Prevalence of antibodies to Bovine Viral Diarrhea Virus (BVDV) in blood and milk serum in dairy cattle in Kars district of Turkey

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Received: 24-11-2015 Accepted: 08-03-2016 DOI:10.18805/ijar.9497

ABSTRACT

The purpose of this study was to detect antibodies against Bovine Viral Diarrhea Virus (BVDV) in blood and milk serum samples. With this aim, a total of 192 blood and milk samples were collected from unvaccinated Holstein cows in Kars district of Turkey. Blood and milk serum samples were tested to determine the presence of antibodies against BVDV by commercial indirect enzyme-linked immunosorbent assay (ELISA). In this study, while 172 of blood serum (89.58%) were found to be positive for the presence of BVDV antibodies, 161 of milk serum samples (83.85%) were positive. In addition, 150 (78.12%) both blood and milk serum samples of same cattle were positive for BVDV antibodies. Only 22 (11.45%) blood serum of cattle was detected positive for BVDV, while only 11 (5.73%) milk serum was seropositive. Data obtained from the study showed the presence of BVDV infection in dairy herds in the Kars region and demonstrate that blood serum and milk serum samples might be consistent with one another in the determination of BVDV seroprevalence.

Key words: Blood, BVDV, ELISA, Milk, Seroprevalence.

Bovine viral diarrhea virus (BVDV) is a member of the genus Pestivirus, a group of small-enveloped, single stranded RNA viruses in the family Flaviviridae. The virus is classified into two main genotypes (BVDV-1 and BVDV-2), based on antigenic and genetic properties, with each genotype further divided into either cytopathic or non-cytopathic biotypes, based on how they replicate in cell culture (Ridpath, 2013). BVDV is a multi-system viral infection which causes significant economic losses and is characterized by clinical and pathological variations that range from reproductive disorders and congenital abnormalities to abortion, birth of calves with persistent infections, early embryo death and mummification (Zoth and Taboga, 2006; Reimann et al., 2007). Persistently infected (PI) animals continually shed a large amount of virus in their body fluids. Furthermore, PI animals are the single most important source of infection for other animals (Vilcek et al., 1997; Passler et al., 2007). Early detection and subsequent removal of PI animals is essential to a successful BVDV control programme (Moenning et al., 2005; Valle et al., 2005; Lindberg et al., 2006; Presi et al., 2011; Ridpath, 2012; Stähl and Alenius, 2012). Although “an” OIE-listed disease, necessary national control programmes for BVDV does not exist in many countries (Ackermann and Engels, 2006; Heffernan et al., 2009).

ELISAs are preferred in studies of strategies for controlling BVDV infection because of their sensitivity and fast that allows the screening of many samples. (Alvarez et al., 2012; Gonzalez et al., 2014). The use of milk serum samples provides advantages in research because they are easier to obtain and cause less stress on the animals (Beaudeau et al., 2001a). Besides, the presence of antibodies detected in tank milk samples with ELISA can demonstrate the existence of BVDV infection and distinguish between infected and uninfected herds (Beaudeau et al., 2001b). Tank milk results not only indicate the presence of infected animals in the herd, but it also shows that there might be PI calves in the herd, and the necessity of conducting individual testing on calves (Bitsch and Ronsholt, 1995).

This study was conducted to detect the antibodies against BVDV by ELISA in blood and milk serum samples from dairy cattle raised in dairy herds around Kars, which is in the Northeastern Anatolia Region and to determine whether or not milk serum samples could be used in place of blood samples.

Clinical Materials: The study material consisted of 192 Holstein cows not vaccinated for BVDV in 5 dairy cattle operations in the province of Kars. The blood samples (n=192) were collected from coccygeal vein in sterile tubes and taken to the laboratory. The samples were centrifuged at 3000 rpm for 20 min to separate the serum and then stored at -20°C until they are tested. A volume of 0.2 ml rennin and 0.1 ml saturated CaCl₂ were added to the 10 ml milk samples placed in sterile glass vials, which were then incubated for 1 hour at 37°C. At the end of the incubation period, they were centrifuged for 20 min. at 3000 rpm and then the layer of cream was removed. After obtaining the
serum, inactivation was achieved by incubating in a double-boiler for 30 minutes at 56°C. The milk serum samples were stored at 37°C until tested.

