

Introduction

Management and control of bovine viral diarrhea (BVD) virus infection in cattle herds must consider two ways that BVD virus passes from one animal to another within a herd. The first is passing of the virus from one animal to another (horizontal transmission) when an animal infected with BVD virus secretes the virus in its nasal and other secretions and the virus enters a susceptible animal through the mouth or respiratory tract; and the second is the passing of BVD virus from an infected dam's bloodstream to her fetus during pregnancy (vertical transmission). Horizontal transmission of BVD virus to calves or adult cattle results in a temporary (transient) infection that is usually mild, but can occasionally result in severe disease. The main negative health effects of BVD virus are that it can inhibit conception and cause abortion in susceptible females; and it suppresses the immune system, making infected animal more susceptible to other diseases. In addition to contributing to disease in infected cattle, horizontal infection from one animal to a pregnant dam can lead to vertical transmission of BVD virus to her fetus during pregnancy. Fetal infection with BVD virus can lead to fetal death, the birth of a normal calf, or the birth of a calf that is persistently infected (PI) with BVD virus – meaning that the infection lasts the entire life of the animal.

The primary source of BVD virus is PI cattle; with transiently infected cattle considered a less important source. Persistently infected animals are a much more efficient transmitter of BVD virus than transiently infected animals because they secrete higher concentrations of virus for a longer period of time. Transiently infected animals experience a short period (7 to 10 days) where virus is shed in body secretions. In contrast, PI animals usually have a very high and persistent amount of virus circulating in their blood and other fluids, and BVD virus is shed continually from virtually all secretions including nasal discharge, saliva, semen, urine, tears, milk, and to a lesser extent, feces. Horizontal transmission of BVD virus to susceptible cattle has been shown to occur after only one hour direct contact with a single PI animal. Over-the-fence contact with a PI animal from a neighboring herd can also introduce BVD virus into a susceptible herd. Horizontal transmission of the virus from either persistently or transiently BVD virus-infected animals to susceptible cattle in direct contact may be via nose or mouth contact with virus-containing body fluids. In addition, air transmission over short distances seems likely.

Reproductive Effects of BVD Virus Infection

Even mild BVD virus infections of breeding females can cause failure to conceive, abortion, or vertical fetal infection. The immune status of the dam, the stage of gestation, and the characteristics of the virus itself are important factors in determining the result of BVD virus infection of pregnant cows and heifers. The BVD virus is able to cross from the dam's bloodstream to the fetus with high efficiency during the pregnancy of susceptible dams. Fetal infection can lead to early embryonic death, abortion, birth defects, stunting, the birth of PI calves, or the birth of normal calves. Persistently infected cattle are the result of fetal exposure to the noncytopathic biotype of BVD virus prior to the development of a fairly mature immune system at about 125 days of gestation

In addition to BVD virus causing conception failure and abortion, reproductive efficiency can be decreased due to fatal birth defects following fetal infection between 100 and 150 days of gestation. The lesions associated with fetal infection with BVD virus include brain

malformations, spinal cord defects, cataracts and other eye abnormalities, sparse haircoats, a short lower jaw, growth retardation and lung immaturity.

Exposure of Beef Herds to Persistently Infected (PI) Cattle

Suckling calves are commonly in contact with the breeding herd during early gestation, prior to the time the bovine fetus develops a competent immune system. As a result, PI suckling calves are considered to be the primary source of BVD virus in breeding herds. The result of an introduction of a PI animal into a beef herd depends on the timing of the introduction relative to the breeding season and the resulting immune status of the herd during early gestation.

Even in the absence of vaccination, the number of PI animals and the amount of BVD virus infection in a herd seems to be self-limiting unless the herd has a lot of additions. A likely scenario for a BVD virus-exposed herd with few additions is to experience an initial peak of disease and then in subsequent months and years, to experience low-level chronic reproductive losses. If a PI animal enters the herd either by birth or by purchase near the start of the breeding season, a high percentage of the herd may not be immunologically protected to the degree necessary to prevent infection, conception failure, abortion, or fetal infection. Once the PI animal is in contact with the breeding herd for a long enough period of time, the majority of the herd should become infected and produce immunity that protects against further disease. Cattle with some immunity to BVD virus are less likely to have conception failures, abortions, or infected fetuses compared to immunologically naïve animals. If no intervention is applied to the herd, the following year, the number of susceptible females should be greatly decreased and the number of abortions and infected fetuses should decrease.

