UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA

Carolina de Oliveira Freitas

ANÁLISE GENÉTICA E ANTIGÊNICA DO ECTODOMÍNIO DA GLICOPROTEÍNA E2 DE PESTIVÍRUS ISOLADOS DE BOVINOS NO RIO GRANDE DO SUL, BRASIL

Santa Maria, RS
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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração em Sanidade e Reprodução Animal, da Universidade Federal de Santa Maria (UFSM), como requisito parcial para obtenção do grau de **Mestre em Medicina Veterinária.**

Orientador: Prof. Rudi Weiblen

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Aprovado em 20 de fevereiro de 2020:

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Santa Maria, RS
2020

AGRADECIMENTOS

A realização deste mestrado só foi possível devido ao apoio e a dedicação de algumas pessoas que fizeram parte diretamente ou indiretamente da execução deste trabalho. Agradeço imensamente a todos que de alguma forma, contribuíram para a realização desta dissertação, em especial:

- Aos meus pais, por todo o apoio, força, confiança, amor incondicional e por sempre acreditarem que tudo é possível, e por não medirem esforços para que se concretizem;

- A minha família, por todo o carinho e força que me proporcionam;

- Ao meu namorado, Matheus, por estar junto comigo, independente do momento e por palavras de força e determinação para que eu conseguisse buscar sempre o melhor;

- Aos professores Eduardo Furtado Flores e Rudi Weiblen agradeço a oportunidade de trabalhar neste laboratório exemplar, por todas as orientações, oportunidades, confiança e por todos os ensinamentos;

- Aos colegas do Setor de Virologia, em especial ao colega Pablo Sebastian Britto de Oliveira por todo o auxílio desde o inicio do desenvolvimento do projeto de mestrado, assim como ao colega José Valter Joaquim Silva Júnior por sempre estar disposto a esclarecer dúvidas e prestar auxílio. E também a amiga e colega Giovana Basso por todo o companheirismo e força. Agradeço aos estagiários, em especial a Nathalia Michelon por toda dedicação, auxílio e conhecimento compartilhado;

- À Universidade Federal de Santa Maria (UFSM), ao Programa de Pós-Graduação em Medicina Veterinária (PPGMV), pelas respectivas estruturas, organização e auxílios que possibilitaram a realização deste trabalho;

- Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Código 001) pelos respectivos apoios financeiros;

A todos que de alguma forma contribuíram para a conclusão desta dissertação.

Lute com determinação, abrace a vida com paixão, perca com classe e vença com ousadia, porque o mundo pertence a quem se atreve e a vida é muito bela para ser insignificante.

(Charles Chaplin)

RESUMO

ANÁLISE GENÉTICA E ANTIGÊNICA DO ECTODOMÍNIO DA GLICOPROTEÍNA E2 DE PESTIVÍRUS ISOLADOS DE BOVINOS NO RIO GRANDE DO SUL, BRASIL

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Os pestivírus de bovinos, vírus da diarreia viral bovina 1 e 2 (*bovine viral diarrhea virus,* BVDV-1, BVDV-2) e pestivírus *Hobi-like* (HoBiPeV) estão associados com várias manifestações clínicas e reprodutivas produzindo perdas importantes para a bovinocultura. Os isolados de campo apresentam uma grande variabilidade genética e antigênica, especialmente na glicoproteína E2 do envelope viral. Essa variabilidade pode comprometer o diagnóstico molecular e/ou imunológico e estratégias de formulação vacinal e imunização. Assim, este estudo realizou uma análise genética e antigênica de 51 isolados de pestivírus obtidos de bovinos de corte no Rio Grande do Sul, Brasil, no ano de 2017. Inicialmente, os isolados foram identificados por PCR e confirmados por sequenciamento e análise filogenética da região 5'UTR do genoma, complementando uma análise realizada anteriormente em uma região mais abrangente da 5'UTR. A seguir, os isolados foram submetidos à testes de reatividade com 11 anticorpos monoclonais (MAbs) para a glicoproteína E2, seguido do sequenciamento e análise do domínio DA do ectodomínio da E2. A partir das sequências de nucleotídeos da região 5'UTR foram identificados 27 isolados de BVDV-1 (22 de -1a e 5 de -1b), 23 isolados de BVDV-2 (todos -2b) e um isolado de HobiPeV(-3a). Essa subtipagem correspondeu a um estudo anterior que caracterizou toda a porçao da 5'UTR. A análise de sequências (nucleotídeos e aminoácidos) do domínio DA da E2 identificou várias alterações de aminoácidos, observadas principalmente nos sítios imunodominantes, locais onde há uma maior exposição aos anticorpos. Os resíduos que apresentaram maior variabilidade foram nas posições: 695 e 782 do genoma do pestivírus bovino. Esses locais já haviam sido descritos anteriormente por apresentarem uma alta taxa de mutações e trocas de aminoácidos, provavelmente por serem importantes epítopos e alvos de anticorpos. Em menor número, foram observadas mutações na posição 701 e 734. Os testes de reatividade com MAbs revelaram uma variabilidade marcante na maioria dos epítopos da E2. Enquanto alguns MAbs reconheceram quase todos os isolados, confirmando a existência de epítopos altamente conservados na E2, vários MAbs reconheceram poucos ou mesmo nenhum isolado. Assim, o presente estudo fornece informações importantes referentes a variabilidade genética e antigênica do domínio DA da E2 entre isolados de campo de pestivírus bovinos. Essas informações, por sua vez, podem ser úteis para o diagnóstico molecular, imunológico e também para embasar as estratégias de elaboração de vacinas.

Palavras-chave: *Pestivirus, BVDV, subtipagem, diversidade antigênica, E2*

ABSTRACT

GENETIC AND ANTIGENIC ANALYSIS OF THE DA ECTODOMAIN OF GLYCOPROTEIN E2 OF PESTIVIRUSES ISOLATED OF CATTLE FROM RIO GRANDE DO SUL, BRAZIL

AUTHOR: Carolina de Oliveira Freitas
ADVISER: Rudi Weiblen

The bovine pestiviruses, *bovine viral diarrhea virus* 1, 2 (BVDV-1, BVDV-2) and HoBi-like (HobiPeV) are associated with several reproductive and clinical manifestations, producing important losses for the cattle industry. Field isolates have a significant genetic and antigenic variability, especially in the envelope glycoprotein E2. This variability may compromise molecular and immunological diagnosis and immunization strategies. Thus, this study performed a genetic and antigenic analysis of 51 pestivirus isolates obtained from beef cattle in Rio Grande do Sul, Brazil, in 2017. Initially, the isolates were identified by PCR and confirmed by sequencing and phylogenetic analysis of the 5'UTR region of the genome, complementing an analysis previously carried out in a more comprehensive region of the 5'UTR. Then, the isolates were submitted to reactivity assays with 11 monoclonal antibodies (MAbs) to the E2 glycoprotein, followed by sequencing and analysis of the domain A (DA) of the E2 ectodomain. Based on the phylogenetic analysis of the 5'UTR, 27 isolates were identified as BVDV-1 (22 -1a and 5 of -1b), 23 as BVDV-2 (all as -2b) and one as HobiPeV(-3a). This subtyping corresponded to a previous study that characterized the entire portion of 5'UTR. Sequence analysis (nucleotide and amino acid) of the E2 DA domain identified several nucleotide and amino acid changes, mainly at immunodominant sites subjected to binding by antibodies. The amino acid residues that presented the highest variability were at sites: 695 and 782 on the bovine pestivirus genome. Fewer aa changes were also observed at position 701 and 734. MAb binding assays revealed marked variability in most E2 epitopes. On the other hand, some MAbs recognized almost all isolates, confirming the existence of highly conserved epitopes in E2, while other MAbs recognized few or no isolates. Thus, the present study provides important information about the genetic and antigenic variability of the E2 DA domain of bovine pestivirus. This information may be useful to guide molecular an immunological diagnosis and for vaccine formulation as well.

