Int J Pharm Bio Sci 2012 Oct; 3(4): (B) 874 - 884

Review Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IDENTIFICATION AND CONTROL STRATEGIES OF BOVINE VIRAL DIARRHOEA VIRUS (BVDV)

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ABSTRACT

Bovine viral diarrhoea virus (BVDV) is a *Pestivirus* in the family Flaviviridae and is closely related to classical swine fever and ovine border disease viruses. Two antigenically distinct genotypes of BVDV exist, types 1 and 2. According to the OIE (2008), bovine viral diarrhoea/mucosal disease (BVD/MD) and classical swine fever (CSF) are notifiable diseases and hence eradication programmes are underway in several countries worldwide. Scandinavian countries were extremely successful in controlling BVDV infection while limited success was achieved in U.S.A. One of the most important aspects of the disease is that, there are no pathognomonic clinical signs of infection with bovine viral diarrhoea virus (BVDV) in cattle. Diagnostic investigations therefore rely on laboratory-based detection of the virus, or of virus-induced antigens or antibodies in submitted samples. The serological evidence that BVDV infection is widespread in India is of utmost practical importance and more focus is to be concentrated in controlling the disease. The present review aims in discussing the methods of detecting the BVD virus in different samples and the strategies required to control the spread of infection.

KEY WORDS: Bovine viral diarrhoea; Diagnosis; Control; Pestivirus; Persistent infection

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INTRODUCTION

Bovine viral diarrhoea (BVD) is caused by bovine viral diarrhoea virus (BVDV), a positive-sense, single-stranded, enveloped RNA virus of the genus *Pestivirus* and family Flaviviridae[1]. Cattle are the animals most commonly infected but the virus can also be found in sheep, goats and wild ruminants, which may serve as reservoirs. Non-cytopathogenic (non-cp) and cytopathogenic (cp) biotypes of the virus exist[2] and persistent infection with non-cp biotype is mainly responsible for the maintenance and transmission of the virus in nature. Calves which become pre-natally infected with non-cp BVDV develop a persistent infection for life and become major reservoirs of BVDV within the herd. When exposed to cp BVDV during the adult stage, these cattle may suffer from fatal mucosal disease (MD). The disease has a world-wide distribution and the BVDV antibody prevalence ranges from 50% to 90% in cattle[3,4]. Prevalence of 56% and 79.2% BVDV antibodies have been recorded, using an enzyme-linked immunosorbent assay (ELISA), in bovine sera from Brazil[5] and Zimbabwe[6] respectively. The mean prevalence of BVDV antibodies in Indian cattle was found to be 15.29%[7].

The economic losses associated with BVDV are due to abortion, still births, congenital abnormalities, increased neonatal mortality, prenatal and postnatal growth retardation and persistently infected calves. Cattle infected with BVDV may develop multiple and diverse clinical manifestations. The wide spectrum of disease associated with BVDV infection includes sub-clinical infections, congenital deformities in calves and acute and chronic MD. The majority of infections (70-90%) in susceptible cattle are sub-clinical with the development of neutralizing antibodies[8]. However, infection with BVD is acute in seronegative, immunocompetent cattle. Clinical signs may include pyrexia, depression, inappetence, occulonasal discharges and occasionally oral lesions characterized by erosions or shallow ulcerations and foul smelling watery diarrhoea; high morbidity is experienced but mortality is low.

The diagnosis of the BVDV based on clinical signs is difficult, as there are no pathognomonic clinical signs of BVDV in cattle. Therefore one should rely on the laboratory based diagnostic investigations for the diagnosis of BVDV[9]. In unvaccinated dairy herds, serological testing of bulk milk is a convenient method for BVDV prevalence screening. Alternatively, serological testing of young stock may indicate if BVDV is present in a herd. In BVDV positive herds, animals persistently infected (PI) with BVDV can be identified by combined use of serological and virological tests for examination of blood samples. ELISA's have been used for rapid detection of both BVDV antibodies and antigens in blood, but should preferably be backed up by other methods such as virus neutralization, virus isolation in cell cultures or amplification of viral nucleic acid. Detailed knowledge of the performance of the diagnostic tests in use, as well as of the epidemiology of BVD is essential for identification of viraemic animals in the affected herds[9]. The detailed description of different diagnostic tests for the identification of BVDV antigen, antibody or the nucleic acid from different samples (serum, blood, milk, tissues etc.) for identification of either acutely infected animals or chronic carriers/persistently infected is addressed below.

