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Full Article

Prevalence of Bovine Viral Diarrhoea Virus antibodies and antigen among the aborted cows in industrial dairy cattle herds in Mashhad area of Iran

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ABSTRACT

The measurement of antibody responses of animals exposed to BVDV either through a natural exposure or an immunization protocol is still a standard procedure. For BVDV, the test formats have been largely limited to ELISA which is a valuable diagnostic test to measure the level of BVDV specific antibodies as well as antigen in blood samples. In the present study, 120 blood samples were collected from the cows with the history of abortion in different period of pregnancy from different industrial dairy cattle herds of Mashhad area of Iran. Also 30 samples were collected from the cows with no history of abortion as control. The presence of antibody against BVDV from the 120 serum samples was investigated by indirect ELISA. From 120 serum samples which were collected from aborted cows, 89 samples were positive (%74.16). From these positive samples, 12(13.48%), 54 (60.68%) and 23 (25.84%) samples belong to the first, second and third trimester of pregnancy, respectively. From 89 positive samples, 12 (13.48%) samples were related to stillbirth and 8 (8.99%) samples were belongs to the mummified fetus. From 89 positive samples, 71 (79.78%) were related to cattle between 2-5 years old and 18 (20.22%) were associated to cattle more than 5 years old. In control group, 20 samples (66.66%) were antibody positive. Also the presence of BVDV antigen in serum samples was investigated by Ag-capture ELISA. From 120 serum samples, 2 samples were positive (1.67%), which belongs to the second period of pregnancy. In control group, none of the samples were antigen positive. The results of this study showed that the prevalence of BVDV infection is high among the aborted cows of Mashhad area. Although this prevalence is higher than the control group, the observed difference is not significant.

Keywords: Bovine virus diarrhoea virus, ELISA, antibody, antigen, abortion

INTRODUCTION

Bovine viral diarrhoea virus (BVDV) infection is a worldwide distributed animal disease characterized by bovine reproductive disorders that can severely affect the developing embryo and fetus (Talebkhan Garoussi 2007). BVDV belongs to the *Pestivirus* genus in the *Flaviviridae* family (Pringle 1999). BVDV infection is listed as a group-B disease (milder infectious disease) on the list of noticeable animal diseases (Valle *et al*

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2001). The disease was first described by Olafson et al (1946) and diagnostic tests for individual animals and test strategies for cattle populations have evolved considerably. Transiently infected animals with high, long-lasting antibody levels and persistently infected (PI) animals with high levels of viremia have provided important targets for diagnostic efforts (Houe *et al* 2006). The accuracy of available diagnostic tests is crucial for the success of a control program. For BVDV infections, several diagnostic tests, aiming either to detect the virus itself or to detect viral-specific antibodies, are available. In general, the analytical sensitivity and specificity of these tests are high (Goyal 2005). The measurement of antibody responses of animals exposed to BVDV either through a natural exposure or an immunization protocol is still a standard procedure. Among different serological assays that have been used for BVD over the years, the most commonly used antibody detection techniques are the virus neutralization test (VNT) and ELISAs. VNT is a labor-intensive and also expensive test (Sandvik 2005). As an alternative to the VNT, indirect and blocking ELISAs are commonly used (Schrijver and Kramps.1998). ELIS As have many advantages over the VNT and for BVDV, the test formats have been largely limited to ELISA which is a valuable diagnostic test to measure the level of BVDV specific antibodies as well as antigen in blood samples. In general, ELISAs have proven to perform well in practical use, with sensitivity and specificity values between 95 and 100% (Sandvik & Krogsrud 1995, Brinkhof *et al* 1996).

