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Original Contribution

COMPARATIVE EVALUATION OF TWO TESTS TO DETERMINED ANTIBODIES AGAINST MUCOSAL DISEASE-VIRAL DIARRHEA

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ABSTRACT
Bovine viral diarrhea virus (BVDV) is one of the most important viral pathogens of cattle worldwide. Because of the insidious and complex nature of BVDV, laboratory diagnosis is critical in preventing and controlling BVDV infections. These same characteristics often make laboratory diagnosis challenging. A firm understanding of the disease is required to select the appropriate diagnostic strategies and samples for diagnostic submission and then make sound interpretations of the results.
This study included comparative analysis of 74 sera samples from non vaccinated dairy cattle by virus neutrilization test (VN) and ELISA method. After different dilutions of samples, virus neutrilization test is the more sensitive test compared to ELISA, which reveal 64 positive results, while ELISA only 60 positive samples out from 74 samples.VN more sensitive in about 6.25 % than ELISA in demonstration of BVDV antibodiesin serum.In the high diluted samples, the ELISA failed to reached positive reaction, which missed four samples give rised fausle negative results.

Key word: Cattle, BVDV, VN, ELISA, Comparative

INTRODUCTION

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 (1 - 3). Two antigenically distinct genotypes of BVDV exist, types 1 and 2, and two subgenotypes (a and b). BVDV of both genotypes may occur in noncytopathogenic and cytopathogenic forms (biotypes), classified according to whether or not it produces visible change in cell cultures. Usually, it is the noncytopathogenic biotype that circulates in cattle populations. Each biotype has a specific role in a variety of clinical syndromes – acute, congenital and chronic infections (4, 5).

BVDV have four major structural antigenic polypeptides (6, 7). The capid protein (C) does not elicit an antibody response in cattle.

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Glycoprotein E^{rns} causes production of significant levels of antibodies in animals. Antibodies to E^{rns} have limited neutralizing activity. Glycoprotein E1is covalently linked to E2. Convalescent cattle serum does not contain significant levels of antibody to E1. Glycoprotein E2 is antigenic targed for antibodies. E2 is highly antigenic the production of neutralizing antibodies in the host after infection. Nonstructural protein 23 has two separate polipeptides – NS2 and NS3. Protein NS3 is marker of citopatic BVDV and is the most conserved protein in the pestivirus family. This polypeptide is very stable in infected cells and highly immunogenic.

The ability of BVDV antibodies to protect (neutralizing) against BVDV infection is dependent on the virus strain, level and isotype of antibody produced. An indirect measure of virus infection is the detection of virus-specific antibodies in the sera of animals. Many tests are available for the detection of anti-BVDV antibodies. —virus-neutralization (VN), indirect immunofluorescence assay, indirect immunoperoxidase, and ELISA tests (8, 9).

The most commonly use are ELISA tests and VN. Several antibody ELISA tests were developed in 1990 (7, 10 - 12) and standard VN test were used. Control positive and negative standard sera must be included in every test. These should give results within predetermined limits for the test to be considered valid.

The virus neutralization, also known as serumneutralization (SN), is considered to be the gold standard test for the detection of anti-BVDV antibodies and is used worldwide (13).

In most situations, citopatic strains of BVDV are used in the test so that the presence of neutralizing antibodies can be detected by inhibition of viral infectivity as detected by the absence of viral cytopathology. Cross-neutralization tests can be used to characterize antigenic differences among pestiviruses (7, 8).

A number of commercial enzyme-linked immunosorbent assay (ELISA) kits have been developed for the detection of anti-BVDV antibodies in serum samples. The antigens used in ELISA tests include whole virus antigen, nonstructural protein, monoclonal antibodies, and peptides. Several factors can influence the results of an ELISA test—antigen, conjugated antibody, test sample, etc. (7, 14 - 17).