Estimates of the prevalence of PI animals in the general U.S. beef cattle population has been reported to range between 0.13% and 2.0%. And about 4% of U.S. beef herds are expected to have at least one PI animal. Persistent infection has a clustered distribution, which means a few herds may contain several PI cattle but most herds contain only normal cattle. Clustering of multiple PI animals in a herd is primarily due to exposure of numerous susceptible dams to a PI or transiently infected source of BVD virus prior to day 125 of gestation. Although a high percentage of PI calves die at or near birth or at least by weaning, in some situations 17% to 50% of PI calves may survive so that they reach the age to enter the breeding pool or to enter a feedlot. Persistently infected females of breeding age not only are a source of horizontal transfer of BVD virus, but will always produce a PI calf themselves.

Effects of BVD Virus Infection in Feeder Cattle

Infection with BVD virus has been associated with respiratory disease outbreaks in feedlot situations. Persistently infected cattle are the primary source of BVD virus transmission to in-contact susceptible cattle during marketing, trucking, and while in feeding pens; and pastures and have been shown to have an impact on health performance of susceptible penmates and cattle in adjacent pens. Ongoing work is investigating the amount of disease in feedlot cattle that can be attributed to BVD virus (both transiently and persistently infected cattle).

Economic Considerations for Diagnostic Testing

The cost of the presence of at least one PI animal in a beef herd has been reported to range from \$14.85 per cow per year to \$24.84 per cow per year. However, the cost of initiating a BVD PI screening protocol on a farm or ranch is significant. The economic value of screening for PI animals in cow-calf herds is influenced by the likelihood of finding at least one PI animal

in the herd, the negative production effects when PI animals are present, the cost of inputs and the value of animals sold (price cycle).

Because of the low prevalence of herds with at least one PI animal, not all producers are economically justified to initiate diagnostic screening protocols for PI cattle. However, if ranch history raises a suspicion of PI cattle being present in the herd, a protocol to screen the herd can be defended based on its likelihood to improve economic return.

Diagnostic Testing Strategies to Identify PI Calves

Because the persistently infected animal is an important reservoir and transmitter of BVD virus, control programs must first identify and remove these animals from the breeding herd. Because of vertical transmission of the virus from viremic dams to their fetuses, PI animals should be removed prior to the start of the breeding season in beef herds with a controlled breeding season. In order to find and remove PI cattle prior to the start of the breeding season, all calves, all replacement heifers, all bulls, and all non-pregnant dams without calves due to not becoming pregnant, aborting, or calf death must be tested for PI status. Any female that is still pregnant at the time the herd is tested should be isolated from the breeding herd and kept isolated until her calf is tested and found to be negative. Once a calf is identified as PI, in most cases, it should be euthanized and the dam should be tested. Most dams of PI calves are not PI themselves and if confirmed as non-PI, it can re-enter the breeding herd because naturally acquired immunity is considered to prevent any future fetal infections. If the dam is identified as a PI, it should be sold to slaughter immediately.

Monitoring Herds for BVD PI Risk

The cost of initiating a BVD PI whole-herd screening protocol on a farm or ranch is significant. In order to decide whether or not whole-herd screening is economically justified, several strategies can be employed to monitor herds for their risk of having PI cattle present.

Use of production records and laboratory evaluation of moribund and dead calves

The minimal level of surveillance for every herd should include monitoring of herd fertility (early breeding season pregnancy proportion, pregnancy per insemination proportion, and total pregnancy proportion), calf sickness and death loss proportions, and percent weaned calf crop. Because of the negative effect of the presence of PI calves in a breeding herd on measures of reproductive efficiency, the presence of physical abnormalities at birth, and calf survivability to weaning, an unacceptable level of these symptoms increases the risk that BVD virus is a problem in the herd and increases the likelihood that whole-herd screening for PI cattle will be economically rewarding. Although, having PI calves in a herd usually decreases production efficiency, relatively closed herds with long-term PI exposure may not have noticeable production losses. In these cases, negative health effects may not become obvious until the PI calves are placed in contact with new herd mates (either by purchasing new herd additions or by commingling PI calves with calves from other herds after weaning).