**Indirect ELISA:** Commercial ELISA kit (IDEXX, ELISA BVD/MD/BD p80, France) was used for the detection of antibodies against BVDV in blood and serum samples and the assay was carried out according to the manufacturer’s instructions. The non-structural protein p80/125 (p80) was coated onto the walls of 96-well microplates included in the test kit.

**BVD serum ELISA:** Briefly, 50 ml of test serum diluted at 1:2 in dilution buffer were added to wells and incubated for 1 hour at room temperature (+21°C ±5°C). Then 100 ml of monoclonal anti-p80 peroxidase conjugate diluted at 1:100 in dilution buffer were added to each well. Following incubation for 30 min at room temperature, unbound conjugate was removed by washing and 100 ml of enzyme substrate 3,3',5,5'-Tetramethylbenzidine (TMB) were added to the wells. After incubation at room temperature for 20 min, the enzymatic reaction was stopped by adding 100 ml of 0.5M H₂SO₄ solution.

**BVD milk ELISA:** Briefly, milk samples (100 µl) were added to each test well and incubated for 2 hour at room temperature. Anti-p80 Ab WB112 coupled to peroxidase (100 µl) was added to each well and incubated for 30 min at room temperature. After washing, 100 µl of the enzyme substrate 3,3',5,5'-Tetramethylbenzidine (TMB) was added to the wells and incubated for 20 min at room temperature. Then 100 µl of 0.5M H₂SO₄ solution was added to each well.

ELISA results were analyzed with an automated ELISA reader at 450 nm. For each tested sample, the percent inhibition (% inhib) was calculated by means of the following formulas:

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\text{% inhibition of sample} = \left( \frac{OD_{sample}}{OD_{neg}} \right) \times 100
\]

It was deemed negative if the % inhibit for blood serum sample was greater than or equal to 50%, suspected if it was between 40% and 50%, and positive if it was less than or equal to 40%. If the % inhibit for milk serum sample was greater than or equal to 80% it was considered negative, and anything lower than 80% was considered positive.

**Statistical analysis:** Differences between blood and milk serum samples were calculated by using the chi-square test (Minitab 14.0 Inc., State College, PA, USA). Differences were considered significant when \( P < 0.05 \).

**Indirect ELISA:** In this study, 172 (89.6%) of blood serum samples were positive for BVDV antibodies, while 161 (83.8%) of milk serum samples were found positive. In 150 (78.1%) of animals studied, both blood and milk serum samples were positive for BVDV antibodies. Only blood serum positive in 22 (11.5%) animals, while only milk serum samples were positive in 11 (5.7%) cases (Table 1).

**Statistical Analysis:** No difference was found between the use of blood and milk serum samples for the purpose of identifying the presence of BVDV antibodies (Table 1).

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**Fig 1:** Geographical positioning of the Kars district in which the study was performed.
BVDV is a major cause of reproductive failure and immunosuppression, and cause substantial economic losses in livestock industries through their impact on reproduction and health. Numerous studies (Yildirim et al., 2011; Bulut et al., 2013; Erol et al., 2014) conducted with regard to the BVDV infection in Turkey have demonstrated that the infection is widespread in cattle. These studies have reported that BVDV seroprevalence varies between 14.3-100% in cattle in various regions of Turkey.

An effective control program against the BVDV infection can be created in dairy cattle operations with the data obtained from epidemiological, diagnostic and virological studies (Lindberg and Alenius, 1999; Presi et al., 2011; Ridpath, 2012; Ståhl and Alenius, 2012).

