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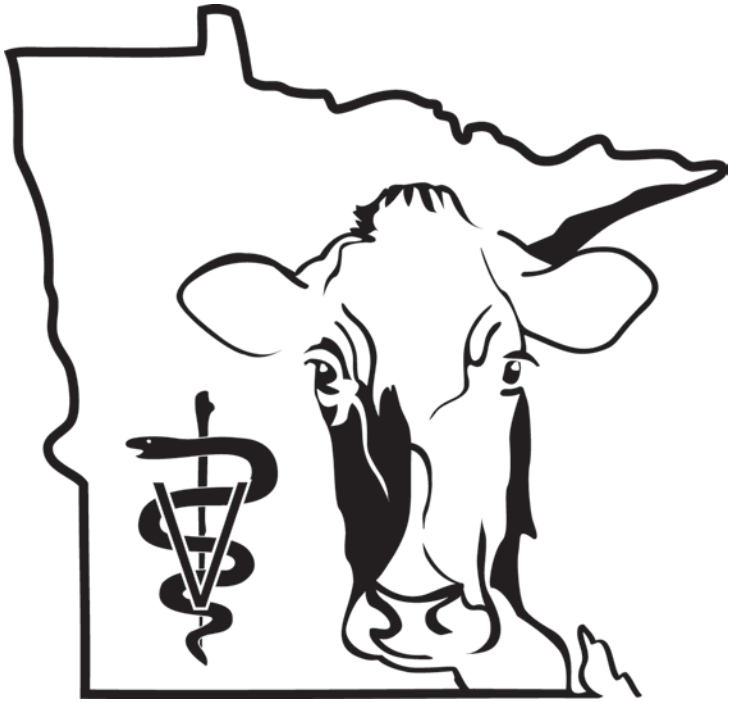

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BVD: The Old and the New

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Historical View: Bovine viral diarrhea (BVD) was first described in the mid 1940's in New York as a new acute disease of cattle manifested by clinical signs that included fever, diarrhea, leukopenia, salivation, nasal discharge, anorexia, dehydration, depression, and abortion. High morbidity and low mortality was seen in affected groups of cattle. The disease was termed bovine viral diarrhea because a bacterial causal agent could not be identified. A few years later, two other new diseases with clinical signs similar to those of bovine viral diarrhea were seen in Iowa and Indiana. Acute onset, extensive erosion and ulceration of the mucosa of the gastrointestinal tract, and death characterized the disease seen in affected cattle in Iowa. Chronic disease with erosion and ulceration of the epithelium of the coronary band and interdigital cleft, lameness, intermittent diarrhea, and wasting were seen in affected cattle in Indiana. In contrast with initial descriptions of bovine viral diarrhea, low morbidity and high mortality were typical of the new diseases seen in Iowa and Indiana. The disease seen in Iowa was named mucosal disease and the disease in Indiana became known as chronic BVD. By the early 1960's, it had been determined that BVD virus cause all three diseases and that two biotypes (noncytopathic and cytopathic) of BVD virus existed. The viral biotypes were differentiated by their effect on cultured cells with noncytopathic BVD having no adverse effect on cell growth and cytopathic BVD causing cytoplasmic vacuolation and cell death. Progress made in understanding BVD and BVD viruses in decades from 1960 is highlighted below.

- ***1960 – 1970*** Virus neutralization and fluorescent antibody diagnostic tests developed, modified live virus vaccines produced, post vaccinal mucosal disease reported
- ***1970 – 1980*** Effects of fetal infection studied, effect of BVD virus on the immune system recognized, role of BVD in mixed infections introduced, persistent infection identified
- ***1980 – 1990*** Inactivated viral vaccines, epidemiology and importance of persistent infection, dual virus theory for cause of mucosal disease and chronic BVD, viral proteins identified and partially characterized, knowledge expanded on effect of BVD virus on immune function and on mixed infections, nucleic acid sequence of BVD virus reported
- ***1990 – 2000*** Mechanism of origin of cytopathic BVD virus, virulent noncytopathic BVD virus identified, viral genotypes and subgenotypes described, molecular diagnostic tests developed, diagnostic tests improved, viral proteins further characterized, antigenic differences among viral isolates defined, new inactivated virus and modified live virus vaccines produced
- ***2000 – 2010*** Effect of BVD virus on cell function, epidemiology of BVD better defined, further improvement and modification of vaccines, control of BVD realized???

