ELSEVIER
SAUNDERS

Origination and consequences of bovine viral diarrhea virus diversity

Steven R. Bolin, DVM, PhD[a],
Daniel L. Grooms, DVM, PhD[b],*

[a]*Department of Veterinary Pathobiology and Diagnostic Investigation,
Michigan State University, East Lansing, MI 48824, USA*
[b]*Department of Large Animal Clinical Sciences, Michigan State University,
A100 VTH, East Lansing, MI 48824, USA*

The purpose of this article is to provide an overview of genetic, antigenic, and biologic diversity among bovine viral diarrhea viruses (BVDV), and to discuss the impact of that diversity on disease manifestations, diagnostic testing, and disease control strategies. The genetic diversity that occurs among isolates of BVDV is characteristic of RNA viruses that exist in nature as quasispecies (a swarm of viral mutants). The basis for the viral quasispecies phenomenon will be discussed briefly and related to recent evidence for the existence of BVDV as a quasispecies. The genetic diversity that occurs among BVDV is reflected in the antigenic diversity found among viral isolates worldwide. The persistently infected (PI) animal is considered important for maintaining BVDV in nature, and as being a primary source of virus for other cattle. PI cattle may also serves as a source of viral genetic variants that may be ''selected'' by non-PI cattle when infected with virus. The emergence and establishment of genetic and antigenic variants of BVDV also is affected by selective pressure applied to the virus by the innate and adaptive host immune responses. The array of disease manifestations seen during infection with BVDV, and the corresponding pathogenic processes, may be attributed to viral diversity; however, the definitive viral markers for tissue tropism or virulence have yet to be identified.

* Corresponding author.
E-mail address: groomsd@cvm.msu.edu (D.L. Grooms).

Basis for diversity

Quasispecies

Compared with DNA viruses, RNA viruses are highly mutable. Positive-strand RNA viruses, like BVDV, are subject to genomic modifications that involve point mutations or recombination of RNA. The latter may be homologous (involving recombination of viral RNA [self-RNA]) or nonhomologous (involving recombination of RNA from another BVDV [nonself] or from the infected host). Point mutations are a regular occurrence in RNA viruses, which have mutation frequencies that approach 10^{-4} base substitutions per base site. This means that any given base in the viral genome is expected to undergo mutation once in every 10,000 replications of the viral RNA. At that frequency of mutation, a 10,000 base RNA virus (BVDV has about 12,300 bases in its genome) is essentially guaranteed at least one point mutation (single base change) per replication cycle of the viral RNA [1–4]. The high frequency of point mutations primarily is attributable to the error-prone viral RNA polymerases responsible for replication of viral RNA.

As an explanation for the above, the parent RNA virus (virus that infects a cell) must undergo two rounds of replication of its RNA to produce viable progeny. The first round of replication produces an RNA that is complementary to the RNA contained in the parent virus. The complementary RNA then serves as the template for a second round of replication that produces the RNA that is packaged into the progeny viruses. Multiple copies of complementary RNA are produced from each virion that infects a cell, and multiple copies of progeny RNA are produced from each strand of complementary RNA. A point mutation that occurs during replication of the complementary strand of viral RNA will carry over to each strand of progeny RNA produced from that template, creating a clone of viral progeny expressing that particular mutation. A point mutation that occurs during replication of the progeny RNA from the complementary RNA template will be unique to that individual progeny (Fig. 1). Because there are two rounds of replication of RNA for each cycle of viral replication, and each round of replication of viral RNA has a mutation frequency of 10^{-4}, the expected number of point mutations per viral genome per replication cycle of virus may exceed 1 (mutation rate >1). Thus, each progeny virus will differ from the parent virus by 1 or more point mutations, and a swarm of viral mutants is created with each cycle of viral replication. The swarm of viral mutants forms a quasispecies.

Virus replicates in many cells in the infected host and several replication cycles may occur in 1 day. The number of times that a point mutation can occur at one base site in 1 day of viral replication can exceed 1 million during the peak of infection, when the viral load may be 10^2 to 10^4 infectious particles per milliliters of plasma [5]. The potential to create new viruses is
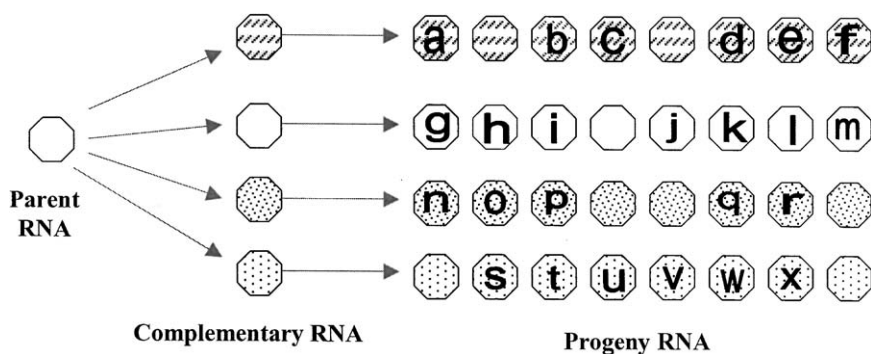
Fig. 1. A swarm of viral mutants generated during viral replication due to the high mutation frequency of an RNA virus. The open octagon to the left represents the parent virus. Octagons with patterns represent complementary RNA that has a point mutation. The point mutation in the complementary RNA carries over to the progeny RNA, forming clones of viruses with like mutations. Additional mutations (a through x) occurred in replication of the progeny RNA from the complementary RNA template.

tremendous; however, under neutral conditions that do not provide a selective advantage for one of the mutants, the mutant swarm tends to maintain a master base sequence that reflects the base sequence of the parent virus. This is because most point mutations are either deleterious for survival of the virus, and are not carried forward, or they do not give the viral mutant a competitive advantage that allows it to dominate the mutant swarm. However, the ability to constantly generate mutants allows RNA viruses to adapt quickly to host responses and, in some cases, establish chronic or persistent infections using a variety of mechanisms [6,7].

Selective forces

The ability to evade the immune response and establish a chronic infection would prolong shedding of virus and enhance viral survival in nature. Viruses that exist as a quasispecies have the potential to create a series of mutants that stay one step ahead of the adaptive immune system, thereby prolonging infection and extending the time when the virus may be transmitted to a new host. Such viruses also have the potential to infect a host that has an existing immune response due to prior exposure with virus. The larger the dose of virus that is in the initial inoculum during transmission to a new host, the greater the chances are that a mutant virus is present that can evade an existing immune response. Interestingly, a swarm of mutant viruses may possess a molecular memory that retains genetic mutants that demonstrated a selective advantage during previous cycles of viral replication [8,9]. Thus, when the viral swarm encounters an exigent circumstance, such as infection of a host with an existing immune response or with a physiologic disturbance like fever [10], mutants already are present that were successful previously in a similar circumstance.

