BVDV: Not just a cattle problem

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Abstract

Bovine viral diarrhea virus (BVDV), the prototypic member of the genus Pestivirus of the family *Flaviviridae*, causes disease in cattle worldwide. A wide range of clinical manifestations from subclinical to fatal disease can occur upon infection with BVDV, resulting in significant losses to the cattle industries. In addition to being one of the most important viruses in cattle, BVDV can infect other species of the family Artiodactyla, including domestic and wild ruminants, camelids, and swine. Infection with BVDV in heterologous hosts can cause similar disease processes as observed in cattle, including persistent infection, respiratory disease, reproductive disease, and immunosuppression. BVDV infection of heterologous hosts can interfere with diagnosing diseases such as Classical Swine Fever (CSF) due to cross-reactivity of antibody assays between BVDV and Classical Swine Fever Virus (CSFV)(1). Additionally, heterologous hosts may be a reservoir for BVDV, leading to decreased success of BVDV eradication programs.

For the better part of the past five decades, researchers have been investigating the prevalence and implications of BVDV in heterologous hosts such as small ruminants, cervids, camelids, and swine. As early as 1973, it was recognized that swine could be a significant "carrier host" of BVDV. Our research group has continued research of BVDV in heterologous hosts, to include the current research of Dr. Shari Kennedy. The aims of the current study are to significantly increase the understanding of BVDV infections in swine, resulting changes in the viral genome in time during acute infections, and between chronically infected and persistent infections in congenitally infected piglets.

Keywords: Bovine viral diarrhea virus, chronic infection, persistent infection, congenital infection, swine/pigs

Introduction

Bovine viral diarrhea virus (BVDV) has a single-stranded positive-sense RNA genome and is the prototypic member of the genus Pestivirus of the family Flaviviridae. The viruses within the genus Pestivirus have been assigned to eleven different species based on the phylogenic analysis of the conserved amino acids sequences of the virus. Pestiviruses A-K include BVDV 1, BVDV 2, Classical Swine Fever Virus, Border Disease Virus, Antelope pestivirus, Porcine pestivirus, Giraffe pestivirus, HoBi-like pestivirus, Aydin-like pestivirus, Rat pestivirus, and Atypical porcine pestivirus, respectively.

BVDV is a bovine disease that results in disease in not only cattle, but wild and domestic animals in the mammalian order Artiodactyla such as sheep, goats, deer, alpacas, and pigs. Infection with BVDV in heterologous hosts can cause similar disease processes as observed in cattle, including persistent infection, respiratory disease, reproductive disease, and immunosuppression.

As BVDV does not possess strict host specificity, heterologous (infections in species other than cattle) provide generation of viral diversity. However, limited research has evaluated the genetic and antigenic changes resulting from infection of heterologous hosts. The consequences of these infections are currently not well understood. It has been proposed by multiple researchers that BVDV infections in heterologous hosts such as in pigs may pose a significant reservoir of infection for cattle.

History of BVDV in Swine

In the early 1960s, cross-reactive antigens between BVDV and CSFV were described, but it was another decade before live BVD viral particles from pigs were able to be recovered. Prior

to this, BVDV antibodies detected on agar gel immunoprecipitation testing for CSFV called the accuracy of this test into question due to false positive results, thus some BVDV infections in swine were credited to unusual asymptomatic CSFV infections. It wasn't until 1988 before researchers were able to describe 19 cases in pigs that were clinically consistent with CSF disease that were determined to be caused by natural BVDV infection(2). That same year, there were detected 8 additional cases of BVDV infection in pigs due to inoculation with hog cholera (CSFV) vaccines that were contaminated with BVDV(3). Since then, numerous studies have been performed determining the efficacy of BVDV vaccination to provide protection against CSFV and to better characterize the different types of BVDV infection in swine.

