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RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

A Serological investigation for Bovine Viral Diarrhea Virus infection in and around Afyonkarahisar province, West Anatolia

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S U M M A R Y

Bovine Viral Diarrhea Virus (BVDV) is one of the most prevalent infections of domestic ruminants and has significant effect on the cattle industry. Afyonkarahisar province is the homeland of well-known some specific products made from cattle milk and meat. Cattle population is high and generally intensive breeding having been preferred due to climatic conditions. High level persistent infection was detected before in big-size herds before. Aim of the study was to investigate the infection in family type small enterprises in the Afyonkarahisar and close provinces. For this purpose, blood serum samples were obtained from small private herds in boroughs of Afyonkarahisar, Burdur and Denizli provinces. Samples were controlled for BVDV specific antibody (Ab) presence and titter values using serum neutralisation test. The samples collected from 9 boroughs of Afyonkarahisar, among 73 and 148 samples from each one. Seropositivity was varied between 73.6% and 95.2%. In total, out of 972 samples, 823 (84.6%) was found to be positive. Proportion in Burdur and Denizli provinces were 35.7% (5/14) and 42.8% (12/28), respectively. Considering determined high values for BVDV infection, consultative support seems quiet necessary for control/eradication of the disease for small scale enterprises.

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Afyonkarahiar İlinde Bovine Viral Diarrhea Virus Enfeksiyonunun Serolojik Olarak Araştırılması

ÖZET

Bovine Viral Diarrhea Virus (BVDV) evcil ruminantların en yaygın enfeksiyonlarından biridir ve sığır endüstrisi üzerinde önemli etkileri vardır. Afyonkarahisar ili sığır eti ve sütünden yapılan iyi tanınan belli spesifik ürünlerin anavatanıdır. Sığır populasyonu yüksektir ve iklim koşulları nedeniyle genellikle intesif yetiştiricilik tercih edilmektedir. Büyük ölçekli sürülerde yüksek seviyede persiste enfeksiyon daha once de belirlenmiş idi. Çalışmanın amacı söz konusu enfeksiyonun Afyonkarahisar ili ve yakın çevresinde aile tipi küçük işletmelerde araştırılmasıdır. Bu amaçla Afyonkarahisar, Burdur ve Denizli illerindeki küçük özel işletmelerden kan serum örnekleri elde edildi. Örnekler serum nötralizasyon test kullanılarak BVDV spesifik antikor varlığı ve titresi yönünden kontrol edildi. Afyonkarahisar ilinden örnekler 9 ilçeden toplandı ve her bir ilçeden 73 ile 148 arasında örnek elde edildi. Seropozitifliğin %73.6 ile %95.2 arasında olduğu görüldü. Toplamda ise 972 örneğin 823'ünün (%84.6) pozitif olduğu belirlendi. Burdur ve Denizli illerindeki oranların %35.7 (5/14) ve %42.8 (12/28) olduğu saptandı. BVDV enfeksiyonu için belirlenen yüksek oranlar dikkate alındığında, küçük ölçekli işletmelerde hastalığın kontrol/eradikasyonu için danışmanlık desteği verilmesinin oldukça gerekli görülmektedir.

INTRODUCTION

Bovine Viral Diarrhea Virus (BVDV) infection is a one of the major pathogen of the cattle with worldwide distribution (Ridpath, 2010) including Turkey. The virus is a member of the genus *Pestivirus* in the family *Flaviviridae*. Pestivirus genus contains four species; Bovine viral diarrhea virus 1, Bovine viral diarrhea virus 2, Border disease virus (BDV) and Classical swine fever virus (CSFV), additionally a tentative virus was isolated from a giraffe (King et al 2012). According to one of the latest study on genotyping of the virus (Giangaspero et al 2013), 16 cluster determined; BVDV1, 5 for BVDV2, 8 for BDV and 3 for CSFV. In the studies in Turkey, Yılmaz et al (2012) were carried out a phylogenetic analysis, based on comparison of the E2 or the 5'UTR coding regions, out of 19 Ag positive samples, they indicated that 17 belonged to the BVDV-1 genotype and 2 to the BVDV-2 genotype. Yeşilbağ et al (2014) were also detected BVDV1 and both study revealed novel clusters.

The BVDV has two biotypes cytopathogen (cp) and non-cytopathogen (ncp). Complex pathogenesis mainly determined by biotype of the virus and age of the foetus in the time of transmission. Clinical findings are quite variable. Beside direct disorders in respiratory, alimentary and reproductive tract, immune system is also effecting; Due to lymphocyte disfunctions (Roth et ark 1981, Brownlie 1990), sensitivity increases to the other infections during acute infection (Reggiardo and Kaeberle 1981, Potgieter et al 1984, Al-Haddawi et al 2007, Lee et al 2009).