Viral mutants that are of low virulence would seem best fit for adaptation to the host and persistence in nature. Those viruses would have few adverse effects on the host, allow host survival, and may have a prolonged period of viral shedding. In the case of BVDV, most viral isolates are of low virulence and noncytopathic in biotype. This is consistent with a virus that is well adapted to its host. However, low virulence and host survival may be selected against in a process that favors viral mutants that are more "fit" for replication in the host [11]. Viral mutants that replicate to a higher titer than the parent virus would have a competitive advantage and soon dominate the mutant swarm. This process could favor emergence of virulent viruses that exhibit enhanced viral replication, which in turn, could lead to extensive tissue damage and a burst of viral shedding in high numbers. The release of high numbers of virus improves the chance of viral transmission, making virulence a positive trait and giving virulent virus a competitive advantage over less virulent viruses [12]. In the case of BVDV, this might explain the periodic emergence of virulent BVDV that produce large outbreaks of disease [13].

Bovine viral diarrhea viruses the quasispecies

Recent studies have shown that BVDV exists as a quasispecies. Those studies have focused on the 5′untranslated region of the viral RNA. This region of the viral genome is relatively conserved, due to its important role as a ribosomal entry site for production of viral protein, as the site for initiation of replication of virion RNA, and for its role in encapsulation of the viral RNA in viral progeny [14–19]. However, altered base sequence in this region of the viral genome has been identified after passage of the virus in cell culture, and has been detected in viral RNA that was extracted from tissues of an infected animal [20,21]. The ability to mutate rapidly allows a virus like BVDV to quickly produce mutants that are better fit to replicate in the host. As an example, mutations were created in the 5′ untranslated region of viral RNA that impaired viral replication. The created mutations were "repaired" within a few rounds of viral replication in cell culture [18]. The repair was done by natural selection of spontaneous mutants that were better fit for replication than the parent virus.

Populations of genetic variants of BVDV also have been identified within individual PI cattle [22]. The genetic variants had different amino acid sequences in the immunologically important E2 envelope glycoprotein, an important target of viral neutralizing antibody. Variation in amino acid sequence of the E2 protein may benefit a virus during an acute infection by allowing escape from the immune response. However, antigenic variation in viral proteins in the PI animal likely would be detrimental to the virus. This is because immunotolerance in a PI animal is specific to the persistent BVDV; hence, antigenic variation would likely trigger an immune response against that population of mutant virus. There would be selective pressure

to maintain antigenic continuity during persistent infection. However, the detection of genetic variants in PI cattle suggests that those animals may enhance the diversity of BVDV by serving as a source of viral variants that can infect other cattle. Also, this may explain the presence of viral neutralizing antibodies that are occasionally found in PI cattle with no known exposure to BVDV from an outside source.

Even though viral variants can be detected in a PI animal, the swarm of viral mutants as a whole maintains a stable master, or consensus, sequence over time. This has been shown for the relatively stable 5′ untranslated region of the viral RNA and for the highly variable E2 envelope glyco-protein [23,24]. Similarly, the master sequence remains relatively stable in individual animals and in groups of animals during outbreaks of acute disease [25]. This relative stability allows use of molecular epidemiology to track a specific virus involved in a series of disease outbreaks. It also suggests that the genetic diversity and multiple viral genotypes found among BVDV in a large geographic area like North America is due to the gradual accumulation of mutations in BVDV of several different origins, as opposed to extremely rapid evolution of a single BVDV.

Although there is a tendency to maintain the master sequence of a virus under neutral conditions, the immune response of the infected host creates a nonneutral condition, and may select viral variants. This has been seen on farms that harbor multiple PI cattle, which likely originated from a single outbreak of acute infection in immunocompetent pregnant cattle. Comparison of the BVDV from those animals showed that the viral isolates were similar; however, antigenic differences could be detected among the viral isolates [26]. The selection of the antigenic variants likely occurred during the acute infection of the dams of those PI cattle and resulted in transplacental transmission of slightly different BVDV to a group of fetuses.

Viral biotypes

In addition to having a high frequency of point mutations, RNA viruses also have a propensity for recombination [5,27,28]. This allows RNA viruses to exchange segments of their genomes and potentially create new viral species. Recombination in BVDV RNA has been shown to be either homologous, involving self-viral RNA, or heterologous, involving nonself-viral RNA or host cell RNA [29–35]. In BVDV, recombination of RNA has not been shown to create new viral species (a new viral genotype), but it can cause a switch in viral biotype. Two biotypes of BVDV (cytopathic and noncytopathic) exist in nature. The viral biotypes are characterized by their ability to cause cytopathic effect and cell death in cultured cells. Cytopathic BVDV induce cytoplasmic vacuolation and death of susceptible cultured cells within a few days of infection. Noncytopathic BVDV establish an inapparent persistent infection in cultured cells.

Noncytopathic BVDV is more prevalent in nature, and serves as the parent virus from which cytopathic BVDV arise after homologous or heterologous recombination in the noncytopathic viral RNA. The recombination usually occurs in the genomic region encoding the NS2-3 nonstructural protein of the noncytopathic BVDV and results in the insertion of either self or foreign RNA into the NS2-3 coding region. The genome of the resulting cytopathic BVDV is essentially identical to that of the parent noncytopathic BVDV except for the insert of additional RNA. The recombination event causes the large NS2-3 protein of noncytopathic virus to split into two smaller proteins that are termed NS-2 and NS-3. The NS-3 protein is considered a molecular marker of cytopathic virus and the cause of cytopathic effect [36]. Reversion of cytopathic BVDV back to the noncytopathic biotype also occurs. The resulting noncytopathic BVDV usually lose the ability to express of the NS-3 protein, but some noncytopathic BVDV have been identified that retain expression of the NS-3 protein without causing cytopathic effect in cultured cells [37,38].

