

Bovine viral diarrhea virus infections in pigs: why is this situation important for Brazilian herds?

A infecção pelo vírus da diarreia viral bovina em suínos: por que essa situação é importante para os rebanhos brasileiros?

Henrique Meiroz de Souza Almeida¹, Igor Renan Honorato Gatto¹, Anne Caroline Ramos dos Santos¹, Daniele Araujo Pereira², Karla Alvarenga Nascimento², Thaís Gasparini Baraldi², Marina Lopes Mechler¹, Luís Guilherme de Oliveira^{2*}

ABSTRACT: Swine can be infected by the bovine viral diarrhea virus (BVDV) under natural conditions. For this reason, further information and divulgation are needed regarding the pathogenicity of this virus in swine. This infection is practically unknown in the realm of pig farming, and, as it shares some similarities with the classical swine fever virus (CSFV), its diagnosis becomes a challenge for official sanitary programs. Studies have shown the absence of clinical signs in piglets and reproductive problems in sows due to BVDV infections. There is little research on the prevalence, risk factors, preventive measures and control of BVDV in pigs around the world. And in Brazil, the data is practically non-existent. At the time of diagnosis, comparing the most efficient laboratory tests such as virus neutralization, ELISA, RT-PCR, and immunofluorescence so as to minimize the risk of cross serological reactions when dealing with a persistent or transient infection, can be an important tool. Moreover, the practical implications for CSFV eradication programs are a main reason for the development of further research against this infection. Therefore, this paper aims to review various aspects of BVDV infection in pigs, and how this information can be important for Brazilian herds.

KEYWORDS: swine production; bovine viral diarrhea virus; pathogenesis; animal health protection; classical swine fever virus.

RESUMO: O suíno pode ser infectado pelo vírus da diarreia viral bovina (BVDV) em condições naturais, por isso são necessárias maiores informações e mais divulgação sobre a ação deste vírus nos suínos. Esta infecção é praticamente desconhecida na suinocultura e, devido a algumas semelhanças com vírus da peste suína clássica (VPSC), torna-se um desafio para os programas sanitários oficiais. Estudos revelam a ausência de sinais clínicos em leitões ao mesmo tempo em que evidenciam problemas reprodutivos em porcas devido à infecção do BVDV. Poucas são as pesquisas sobre a prevalência, fatores de riscos, medidas de prevenção e controle do BVDV em suínos no mundo e, no Brasil, os dados são praticamente inexistentes. No diagnóstico, comparar os exames laboratoriais mais eficientes como a virusneutralização, ELISA, RT-PCR e imunofluorescência, diante de uma infecção persistente ou transitória, e assim minimizar o risco de reações sorológicas cruzadas pode ser uma ferramenta fundamental. Ademais, as implicações práticas em programas de erradicação da PSC são um grande motivo para o desenvolvimento de mais pesquisas frente a esta infecção. Portanto, este trabalho pretende revisar diversos aspectos da infecção do BVDV em suínos evidenciando o quanto essa situação pode ser importante para os rebanhos brasileiros.

PALAVRAS-CHAVE: suinocultura; vírus da diarreia viral bovina; patogenia; defesa sanitária animal; vírus da peste suína clássica.

¹Department of Preventive Veterinary Medicine and Animal Reproduction, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP) – Jaboticabal (SP), Brazil.

²Department of Veterinary Clinic and Surgery, Faculdade de Ciências Agrárias e Veterinárias, UNESP - Jaboticabal (SP), Brazil.

*Corresponding author: luis.guilherme@fcav.unesp.br

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INTRODUCTION

Among the diseases affecting animal production, there are viral infections, which can be caused by the genus *Pestivirus*. These infections are extremely important, causing major economic and production losses worldwide. The genus *Pestivirus* has four species of viruses that are formally recognized: classical swine fever virus (CSFV), bovine viral diarrhoea viruses 1 and 2 (BVDV-1 and BVDV-2), and border disease virus (BDV), in addition to one tentative species represented by an isolated from a giraffe (BECHER et al., 2003).

Recently BVDV-1 and BVDV-2 have become relevant to pigs, mainly because of classical swine fever, a foreign animal disease with serious consequences for the pig industry and the economy. The antigenic similarity between BVDV and CSFV can generate cross-reactivity in diagnostic tests and, altogether with similarity in clinical signs makes it very challenging to have a reliable diagnosis. Furthermore, data about the occurrence and prevalence of BVDV infections in pigs in Brazil are lacking, both in the pig industry and in non-technified swineherds, which can present several risk factors, including the lack of biosecurity measures and interspecies transmission.

