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

José Conrado dos Santos Jardim

INFECÇÃO EXPERIMENTAL DE BEZERROS COM ISOLADOS BRASILEIROS DE PESTIVÍRUS *HoBi-like*

Santa Maria, RS, Brasil
2018

José Conrado dos Santos Jardim

INFECÇÃO EXPERIMENTAL DE BEZERROS COM ISOLADOS BRASILEIROS DE PESTIVÍRUS *HoBi-like*

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, RS), como requisito parcial para obtenção do grau de **Mestre em Medicina Veterinária.**

Orientador: Prof. Eduardo Furtado Flores

Santa Maria, RS, Brasil
2018

José Conrado dos Santos Jardim

INFECÇÃO EXPERIMENTAL DE BEZERROS COM ISOLADOS BRASILEIROS DE PESTIVÍRUS *HoBi-like*

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, RS), como requisito parcial para obtenção do grau de **Mestre em Medicina Veterinária.**

Aprovado em 27 de fevereiro de 2018:

_____

Eduardo Furtado Flores, PhD. (UFSM)

(Presidente/Orientador)

_____

Juliana Felipetto Cargnelutti, Dra. (UFSM)

(Co-orientadora)

_____

Mário Celso Sperotto Brum, Dr. (UNIPAMPA)

Santa Maria, RS

2018

RESUMO

INFECÇÃO EXPERIMENTAL DE BEZERROS COM ISOLADOS BRASILEIROS DE PESTIVÍRUS *HoBi-like*

AUTOR: José Conrado dos Santos Jardim
ORIENTADOR: Eduardo Furtado Flores

Os vírus *HoBi-like* pertencem a um grupo ainda não classificado oficialmente de pestivírus bovino, originalmente identificados em soro fetal bovino (SFB) comercial de origem brasileira e, subsequentemente, isolados de SFB e de animais doentes em vários países. Embora frequentemente isolados de casos clínicos, a maioria dos isolados de pestivírus *HoBi-like* não reproduz sinais clínicos quando inoculados experimentalmente. Neste estudo, foi realizada a infecção experimental de bezerros com dois isolados brasileiros de pestivírus *HoBi-like*. Bezerros inoculados com o isolado SV757/15 pela via intranasal (IN), apresentaram viremia entre os dias 4 e 12 pós-infecção (pi) além de excreção viral em secreções nasais até o dia 12 pi. Clinicamente, os animais inoculados apresentaram hipertermia transitória (dias 4 a 8 pi) e linfopenia entre os dias 4 e 8 pi. Em um segundo experimento bezerros inoculados com o isolado SV478/07 apresentaram apatia, anorexia, sinais respiratórios leves e diarreia pastosa nos dias seguintes à inoculação. Estes animais desenvolveram hipertermia (dia 4 a 9 pi), linfopenia (dia 4 a 7pi), além de viremia entre os dias 2 e 9, e excretaram vírus em secreções nasais até dia 14 pi. Ambos os grupos soroconverteram para os vírus inoculados, desenvolvendo títulos de anticorpos neutralizantes entre 40 e 1280 no dia 30 pi. Esses resultados confirmam e estendem os achados anteriores mostrando que as infecções de bovinos susceptíveis com pestivírus tipo *HoBi-like* são predominantemente subclínicas ou acompanhadas de sinais clínicos leves. Não obstante, esses resultados também indicam a existência de diferenças de virulência entre os isolados de campo.

Palavras-chaves: BVDV, pestivírus, infecção experimental, patogenia.

ABSTRACT

EXPERIMENTAL INFECTION OF CALVES WITH BRAZILIAN ISOLATES OF *HoBi-like* PESTIVIRUSES

AUTHOR: José Conrado dos Santos Jardim
ADVISER: Eduardo Furtado Flores

HoBi-like viruses belong to a yet unclassified group of bovine pestiviruses of cattle, originally identified in commercial fetal bovine serum (FBS) of Brazilian origin and, subsequently, isolated from FBS and from sick animals in several countries. Although frequently isolated from overt, severe disease, most HoBi-like isolates do not reproduce marked clinical signs when inoculated experimentally. In this study, the experimental infection of calves with two Brazilian isolates of HoBi-like pestivirus was carried out. Four to six months-old, seronegative calves inoculated by the intranasal route (IN) with isolate SV757/15 presented viremia between days 4 and 12 post-infection (pi) and excreted virus in nasal secretions until the day 12 pi. Clinically, the inoculated animals presented transient hyperthermia (days 4 to 8 pi) and lymphopenia between days 4 and 8 pi. In a second experiment, calves inoculated with isolate SV478/07 showed apathy, anorexia, mild respiratory signs and pasty diarrhea on the days following inoculation. These animals developed hyperthermia (day 4 to 9 pi), lymphopenia (day 4 to 7pi), viremia between days 2 and 9 and virus excreted in nasal secretions up to day 14 pi. Both groups seroconverted to the inoculated viruses, developing neutralizing antibody titers between 40 and 1280 on day 30 pi. These results confirm and extend previous findings showing that infections of susceptible cattle with HoBi-like pestiviruses are predominantly subclinical or accompanied by mild clinical signs. Nonetheless, these results also indicate the existence of differences in virulence among field isolates.

Key words: BVDV, pestivirus, experimental infection, pathogenesis.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1 REVISÃO BIBLIOGRÁFICA

O vírus da diarreia viral bovina (BVDV) é um dos principais patógenos de bovinos, sendo responsável por grandes perdas econômicas em rebanhos de produção (HOUE, 1999). As manifestações clínicas da infecção podem variar desde distúrbios respiratórios, gastrointestinais e reprodutivos até uma doença fatal chamada doença das mucosas. Não obstante, as formas subclínicas ou clínicas leves são consideradas as mais frequentes. Estratégias de controle e erradicação da doença vem sendo empregadas em diversos países, tais como identificação e eliminação de animais persistentemente infectados (PI), monitoramento sorológico e vacinação (HOUE, LINDBERG & MOENNIG, 2006; LINDBERG et al., 2006; RIDPATH, 2012b). Nos últimos anos, o sugimento de uma nova variante viral, isolada de soro fetal bovino, colocaram em dúvida a eficácia dos programas de controle, assim como das vacinas disponíveis no mercado. Esta nova espécie viral ainda permanece sem classificação oficial, podendo ser denominada de BVDV-3, vírus *HoBi-like* ou pestivírus atípico bovino (SMITH et al., 2017). Após sua identificação, em 2004, diversos relatos de infecções naturais por este novo grupo de vírus vem sendo descritas no Brasil, Itália e Sudeste Asiático (CORTEZ et al., 2006; DECARO et al., 2011; KAMPA et al., 2009)

A família *Flaviviridae* é composta por quatro gêneros: *Flavivirus*, *Pestivirus*, *Hepacivirus* e *Pegivirus*. O gênero *Pestivirus* abriga vírus de grande importância veterinária, como o vírus da peste suína clássica (CSFV), o vírus da doença das fronteiras de ovinos (BDV) e os BVDV-1 e BVDV-2. Os vírus classificados nesta família são envelopados, possuem um diâmetro aproximado de 40 a 60 nm, e contêm genoma RNA de fita simples, polaridade positiva, com aproximadamente 12,5 quilobases (kb) (DONIS; CORAPI; DUBOVI, 1991). Os isolados de BVDV podem ser classificados, de acordo com sua capacidade de causar citopatologia em cultivo celular, em citopáticos (cp) e não citopáticos (ncp) (GILLESPIE et al., 1961), sendo que mais de 95% dos isolados de BVDV circulantes nos rebanhos bovinos pertencem ao biotipo ncp. Os pestivírus são classificados de acordo com a homologia de regiões conservadas no genoma, principalmente a região não traduzida 5’ (5’UTR) e, assim, os pestivírus de bovinos são subdivididos em espécies (BVDV-1 e BVDV-2) (VILCEK et al., 2005).

Os primeiros relatos de BVDV datam de 1946 por pesquisadores da Universidade de Cornell, em um pequeno rebanho em Ithaca, EUA, sendo inicialmente caracterizada como uma infecção causada por vírus associada à diarreia e a lesões no trato gastrointestinal de bovinos

(OLAFSSON et al., 1946). Os sinais clínicos incluíam leucopenia, febre, desidratação, diarreia, anorexia, erosões gastrointestinais e hemorragias em diversos órgãos. Alguns anos mais tarde, entre as décadas de 40 e 50, surtos da doença foram descritos, porém com sinais agudos mais severos, incluindo diarreia profusa hemorrágica. Esta apresentação foi denominada de doença das mucosas (DM), que apesar de suas características clínicas serem idênticas ao BVDV, não era reproduzida experimentalmente (RAMSEY & CHIVERS, 1953). O desenvolvimento de animais persistentemente infectados (PI) e os mecanismos que levam a DM só foram elucidados anos mais tarde (BROWNLIE, CLARKE & HOWARD, 1984).

