Effect of experimental infection with Bovine Viral Diarrhea virus in pregnant sows and newborn piglets

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Abstract

Bovine viral diarrhea virus (BVDV) is genetically and serologically related to other members of the *Pestivirus* genus, such as classical swine fever virus (CSFV), and may cause reproductive disroders, there is a lack of research to clarify the pathogenicity of BVDV in different gestational periods of sows and potential consequences to newborn piglets. The objective this research was to assess the effect of bovine viral diarrhea virus infection during the gestational period of sows and newborn piglets.

Introduction

The biology of *Pestivirus* is characterized by unique and interesting features that are both crucial for their success as pathogens and challenging from a scientific point of view. Many features of the *Flaviviridae* family members are quite diverse and reflect their adaptation to different propagation strategies and hosts (1). Bovine Viral Diarrhea virus (BVDV) is genetically and serologically related to other members of the genus *Pestivirus*, such as CSFV, and may cause reproductive disroders in sows. Timely, the BVDV when infecting swine can cause clinical signs resembling those of the Classic Swine Fever (CSFV), hindering the differential diagnosis, prevention and control of these diseases (8). Intrauterine infection of BVDV can lead to reproductive disorders similar to those seen in CSFV infections with low or medium pathogenic strains (6). Therefore, control measures should be based on full knowledge of the epidemiology of BVDV including awareness of other potential sources of the virus, such as swine. This research aimed to assess the effect of an experimental infection with strain BVDV-2 (VS253) during different gestational periods of sows and the consequences in neonate piglets.

Methods and Materials

Twelve pregnant sows were allocated into five groups, which were inoculated experimentally with the BVDV-2(VS253) 6ª in MDBK 30/10/15 with titer $10^{5.5}$ TCID 50/mL strain, one group was infected 30 days before insemination (G0;n=2) and three groups during gestation, first third (G1;n=2), second third (G2;n=3) and last third (G3;n=3) and the fifth was mock infected as a control group (G4;n=2) (Figure 2). Blood and nasal swab samples were collected every three days starting at the inoculation until the farrowing and were used for virusneutralization test, RT-qPCR (Figure 3) and hemogram. At birth, half of the neonates were euthanized to obtain tissue samples for histopathology (Figure 1) and RT-qPCR.

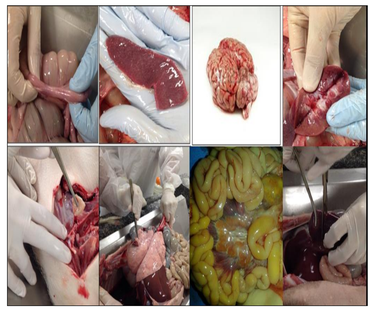

Figure 1. Tissues of the piglets.

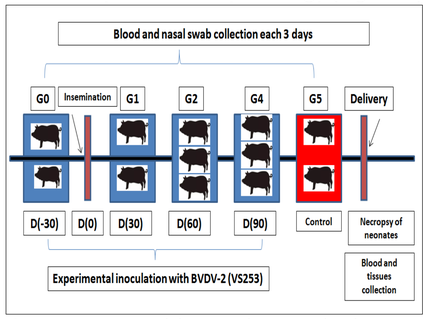

Figure 2. Experimental design with sows and piglets.

Figure 3. *Gold BVDV Detection Kit* commercial used in RT-qPCR.

Results

All sows seroconverted during the gestational period, except the control group (G4) and G3. G0, G1 and G2 sows seroconverted, between 12 days and 33 days after inoculation. The highest titer was 160 (G0). Serial dilutions of BVDV-2 (VS253) were performed starting at 10^0 to 10^7 , in roder to obtain a RT-qPCR standard curve (Table 1). The experimental inoculation with BVDV-2 was carried out successfully, the results showed that the sows presented a transient viremia once BVDV-2 genetic material was detected in blood and nasal swabs in G0, G1, G2 and G3 sows, with Ct <36 (Table 2). The viremia was detected from day 3 post infection (dpi) through 12 dpi and viral shedding was detected starting at 6 dpi through 23 dpi. In the histopathology piglets born from infected sows presented gliosis lesions. G1 sows had thrombocytopenia in day 36 dpi. In this study, a challenged G0 (323) sow aborted at 60 days of gestation, histopathology analysis of the placentae was done and showed no lesions. Thus, differential diagnosis for Parvovirus and Circovirosis were performed resulting in negative for both diseases. Stillborn piglets were present in all infected groups, while only one sow (G3) had a mummified fetus. One sow (G2) returned to estrus after 40 days of gestation, but no abortion sign was noticed. Regardless of the gestation phase the inoculation occured, the viral strain was not able to cross the placentae, which served as a protective barrier for the fetuses.

Table 1. Serial dilutions of the BVDV-2 VS253 strain used in the RT-qPCR standard curve demonstrating the Ct obtained for each dilution (10^0 to 10^{-7})

Viral Concentration	Ct
10^0	13.94
10^{-1}	22.38
10^{-2}	26.35
10^{-3}	31.45
10^{-4}	32.21
10^{-5}	33.13
10^{-6}	34.16
10^{-7}	35.19

Table 2. Reproducibility of RNA (BVDV-2) in RT-qPCR performed on blood and swab samples of sows.

Value Ct of samples Blood		Value Ct of samples Swab	
Sows	Ct	Sows	Ct
392 (G0)	34.940	323 (G0)	33.154
729 (G2)	35.094	1386 (G1)	34.915
729 (G2)	34.136	355 (G1)	35.487
427 (G1)	34.524	392 (G0)	34.884
348 (G3)	35.697		

Discussion

After oronasal inoculation of the sows with the BVDV-2 isolate (VS253), these seroconverted and supported BVDV replication, which detected viral RNA in the blood and nasal secretion in G0, G1, G2 and G3 sows. Sows seroconverted between 12 and the 33 day after infection. In another study, with experimental inoculation of BVDV-1 in sows inoculated with 65 days of gestation, seroconversion was found 21 days after inoculation, with a titer ranging from 64 to 512 (2). Other studies have described BVDV inducing viremia with one week and seroconversion with three weeks after experimental inoculation in pigs (3,4,5). the results confirm the non-infection by the vertical route in piglets, agreeing with the results of (2) which states that transplacental infection in pigs was not efficient, however they contrast with studies demonstrating transplacental infection (6,7).

Conclusions

The effects of BVDV infection in swine are less aggressive when compare with the bovine, but depend of strain and there are few studies for comparison. The viral strain used was not able to cross the placenta, which acted as a protective barrier, regardless the gestational period the experimental infection was done. On the other hand, BVDV-2 was able to replicate in the sows, that shed the virus and presented seroconversion. Thus, the highest titers were observed during viremia and did not prevent viral shedding. Despite the advancement of molecular biology and detailed investigation of several aspects related to pestiviruses, working with these agents still very demanding.

Future Directions

Pestiviruses are highly interesting and economically important pathogens to be analyzed and studied. In the last years, associated with the evolution of the molecular tools, several works the area elucidating surprising features of those virus and its interaction with different hosts.

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