Keywords: *Pestivirus, BVDV, subtyping, antigenic diversity, E2.*

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1. INTRODUÇÃO

O vírus da diarreia viral bovina (*bovine viral diarrhea virus,* BVDV) é um dos principais patógenos de bovinos responsável por perdas significativas na bovinocultura e tem sido detectado no Brasil desde os anos 60 (VIDOR, 1974). O agente pode provocar uma grande variedade de manifestações que incluem a imunossupressão dos animais, infecção subclínica, respiratória e digestiva. Em vacas pode causar retorno ao cio, abortos, reabsorção embrionária, natimortos e também o nascimento de animais persistentemente infectados (PI) (BAKER, 1995; HOUE, 2003; PETERHANS et al., 2010; LANYON et al., 2014; RIDPATH et al., 2017).

O gênero *Pestivirus,* família *Flaviviridae*, é classificado em 11 espécies virais, *Pestivirus A* a *K.* Desses, três espécies possuem importância para bovinos: *Pestivirus A* (vírus da diarreia viral bovina 1, BVDV-1), *Pestivirus B* (BVDV-2) e *Pestivirus H* (*Pestivirus Hobi-like*, HoBiPeV). Os pestivírus possuem vírions com envelope, 40 a 60 nanômetros de diâmetro e nucleocapsídeo icosaédrico. O genoma é constituído por uma fita simples de RNA com polaridade positiva de aproximadamente 12,3 kilobases (kb) (ICTV, 2019).

O RNA genômico apresenta apenas uma fase aberta de leitura (*open reading frame* – ORF), que é traduzida em uma poliproteína de aproximadamente 4.000 aminoácidos (aa), essa é clivada em 11 a 12 proteínas funcionais, dependendo do biotipo do vírus (NEILL et al., 2013). A ordem de tradução das proteínas virais na poliproteína é: N^{pro}-C-E^{rns}-E1-E2-p7-NS2/3-NS4a-NS4b-NS5a-NS5b, sendo a C, E^{rns}, E1 e E2 as proteínas estruturais (COLLETT et al., 1988; NEILL et al., 2013). O genoma apresenta duas regiões não traduzidas, 5'UTR e 3'UTR, a primeira dessas é altamente conservada e usualmente utilizada para a classificação dos pestivírus bovinos em tipos e subtipos (RIDPATH et al., 1994; VILCEK et al., 2001; NEILL et al., 2013). Outras regiões, como N^{pro}, E2 e E^{rns}, além de genomas inteiros, também podem ser utilizadas para a classificação de pestivírus em espécies e subespécies (COLLETT et al., 1988; NEILL et al., 2013). Com base nesses critérios, o BVDV-1 tem sido classificado em subespécies/subtipos 1a ao 1u, o BVDV-2 em 2a à 2d e os isolados de HobiPeV sugere-se que sejam classificados em quatro subespécies, 3a ao 3d (GIANGASPERO et al., 2008; SIMMONDS et al., 2011; GIAMMARIOLI et al., 2015).

As espécies e subespécies de pestivírus bovinos apresentam diferentes distribuições geográficas e prevalências nos continentes (YESILBAG et al., 2017). Diversos relatos sorológicos, clínico-patológicos e de isolamento do agente demonstram a ampla disseminação

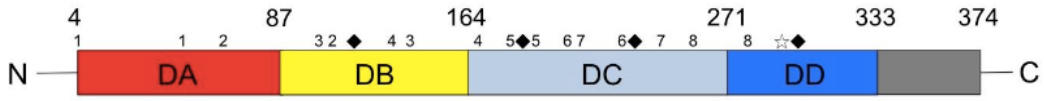
dos pestivírus bovinos no rebanho bovino brasileiro (RIDPATH et al., 2017; FLORES et al., 2018) e um ou mais de seus subgenótipos já foram isolados em todos os continentes (RIDPATH et al., 2010; YESILBAG et al., 2017; RICHTER et al., 2019). Na América do Sul e América do Norte a prevalência de BVDV-2 é maior do que na e Europa. Já no Brasil, diversos estudos indicam uma alta prevalência do BVDV-1 (57%), principalmente do subtipo -1a, seguido de -1b, -1d, -1c e -1i (WEBER et al., 2014; YESILBAG et al., 2017; FLORES et al., 2018; MONTEIRO et al., 2019). Sobre os isolados de BVDV-2 (42%), o subtipo mais circulante no Brasil é o -2b, seguido do -2a (GIANGASPERO et al., 2008; WEBER et al., 2014; FLORES et al., 2018; MONTEIRO et al., 2019). Interessantemente, esses resultados são similares aos obtidos em estudos no Chile (PIZARRO-LUCERO et al., 2006).

Os pestivírus apresentam altas taxas de mutações, o que leva a grande diversidade genética, sendo que o Brasil está entre os países que apresentam a maior diversidade genética de pestivírus (SILVEIRA et al., 2017). Tal variabilidade dificulta o diagnóstico molecular, assim como a variabilidade antigênica compromete o diagnóstico sorológico e a eficácia das vacinas (PELLERIN et al., 1994; RIDPATH et al., 1994; BAUERMANN et al., 2012). A maioria das vacinas disponíveis no Brasil possui em sua composição cepas europeias e americanas, que podem ser antigenicamente diferentes das circulantes no Brasil (BOTTON et al., 1998; BIANCHI et al., 2011; ANZILIERO et al., 2015). Além disso, nenhuma dessas vacinas possui cepas de HoBiPeV. Nesse sentido, a caracterização genética dos isolados de campo tem se mostrado relevante e útil para o diagnóstico e controle dos pestivírus (PELLERIN et al., 1994; LINDBERG et al., 2005).

A grande variabilidade antigênica dos isolados de pestivírus deve-se, sobretudo, à região hipervariável da glicoproteína E2, uma das principais glicoproteínas do envelope viral, a qual está envolvida na ligação dos vírions aos receptores celulares. Essa região hipervariável é imunogênica e induz a produção de anticorpos neutralizantes no hospedeiro (DONIS, 1995). A glicoproteína E2 é constituída de quatro domínios, DA, DB, DC e DD, sendo que os domínios DA e DB, localizados na posição amino-terminal (N-terminal), são os mais expostos na superfície viral. Por essa razão, esses domínios são os principais sítios de ligação de anticorpos neutralizantes e também a região onde ocorre maior variabilidade antigênica (OMARI et al., 2013). As trocas de aminoácidos e as variações nas estruturas antigênicas podem influenciar na virulência dos pestivírus (SHEN et al., 2011). Adicionalmente, com as mutações provocadas pela pressão de seleção, as diferenças antigênicas nas glicoproteínas podem influenciar na eficácia dos diagnósticos laboratoriais assim como na eficácia das

vacinas. Já os domínios DC e DD, teoricamente, não possuem epítopos de ligação de anticorpos, sugerindo que não são expostos na superfície viral, figura 1 (WANG et al., 2015).