Em 1990 nos Estados Unidos (EUA), uma nova doença foi relatada com características de diarreia hemorrágica severa. Em 1994, constatou-se ser causada por um BVDV genética e antigenicamente diferente dos anteriormente identificados, sendo denominado de BVDV-2 (RIDPATH; BOLIN; DUBOVI, 1994). Estes isolados de BVDV-2 demonstraram ser mais virulentos, principalmente quando introduzidos em rebanhos soronegativos, causando altos índices de morbidade e mortalidade (VAN RIJN et al., 1997). Entretanto, amostras de baixa a moderada virulência foram isolados nos anos seguintes, caracterizados por induzir uma ampla variedade de sinais clínicos, desde doença aguda leve (gastroentérica e respiratória), até doença aguda severa (gastroentérica, respiratória e hemorrágica) (WEBER et al., 2014).

Por se tratar de um agente com grande impacto econômico, o BVDV vem sendo muito estudado ao longo das décadas, com o objetivo de investigar a patogenicidade e virulência de isolados de campo. Atualmente, sabe-se que estes vírus possuem distribuição mundial e grande parte dos seus isolados apresenta baixa virulência (RIDPATH, 2012a). A transmissão pode ocorrer por contado direto entre os animais ou de forma indireta através de secreções, restos placentários, fetos abortados ou ainda verticalmente com a infecção de fêmeas prenhes (RIDPATH, 2010a). Após uma replicação inicial no epitélio do trato respiratório superior, orofaringe e linfonodos regionais, o vírus faz viremia associado a linfócitos, atingindo vários órgãos, como pulmão, língua, esôfago, coração, baço, abomaso e intestinos grosso e delgado (HAMERS et al., 2000).

A infecção persistente ocorre quando a fêmea é infectada no período de 40 a 120 dias de gestação (RIDPATH; NEILL, 2000). Durante esta fase, o feto ainda não é imunocompetente, e a infecção neste período faz com que o feto não reconheça o vírus e se torne tolerante ao mesmo, não sendo capaz de desenvolver uma resposta imune. Os animais PI excretam BVDV ncp de forma contínua, sendo assim a principal fonte de infecção do rebanho. Esses animais também podem apresentar retardado no crescimento, morrer em poucos dias ou desenvolver a

doenças das mucosas (DM) (DECARO et al., 2014). A DM é uma manifestação clínica que ocorre quando um animal PI (infectado pelo biótipo ncp) sofre a infecção por um BVDV cp. Essa coinfecção leva a mutações e alterações moleculares no vírus não citopático que passa a expressar a proteína NS3, resultando no desenvolvimento da DM. Neste caso a doença tem curso fatal, e o animal desenvolve grave doença gastroentérica, com erosões, úlceras e hemorragia na mucosa digestiva (BROWNLIE; CLARKE; HOWARD, 1984).

A forma clínica da infecção por BVDV pode cursar com doença aguda leve com tosse, secreção nasal, imunossupressão, hipertermia e diarreia, até doença aguda severa (gastroentérica, trombocitopenia e pneumonia) (BAKER, 1995; BOLIN; GROOMS, 2004; CARMAN et al., 1998). Os animais PI podem nascer aparentemente normais, porém frequentemente apresentam malformações congênitas ou retardado no crescimento (HOUE; LINDBERG; MOENNIG, 2006; WEBER et al., 2014). Somente animais PI podem desenvolver DM apresentando lesões ulcerativas em órgãos do sistema gastrointestinal, sendo em 100% dos casos fatais (BROWNLIE; CLARKE; HOWARD, 1984). Esta diversidade de sinais clínicos está diretamente relacionada com a virulência do agente e fatores relacionados ao hospedeiro, como o estado de imunológico e/ou fase da gestação em que ocorre a infecção (BAKER, 1995; BOLIN; GROOMS, 2004). Apesar da grande manifestação de sinais clínicos, as infecções subclínicas possuem o maior impacto econômico, reduzindo os índices reprodutivos em animais de produção, cursando com linfopenia e imunossupressão severa, e facilitando a disseminação de outros agentes no rebanho (RIDPATH, 2010b). Em fêmeas prenhes, o vírus pode causar reabsorção embrionária, retorno ao cio, abortos, mumificação fetal, nascimento de natimortos ou animais persistentemente infectados dependendo do estado da gestação (CARMAN et al., 1998).

Após a replicação inicial no epitélio do trato respiratório superior e nos gânglios linfáticos regionais, as partículas víricas atingem a corrente sanguínea disseminando-se para outros órgãos, como pulmão, coração, baço, abomaso e intestinos (LIEBLER-TENORIO; RIDPATH; NEILL, 2004) O período de viremia pode durar de 5 a 7 dias, entretanto pode se estender por até 15 dias, dependendo do nível de estresse do hospedeiro e da presença de outros patógenos (RIDPATH, 2010a). O dano tecidual, causado pela replicação viral atrai para o local células de defesa que secretam fatores de inflamação, podendo ser observadas lesões caracterizadas por edema pulmonar, úlceras e erosões na língua/esôfago, petéquias e equimoses no baço/coração, e áreas multifocais nos intestinos (KHODAKARAM-TAFTI; MOHAMMADI; FARJANI KISH, 2016; LUNARDI et al., 2008).

Em 2004 na Alemanha, uma nova variante de BVDV foi identificada em amostras de soro fetal bovino comercial proveniente do Brasil. Esse vírus é distinto geneticamente dos subgenótipos já conhecidos, e por isso foi denominado de *HoBi*, em referência às iniciais dos pesquisadores que identificaram esse pestivírus atípico (Horst Schirrmeier, Gunther Strebelow, Klaus Depner, Bernd Hoffmann e Martin Beer) (SCHIRRMEIER et al, 2004). A partir de então, inúmeros isolados de *HoBi-like*/pestivírus atípicos tem sido identificados em amostras de soro fetal bovino e também de doença em animais em vários países, como no Brasil, na Itália e no Sudoeste Asiático (CORTEZ et al., 2006; DECARO et al., 2011; KAMPA et al., 2009; LIU et al., 2009b; SILVEIRA et al., 2017; STALDER et al., 2005; WEBER et al., 2014)

Esta nova espécie viral ainda permanece sem classificação oficial, e tem sido denominada de BVDV-3, vírus *HoBi-like* ou pestivírus atípico bovino (SMITH et al., 2017). O vírus *Hobi-like* apresenta carcaterísticas moleculares que demonstram sua relação filogenética com o BVDV-1 e 2, embora sejam distintos antigenicamente. Na Europa já foram descritas doença respiratória aguda e morte de bovinos, dois quais foram isolados *HoBi-like* ncp e cp de um mesmo animal (DECARO et al., 2012b). O vírus *HoBi-like* pode ainda causar abortos, malformações (BIANCHI et al., 2011; DECARO et al., 2012b) e gerar animais PI (DECARO et al., 2013a). No Brasil, já foram relatados quadros compatíveis com DM, onde foram observados sinais respiratórios e digestivos, além de lesões cutâneas e opacidade da córnea (WEBER et al., 2014).

Em outro estudo, animais experimentalmente infectados com cepas *HoBi-like* de baixa e alta virulência apresentaram sinais clínicos semelhantes aos observados em animais naturalmente infectados; estes sinais clínicos são indistinguíveis das demais cepas de BVDV, sendo observada principalmente febre e decréscimo no número de linfócitos circulantes (RIDPATH et al., 2013). A infecção natural de bezerros jovens (aproximadamente 6 meses de idade) pelo vírus *HoBi-like* foi descrita pela primeira vez em 2010 na Itália. Os animais apresentaram sinais clínicos leves, caracterizados por tosse e secreção nasal, além de hipertermia e leucopenia leve, com duração de, aproximadamente, 21 dias. Neste mesmo estudo, na tentativa de reproduzir a doença, o vírus isolado foi inoculado experimentalmente em seis bezerros, sendo que apenas dois desenvolveram sinais clínicos, consistindo em descarga nasal, hipertermia e leucopenia leve (DECARO et al., 2011).

A comparação da infecção experimental de bovinos com *HoBi-like* e cepas BVDV de baixa e alta virulência foi realizada em bezerros nos Estados Unidos (RIDPATH et al., 2013). Animais infectados com o vírus *HoBi-like* apresentaram sinais clínicos semelhantes aos

observados em animais naturalmente infectados, cursando, principalmente, com febre e decréscimo no número de linfócitos circulantes. Estes sinais clínicos também foram semelhantes aos desenvolvidos por bezerros inoculados com BVDV de baixa e alta virulência, sendo que estes animais também desenvolveram diarreia (RIDPATH et al., 2013). Desde sua primeira descrição em 2004, pesquisadores buscam reproduzir os sinais clínicos e patológicos causados pela infecção do *HoBi-like* em bovinos com o objetivo de compreender a patogenia deste pestivírus. Entretanto, a infecção experimental de bovinos muitas vezes não reproduz os achados de campo. Isolados de campo com histórico de doença aguda grave, quando inoculados experimentalmente em terneiros, apresentam baixa ou nenhuma virulência (RIDPATH et al., 2013). A falha em reproduzir a infecção por *HoBi-like* pode ser decorrente de diversos fatores como a idade, estado nutricional e imunológico do animal, bem como a virulência do agente (RIDPATH et al., 2013).