VIRUS ISOLATION

It has been the backbone of virology, but has been losing favor recently because of the expense involved and the perceived slowness of the process. It will remain an integral part of the diagnostic process because it is the only way to discover new viruses or old viruses in new animal species. Also, it provides new isolates for comparison with current vaccines

or old field strains (BVDV type 1 vs. BVDV type 2). Viruses are not static entities; changes in them need to be tracked. The virus may be isolated in a number of bovine monolayer cell cultures (e.g. kidney, lung, testis or turbinate). Growth of both biotypes is usually satisfactory. Non cytopathogenic BVDV is a common contaminant of fresh bovine tissue, and cell cultures must be checked for freedom from adventitious virus by regular testing[10,11]. Primary or secondary cultures can be frozen as cell suspensions in liquid nitrogen. These can then be tested over a series of passages, or seeded to other susceptible cells and checked before routine use. Such problems may be overcome by the use of continuous cell lines, which can be obtained BVD-free[10].

ANTIGEN DETECTION TESTS

These are a second way of detecting viruses in clinical samples. The most common of these is the fluorescent antibody test done on frozen tissues. A similar test is the immunoperoxidase test usually done on formalin-fixed tissue. Other antigen detection tests are those that capture antigen on some type of solid support (antigen-capture ELISA or ACE tests). These processes are quick, specific, and thus preferred when sufficient viral protein is present such as in BVDV persistently infected animals. A lack of sensitivity in the ACE assay can, however, be a problem and should never be used to detect acutely infected animals.

ANTIBODY DETECTION TESTS

These are the tests that identify antibody as an indirect means of inferring the presence of a viral infection. There is a whole host of antibody detection tests, and the type of test selected will be determined by the type of virus that one wishes to detect and the types of tests available. For BVDV, the standard antibody detection test is the serum (virus) neutralization assay. For non-vaccinated animals, both a type 1 and type 2 SN should be requested.

NUCLEIC ACID DETECTION TESTS

These are the new techniques for finding viruses. Of the various methods used for this purpose, the polymerase chain reaction or PCR is the one most favored. While this test is very sensitive, it is fraught with technical difficulties and one must be very careful in the selection of the laboratory doing the testing. It is very critical to question the type and number of controls run with the test sample. If the test result is reported as negative, did the lab run the appropriate positive controls? Likewise, have false positive results been ruled out by use of appropriate controls. The strength of PCR (its exquisite sensitivity) is also its downfall because tiny amounts of contaminating nucleic acid can be amplified to produce a false positive test result.

DIFFERENT TESTS PERFORMED FOR IDENTIFICATION OF BVD :

1. Bovine Viral Diarrhea Immune Peroxidase test (IP)
2. Bovine Viral Diarrhea Serum Isolation (with IP detection)
3. Bovine Viral Diarrhea Fluorescent Antibody test (FA)
4. Bovine Viral Diarrhea Virus Isolation
5. Bovine Viral Diarrhea Virus Serum Neutralization test (SN)
6. Bovine Viral Diarrhea Serum Isolation
7. Bovine Viral Diarrhea Whole Blood Isolation
8. PCR Detection Using (Bulk) Milk Samples
9. Bovine Viral Diarrhea Antigen Capture ELISA test [ACE] (serum or milk)
10. Bovine Viral Diarrhea Antigen Capture ELISA test [ACE] (skin)
11. Pooled PCR testing for herd screening
12. BVDV PCR test

Tests 2, 4, 6 and 7 are all variations on virus isolation tests. Test 5 is the only one that detects antibody to BVDV. Tests1, 3, 9 and 10 are antigen detection tests while test 8, 11and 12 are nucleic acid detection tests.

The choice of test depends on the current clinical problem and on the past test data from

the herd or animal. Examples of how these tests should be used to detect and identify a BVDV problem are given along with a few details about each test.

1. Bovine Viral Diarrhea IP

This immunoperoxidase (IP) method provides an antigen detection test that uses formalin-fixed tissue. It is generally done in conjunction with a necropsy or histopathology submission. It is a very reliable antigen detection test and it can be used to detect BVDV in acute infections as well as animals persistently infected with BVDV[12]. This test is the basis of the skin biopsy IHC/IP detection test for detecting PI calves. As a skin biopsy test, it does not reliably detect acutely infected animals.

