV-2a (2) BVDV-2b (3) HoBiPeV (4)	Monteiro *et al.* (2018*a*, 2018*b*)
2007	RS (1)	3* (1)	PI animals *All sequences had 100% of nt identity	5′UTR	BVDV-2b (1)	Santos *et al.* (2011)
2007–2009	PR (4)	4	Serum samples from transiently and persistently infected animals from a vaccinated dairy herd with reproductive problems	5′UTR N^{pro}	BVDV-1a (1) BVDV-1b (2) BVDV-1d (1)	Otonel *et al.* (2014)
2010	RS (25)	33* (25)	Serum of 6–12 months-old cattle screened for FMDV antibodies *Eight sequences had 100% nt identity with genotyped sequences	5′UTR, N^{pro}	BVDV-1a (15) BVDV-1b (3) BVDV-1d (1) BVDV-2b (14)	Weber *et al.* (2014)
2011	PR (2)	2	Beef cattle that died due to concomitant BVDV infection, mycotoxicosis and seneciosis	5′UTR	BVDV-1d (2)	Headley *et al.*, (2014)
2011	PA (1)	22* (1)	Serum of cattle with vesicular disease Only one virus of the outbreak has been sequenced	5′UTR	BVDV-1a (1)	Alves *et al.* (2016)

(*Continued*)

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Table 1. (*Continued.*)

Year isolation/ detection	State	*n*	History	Target	Genotypes/ subgenotypes	Reference
2011–2012	PB (4)	4	Herd with reproductive problems; animals affected by a MD-like disease	5′UTR, N^{pro}, E2	HoBiPeV (4)	Weber *et al.* (2016*a*, 2016*b*)
2012	RS (1)	1	Lung samples from a farmed wild boar obtained in a slaughterhouse	5′UTR	BVDV-2b (1)	Weber *et al.* (2016*a*, 2016*b*)
2012	PB (1)	1	Three-months-old female calf with signs of acute BVD	5′UTR	HoBiPeV (1)	Marques *et al.* (2016)
2012–2013	MA (10) RN (7)	17	Serum of 16.621 cattle from 569 herds screened for FMDV antibodies	5′UTR, N^{pro}, E2	HoBiPeV (17)	Silveira *et al.* (2018)
2012–2014	RS (10) SC (1)	11	PI animals, fetal serum, clinical BVD cases, calves with retarded growth	5′UTR	BVDV1a (3) BVDV 1b (5) BVDV-2b (1) BVDV-2* (2)	Unpublished
2015	SP	1	Virus recovered from a heifer with MD-like disease	Full genome	HoBiPeV (1)	Cortez *et al.* (2017)
2015	RS (1)	22* (1)	Outbreak of MD-like disease without classical intestinal lesions *Only one isolate of the outbreak has been sequenced	5′UTR N^{pro}	BVDV-1d (22)	Bianchi *et al.* (2017)
2016	RS (1)	1	Four-year-old cow with signs of BVD	5′UTR N^{pro}	BVDV-1i (1)	Mósena *et al.* (2017*a*, 2017*b*)
2016	MA (2)	2	Serum of buffaloes from two herds with high BVDV serology	5′UTR	BVDV-1b (2)	Paixão *et al.* (2018)
2017	RS (90)	90	Screening of sera of beef calves destined to export to Europe	5′UTR	BVDV-1a (28) BVDV-1b (10) BVDV-2b (31) HoBiPeV (21)	Monteiro *et al.* (2018*a*, 2018*b*)

[a]No information available.

pestiviruses' from cattle in Brazil, as opposed to FBS, and indicated the circulation of HoBiPeV in Brazil as early as by 2000. However, HoBiPeV may have been present even earlier in Brazil based on the isolation of strain BrazBuf930 in the late 1990′s, in an outbreak of disease in water buffaloes (Canal, unpublished data; Bauermann *et al.*, 2013).

Bianchi *et al.* (2011) performed a genetic and antigenic characterization of 20 pestivirus isolates obtained from PI animals, calves with retarded growth, clinical cases of BVD, aborted fetuses and semen collected between 2000 and 2010 in RS. Phylogenetic analysis based on the 5′UTR identified nine BVDV-2 (three BVDV-2a and six BVDV-2b), seven BVDV-1 (six BVDV-1a and one BVDV-1b) and three isolates were classified as 'atypical pestiviruses' (Table 1). These viruses, subsequently classified as HoBiPeV, were isolated from commercial bull semen associated with the birth of blind calves (SV713/00), from a calf of a herd with reproductive problems (SV241/10) and from an aborted fetus (SV311/10).