Bovine viral-diarrhoea virus (BVDV), endemic in most cattle-raising countries, also causes reproductive failure. Infections during pregnancy can result in embryonic death, abortions, birth of stillborn or weak calves, or can lead to birth of persistently infected (PI) calves that will shed virus throughout their lifetime (Grooms 2004). Infection with BVDV is generally subclinical, however, when a dam is infected with BVDV during pregnancy, transplacental infection may occur, and as a result, fetal abortion, mummification or congenital defects may occur depending on the gestation stage (Kozasa *et al* 2005). More importantly, infection in the first trimester of pregnancy can result into the birth of immunotolerant calves that are persistently infected (PI) with BVDV. The PI animals are a major source of virus spread and thus, it is very important to identify and remove them from the cattle herd (Lindberg 2003). In general, PI cattle show varied clinical manifestations such as diarrhea, pneumonia (as a result of immunosuppression), poor growth, some succumb to mucosal disease, and some PI cattle indicate no clinical manifestations. The PI cattle on dairy farms are suspected as the cause of milk production loss and/or increase in occurrence of secondary or opportunistic infections (Baker 1995, Chi *et al* 2002, Kelling *et al* 2002). Although detecting animals carrying virus is essential for identification and removal of PI animals from an infected herd, screening herds for antibody carriers is also important to identify PI animals (usually seronegative) and to determine the herd's infection status and susceptibility (Mainar-Jaime *et al* 2001**.** Seroprevalence in non-vaccinated herds differs among areas or countries, ranging between 20 and 90% (Alenius *et al* 1986, Loken *et al* 1991). Area differences could in part be explained by factors such as cattle density, herd size or livestock trade (Houe *et al* 1995).

Mashhad is the capital of north-eastern province of Iran with high agricultural economic values. Previous studies in this region have shown a high prevalence of BVDV infection among industrial dairy cattle herds (Talebkhan Garoussi *et al* 2008 & 2009). However, none of the previous studies have addressed the potential risk factors contributing to BVDV infection in this region. Abortion is a major problem in herds of Mashhad area and so far, no study has been undertaken to show any relation between BVDV infections in aborted cows in Iran. Therefore, the purpose of the present study was to evaluate the prevalence rate of BVDV infection among cows with the history of abortion in Mashhad area of Iran using ELISA technique and to determine any association between the rate of BVDV infection and abortion.

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MATERIALS AND METHODS

Collecting sera. In total, 120 Holstein aborted cows blood samples plus 30 blood samples from cows with no abortion history as control were obtained from different industrial dairy cattle herds in Mashhad area of Iran. The samples were centrifuged at 2000 × g at room temperature for five min to separate sera. Sera were stored at -20ºC until used.

Indirect ELISA. The assay was performed by Bovine Viral Diarrhoea Virus (BVDV) Antibody Test kit manufactured by IDEXX (HerdChek, IDEXX Laboratories, Westbrook, ME, USA), in a 96-well micro titration plates which were coated with BVDV antigen. The sensitivity and specificity of the test as manufacture instruction were mentioned 96.3% and 99.5%, respectively. The serum samples were diluted (1:1) by wash solution. One-hundred µl of sera was loaded into wells and incubated for 90 minutes at room temperature. Positive and negative control sera were used as indicated in the kit. The wells were washed five times with 300 µl of wash solution. Following the final washing, the plate slapped vigorously, well down on a bench top which covered with paper towels. Then, 100 µl of anti-bovine HRP conjugated was loaded into all the wells and incubated for 30 minutes at room temperature. The plate was washed as described above to remove the excess conjugate. For colour development, 100 µl of TMB was added to each well as a substrate and incubated for 10 minutes at room temperature at darkness. The reaction was terminated by the addition of 100 µl of stop solution to each well. The absorbance at 450 nm was monitored in ELISA reader.

Detection of BVDV E^{rns} antigen **by antigen-capture ELISA**. All samples were tested using commercial BVDV Antigen Test Kit /Serum Plus (HerdChek, IDEXX Laboratories, Westbrook, ME, USA), in which microtitre plates were coated with anti- E^{rns} monoclonal antibodies. The kit is based on the detection of the E^{rns} (gp44-48) glycoprotein of the BVD virus. The sensitivity and specificity of the test as manufacture instruction were mentioned 100% and 100%, respectively. The serum samples were diluted (1:1) by wash solution. Fifty µl of sera was loaded into wells and incubated for 2 hours at 37º C. The rest of the test was followed as mentioned in serum antibody assay and finally the absorbance at 450 nm was monitored in ELISA reader.