2. Bovine Viral Diarrhea Serum Isolation and Bovine Viral Diarrhea Serum Isolation (with IP detection)

These tests are virtually identical virus isolation tests with the exception of the method used to detect the presence of the virus. Normally, the virus is detected using fluorescent antibody staining of the test cells. The test "with IP detection" uses an immunoperoxidase system to detect the presence of BVDV. These tests are used to detect persistently infected animals when a small number of animals are to be tested or when the level of certainty of a negative status is of paramount importance, i.e. exports or animals qualifying for AI centers.

3. Bovine Viral Diarrhea FA

This is an antigen detection test run on fresh frozen tissue as contrasted with the IP test which uses formalin-fixed tissues. This test can be done more rapidly than the IP test, but the reliability of the FA test is less than the IP test. It is most useful in detecting acute infections.

4. Bovine Viral Diarrhea Virus Isolation

Virus isolation has been the "gold standard" for BVDV detection and will continue to be an important diagnostic test. This test is done primarily on tissue samples or swabs from acutely infected animals as well as from persistently infected animals. For animals suspected of being acutely infected, a whole blood sample should be submitted if possible (see item 7). For BVDV isolation, feces are never the sample of choice.

5. Bovine Viral Diarrhea Virus SN

This is the only test routinely used to detect and quantify antibodies specific for BVDV. In the classical use of this test, acute and convalescent sera are tested to determine whether a recent infection has occurred. This test is also valuable using single serum samples from a group of animals to determine the infection status of a herd. It can also be used to evaluate the vaccination program. For non-vaccinated animals, both type 1 and type 2 SN should be requested.

***6. Bovine Viral Diarrhea Serum Isolation*:** See item 2 above.

7. Bovine Viral Diarrhea Whole Blood Isolation

This test is a virus isolation which uses the mononuclear cells in unclotted whole blood as the test inoculum for cell culture. This test is reliable even in animals with both virus and antibodies, as the buffy coat is separated from the serum and the mononuclear cells are cultured live on cells.

8. PCR Detection Using Bulk Milk Samples

This test uses the sensitivity of the PCR test to screen several hundred lactating animals for PI status with a single sample. A 200 ml sample of bulk tank milk is sent cold but not frozen to the lab for testing. The number of animals represented in the sample should ideally be less than 400 even though dilution tests suggest that positive results can be obtained with 1 animal in 600. If the number of animals is large, taking several samples representing different groups of animals is recommended. This test should not be used to

assess PI status of the entire herd because most PI animals do not survive to produce milk. A negative bulk tank test tells you nothing about the non-lactating animals.

9. Bovine Viral Diarrhea Antigen Capture ELISA

(Serum or milk) : This test detects BVDV antigen in serum from persistently infected animals. Antibody will interfere with the test. As a result, it should not be used with animals less than about three months of age[12].

10. Bovine Viral Diarrhea Antigen Capture ELISA

(Skin) : This test detects BVDV antigen from skin biopsy samples. It is a reliable antigen detection test for the detection of persistently infected animals. Because it occasionally detects acute infections, animals with positive test results should be retested with viral or antigen detection tests 3 weeks later, to confirm PI status prior to culling[12]. This test performs well in animals with colostral antibodies, so it can be applied to samples from any age animals.

11. Pooled PCR Testing for Herd Screening

For herd screening purposes, pooling of whole blood/serum samples is an economical way to detect PI animals. Testing can be done on the whole herd including calves with colostral antibodies (whole blood). Individual samples are submitted to the laboratory which does the pooling. Testing strategies will identify the individual animals that are persistently infected[12].

12. BVDV PCR Test

PCR testing can be used in any situation where virus isolation would be appropriate. Because of the sensitivity of the test, interpretation of positive test results can be problematic when MLV vaccines have been administered close to sample collection. In addition, a positive PCR test on a single sample cannot distinguish between an acutely infected animal and a PI animal[12].