Assim, com a necessidade de caracterizar a infecção deste vírus em bovinos, este estudo teve como objetivo caracterizar os aspectos virológicos e patológicos da infecção por dois isolados brasileiros de *HoBi-like* (SV478/07 e SV757/15), a fim de utilizar em outros estudos, como em testes de vacinas.

2 CAPÍTULO 1

Respiratory signs, fever and lymphopenia in calves inoculated with Brazilian HoBi-like pestiviruses

*José Conrado dos Santos Jardim[1]; Bruna Portolan Amaral.[1];Mathias Martins.[1]; Pablo Sebastian Britto de Oliveira[1]; Marcos Bryan Heinemann.[2]; Adriana Cortez.[3];Rudi Weiblen.[1];Eduardo Furtado Flores.[1]**

(Artigo submetido à revista *Microbial Pathogenesis – 2017)*

[1]Programa de Pós-Graduação em Medicina Veterinária e Setor de Virologia, Universidade Federal de Santa Maria, Avenida Roraima, 1000, prédio 63A, Centro de Eventos, Santa Maria, RS. Brazil. 97105-900.

[2]Departamento de Medicina Veterinária Preventiva e Saúde Animal, Universidade de São Paulo, USP.

[3]Curso de Medicina Veterinária, Universidade Santo Amaro, São Paulo, Brasil.

*Corresponding author: eduardofurtadoflores@gmail.com

Abstract

Hobi-like viruses comprise an unclassified group of bovine pestiviruses originally identified in commercial fetal bovine serum of Brazilian origin and, subsequently, isolated from diseased animals in several countries. Although frequently isolated from clinical cases, most HoBi-like isolates failed to reproduce overt disease upon experimental inoculation. Herein, we describe the outcome of experimental infection of four to six months-old seronegative calves with two Brazilian HoBi-like isolates. Calves inoculated intranasally with isolate SV478/07 developed viremia between days 2 and 9 post-inoculation (pi) and shed virus in nasal secretions up to day 11pi. These animals presented hyperthermia (day 7 to 10-11 pi) and lymphopenia from days 4 to 8pi. Clinically, all four calves developed varied degrees of apathy, anorexia, mild to moderate respiratory signs (nasal secretion, hyperemia), ocular discharge and pasty diarrhea in the days following virus inoculation. In contrast, calves inoculated with isolate SV757/15 presented only hyperthermia (days 3 to 10-11 pi) and lymphopenia (days 4 to 8 pi), without other apparent clinical signs. In these animals, viremia was detected up to day 9 pi and virus shedding in nasal secretions lasted up to day 12-14 pi. Both groups seroconverted to the inoculated viruses, developing virus neutralizing (VN) titers from 320 to 5120 at day 28pi. These results extend previous findings that experimental infections of calves with HoBi-like viruses are predominantly mild, yet they also indicate differences in virulence among field isolates.

Key words: bovine pestivirus, atypical pestivirus, experimental infection, pathogenesis.

1 Introduction

Hobi-like pestiviruses comprise an unclassified group of bovine pestiviruses initially identified as contaminant of fetal bovine serum of Brazilian origin [1]. These viruses are genetically and antigenically related to *Bovine viral diarrhea virus* 1 (BVDV-1) and 2 (BVDV-2), the prototypes of the genus *Pestivirus*, family *Flaviviridae*. The genus *Pestivirus* also includes *Border disease virus* (BDV), *Classical swine fever virus* (CSFV) and four other putative species of veterinary relevance [2]. A new classification and nomenclature of pestiviruses has been proposed, including seven new viral species, e.g. *Pronghorn pestivirus*, *Bungowannah virus*, *Giraffe pestivirus*, *Hobi-like pestivirus*, *Aydin-like pestivirus*, *Rat pestivirus* and *Atypical porcine pestivirus* [2]. Pestiviruses are small (40-50nm), enveloped, single-stranded RNA viruses whose genome encodes 11-12 mature polypeptides [3]. The prototype pestiviruses BVDV-1 and BVDV-2 are distributed worldwide and have been associated with a variety of clinical manifestations in cattle [4]. The HoBi-like viruses, also referred to as atypical bovine pestiviruses or BVDV-3 have also been recognized as bovine pathogens, causing clinical presentations similar to those observed in BVDV-1 or BVDV-2 infections [5,6].

HoBi-like viruses are genetically and antigenically related to BVDV-1 and BVDV-2, yet significant antigenic differences do exist among these groups of viruses [1,7–9]. In particular, the low serological cross-reactivity between BVDV species and HoBi-like viruses has raised concerns regarding to protection by BVDV vaccines against these novel [6,7,9,10].

The first Hobi-like virus, isolate D32/00, was detected in a batch of fetal bovine serum (FBS) imported from Brazil [1]. Subsequently, genetically similar viruses were detected in lots of FBS collected from different origins over several years and thereafter called *HoBi-like*

viruses [5,11–15]. In addition, a significant part of Brazilian FBS batches are contaminated with pestiviruses [16], including HoBi-like [17,18]. Following their identification in FBS, viruses belonging to the Hobi-like group have been subsequently associated with a variety of clinical manifestations in cattle in Brazil [19–21], Italy [22], Thailand [5], India [23], and Bangladesh [24]. A t report indicates the involvement of HoBi-like viruses in respiratory diseases in small ruminants from China [25].

Most clinical manifestations associated with natural HoBi-like virus infection resemble those classically associated with BVDV infection, including moderate to severe fever, leukopenia and respiratory disease [22], mucosal-like disease [21,26,27], reproductive failure [19,28,29] and persistent infections [10]. However, experimental inoculation of calves with HoBi-like isolates have failed in reproducing the severe disease reported in some field outbreaks [22]. Experimentally infected calves may present fever, slight decrease in white blood cells, moderate conjunctivitis, watery-mucoid nasal and ocular discharge and, sometimes, cough [1,28,30,31],. Viremia, virus shedding in nasal secretions and seroconversion at different extents has also been observed in inoculated animals [1,28,30,31]. A study demonstrated several reproductive failures (abortions, stillbirths, weal born calves, persistently infected offspring) in sheep experimentally infected with an Italian HoBi-like isolate, suggesting sheep as a model to study the protection conferred by vaccines [30].

Atypical pestiviruses, subsequently characterized as HoBi-like, have been identified in many Brazilian regions associated with a variety of clinical manifestations [19–21]. These data indicate that HoBi-like viruses are endemic among Brazilian cattle. Virus identification over two decades indicate that approximately 6 to 12% of bovine pestiviruses circulating in Brazil belong to the HoBi-like group [18–20,27]

In this study, we performed an experimental infection of calves with two Brazilian HoBi-like virus isolates, to better understand their biology and pathogenesis and to identify virulent isolates to be used as challenge viruses in vaccine-challenge experiments.

2 Materials and Methods

2.1 Experimental design

Groups of four to six-months-old calves were inoculated intranasally (IN) with each of two Brazilian HoBi-like isolates and monitored regarding to clinical, virological and serological aspects following inoculation. Clinical monitoring included daily observation for systemic and respiratory signs of infection, body temperature and leukocyte counts. Virus shedding in nasal secretions and viremia were investigated by virus isolation in cell culture. The serological response to virus infection was assayed by virus-neutralizing (VN) tests performed in serum samples collected at 28 days post-infection (pi). This study was approved by the board of ethics of the institution registered with nº 7262170717.

2.2 Viruses and cells

The HoBi-like pestiviruses SV478/07 [32] and SV757/15 [9,27] were isolated at the Virology Section, Federal University of Santa Maria (SV/UFSM). SV478/07 was isolated from the blood of a persistently infected (PI) from a herd with reproductive problems in Midwestern Brazil; SV757/15 was isolated from intestinal segments of a heifer dying of gastro-intestinal disease resembling mucosal disease (MD) in Southeastern Brazil (Cortez et al. 2017). BVDV-1 Singer strain and BVDV-2 VS-253 used in cross-VN assays were from our laboratory collection.

All procedures of virus amplification, quantitation and isolation from clinical specimens used pestivirus-free Madin Darby bovine kidney cells (MDBK, ATCC – CCL22). Cells were

maintained in minimum essential medium (MEM, ThermoFisher Scientific, Massachusetts, USA), supplemented with 10% equine serum, 100 U/mL of penicillin and 100 µg/mL of streptomycin (ThermoFisher Scientific, Massachusetts, USA). Virus stocks were titrated by limiting dilution in 96-well microtiter plates and virus titers were calculated according to Reed & Muench (1938) [33] and expressed as $\log_{10}$ median tissue culture infective dose ($TCID_{50}$/mL). Viruses were used for animal inoculation at passages # 6 (SV478/07) or # 8 (SV757/15). The inoculum consisted of 10 mL of a virus suspension containing 10^7 $TCID_{50}$/mL.

2.3 Animals, virus inoculation and monitoring

Two groups of four to six-months-old calves, seronegative to BVDV, were used for virus inoculation. Group 1 consisted of four Jersey male calves inoculated with isolate SV478/07; group 2 consisted of four male Holstein calves inoculated with isolate SV757/15. The groups were kept in separate barns without contact with each other, feeding on natural grass and receiving alfalfa and pelletized food and water *at libitum*. Each calf was inoculated by intranasal (IN) vaporization of 10 mL of a virus suspension containing 10^7 $TCID_{50}$/mL of the respective virus, followed by swabbing the virus suspension against the nasal mucosa. Two calves of the same age and genetic background were inoculated with MEM and served as mock-inoculated controls.