Genetic typing of viruses associated with clinical cases/ outbreaks

A number of studies reported the genetic identification of pestiviruses associated with clinical cases, disease outbreaks or, simply, of viruses detected in the sera of cattle – or water buffaloes – from herds with positive BVDV serology and/or with reproductive problems (Table 1). Lunardi *et al.* (2008) performed the genetic identification of a pestivirus involved in an outbreak of gastroenteric disease in Parana state (PR), southern Brazil, in which six steers developed signs of acute BVD (depression, anorexia, watery diarrhea, sialorrhea, and weakness) and died 24 h to 15 days after observation of the first clinical signs. Samples of different organs obtained from a necropsied steer were positive in a 5′UTR-based RT-PCR and genetic analysis identified a BVDV-1b genome.

Dias *et al.* (2013) identified two BVDV isolates obtained from the serum of PI calves from a crossbred herd in Southeastern Brazil (state of Minas Gerais, MG) as BVDV-1b, based on phylogenetic analysis of the 5′UTR. The herd had no reports of clinical signs or reproductive failure suggestive of BVDV infection.

Otonel *et al.* (2014) performed a RT-PCR screening in sera from vaccinated cattle of a dairy herd with reproductive problems in PR state, detecting the BVDV genome in 27 cows and two PI calves. Sequencing of 5′UTR and/or N^{pro} amplicons obtained from four animals revealed three different BVDV subgenotypes (BVDV-1a, BVDV-1b, and BVDV-1d), calling attention for the possible infection of open herds with multiple BVDV subgenotypes.

A HoBiPeV strain – identified by analysis of the 5′UTR – was associated with a case of acute, fatal gastroenteric disease in the semi-arid region of Paraiba state (PB), northeastern Brazil (Marques *et al.*, 2016). A HoBiPeV was also identified in a spleen sample from a dead calf presenting signs of acute BVDV in a beef cattle herd in Mato Grosso (MT). In this study, phylogenetic

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analysis was based on comparison of both 5′UTR and N^{pro} gene sequences (Rodrigues *et al.*, 2011).

Paixão *et al.* (2018) reported the genetic identification of BVDV isolated from the serum of water buffalo calves from two herds with high levels of BVDV antibodies in Maranhão state, northeastern Brazil. Phylogenetic analysis based on the 5′UTR revealed two BVDV-1b isolates circulating in each herd. Apparently, this was the first identification of active BVDV infection in water buffaloes in Brazil.

Bianchi *et al.* (2017) reported the isolation of a noncytopathic BVDV from 22 affected animals in an outbreak of MD in RS state. This outbreak was unusual in that there were no intestinal lesions. Phylogenetic analysis based on 5′UTR and N^{pro} sequences revealed that all 22 animals were infected with the same BVDV-1d virus. Headley *et al.* (2014) identified a BVDV-1d in specimens from two cows that died after clinical manifestations of uncoordinated gait, fever, transient bloody diarrhea, dyspnea, and lateral decumbency, probably concomitantly intoxicated by mycotoxins and *Senecio brasiliensis*.

In RS state, five calves PI with BVDV were submitted to necropsy after developing clinical signs characterized by growth impairment, nasal and ocular discharge, and congenital cataract. Amplification of the 5′UTR followed by phylogenetic analysis revealed that all five calves were infected with a BVDV-2b strain (Santos *et al.*, 2011). A BVDV-2b isolate was also detected in lung samples of a wild boar collected in a commercial slaughterhouse in RS in 2012 (Weber *et al.*, 2016*a*, 2016*b*). Alves *et al.* (2016) detected a BVDV-1a genome in the sera of cattle during an outbreak of vesicular disease by pseudocowpox virus in Pará (PA), northern Brazil. Nucleotide sequencing and phylogeny based on the 5′UTR demonstrated a high similarity with Oregon C24V BVDV strain.