Figura 1- Domínio DA da glicoproteína E2, compreendendo os resíduos de aminoácidos de posição 4-87 está em vermelho e o domínio DB está em amarelo compreendendo os resíduos 88–164. O domínio DC, em azul claro, compreendendo os resíduos 165–271 e o domínio e o domínio DD os resíduos 272-333, o qual está em azul escuro.



Fonte: (WANG et al., 2015).

Com base na alta diversidade de pestivírus circulantes no território brasileiro e na importância da glicoproteína E2 para o diagnóstico e eficácia das vacinas, o presente estudo realizou uma análise genética e antigênica do domínio DA de isolados de pestivírus obtidos de bovinos de corte no Rio Grande do Sul, identificando as espécies e subtipos circulantes, as principais alterações de sequência no DA e o perfil de reatividade com anticorpos monoclonais contra a E2.

Capítulo 1

Genetic and antigenic analysis of the DA ectodomain of glycoprotein E2 of pestiviruses isolated of cattle from Rio Grande Do Sul, Brazil

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(Artigo submetido para a revista *Ciência Rural*)

Genetic and antigenic analysis of the DA ectodomain of glycoprotein E2 of pestiviruses isolated of cattle from Rio Grande Do Sul, Brazil

Análise genética e antigênica do ectodominio DA da glicoproteína E2 de pestivírus isolados de bovinos do Rio Grande do Sul

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ABSTRACT

The bovine pestiviruses, *bovine viral diarrhea virus* 1, 2 (BVDV-1, BVDV-2) and *HoBi-like* (HobiPeV) are associated with several clinical manifestations and reproductive failure and produce important losses for the cattle industry. Field pestivirus isolates present a significant genetic and antigenic variability, especially in the envelope glycoprotein E2. This variability may compromise molecular and immunological diagnosis and immunization strategies. Thus, this study performed a genetic and antigenic analysis of 51 pestivirus isolates obtained from beef cattle in Rio Grande do Sul, Brazil, during 2017. Initially, the isolates were identified by PCR and confirmed by sequencing and phylogenetic analysis of the 5'UTR

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region of the genome, complementing an analysis previously carried out in a more comprehensive region of the 5'UTR. Then, the isolates were submitted to reactivity assays with 11 monoclonal antibodies (MAbs) to the E2 glycoprotein, followed by sequencing and analysis of the domain A (DA) of the E2 ectodomain. Based on the phylogenetic analysis of the 5'UTR, 27 isolates were identified as BVDV-1 (22 -1a and 5 of -1b), 23 as BVDV-2 (all as -2b) and one as HobiPeV (-3a). This subtyping confirmed a previous classification based on a longer 5'UTR sequence. Sequence analysis (nucleotide and amino acid) of the E2 DA domain identified several nucleotide and amino acid(aa) changes, mainly at immunodominant sites subjected to binding by antibodies. The amino acid residues that presented the highest variability were at sites: 695 and 782 on the bovine pestivirus genome, sites previously described for having a high rate of mutations and amino acid changes. Fewer aa changes were observed at position 701 and 734. MAb binding assays revealed marked variability in most E2 epitopes. Whereas a few MAbs recognized almost all isolates, confirming the existence of highly conserved epitopes in E2, many MAbs recognized few or no isolates. Thus, the present study provides important information about the genetic and antigenic variability of the E2 DA ectodomain of bovine pestiviruses. This information may be useful to guide molecular an immunological diagnosis and for vaccine formulation as well.

Keywords: BVDV, pestivirus, antigenic diversity, E2, ectodomain, monoclonal antibody.

RESUMO

Os pestivírus bovinos, o vírus da diarréia viral bovina 1, 2 (BVDV-1, BVDV-2) e o tipo HoBi (HobiPeV) estão associados a várias manifestações clínicas e falhas reprodutivas e produzem importantes perdas para a indústria pecuária. Os isolados de pestivírus de campo apresentam uma variabilidade genética e antigênica significativa, principalmente na glicoproteína E2 do

envelope. Essa variabilidade pode comprometer o diagnóstico molecular e imunológico e estratégias de imunização. Assim, este estudo realizou uma análise genética e antigênica de 51 isolados de pestivírus obtidos de bovinos de corte no Rio Grande do Sul, Brasil, em 2017. Inicialmente, os isolados foram identificados por PCR e confirmados por sequenciamento e análise filogenética da região 5'UTR do genoma, complementando uma análise anteriormente realizada em uma região mais abrangente do 5'UTR. Em seguida, os isolados foram submetidos a ensaios de reatividade com 11 anticorpos monoclonais (MAbs) contra a glicoproteína E2, seguidos de sequenciamento e análise do domínio A (DA) do ectodomaco E2. Com base na análise filogenética do 5'UTR, 27 isolados foram identificados como BVDV-1 (22 -1a e 5 de -1b), 23 como BVDV-2 (todos como -2b) e um como HobiPeV (-3a). Essa subtipagem confirmou uma classificação anterior com base em uma sequência 5'UTR mais longa. A análise de sequência (nucleotídeo e aminoácido) do domínio DA E2 identificou várias alterações de nucleotídeo e aminoácido (aa), principalmente em locais imunodominantes sujeitos à ligação por anticorpos. Os resíduos de aminoácidos que apresentaram maior variabilidade foram nos locais: 695 e 782 no genoma do pestivírus bovino, locais previamente descritos por apresentarem uma alta taxa de mutações e alterações de aminoácidos. Foram observadas menos alterações aa nas posições 701 e 734. Os ensaios de ligação ao MAb revelaram uma variabilidade acentuada na maioria dos epítopos E2. Enquanto alguns MAbs reconheceram quase todos os isolados, confirmando a existência de epítopos altamente conservados em E2, muitos MAbs reconheceram poucos ou nenhum isolado. Assim, o presente estudo fornece informações importantes sobre a variabilidade genética e antigênica do ectodomínio E2 DA de pestivírus bovinos. Esta informação pode ser útil para orientar o diagnóstico molecular e imunológico e também para a formulação de vacinas.

Palavras chave: BVDV, pestivírus, diversidade antigênica, E2, ectodomínio, anticorpos monoclonais.

INTRODUCTION

The genus *Pestivirus*, family *Flaviviridae,* comprises three important pathogens of cattle, *Bovine viral diarrhea virus 1* (BVDV-1; *Pestivirus A*), BVDV-2 (*Pestivirus B*) and *HoBi-like pestivirus* (*Pestivirus H,* HoBiPeV) (SIMMONDS et al., 2011; ICTV, 2019). Pestiviruses are small (50nm), enveloped, single-stranded, positive-sense RNA viruses, whose genome is approximately 12.3kb in length and contains a unique open-reading frame (ORF) flanked by two untranslated regions (5 and 3 UTRs). 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). Nucleotide sequencing and comparison of the highly conserved 5'UTR, in addition to N^{pro} and E2, has served for pestivirus phylogeny and classification into subtypes (RIDPATH et al., 1994; VILCEK et al., 2001; BECHER et al, 2003; LIU et al., 2009).