In addition to monitoring production records, minimal surveillance should include the necropsy examination of as many aborted fetuses, stillborn calves, and calves that die pre-weaning as possible. In addition, sick calves from clusters of pneumonia, neonatal scours, or septicemia outbreaks that are not easily explained by sanitation or other problems should also be tested for BVD virus exposure and PI status. If most pre-weaning deaths are examined for BVD virus and found to be negative, it is not likely that PI animals are present in the herd. The presence of PI animals in the herd will be established by a single confirmed PI test.

The advantage of utilizing production measures and necropsies to determine if herds have either a high or low risk for the presence of BVD virus PI animals is that minimal expense is involved and these management tactics are also used to monitor for other disease and production problems. This level of monitoring is probably appropriate in herds with no evidence for the presence of PI animals and that are at low risk of PI introduction. The disadvantage is that at least one PI animal is allowed into the herd before production losses are identified, and production losses will continue for at least one year after intervention is initiated.

Use of pooled samples of blood or skin biopsies for PCR testing

Herd monitoring for the introduction of PI animals can also be accomplished with pooled blood or skin biopsy (usually ear notch) samples for PCR testing. PCR is a relatively new testing technology that can detect very small amounts of virus even when highly diluted. By pooling samples, the expense of screening herds with few PI animals is minimized. A single PI animal is detectable in pools of 50 to 250 negative samples. Animals contributing to negative samples are all assumed to be non-PI, whereas positive pools may contain samples from PI animals or transiently infected animals. If the initial pool is PCR-positive, it must be split and retested to find the one or more individuals in the pool that are infected with BVD virus. The best size of the initial pool is determined by the balance between the cost savings of having large numbers of individuals represented in negative pools and few individuals represented in positive pools that require further diagnostics. If pool size is too large, there is an increased chance that any single pool will test positive, requiring additional testing to identify the few truly PI individuals in the pool. If the samples are grouped in unnecessarily small pools, the cost benefit of pooling samples is lost to the large number of negative pools tested for each positive pool identified.

If samples are collected for pooled PCR from all suckling calves prior to the start of the breeding season, PI cattle can be identified and removed prior to possible contact with pregnant females, thereby eliminating the opportunity for a PI animal within the herd to cause reproductive failure and the to create more PI animals in the next calf crop. If samples are taken at a later time, such as at weaning, although PI cattle can be removed from the herd, those PI calves were shedding BVD virus and were in contact with pregnant females throughout much of gestation and can cause reproduction and production losses including the creation of PI cattle in the next calf crop.

Use of annual whole-herd testing

Certain high biosecurity herds, such as herds selling or developing replacement breeding animals, may elect to undergo a high level of surveillance even in the absence of evidence that PI animals are present. This high level of biosecurity may be important to their marketing plan or may indicate a high value placed on avoiding the small, but real risk of introducing BVD virus into the herd with subsequent negative reproductive, health, and marketing consequences. The first year that a beef herd adopts this strategy, all suckling calves, all females that were bred that failed to present a calf on test-day, all replacement heifers, and all bulls should be tested. If any calf is confirmed as a PI animal, his dam should be tested as well. In subsequent years, only suckling calves and any purchased animals need to be tested. Testing for PI status is a once-in-a-lifetime event. If an animal is PI-negative, it will be always be PI-negative, and if an animal is PI-positive, it will always be positive. Therefore cattle need only one test for PI status in its life.

If pregnant animals are purchased, the dam should be tested prior to or at arrival and the calf should be tested immediately after birth. In beef herds with a confined breeding season, this testing must occur before the start of the breeding season to ensure that no PI animals are in

contact with pregnant females during gestation. Heifer development operations should test every heifer prior to or at arrival at the facility.

Biosecurity - Other Potential Sources of BVD Virus

Bulls (PI and transiently infected)

Male PI calves will occasionally be selected for use as breeding bulls. The amount of BVD virus excreted in the semen of PI bulls is very high. BVD virus-contaminated semen is an efficient horizontal transmitter of disease from bull to cow. If PI bulls are used, all or most susceptible females bred with the semen will become infected with BVD virus although most will not produce a PI calf.