Genetic and antigenic diversity

Viral genotypes and genetic diversity

The high frequency of mutation, propensity for recombination, and selective pressure from immune responses stimulated by natural infection or vaccination has led to the creation of a large assortment of genetic and antigenic variants of BVDV. The genetic variants can be grouped based on the homology of aligned nucleic acid sequences from various segments of the viral genome [39–45]. The segments of the viral genome used most frequently for phylogenetic analyses (typing or grouping) of BVDV are the 5″ untranslated region and the immediately adjacent region that encodes the N^{pro} viral protein. The array of BVDV form genotypes, subgenotypes within genotypes, and isolates within subgenotypes. Current viral taxonomy places BVDV in the genus Pestivirus in the family Flaviviridae. Pestiviruses segregate into at least five (possibly six) viral genotypes [42,43]. Those genotypes are classical swine fever virus, bovine viral diarrhea virus type 1, bovine viral diarrhea virus type 2, border disease virus, and a genotype represented by a single viral isolate termed Giraffe-1.

The viral genotypes are about 60% similar to each other in their base sequence. Subgenotypes within a genotype are designated by a number followed by a lower case letter (BVDV type1a, 1b, 1c, etc.). Subgenotypes are about 80% to 85% similar to each other. Each subgenotype includes a group of viral isolates that usually are 90 + % similar to each other. Currently, 11 subgenotypes of BVDV type 1 and two subgenotypes of BVDV type 2 have been identified [42,44,45]. Recent phylogenetic surveys suggest that there are regional differences in the distribution of viral genotypes and subgenotypes [44,46–49]. The regional distribution of viral

genotypes and subgenotypes likely reflects historic routes for movement of cattle, vaccine usage over time, and geographic isolation of cattle populations. As the result of different selective pressures and management practices, BVDV has evolved into the array of genotypes and subgenotypes present today. There is some linkage of viral genotypes and subgenotypes with clinical manifestations of disease including thrombocytopenia, reproductive failure, or pneumonia [13,39,40,49–51]. Also, regional bias may occur as to the viral genotype or subgenotype involved with the various disease forms that are seen after infection with BVDV.

Antigenic diversity

The genetic diversity seen among BVDV results in extensive antigenic diversity. Monoclonal antibodies raised against a diverse array of BVDV identify a fairly large number of epitopes (single antigenic sites) on the immunologically important viral proteins. The large number of epitopes allows use of panels of monoclonal antibodies to differentiate BVDV based on their antigenic profile [40,41,52,53]. Most field isolates of BVDV show unique patterns of monoclonal antibody binding when reacted with a large panel of monoclonal antibodies raised against several different viruses. In fact, BVDVs that are antigenically alike in monoclonal antibody assays are difficult to find.

Viruses are readily segregated into genotypes by patterns of monoclonal antibody binding. Similarly, segregation of BVDV into genotypes can be done using convalescent serum or postvaccinal serum in viral neutralization assays [39–41,54–56]. Separation of viruses into subgenotypes using polyclonal serum and viral neutralization assays has proven difficult. Even though antigenic differences likely exist between subgenotypes, the variable antibody response that occurs among cattle after infection or vaccination makes separating viruses into subgenotypes using polyclonal antibody uncertain. In contrast to antibody raised in response to viral infection, polyclonal antibody raised in cattle, sheep, or mice against the E2 envelope glycoprotein expressed in either baculovirus or vaccinia virus appears to be extremely virus specific, and may be able to separate viral isolates into different subgenotypes [57,58]. In summary, BVDV exists as an antigenically diverse array of viruses that manage to retain some antigenic similarity with each other and with other pestiviruses. Thus, all BVDV are serologically related, but the strength of that relationship will vary.

Consequences of diversity

Diversity of clinical disease

The clinical outcome following infection with BVDV is complex and dependent on multiple factors that are agent, host, and environmentally

related. Host factors that can influence the clinical outcome of infection include whether the host is immunotolerant or immunocompetent to BVDV, immune status (passive from colostral antibodies or active from exposure or vaccination), pregnancy status in females, gestational age of the fetus at the time of infection, level of environmental stress at the time of infection, and concurrent infection with other pathogens. It is well established that variation in virulence exists between different BVDV isolates. However, the basis for clinical variation at the virus level is not understood.

It is important to realize that despite wide genetic and antigenic diversity of BVDV isolates, most viral isolates are capable of inducing some common clinical syndromes. All noncytopathic BVDV isolates appear capable of infecting the fetus resulting in abortion, congenital defects, or the development of immunotolerance and subsequent persistent infection (it should be noted that persistent infection has not been observed with cytopathic isolates of BVDV). The virus is known to have an affinity for cells involved in immunity, and is capable of inducing some degree of immunosuppression. The majority of both type 1 and type 2 BVDV isolates are of low virulence and induce subclinical to very mild disease [59]. Most animals infected with BVDV undergo subclinical infections that result in mild fever, leukopenia, and development of serum-neutralizing antibodies. Subclinical infections explain the positive serum neutralization titers to BVDV that is found in the majority of unvaccinated cattle. It has been previously estimated that 70% to 90% of BVDV infections occur without manifestation of clinical signs [60].

When BVDV infections result in clinical disease, it has historically been referred to as BVD. Most clinical presentations of BVDV infection are mild, consisting of lethargy, anorexia, fever, diarrhea, and decreased milk production in lactating cows. Beginning in 1993, an atypical form of BVDV infection, referred to as severe BVD, was recognized in Canada [13,39]. The disease had a peracute course, caused high morbidity, and resulted in a substantial number of deaths in all age groups. This new form of BVDV infection killed approximately 25% of veal calves in certain regions of Canada [39]. Clinical disease in the Canadian outbreak was characterized by fever, pneumonia, and sudden death in all age groups of cattle [13]. Viral isolates obtained from these severe acute outbreaks were genotype 2 BVDV. Acute BVDV infections in cattle also can cause a hemorrhagic syndrome [61,62]. These infections are characterized by severe thrombocytopenia, bloody diarrhea, epistaxis, hemorrhages on mucosal surfaces, hyphema, bleeding from injection sites, pyrexia, leukopenia, and death [61]. Thus far, only noncytopathic type 2 BVDV has been associated with the hemorrhagic syndrome [40,61].

Although type 2 BVDV has been associated with many of the documented outbreaks of severe BVD and hemorrhagic syndromes, it should be emphasized that type 1 BVDV isolates are capable of resulting in

severe disease [44]. Outbreaks of severe clinical disease consisting of diarrhea, rapid dehydration, and death have been observed in association with isolation of type 1 BVDV (Dr. Kenny Brock, personnel communication). Additionally, type 2 BVDV isolates of low virulence are common, and are likely to predominate over virulent type 2 isolates [63]. Regardless of viral genotype, there is a continuum of virulence among the different BVDV isolates that ranges from causing subclinical to mild disease to isolates that cause severe, life-threatening clinical syndromes.