Emerged disorders and persistent character of the virus cause to significant economic looses in cattle industry. The infection can be assumed as a treat for sustainable cattle breeding (Gunn et al 2005, Houe et al 2006).

Afyonkarahisar province has significant cattle population in both breeding aim. Specific milk and meat product like kaimak and soujouk are province's one of the a few economic sectors (Pamuk and Gürler 2010). Intensive breeding are the most preferred breeding style due to relatively harsh climate in the region (Günlü et al 2001).

Control or eradication for BVDV mostly carried out in big or medium scale cattle herds in Turkey. However, family type small enterprises are still common nationwide and vast majority of them are lack of veterinary consultancy. BVDV is also not takes places among national control program carried out infections by ministry of agriculture

However, the BVDV is one the widespread infection cattle in like many parts of the Turkey. Persistent and acute infection was determined in big size enterprises, persistently infected cattle ratio can be reach to 5% in some of them (S. Gür, Unpublished data).

Purpose of this study is to reveal a profile of the BVDV infection via serological controls in small family type enterprises especially in Afyonkarahisar, the samples from Burdur and Denizli provinces were also examined.

MATERIAL AND METHODS

Cell culture

Madin Darby bovine kidney (MDBK) cell culture (ATCC, CCL-22) was preferred for virus propagation, titration and microneutralisation tests. Dulbecco's Minimal Essential Medium (DMEM) and 2-10% foetal calf serum (FCS) were used as a cell culture medium in the tests.

Virus

Bovine Viral Diarrhea Virus, NADL strain ($10^{-4.5}$ $TCID_{50}$/0,1ml) was used as control virus. Titter value ($100TCID_{50}$) of the virus suspension was calculated by the Kaerber method (1964).

Blood serum samples

Out of 17 boroughs, blood serum samples (n=972) were collected from small or medium scale private enterprises in 9 boroughs of Afyonkarahisar province. Rest of the samples were obtained from Burdur and Denizli provinces (n=42). Total of 1.014 adult cattle were sampled for the study. Ages of the cattle were between 1 and 7. Sex of the animals was ignored, however all of the studied farms were dairy enterprises, by the way all of the animals were female.

The samples that have been taken from *vena jugularis* were centrifuged at 3.000rpm/10min. (+4°C) and serum fractions were separated into a sterile tube. After inactivation at 30°C for 30min., the samples were kept at -20°C until to the test.

Microneutralisation test

Microneutralization test was carried out for detection of pestivirus specific antibodies. Initially, the cell culture medium was controlled for ncp pestivirus contamination using direct BVDV antigen

(Ag) ELISA (Idexx, USA). All serum samples were diluted 1/5 with cell culture medium and placed into two wells of the tissue culture plates as a 50 µl with same volume of virus suspension containing 100 $TCID_{50}$. After 1h of incubation, 50 µl cell suspensions (300.000 cells/ml) were added and incubated at 37 ˚C, %5 CO_2 for three day. Test results were determined on the basis of micromorphology of cells using inverted microscope. All positive serum samples were subsequently diluted in rates of 1/5, 1/10, 1/20, 1/40, 1/80, 1/160 and tested again for the determination of titter values.

RESULTS

Microneutralisation test

As a result of the test, 1/5 above dilutions were accepted as positive. Out of 1.014 samples, 972 were collected from 9 boroughs (varied between 73 and 148 samples from each of them) of Afyonkarahisar province (table 1). Proportions were among 53.4% and 95.2%. Out 972 samples, 823 (84.6%) were found to be positive for pestivirus specific antibodies. Small number of samples were obtained from two enterprises of Burdur and Denizli, seropositivity were detected as 35.7% (5/14) and 42.8% (12/28), respectively. In total, %82.8 of the samples was detected as positive.

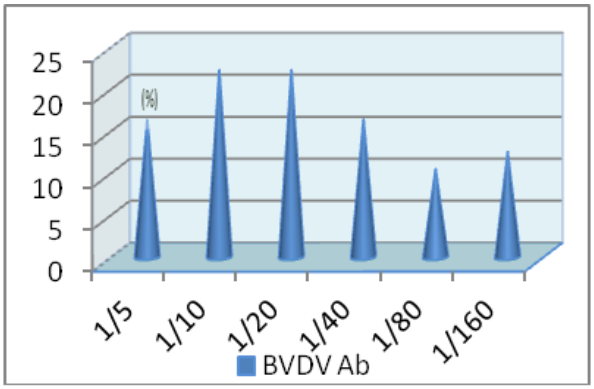
Table 1. Samples and BVDV Antibody (Ab) test results.