Current topics of importance for practitioners: Several topics of importance relative to control of BVD have emerged in the literature over the last decade. Other important topics that were studied decades ago are being investigated again, using tests and reagents developed

recently. The following paragraphs provide brief summaries on areas of interest on the biology of BVD virus and on diseases induced by BVD virus.

Viral taxonomy – BVD virus is now classified as a member of the Flaviviridae viral family, which includes flaviviruses (west Nile virus, yellow fever virus), pestiviruses (BVD, classical swine fever [hog cholera], and border disease virus), and hepatitis C virus. The flaviviruses are serologically related to each other but not serologicially related to the pestiviruses or hepatitis C virus. Similarly, the pestiviruses are serologically related to each other but are not serologically related to the flaviviruses or to hepatitis C virus. Members of the Flaviviridae family have a lipid envelope and a genome that consists of a single strand of positive sense RNA.

Viral genotypes – The ability to obtain the nucleic acid sequence of viruses has allowed grouping of viruses according to sequence similarity over select regions of the genome. Thus, a viral genotype is a group of viruses that share a high level of sequence similarity in what is often a small and relatively conserved region of the genome. Viral genotype does not have to relate to phenotype, an example of phenotype being viral serotypes. BVD genotype 1 and genotype 2 viruses do form separate serotypes. Subgenotypes may be identified within a viral genotype. Genotype 1 and genotype 2 BVD viruses contain several subgenotypes. An example of viral genotypes and sub genotypes is given below in the alignment of a 50 base sequence from each of 4 BVD viruses. A dot means the base located in that position matches the reference sequence that is in the top line. If the base does not match, the base in that position appears as the letter a, g, c, or t.

tgtcctcagatggatatacaacagattggattgcccattatgggtcacca
.....g.....a.....
a..a..t..g...g.cc.....a.c.ca....t..cc.g.....ag.a.
a..g..t.....g.t.....a.c.c..c.....cc.g.....ag.a.

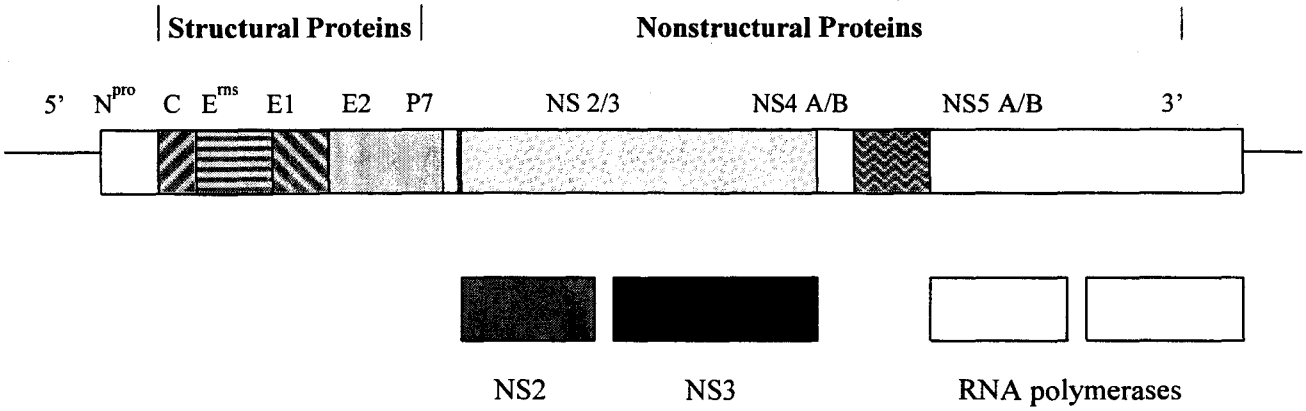
The top virus and the second virus differ by only 2 of 50 bases; therefore they are 96% similar and belong to the same genotype (less than 80% similarity would indicate the viruses are in different genotypes). The third virus is only 64% similar to the top virus and 66% similar to the second virus. The fourth virus is 70% similar to the top virus, 72% similar to the second virus, and 86% similar to the third virus. Because the third and fourth viruses are less than 80% similar to the first or second virus, they are in a different genotype. In fact, they represent 2 subgenotypes (genotype 1a and genotype 1b).

Antigenic variation – BVD virus exhibits considerable antigenic variability when assayed using large panels of monoclonal antibodies. Antigenic differences are sufficient between viral genotypes that serology can be used to deduce the viral genotype involved in outbreaks of disease. However, serology is not a reliable means to differentiate viruses that belong to the same genotype.