It is well established that acute BVDV infection can result in immunosuppression [64]. BVDV-induced immunosuppression increases the host's susceptibility to other pathogens, and may enhance the pathogenicity of coinfecting pathogen. Concurrent stress on the host at the time of BVDV infection is undoubtedly additive to the viral-induced immunosuppression. Synergistic effects of BVDV infection have been demonstrated with *Mannheimia haemolytica*, bovine herpesvirus-1, and bovine respiratory syncytial virus. Bovine viral diarrhea virus infections also have been associated with concurrent salmonellosis, *Escherichia coli*, bovine papular stomatitis, rotavirus, and coronavirus infections. Comparative experimental studies demonstrate that differences in the effect of BVDV isolates on cells of the immune system can be significant [65–68]. In calves experimentally inoculated with either a low virulence type 1 virus, a low virulence type 2 virus (7937) or a high virulence type 2 virus (890), a corresponding 21%, 49%, and 65% drop in white blood cell count was observed between day of infection and day 12 postinfection [67]. These differences are most important when combined with other disease exposures such as those that may occur in a commingle feedlot environment.

The role of BVDV in the bovine respiratory disease has been reviewed recently [69]. In the United States, BVDV has been reported as the most common virus isolated from outbreaks of bovine respiratory disease. Experimentally, it has been difficult to reproduce respiratory disease with BVDV alone, but synergistic effects have been documented between BVDV and *M haemolytica* [70], bovine herpesvirus-1 [71], and bovine respiratory syncytial virus [72]. Experimental studies have suggest that some BVDV isolates have more pulmonary tropism and are more likely to be associated with bovine respiratory disease than others [50,73,74].

The reproductive consequences of BVDV are reviewed elsewhere in this publication. In brief, BVDV infections have been associated with infertility, early embryonic deaths, a variety of congenital defects, and fetal infection with seroconversion. Most importantly, fetal infection between 30 and 125 days of gestation can result in the development of immunotolerance to the virus and the subsequent birth of calves that are PI with BVDV. Cattle PI with BVDV serve as the major virus reservoir and source of virus transmission within and between farms. Differences in reproductive outcomes are most dependent on time of infection. Differences in the ability of individual BVDV isolates to cause reproductive failure have not

been well documented, although it has been speculated that differences exist [41]. Review of diagnostic laboratory data by Evermann supports this conclusion by finding that type 1 BVDV isolates were more commonly associated with persistent infections, congenital defects, and weak calves, while type 2 BVDV isolates were more commonly found in aborted fetuses [49]. Experimental studies provide evidence that different BVDV isolates have different fetal tissue tropisms, and this difference may result in different fetal pathologies and clinical outcomes [75,76]. In a dose titration study comparing the ability of a type 1 and type 2 isolate to cross the placenta and infect the fetus, fetal infection occurred in four of four heifers challenged with $10^7, 10^5, 10^3$, and 10^1 CCID$_{50}$/dose of type 2 virus while occurring in four of four, four of our, three of four, and zero of four heifers challenged with $10^8, 10^6, 10^4$, and 10^2 CCID$_{50}$/dose of type 1 virus, respectively [77]. Results form this study suggested that some BVDV isolates might be more likely to cross the placenta than others, although the reason for this is unknown.

Mucosal disease (MD) is a unique clinical syndrome that occurs in cattle PI with BVDV [78]. MD occurs when cattle that are immunotolerant to, and PI with a NCP biotype of BVDV, become infected with a CP biotype of BVDV that shares close homology with the persistently infecting non-cytopathic virus. Thus, not every combination of NCP and CP virus will result in MD. It is believed that the CP-BVDV most commonly arises de novo from the NCP, persistently infecting BVDV by molecular rearrangement. External sources of the CP virus can occur as demonstrated by the documented occurrence of MD following the use of modified-live BVDV vaccines and experimental studies where MD was produced by superinfection with CP-BVDV. Cytopathic and noncytopathic biotypes are represented in type 1 and type 2 genotypes, and MD has been documented to occur in both genotypes. Differences between viral genotypes in MD presentation has not been documented.

Diagnostic challenges

Both organism and immune response detection methods are used to diagnose BVDV. Diagnostics target viral antigens (immunoperoxidase microtiter assay, antigen ELISA, immunohistochemistry, fluorescent antibody), genomic material (PCR, in situ hybridization) or BVDV specific antibodies (virus neutralization, antibody ELISA). These assays have varying risks for failure when used to detect an organism with the capability of having a diverse genetic and antigenic makeup, such as BVDV.

Antigen detection assays rely on either monoclonal or polyclonal antibodies to detect BVDV specific antigens. Polyclonal antibodies derived from hyperimmunized swine or calves are generally broadly reactive as they contain antibodies directed against multiple epitopes, many of which are conserved among viruses. Monoclonal antibodies are specific for one

epitope, and if that epitope varies between viruses, binding of the monoclonal antibody can fail. Most antigen detection assays use polyclonal antibodies or a pool of monoclonal antibodies to provide the broadest reactivity and capability of detecting a diverse population of BVDV isolates.

PCR detects and amplifies genetic sequences that are unique to the organism of interest. The accuracy of PCR is dependent on the ability of PCR primers to specifically bind to target genetic material unique to the organism of interest. The difficulty that can arise with PCR is identifying genetic material that is unique to the organism of interest yet stable enough that it does not change significantly over time. Many PCR methodologies have been reported for detecting BVDV [79–83]. Primers have been designed that are capable of detecting a wide variety of field samples [84–87]. Diagnostic PCR primers primarily have been directed against the 5′ untranslated region of BVDV where nucleotide homology can be as high as 95% between isolates [88], thus allowing for high epidemiologic sensitivity.