Calculation and statistical analysis. The result could be read visually where the OD was measured at 450 nm. Calculations for test samples were analyzed as follow for BVDV antibody:

The presence or absence of BVDV antibodies in the sample is determined by S/P ratio for each sample.

$$S/P = \frac{\text{Sample A450 - NC}\bar{x}\text{ A450}}{\text{PC}\bar{x}\text{ A450 - NC}\bar{x}\text{ A450}}$$

$PC\bar{x}$ and $NC\bar{x}$ represent positive and negative control mean respectively. According to manufacture instructions, samples with S/P values less than 0.2 were classified as negative and samples with S/P values equal or greater than 0.3 were classified as positive for BVDV antibody. For BVDV antigen, the presence or absence of BVDV antigen in the sample is determined by the corrected OD value (S-N) for each sample as follow: S-N= Samples A450 - $NC\bar{x}$

Samples with S-N values less or equal to 0.3 were classified as negative and samples with S-N values higher than 0.3 were classified as positive for BVDV antigen.

Statistical analysis. Proportion of seropositivity was compared between aborted and healthy cows using Chi-square test.

RESULTS

For antibody detection, 89 (74.17%) out of 120 serum samples were interpreted BVDV seropositive (S/P≥0.3) (Table 1). From these positive samples, 12(13.48%), 54 (60.68%) and 23 (25.84%) samples were associated to the first, second and third trimester of pregnancy, respectively (Table 1). 31 (25.83%) of serum samples of aborted cows had S/P <0.2 values and were interpreted BVDV seronegative. In control

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group, 20 samples (66.66%) were antibody positive and 10 samples(33.34%)were negative (Table 1).

Table 1. The numbers of positive samples to BVDV antibody in first, second and third trimester of pregnancy and also in control group.

			Distribution of Positive cases in 3 Trimesters		
Total number of samples	Positive samples	Negative samples	First trimester	Second trimester	Third trimester
Aborted cows (120)	89 (74.17%)	31 (25.83%)	12(13.48%)	54 (60.68%)	23 (25.84%)
Control cows (30)	20 (66.66%)	10 (33.34%)	-------	-------	--------

The observed difference between aborted cows and control group is not significant (P=0.41). From 89 seropositive samples in aborted cows, 12 (13.48 %) samples were related to stillbirth. From these, 2 (16.67%), 7 (58.33%) and 3(25%) samples were related to first, second and third trimester of pregnancy. Also, 8 (8.99%) samples associated with mummified fetus. From these samples, 3 (37.50%), 3 (37.50%) and 2 (25%) were associated to the first, second and third trimester of pregnancy (Table 2).

Table 2. Distribution of stillbirth and mummified fetus in 3 trimester of pregnancy.

Fetus characteristic	Total n. of positive samples	First trimester	Second trimester	Third trimester
Stillbirth	12 (14.28%)	2 (16.67%)	7 (58.33%)	3 (25%)
Mummified	8 (8.99%)	3 (37.50%)	3 (37.50%)	2 (25%)

From 89 positive samples, 71 (79.78%) were related to cattle between 2-5 years old and 18 (20.22%) were associated to cattle more than 5 years old. Also the presence of BVD antigen in serum samples was investigated by ELISA. From 120 sera samples, 2 samples were positive (1.67%), which were belongs to the second period of pregnancy and in control group, none of the samples were antigen positive (Table 3).