TESTING STRATEGIES

The testing strategies discussed here are at herd level. These strategies may not be optimal to detect an individual animal, or to diagnose BVDV in for example aborted fetuses. The choice of test depends on the current clinical problem and on the past test data from the herd or animal. Examples of how these tests should be used to detect and identify a BVDV problem are given along with a few details about each test. Below we have identified three scenarios; the first is identification of persistently infected cattle. These are the carriers of the BVD virus, and the main source of infection. The second scenario is the long term maintenance of low BVDV risk in herds. The third scenario is the herd evaluation of the vaccination program.

1) For identifying persistently infected cattle - Herd screening is required which is done by the following tests.

1.1 Pooled PCR Test : already discussed

1.2 ACE Test (Serum or milk) : already discussed

1.3 ACE Test (skin) : already discussed

1.4 Viral Diarrhea Whole Blood Isolation: already discussed

2) Long term Maintenance of low BVDV risk in herds: the following tests are useful.

2.1 Bovine Viral Diarrhea Virus SN : already discussed

2.2 Pooled PCR Test: already discussed

2.3 Sentinel Animal Antibody Surveillance

One innovative method of monitoring BVD circulation within a group of animals is to introduce a sentinel animal to that group and monitor its antibody status utilizing the BVD SN test above. The sentinel must be evaluated as a demonstrated non-PI animal and must remain unvaccinated throughout its life within the herd. One example of the use of this animal would be to introduce him to a calf cohort and test him at times critical to the transmission of BVD virus within the cohort.

One strategic testing strategy could include three weeks post introduction, at 3 months of age for the cohort, cohort prefreshening, and annually once introduced to the milking herd. Utilize the BVD SN test for this evaluation.

2.4 For continued PI calf surveillance, the following tests are useful

2.4.1 ACE Test (serum or milk) : already discussed

2.4.2 ACE test (skin) : already discussed

2.4.3 Bovine Viral Diarrhea Whole Blood Isolation: already discussed

3) Evaluation of Vaccination Program Surveillance at Breeding time or Pregnancy Check **:** the following tests are useful

3.1 Bovine Viral Diarrhea Virus SN

Evaluation of the level of protection in the herd consequent to a comprehensive vaccination program may be necessary to assess the efficacy of the vaccination strategy. The SN test can be employed for this purpose. Testing a random percentage of the herd or defined risk groups are two strategies that may be employed. The detection of adequate antibody level is the goal of this vaccination strategy evaluation process. The BVD SN test described above is appropriate for this purpose. The test should be used in vaccinated animals greater than three months of age.

3.2 Regulatory Testing for Sale, Export or entry into an Artificial Insemination Center**:** the following tests are useful

3.2.1 Bovine Viral Diarrhea Serum Isolation and Bovine Viral Diarrhea Serum Isolation (with IP detection): already discussed

3.2.2 Bovine Viral Diarrhea Whole Blood Isolation : already discussed

3.2.3 ACE Test: Depending on age (presence of colostral antibodies) and convenience, either serum or skin samples can be submitted for pre-purchase or pre-sale screening to detect persistently infected individuals.

3.2.4 Pooled PCR Test : already discussed

CLINICAL SCENARIOS AND BVD TESTS OF CHOICE

At least for the foreseeable future, BVDV will continue to be a problem for the cattle industry. The selection of the proper tests for the correct reasons is paramount for success in diagnosing a BVDV problem. One should never rely on a single test especially when confronted with an acute episode of clinical disease. Veterinarians should work with producers to design a testing scheme appropriate to the specific needs of a farm.

Live animal with clinical signs of an acute infection

Virus isolation (test no. 7) is the preferred test and an unclotted whole blood sample is the specimen of choice. Serum should not be submitted for virus isolation when an acute infection is suspected, regardless of the test format used. A PCR test (no.12) would also be appropriate. Serum should be collected to determine the antibody status (test no. 5) of the animal at the time of the clinical signs and several weeks later. The four-fold rule applies, i.e. the change in antibody titer must be at least a factor of four before one can consider BVDV (or any infectious agent) as a potential instigator of the clinical episode.

Animal found dead with gross lesions suggestive of BVDV

In these cases, it is always best to submit the entire animal for a necropsy. If this is not possible, then tissues should be collected for histopathology and immunoperoxidase testing for BVDV (formalin fixation) (test no. 1); fresh tissue should be collected and shipped overnight on ice for virus isolation (test no. 4), fluorescent antibody testing (test no. 3), or PCR (test no 12).