Viral quasispecies (mutant swarms) – Positive strand RNA viruses enjoy a high mutation rate. It is estimated that the mutation rate of a given base in the genome of a positive strand RNA virus is 10^{-4} per replication of the viral genome. That means that an RNA virus that has a genome of 10,000 bases (BVD virus is about 12,300 bases) will have at least one point mutation

somewhere in its genome with each replication of its RNA. Hence, a single parent virus infecting a cell produces 100 progeny and all are different from the parent. A mutant swarm has been created. This allows the virus to be responsive to selective pressure (antibody) and survive longer in the host. It also accounts for the many different BVD viruses encountered in the field. Fortunately, all BVD viruses are serologically related, so even though viral variants emerge quickly, immunization or natural infection will afford at least partial protection from disease.

Viral biotypes – As mentioned above, BVD virus exists as either of two biotypes, noncytopathic or cytopathic. The noncytopathic virus is the parent virus and the cytopathic virus is the offspring. Cytopathic BVD virus is created as the result of an error in viral RNA replication occurring in the region of the viral genome that encodes a nonstructural (not part of the mature virus particle) viral protein. The error often is a



recombination of host cell RNA with viral RNA in the genomic region encoding the NS2/3 nonstructural protein. Noncytopathic viruses make the large NS2/3 protein. Recombination of viral RNA with host cell RNA in the NS2 region causes the large NS2/3 protein to split into two proteins, NS2 and NS3. The NS3 protein is a biochemical marker for cytopathic virus and its formation induces cytopathic effect in cells. Note that the recombination event that leads to creation of cytopathic virus does not affect the viral structural proteins that react with viral neutralizing antibody. Thus, the cytopathic virus is antigenically similar to its parent noncytopathic virus. Cytopathic viruses may be genotype 1 or genotype 2.

Viral reservoirs – BVD virus infects most, if not all, even toed ungulates (deer, swine, cattle, sheep, goats etc). The most important reservoir is the persistently infected bovine. Persistently infected cattle shed virus for life, primarily in nasal secretions. Fetal infection with noncytopathic BVD virus in the first 4 months of gestation leads persistent infection. Persistently infected cattle usually do not have neutralizing antibody against BVD virus in their serum. However, they may make neutralizing antibody if vaccinated and some persistently infected cattle make neutralizing antibody against their own noncytopathic virus. There is some indication that BVD virus may persist in gonads after postnatal infection of healthy cattle.

Diseases induced by BVD virus –

- Acute BVD – clinically mild, most common form of disease caused by either type 1 or type 2 BVD virus
- Acute BVD – clinically severe, usually associated with type 2 BVD virus
- Reproductive failure – BVD virus is an important cause of reproductive failure at all stages of gestation, regardless of genotype or biotype
- Congenital malformations – usually seen after fetal infection between 4 and 6 months of gestation
- Persistent infection – always a noncytopathic virus, fetal infection in first 4 months of gestation; calf may be normal, stunted, and/or weak at birth; life span often less than 6 months but some survive for years
- Acute mucosal disease – acute onset, short course, leukopenia, diarrhea with or without blood and mucus, dehydration, death – both noncytopathic and cytopathic virus isolated
- Chronic mucosal disease – intermittent diarrhea, lameness, wasting, disease lasts weeks to months, death – often only noncytopathic virus isolated and antibody may be detected
- Late onset mucosal disease – result of recombination of RNA between 2 BVD viruses, has been seen several weeks to months after exposure of persistently infected cattle to cytopathic virus

Mixed Infections – BVD virus replicates in cells of the immune system and may cause immunodepletion and immunosuppression. Numerous studies have shown that BVD virus can decrease the number of T-lymphocytes in circulating blood, suppress lymphocyte blastogenesis, suppress bacterial killing by phagocytic cells, suppress interferon activity, and suppress memory T-lymphocyte responses. Enhanced replication and expanded tissue distribution of other bacterial and viral pathogens is the end effect of BVD virus induced immunosuppression. An increase in pathogen load within an animal often leads to enhanced severity of disease. In particular, BVD virus appears to play an important role in polymicrobial bovine respiratory disease in weaned calves and in polymicrobial enteric disease in young calves.

Diagnostic tests and vaccination strategies – Always controversial subjects for which there is no right answer.