DISCUSSION

In this study we showed the prevalence of BVDV infection among cows with the history of abortion in industrial dairy cattle herds of Mashhad area of Iran. Our results showed that BVDV infection present widely (74.17%) in aborted cows in these herds. The rate of seropositive cows was also high in the control group (66.66%), but lower than the cows with no history of abortion. However the difference was not significant. Therefore we can not conclude that the abortion is a direct consequence of BVDV infection in the herds studied. Although no significant association was found between BVDV infection and abortion, BVDV infection could be related to other reproductive parameters (such as infertility or embryonic death) which were not studied in this research. Other recent studies in Mashhad area of Iran have shown that the BVDV seroprevalence is 72.25% (Talebkhan Garoussi *et al* 2009). All of the herds in this study were antibody positive against BVDV and the prevalence ranged from 66 to 100% within the herds of Mashhad area of Iran.

Table 3. Distribution BVDV antigen in second trimester of pregnancyin 2 aborted cases.

Total number of samples	Positive samples	Negative samples	First trimester	Second trimester	Third trimester
Aborted cows (120)	2 (1.67%)	118 (98.33%)	0	2 (1.67 %)	0
Control cows (30)	-------	30 (100%)	-------	-------	--------

Our results are in agreement with this study and since vaccination against BVDV is not practiced in the cattle herds of Iran, serological response reflected natural infection. Most probably, these herds have had a recent or an ongoing infection most likely due to the presence of PI animal(s) (Houe & Meyling, 1991). BVDV infection can cause abortion at any time during gestation, but only in dams not previously exposed to the infection (Grooms 2004). Thus, in our study, BVDV seroprevalence of 74.17% among cows with the history of abortion reflects previous exposure to BVDV infection. Our data showed that seroprevalence of 66.66% in control group with no history of abortion. Therefore, we could not attribute this slight difference between aborted and control group to BVDV infection.

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Bovine viral diarrhea virus contributed significantly and substantially to economic loss of dairy herds in many parts of the world and associated to increased abortion rates, extended calving-to-conception intervals, and reduced milk production (Heuer *et al* 2007). Therefore, it must be studied more for the prevalence and different epidemiological aspects and risk factors of BVDV in Mashhad as an important pole of dairy production in Iran. Research studies based on the BVDV antibodies detection, either in individual animals or bulk milk, have shown that the prevalence of infected herds ranged 70% to 100% in many parts of the world (Edwards *et al* 1987, Reinhardt *et al* 1990, Niskanen *et al* 1993, Obando *et al* 1999). It was shown that the herds with high cattle population density had higher prevalence of infection than the herds which were smaller (Loken *et al* 1991). In samples from PI calves, BVDV-specific maternal antibodies may block viral infectivity or detection of viral antigens, usually up to an age of around three months (Palfi *et al* 1993), and this is why the percentage of positive samples in our antigen test is low. There is a clear relationship with the maternal antibody titre; the higher the average antibody titre, the lower the frequency of virus isolation test positives. Thus, in the presence of high levels of maternal antibodies, the virus isolation and the antigen ELISA tests were shown to be unreliable indicators of the presence of persistent infections with BVD virus. These findings confirm the results of the experiment published by Palfi *et al* (1993). Adherence of antibodies to the virus surface may also explain the false negative results using the antigen ELISA test in the presence of high levels of antibodies. In our study, only 2 out of 120 samples were BVDV antigen positive in aborted cows and at the same time BVDV antibody titers were high in these cows. To make most of the information obtained from laboratory diagnostic investigations, a thorough understanding of both the epidemiology of BVD as well as the performance of diagnostic tests is essential. For example, typical values for the sensitivity and specificity of an excellent Ag ELISA for BVDV may be 97% and 99%, respectively, which means that 3% of the PI animals in a population are not detected (Sandvik 1999). The Sandvik›s results, clearly showed that in a high proportion of the PI animals, the virus isolation test and the antigen ELISA test, when run at day 7 after the ingestion of colostrum, were both negative. Taken together our results clearly demonstrate a high prevalence rate of BVDV infection in dairy cattle herds with the history of abortion in Mashhad area of Iran. The results presented in this study confirm previous reports of high incidence of BVDV infection in this region of Iran. Therefore preventive measures should be taken in consideration in order to control the level of infection and subsequently reduce the economic impact of BVDV infection.

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