The lack of data about the occurrence and prevalence of the disease in Brazil in conjunction with the great importance of the cross-reactivity between BVDV and CSFV, justify a paper like this, which aims to provide further information regarding the epidemiology of BVDV infections in pigs to contribute to the literature, and thus offer more technical data that is useful for surveillance and the classical swine fever eradication program.

THE PESTIVIRUS GENUS AND ITS MOLECULAR BASIS FOR ANTIGENIC LIKENESS

The genus *Pestivirus* from the *Flaviviridae* family consists of several viruses that are of great economic importance to livestock, and which were named according to the species of preferential infection (ASFOR et al., 2014). Some viruses infect different host species relatively easily (MOENNIG, 1990). The Bovine Viral Diarrhoea Virus (BVDV) belongs to this genus and may present antigenic variations. In this case, phylogenetic analyses based on region 5' UTR and encoding the polyprotein gene NS23/p125 suggest the existence of two genotypes, BVDV-1 and BVDV-2. Furthermore, differences have been reported in the pathogenesis and antigenicity between genotypes. The BVDV-2 is more related to the most virulent and hemorrhagic form of the disease (RIDPATH et al., 1994).

Recently, a new species was isolated from fetal bovine serum in Brazil and currently it is being called "Hobi-like" worldwide. Due to its genetic and antigenic similarity to BVDV-1 and BVDV-2, some researchers have suggested the virus be

named BVDV-3, however there is no consensus yet among the scientific community (BAUERMANN et al., 2013). To the best of our knowledge, the presence of different genotypes of CSFV has not been reported in the scientific literature.

High genome similarity, high homology and serological cross-reactivity against monoclonal antibodies (MABs) indicate that there is an important resemblance between the *Pestiviruses* (VAN RIJN, 2007) and it is suggested that all of them emerged from a common ancestor virus (LIESS; MOENNIG, 1990). However, the genetic diversity of the *Pestivirus* genus results from successive mutations that occur because of the viral replication process and recombination with other homologous or heterologous RNAs (NAGAI et al., 2004). A phylogenetic analysis of a 268 nucleotide sequence within the 5'UTR of the viral genome resulted in the division of the BVDV into two genotypes: BVDV-1 and BVDV-2 (FLORES et al., 2000).

Envelope glycoprotein E2 of *Pestivirus* is the dominant protein in the host immune response, and the antibodies produced against this antigen are essential for diagnostic tests and for immunity, which is induced by vaccination (JELSMA et al., 2013). Molecular studies of the E2 glycoprotein help to unveil the origin of the serological cross-reactivity among the viruses of this genus. JELSMA et al. (2013) showed that the B/C domains of the E2 glycoprotein in CSFV are analogous to the N-terminal portion of the A domain of the E2 glycoprotein of BVDV, as the BVDV Z2 region is similar to part of the CSFV D/A domain, proving that despite being different species, the antigenic structure of these two pestivirus specimens is similar.

According to SANDVIK (2005), the E2 envelope glycoprotein is mainly responsible for antigenic similarity and difference between the *Pestivirus*. Research conducted by FLORES et al. (2000) indicated that the source of the antigenic differences between the strains and the BVDV sub-genotypes relates to variants of the glycoprotein gp53/E2 in the viral envelope. In spite of the fact that the information is relatively limited, it is believed that there is a great similarity between the antigenic sites of BVDV-1, BVDV-2 and CSFV (NEWCOMER; GIVENS, 2013).

Studies based on cross-reaction in virus neutralization tests identified six antigenic groups of *Pestivirus* [BVDV-1, BVDV-2, BDV, CSFV, H138 (Giraffe-1), and V60 (Reindeer-1)], with the most important being group I, which comprises four BVDV strains isolated from cattle and two BVDV strains isolated from pigs (DEKKER et al., 1995). RIDPATH et al. (2000) carried out virus neutralization tests using hyperimmune sera produced for different species of *Pestivirus* (BVDV-1, BVDV-2, BDV and CSFV), and tested each of these sera in the same species, noting the occurrence of neutralization between heterologous species with titers up to 1024 (sera anti-CSFV for BVDV-1) and 512 (sera anti-CSFV for BVDV-2).