The inoculated animals were monitored for respiratory signs (nasal secretion, aspect and color of the nasal mucosa, breathing) and systemic signs (body temperature, alertness and appetite) through two daily examinations up to day 15 post-inoculation (pi). Before virus inoculation, the baseline temperature for each animal and group was determined by daily measurements starting at day -7. Blood samples for leukocyte counts were collected three times in the week before virus inoculation (days -4, -2 and 0) and every two days following virus inoculation up to day 10. The samples were submitted to total leukocyte counts in an automatic

Sysmex KX-21N. Heparinized blood collected daily up to day 14 pi was submitted to virus isolation according to Dias et al. (2017) [9]. Mock-infected and MDBK cells infected with BVDV Singer strain were used as controls. Beginning at day 2 pi, nasal swabs were collected daily in 1 mL of culture medium and submitted to virus isolation in cell culture as described above.

Sera obtained from blood collected at days 0 and 28 pi were submitted to virus-neutralizing (VN) assays. VN assays were performed in 96-well plates, by incubating two-fold dilutions (starting at 1:5) of each serum with approximately 100-200 $TCID_{50}$ of the respective HoBi-like isolate for 2 h at 37°C in a 5% CO_2 incubator, followed by addition of a suspension of MDBK cells and incubation for 96 h. Readings were realized by monitoring indicator cells for viral antigens by fluorescent antibody assay (FA) [9]. VN assays with sera collected at day 28pi were also tested against representative BVDV-1 (Singer strain) and BVDV-2 (VS-253) to investigate serological cross-reactivity. The antibody titers were expressed as the reciprocal of the highest serum dilution that prevented virus replication.

3 RESULTS

3.1 Clinical monitoring

Following virus inoculation, animals of both groups presented an increase in body temperature comparing to baseline levels. Group 1 (SV478/07) calves presented a mean increase higher than 0.5 degree between days 7 and 10-11 pi. Temperatures returned to physiological levels at days 12-13 pi. Group 2 (SV757/15) calves presented a mean increase of up to 1.3 degree between days 3 and 9 pi, with a secondary peak at days 11 and 12 pi; the temperatures returned to baseline levels at day 13 pi. In general, the temperatures peaked earlier and to highest values in animals of group 2 (Figure 1). The body temperature of control calves remained within the baseline limits.

Upon daily clinical examination, animals inoculated with isolate SV478/07 (group 1) presented apathy, reduction in food intake, nasal and ocular discharge and diarrhea. Nasal discharge was mild to moderate and predominantly mucosal or mucopurulent. Diarrhea was mild and predominantly mucous. These clinical signs started around days 5 - 6 pi and lasted for 5 to 6 days, subsiding spontaneously without therapy (Table 1). Group 2 (SV757/15) calves did not present apparent clinical signs throughout the observation period. No reductions in food intake, apathy or respiratory/digestive signs were apparent. The control animals remained healthy throughout the observation period. One calf from group 2 (#71) died of unrelated causes at day 12 pi. These results demonstrate different clinical outcomes between the groups. Whereas group 2 calves remained clinically healthy, group 1 developed mild to moderate, transient systemic and respiratory/digestive disease.

3.2 Lymphocyte counts

Group 1 calves (SV478/07) presented a progressive decrease in lymphocyte counts, beginning at day 2 pi and reaching the lowest levels at day 6 pi (26.9% of the baseline levels). A slight lymphopenia (18.9% reduction) was still observed at day 8 pi, with values returning near to basal levels at day 10 pi (Figure 2). Group 2 calves (SV757/15) also presented a decrease in lymphocyte counts, beginning at day 4 pi and reaching minimum levels at day 6 pi (an approximately 24.8% reduction comparing to basal levels). The values returned to nearly basal levels at day 10 pi. Thus, both isolates induced a slight to moderate, transient reduction in lymphocyte counts in inoculated animals. Control animals presented the lymphocyte counts with no significant changes comparing to baseline levels.

3.3 Viremia

All animals of both groups developed viremia detected by virus isolation from blood leucocytes. Viremia was initially detected in animals from Group 1 (SV478/07), starting at day 2 pi ending at days 8-9 pi. In animals from Group 2 (SV757/15), viremia was detected from day 4 pi and lasted up to days 8-9 pi (Table 1). Minor differences in the duration of viremia were observed between the groups. No infectious virus was detected in the blood of control calves upon inoculation of cell cultures.

3.4 Virus shedding in nasal secretions

Virus shedding in nasal secretions was detected in two calves from group 1 (SV478/07) from day 3 to 11 pi and in all animals of group 2 (SV757/15) from day 4 to 8-9 pi. Virus titers in nasal secretions reached up to $10^{5.1}$ $TCID_{50}$/ml between days 4 and 8 pi (not shown). Interestingly, no infectious virus was detected in nasal secretions of two animals from group 1 (#74 and 75). Control calves did not shed virus in nasal secretions during the monitoring period.

3.5 Serology

Neutralizing antibodies were detected in titers from 320 to 5120 in animals of both groups at day 30 pi. In general titers of group 2 calves (SV747/15) were higher than those of group 1 calves (SV478/07) (Table 1). Control animals remained seronegative at the end of the experiment. VN titers against BVDV-1 and BVDV-2 at day 28pi in both groups were significantly lower than those to the respective homologous viruses. These results confirm the antigenic differences between HoBi-like viruses and either BVDV-1 or BVDV-2.

4 Discussion

Our results corroborate and extend previous findings that experimental infection of seronegative calves with Hobi-like pestiviruses are frequently subclinical or accompanied by mild systemic and/or respiratory signs. In addition, our results indicate the existence of differences in virulence among field HoBi-like isolates, since the clinical outcomes varied between the groups. Calves inoculated with SV478/07 – in addition to fever and lymphopenia - developed apathy, anorexia, mild to moderate respiratory signs and diarrhea. In contrast, calves inoculated with SV757/15 developed only mild fever and lymphopenia. The extent (magnitude and duration) of viremia and virus shedding also varied between the experimental groups, suggesting different abilities/capacities of the two isolates to replicate in their hosts. In anyway, additional studies are needed to better understand the pathogenesis of HoBi-like virus infections and to indicate whether highly virulent HoBi-like viruses do exist, as demonstrated for BVDV-2 and, to a much lesser extent, for BVDV-1.

Following their initial identification in FBS [1], viruses belonging to the Hobi-like group have been subsequently associated with a variety of clinical manifestations in cattle in several countries. Many of the clinical manifestations attributed to HoBi-like viruses – including fever, lymphopenia, respiratory, digestive disease and reproductive failures - resemble those classically associated with BVDV-1 and BVDV-2 infections. Although many of the reported cases/outbreaks were associated with moderate to severe clinical presentation, experimental inoculation of susceptible calves with HoBi-like viruses isolated from these cases have failed to reproduce overt disease, especially the severe respiratory disease first described in Italy. Even the isolate recovered from this outbreak failed to reproduce respiratory disease upon inoculation of susceptible calves [22].

Under experimental conditions, most animals inoculated with HoBi-like viruses to date presented only elevated body temperature and a slight decrease in white blood cells. Decaro et

al [28] inoculated calves with an European HoBi-like strain (Italy – 1/10-1), observing mild respiratory signs (nasal discharge), moderate hyperthermia and leukopenia, viremia and viral shedding through the nasal and fecal routes. Later, experimental infection of calves and sheep and pigs through direct inoculation or exposure to PI calves resulted in lymphopenia (in 35% of the animals), fever, viremia and seroconversion. Moderate conjunctivitis, watery-mucoid nasal, ocular discharge and cough were observed in some exposed calves [28].

The number of experimental studies performed and the number of HoBi-virus isolates tested to date are still low to draw conclusions on the virulence potential of this novel group of bovine pestiviruses. Clinical observations in natural outbreaks have demonstrated that these viruses are able to produce overt, severe respiratory [22] and gastroenteric disease [27] during natural acute infections. In addition, severe gastroenteric disease resembling MD has been associated with co-infection by cytopathic (cp) and non-cytopathic HoBi-like isolates [21,22]. Nonetheless, the initial studies – including the current – indicate that experimental infections of susceptible calves with HoBi-like virus infections are frequently subclinical or accompanied by mild systemic and respiratory signs. The failure to reproduce overt/severe disease under experimental conditions – even with viruses isolated from cases of severe disease - clearly indicate that other factors – including host-derived – likely influence the outcome of natural infections.

On the other hand, the different clinical outcomes in the experimental groups indicate the existence of differences in virulence among HoBi-like isolates. Isolate SV478/07 - in addition to viremia and virus shedding - produced fever, lymphopenia, apathy/anorexia, mild to moderate respiratory signs and transient mucoid diarrhea. This virus was isolated from the blood of a healthy calf in a herd suffering from reproductive disorders, in a trial for identifying PI animals. Isolate SV757/15 produced only a transient and slight fever and lymphopenia, accompanied by a 5-7 days lasting viremia and virus shedding in nasal secretions. This virus

was isolated from tissues of a heifer suffering from a severe gastroenteric disease, whose clinical and pathological findings resembled those of mucosal disease (MD). Nevertheless, only a non-cytopathic virus was isolated, indicating an acute infection rather than MD. Hence, under experimental conditions neither isolate reproduced the clinical disease from which they have been associated.