Clinical Signs of BVDV infection in Pigs

Postnatal natural infections with BVDV in pigs most commonly are asymptomatic or have very mild clinical signs (4-8). These clinical signs can include slightly elevated rectal temperature, reduced appetite, or diarrhea. Similar to natural infections, experimental inoculation of pigs postnatally with BVDV typically yield no clinical signs, but may result in mild transient fever, chronic gastroenteritis, reduced appetite, or diarrhea depending on the strain of BVDV used to inoculate. Additional clinicopathologic abnormalities may also be observed in animals with or without clinical signs. These clinicopathologic abnormalities may include leukopenia, thrombocytopenia, septicemia with hemorrhages in the lymph nodes, epicardium, and kidneys, and thymic atrophy. While reported in the early literature, more contemporary reports often do not document the occurrence of these clinical signs, which may be due to virulence of the infecting BVDV isolate, dose and route of inoculum, or life stage of animals used in the studies.

Reproductive disorders in sows and gilts have been reported (can occur) following natural and experimental infections with BVDV and are characterized by birth of weak piglets,

abortion, mummification, and the birth of persistently infected piglets (3, 7, 9, 10). Congenital infection of piglets with BVDV has been shown to occur in experimental and natural infections (11-13). In utero infections prior to the development of a competent immune system could lead to the creation of a CI or PI animal. There are many factors to consider when trying to understand congenital infection in swine. BVDV species and subspecies as well as biotype (noncytopathic versus cytopathic), age of gestation of inoculation of the dam, and prior exposure to BVDV all play a role in the development of transplacental infection. While they may appear clinically normal, congenitally infected pigs may be born small, can be weak or fail to thrive may have rough haircoat, show signs such as diarrhea, hemorrhage, or even apparently normal. Pregnant gilts or sows infected with a noncytopathic biotype of BVDV within the first trimester of gestation, or prior to a competent immune system, may create a persistently infected piglet.

It is currently unknown whether these persistently or chronically infected piglets have the potential to pose the same herd health risk as persistently infected cattle. These risks may include high levels of viral shedding, which may act as a source of infection for naïve herd-mates, other pregnant animals, or even possibly wildlife. These piglets may shed the virus in the bodily secretions such as ocular, nasal, or oral fluids, urine, feces, and blood. Persistently infected (PI) piglets may die shortly after birth, but a few reports have shown PI pigs survive to slaughter age and beyond (12, 13)

Sources of BVDV in swine

In recent publications from major swine producing countries, the seroprevalence rate of BVDV in swine herds appear to be high. Reported rates of up to 64% (21/33 swine herds) across seven Brazilian states and 23.6% (50/212 positive samples) of pig herds from 11 providences throughout China (14-16). While PI cattle are the most commonly implicated source of BVDV

infection in swine, contaminated culture media, vaccines, milk, bovine offal fed to pigs, contaminated needles or equipment, and close contact with small ruminants have also been documented or are postulated to be possible sources of infection. A significant association has been demonstrated between detection of BVDV antibodies in Brazilian swine herds and improperly cleaned and disinfected trucks and visitors that do not respect biosecurity rules of time between farm visits (16).

Implications of BVDV in swine

Due to the close genetic relationship and cross-reactivity of Classical Swine Fever Virus (CSFV) and BVDV, many serologic surveillance programs have been negatively impacted by infection of swine with BVDV. Typical CSFV clinical signs and a positive pestivirus antibody titer has falsely resulted in the diagnosis of Classical Swine Fever diagnosis (4, 14, 17, 18). This false positive result lead to unnecessary depopulation measures of swine herds. Prior infection with BVDV in swine has shown to result in higher titers to BVDV than CSFV, thus leading to a false-negative diagnosis of CSFV when neutralization peroxidase linked assay was used following a CSFV challenge study (19)These scenarios have led to inaccurate diagnosis of both BVDV and CSFV in swine, decreased the effectiveness of CSFV control and eradication programs, and further complicated understanding and control BVDV infections in swine.