Sporadic abortions, calf pneumonias, poor reproductive performance

These types of problems are suggestive of a chronic BVDV problem, but no convincing evidence is available. In this situation, the BVDV SN (test no. 5) is the test of choice to determine whether the virus is in the herd. A BVDV SN test on a single sample from 6-10 animals, which have not been vaccinated for BVD (request type 1 and type 2 SN), or on animals with defined vaccination histories can be used to detect the presence of BVDV in a herd. In addition, appropriate diagnostic testing on sick animals and fetuses should be done. Once evidence is obtained for the presence of BVDV in the herd, then other tests can be done to manage and monitor the problem, such as bulk milk tests and individual animal tests.

IDENTIFYING PERSISTENTLY INFECTED ANIMALS

Six month old poor doing calf with no acute signs

A serum virus isolation test (tests no. 2 and no. 6) or an Ace test (test no 9) are the best tests for the determination of a PI animal of this age. A whole blood virus isolation (no. 7), PCR (no 12) or IP test on skin (no. 1) are also acceptable in this circumstance.

Eight month old bull going to an AI center

The serum virus isolation test (no. 2 and no. 6) is adequate to detect PI animals in this age group. It should always be used when the consequences of a false negative test result are substantial. Whole blood virus isolation (test no 8) or PCR (test no 12) can also be used but at higher cost.

Firm evidence of BVDV in herd with possible persistently infected animals

While one would not go wrong using a serum virus isolation test, the economics of the situation dictate that the serum or skin ACE tests (tests no 9 & 10) or pooled PCR test (no 11) be used. If animals are under 3 months of age, then the serum ACE test is inappropriate because of potential interference by colostral antibodies.

CONTROL OF BVDV

Vaccination to control BVDV-induced disease and production loss

In addition to removal of PI reservoirs, it is theorized that BVDV transmission to and within the herd can be reduced with an appropriate vaccination program. To date, using information from *in vitro* studies and limited field trials, one can only make empirical recommendations regarding what constitutes an effective vaccination program to limit postnatal and gestational BVDV transmission.

In vitro evidence of vaccine effects

In vitro 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 days between doses) of an inactivated BVDV vaccine or a modified live, temperature sensitive BVDV vaccine, serum from each animal was capable of neutralizing a wide range of antigenically diverse European and American isolates of BVDV, including genotypes I and II[13,14]. Other work has shown that administration of a single dose of a modified live vaccine against BVDV 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 BVDV[15,16].

Colostral immunity and vaccination of young calves

Adequate intake of colostrum from BVDV seropositive dams can provide protection from clinical disease in young calves[15,16,17]. BVDV vaccination of young calves has also been demonstrated to reduce clinical disease and mortality compared to colostrum-deprived, unvaccinated calves when experimentally challenged[15,16]. Calves that did or did not receive colostral antibodies to BVDV that were vaccinated with a single dose of MLV vaccine that contained type-I BVDV isolate at 10 to 14 days of age were protected from clinical

disease when experimentally challenged with a virulent type-II BVDV 21 days after vaccination. In contrast, calves that did not receive colostral antibodies to BVDV and did not receive the MLV vaccine suffered severe clinical disease[15,16]. Clinical scores were not significantly different between seropositive-vaccinated and seropositive-unvaccinated calves after viral inoculation. Most of the vaccinated calves that were seronegative prior to vaccination did not have measurable serum antibody response 21 days following vaccination at the time of experimental BVDV inoculation, even though these calves were protected from clinical disease[15,16]. Similarly, Ridpath et. al. demonstrated that an active protective response was mounted in young calves in the presence of colostral-derived passive immunity that were experimentally challenged with virulent BVDV even though serum antibody titers had decayed to low levels[17]. Cortese et al., and Ridpath et al, point out that serum antibody titers are an inadequate measure of vaccine or natural protection[15,16,17].