Embryo transfer

Embryo transfer is a potential route of transmission of BVD virus. If the embryo recipient is PI, vertical transmission to the transferred embryo will occur with the creation of a PI fetus. Although there is no evidence to suggest that BVD virus is present inside the embryos of infected females, the virus can be present on the intact zona pellucida of PI and transiently infected females and the virus is present at high levels in the uterine environment of PI donors. Established washing procedures will remove contaminating virus, but if these procedures are not followed, BVD virus from the collection fluids or virus present on the zona pellucida can be horizontally transferred to a susceptible recipient cow. Vertical transmission from the recipient cow to the fetus can occur resulting in fetal death or the birth of a PI calf. BVD virus infection of the recipient cow and fetus can also occur when both the donor and recipient are free of BVD virus if BVD virus-contaminated fetal serum is used in the embryo transfer process or if contaminated liquid nitrogen is in direct contact with embryos.

Other species (domestic and wildlife)

Other species may be potential sources of BVD virus to susceptible cattle herds. Transmission of BVD virus between sheep and cattle has been demonstrated, but the importance of this transmission has not been established. BVD virus has also been isolated from pigs, but again, the importance of pigs as a source of the virus to susceptible herds is not established. Deer seropositive to BVD virus have been identified in North America and Europe. However, the existence of PI deer appears to be a rare event and cattle are assumed to be the source of BVD virus infection for free-ranging ruminants.

Mechanical spread

Human activities may serve in the transmission of BVD virus from PI cattle to susceptible animals. A 19-gauge needle was able to infect susceptible cattle with BVD virus when used IV within 3 minutes of drawing blood from a PI animal. Nose tongs were able to infect susceptible cattle with BVD virus when used for 90 seconds within 3 minutes of being used in a PI animal. And a palpation sleeve was able to transmit the virus from a PI heifer to susceptible heifers that were palpated after her.

No evidence has been presented that insects are a source of BVD virus transmission in field outbreaks. However, a role is possible in that BVD virus was isolated from non-biting flies collected from the face of a PI animal, and experimental BVD virus transmission between a PI animal and susceptible animals occurred when 50 biting flies were fed on the PI animal for 5 minutes and 15 minutes later fed on susceptible animals.

Vaccination to Control BVD Virus-Induced Disease and Production Loss

In addition to removal of PI reservoirs, BVD virus transmission to and within the herd can be reduced with an appropriate vaccination program. To date, using laboratory information and limited field trials, one can make recommendations regarding what constitutes an effective vaccination programs to limit postnatal and gestational BVD virus transmission.

Laboratory evidence of vaccine efficacy

Laboratory work has indicated that although there were large variations in the vaccine-induced virus neutralizing titers of individual colostrum-deprived calves vaccinated with two doses (21 day separation between doses) of an killed BVD virus vaccine or a modified live BVD virus vaccine, serum from each animal was capable of neutralizing a wide range of antigenically diverse European and American isolates of BVD virus, including genotypes I and II. Other work has shown that administration of a single dose of a modified live vaccine against BVD virus stimulated an antibody response in seronegative cows that was detectable for at least 18 months. These antibodies were able to cross neutralize 12 antigenically diverse strains of BVD virus.

Ability of vaccines to provide fetal protection

Cowherd vaccination programs are primarily designed to prevent fetal infection, which is immunologically more difficult than protection from clinical disease. In order to prevent fetal infection, vaccination of an exposed herd would have to prime the immune system to effectively neutralize circulating virus before they can cross the placenta and cause fetal infection. Evidence from earlier as well as recently reported trials indicate that vaccination provides some protection of the fetus when the dam is experimentally challenged, but that protection does not extend to 100% of fetuses of exposed dams.

Control Programs to Limit Losses Due to BVD Virus

Control program for BVD Virus in beef cowherds

The primary goals of BVD virus control in breeding herds are to prevent fetal infection in order to eliminate BVD virus-associated reproductive losses (thereby preventing the birth of PI calves) and to reduce losses from transient BVD virus infections. Cattle that have been infected with BVD virus after birth and recovered are considered to be protected from clinical disease following subsequent exposure but protection may not be 100%. While vaccination does provide some protection from fetal infection, BVD virus control is generally achieved by a combination of: removal of PI cattle, vaccination, and a biosecurity system that prevents the introduction of PI animals into the herd and minimizes the contact with potentially viremic cattle or wildlife.