Interestingly, research conducted *in vivo* showed that pigs infected with BVDV had clinical resistance to the CSFV infection, likewise there was no detectable transmission of CSFV among pig herds due to the occurrence of a cross serological reaction (WIERINGA-JELSMA et al., 2006). As such, MENGELING et al. (1963a) demonstrate that bovine kidney cells infected with BVDV in cell culture showed fluorescence when brought into contact with anti-CSFV antibodies conjugated with fluorescein, confirming the antigenic similarity between the viruses of this genus.

Cross-reactivity in serological tests also occurs within the same species, since there is evidence that animals immunized with the vaccine for BVDV-1 can be protected against BVDV-2 and “Hobi-like” (DECARO et al., 2013). On the other hand, antigenic variation can occur within the same genotype (BVDV-1). When testing against a hyperimmune lamb serum, antigenic similarity rates (R) ranged from 1.1 to 50 among the subgenotype of BVDV-1 (ALPAY; YESILBAG, 2015).

DIAGNOSTIC TESTS AND SEROLOGICAL CROSS-REACTIONS

Laboratory tests are crucial in performing diagnoses of diseases that are caused by pestiviruses. They are also important in differentiating the etiologic agents of this genus. Diagnostic techniques that detect the presence of anti-BVDV antibodies in serum are considered more efficient, faster and cheaper when it comes to identifying animal exposure to the virus.

Viral isolation techniques can be used, but due to their laborious nature, the use of PCR can be a good alternative for detecting the viral agent (HOUE et al., 2006). Samples such as blood, milk, saliva and tissue can be successfully tested using RT-PCR (KLIUCINSKAS et al., 2008), and can be stored for prolonged periods of time with minimal effect (VILCEK et al., 2001).

A direct fluorescent antibody test (DFA) has been highlighted as an important diagnostic test for CSF because it can easily identify cells that are infected with CSFV in the cell culture, which is isolated from serum and blood samples from infected pigs (MENGELING et al., 1963b). However, pigs infected with BVDV may have false-positive DFA results, requiring the use of laborious and time-consuming confirmatory tests. Positive cases of CSF require the slaughter of the entire swine herd (WENSVOORT et al., 1989).

The virus neutralization test (VNT) is based on the identification and quantification of antibodies against the E2 envelope glycoprotein (SANDVIK, 2005). This technique is considered the reference test for the diagnosis of bovine viral diarrhea (OIE, 2015c) because of the several advantages it offers such as the ability to detect and quantify antibodies, its ability to test sera from different animal species, and its flexibility to use different genotypes/sub-genotypes of BVDV, thus increasing the power of the diagnostic test (DUBOVI, 2013).

According to DUBOVI (2013), the possibility of using any kind of serum in virus neutralization is essential. Because BVDV infects several animals in addition to cattle, there is the need for a test that assesses different types of serum. This is possible in VNT, which is considered the best test for the diagnosis of bovine viral diarrhea. For VNT, it is essential to test the serum for BVDV-1 and BVDV-2, since low titers of antibodies of BVDV-2 cannot be detected when performing VNT with the BVDV-1, and vice versa (OIE, 2015a). When performed according to standard protocols of OIE, virus neutralization is a very sensitive and specific test (SANDVIK, 2005), although these values differ between laboratories.

ELISA tests for the diagnosis of CSF have been developed using MABs, which are based on the detection of antibodies produced for E2 glycoprotein. In tests performed in seven ELISA commercial kits, with specificity ranging from 92 to 100% and sensitivity ranging from 51 to 100%, only three commercial kits were able to differentiate anti-BVDV antibodies from anti-CSFV antibodies in the samples (SCHROEDER et al., 2012).

Reports of CSF outbreaks in the field showed that when ELISA is used as a primary diagnostic test, it can have false positive results, mainly in low antibody titer samples (DE SMIT et al., 1999). In all cases, it is necessary to use a second confirmatory test, which increases the time of action needed to focus contention.

The use of MABs has emerged as a possible solution to prevent cross reactions in serological tests. When testing polyclonal anti-CSFV front sera with 31 strains of BVDV and BDV and 94 strains of CSFV, all were reactive. While using anti-CSFV MABs there was no reaction with strains of BVDV and BDV (WENSVOORT et al., 1989). On the other hand, studies conducted at the same time claim that anti-BVDV MABs were reactive to 40% of the CSFV strains tested, however anti-CSFV MABs did not react to any other kind of *Pestivirus*, indicating a possible solution for the diagnosis of CSF. (CAY et al., 1989; EDWARDS et al., 1991). Regardless, further studies are needed.