The different clinical outcomes between the inoculated groups likely reflects differences in virulence between the isolates. Low and high virulence isolates have been well recognized among BVDV isolates, especially BVDV-2 [7,24,31]. Nonetheless, the genetic background of the calves might have also influenced the clinical outcomes, since they were from different genetic backgrounds (Holstein x Jersey). Regardless, both groups seroconverted to inoculated viruses to high titers, demonstrating an efficient virus replication and strong immunological response.

HoBi-like viruses are genetically and antigenically related to BVDV-1 and BVDV-2, yet marked antigenic differences have been demonstrated between these groups of viruses [1,7–9,31]. In the present study, the VN assays performed against representative BVDV-1 (Singer) and BVDV-2 (VS-253) strains with sera collected from calves at day 28pi confirmed the lower neutralizing activity – to different extents - of HoBi antisera against these viruses. The low serological cross-reactivity between BVDV species and HoBi-like viruses represents a major concern regards to vaccine protection [6,7,9,34]. To date, no commercial vaccine includes HoBi-like viruses in their formulation. Considering the low serological reactivity of BVDV-1/BVDV-2 antisera against HoBi-like viruses, it seems unlikely that the current vaccines containing BVDV-1 and BVDV-2 (or even vaccines containing only BVDV-1) would protect against natural exposure to HoBi-like virus isolates. Thus, inclusion of HoBi-like strains in vaccines to be used in regions where these viruses circulate seem mandatory [9].

In addition to investigate the virulence of field isolates and study their pathogenesis, our study aimed at identifying a virulent strain to be used in vaccine-challenge experiments. In this sense, our results suggest that isolate SV478/07 may be adequate for such studies since it produced mild to moderate clinical signs in all inoculated calves.

Acknowledgement

This study was supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq). J.C. Jardim, B.P. Amaral, M. Martins, P.S.B. de Oliveira, E.F. Flores and R. Weiblen are CNPq research fellows.

References

[1] H. Schirrmeier, Genetic and antigenic characterization of an atypical pestivirus isolate, a putative member of a novel pestivirus species, J. Gen. Virol. 85 (2004) 3647–3652. doi:10.1099/vir.0.80238-0.

[2] D.B. Smith, G. Meyers, J. Bukh, E.A. Gould, T. Monath, A. Scott Muerhoff, A. Pletnev, R. Rico-Hesse, J.T. Stapleton, P. Simmonds, P. Becher, Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae, J. Gen. Virol. 98 (2017) 2106–2112. doi:10.1099/jgv.0.000873.

[3] R.O. Donis, Molecular biology of bovine viral diarrhea virus and its interactions with the host., Vet. Clin. North Am. Food Anim. Pract. 11 (1995) 393–423. doi:10.1016/S0749-0720(15)30459-X.

[4] J.C. Baker, The clinical manifestations of bovine viral diarrhea infection., Vet. Clin. North Am. Food Anim. Pract. 11 (1995) 425–45. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8581856.

[5] L. Liu, J. Kampa, S. Belák, C. Baule, Virus recovery and full-length sequence analysis of atypical bovine pestivirus Th/04_KhonKaen, Vet. Microbiol. 138 (2009) 62–68. doi:10.1016/j.vetmic.2009.03.006.

[6] F. V Bauermann, J.F. Ridpath, R. Weiblen, E.F. Flores, HoBi-like viruses, J. Vet. Diagnostic Investig. 25 (2013) 6–15. doi:10.1177/1040638712473103.

[7] F. V Bauermann, E.F. Flores, J.F. Ridpath, Antigenic relationships between Bovine viral diarrhea virus 1 and 2 and HoBi virus, J. Vet. Diagnostic Investig. 24 (2012) 253–261. doi:10.1177/1040638711435144.

[8] M. Larska, M.P. Polak, V. Riitho, R. Strong, S. Belák, S. Alenius, Å. Uttenthal, L. Liu, Kinetics of single and dual infection of calves with an Asian atypical bovine pestivirus and a highly virulent strain of bovine viral diarrhoea virus 1, Comp. Immunol. Microbiol. Infect. Dis. 35 (2012) 381–390. doi:10.1016/j.cimid.2012.03.003.

[9] R.K. Dias, J.F. Cargnelutti, M.N. Weber, C.W. Canal, F. V. Bauermann, J.F. Ridpath, R. Weiblen, E.F. Flores, Antigenic diversity of Brazilian isolates of HoBi-like pestiviruses, Vet. Microbiol. 203 (2017) 221–228. doi:10.1016/j.vetmic.2017.03.021.

[10] N. Decaro, M. Losurdo, M.S. Lucente, R. Sciarretta, V. Mari, V. Larocca, G. Elia, N. Cavaliere, V. Martella, A. Fasanella, C. Buonavoglia, Persistent infection caused by hobi-like pestivirus, J. Clin. Microbiol. 51 (2013) 1241–1243. doi:10.1128/JCM.03134-12.

[11] H.P. Stalder, P. Meier, G. Pfaffen, C. Wageck-Canal, J. Rüfenacht, P. Schaller, C. Bachofen, S. Marti, H.R. Vogt, E. Peterhans, Genetic heterogeneity of pestiviruses of ruminants in Switzerland, Prev. Vet. Med. 72 (2005) 37–41. doi:10.1016/j.prevetmed.2005.01.020.

[12] K. Ståhl, M. Beer, H. Schirrmeier, B. Hoffmann, S. Belák, S. Alenius, Atypical "HoBi"-like pestiviruses-Recent findings and implications thereof, Vet. Microbiol. 142 (2010) 90–93. doi:10.1016/j.vetmic.2009.09.048.

[13] S. Peletto, F. Zuccon, M. Pitti, E. Gobbi, L. De Marco, M. Caramelli, L. Masoero, P.L. Acutis, Detection and phylogenetic analysis of an atypical pestivirus, strain IZSPLV_To, Res. Vet. Sci. 92 (2012) 147–150. doi:10.1016/j.rvsc.2010.10.015.

[14] M. Giammarioli, L. Ceglie, E. Rossi, M. Bazzucchi, C. Casciari, S. Petrini, G.M. De Mia, Increased genetic diversity of BVDV-1: recent findings and implications thereof, Virus Genes. 50 (2015) 147–151. doi:10.1007/s11262-014-1132-2.

[15] H. Xia, M. Larska, M. Giammarioli, G.M. De Mia, G. Cardeti, W. Zhou, S. Alenius, S. Belák, L. Liu, Genetic Detection and Characterization of Atypical Bovine Pestiviruses

in Foetal Bovine Sera Claimed to be of Australian Origin, Transbound. Emerg. Dis. 60 (2013) 284–288. doi:10.1111/j.1865-1682.2012.01341.x.

[16] T.F. Pinheiro de Oliveira, A.A. Fonseca, M.F. Camargos, A.M. de Oliveira, A.C. Pinto Cottorello, A. dos R. Souza, I.G. de Almeida, M.B. Heinemann, Detection of contaminants in cell cultures, sera and trypsin, Biologicals. 41 (2013) 407–414. doi:10.1016/j.biologicals.2013.08.005.

[17] H. Xia, B. Vijayaraghavan, S. Belák, L. Liu, Detection and Identification of the Atypical Bovine Pestiviruses in Commercial Foetal Bovine Serum Batches, PLoS One. 6 (2011) e28553. doi:10.1371/journal.pone.0028553.

[18] F.L. Monteiro, J.F. Cargnelutti, M. Martins, D. Anziliero, M.M. Erhardt, R. Weiblen, E.F. Flores, Detection of respiratory viruses in shelter dogs maintained under varying environmental conditions, Brazilian J. Microbiol. 47 (2016) 876–881. doi:10.1016/j.bjm.2016.07.002.

[19] A. Cortez, M.B. Heinemann, A.M.M.G. de Castro, R.M. Soares, A.M. V. Pinto, A.A. Alfieri, E.F. Flores, R.C. Leite, L.J. Richtzenhain, Genetic characterization of Brazilian bovine viral diarrhea virus isolates by partial nucleotide sequencing of the 5'-UTR region, Pesqui. Veterinária Bras. 26 (2006) 211–216. doi:10.1590/S0100-736X2006000400005.

[20] E. Bianchi, M. Martins, R. Weiblen, E.F. Flores, Perfil genotípico e antigênico de amostras do vírus da diarréia viral bovina isoladas no Rio Grande do Sul (2000-2010), Pesqui. Veterinária Bras. 31 (2011) 649–655. doi:10.1590/S0100-736X2011000800003.

[21] M.N. Weber, A.C.S. Mósena, S.V.D. Simões, L.L. Almeida, C.R.M. Pessoa, R.F. Budaszewski, T.R. Silva, J.F. Ridpath, F. Riet-Correa, D. Driemeier, C.W. Canal, Clinical Presentation Resembling Mucosal Disease Associated with "HoBi"-like Pestivirus in a Field Outbreak, Transbound. Emerg. Dis. 63 (2014) 92–100. doi:10.1111/tbed.12223.