Removal of PI animals

Herds should be monitored to determine the risk that one or more PI cattle are present. If the presence of PI cattle is confirmed or strongly suspected, a whole-herd screening protocol, should be undertaken to identify and remove PI individuals.

Biosecurity to prevent herd exposure to PI animals

Biosecurity to prevent herd exposure to PI or transiently infected animals is important, especially after the removal of PI cattle, because with the removal of PI BVD virus shedders, the percentage of naturally protected animals in a herd decreases. All replacement heifers and bulls that enter the breeding herd, whether raised or purchased, should be tested and confirmed to not be PI prior to the start of breeding. If a pregnant animal is purchased, it should be segregated from the breeding herd until both the dam and the calf is confirmed to not be PI. Fence line contact with neighboring cattle should be managed so that stocker cattle are not adjacent to the

breeding herd during early gestation, and other cowherds are not adjacent unless they also have a strict biosecurity and vaccination program in place.

Vaccination as a component of biosecurity

Biosecurity also involves application of a vaccination protocol to reduce the risk of fetal infection in the event of cowherd exposure to an animal shedding BVD virus. Modified live vaccines (MLV) have inherent properties that may enable them to stimulate more complete protection against transplacental infection. For that reason, one recommendation is to vaccinate unstressed, healthy heifers with MLV vaccine. Vaccine administration should be timed so that a protective immune response coincides with the first four months of gestation. This is done to maximize the potential for adequate immunity to protect against fetal infection and reproductive failure or the birth of persistently infected calves. In heifers not previously vaccinated, the primary series should consist of two administrations. The first dose should be given when the heifers are 6 months of age or older, and the second dose should be given two months before breeding. Beef cows should be revaccinated annually before breeding according to label directions.

Control program for BVD VIRUS in stocker/feedlot operations

Vaccination is currently the primary control intervention for BVD virus in stocker and feedlot operations. Research to determine the economic value of screening feeder cattle for the presence of PI individuals prior to purchase or at arrival is just beginning. The economic return will depend on the prevalence of PI cattle, the sensitivity and specificity of the test used, and the economic cost of the disease to the operation.

References:

- Baker JC: 1987 *J Am Vet Med Assoc* 190:1449-1458.
- Barber DML, Nettleton PF, Herring JA: 1985 *Vet Rec* 117:459-464.
- Baszler TV, Evermann JF, Kaylor PS, Byington TC, Dilbeck PM: 1995 *Vet Pathol* 32:609-618.
- Bezek DM, Stofregen D, Posso M: 1995 *J Vet Diagn Invest* 7:395-397.
- Bielanski A, Nadin-Davis S, Sapp T, Lutze-Wallace C: 2000 *Cryobiology* 40:110-116.
- Binkhorst GJ, Journee DLH, Wouda W, Straver PJ, Vos JH: 1983 *Vet Q* 5:145-155.
- Bolin SR: 1990 *Vet Med* 85:1124-1132.
- Bolin SR, McLurkin AW, Cutlip RC, Coria MF: 1985 *Am J Vet Res* 46:2385-2387.
- Brock KV, Cortese VS: 2001 *Veterinary Therapeutics* 2:354-360.
- Brock KV, Grooms DL, Ridpath, J, Bolin SR: 1998 *J Vet Diagn Invest* 10:22-26.
- Brock KV, Redman DR, Vickers ML, Irvine NE: 1991 *J Vet Diagn Invest* 3:99-100.
- Brownlie J: 1990 *Rev Sci Tech Off Int Epiz* 9:43-59.
- Brownlie J, Clarke MC, Hooper LB, Bell GD: 1995 *Vet Rec* 137:58-62.
- Brownlie J, Clarke MC, Howard CJ, Pocock DH: 1987 *Annl's Rech vet* 18:157-166.
- Callan RJ, Schnackel JA, Van Campen H, et al.: 2002 *J Am Vet Med Assoc* 220:1348-1352.
- Carlsson U, Belak K: 1987 *Acta Vet Scand* 35:79-88.
- Casaro APE, Kendrick JW, Kennedy PC: 1971 *Am J Vet Res* 32:1543-1562.
- Cherry BR, Reeves MJ, Smith G: 1998 *Prev Vet Med* 33:91-108.
- Cortese VS, Grooms DL, Ellis J, Bolin SR, Ridpath JF, Brock KV: 1998 *Am J Vet Res* 59:1409-1413.
- Cortese VS, West KH, Hassard LE, Carman S, Ellis JA: 1998 *J Am Vet Med Assoc* 213:1312-1319.
- Cortese VS, Whittaker R, Ellis J, Ridpath JF, Bolin SR: 1998 *Am J Vet Res* 59:848, 850.