Genotyping of laboratory pestivirus collections

A few studies reported the genetic identification of laboratory collections of pestiviruses (Table 1). Silveira *et al.* (2017) genetically characterized a collection of 89 pestiviruses obtained from different sources in Brazil, between 1995 and 2014. The 5′UTR, N^{pro} and E2 regions were used for genotyping isolates/sequences from cattle (25), contaminated cell cultures (9), FBS (3), or from unknown origin (4). Forty-eight of these isolates had been genotyped in previous studies (Cortez *et al.*, 2006; Bianchi *et al.*, 2011; Weber *et al.*, 2014). Out of the 41 viruses not been previously genotyped (Supplementary file), 28 (68.3%) were identified as BVDV-1, seven as BVDV-2 (17%), and six as HoBiPeV (14.6%). Eighteen viruses were identified as BVDV-1a (43.9%), five as BVDV-2b (12.2%), five as BVDV-1b (12.2%), four BVDV-1d (9.75%), one BVDV-1e (2.4%), and two BVDV-2c (4.9%). Among all 89 sequences, 48 (53.9%) were classified as BVDV-1, 30 as BVDV-2 (33.7%), and 11 (12.4%) as HoBiPeV (*HoBi-like*). Nineteen of the 89 isolates, mainly BVDV-1, had identical sequences, possibly reflecting laboratory contamination. This study included a considerable number (9.75%) of viruses/genomes of unknown origin ($n = 4$) and contaminants of cell cultures (9), including two BVDV-2c genomes. The geographical origin of these viruses is uncertain and, as such, these sequences should not be considered as part of Brazilian BVDV genotypes.

Vilcek *et al.* (2004) characterized BVDV isolates from different countries, including ten from Southern Brazil. From these, five had been genotyped previously (Gil *et al.*, 1998; Cortez *et al.*, 2006). The remaining five isolates included three BVDV-1a, one BVDV-1b, and one BVDV-1d. These viruses were originally isolated at our laboratory (Virology Section of the Federal University of Santa Maria, RS) from sera of bovine fetuses or from the blood of animals from herds with reproductive failure. The correct identification of these isolates is in the Supplementary file.

Genetic identification of pestiviruses in cattle sera

Some studies reported the genetic detection and identification of pestiviruses present in serum of cattle collected for diverse purposes (Table 1). Weber *et al.* (2014) performed a screening and genetic identification of pestiviruses in sera of 9,078 young cattle submitted to an official serological survey for foot and mouth disease virus (FMDV) in RS (2010). Serum samples were mixed in 227 pools of up to 44 samples and submitted to RT-PCR, followed by individual testing of samples composing the positive pools. Genomes of 25 different viruses were detected and analyzed based on 5′UTR and N^{pro} (Supplementary file). BVDV-2b was the predominant subgenotype (48%), followed by −1a (36%), −1b (12%), and −1d (4%). Similar to the previous results of Bianchi *et al.* (2011), an unexpectedly high frequency of BVDV-2 was observed in RS state.

Silveira *et al.* (2018) performed a screening for pestivirus genomes in the sera of cattle, collected from 2012 to 2013, as part of an FMDV vaccination monitoring program, from two northeastern states [Maranhão (MA) and Rio Grande do Norte (RN)]. Serum samples from 16,621 cattle from 569 herds in both states were pooled in up to 45 samples/pool and tested by RT-PCR. Individual samples from positive pools were then submitted to RT-PCR followed by sequencing using 5′UTR, N^{pro}, and E2 primers. A total of 17 pestivirus genomes (0.1%) were detected in cattle from 15 herds (2.64%). All isolates were classified as HoBiPeV based on phylogenetic analysis (5′UTR, N^{pro}, E2). Interestingly, no BVDV-1 or BVDV-2 genomes were detected in the sampled population, in spite of several studies reporting the circulation of these viruses in the region. These results suggested that HoBiPeV viruses are the predominant pestiviruses in these northeastern Brazilian states. These findings – although potentially interesting – should be taken cautiously because RT-PCR testing of pooled samples containing mixed genomes or contamination may result in preferential detection of the most abundant genomes or the genomes with highest primer complementarity (Monteiro *et al.*, 2018*a*).