[22] N. Decaro, M.S. Lucente, V. Mari, F. Cirone, P. Cordioli, M. Camero, R. Sciarretta, M. Losurdo, E. Lorusso, C. Buonavoglia, Atypical Pestivirus and Severe Respiratory Disease in Calves, Europe, Emerg. Infect. Dis. 17 (2011) 1549–1552. doi:10.3201/eid1708.101447.

[23] N. Mishra, K. Rajukumar, A. Pateriya, M. Kumar, P. Dubey, S.P. Behera, A. Verma, P. Bhardwaj, D.D. Kulkarni, D. Vijaykrishna, N.D. Reddy, Identification and molecular characterization of novel and divergent HoBi-like pestiviruses from naturally infected cattle in India, Vet. Microbiol. 174 (2014) 239–246. doi:10.1016/j.vetmic.2014.09.017.

[24] N. Haider, M.S. Rahman, S.U. Khan, A. Mikolon, E.S. Gurley, M.G. Osmani, I.S. Shanta, S.K. Paul, L. Macfarlane-Berry, A. Islam, J. Desmond, J.H. Epstein, P. Daszak, T. Azim, S.P. Luby, N. Zeidner, M.Z. Rahman, Identification and Epidemiology of a Rare HoBi-Like Pestivirus Strain in Bangladesh, Transbound. Emerg. Dis. 61 (2014) 193–198. doi:10.1111/tbed.12218.

[25] H. Shi, Y. Kan, L. Yao, C. Leng, Q. Tang, J. Ji, S. Sun, Identification of Natural Infections in Sheep/Goats with HoBi-like Pestiviruses in China, Transbound. Emerg. Dis. 63 (2016) 480–484. doi:10.1111/tbed.12551.

[26] N. Decaro, G. Lanave, M.S. Lucente, V. Mari, K. Varello, M. Losurdo, V. Larocca, E. Bozzetta, N. Cavaliere, V. Martella, C. Buonavoglia, Mucosal Disease-Like Syndrome in a Calf Persistently Infected by Hobi-Like Pestivirus, J. Clin. Microbiol. 52 (2014) 2946–2954. doi:10.1128/JCM.00986-14.

[27] A. Cortez, J.P. Araújo, E.F. Flores, M.G. Ribeiro, J. Megid, A.C. Paes, J.P. de Oliveira Filho, L.S. Ullmann, C.D. Malossi, M.B. Heinemann, Complete genome sequence of a Hobi-like virus isolated from a Nelore cow with gastroenteric disease in the state of

São Paulo, Brazil, Genome Announc. 5 (2017) e00767-17. doi:10.1128/genomeA.00767-17.

[28] N. Decaro, V. Mari, M.S. Lucente, R. Sciarretta, A. Moreno, C. Armenise, M. Losurdo, M. Camero, E. Lorusso, P. Cordioli, C. Buonavoglia, Experimental infection of cattle, sheep and pigs with “Hobi”-like pestivirus, Vet. Microbiol. 155 (2012) 165–171. doi:10.1016/j.vetmic.2011.08.030.

[29] F. V Bauermann, S.M. Falkenberg, B. Vander Ley, N. Decaro, B.W. Brodersen, A. Harmon, B. Hessman, E.F. Flores, J.F. Ridpath, Generation of Calves Persistently Infected with HoBi-Like Pestivirus and Comparison of Methods for Detection of These Persistent Infections, J. Clin. Microbiol. 52 (2014) 3845–3852. doi:10.1128/JCM.01563-14.

[30] F.V. Bauermann, S.M. Falkenberg, N. Decaro, E.F. Flores, J.F. Ridpath, Experimental infection of calves, sheep, goats and pigs with HoBi-like viruses by direct inoculation or exposure to persistently infected calves, Vet. Microbiol. 181 (2015) 289–293. doi:10.1016/j.vetmic.2015.10.011.

[31] J.F. Ridpath, S.M. Falkenberg, F. V Bauermann, B.L. VanderLey, Y. Do, E.F. Flores, D.M. Rodman, J.D. Neill, Comparison of acute infection of calves exposed to a high-virulence or low-virulence bovine viral diarrhea virus or a HoBi-like virus, Am. J. Vet. Res. 74 (2013) 438–442. doi:10.2460/ajvr.74.3.438.

[32] S. Silveira, M.N. Weber, A.C.S. Mósena, M.S. da Silva, A.F. Streck, C.A. Pescador, E.F. Flores, R. Weiblen, D. Driemeier, J.F. Ridpath, C.W. Canal, Genetic Diversity of Brazilian Bovine Pestiviruses Detected Between 1995 and 2014, Transbound. Emerg. Dis. 64 (2017) 613–623. doi:10.1111/tbed.12427.

[33] P.J. Pappas, The American Venous Forum and the lessons learned from the battle of Thermopolae, J. Vasc. Surg. 54 (2011) 1187–1192. doi:10.1016/j.jvs.2011.05.096.

[34] N. Decaro, V. Mari, M.S. Lucente, R. Sciarretta, G. Elia, J.F. Ridpath, C. Buonavoglia, Detection of a Hobi-like virus in archival samples suggests circulation of this emerging pestivirus species in Europe prior to 2007, Vet. Microbiol. 167 (2013) 307–313. doi:10.1016/j.vetmic.2013.09.006.

Table 1 – Virus shedding, viremia, clinical signs and neutralizing antibodies presented by calves experimentally inoculated with HoBi-like viruses.

Group	Animal (#)	Virus shedding (dpi)	Viremia (dpi)	Clinical signs	Neutralizing antibodies		
					BVDV-1	BVDV-2	Homologous
SV 478/07	49	3-11	3-8	Purulent nasal discharge and diarrhea (days 5-10)	10	80	640
	50	3-11	2-8	Nasal/ocular discharge and diarrhea (days 6-9)	20	160	640
	74	nd	2-8	Apathy, diarrhea, and nasal discharge (days 6-12)	10	160	320
	75	nd	2-9	Nasal discharge and apathy (days 8-12)	10	80	320
SV 757/15	69	2-12	4-7	none	160	10	1280
	70	2-12	4-8	none	80	<5	5120
	71*	2-12	4-6; 8-9	none			Nt
	72	2-14	4-8	none	80	<5	640

dpi: days post-inoculation; np: not performed; *animal #71 died at 12 dpi.

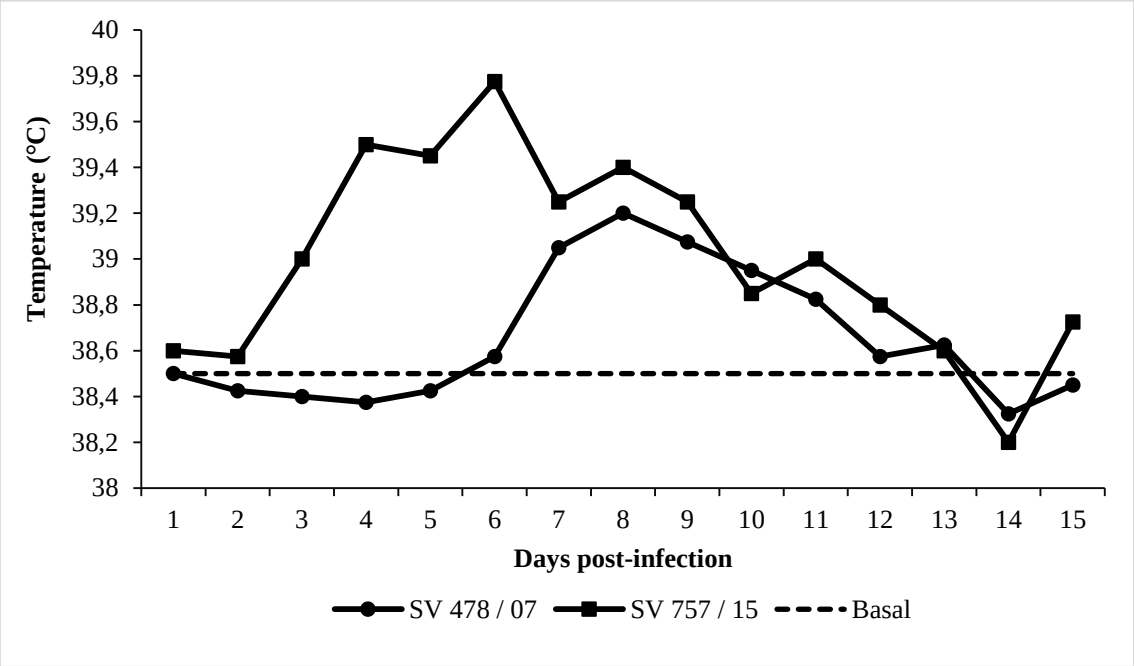

Figure 1. Mean daily temperatures of calves inoculated with HoBi-like isolates SV478/07 and SV757/15. The dashed line represents the baseline temperatures obtained from the animals in the days prior to virus inoculation.

