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Serological and Virological Investigation of Bovine Viral Diarrhea Virus (BVDV) Infection in Candidate Bulls Before Taken in Artifical Insemination Centers by Enzyme Linked Immunosorbent Assay (ELISA)

Suni Tohumlama Merkezlerine Alınma Öncesi Aday Boğalarda Enzyme Linked Immunosorbent Assay (ELISA) ile Bovine Viral Diarrhea Virus (BVDV) Enfeksiyonunun Serolojik ve Virolojik Olarak Araştırılması

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Abstract: Bovine Viral Diarrhea (BVD) is a common infection all over the world. It causes important economical losses in cattle breeding. In this study, a total of 46 blood samples were examined taken into tubes with and without EDTA from candidate bulls in Artifical Insemination Centers. Blood serum samples were tested to detect for antibodies against Bovine Viral Diarrhea Virus (BVDV) and leukocyte samples were tested for BVDV antigens by ELISA methods. Eight (17.3%) out of 46 serum samples were found as positive by the means of antibodies against BVDV while 3 (6.5%) out of 46 leukocyte samples were detected as positive for BVD antigens. One (2.1%) of the seropositive bulls was detected as positive for BVD antigen. Two (4.3%) of the seronegative bulls were detected as positive for BVD antigens.

Key words*:* BVDV, Bull, ELISA.

Öz: Bovine Viral Diarrhea (BVD) tüm dünya üzerinde görülen yaygın bir enfeksiyondur. Sığır yetiştiriciliğindeki en önemli ekonomik kayıpların nedenidir. Bu çalışmada suni tohumlama merkezlerinde kullanılmak üzere seçilen toplam 46 adet aday boğadan EDTA'lı ve EDTA'sız tüplere kan örnekleri alınarak incelendi. Kan serum örnekleri BVDV'ye karşı oluşan antikorlar yönünden, lökosit örnekleri ise BVDV antijen varlığı yönünden ELISA ile test edildi. 46 adet serum örneğinden 8 (%17.3) adedi BVDV antikorları yönünden seropozitif olarak tespit edilirken, lökosit örneklerinden 3 adedi (%6.5) ise BVDV antijen varlığı yönünden pozitif bulundu. 3 adet BVDV antijen pozitif boğanın 2 adedi (%4.3) seronegatif iken 1 adedi (%2.1) ise seropozitif olarak tespit edildi.

Anahtar sözcükler*:* BVDV, Boğa, ELISA.

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Introduction

Bovine Viral Diarrhea-Mucosal Disease (BVD/MD) is a common infection among cattle all over the world (Zemke et al. 2010). It causes important economical losses in cattle breeding, e.g. fertility (Kale et al. 2011). Infection is characterized with depression, temperature, mild diarrhea and temporary leucopenia (Radostits and Littlejohns 1988, Peterhans and Schweizer 2010).

The causative agent, BVD virus (BVDV), is a member of the genus Pestivirus in the Flaviviridae family. On the basis of growth characteristics in cell cultures, naturally occurring BVDV strains are divided into cytopathic (cp) and noncytopathic (ncp) biotypes; cp strains cause visual cytopathic effects, while ncp strains grow in cells without visual cytopathic effects (Wasteway et al. 1985, Fray et al. 2000).

Several ways of indirect, vehicle or vector transmission of BVDV have been reported, i.e. reusing needles and nose tongs, rectal gloves or by using live or contaminated vaccines. Transmission can also occur through blood feeding flies (Tarry et al. 1991, Passler et al. 2007).

Persistently infected (PI) animals result from the infection of the bovine foetus with a ncp-BVDV biotype early in gestation. These animals show specific immunological tolerance to the carrier virus and maybe born apparently healthy. PI animals are the main source of virus transmission as they continuously shed large amounts of virus in the environment. Virus is excreted in smaller amounts from acutely infected animals and for only a few days during the acute infection (Passler et al. 2007, Brownlie 1991).

Semen produced by PI bulls often contains virus and may transmit infection by either natural service or artificial insemination (AI). Bulls may acquire BVDV infection from infected herdmates with subsequent seminal shedding of the virus. However, in such cases the excretion of BVDV in the semen is transient and the amount of virus is lower than that in PI bulls. Detection and elimination of PI bulls are essential compenents of BVDV control programs, especially in AI centers (Revell et al. 1988, Marley et al. 2009).

Fetal Mucosal Disease (MD) occurs only in PI animals and is induced by the cp-BVDV. These event may take plays by mutation to the cp biotype or superinfection with a cp-BVDV (Brownlie et al. 1984, Westenbrink et al. 1989).