EPIDEMIOLOGICAL ISSUES OF BVDV INFECTIONS IN PIGS

Despite the fact that ruminant *Pestivirus* infections in pigs are not as problematic as CSFV infections, distinguishing between these two diseases can sometimes be very difficult (PATON; DONE, 1994). *Pestivirus* infections in pigs are responsible for raising sanitary barriers between countries. Infection caused by BVDV in pigs have been reported in countries such as China (DENG et al., 2012), the Netherlands (LOEFFEN et al., 2009), Brazil (GATTO, 2015; ALMEIDA, 2015) and others, which draws up concerns about the existence of accurate diagnostic tests, questions about risk factors involved, and the BVDV's clinical form.

The first report of isolation ruminant pestiviruses in naturally infected pigs occurred in 1973 (FERNELIUS et al., 1973). BVDV infections in pigs are usually asymptomatic, although in some cases, reproductive problems have been observed, such as birth of weak piglets, abortion, fetal mummification, hyperthermia and intestinal cramps (KIRKLANT et al., 2012; TAO et al., 2013). In pregnant sows, transplacental infection can cause abortions, stillbirths, the birth of weak piglets, malformations and even the birth of persistently infected piglets (PATON; DONE, 1994; BECHER et al., 2003).

Cases in which the BVDV infection induced a lot of injuries in pigs were caused by viral strains after serial passages and infections in this species. In piglets, BVDV infection has clinical signs, such as anemia, delayed development, rough coat, polyarthritis, congenital tremors, petechiae on the skin, diarrhea, conjunctivitis and cyanosis (TERPSTRA; WENSVOORT, 1988). Although, pigs' postnatal BVDV infection is considered harmless, the CSFV infection affects herds with high mortality (MOENNIG, 1990).

In England, there was an outbreak of sudden death in piglets with clinical signs that are similar to CSF, but the etiologic diagnosis was BVDV infection. The same agent was isolated from cattle on the same farm. The isolated virus was equally susceptible to neutralization from swine and cattle antibodies (PATON et al., 1992).

The prevalence of BVDV infection in pigs varies according to regions. In 11 Chinese provinces, the prevalence of sows with reproductive disorder was 20–30%, and they had BVDV-1, the most prevalent genotype (DENG et al., 2012). In the Netherlands, the prevalence of positive cases was 0.42% in finishing pigs, 2.5% for sows and 11% of swine herds (LOEFFEN et al., 2009). In a study conducted in Poland, 14,608 pig sera collected between 2008 and 2011 were tested with ELISA, and BVDV was detected in 11 (68.75%) out of the 16 provinces, and the seroprevalence varied from 0.1% to 1.04% (with an average of 0.31%) (LIPOWSKI, 2014). Older data affirm that in countries declared free of CSF, the prevalence of BVDV infected pigs ranged from 1.6% to 43.5% (JENSEN, 1985). However, a study of pig herds in the province of Ontario, Canada, did not identify any animal that was positive for BVDV (O'SULLIVAN et al., 2011).

Studies in Brazil are being conducted by our team, and preliminary results are very interesting. GATTO (2015) developed a study that aimed to detect anti-BVDV antibodies in finishing pigs slaughtered in the state of São Paulo. 817 swine blood samples were collected from animals in several Brazilian states. Virus neutralization tests were applied and the seroprevalence result of neutralizing anti-BVDV-1-Singer antibodies was 2.32%. Taken together, two other studies were performed in swine from non-technified rearing farms (GATTO, 2015; ALMEIDA, 2015). In the first one, 412 samples of swine blood were collected in the city's slaughterhouse during the bleeding from pigs of 20 different small farms in the city of

Mossoró – Rio Grande do Norte State. Results showed that 9 out of 20 (45%) farms had at least one positive animal. 4.13% of the animals (17 pigs) were positive in the virus neutralization test (GATTO, 2015).

ALMEIDA (2015) focused on establishing the prevalence of non-technified rearing farms in the state of São Paulo. Serum samples of 360 swine, from 56 farms located in the northeast regions were collected and tested for virus neutralization using BVDV-1 strain Singer and BVDV-2 strain VS253 as the standard antigen. Only 17 samples were positive, presenting a prevalence of 4.72%, and 15 farms had at least one positive animal, showing 26.79%. When analyzing the genotypes separately, 1.94% was positive for BVDV-1 and 3.06% for BVDV-2 strains.