Currently, BVDV 1 and 2 are among the most important pathogens of cattle, with a global distribution and are associated with a variety of clinical manifestations in cattle (HOUE 2003). HoBiPeV were initially identified as contaminants of fetal bovine serum (FBS) from Brazilian origin (SCHIRRMEIER et al., 2004) and subsequently isolated in several continents from FBS and from cattle presenting clinical conditions similar to those classically attributed to BVDV-1 and BVDV-2 [LIU et al., 2009; DECARO et al., 2011; DECARO et al., 2012; BAUERMANN et al., 2013; WEBER et al., 2016; MONTEIRO et al., 2018].

The three pestivirus species are genetically and antigenically closely related, which may influence both diagnostic and control strategies (RIDPATH 2005; BAUERMANN et al., 2013). Pestiviruses show great genetic and antigenic variability, the main responsible for the antigenic variability is glycoprotein E2, an immunodominant envelope glycoprotein involved in virus binding to cellular receptors and a major important target for neutralizing antibodies (DONIS et al., 1988; WEILAND, et al., 1990; DEREGT et al., 1998). Glycoprotein E2 is a type I transmembrane protein with an N-terminal ectodomain and a C-terminal helix anchored in the viral membrane. In the viral envelope, E2 forms disulfide-linked homodimers and heterodimers with E1 (WEILAND, et al., 1990), this heterodimer is crucial for BVDV attachment and cell entry (RONECKER et al., 2008). The amino-terminal region of the E2 ectodomain is exposed at the virion surface and contains the major antigenic epitopes, eliciting a humoral response (DEREGT et al., 1998; NEILL 2013).

The E2 protein consists of four domains, DA, DB, DC and DD. The DA and DB domains, located at the amino-terminal (N-terminal) end, are the most exposed on the viral surface. Due to this, these domains are the main sites of neutralizing antibody binding and the region where the prominent antigenic variability occurs (OMARI et al., 2013). The DC and DD domains apparently do not harbor antibody binding epitopes, suggesting that they are not exposed on the viral surface (WANG et al., 2015).

The variability of BVDV E2 has been extensively demonstrated by nucleotide sequence analysis (BECHER et al., 1999; SILVEIRA et al., 2015) and, indirectly, by monoclonal antibody binding (BOLIN et al., 1988; DEREGT et al., 1990; DUBOVI 1992; DEREGT et al., 1998). In addition, virus-neutralizing (VN) assays provides an indirect means of assessing the antigenic variability of BVDV as well as for other pestiviruses (RIDPATH et al., 1994; BECHER et al, 2003; BOTTON et al., 1998).

Bovine pestiviruses are widely distributed among Brazilian cattle and a recent review pointed out for a clear predominance of BVDV-1 (54.4%), followed by BVDV-2 (25.7%) and HoBiPeV (19.9%) (FLORES et al., 2018). Continuous monitoring and genetic/antigenic characterization of isolates have proven relevant and useful for diagnostic and control of pestiviruses in Brazil, providing basis for designing/adaptation of diagnostic tools and new vaccine formulation. In this context, the present study provides additional information on the antigenic and genetic properties of pestiviruses circulating in Southern Brazil, through antigenic and sequence analysis of the E2 ectodomain of viruses isolated from beef cattle.

MATERIALS AND METHODS

Viruses and cells

The pestiviruses analyzed in this study were isolated from sera of beef cattle in Rio Grande do Sul, Brazil (2017). Fifty-one pestiviruses were isolated and amplified in MDBK cells (ATCC, VA, USA). Cells were maintained in MEM (minimum essential medium, ThermoFisher Scientific, MA, USA), supplemented with 10% equine serum, 100U/mL of penicillin and 100µL of streptomycin (ThermoFisher Scientific, MA, USA). BVDV-1 Singer strain and BVDV-2 890 were used as controls in fluorescent antibody assays.

Genetic identification

The isolates were initially submitted to genetic identification through nucleotide sequence analysis of 5'UTR region. For this, 300µL of MDBK cell supernatant infected with each isolate were submitted to RNA extraction using TRIzol® reagent (ThermoFisher Scientific, MA, USA), according to the manufacturer's instructions. After RNA extraction, the cDNA was synthesized using Go Script Reverse Transcription Mix (Promega, WI, USA). For the identification of viral species and subtypes, the cDNA was subjected to a PCR assay targeting the 5'UTR of the viral gene using primers BP189-389, according to the instructions

described in MONTEIRO et al. (2019a), generating a 201pb product. Some samples had been previously identified genetically by MONTEIRO et al. (2019b), analyzing the sequences amplified by primers HCV 90-368, BVDV-2#3 and N2-R5. Total RNA was extracted from MDBK infected cells with BVDV-1 Singer (cp), BVDV-2 890 (ncp) and HoBiPeV LV01/12 (SILVEIRA et al, 2018) and were used as controls. Ultrapure water was used as negative control. The phylogenetic analysis was conducted in the Molecular Evolutionary Genetics Analysis (MEGA) software 7 (TAMURA et al., 2011), using the Neighbor-joining method, and the evolutionary distances were computed using the p-distance method. The bootstrap values were calculated using 1000 replicates.

Sequence analysis of domain DA

Amplification of the DA domain of E2 was performed using degenerate primers, B11-B32 (COUVREUR et al., 2002). The cycling conditions used were: 94 ° C for 3 min, following by 35 cycles of 45 s 94 ° C for 45 s, 55 ° C for 45 s, 72 ° C for 45 s and final incubation of 7 min for 72 ° C, generating a 606bp product. For nucleotide sequencing, PCR products were purified using QIAquick PCR Purification Kit (QIAgen, Hilden, Germany). All samples were sequenced in duplicates using BigDye kit. The nucleotide sequences were analyzed by Staden program (STADEN 1996) to obtain the consensus sequence. The alignment was performed by ClustalW method and the translation of the nucleotides were performed using the program BioEdit Sequence Alignment Editor Software (7.0.5.3). MatGAT2.1 (CAMPANELLA et al., 2003) program was used to calculate pairwise identities using the BLOSUM62 matrix, alignments for E2 nucleotide/amino acids sequences of viruses and shown in matrix format, using twenty-five reference sequences of each subtype were used from GenBank.

MAb binding assays

MDBK cells inoculated with each isolated, m.o.i of approximately 0.5 - 1, were individualized with trypsin at 20 – 30 h post-inoculation, resuspended and allowed to attach to multispot glass slides. Cell monolayers were fixed in cold acetone (5min), washed in PBS and distilled water and submitted to IFA, according to BOTTON et al. (1998). The fixed cells were individually incubated with 11 MAbs anti-BVDV (Table 1) by 1h at 37º C, followed by washes and incubation with FITC-conjugated mouse anti-IgG antibody (1h at 37º C). The slides were stained with Evans blue (5 min) and observed under epifluorescence microscopy (Carl Zeiss, Inc., New York, USA). MDBK cells free of pestiviruses and cells infected with BVDV-1 Singer or BVDV-2 890 were used as positive and negative controls, respectively.