Chronic Infection of BVDV in swine

Currently, swine are among the two species in which viral clearance following prolonged infection has been documented. This chronically infected (CI) animal begins much the same as a persistently infected (PI) animal by intrauterine infection with a noncytopathic BVDV strain. They are infected prior to full competency of the immune system; therefore, their immune

system recognizes the virus as self and does not mount an appropriate response. After birth, these animals excrete virus in bodily secretions such as saliva, nasal fluid, urine, feces, semen and blood. They can develop leukopenia, viremia, but may also have normal white blood cell counts or thrombocytopenia. There are reports that indicate that congenitally infected pigs often die within 2-16 weeks after birth, with signs resembling those of Classical Swine Fever, but few others show PI and CI piglets surviving till slaughter age and further into adulthood.

The determining difference between the two congenital infectious states depend on the eventual clearance of the virus and the presence of serum neutralizing antibodies to the infecting BVDV strain. As the name states, persistently infected animals are infected for life, whereas, chronically infected animals begin identical to PI animals, but for a currently unknown reason, clear the virus after birth. A paper by Terpstra in 1997 details a congenital PI infection of BVDV in two boars and an intersex pig that survived beyond three months(2). One piglet seroconverted at one month of age, a second between six and eight months, and the third remained viremic and immunotolerant to BVDV until slaughter at 26 months. To date, there is not a well determined cut-off point for this clearance of virus during seroconversion. More importantly, it is not very well understood the how or why clearance of the BVDV occurred.

Current research of BVDV genomics in swine

While the immune recognition of the virus is central to the ability to clear BVDV in CI pigs, the exact mechanisms of viral clearance are currently unknown, prompting the proposed experiments being currently carried out by our research group. It is plausible that sufficient viral genetic change occurs in some congenitally infected piglets, allowing recognition of the virus as foreign, resulting in an effective immune response, thus seroconverting and clearing the virus. We have previously demonstrated that there are minimal viral genetic changes in PI cattle over

time (20-23), but there are no studies that have investigated the quantity and location of BVDV genomic changes of nucleotide substitutions in BVDV over time and between PI and CI piglets to elucidate which mechanisms allow viral clearance.

To perform this study, we have inoculated 4 BVDV naïve gilts at 26 and 27 days of gestation with a noncytopathic BVDV-1b strain of porcine origin that our group has used before. BVDV infection status of the piglets will be assessed by virus isolation at the time of birth. Blood samples will be collected weekly until approximately 6 months of age or until the time of seroconversion from all surviving piglets to assess infection status by virus isolation and antibody status by virus neutralization. Full-length viral genome sequencing will be performed on viruses from the initial inoculum, sera from gilts on day 7 after inoculation, progeny piglets at birth and the time point prior to developing neutralizing antibodies (CI piglets) and age-matched controls (PI piglets). The nucleotide and amino acid sequences will be evaluated for number and location of changes over time and between the groups (CI and PI piglets).

The long-term goal of this research is to further understand BVDV infections in heterologous species. We have previously demonstrated that heterologous infections result in greater genetic variability than infections in cattle. Based on this previous work, we hypothesize that change in the BVDV allows some congenitally infected piglets to clear BVDV, a remarkable phenomenon, not known to occur in PI ruminants. To test the research hypothesis, we will evaluate specific objectives: 1. Quantify and characterize the nucleotide changes that occur in the genome of viruses isolated from acutely infected pregnant gilts and their progeny following BVDV-1b infection; 2. Compare the nucleotide changes in the infecting BVDV-1b viruses over time and between CI and PI piglets. Consequently, the purpose of this study is to expand on our

previous work by studying viral changes that result from BVDV infections in heterologous hosts such as swine and determine if there is a genetic basis for clearance of chronic infections.

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Conflict of Interest:

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Animal Use and Care:

Dr. Shari Kennedy's research project is under the approval of IACUC protocol 2019-3527