Davidson WR, Crow CB: 1983 *J Wildlife Dis* 19:345-348.

Done JT, Terlecki S, Richardson C, et al.: 1980 *Vet Rec* 106:473-479.

DuBois WR, Cooper VL, Duffy JC, Dean DD, Ball RL, Starr BD, Jr: 2000 *Bov Pract* 34:98-100.

Dubovi EJ: 1996, *Vet Med* 91:867-872.

Duffell SJ, Harkness JW: 1985 *Vet Rec* 117:240-245.

Ellis JA, Martin K, Norman GR, Haines DM: 1995 *J Vet Diagn Invest* 7:433-436.

Frolich K, Thiede S, Kozikowski T, Jakob W: 2002 *Ann New York Acad Sci* 969:4-13.

Fulton RW, Purdy CW, Confer AW, Saliki JT, Loan RW, Briggs RE, Burge LJ: 2000 *Can J Vet Res* 64:151-159.

Fulton RW, Ridpath JF, Saliki JT, et al.: 2002 *Can J Vet Res* 66:181-190.

Grooms DL, Brock KV, Norby B: *Proceedings of the 83rd Annual Meeting of the Conference of Research Workers in Animal Diseases*, St. Louis, MO. November 11, 2002. Abstract #186.

Gunn HM: 1993 *Vet Rec* 132:584-585.

Hamers C, Couvreur B, Dehan P, Letellier C, Lewalle P, Pastoret PP, Kerkhofs P: 2000 *Vet J* 160:250-258.

Hamers C. di Valentin E, Lecomte C, Lambot M, Joris E, Genicot B, Pastoret PP: 2000 *J Vet Med B* 47:721-726.

Hamers C. di Valentin E, Lecomte C. Lambot M, Joris E, Genicot B, Pastoret PP: 2002 *Vet J* 163:61-67.

Harkness JW: 1987 *Ann Rech Vet* 18:167-174.

Harkness JW, Roeder PL, Drew T, Wood L, Jeffrey M: (1987) *Pestivirus Infection of Ruminants*. Ed J.W. Harkness. Brussels, CEC. p. 233.

Houe H: 1992 *Res Vet Sci* 53:320-323.

Houe H: 1993 *Prev Vet Med* 15:275-283.

Houe H: 1995 *Vet Clin N Amer: Food An Pract* 11:521-547.

Houe H: 1999 *Vet Micro* 64:89-107.

Houe H, Baker JC, Maes RK, Wuryastuti H, Wasito R, Ruegg PL, Lloyd JW: 1995 *J Vet Diagn Invest* 7:321-326.

Houe H, Meyling A: 1991 *Arch Virol Suppl* 3:157-164.

Howard CJ, Brownlie J, Thomas LH: 1986 *Vet Rec* 119:628-629.

Kahrs RF: 1973 *J Am Vet Med Assoc* 163:877-878.

Kelling CL: 1996 *Vet Med* 91:873-877.

Kelling CL, Grotelueschen DM, Smith DR, Brodersen BW: 2000 *Bov Prac* 34:13-22.

Kelling CL, Steffen DJ, Topliff CL, Eskridge KM, Donis RO, Higuchi DS: 2002 *Am J Vet Res* 63:1379-1384.

Kirkland PD, Richards SG, Rothwell JT, Stanley DF: 1991 *Vet Rec* 128:587-590.

Larson RL, Pierce VL, Grotelueschen DM, and Wittum TE: 2002 *Bov Pract* 36:106-112.

Liess B, Moenning V: 1990 *Rev Sci Tech Off Int Epiz* 9:151-161, 1990.

Lindberg A, Groenendaal H, Alenius S, Emanuelson U: 2001 *Prev Vet Med* 51:199-214.