RESULTS

Genetic identification

The viral isolates were identified as pestiviruses based on phylogenetic analysis of a part of the 5'UTR amplified with primers BP189-389. Nucleotide sequencing of the amplicons revealed 27 isolates of BVDV-1. Most isolates were classified as BVDV-1a (22 isolates) and some (5 isolates) as -1b (Figure 1) totaling 27 isolates. Twenty-three isolates were classified as BVDV-2, all them identified as BVDV-2b (Figure 2). The isolate of HoBiPeV grouped next to subtype -3a (Figure 3). The phylogenetic relations of these isolates among them and with other pestiviruses are shown in Figures 1 to 3.

Sequence analysis of E2 domain DA

Analysis of the DA sequences revealed significant variability in amino acid (aa) sequences, especially at particular positions. In general, scattered aa change were observed throughout the sequence, with some specific positions displaying more frequent changes. The positions, in the bovine pestivirus genome, displaying the highest variation were analyzed in

more detail and are described in Table 2. In general, BVDV-1 isolates displayed a higher degree of variability, noticeably at positions 782 and 695 in the bovine pestivirus genome. At position 782, 16 out of 27 sequences (59.2%) presented aa change comparing to the standard viruses (Singer, Oregon/C24V/Osloss). Twelve of these (44,4%) were Glu(E)→Gly(G), but also three mutations (3.5%) were Glu(E)→Pro(P). Twelve changes (44.4%) were observed at position 695, being nine (33.3%) Asp(D)→Asn(N) and three (1.1%) Asp(D)→Glu(E), less frequent changes were observed at residues 701 and 734 (five changes), 781 (4), 697 (3) and 724 (2). Position 724 also showed an aa deletion in three BVDV-1 isolates.

The aa deletion at position 724 was a consistent finding among BVDV-2 isolates (19 out of 23) including the reference strains. Noticeably, BVDV-2 isolates showed a lower frequency of changes in most positions, comparing to BVDV-1. Four aa change (17.4%) were observed at positions 695 and 781.

The identity in nucleotide(nt) and amino acid sequences among the isolates analyzed in this study are shown in Table 3. In general, the aa similarity in DA sequences among BVDV-1 was 73.09% (nt 78.74%) and in BVDV-2 was 71.80% (nt 78.33%). The identity dropped significantly when comparing BVDV1 with BVDV-2 amino acids (71.84%).

3.3 MAb binding assays

The reactivity of E2 MAbs with BVDV isolates in IFA test is presented in Tables 4 and 5. The MAbs directed to glycoprotein E2 showed a high variability in reacting with antigens of fields isolates. A few MAbs reacted with most or several isolates (3.1C4 and 18D4), demonstrating the existence of conserved epitopes in E2, even between BVDV-1 and BVDV-2. On the other hand, most MAbs showed a scattered pattern of binding, failing to recognize a variable (or most) number of isolates. As expected, the MAb reactivity was more consistent/frequent with BVDV-1 than with BVDV-2.

DISCUSSION

The bovine pestiviruses BVDV-1, BVDV-2 and HoBiPeV are among the most important pathogens of cattle (HOUE 2003). These viruses are genetically and antigenically closely related, however genetic and antigenic differences observed among these groups of viruses, and even within each viral species, may influence diagnostic and vaccine efficacy (PELLERIN et al., 1994; RIDPATH 2005; BAUERMANN et al., 2013). The main responsible for the variability is the glycoprotein E2 gene, with contains regions highly variable. This gene encodes an immunodominant envelope glycoprotein involved in virus binding to cellular receptors, virus penetration and also a major important target for neutralizing antibodies (DONIS et al., 1988; WEILAND et al., 1990; DEREGT et al., 1998).

This study provides additional information on the genetics and antigenic properties of the E2 ectodomain of pestiviruses (mostly BVDV-1 and BVDV-2) isolated from beef cattle in Southern Brazil. The isolates were identified as pestiviruses based on phylogenetic analysis of a part of the 5'UTR, using just the primers BP189-389. Most of these isolates have been previously classified by MONTEIRO et al. (2019b), however, using a different set of primers (HCV90-368). Both sets of primers amplify almost equivalent sequences within the 5'UTR, a conserved genome region largely used to genetically identify pestivirus species and subtypes (RIDPATH et al., 1994; XIA et al., 2007; LIU et al., 2009; WEBER et al., 2014). The genetic subtyping of isolates using primers BP189-389 totally agreed with the previous genetic classification by MONTEIRO et al. (2019b). This agreement indicates that analysis of a smaller region of the 5'UTR (amplified by primers BP189-389) may be reliably used for species and subtype identification of pestiviruses. This is especially relevant considering that primers BP189-389 present a broader range of pestivirus detection than primers HCV90-368 MONTEIRO et al. (2019b). Thus, primers BP189-389 may be used both for diagnostic/virus

detection and, subsequently, for genetic identification by nucleotide sequencing and phylogenetic analysis of the amplicons.

Nucleotide sequencing of the amplicons revealed 27 BVDV-1 (22 isolates of -1a subtype and 5 isolates of -1b), 23 of BVDV-2 (all isolates classified as -2b) and one HoBiPeV. Several studies have characterized genetically bovine pestiviruses from Brazil, demonstrating that both BVDV-1 and BVDV-2 are circulating in the country, with a predominance of BVDV-1 [CANAL et al., 1998; BIANCHI et al., 2011; YESILBAG et al., 2017; MONTEIRO et al., 2018; MONTEIRO et al., 2019a; 2019b;). As in this study, other researchers have shown a highest prevalence of BVDV-1, with a significant predominance of subtype -1a, followed by -1b and -1d (EVERMANN & RIDPATH, 2002; GIANGASPERO et al., 2008; WEBER et al., 2014; FLORES et al., 2018; MONTEIRO et al., 2019b). For BVDV-2 isolates, the most circulating subtype is -2b followed by -2a (XIA et al., 2007; FLORES et al., 2018; MONTEIRO et al., 2019b); in the present study only BVDV-2b was identified. HoBiPeV isolates found in Brazil are mostly subtype -3a (MONTEIRO et al., 2019b). This was the only subtype found in this search. In this sense, the Brazilian cattle herd contains a unique mixture of pestivirus species and subtypes, unlike those seen in other countries (FLORES et al., 2018). BVDV-1a is the predominant subgenotype in the Americas and also worldwide, followed by BVDV-1b. BVDV-2 is more prevalent in North America than in other continents, BVDV-2a being the most prevalent in all continents (YESILBAG et al., 2007; GIANGASPERO et al., 2008). The continuous monitoring of genetic properties of viruses circulating in the field has been of pivotal importance towards diagnostic and control of pestiviruses in Brazil (CANAL et al., 1998; FLORES et al., 2018).

The degree of similarity between BVDV isolates demonstrates a wide variability within and outside the groups. In general, the aa similarity in DA sequences among BVDV-1 was 73.09% and in BVDV-2 was 71.80%, whereas it was 71.84% comparing BVDV-1 and

BVDV-2. These results corroborate those already found in other studies reaffirming that BVDV-2 is less variable (PATON et al., 1992; VAN RIJN et al., 1997; JELSMA et al., 2013; LANG et al., 2014; RIDPATH et al., 2015). The homology percentage among our isolates was lower compared to the GenBank reference sequences.