The procedure used to prepare whole virus antigen can also affect the specificity and sensitivity of the ELISA test. For example, Pilinkiene et al. (1999) found that antigens prepared by mild treatment showed the most specificity and activity. Cho et al. (1991) prepared antigen from MDBK-grown BVDV. The antigen was solubilized with MEGA-10 (decanonyl-N-methylglucamide) followed by the removal of hydrophobic proteins with Triton X-100 treatment. Compared to VN, this test was 100% specific and 97% sensitive. Moennig et al. (1991) described the development of an ELISA using the nonstructural protein p125/80 of BVDV as antigen. The results were comparable to those obtained by the VN test.

The specificity of serodiagnosis has been enhanced greatly by the use of monoclonal antibody in competitive ELISA systems. Competitive (blocking) enzyme-linked immunosorbent assays for antibodies against BVDV were satisfied with NS3 monoclonal antibody (10, 19). Paton et al. (1991) uses two NS3 monoclonal antibodies reactive to 157 different pestiviruses development of a blocking ELISA. They examined the ability of analysis for antibodies against pestiviruses in cattle, sheep and pigs. The relative sensitivity of the blocking ELISA compared with VN was higher for cattle and sheep sera (94.7% and 99.1%, respectively) but lower for pigs (76%), whereas the specificity is high in each case (96%).

Several tests capture ELISA for detection of NS2-3 protein in infected animals have been continuously developed and marketed (20). In NS3/NS2-3 proteins are highly immunogenic and NS2-3 protein is produced in large quantities constant in infected animals.

Beaudeau et al. (2001) developed a blocking ELISA using monoclonal antibody against NS2- 3 and used this test for mass screening of milk and serum samples with sensitivity and specificity of approximately 97% as compared to VN.

Langedijk et al. (2001) developed a solid phase antibody ELISA using peptides deduced from the C terminal end (residues 191–227) of pestivirus envelope protein E ^{rns}. This ELISA was cross-reactive for several types of pestiviruses and could be used for general detection of pestivirus antibodies. To detect type-specific antibody, a liquid phase ELISA using a labeled specific CSFV peptide, and an unlabeled BVDV peptide (to block cross-reactivity) was used. This test can potentially be used for the differentiation of vaccinated animals from infected ones if vaccination is based on another envelope protein (E2).

A single serum dilution can be used in an ELISA test to quantitate antibodies. Graham et al. (1997) standardized a commercial ELISA test for detection of serum antibodies to BVDV so that a single serum dilution could be tested and the results expressed quantitatively using a standard curve. Various dilutions of known sera were tested and their endpoint titers calculated by an algebraic method directly from a plot of each titration series and also from a regression line fitted to this plot.

MATERIAL AND METODS

1. Sera. We received a total of 74 blood samples from the number of cows and heifers from two farms with spontaneous infection.

2. Enzyme-linked immunosorbent assay (ELISA). We used a kit (Bio-X BVDV ELISA

Kit, Herd Laboratories) for detection of antibodies in blood sera. Test is constructed based on monoclonal antibodies specific to NS3 protein.

3. Virus neutralisation test (VN). We used the method described in the OIE Terrestrial Manual 2008, Chapter 2.4.8, Bovine viral diarrhea, 704 (1) with some differences:

a. We used isolated cytopathic strain of BVDV Kableshkovo one serotype of infectious titer 104 EID50/0.1 ml.

b. We used stable cell line fetal cow trachea (FTr).

c. Twofold dilutions of sera starting at 1:2, equivalent to a final dilution 1:4. For the performance included a fetal calf serum (FTS, cat. № DE14-801E, lot № 4SB 000502/2010, Cambrex) and polivinil hlopidni plates with 96 outlet - Limbro

4. Statistical methods

The comparative results for sensitivity, specificity, coverage of diagnostic methods performed by Courtney et al. (1990). Compare the results of serological tests (MFA and ELISA). Determination:

- Sensitivity (Se): the frequency of positive samples obtained from individual tests on all samples with positive result (of the two tests).