The definitive etiological diagnosis of BVD by virus isolation, can be time-consuming, expensive, and elusive, however there is continuing progress being made in resolving these difficulties. It can be attempted by inoculation of nasopharengeal swabs, ocular swabs, intestinal tissues, spleen, or most other tissues, or any fraction of blood into cell cultures. Recovery of virus from feces is generally difficult. Isolation of virus from any source, in cell culture may require more than one passage before the virus is detectable. It is then recognized by cytopathic effects or, in the case of ncp strains, either interference with a cytopathic virus or various serological methods may be used to demonstrate the presence of virus or virus-associated antigens. Both cp and ncp pestiviruses have been isolated from spleen or blood of individual cattle with BVD and it has been suggested that both should be present in cases of mucosal disease. The serological methods used to detected ncp virus or antigen in cell culture or tissues, such as intestine, kidney or spleen from affected animals or aborted foetal tissue, include direct or indirect immunofluorescent antibody staining, immunoperoxidase staining, and gel diffusion tecniques (Lamm et al. 2009). Nasal epithelial cells collected on cotton swabs were stained by fluorescent antibody for the diagnosis of field cases of BVD in calves and, using a similar technique, the detection virus antigen in cells, obtained from the nasopharynx using swabs, was shown to be a rapid and efficient method for identifying carriers, agreeing perfectly with virus isolation from leukocyte and clotted blood (Radostits and Littlejohns 1988, Cornish et al. 2005).

Serological techniques are also used to detect antibody. Currently, the serologial methods employed in the detection of BVDV antibodies have been confined to the serum neutralisation test (SNT), immundiffusion test and complement-fixation test (Tsvetkov et al. 1982). However, these 3 methods still have some undesirable features; hence, the need exist for a simple, rapid, sensitive and spesific test. Recently, the enzyme-linked immunosorbent assay (ELISA) -first established by Engvall and Perlmann (1971)- has been used extensively for the measurement of antibody in viral diseases because of its simplicity, rapidity, economy and high sensitivity (Cornish et al. 2005, Chu et al. 1985, Dehkordi 2011).

The aim of this study was to determine, whether or not BVDV antigen and antibodies against to BVDV in candidate bulls before taken in Artifical Insemination Centers in Turkey.

Materials and Methods

Samples

In this study, total 46 bulls' blood samples were taken into normal tubes to obtained serum and into tubes with EDTA to determined BVDV antigens.

Blood samples which were taken into normal tubes were santrifuged 2000 rpm/20 min. Serum were obtained. The serums were kept in deepfreezer under -25 ºC. The serum were inactivated in 30 min at 56ºC before used.

Leucocyte samples were prepared from blood samples taken into tubes with EDTA by a standard method. Theleukocytesamples were kept in deepfreezer under -25 ºC until used.

Methods

Blood serum samples were tested to detect for antibodies against BVDV and leukocyte samples were tested for BVDV antigens by ELISA methods as described in test procedure. Commercial direct and indirect ELISA kits (Institut Pourquier, France) were used for detection BVDV antigens and antibodies against BVDV.

Results

Eight out of 46 (17.3%) serum samples were found as positive by the means of antibodies against BVDV while 3 out of 46 (6.5%) leukocyte samples were detected as positive for BVD antigens (Table 1).

One (2.1%) of the seropositive bulls was detected as positive for BVD antigen. Two (4.3%) of the seronegative bulls were detected as positive for BVD antigens (Table 1). BVD antigen positive candidate bulls were removed from Artifical Insemination Centers.

Table 1. Distribution of BVDV antibody and antigen of bulls

BVDV		**Antibody**		**Total**
		+	**-**	
Antigen	**+**	1	2	**3**
	-	7	36	**43**
Total		**8**	**38**	**46**

Discussion

BVDV infection normally results in an acute, transient and subclinical disease, which is the most common form and is probably due to the widespread presence of serum antibodies

to BVDV in bovine populations. Although the detection of neutralizing antibodies to BVDV is frequent, the patogenesis and the alteration of the immune response brought on by the infection often results in little or no neutralizing antibody (Justewicz et al. 1987).

An ELISA would have several advantages over the neutralization test for detecting antibody. These include: economy of labour; more rapid availability of results; and no requirement for cells and cell culture during the assay. However, earlier studies with BVDV indicated that most virus neutralizing activity was in the IgM fraction from bovine serum and there was an unusually persistent IgM antibody response following infection (Fernelius 1966). Furthermore a restricted isotype response has beeen reported for certain other infections of cattle (Musoke et al. 1981).

Serological diagnosis is very important for the detection of BVDV, an important pathogen related to reproductive failure. Researchers (Cornish et al. 2005, Dehkordi 2011, Pacheco and Lager 2003) estimated that ELISA-BVDV is good sensitivity, specificity and repeatability method for detecting antibodies against BVDV and İt is easy to transfer, economical, and easy to perform.