The analysis of aa sequences of E2 DA showed some scattered nucleotide changes over the region and, as expected, a significant variability in some specific sites. BVDV-1 isolates displayed the highest degree of variability at position 782 in the bovine pestivirus genome. Sixteen out of 27 sequences (59.2%) presented aa changes comparing to the standard viruses (Singer, Oregon/C24V/Osloss). Twelve of these (44.4%) were Glu(E)→Gly(G), but also three mutations (3.5%) were Glu(E)→Pro(P). Four aa changes (all Pro(P)→Gly(G)) in this position were observed among BVDV-2 isolates. Although to a lesser extent, residue 781 also showed an important mutation rate among our BVDV-1 and BVDV-2 isolates. These findings agree with previous observation that residues 771-783 are the sites displaying the highest aa mutation rates (SHEN et al., 2011). Not surprisingly, these sites have been reported to be immunodominant and harbor major antibody binding sites (DEREGT et al, 1998; COUVREUR et al., 2002; SHEN et al., 2011).

Position 695 also showed a high variability among BVDV-1 isolates and, to a lesser extent among BVDV-2. While the reference strains presented the residue Asp or Val, the isolates presented Asp, Val or Asn. In contrast, few alterations were observed in this position among BVDV-2 isolates (Glu(E)→Asn(N)). The HoBiPeV isolate showed a change Ser(S)→Val(V). In this sense, two highly antigenic regions have been mapped between positions 691-761 and 762-765 of E2, which are believed to be important antibody binding sites (DEREGT et al, 1998). Thus, the variability in position 695 may also be a result of selective pressure by the host immune system, leading to viral escape from antibodies with neutralizing activity.

Residues 695, 697 and 701 seem to be of utmost importance for antibody binding, as this region has been shown to frequently harbor aa changes, according to viral species and subtype, and this variation may serve for viral escape from antibody binding (DEREGT et al, 1998; DONIS 1995). In fact, changes in residues 697 and 701 indicate important antibody escape mutations, as these two regions are relevant antibody binding points (DEREGT et al, 1998). In our study, positions 697 and 701 also showed aa changes among BVDV-1, yet in lower frequency than position 695.

Position 714 did not present changes comparing to reference strains, contrasting with some previous studies (COUVREUR et al., 2002; VAN RIJN et al., 1997). Frequent aa changes were also present in position 734 of BVDV-1 (Thr(T)→Ile(I)), BVDV-2 (Ser(S)) and HoBiPeV (Ser(S)→ Thr(T)). Mutations in this position have also been described elsewhere and may also be involved in antibody escape (VAN RIJN et al., 1997). An aa deletion at position 724 was observed in a few BVDV-1 and in most BVDV-2 isolates. Similar findings were also described among U.S (United States) isolates, leading to suggestion that it may be a signature of BVDV-2 isolates (COUVREUR et al., 2002). Our data indicates that, although consistently found among BVDV-2, this deletion may also be present in some BVDV-1. Thus, it should not be considered a signature of BVDV-2. Positions 765, 767 and 778 were unchanged among BVDV-1 and had only one change (767) among BVDV-2. Interestingly, these positions have been reported as immunodominant and potential sites for antibody binding and escape (PATON et al., 1992; DEREGT et al., 1998; COUVREUR et al., 2002; BAUERMANN et al., 2012).

As a whole, the most significant aa changes observed among our isolates are similar to what has been generally described for BVDV (DEREGT et al., 1998; VAN RIJN et al., 1997; COUVREUR et al., 2002; BAUERMANN et al., 2012). Nonetheless, some positions described to harbor aa changes in previous studies remained almost unaltered among our

isolates (e.g. 714, 765, 767 and 778) (VAN RIJN et al., 1997; DEREGT et al., 1998; COUVREUR et al., 2002; SHEN et al., 2011; BAUERMANN et al., 2012).

Thus, it seems that the pattern and degree of aa changes in E2 may not be universal among pestivirus isolates. Rather, it may slightly change among different populations of viruses, reflecting different patterns of selective pressure and/or particular evolutionary trends. In this regard, the pattern observed in the present study should not be extended/extrapolated to the overall population of pestiviruses circulating in Brazil since it represents a sampling from beef cattle in Southern Brazil.

The antigenic analysis of E2 by binding to a panel of eleven MAbs illustrated the high variability of this protein, as indicated by differential binding of several MAbs and lack of binding by a number of MAbs (Table 4 and 5). Unfortunately, the epitopes recognized by the individual MAbs have not been mapped, precluding any attempt to correlate the sequence analysis with MAb binding. In addition to antigenic differences between BVDV-1 and BVDV-2, the MAb binding assays also showed antigenic variability within these groups of viruses, demonstrated by differential binding by some MAbs. Interestingly, MAbs 3.1C4 and, to a lesser extent MAbs 18D4 and 7.1.8 recognized a high proportion of BVDV-1 and BVDV-2 isolates, confirming the existence of well conserved epitopes within E2, shared by viruses of the same genotype as well as by viruses from different species. In particular, these MAbs may be useful for diagnostic purposes. Unfortunately, no single MAbs was able to distinguish between BVDV-1 and BVDV-2 isolates. These results largely corroborate with those already found in other studies (BOTTON et al., 1998; BIANCHI et al., 2011; PECORA et al., 2014; DIAS et al., 2017).

CONCLUSION

A significant antigenic and genetic variability was observed in the E2 ectodomain of BVDV isolates, the Brazilian cattle herd contains a unique mixture of pestivirus species and subtypes. Most aa changes detected were similar to those described previously, however, the degree and pattern of changes in E2 were slightly different from other reports, the analysis of aa sequences of E2 DA showed a significant variability in some specific sites. Thus, it seems that the pattern and degree of aa changes in E2 may not be universal among pestivirus isolates. In this regard, the pattern observed in the present study should not be extended/extrapolated to the overall population of pestiviruses circulating in Brazil since it represents a sampling from beef cattle in Southern Brazil. These findings reinforce the need of a continuous monitoring of genetic and antigenic properties of pestivirus isolates, as to provide basis for diagnostic and control.

ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarships. Carolina de Oliveira Freitas (process 131282/2018-2**)** received Master Scholarship, Eduardo F. Flores (process 301414/2010-6) and Rudi Weiblen (process 304153/2014-1) were supported by CNPq research fellowships. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001. C.O.F is graduate students of the Programa de Pós-graduação em Medicina Veterinária – PGMV/UFSM.

CONFLICT OF INTEREST STATEMENT

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

COF, FLM, JFC, PBSO, JVJSJ, EFF, RW: designed and performed experiments, and analyzed data. COF, PBSO, J: conceived the experiments and wrote the paper.

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1 Table 1- Monoclonal antibodies used in antigenic characterization of pestivirus isolates.