A comprehensive genetic identification of bovine pestiviruses in southern Brazil was recently conducted by Monteiro *et al.* (2018*b*). The authors reported the genetic detection and identification of 90 pestivirus genomes from sera of beef calves from RS state destined to export to Europe (2017). Screening of 15,684 serum samples of beef calves from hundreds of herds by an antigen capture ELISA and, subsequently, by RT-PCR revealed 135 samples containing pestivirus RNA. Genetic typing of these viruses based on the 5′UTR revealed 90 different viruses. Thirty-eight were identified as BVDV-1 (42.2%), 31 as BVDV-2 (34.4%), and 21 as HoBiPeV (23.3%). Among BVDV-1, only subtypes −1a ($n = 28$, 31.1%) and −1b ($n = 10$, 11.1%) were identified. All 31 BVDV-2 isolates belonged to BVDV-2b subtype and the 21 HoBiPeV viruses clustered to subtype 3a. Even though it focused on a single state, this study provided an approximate genetic profile of pestiviruses circulating in a geographically defined cattle population in an important Brazilian beef cattle-raising state.

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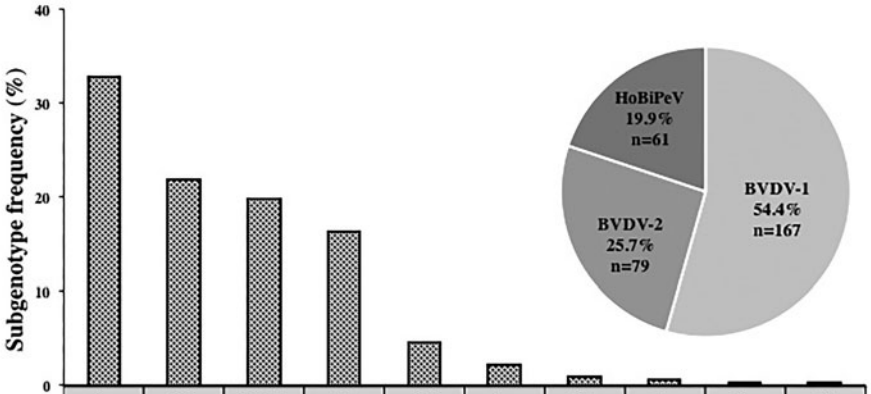

	1a	2b	HoBi-*like*	1b	1d	2a	2 *	2c	1c	1i
%	32.9	21.8	19.9	16.3	4.6	2.3	1.0	0.7	0.3	0.3
Total n=307	101	67	61	50	14	7	3	2	1	1

Fig. 1. Frequency of bovine pestivirus species/genotypes and subgenotypes in Brazil (1998–2018).

Pestivirus investigation in FBS

A study by Monteiro *et al.* (2018*a*) reported the screening and genetic identification of pestiviruses in 73 lots of FBS produced in Brazil between 2006 and 2014. Forty-six lots consisted of pooled fetal sera collected in slaughterhouses in Southeastern and Midwestern states. Twenty-seven samples represented commercial batches of FBS produced in Brazil, whose geographic origin within the country was unavailable (Table 1). Thirty-nine lots (53.4%) were positive for pestivirus RNA by RT-PCR. Nucleotide sequencing and phylogenetic analysis of the 5′UTR revealed 34 lots (46.6%) containing BVDV-1 (23 BVDV-1a, eight BVDV-1b, and three BVDV-1d). Six batches (8.2%) contained BVDV-2 (two BVDV-2a, three BVDV-2b, and one undetermined due to short nucleotide sequence) and four FBS batches (5.5%) harbored HoBiPeV virus genomes. Five batches (6.8%) contained more than one pestivirus. The lack of the geographical origin of most FBS batches somewhat restricts the epidemiological value of some of these findings.