Virus isolation techniques employing susceptible cell lines have been used for the detection of viraemic animals. These methods are time consuming and expensive, and many laboratories have experienced difficulties with pestivirus isolations due to adventitous viral contamination of both cell lines and the bovine serum added to media used for propagating the cells (Rossi et al. 1980, Xia et al. 2011). One way to overcome these problems has been the recent development of ELISA techniques for detecting viral antigen (Shannon et al. 1991).

In this study, 46 bull serum and leucocyte samples were studied to detect BVDV antigens and antibodies against BVDV. Eight out of 46 bulls were detected seropositive while 38 out of 46 bulls were seronegative.

Westenbrink et al. (1986) comparatively examined to detect antibodies against BVDV by blocking enzyme linked immunosorbent assay (ELISA) and virus neutralization (VN) tests. The researchers stated that they found a positive correlation (91%) between the BVD virus antibody titres measured with the two tests and an equal effectiveness in measuring maternal antibodies. Bock et al. (1986) comparatively studied in the 886 blood sera and another blood serum from 6 calves experimentally infected with BVDV for antibodies against BVDV by SN and ELISA. Bock et al. (1986) were detected 96.3% similar result between the

two tests of 886 cattle blood serum while they stated that they could not detect any antibodies by SN test in infected calves on the 14th day. Polak and Zmudzinski (1995) checked 3216 serum samples from 81 herds located in Western and Northern Poland for presence of antibodies against BVDV and they reported that they detected 2672 serum samples as positive (83%) at the end of their study. Obando et al. (1999) applied ELISA for 615 cattle blood serum samples taken in Apure state in Venezuella in order to detect antibodies against BVDV and reported that they detected seropositive results as a rate of 36.7%. Chu et al. (1985) studied 50 cattle blood serum for BVDV antibodies by ELISA and SN. Researchers (Chu et al. 1985) reported that they detected 2 of 50 cattle by ELISA and that ELISA is a much sensitive method to detect BVDV antibodies.

Mockeliuniene et al. (2004) used ELISA to estimate the level of BVDV infection in cattle herds at the different Lithuanian districts and to determine factors influencing the course of BVDV infection. In addition, they obtained cattle blood samples came from Lithuanian Artificial Insemination (AI) Centers among the breeding bulls' sperm donors. The researchers were investigated in totally 4098 blood serum samples from animals of different age and sex. They reported that the number of seropositive animals ranged from 11.9 to 100% and they pointed out that 29.9% of the herds were not infected with BVDV, in 32.7% of the herds from 70 to 100% of cattle were seropositive to BVDV and determined that a positive correlation between the number of seropositive cattle, and the size of herds and age of animals. Sex of animal had no influence on the prevalence of BVDV. The researchers estimated that the annual incidence risk of infection with BVDV decreases with the animal age.

In this study, 3 out of 46 bull were detected BVDV antigen positive while 43 out of 46 bull were negative. Two out of 3 bulls detected antigen positive were had no antibodies against BVDV and 1 out of 3 bulls detected as antigen positive and antibody positive. When seronegative cattle is infected with a ncp BVDV biotype, virus can be transfered easily into the foetus and infection in early period of gestation may produce PI calf. PI calves show specific immunological tolerance to the carrier virus and maybe born apparently healthy.

Simsek (1997) reported 2 acut persistent infections by BVDV in 142 healty cows by monitoring the leucocyte samples with direct immunofluorescence test (DIFT). Shannon et al. (1991) examined 418 animals by different methods for detecting BVD virus infection, at the end of the study they reported that 108 of 418 animals BVD antigen positive by ELISA

following inoculated blood samples of 108 positive animals analysed in the ELISA into cell culture. They declared BVDV virus in 55 of 108 animals using an immunperoxidase staining technique and ELISA has been validated on field samples and is suitable for routine diagnostic. Sandvik et al. (1997) experimentally inoculated 24 calves with BVDV and examined for viral antigen in peripheral leukocyte with an ELISA. The researchers stated that they detected positive results inleukocytesamples on days 3-4 from two of 8 animals inoculated intranasally, and on days 11-13 from three of 16 animals inoculated intramuscularly by antigen ELISA. Ozturk et al. (2012) analysed 92 samples for antibodies to BVDV in Burdur province, Southwest region of Turkey. They found BVDV seropositivity as 81.5% (75/92) and BVDV antigen was determined in 2.2% (2/92) of the samples at same time.

In conclusion, 2 out of the 8 bulls' dams were infected with ncp BVDV for the first time during the early period of pregnancy and they were detected antigen positive and antibody negative. Because not to be done a second sampling of these bulls, this condition could not be determined whether as a result of an acut or persistent infection or not. It was interesting that the other bull detected antigen positive and antibody positive. It was also infected by introutero in early period of gestation and it was detected antigen positive, but it was detected antibody positive. Maybe this bull was infected another ncp biotype of BVDV during a point of its lifespan and formed antibodies against BVDV.

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