2

Monoclonal antibody	Protein	BVDV species*
3.1C4	E2 [a]	BVDV-1
7.1.8	E2 [b]	BVDV-1
10F9	E2 [b]	BVDV-1
18D4	E2 [b]	BVDV-1
27B3	E2[b]	BVDV-1
20G7	E2[b]	BVDV-1
19F9	E2 [b]	BVDV-1
F114D8	E2 [b]	BVDV-1
6D11	E2 [b]	BVDV-1
6C5	E2 [b]	BVDV-1
32B3	E2 [b]	BVDV-1

3

4 [a] (KREUTZ et al., 2000), [b] (CORAPI ET AL., 2000). * Viral species for which it was produced.

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1 Table 2- Analysis of amino acid sequences of domain A of glycoprotein E2 of pestivirus

2 isolates.

3

Identification of isolate/strain	Amino acid position						
	695	697	701	724	734	781	782
BVDV-1_Singer Arg (MH133206.1)	D	K	S	D	T	Q	E
BVDV-1_Oregon (AF091605.1)	D	K	S	D	T	Q	E
BVDV-1_CP7 (U63479.1)	D	K	S	E	S	Q	E
BVDV-1_Osloss (M96687.1)	V	K	Y	E	T	Q	E
BVDV-1_10JJ-SKR (KC757383.1)	D	K	S	D	T	S	A
BVDV-1_NADL (M31182.1)	D	K	S	E	T	Q	E
SV 605/17-55	N	.	.	.	.	.	G
SV 605/17-108	[a]	.	Q	.	.	.	.
SV 125/17-415	.	.	.	.	.	.	G
SV 256/17-1177	.	.	.	.	.	.	G
SV 256/17-1178	.	.	.	.	.	.	G
SV 256/17-1192	.	.	.	.	.	.	G
SV 256/17-1258	.	.	.	.	.	.	G
SV 508/17-1264	.	.	Q	.	.	.	.
SV 125/17- 1337	.	.	.	.	.	.	G
SV 125/17-1368	.	.	.	.	.	.	.
SV 256/17-1568	.	.	.	.	.	.	G
SV 508/17-1687	.	.	.	.	.	.	.
SV 508/17-1960	N	.	.	.	I	.	.
SV 508/17-2009	N	.	.	.	I	.	.
SV 125/17-2370	.	.	.	.	.	.	.
SV 125/17-2526	.	.	.	.	.	.	.
SV 508/17-2841	N	.	.	.	I	.	.
SV 508/17-2890	N	.	.	.	I	.	.

SV 508/17-2920	N	.	.	.	.	.	.
SV 508/17-3005	E	S	Q	[b]	.	G	P
SV 508/17-3036	N	.	.	.	I	.	.
SV 605/17-3377	N	.	.	.	.	.	G
SV 125/17-3581	E	S	H	[b]	.	G	P
SV 125/17-3656	.	.	.	.	.	.	G
SV 125/17-4023	E	T	Q	[b]	.	G	P
SV 508/17-4506	N	.	.	.	.	.	G
SV 256/17-5289	.	.	.	S	.	.	G
BVDV-2_CN 10.2015.821 (MG879027.1)	E	K	Q	[b]	S	G	T
BVDV-2_MadSpl (MH231137.1)	E	K	Q	[b]	S	G	P
BVDV-2 RS886(MH231143.1)	E	K	Q	[b]	S	G	P
BVDV-2_New York'93(AF502399.1)	E	K	Q	[b]	S	G	P
BVDV-2(AB567658.1)	E	T	Q	[b]	T	G	P
BVDV-2 VOE 4407(HG426495.1)	E	K	Q	[b]	S	G	P
BVDV-2_890(U18059.1)	E	K	Q	[b]	S	G	P
SV 605/17-42	.	.	.	[b]	.	.	.
SV 605/17-75	N	.		D	.	Q	G
SV 125/17-382	.	.	.	[b]	.	.	.
SV 256/17-426	.	.	.	[b]	.	.	.
SV 125/17-430	.	.	.	[b]	.	.	.
SV 605/17-1150	.	.	.	[b]	.	.	.
SV 125/17-1182	.	.	.	[b]	.	.	.
SV 508/17-1832	.	.	.	[b]	.	.	.
SV 125/17-2139	.	.	.	[b]	.	.	.
SV 125/17-2140	.	.	.	[b]	.	.	.
SV 605/17-2368	.	.	.	[b]	.	.	.
SV 605/17-2825	N	.	.	D	.	Q	G
SV 508/17-3075	.	S	.	[b]	.	.	.
SV 125/17-3267	.	.	.	[b]	.	.	.

SV 605/17-3499	.	.	H	[b]	.	.	.
SV 508/17-3978	.	.	.	[b]	.	.	.
SV 508/17-4106	.	.	.	[b]	.	.	.
SV 125/17-4271	.	S	.	[b]	.	.	.
SV 125/17-4437	.	.	.	[b]	.	.	.
SV 508/17-4446	.	.	.	[b]	.	.	.
SV 508/17-4544	N	.	.	D	.	Q	G
SV 125/17-4558	D	.	.	D	.	Q	G
SV 508/17-4896	.	.	.	[b]	.	.	.
Italy 68/13(KJ627180.1)	S	K	Q	A	S	E	D
SV478/07(KY767958.1)	S	K	Q	A	S	E	D
LV03/12(MH410816.1)	S	K	Q	A	S	E	D
SV508/17-1339	V	.	.	E	T	.	E

[a] Identical to reference sequence; [b] Amino Acid Absence; A(Ala): Alanine, C(Cys): Cysteine, D(Asp): Aspartc acid, E(Glu): Glutamic acid, F(Phe): Phenylalanine, G(Gly): Glycine, H(His): Histidine, I(Ise): Isoleucine, K(Lys): Lysine, L(Leu): leucine, M(Met): Methionine, N(Asn): Asparagine, P(Pro): Proline, Q(Gln): Glutamine, R(Arg): Arginine, S(Ser): Serine, T(Thr): Threonine, V(Val): Valine, W(Trp): Tryptophan, Y(Tyr): Tyrosine.

Table 3- Identity in domain A (DA) among BVDV isolates.

Virus	Mean nt	Mean aa
BVDV-1	78.74 (14.43)	73.09 (17.03)
BVDV-2	78.33 (14.43)	71.80 (17.87)
BVDV-1 x BVDV-2	72.92 (12.36)	71.84 (15.91)

1 Table 4- Reactivity of monoclonal antibodies with isolates of bovine viral diarrhea virus 1.

2

	3.1C4	18D4	7.1.8	10F9	20G7	27B3	19F9	F114D8	6D11	32B3	6C5
Singer	+	+	+	+	+	+	+	+	+	+	+
2841	+	+	+	+		+	+	+		+	+
2920	+	+	+	+			+	+	+	+	+
2526	+	+	+	+			+	+		+	+
1960	+	+	+	+			+	+		+	+
1368	+	+	+				+		+		+
108	+	+	+				+		+	+	
3581	+	+			+			+			+
5289	+	+			+			+			+
2009	+	+	+				+	+		+	
2890	+	+	+				+	+		+	
3005	+	+	+			+			+		+
1177	+	+	+				+				+
2370	+	+	+				+			+	+
1687	+	+	+	+							+
3377	+	+	+		+						
3036		+	+				+	+			+
1178	+	+	+				+				
1337	+	+	+		+		+				

4506		+		+	+			+	+		
3656	+	+	+			+		+		+	
1192	+	+	+			+		+			
1258	+	+	+			+					
55	+	+	+							+	
1568	+	+	+			+					
415	+	+	+							+	
1264	+		+								
4023	+										

+: Fluorescence emission.