Complete genome sequencing

In addition to D32/00_'HoBi' (Schirrmeier *et al.*, 2004), at least four full genomic sequences of pestiviruses isolated in Brazil have been published: three HoBiPeV and one BVDV-1i. Mósena *et al.* (2017*a*, 2017*b*) performed a full genomic sequencing and characterization of a BVDV-1i isolated from cattle in RS state. BVDV-1i is an uncommon subgenotype and has been detected in few regions (Yeşilbağ *et al.*, 2017). Phylogenetic analyses based on the whole genome, 5′UTR, and N^{pro} sequences showed that strain was closely related to previously characterized BVDV-1i (JQ920104.1, JQ920215.1, and FJ493484.1) from the UK (90.5% highest nt identity at 5′UTR) and Uruguay (KT833795.1) (90.6 high identity at N^{pro}). Mósena *et al.* (2017*a*, 2017*b*) performed the full genomic sequencing and antigenic characterization of two previously described Brazilian HoBiPeV isolates (Bianchi *et al.*, 2011; Weber *et al.*, 2016*a*, 2016*b*). According to the authors, the presented data provided evidence that HoBiPeVs are members of the genus *Pestivirus* and should be formally recognized as a novel species. Lastly, Cortez *et al.* (2017) published the complete genome sequence of a HoBiPeV (strain SV757/15) isolated from a Nelore heifer with gastroenteric disease in SP state.

In addition to the above studies, some studies by groups outside Brazil included Brazilian pestiviruses, including some already characterized (Schirrmeier *et al.*, 2004; Yeşilbağ *et al.*, 2017). In addition to published studies, unpublished data from our laboratory includes the genotyping of BVDV isolates from cases of gastroenteric and respiratory disease, PI animals, and fetal serum samples from RS and SC states (2014–2014). Phylogenetic analysis revealed BVDV-1a (3), BVDV-1b (5), and BVDV-2b (1) strains. Phylogenetic analysis of two remaining BVDV-2 isolates has not been performed yet.

The genetic profile of Brazilian bovine pestiviruses

Considering the published data and a few unpublished sequences from our laboratory, some 307 distinct pestivirus isolates were genotyped in the last two decades, including four full genomic sequences (Table 1). These numbers included only viruses unequivocally isolated from or detected in Brazilian sources, e.g. obtained from clinical specimens, FBS, or cattle serum of Brazilian origin. Thus, this calculation excluded viruses from uncertain origin, e.g. viruses detected in FBS of uncertain origin and viruses contaminating cell cultures. This total number also excluded multiple results for the same virus genotyped in different studies (Cortez *et al.*, 2006; Bianchi *et al.*, 2011; Silveira *et al.*, 2017) and multiple results for the same virus isolated from different animals during an outbreak (Otonel *et al.*, 2014; Bianchi *et al.*, 2017; Silveira *et al.*, 2017; Monteiro *et al.*, 2018*a*). The reviewed studies included genotyping of viruses involved in clinical cases and outbreaks, detected in PI animals in herds with reproductive failures, detected in pooled FBS and packed commercial FBS, detection of virus sequences in pooled or individual serum samples of cattle collected for diverse purposes, and laboratory collections of viruses from diverse clinic-pathological and epidemiological backgrounds. We also included four full genomic sequences of pestiviruses isolated in Brazil published by Brazilian researchers, excluding Brazilian isolates sequenced abroad.

Based on this inclusion criteria, the relative frequencies of pestivirus species and subgenotypes identified in Brazil (1998–2018) are presented in Fig. 1. The profile indicates a clear predominance of BVDV-1 (54.4%), with high frequency of subgenotypes −1a (33.9%) and −1b (16.3%). BVDV-1d was detected at low

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frequency (4.6%) and subgenotypes −1e and 1i were identified in one sample each. A number of studies have shown that various BVDV subgenotypes predominate in different countries (Yeşilbağ *et al.*, 2017). Thus, according to published data, BVDV-1b is the predominant subgenotype in North America (53%) and worldwide (31.6%), followed by BVDV-1a (19.9% in North America and 20.8% worldwide) (Yeşilbağ *et al.*, 2017). In contrast, our data indicated a predominance of BVDV-1a among Brazilian isolates.