Regarding transmission, epidemiological studies indicate that cattle are natural BVDV hosts, and are the major source of infection for pigs and other ruminants (KIRKLANT et al., 2012; RIDPATH, 2010). Direct contact with cattle on the same farm is considered the main source of BVDV transmission for pigs (KIRKLANT et al., 2012; LIESS; MOENNIG, 1990). Transmission can occur due to milk from infected cattle and other dairy products being fed to the pigs, from the use of contaminated CSF vaccines, and through fomites (CARBREY et al., 1976; TERPSTRA; WENSVOORT, 1988). Contrary to what was previously believed, transmission also occurs from one pig to another, although rarely (WIERINGA-JELSMA et al., 2006).

As such, DENG et al. (2012) claim that the prevalence of BVDV in pig herds is closely linked with the prevalence of the disease in cattle herds. Thus, they corroborate the conclusions of LOEFFEN et al. (2009) and O'SULLIVAN (2011), who attribute the low prevalence of BVDV in swineherds with the high level of animal production, which leads to the decline of farms with more than one animal species and reduces the contact between cattle and pigs. In addition to contact with cattle, others risk factors exist, as GATTO (2015) demonstrated in a study focused on evaluating the occurrence of anti-BVDV-2 antibodies and the associated risk factors in finishing pigs. Significant association was observed in the logistic regression with the risk factors: trucks were not washed and disinfected ($p = 0.0077$). Ultimately, more data is necessary to clarify important information about epidemiological issues of BVDV transmission in pig herds.

IMPLICATIONS TOWARDS CLASSICAL SWINE FEVER ERADICATION PROGRAMMS

Classical Swine Fever (CSF) is a foreign animal disease according to OIE (2014), since its occurrence leads to serious consequences for animal welfare, pig farming and the export of animals and animal products. It is a high mortality and morbidity disease and its severity often extends beyond national

borders, bringing socio-economic losses, and making the international trade of pigs and their products difficult or impossible (BRASIL, 2004b). The Brazilian states of Rio Grande do Sul and Santa Catarina achieved recognition as Classical Swine Fever (CSF) free areas by the World Organisation for Animal Health in 2015 (OIE, 2015b).

In Brazil, CSF is a disease that activates animal health protection measures, and, as a result, the infected animals are euthanized. There is a contingency plan for if CSF were to reach the whole country, and all actions to be taken in an outbreak are established (BRASIL, 2004b). On the other hand, for states where CSF is controlled, there is a surveillance plan to perform in the swine productive system (BRASIL, 2015). Vaccination is prohibited throughout national territory. It is allowed only in specific cases, where there is a high risk of spreading the disease, and it requires authorization from the animal health authorities (BRASIL, 2004a).

Due to the antigen structure's similarity among the etiologic agents of CSF and BVD, cross-reactions in serological tests are likely to occur. Thus, the presence of anti-BVDV antibodies in pig serum can lead to false positive results of serologic tests for the diagnosis of CSF, causing problems in eradication programs of CSF or even in epidemiological surveys of this disease (LOEFFEN et al., 2009; TAO et al., 2013).

Although it is banned in Brazil (BRASIL, 2004a), the vaccination is a widely used strategy for CSF control and eradication programs. However, studies show that the presence of anti-BVDV antibodies in herds due to previous animal infection reduces the effectiveness of the vaccine against CSF (VAN RIJN, 2007), which results in a poor performance from the control and eradication programs. According to DE SMIT et al. (1999), eradication programs with poor diagnostic accuracy can lead to a delay in decision making, which leads to further spread of the disease and increased economic loss.

The presence of anti-BVDV antibodies in pig herds in the Netherlands hindered the control and diagnosis of positive animals during an outbreak of CSF in the 1990s, due to the occurrence of false-positives (DE SMIT et al., 1999). The main measure taken to prevent positive cases of CSF is the slaughtering of the animals. Even though it results in losses for the producer, a correct diagnosis is of utmost importance for disease control and eradication (WENSVOORT et al., 1989).

CONCLUSION

Among the items discussed in this review, the issue of Bovine Viral Diarrhea Virus infection in pigs is poorly known. It is believed that natural and experimental infection in pigs with BVDV can present similar pathogenesis in swine, cattle and sheep. However, there is no information that support this affirmation. Other points regarding seroconversion, prevalence, and diagnostics are being clarified. Finally, this review showed further work on BVDV infection in swine in order to elucidate specific information about this ruminant pathogen, which has still been poorly studied in pig farming. Additionally, it demonstrated the extent of BVDV particularities in pigs in order to differentiate them from CSF cases.

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