1 Table 5- Reactivity of monoclonal antibodies with isolates of bovine viral diarrhea virus 2 and

2 HoBiPeV.

3

	3.1C4	18D4	7.1.8	10F9	20G7	27B3	19F9	F114D8	6D11	32B3	6C5
Singer	+	+	+	+	+	+	+	+	+	+	+
SV890	+	+	+				+	+			+
3978	+	+	+	+	+		+	+	+	+	+
2368	+	+	+	+	+	+	+	+			+
4896	+	+	+	+	+		+	+	+	+	+
1832	+	+	+	+	+	+	+			+	
42	+	+	+	+	+		+		+	+	
4446	+	+	+	+	+				+	+	+
1150	+	+	+	+	+		+	+		+	
75	+			+	+			+			+
3075		+	+			+	+	+			
3499	+				+	+	+				
1339*	+	+	+				+				
2825	+		+		+	+					
426	+						+				
1182	+		+								
382	+	+								+	
4544	+	+				+					

4106	+				+
4558	+				
2139	+	+			
4437	+				
3267	+				
2140	+				
4271	+				
430	+				

+: Fluorescence emission; * HoBiPeV isolated.

Figure 1- Phylogenetic tree based on 5'UTR sequences of BVDV-1 amplified using primers BP189-389. Sequences were analyzed by Neighbor-joining method and the p-distance. The length of each pair of branches represents the distance between sequence pairs and the scale bar represents the percentage of nucleotide differences in the rectangular tree. Isolates detected in this study are highlighted with symbols, the isolates that were used in previous studies (hollow square) (MONTEIRO et al., 2019b). and those only used in this study (black squares).

Figure 2- Phylogenetic tree based on 5'UTR sequences of BVDV-2 amplified using primers BP189-389. Sequences were analyzed by Neighbor-joining method and the p-distance. The length of each pair of branches represents the distance between sequence pairs and the scale bar represents the percentage of nucleotide differences in the rectangular tree. Isolates detected in this study are highlighted with symbols, the isolates that were used in previous studies (hollow circle) (MONTEIRO et al., 2019b). and those only used in this study (black circles).

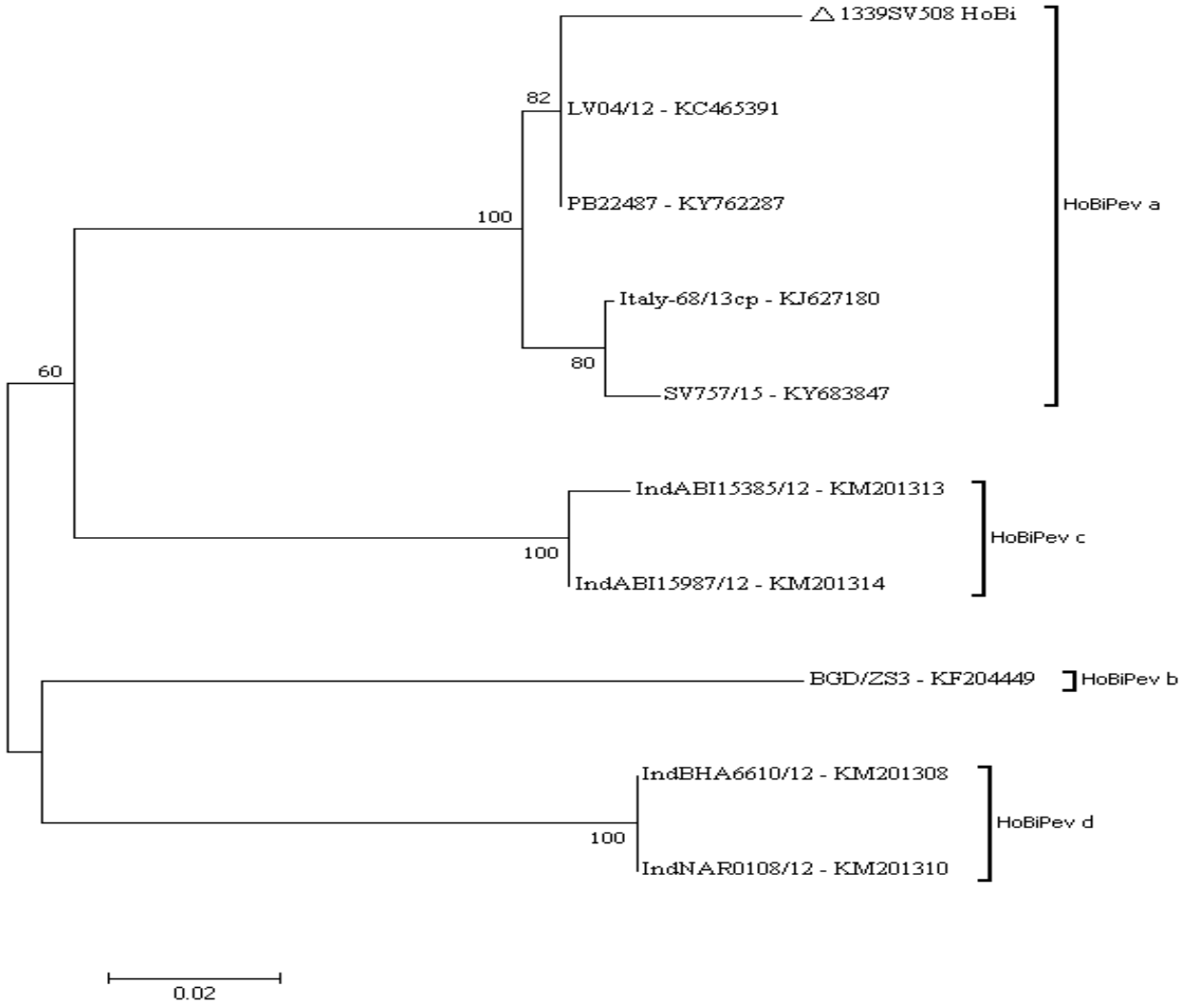

Figure 3- Phylogenetic tree based on 5'UTR sequences of HoBiPeV using primers BP189-389. Sequences were analyzed by Neighbor-joining method and the p-distance The length of each pair of branches represents the distance between sequence pairs and the scale bar represents the percentage of nucleotide differences in the rectangular tree. The isolate detected in this study are highlighted with symbol, the isolates that were used in previous studies (hollow rectangle) (MONTEIRO et al., 2019b).

2. CONCLUSÃO

Foi observada uma variabilidade antigênica e genética significativa no ectodomínio E2, DA, de isolados de BVDV. A maioria das alterações aa detectadas foram semelhantes às descritas anteriormente, no entanto, o grau e o padrão das alterações no E2 foram ligeiramente diferentes de outros relatórios. Esses achados reforçam a necessidade de um monitoramento contínuo das propriedades genéticas e antigênicas de isolados de pestivírus como base para diagnóstico e controle.

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