Loneragan GH, Thomson DU, Montgomery DL, et al: *Proceedings of the 83rd Annual Meeting of the Conference of Research Workers in Animal Diseases*, St. Louis, MO. November 11, 2002. Abstract #32.

Martin SW, Bateman KG, Shewen PE, Rosendal S, Bohac JE: 1989 *Can J Vet Res* 53:355-362.

McClurkin AW, Coria MF, Cutlip RC: 1979 *J Am Vet Med Assoc* 174:1116-1119.

McClurkin AW, Littledike ET, Cutlip RC, Frank GH, Coria MF, Bolin SR: 1984 *Can J Comp Med* 48:156-161.

Meyling A., Houe H, Jensen AM: 1990 *Rev. sci. tech. Off. Int Epiz.* 9:75-93.

Meyling A, Jensen AM: 1988 *Vet Microbiol* 17:97-105.

Meyling A, Ronsholt L, Dalsgaard K, Jensen AM: 1987 *Pestivirus Infection of Ruminants*. Ed J.W. Harkness. Brussels, CEC. p. 225.

Miller MA, Ramos-Vara JA, Kleiboeker SB, Turnquist SE, Rosenkrans KS, Larson RL: 45th Annual Meeting, American Association of Veterinary Laboratory Diagnosticians, St. Louis, MO, Oct 19-21, 2002. pp. 69.

Moerman A, Straver PJ, de Jong MCM, Quak J, Baanvinger Th, van Oirschot JT: 1993 *Vet Rec*: 622-626.

Muñoz-Zanzi CA, Johnson WO, Thurmon MC, Hietala SK: 2000 *J Vet Diagn Invest* 12:195-203.

Nielsen SS, Roensholt L, Bitsch V: 2000 *J Wildlife Dis* 36:584-587.

Niskanen, R, Lindberg A, Larsson B, Alenius S: 2000 *Acta vet scand* 41:93-99.

Niskanen R, Lindberg A, Traven M: 2002 *The Vet J* 163:251-259.

Njaa BL, Clark EG, Janzen E, Ellis JA, Haines DM: 2000 *J Vet Diagn Invest* 12:393-399.

Orban S, Liess B, Hafez SM Frey HR, Blindow H, Sasse-Patzer B: 1983 *Zbl Vet Med B* 30:619-534.

Palfi V, Houe H, Philipsen J: 1993 *Acta vet scand* 34:105-107.

Paton DJ, Goodey R, Brockman S, Wood L: 1989 *Vet Rec* 124:63.

Pillars RB, Grooms DL: 2002 *Am J Vet Res* 63:499-505.

Rae AG, Sinclair JA, Nettleton PF: 1987 *Vet Rec* 120:504.

Revell SG, Chasey D, Drew TW, Edwards S: 1988 *Vet Rec* 123:122-125.

Ridpath JF, Neill JD, Endsley J, Roth JA: 2003 *Am J Vet Res* 64:65-69.

Roeder PL, Harkness JW: 1986 *Vet Rec* 118:143-147.

Roeder PL, Jeffrey M, Cranwell MP: 1986 *Vet Rec* 118:44-48.

Singh EL: 1985 *Rev Sci Tech Off Int Epiz* 4:867-872.

Singh EL, Eaglesome MD, Thomas FC, Papp-Vid G, Hare WCD: 1982 *Theriogenology* 17:437-444.

Tarry DW, Bernal L, Edwards S: 1991 *Vet Rec* 128:82-84.

Terpstra C, Wensvoort G: 1988 *Res Vet Sci* 45:137-142.

Traven M, Alenius S, Fossum C, Larsson B.: 1991 *J Vet Med B* 38:453-462.

Voges H, Horner GW, Rowe S, Wellenberg GJ: 1998 *Vet Microbiol* 61:165-175.

Wentink GH, Van Exsel ACA, Goey I, van Lieshout JA: 1991 *Vet Quart* 13:233-236.

Whitmore HL, Zemanis R, Olson J: 1981 *J Am Vet Med Assoc* 178:1065-1067.

Wittum TE, Grotelueschen DM, Brock KV, et al.: 2001 *Prev Vet Med* 49:83-94.

Zimmer G, Schoustra W, Graat EAM: 2002 *Res Vet Sci* 72:75-82.