Assays to detect virus neutralizing (VN) antibodies also are affected by BVDV diversity. As a result of nonstandard assay procedures, neutralizing BVDV antibody titers reported by different laboratories can vary significantly [89]. A significant variable that can differ from laboratory to laboratory is the reference virus used in the neutralizing assay. In a study by Vaughn, 14 animal diagnostic laboratories were asked to run BVDV VN antibodies on split serum samples collected from 11 calves [89]. The average of the 11 calves reported by each lab ranged from 1:18 to 1:2028. It was concluded that different BVDV reference strains being used in the VN assays likely account for some of the observed lab-to-lab variation. VN antibody titers may be dramatically different depending on the viral genotype with which the animals are exposed to. In a study using seroconversion in unvaccinated heifers as an indicator for circulating BVDV, it was observed that type 2 VN antibody titers were always highest if the actual virus circulating on the farm was type 2 BVDV (Table 1) [90]. The same observation was made for type 1 VN antibodies and type 1 virus. Therefore, using a VN assay designed to detect type 1 antibodies (assay using a type 1 reference virus) in a herd where type 2 virus is circulating may yield significantly different results than a VN assay using a type 2

Table 1
Bovine viral diarrhea virus type 1 and 2 virus neutralizing antibody titers of five 12-month-old unvaccinated heifers from a farm where type 2 bovine viral diarrhea virus was found circulating

Animal ID	Type 1 VN titer	Type 2 VN titer
216	<4	<4
219	8	>4096
224	16	>4096
224	4	204
226	4	1024

reference virus. When using virus VN assays as a BVDV diagnostic tool, requesting both type 1 and 2 viral neutralizing antibody titers should be considered.

Vaccination failure

Diversity among BVDV is a suspected cause of vaccination failure [91–96]. However, several studies have shown that a BVDV type 1 immunization induces clinical protection against a type 2 challenge [91,97–101]. Although protecting cattle against clinical disease is important, it may not be sufficient in terms of controlling reproductive failure. A key component in controlling BVDV is preventing fetal infections that result in the birth of calves PI with the virus. Preventing fetal infection and the subsequent sequela involves controlling virus exposure and enhancing BVDV specific immunity in susceptible dams. An important issue is the ability of immunity developed against one virus strain to crossprotect against heterologous BVDV strains effectively enough to prevent fetal infection. Several field studies suggest that immunologic protection against heterologous BVDV challenge may be incomplete with respect to fetal protection [93–96]. Early vaccines were developed with little knowledge of their ability to provide fetal protection. Currently, efficacy data on fetal protection is not required for approval of vaccines for BVDV in the United States [102]. Experimental studies attempting to address fetal protection are limited, and have focused primarily on immunity developed following vaccination. Results of vaccine fetal protection studies have been mixed, and are often dependent on the challenge model (see article on Reproductive Consequences of BVDV for further details). Most trials have involved killed vaccines, and efficacy has ranged from 25% to 100%. In studies evaluating the fetal protection efficacy of a modified-live vaccine, Cortese and Brock demonstrated 88% and 58% fetal protection in heifers immunized one time with a commercially available type 1 modified-live BVDV vaccine and challenged at 75 days in gestation with type 1 or type 2 BVDV, respectively [103,104]. Except for different challenge viruses, these studies were conducted similarly, suggesting that the vaccine was less likely to stimulate a fetal protective immunity against type 2 viruses compared with type 1 viruses. Other studies have not fully evaluated protection against multiple viruses.

Summary

The potential consequences of BVDV genetic and antigenic diversity are far ranging. The complexity of clinical presentations associated with BVDV likely arises from factors encoded by the virus genome. More importantly, prevention and control of BVDV may be complicated by diagnostic and

immunization failure resulting from virus diversity. Evolutionary pressures will continue to drive further diversity, making control of BVDV challenging. Current and the potential for future BVDV strain diversity should be considered when designing BVDV control programs both at the individual farm and national herd level.

References

[1] Holland J, Spindler K, Horodyski F, Grabau E, Nichol S, Vandepol S. Rapid evolution of RNA genomes. Science 1982;215:1577–85.

[2] Holland JJ, de la Torre JC, Clarke DK, Duarte E. Quantitation of relative fitness and great adaptability of clonal populations of RNA viruses. J Virol 1991;65:2960–7.

[3] Domingo E, Holland JJ. RNA virus mutations and fitness for survival. Annu Rev Microbiol 1997;51:151–78.

[4] Drake JW, Holland JJ. Mutation rates among RNA viruses. Proc Natl Acad Sci USA 1999;96:13910–3.

[5] Figlerowicz M, Alejska M, Kurzynska-Kokorniak A. Genetic variability: the key problem in the prevention and therapy of RNA-based virus infections. Med Res Rev 2003;23:488–518.

[6] Domingo E, Baranowski E, Ruiz-Jarabo CM, Martin-Hernandez AM, Saiz JC, Escarmis C. Quasispecies structure and persistence of RNA viruses. Emerg Infect Dis 1998;4:521–7.

[7] Chaston TB, Lidbury BA. Genetic "budget" of viruses and the cost to the infected host: a theory on the relationship between the genetic capacity of viruses, immune evasion, persistence and disease. Immunol Cell Biol 2001;79:62–6.

[8] Ruiz-Jarabo CM, Arias A, Baranowski E, Escarmis C, Domingo E. Memory in viral quasispecies. J Virol 2000;74:3543–7.

[9] Domingo E. Viruses at the edge of adaptation. Virology 2000;270:251–3.

[10] Quer J, Hershey CL, Domingo E, Holland JJ, Novella IS. Contingent neutrality in competing viral populations. J Virol 2001;75:7315–20.

[11] Novella IS, Duarte EA, Elena SF, Moya A, Domingo E, Holland JJ. Exponential increases of RNA virus fitness during large population transmissions. Proc Natl Acad Sci USA 1995;92:5841–4.

[12] Saiz JC, Domingo E. Virulence as a positive trait in viral persistence. J Virol 1996;70:6410–3.

[13] Carman S, van Dreumel T, Ridpath J, et al. Severe acute bovine viral diarrhea in Ontario, 1993–1995. J Vet Diagn Invest 1998;10:27–35.

[14] Poole TL, Wang C, Popp RA, Potgieter LN, Siddiqui A, Collett MS. Pestivirus translation initiation occurs by internal ribosome entry. Virology 1995;206:750–4.

[15] Rijnbrand R, van der Straaten T, van Rijn PA, Spaan WJ, Bredenbeek PJ. Internal entry of ribosomes is directed by the 5′ noncoding region of classical swine fever virus and is dependent on the presence of an RNA pseudoknot upstream of the initiation codon. J Virol 1997;71:451–7.

[16] Chon SK, Perez DR, Donis RO. Genetic analysis of the internal ribosome entry segment of bovine viral diarrhea virus. Virology 1998;251:370–82.

[17] Frolov I, McBride MS, Rice CM. cis-Acting RNA elements required for replication of bovine viral diarrhea virus-hepatitis C virus 5′ nontranslated region chimeras. RNA 1998;4:1418–35.