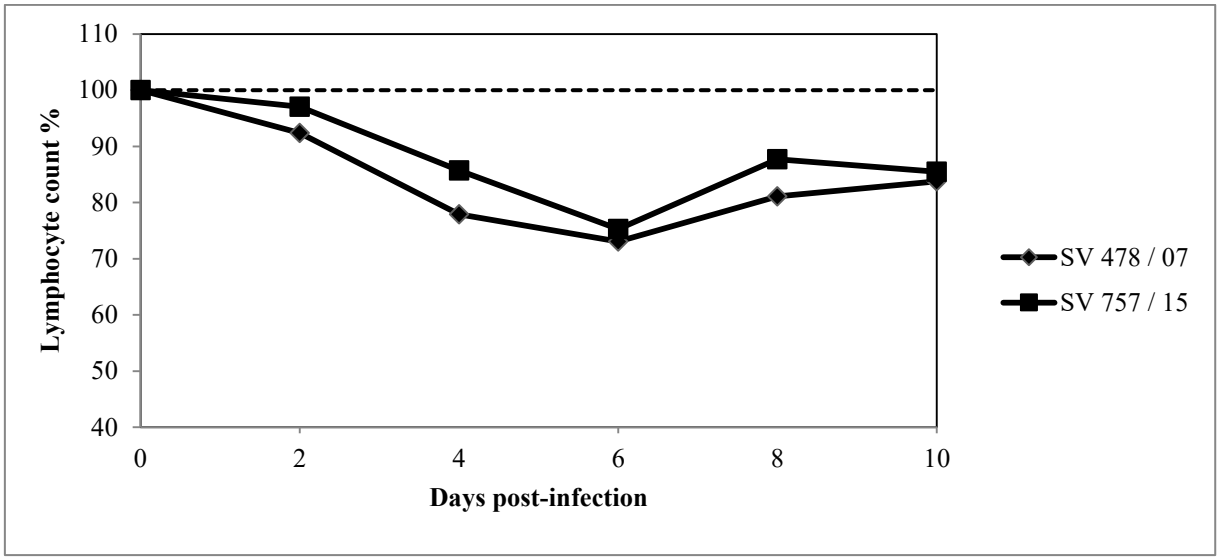

Figure 2. Lymphocyte counts in calves inoculated with HoBi-like isolates SV478/07 and SV757/15. Each value represents the mean of daily lymphocyte counts for each group. The dashed line represents the baseline lymphocyte obtained from the animals in the days prior to virus inoculation.

3 CONCLUSÕES

Os resultados apresentados nesta dissertação permitem concluir que:

- A infecção experimental de terneiros soronegativos com isolados brasileiros de pestivírus *HoBi-like* resultou em sinais clínicos leves, com severidade maior nos animais inoculados com o SV478/07.

- Diferenças de virulência provavelmente existem entre isolados de pestivírus *HoBi-like.*

4 REFERÊNCIAS

BAKER, J. C. The clinical manifestations of bovine viral diarrhea infection. **The Veterinary clinics of North America. Food Animal Practice**, [s. l.], v. 11, n. 3, p. 425–45, 1995. Disponível em: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8581856>

BAUERMANN, F. V. et al. Experimental infection of calves, sheep, goats and pigs with HoBi-like viruses by direct inoculation or exposure to persistently infected calves. **Veterinary Microbiology**, [s. l.], v. 181, n. 3–4, p. 289–293, 2015. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0378113515300511>

BAUERMANN, F. V et al. Generation of calves persistently Infected with HoBi-Like Pestivirus and Comparison of Methods for Detection of These Persistent Infections. **Journal of Clinical Microbiology**, [s. l.], v. 52, n. 11, p. 3845–3852, 2014. Disponível em: <http://jcm.asm.org/cgi/doi/10.1128/JCM.01563-14>

BAUERMANN, F. V et al. HoBi-like viruses. **Journal of Veterinary Diagnostic Investigation**, [s. l.], v. 25, n. 1, p. 6–15, 2013. Disponível em: <http://journals.sagepub.com/doi/10.1177/1040638712473103>

BAUERMANN, F. V; FLORES, E. F.; RIDPATH, J. F. Antigenic relationships between Bovine viral diarrhea virus 1 and 2 and HoBi virus. **Journal of Veterinary Diagnostic Investigation**, [s. l.], v. 24, n. 2, p. 253–261, 2012. Disponível em: <http://journals.sagepub.com/doi/10.1177/1040638711435144>

BIANCHI, E. et al. Perfil genotípico e antigênico de amostras do vírus da diarréia viral bovina isoladas no Rio Grande do Sul (2000-2010). **Pesquisa Veterinária Brasileira**, [s. l.], v. 31, n. 8, p. 649–655, 2011. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-736X2011000800003&lng=pt&tlng=pt>

BOLIN, S. R.; GROOMS, D.l L. Origination and consequences of bovine viral diarrhea virus diversity. **Veterinary Clinics of North America: Food Animal Practice**, [s. l.], v. 20, n. 1, p. 51–68, 2004. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0749072003000811>

BROWNLIE, J.; CLARKE, M.; HOWARD, C. Experimental production of fatal mucosal disease in cattle. **Veterinary Record**, [s. l.], v. 114, n. 22, p. 535–536, 1984. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/6087539>

CARMAN, S. et al. Severe Acute Bovine Viral Diarrhea in Ontario, 1993–1995. **Journal of Veterinary Diagnostic Investigation**, [s. l.], v. 10, n. 1, p. 27–35, 1998. Disponível em: <http://journals.sagepub.com/doi/10.1177/104063879801000106>

CORTEZ, A. et al. Genetic characterization of Brazilian bovine viral diarrhea virus isolates by partial nucleotide sequencing of the 5'-UTR region. **Pesquisa Veterinária Brasileira**, [s. l.], v. 26, n. 4, p. 211–216, 2006. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-736X2006000400005&lng=en&tlng=en>

CORTEZ, A. et al. Complete genome sequence of a Hobi-like virus isolated from a Nelore cow with gastroenteric disease in the state of São Paulo, Brazil. **Genome Announcements**, [s. l.], v. 5, n. 33, p. e00767-17, 2017. Disponível em: <http://genomea.asm.org/lookup/doi/10.1128/genomeA.00767-17>

DECARO, N. et al. Atypical Pestivirus and Severe Respiratory Disease in Calves, Europe. **Emerging Infectious Diseases**, [s. l.], v. 17, n. 8, p. 1549–1552, 2011. Disponível em: <http://wwwnc.cdc.gov/eid/article/17/8/10-1447_article.htm>

DECARO, N. et al. Experimental infection of cattle, sheep and pigs with "Hobi"-like pestivirus. **Veterinary Microbiology**, [s. l.], v. 155, n. 2–4, p. 165–171, 2012a. Disponível em: <http://dx.doi.org/10.1016/j.vetmic.2011.08.030>

DECARO, N. et al. Hobi-like pestivirus: both biotypes isolated from a diseased animal. **Journal of General Virology**, [s. l.], v. 93, n. Pt_9, p. 1976–1983, 2012b. Disponível em: <http://jgv.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.044552-0>

DECARO, N. et al. Persistent infection caused by hobi-like pestivirus. **Journal of Clinical Microbiology**, [s. l.], v. 51, n. 4, p. 1241–1243, 2013a.

DECARO, N. et al. Detection of a Hobi-like virus in archival samples suggests circulation of this emerging pestivirus species in Europe prior to 2007. **Veterinary Microbiology**, [s. l.], v. 167, n. 3–4, p. 307–313, 2013b. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0378113513004458>

DECARO, N. et al. Mucosal Disease-Like Syndrome in a Calf Persistently Infected by Hobi-Like Pestivirus. **Journal of Clinical Microbiology**, [s. l.], v. 52, n. 8, p. 2946–2954, 2014. Disponível em: <http://jcm.asm.org/cgi/doi/10.1128/JCM.00986-14>

DIAS, R. K. et al. Antigenic diversity of Brazilian isolates of HoBi-like pestiviruses. **Veterinary Microbiology**, [s. l.], v. 203, p. 221–228, 2017. Disponível em: <http://dx.doi.org/10.1016/j.vetmic.2017.03.021>

DONIS, R. O. Molecular biology of bovine viral diarrhea virus and its interactions with the host.**The Veterinary clinics of North America. Food animal practice**, 1995.

DONIS, R. O.; CORAPI, W. V; DUBOVI, E. J. Bovine viral diarrhea virus proteins and their antigenic analyses. **Archives of virology Supplementum**, [s. l.], v. 3, p. 29–40, 1991.

GIAMMARIOLI, M. et al. Increased genetic diversity of BVDV-1: recent findings and implications thereof. **Virus Genes**, [s. l.], v. 50, n. 1, p. 147–151, 2015. Disponível em: <http://link.springer.com/10.1007/s11262-014-1132-2>

HAIDER, N. et al. Identification and Epidemiology of a Rare HoBi-Like Pestivirus Strain in Bangladesh. **Transboundary and Emerging Diseases**, [s. l.], v. 61, n. 3, p. 193–198, 2014. Disponível em: <http://doi.wiley.com/10.1111/tbed.12218>

HAMERS, C. et al. Differences in Experimental Virulence of Bovine Viral Diarrhoea Viral Strains Isolated from Haemorrhagic Syndromes. **The Veterinary Journal**, [s. l.], v. 160, n. 3, p. 250–258, 2000. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S1090023300905008>

HOUE, H.; LINDBERG, A.; MOENNIG, V. Test strategies in bovine Viral Diarrhea Virus

Control and Eradication Campaigns in Europe. **Journal of Veterinary Diagnostic Investigation**, [s. l.], v. 18, n. 5, p. 427–436, 2006. Disponível em: <http://vdi.sagepub.com/lookup/doi/10.1177/104063870601800501>

HOUE, H. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. **Veterinary Microbiology**, [s. l.], v. 64, n. 2–3, p. 89–107, 1999. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0378113598002624>.