Ability of vaccines to provide fetal protection

The benefit of preventing clinical disease in vaccinated cattle exposed to BVDV is inadequate in the management and complete control of the disease, since infection is perpetuated from one generation to the next through infection of the fetus. Cowherd vaccination programs are primarily designed to prevent fetal infection, which is immunologically more difficult than protection from clinical disease[18]. In order to prevent fetal infection, vaccination of an exposed herd would have to prime the immune system to effectively neutralize circulating virus before it can cross the placenta and cause fetal infection. Evidence from earlier work 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[18]. Efficacy of maternal vaccination to provide fetal protection when the dams were experimentally challenged has been reported to range from 25% to 100% for inactivated vaccines[19,20,21] and from 58% to 92% for modified live vaccines[22,23,24]. Dams have measurable levels of anti-BVDV antibody following vaccination and fetal protection appears to be improved by vaccination, making a planned vaccination program important for BVDV control. However, a sufficient amount of virus is able to escape inactivation by circulating antibodies in some dams to cause transplacental infection, abortion, and the development of persistent fetal infection, making vaccination programs inadequate to control BVDV by themselves[21,22,16].

Control programs to limit losses due to BVDV

The primary goals of BVDV control in breeding herds are to prevent fetal infection in order to eliminate BVDV-associated reproductive losses (thereby preventing the birth of PI calves) and to reduce losses from transient BVDV infections[25]. Cattle that have been infected with BVDV after birth and recovered appear to be protected from clinical disease following subsequent exposure to the virus even if they are seronegative[17]. Seropositive animals due to natural exposure are also considered to have a degree of protection from future fetal transmission of the virus, but the protection may not be complete. While vaccination does provide some protection from fetal infection, the herd level protection is not likely to be complete. As a result, BVDV control is generally achieved by a combination of removal of PI cattle, vaccination, and a bio-security system that prevents the introduction of PI animals into the herd and minimizes the contact with potentially viremic cattle[26].

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. A second whole-herd screening the following year may be advisable in some herds where risk of continued fence-line or other exposure to PI animals is high.

Bio-security to prevent herd exposure to PI animals

Bio-security 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 BVDV shedders, the percentage of naturally protected seropositive animals in a herd decreases[26]. 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[27]. If a pregnant animal is purchased, it should be segregated from the breeding herd until both the dam and the calf are 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 bio-security and vaccination program in place.

Vaccination as a component of bio-security

Bio-security also involves application of a vaccination protocol to reduce the risk of fetal infection in the event of cowherd exposure to a viremic and shedding animal. Modified live vaccines (MLV's) have inherent properties that may enable them to stimulate more complete protection against transplacental infection[26]. 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 PI calves. In heifers not previously vaccinated, the primary series should consist of two administrations. The first dose should be given when the heifers are six 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[26].

SUMMARY

Bovine Viral Diarrhea Virus has important characteristics such as its genetic diversity and ability to induce a persistently infected carrier state that makes its control in cattle populations a challenge. A systematic control program that utilizes diagnostic testing strategies to find and remove PI cattle, vaccination to increase fetal protection from infection, and bio-security to reduce the risk of exposure to animals persistently (or transiently) infected with the virus is necessary for control of BVD.

REFERENCES

1. Wengler G., Bradley D.W., Collett M.S., Heinz F.X.,Schlesinger R.W. & Strauss J.H. Flaviviridae In Virus taxonomy (F.A. Murphy, CM. Fauquet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G.P. Martelli, M.A. Mayo & M.D. Summers, eds). Sixth Report of the International Committee on Taxonomy of Viruses. Springer-Verlag, Vienna, 415-427, (1995).
2. Thiel H.J., Plagemann P.G.W. & Moennig V. Pestiviruses. In Fields Virology, 3rd Ed., Vol. 1. (B.N. Fields,D.M. Knipe & P.M. Howley, eds). Lippincott-Raven, Philadelphia, 1059-1073, (1996).
3. Ernst P.B., Baird J.D. & Butler D.G. Bovine viral diarrhoea: an update. Compend. cont. Educ. pract. Vet, 5, S581-S589, (1983)