[18] Becher P, Orlich M, Thiel HJ. Mutations in the 5′ nontranslated region of bovine viral diarrhea virus result in altered growth characteristics. J Virol 2000;74:7884–94.

[19] Yu H, Isken O, Grassmann CW, Behrens SE. A stem-loop motif formed by the immediate 5′ terminus of the bovine viral diarrhea virus genome modulates translation as well as replication of the viral RNA. J Virol 2000;74:5825–35.

[20] Jones LR, Weber EL. Application of single-strand conformation polymorphism to the study of bovine viral diarrhea virus isolates. J Vet Diagn Invest 2001;13:50–6.

[21] Jones LR, Zandomeni R, Weber EL. Quasispecies in the 5′ untranslated genomic region of bovine viral diarrhoea virus from a single individual. J Gen Virol 2002;83:2161–8.

[22] Collins ME, Desport M, Brownlie J. Bovine viral diarrhea virus quasispecies during persistent infection. Virology 1999;259:85–98.

[23] Paton DJ, Lowings JP, Ramirez GC. Stability of the gp53 gene of a bovine viral diarrhoea virus isolated at different times from a persistently infected steer. Br Vet J 1994; 150:603–7.

[24] Vilcek S, Alenius S, Paton DJ, Mittelholzer C, Belak S. Genetic clustering of bovine viral diarrhoea viruses in cattle farms: genetic identification and analysis of viruses directly from cattle sera. Vet J 1999;158:33–8.

[25] Paton DJ, Carlsson U, Lowings JP, Sands JJ, Vilcek S, Alenius S. Identification of herd-specific bovine viral diarrhoea virus isolates from infected cattle and sheep. Vet Microbiol 1995;43:283–94.

[26] Hamers C, Lambot M, Onclin M, Lecomte C, Pastoret PP. Antigenic diversity of bovine viral diarrhoea viral isolates contradicts the concept of herd specific strain. Epidemiol Infect 1998;121:451–7.

[27] Alejska M, Kurzyniska-Kokorniak A, Broda M, Kierzek R, Figlerowicz M. How RNA viruses exchange their genetic material. Acta Biochim Pol 2001;48:391–407.

[28] Aaziz R, Tepfer M. Recombination in RNA viruses and in virus-resistant transgenic plants. J Gen Virol 1999;80:1339–46.

[29] Meyers G, Tautz N, Dubovi EJ, Thiel HJ. Viral cytopathogenicity correlated with integration of ubiquitin-coding sequences. Virology 1991;180:602–16.

[30] Qi F, Ridpath JF, Lewis T, Bolin SR, Berry ES. Analysis of the bovine viral diarrhea virus genome for possible cellular insertions. Virology 1992;189:285–92.

[31] Ridpath JF, Bolin SR. Delayed onset postvaccinal mucosal disease as a result of genetic recombination between genotype 1 and genotype 2 BVDV. Virology 1995;212:259–62.

[32] Desport M, Collins ME, Brownlie J. Genome instability in BVDV: an examination of the sequence and structural influences on RNA recombination. Virology 1998;246: 352–61.

[33] Becher P, Orlich M, Konig M, Thiel HJ. Nonhomologous RNA recombination in bovine viral diarrhea virus: molecular characterization of a variety of subgenomic RNAs isolated during an outbreak of fatal mucosal disease. J Virol 1999;73:5646–53.

[34] Becher P, Thiel HJ, Collins M, Brownlie J, Orlich M. Cellular sequences in pestivirus genomes encoding gamma-aminobutyric acid (A) receptor-associated protein and Golgi-associated ATPase enhancer of 16 kilodaltons. J Virol 2002;76:13069–76.

[35] Vilcek S, Greiser-Wilke I, Nettleton P, Paton DJ. Cellular insertions in the NS2–3 genome region of cytopathic bovine viral diarrhoea virus (BVDV) isolates. Vet Microbiol 2000;77: 129–36.

[36] Donis RO, Dubovi EJ. Differences in virus-induced polypeptides in cells infected by cytopathic and noncytopathic biotypes of bovine virus diarrhea-mucosal disease virus. Virology 1987;158:168–73.

[37] Qu L, McMullan LK, Rice CM. Isolation and characterization of noncytopathic pestivirus mutants reveals a role for nonstructural protein NS4B in viral cytopathogenicity. J Virol 2001;75:10651–62.

[38] Harding MJ, Cao X, Shams H, et al. Role of bovine viral diarrhea virus biotype in the establishment of fetal infections. Am J Vet Res 2002;63:1455–63.

[39] Pellerin C, van den Hurk J, Lecomte J, Tussen P. Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities. Virology 1994;203:260–8.

[40] Ridpath JF, Bolin SR, Dubovi EJ. Segregation of bovine viral diarrhea virus into genotypes. Virology 1994;205:66–74.

[41] Paton DJ, Sands JJ, Lowings JP, Smith JE, Ibata G, Edwards S. A proposed division of the pestivirus genus using monoclonal antibodies, supported by cross-neutralisation assays and genetic sequencing. Vet Res 1995;26:92–109.

[42] Becher P, Orlich M, Kosmidou A, Konig M, Baroth M, Thiel HJ. Genetic diversity of pestiviruses: identification of novel groups and implications for classification. Virology 1999;262:64–71.

[43] Harasawa R, Giangaspero M, Ibata G, Paton DJ. Giraffe strain of pestivirus: its taxonomic status based on the 5′-untranslated region. Microbiol Immunol 2000;44: 915–21.

[44] Vilcek S, Paton DJ, Durkovic B, et al. Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. Arch Virol 2001;146:99–115.

[45] Flores EF, Ridpath JF, Weiblen R, Vogel FS, Gil LH. Phylogenetic analysis of Brazilian bovine viral diarrhea virus type 2 (BVDV-2) isolates: evidence for a subgenotype within BVDV-2. Virus Res 2002;87:51–60.

[46] Tajima M, Frey HR, Yamato O, et al. Prevalence of genotypes 1 and 2 of bovine viral diarrhea virus in Lower Saxony, Germany. Virus Res 2001;76:31–42.

[47] Jones LR, Zandomeni R, Weber EL. Genetic typing of bovine viral diarrhea virus isolates from Argentina. Vet Microbiol 2001;81:367–75.

[48] Couvreur B, Letellier C, Collard A, et al. Genetic and antigenic variability in bovine viral diarrhea virus (BVDV) isolates from Belgium. Virus Res 2002;85:17–28.