ELISA tests are frequently used in comprehensive prevention-control study of BVDV infections. ELISA is a quite reliable test in terms of sensitivity and specificity on both blood and milk serum samples. ELISA plays a very important role in identifying herds that are free of BVDV and/or those suspected of active infection and identifying the presence of the antibodies that form in response to BVDV (Niskanen et al., 1991, Bitsch and Ronsholt, 1995). The use of milk serum samples provides advantages in research because they are easier to obtain and cause less stress on the animals (Beaudeau et al., 2001a). In BVDV control and eradication programs in countries like Switzerland, Denmark and Norway, milk samples are predominantly used in ELISA tests conducted for the purpose of identifying infection and observing uninfected herds (Lindberg and Alenius, 1999). Research conducted in past years (Niskanen et al., 1991; Kramps et al., 1999; Beaudeau et al., 2001a; Beaudeau et al., 2001b; Stahl et al., 2002) has indicated that milk ELISA is a rapid and reliable method for identifying BVDV antibodies in individual milk samples and determining the titer. A close relationship has also been reported between antibody titers in blood serum and milk serum. Yavru et al. (2013) tested the blood and milk serum of 202 cows for the presence of the antibodies formed in response to BVDV using indirect ELISA, and they determined that both the blood serum and milk serum taken from 148 (73.2%) animals were consistently seropositive. Because it was determined that the ELISA antibody values for blood and milk were similar at

The conclusion of this study, this established that milk serum samples could be used instead of blood serum in the serological diagnosis of BVDV Stahl et al. (2002) claimed that milk samples could be used to determine BVDV antibody levels using ELISA for entire herds in cattle operations suspected of being infected with BVDV. Researchers (2002) reported that the sensitivity of the test was 85% while the specificity was 97%. Niskanen (1993) reported a close relationship between herd prevalence of antibody positive cows and the presence of antibodies in tank milk in a herd consisting of 123 dairy cows. It has been reported that bulk tank samples in particular are quite numerous studies (Yildirim et al., 1999; Erol et al., 2014) conducted with regard to the BVDV infection in Turkey have demonstrated that the infection is widespread in cattle. These studies have reported that BVDV seroprevalence varies between 14.3-100% in cattle in various regions of Turkey.

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The antibodies found in blood circulation (IgG) pass into milk and therefore, the titres of antibodies in milk may be lower than blood. Niskanen et al. (1989) reported that the BVDV antibody titer in milk is less than that in blood serum, especially during the lactation period. Researchers have explained the fact that the seropositivity ratio (79.2%) in milk was lower than in blood serum (82.6%) based on the fact that the animals in the operation where the sampling took place had high milk yields.
BVDV control programs must first identify and remove PI animals from the breeding herd and prevent the infection of susceptible pregnant cows. As a control procedure, it may be advised that persistently infected animals can be kept together with other animals in the herds previous to gestation to reveal herds immunity and to prevent exposing cattle to infection during pregnancy. Immunization efforts have been successfully carried out in many countries to bring BVDV infections under control. An ideal vaccine should be suitable for administration to females before breeding for the prevention of transplacental infection. The vaccines were based on different combinations of identified variations among BVDV isolates in the country. Most BVDV vaccines on the market in many countries, including in Turkey, contained BVDV 1-a and 1-b strains, some of them in combination with a BVDV2 strain (Ridpath, 2005).

Ideally, a pestivirus control programme for Turkey should use a vaccine that contains not only BVDV1 strains but also BVDV2. For efficacious BVDV immunization and protection in Turkey, predominant BVDV 1 subgenotypes (1-l and 1-f) are clear vaccine candidates (Oguzoglu et al., 2011).

In conclusion, the results obtained in the present study showed that BVDV infection is common in sampled herds in the North-Eastern Anatolia region, which is the most important cattle and sheep production area in Turkey. The fact that the ELISA results for blood and milk were similar (P > 0.05) demonstrates that milk serum samples could be used instead of blood serum in the introduce of BVDV infection in herds considering the ease of collection. PI animals should be identified by testing seronegative animals at specified intervals for the presence of viral antibodies, and should be removed from the herd. The importance of selecting animals in the proper lactation period for sampling should be noted in controls conducted with milk samples to determine the status of animals with regard to BVDV infection.

REFERENCES