In our review, 25.7% of the published sequences were identified as BVDV-2, with a clear predominance of BVDV-2b (85.2%) (Fig. 1). BVDV-2a were detected in low frequencies (2.2%) and BVDV-2c ($n = 2$) were only detected in contaminated cell cultures of unknown origin. This high BVDV-2 frequency overall is due, in part, to the unique high frequency of this genotype in Southern states, where it makes up to 48% of the genotyped viruses (Bianchi *et al.*, 2011; Weber *et al.*, 2014). Because sequences from southern Brazil contributed approximately 50% of the analyzed sequences, the overall frequency of BVDV-2 in Brazilian cattle may be somewhat lower than that reported herein. Considering only published sequences, BVDV-2 accounts for approximately 11.8% of BVDV sequences genotyped worldwide (Yeşilbağ *et al.*, 2017). A recent review indicated that BVDV-2a is the most prevalent subgenotype of BVDV-2 on all continents (Yeşilbağ *et al.*, 2017). BVDV-2c has been detected only in Europe and the Americas and a single contaminating BVDV strain from Argentina was classified as BVDV-2d (Giangaspero *et al.*, 2008). Thus, the observed high BVDV-2 prevalence seems somehow unique to Brazil, especially to southern states, compared to overall frequencies detected in other countries and continents (Yeşilbağ *et al.*, 2017). As BVDV vaccination is not a usual practice in Brazil, and only a small part of the herd is currently vaccinated (<5%), it is very unlikely that vaccination immunity would have influenced the frequencies of different viral species and subgenotypes.

HoBiPeV accounted for 19.9% (61/307) of the genotyped viruses (Fig. 1). This number was influenced by the high frequency of these viruses detected in some northeastern states. In fact, HoBiPeV was the only pestivirus detected in pools of cattle sera submitted to an official serological survey for FMDV antibodies (Silveira *et al.*, 2018). Thus, it is conceivable that the overall frequency of HoBiPeV circulating in Brazil may be somewhat lower than this number, as demonstrated by other studies (Cortez *et al.*, 2006; Bianchi *et al.*, 2011; Silveira *et al.*, 2017; Monteiro *et al.*, 2018*a*, 2018*b*). On the other hand, these data corroborate previous findings indicating that HoBiPeVs are endemic and contribute significantly for the pool of bovine pestiviruses circulating in Brazilian cattle.

The estimated prevalence of viral species and subgenotypes, presented herein, may be skewed. First, because more than 50% of the examined viruses were obtained in southern states and roughly 20% from southeastern and midwestern Brazil. Few sequences from northeastern and northern states were genotyped and published to date. Second, the high BVDV-2 frequency in Southern states certainly influenced its high frequency overall. Third, the high number of HoBiPeV genomes detected in some northeastern states likewise influenced their final numbers. Fourth, some studies performed genetic identification of viruses detected from pooled samples. In our experience, RT-PCR detection of viruses in pooled samples such as FBS and pooled sera frequently favors amplification of the most abundant genomes and those genomes with highest primer complementarity, missing viruses present in lower amounts or sequences with lower primer complementarity (Bauermann *et al.*, 2014; Monteiro *et al.*, 2018*a*, 2018*b*). In summary, the use of the published data to assemble a profile of Brazilian pestiviruses should be taken with caution because it may not represent the real frequencies and relative proportions of the viruses circulating in the field. As a consequence, the genetic profile of Brazilian bovine pestiviruses based on these studies seems to be more of a sketch than a well-finished picture, and should, therefore, be considered a provisional rather than a definitive profile.

Genetic and antigenic characterization of isolates is an ongoing process, which has proven relevant and useful for pestivirus diagnosis and control in Brazil. For instance, the initial identification of BVDV-2 in the country in the early years (Canal *et al.*, 1998; Gil *et al.*, 1998; Flores *et al.*, 2002) paved the way for the introduction of BVDV-2 strains in current vaccines. Currently, most BVDV vaccines available in the country contain both BVDV-1 and BVDV-2. The identification of BVDV-2 in Brazil also led to an appraisal and adaptation of molecular and immunodiagnostic tools of pestivirus detection (Canal *et al.*, 1998; Monteiro *et al.*, 2018*a*, 2018*b*). More recently, the identification of HoBiPeV among Brazilian cattle has called attention to the need for evaluation of the molecular and immunodiagnostic assays (Bauermann *et al.*, 2013; Weber *et al.*, 2016*a*, 2016*b*; Silveira *et al.*, 2017; Monteiro *et al.*, 2018*a*, 2018*b*). The need of inclusion of these viruses in current vaccines has also became a subject of relevant debate (Bauermann *et al.*, 2013; Silveira *et al.*, 2017; Monteiro *et al.*, 2018*a*, 2018*b*). Thus, we expect that this review to shed more light on the epidemiology of pestiviruses in Brazil, thus contributing to diagnostics and control efforts.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1466252318000130

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