[49] Evermann JF, Ridpath JF. Clinical and epidemiologic observations of bovine viral diarrhea virus in the northwestern United States. Vet Microbiol 2002;89:129–39.

[50] Baule C, Kulcsar G, Belak K, et al. Pathogenesis of primary respiratory disease induced by isolates from a new genetic cluster of bovine viral diarrhea virus type I. J Clin Microbiol 2001;39:146–53.

[51] Fulton RW, Ridpath JF, Saliki JT, et al. Bovine viral diarrhea virus (BVDV) 1b: predominant BVDV subtype in calves with respiratory disease. Can J Vet Res 2002;66:181–90.

[52] Bolin S, Moennig V, Kelso Gourley NE, Ridpath J. Monoclonal antibodies with neutralizing activity segregate isolates of bovine viral diarrhea virus into groups. Brief report. Arch Virol 1988;99:117–23.

[53] Edwards S, Sands JJ, Harkness JW. The application of monoclonal antibody panels to characterize pestivirus isolates from ruminants in Great Britain. Arch Virol 1988;102: 197–206.

[54] Hamers C, di Valentin E, Lecomte C, et al. Virus neutralizing antibodies against a panel of 18 BVDV isolates in calves vaccinated with Rispoval RS-BVD. J Vet Med B Infect Dis Vet Public Health 2000;47:721–6.

[55] Hamers C, Dehan P, Couvreur B, Letellier C, Kerkhofs P, Pastoret PP. Diversity among bovine pestiviruses. Vet J 2001;161:112–22.

[56] Fulton RW, Burge LJ. Bovine viral diarrhea virus types 1 and 2 antibody response in calves receiving modified live virus or inactivated vaccines. Vaccine 2000;19:264–74.

[57] Bolin SR, Ridpath JF. Glycoprotein E2 of bovine viral diarrhea virus expressed in insect cells provides calves limited protection from systemic infection and disease. Arch Virol 1996;141:1463–77.

[58] Toth RL, Nettleton PF, McCrae MA. Expression of the E2 envelope glycoprotein of bovine viral diarrhoea virus (BVDV) elicits virus-type specific neutralising antibodies. Vet Microbiol 1999;65:87–101.

[59] Ridpath JF, Neill JD. Detection and characterization of genetic recombination in cytopathic type 2 bovine viral diarrhea viruses. J Virol 2000;74:8771–4.

[60] Ames T. The causative agent of BVD: its epidemiology and pathogenesis. Vet Med 1986; 81:848–69.

[61] Corapi WV, Elliott RD, French TW, Arthur DG, Bezek DM, Dubovi EJ. Thrombocytopenia and hemorrhages in veal calves infected with bovine viral diarrhea virus. J Am Vet Med Assoc 1990;196:590–6.

[62] Rebhun WC, French TW, Perdrizet JA, Dubovi EJ, Dill SG, Karcher LF. Thrombocytopenia associated with acute bovine virus diarrhea infection in cattle. J Vet Intern Med 1989;3:42–6.

[63] Ridpath JF, Neill JD, Frey M, Landgraf JG. Phylogenetic, antigenic and clinical characterization of type 2 BVDV from North America. Vet Microbiol 2000;77:145–55.

[64] Potgieter LN. Immunology of bovine viral diarrhea virus. Vet Clin North Am Food Anim Pract 1995;11:501–20.

[65] Bolin SR, Ridpath JF. Differences in virulence between two noncytopathic bovine viral diarrhea viruses in calves. Am J Vet Res 1992;53:2157–63.

[66] Hou L, Wilkerson M, Kapil S, et al. The effect of different bovine viral diarrhea virus genotypes and biotypes on the metabolic activity and activation status of bovine peripheral blood mononuclear cells. Viral Immunol 1998;11:233–44.

[67] Walz PH, Bell TG, Wells JL, et al. Relationship between degree of viremia and disease manifestation in calves with experimentally induced bovine viral diarrhea virus infection. Am J Vet Res 2001;62:1095–103.

[68] Kelling CL, Steffen DJ, Topliff CL, Eskridge KM, Donis RO, Higuchi DS. Comparative virulence of isolates of bovine viral diarrhea virus type II in experimentally inoculated six- to nine-month-old calves. Am J Vet Res 2002;63:1379–84.

[69] Grooms D. Role of bovine viral diarrhea virus in the bovine respiratory disease complex. Bovine Practitioner 1998;32:7–12.

[70] Potgieter LN, McCracken MD, Hopkins FM, Walker RD, Guy JS. Experimental production of bovine respiratory tract disease with bovine viral diarrhea virus. Am J Vet Res 1984;45:1582–5.

[71] Potgieter LN, McCracken MD, Hopkins FM, Walker RD. Effect of bovine viral diarrhea virus infection on the distribution of infectious bovine rhinotracheitis virus in calves. Am J Vet Res 1984;45:687–90.

[72] Brodersen BW, Kelling CL. Effect of concurrent experimentally induced bovine respiratory syncytial virus and bovine viral diarrhea virus infection on respiratory tract and enteric diseases in calves [published erratum appears in Am J Vet Res 1999 Jan;60(1):13]. Am J Vet Res 1998;59:1423–30.

[73] Potgieter LN, McCracken MD, Hopkins FM, Guy JS. Comparison of the pneumo-pathogenicity of two strains of bovine viral diarrhea virus. Am J Vet Res 1985;46:151–3.

[74] Hamers C, Couvreur B, Dehan P, et al. Differences in experimental virulence of bovine viral diarrhoea viral strains isolated from haemorrhagic syndromes. Vet J 2000;160:250–8.

[75] Brock KV, Chase CC. Development of a fetal challenge method for the evaluation of bovine viral diarrhea virus vaccines. Vet Microbiol 2000;77:209–14.

[76] Hewicker-Trautwein M, Trautwein G, Frey HR, Liess B. Variation in neuropatho-genicity in sheep fetuses transplacentally infected with non-cytopathogenic and cytopathogenic biotypes of bovine-virus diarrhoea virus. Zentralbl Veterinarmed B 1995;42:557–67.

[77] Ficken M, Jeevaerathanam S, Wan Welch S, Nelson L, O'Hare M. BVDV fetal infection with selected isolates. International symposium: bovine viral diarrhea virus, a 50 year review, Ithaca (NY); 1995. p. 110–11.

[78] Bolin SR. The pathogenesis of mucosal disease. Vet Clin North Am Food Anim Pract 1995;11:489–500.