4. Harkness J.W.; Sands J.J. & Richards M.S. Serological studies of mucosal disease virus in England and Wales. Res. vet. Sci., 24, 98-103, (1978).
5. Canal C.W., Strasser M., Hertig C, Masuda A. & Peterhans E. Detection of antibodies to bovine viral diarrhoea virus and characterisation of genomes of BVDV from Brazil. Vet. Microbiol., 63 (2-4), 85-97, (1998).
6. Muvavarirwa P., Mudenge D., Moyo D. & Jawangwe S. Detection of bovine virus diarrhoea virus antibodies in cattle with an enzyme-linked immunosorbent assay Onderstepoort J. vet Res., 62 (4), 241-244, (1995).
7. K.J. Sudharshana, K.B. Suresh & M. Rajasekhar. Prevalence of bovine viral diarrhoea virus antibodies in India. Rev. sci. tech. Off. int. Epiz., **18** (3), 667-671, (1999).
8. Ames T.R. The causative agent of BVD: its epidemiology and pathogenesis. Vet. Med., 81, 848-869, (1986).
9. Sandvik T. Laboratory diagnostic investigations for bovine viral diarrhoea virus infections in cattle. J Vet Diagn Invest. Sep;18(5):427-36, (2006).
10. Bolin S.R., Ridpath J.F., Black J., Macy M. & Roblin R. Survey of cell lines in the American Type Culture Collection for bovine viral diarrhea virus. J. Virol. Methods, 48, 211– 221, (1994).
11. Edwards S. Bovine viral diarrhea virus in Cell & Tissue Culture: Laboratory Procedures, Doyle A., Griffiths J.B. & Newell D.G., eds. John Wiley & Sons, Chichester,
UK. Module 7B:5, 1–8, (1993).
12. Fulton RW, Hessman BE, Ridpath JF, Johnson BJ, Burge LJ, Kapil S, Braziel B, Kautz K, Reck A. Multiple diagnostic tests to identify cattle with Bovine viral diarrhea virus and duration of positive test results in persistently infected cattle. Canadian Journal of Veterinary Research. Apr;73(2):117-24, (2009).
13. 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* 47:721-726, (2000).
14. Hamers C, di Valentin E, Lecomte C. *et al*: Virus neutralizing antibodies against 22 bovine viral diarrhoea virus isolates in vaccinated calves. Vet J 163:61-67, (2002).
15. Cortese VS, Whittaker R, Ellis J, *et al*: Specificity and duration of neutralizing antibodies induced in healthy cattle after administration of a modified-live virus vaccine against bovine viral diarrhea. Am J Vet Res 59:848-850, (1998).
16. Cortese VS, West KH, Hassard LE, *et al*: 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 213:1312-1319, (1998).
17. Ridpath JF, Neill JD, Endsley J, *et al*: Effect of passive immunity on the development of a protective immune response against bovine viral diarrhea virus in calves. Am J Vet Res 64:65-69, (2003).
18. R.L. Larson, D.M. Grotelueschen, K.V. Brock, B.D. Hunsaker, *et al.* Bovine Viral Diarrhea (BVD): Review for Beef Cattle Veterinarians. Bov Pract 38:93-102, (2004).
19. Harkness JW, Roeder PL, Drew T, Wood L, Jeffrey M. Pestivirus Infection of Ruminants. Ed J.W. Harkness. Brussels, CEC. p. 233, (1987).
20. Meyling A, Ronsholt L, Dalsgaard K, Jensen AM. Pestivirus Infection of Ruminants. Ed J.W. Harkness. Brussels, CEC. p. 225, (1987).
21. Brownlie J, Clarke MC, Hooper LB, *et al*: Protection of the bovine fetus from bovine viral diarrhea virus by means of a new inactivated vaccine. Vet Rec, 137:58-62, (1995).

22. Brock KV, Cortese VS: Experimental fetal challenge using type II bovine viral diarrhea virus in cattle vaccinated with modified-live vaccine. Veterinary Therapeutics, 2:354-360, (2001).
23. Cortese VS, Grooms DL, Ellis J, *et al*: 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, 59:1409-1413, (1998).
24. Dean HJ, Hunsaker BD, Bailey DO, *et al*: Prevention of persistent infection in calves by vaccination of dams with noncytopathic type-1 modified-live bovine viral diarrhea virus prior to breeding. Am J Vet Res, 64:530-537, (2003).
25. Harkness JW: The control of bovine viral diarrhea virus infection. Ann Rech Vet, 18:167-174, (1987).
26. Kelling CL: Planning bovine viral diarrhea virus vaccination programs. Vet Med, 91:873-877, (1996).
27. Paton D.J., Christiansen K.H., Alenius S., Cranwell M.P.,Pritchard G.C. & Drew T.W. Prevalence of antibodies to bovine virus diarrhoea virus and other viruses in bulk tank milk in England and Wales. Vet. Rec, 142 (15),385-391, (1998).