KAMPA, J. et al. Bovine herpesvirus type 1 (BHV-1) and bovine viral diarrhoea virus (BVDV) infections in dairy herds: Self clearance and the detection of seroconversions against a new atypical pestivirus. **The Veterinary Journal**, [s. l.], v. 182, n. 2, p. 223–230, 2009. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2008.07.006>

KHODAKARAM-TAFTI, A.; MOHAMMADI, A.; FARJANI KISH, G. H. Molecular characterization and phylogenetic analysis of bovine viral diarrhea virus in dairy herds of Fars province, Iran. **Iranian Journal of Veterinary Research**, [s. l.], v. 17, n. 2, p. 89–97, 2016. Disponível em: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5090137/>

LARSKA, M. et al. Kinetics of single and dual infection of calves with an Asian atypical bovine pestivirus and a highly virulent strain of bovine viral diarrhoea virus 1. **Comparative Immunology, Microbiology and Infectious Diseases**, [s. l.], v. 35, n. 4, p. 381–390, 2012. Disponível em: <http://dx.doi.org/10.1016/j.cimid.2012.03.003>

LIEBLER-TENORIO, E. M.; RIDPATH, J. F.; NEILL, J. D. Distribution of Viral Antigen and Tissue Lesions in Persistent and Acute Infection with the Homologous Strain of Noncytopathic Bovine Viral Diarrhea Virus. **Journal of Veterinary Diagnostic Investigation**, [s. l.], v. 16, n. 5, p. 388–396, 2004. Disponível em: <http://vdi.sagepub.com/lookup/doi/10.1177/104063870401600504>

LINDBERG, A et al. The control of bovine viral diarrhoea virus in Europe: today and in the future. **Revue scientifique et technique (International Office of Epizootics)**, [s. l.], v. 25, n. 3, p. 961–79, 2006. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/17361763>

LIU, L. et al. Virus recovery and full-length sequence analysis of atypical bovine pestivirus Th/04_KhonKaen. **Veterinary Microbiology**, [s. l.], v. 138, n. 1–2, p. 62–68, 2009. a.

LIU, L. et al. Maximum likelihood and Bayesian analyses of a combined nucleotide sequence dataset for genetic characterization of a novel pestivirus, SVA/cont-08. **Archives of Virology**, [s. l.], v. 154, n. 7, p. 1111–1116, 2009. b. Disponível em: <http://link.springer.com/10.1007/s00705-009-0419-4>

LUNARDI, M. et al. Outbreak of acute bovine viral diarrhea in Brazilian beef cattle: Clinicopathological findings and molecular characterization of a wild-type BVDV strain subtype 1b. **Research in Veterinary Science**, [s. l.], v. 85, n. 3, p. 599–604, 2008. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0034528808000039>

MISHRA, N. et al. Identification and molecular characterization of novel and divergent HoBi-like pestiviruses from naturally infected cattle in India. **Veterinary Microbiology**, [s. l.], v. 174, n. 1–2, p. 239–246, 2014. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0378113514004441>

MONTEIRO, F. L. et al. Detection of respiratory viruses in shelter dogs maintained under varying environmental conditions. **Brazilian Journal of Microbiology**, [s. l.], v. 47, n. 4, p.

876–881, 2016. Disponível em: <http://dx.doi.org/10.1016/j.bjm.2016.07.002>

PELETTO, S. et al. Detection and phylogenetic analysis of an atypical pestivirus, strain IZSPLV_To. **Research in Veterinary Science**, [s. l.], v. 92, n. 1, p. 147–150, 2012.

OLIVEIRA, P. et al. Detection of contaminants in cell cultures, sera and trypsin. **Biologicals**, [s. l.], v. 41, n. 6, p. 407–414, 2013. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S1045105613001061>

RIDPATH, J. F.; BOLIN, S. R.; DUBOVI, E. J. Segregation of Bovine Viral Diarrhea Virus into Genotypes. **Virology**, [s. l.], v. 205, n. 1, p. 66–74, 1994. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0042682284716205>

RIDPATH, J. F.; NEILL, J. D. Detection and characterization of genetic Recombination in Cytopathic Type 2 Bovine Viral Diarrhea Viruses. **Journal of Virology**, [s. l.], v. 74, n. 18, p. 8771–8774, 2000. Disponível em: <http://jvi.asm.org/cgi/doi/10.1128/JVI.74.18.8771-8774.2000>

RIDPATH, J.. The contribution of infections with Bovine Viral Diarrhea Viruses to Bovine Respiratory Disease. **Veterinary Clinics of North America: Food Animal Practice**, [s. l.], v. 26, n. 2, p. 335–348, 2010a. Disponível em: <http://dx.doi.org/10.1016/j.cvfa.2010.04.003>

RIDPATH, J. F. Bovine Viral Diarrhea Virus: Global Status. **Veterinary Clinics of North America: Food Animal Practice**, [s. l.], v. 26, n. 1, p. 105–121, 2010b. Disponível em: <http://dx.doi.org/10.1016/j.cvfa.2009.10.007>

RIDPATH, J. **Preventive strategy for BVDV infection in North AmericaJapanese Journal of Veterinary Research**, 2012a.

RIDPATH, J. Preventive strategy for BVDV infection in North America. **The Japanese journal of veterinary research**, [s. l.], v. 60 Suppl, n. SUPPL, p. S41-9, 2012b. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22458199>

RIDPATH, J. F. et al. Comparison of acute infection of calves exposed to a high-virulence or low-virulence bovine viral diarrhea virus or a HoBi-like virus. **American Journal of Veterinary Research**, [s. l.], v. 74, n. 3, p. 438–442, 2013. Disponível em: <http://avmajournals.avma.org/doi/abs/10.2460/ajvr.74.3.438>

SCHIRRMEIER, H. Genetic and antigenic characterization of an atypical pestivirus isolate, a putative member of a novel pestivirus species. **Journal of General Virology**, [s. l.], v. 85, n. 12, p. 3647–3652, 2004. Disponível em: <http://jgv.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.80238-0>

SHI, H. et al. Identification of natural infections in Sheep/Goats with HoBi-like Pestiviruses in China. **Transboundary and Emerging Diseases**, [s. l.], v. 63, n. 5, p. 480–484, 2016. Disponível em: <http://doi.wiley.com/10.1111/tbed.12551>

SILVEIRA, S. et al. Genetic diversity of Brazilian bovine Pestiviruses Detected Between 1995 and 2014. **Transboundary and Emerging Diseases**, [s. l.], v. 64, n. 2, p. 613–623, 2017. Disponível em: <http://doi.wiley.com/10.1111/tbed.12427>

SMITH, D. B. et al. Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae. **Journal of General Virology**, [s. l.], v. 98, n. 8, p. 2106–2112, 2017.

Disponível em:
<http://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.000873>

STÅHL, K. et al. Atypical “HoBi”-like pestiviruses-recent findings and implications thereof. **Veterinary Microbiology**, [s. l.], v. 142, n. 1–2, p. 90–93, 2010. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0378113509004659>

STALDER, H. P. et al. Genetic heterogeneity of pestiviruses of ruminants in Switzerland. **Preventive Veterinary Medicine**, [s. l.], v. 72, n. 1–2, p. 37–41, 2005. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0167587705002345>

VAN RIJN, P. A. et al. Subdivision of the pestivirus genus based on envelope glycoprotein E2. **Virology**, [s. l.], v. 237, n. 2, p. 337–348, 1997. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/9356345>

VILCEK, S. et al. Characterization of a novel pestivirus originating from a pronghorn antelope. **Virus Research**, [s. l.], v. 108, n. 1–2, p. 187–193, 2005.

WEBER, M. N. et al. Clinical presentation resembling Mucosal Disease Associated with “HoBi”-like Pestivirus in a Field Outbreak. **Transboundary and Emerging Diseases**, [s. l.], v. 63, n. 1, p. 92–100, 2014.

XIA, H. et al. Genetic detection and characterization of Atypical Bovine Pestiviruses in Foetal Bovine Sera Claimed to be of Australian Origin. **Transboundary and Emerging Diseases**, [s. l.], v. 60, n. 3, p. 284–288, 2013. Disponível em: <http://doi.wiley.com/10.1111/j.1865-1682.2012.01341.x>

XIA, Hongyan et al. Detection and identification of the atypical Bovine Pestiviruses in Commercial Foetal Bovine Serum Batches. **PLoS ONE**, [s. l.], v. 6, n. 12, p. e28553, 2011. Disponível em: <http://dx.plos.org/10.1371/journal.pone.0028553>