[79] Belak S, Ballagi-Pordany A. Bovine viral diarrhea virus infection: rapid diagnosis by the polymerase chain reaction. Arch Virol Suppl 1991;3:181–90.

[80] Hooft van Iddekinge BJ, van Wamel JL, van Gennip HG, Moormann RJ. Application of the polymerase chain reaction to the detection of bovine viral diarrhea virus infections in cattle. Vet Microbiol 1992;30:21–34.

[81] Letellier C, Kerkhofs P, Wellemans G, Vanopdenbosch E. Detection and genotyping of bovine diarrhea virus by reverse transcription-polymerase chain amplification of the 5′ untranslated region. Vet Microbiol 1999;64:155–67.

[82] Mahlum CE, Haugerud S, Shivers JL, et al. Detection of bovine viral diarrhea virus by TaqMan reverse transcription polymerase chain reaction. J Vet Diagn Invest 2002;14: 120–5.

[83] Stadejek T, Pejsak Z, Kwinkowski M, Okruszek A, Winiarczyk S. Reverse transcription combined with polymerase chain reaction as a detection method for pestiviral infections. Rev Sci Tech 1995;14:811–8.

[84] Renshaw RW, Ray R, Dubovi EJ. Comparison of virus isolation and reverse transcription polymerase chain reaction assay for detection of bovine viral diarrhea virus in bulk milk tank samples. J Vet Diagn Invest 2000;12:184–6.

[85] el-Kholy AA, Bolin SR, Ridpath JF, et al. Use of polymerase chain reaction to simultaneously detect and type bovine viral diarrhoea viruses isolated from clinical specimens. Rev Sci Tech 1998;17:733–42.

[86] Fulton RW, d'Offay JM, Saliki JT, et al. Nested reverse transcriptase-polymerase chain reaction (RT-PCR) for typing ruminant pestiviruses: bovine viral diarrhea viruses and border disease virus. Can J Vet Res 1999;63:276–81.

[87] Laamanen UI, Neuvonen EP, Yliviuhkola EM, Veijalainen PM. Comparison of RT-PCR assay and virus isolation in cell cultures for the detection of bovine viral diarrhoea virus (BVDV) in field samples. Res Vet Sci 1997;63:199–203.

[88] Donis RO. Molecular biology of bovine viral diarrhea virus and its interactions with the host. Vet Clin North Am Food Anim Pract 1995;11:393–423.

[89] Vaughn M. SN titer response comparison among fourteen diagnostic laboratories for antibodies protecting against IBR, BVD and BRSV virus following the adminastration of modified live vaccine to weaning aged calves. Proceedings of the 30th annual convention of the American Association of Bovine Practitioners, Montreal, Canada; 1997. p. 141–44.

[90] Pillars RB, Grooms DL. Serologic evaluation of five unvaccinated heifers to detect herds that have cattle persistently infected with bovine viral diarrhea virus. Am J Vet Res 2002; 63:499–505.

[91] Meyling A, Ronsholt L, Dalsgaard K, Jensen A. Experimental exposure of vaccinated and non-vaccinated preganant cattle to isoates of bovine viral diarrhoea virus (BVDV). In: Harkness J, editor. CEC seminar on research on animal husbandry. Brussels, Belgium: Commission of The European Communities; 1987. p. 225–31.

[92] Harkness J, Roeder P, Drew T, Wood L, Jeffery M. The efficacy of an experimental inactivated BVD-MD vaccine. In: Harkness J, editor. CEC seminar on research on animal husbandry. Brussels, Belgium: Commission of The European Communities; 1987. p. 233–50.

[93] Bolin SR, Littledike ET, Ridpath JF. Serologic detection and practical consequences of antigenic diversity among bovine viral diarrhea viruses in a vaccinated herd. Am J Vet Res 1991;52:1033–7.

[94] Kelling CL, Stine LC, Rump KK, et al. Investigation of bovine viral diarrhea virus infections in a range beef cattle herd. J Am Vet Med Assoc 1990;197:589–93.

[95] Van Campen H, Vorpahl P, Huzurbazar S, Edwards J, Cavender J. A case report: evidence for type 2 bovine viral diarrhea virus (BVDV)-associated disease in beef herds vaccinated with a modified-live type 1 BVDV vaccine. J Vet Diagn Invest 2000;12:263–5.

[96] Sockett D, Modderman E. Fetal exposure to bovine viral diarrhea virus despite vaccination in a Wisconsin diary herd. In: Proceedings of the National Animal Disease Center first annual international virtual conference, Ames (IA); 1997. Available from: URL: http://www.nadc.ars.usda.gov/virtconf/main.htm.

[97] Cortese VS, West KH, Hassard LE, Carman S, Ellis JA. Clinical and immunologic responses of vaccinated and unvaccinated calves to infection with a virulent type-II isolate of bovine viral diarrhea virus. J Am Vet Med Assoc 1998;213:1312–9.

[98] Bolin SR, Ridpath JF. Assessment of protection from systemic infection or disease afforded by low to intermediate titers of passively acquired neutralizing antibody against bovine viral diarrhea virus in calves. Am J Vet Res 1995;56:755–9.

[99] Dean HJ, Leyh R. Cross-protective efficacy of a bovine viral diarrhea virus (BVDV) type 1 vaccine against BVDV type 2 challenge. Vaccine 1999;17:1117–24.

[100] Makoschey B, Janssen MG, Vrijenhoek MP, Korsten JH, Marel P. An inactivated bovine virus diarrhoea virus (BVDV) type 1 vaccine affords clinical protection against BVDV type 2. Vaccine 2001;19:3261–8.

[101] Fairbanks K, Schnackel J, Chase CC. Evaluation of a modified live virus type-1a bovine viral diarrhea virus vaccine (Singer strain) against a type-2 (strain 890) challenge. Vet Ther 2003;4:24–34.

[102] United States Code of Federal Regulations. Animal and animal products, C.F.R. 9, Sect. 113.215 and 113.311 (2003).

[103] Cortese VS, Grooms DL, Ellis J, Bolin SR, Ridpath JF, Brock KV. Protection of pregnant cattle and their fetuses against infection with bovine viral diarrhea virus type 1 by use of a modified-live virus vaccine. Am J Vet Res 1998;59:1409–13.

[104] Brock K, Cortese V. Experimental fetal challenge using type II bovine viral diarrhea virus in cattle vaccinated with modified-live virus vaccine. Vet Ther 2001;2:354–60.