$$Se\ (test\ A) = \frac{\text{positive result test A}}{\text{all positive}},$$

$$Se\ (test\ Б) = \frac{\text{positive result test B}}{\text{all positive}}$$

False negative results (FN): the frequency of negative samples in one test showed positive result for the second test.

$$FN\ (test\ A) = \frac{\text{negative result test A}}{\text{positive result test B}},$$

$$FN\ (test\ B) = \frac{\text{negative result test B}}{\text{positive result test A}}$$

- coverage of the tests: number of cases with identical results on two tests to the total number of analyzed samples

$$\text{Coverage of the tests} = \frac{\text{positive result test A and B + negative result test A and B}}{\text{All tested samples}}$$

RESULTS

Of all the tested serum positive for antibodies against non-structural proteins - NP3 virus mucosal disease-viral diarrhea (BVDV) detected by ELISA in 60 units (81 %) - **Table 1.** Positive for antibodies by VN against the mucosal disease-viral diarrhea (BVDV) found in 64 sera number - 86.5 percent.

Table 1. *Comparative results of the ELISA and VN at the titers of VN*

Virus neutralization test			ELISA results	
Titres (final dilution)	Negative	Positive	Negative	Positive
<1:4	10	0	10	0
1:4	0	3	3	0
1:8	0	19	1	18
1:16	0	20	0	20
1:32	0	8	0	8
1:64	0	7	0	7
1:128	0	5	0	5
1:256	0	2	0	2

Difference found in 4 sera (5.4% of all sera). They were negative by ELISA and positive by VN. ELISA was negative in samples with low titers in VN - 1:4 (3 pieces of 3 pieces – 100 %) and 1:4 (1 of 13 samples - 7.7 %).

Comparative data between the two tests are presented in **Table 2**. In response to VN 100% for the ELISA it was 93.75 percent. False negative results found for the ELISA at 6.25% of positive samples. Coverage of the tests found in 94.6%.

Table 2. *Possibilities of ELISA test compared with VN for detection of antibodies in blood sera of cows and heifers from two farms failures*

Test	Positive samples (n=64)	Sensitivity (Se) False negative (FN)
VN +	64	Frequency of positive results: 100 %
VN -	0	False negative results (FN): 0 %
ELISA +	60	Frequency of positive results: 93.75 %
ELISA -	4	False negative results (FN): 6.25 %

DISCUTION

The sensitivity of a given test is expressed as a percentage of positive animals to the test divided to really all infected animals, for example sensitvity of ELISA to bovine leukemia virus is 98% that mean positive samples 98 while faulse negative are only 2.

The VN test more sensitive than ELISA because and consider gold standard in diagnosis but it is take time, and more expensive.

ELISA method need a properiate titer and concentration of antibodies to give the positive result or it will lead to faulse negative diagnosis (23). For this resons must be wait three weeks intervals between taking samples from diseased animals if we need to diagnostic by ELISA to get high titer of antibodies to increase snsitivity of test. Obviously that low titer of Abs. give faulse negative as occure in our study four samples positive in VN test and negative in ELISA test.

There are many researches reveal that VN is best method, in one serological evaluation of precolostral serum samples to detect BVDV in large commercial dairy herds demonstrated that 7.4% by VN test while by ELISA test the value was 6.2% (24).

In study on alpaca animals infected with BVDV,ELISA was a moderately sensitive test and but, the benificial value that results can be obtained in a rapid fashion (internet).

From all above researches discussed, that agreed with our results. In the low diluted samples VN and ELISA method revealed same positive and negative values, but in high dilution sample ELISA gave faulse negative while VN test gave positive values, that make conclusion from our study VN method was more senitive in diagnosis of BVDV, but it need long time and moderately high expensive.

Virus neutralizing antibodies usually appear 3–4 weeks after infection and persist for years. Titers induced by vaccination may also persist for a long time (22).

Passive antibodies decline at 105–230 days (but may persist for more than a year).

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