Tablo 1. Örnekler ve BVDV Antikor(Ab) test sonuçları

No	Localisations	NS*	BVDV	
			Ab(+)	(%)
1	Sinanpaşa**	138	128	92.7
2	Şuhut**	107	78	72.8
3	İhsaniye**	84	77	91.6
4	Hocalar**	73	39	53.4
5	Dazkırı**	110	81	73.6
6	Sandıklı**	123	113	91.8
7	Emirdağ**	148	141	95.2
8	Evciler**	100	88	88
9	Bolvadin**	89	78	87.6
10	Burdur	14	5	35.7
11	Denizli	28	12	42.8
	Total	1.014	840	82.8

**Number of samples*
***Boroughs of Afyonkarahisar province*

The samples that was found to be seropositive were (n=840), re-tested for detection of Ab titter values. Proportional range was highest in 1/10 and 1/20 titters (Graphic 1).



Graphic 1. BVDV Ab titter distribution

Grafik 1. BVDV Ab titre dagılımı

DISCUSSION

Serologic and virologic studies carried out many parts of the world showed high proportions (Houe 1995). Since first detection of BVDV infection in 1964 in Turkey (Öncul et al 1964), prevalence of the infection was reported by many researchers (Burgu and Ozkul, 1993; Karaoglu, 1998; Çabalar and Karaoğlu, 1999, Yavru et al 2013). Beside antigenic studies the corried out in latest years (Yeşilbağ ve ark 2008, Oğuzoğlu et al 2012, Yılmaz et al 2012), serological reports states variable data but not low values in many parts of the Turkey. Burgu et al (2003) were controlled 3.360 cows from various provinces and detected 64.2% Ab and 0.25% Ag positivity. Similar proportions were reported in different parts of Turkey; 58.8% in the Northeast Anatolia (Yıldırım et al 2009), 14.2% in West-Anatolia (Kale et al 2011).

In this study, micronetralisation test has been preferred due to high sensitivity and specifity (Carbrey et al 1971). The neutralising Ab induced by E2 after natural infection is accepted as main protector mediator despite variability of field isolates (Howard et al 1989, Ridpath et al 2003). Cell culture and medium additive foetal serum were controlled in the aspect of contaminant pestivirus in every step of the test process.

According to the test results, seropositivity for BVDV infection was determined in all of the studied herds in different proportions. Out of 1.014 samples, 972 were collected from 9 boroughs of Afyonkarahisar province and determined proportions were between 53.4% (39/73) and 95.2% (141/148).

Out 972 samples, 823 (84.6%) was found to be positive for BVDV specific antibodies. Small number of samples were obtained from two enterprises of Burdur and Denizli, positivity were detected as 35.7% (5/14) and 42.8% (12/28), respectively. Proportions in these provinces were relatively low comparing Afyonkarahisar province but number of the samples is not sufficient to make trustable evaluation. In total, 840 of 1.014 samples 82.8% were positive.

Studied herds were not controlled in the aspect of Ag, by the way persistent animal status are unknown. However, considering high seropositivity it is possible to say that PI animal presence could be important possibility. According to field veterinary clinicians, newborns diarrhoea, fertility disorders and abort cases with "unknown aetiology" (especially due to lack of laboratory confirmation) have been increasing in the Afyonkarahisar province. Standard therapy methods are generally not satisfactory responded, correspondingly economic losses have been gaining more importance and it seems will be continued.

There is serologically cross reaction between cp and ncp biotypes but we know that field isolates are mostly belong to ncp. Economic results of acute infections are significant due to various health disorders (Roth et al 1981, Reggiardo and Kaeberle 1981, Potgieter ve ark., 1984, Kale et al 2011), but persistency related problems are much more serious and accepted as an obstacle in front of the herd future.

Cost of BVDV is estimated as a $20 to $160 per adult years; however tests and supplies are $5-10 for eradication for every individual. Eradication seems quite cheaper. In the control program implemented regions, number of PI animals has been decrease up to five to ten fold in a few years (Walter et al 2005). Vaccination is an important tool in the elimination or decrease the economic burden. However, laboratory evaluation is a necessity in the initial test to determine the herd status, follow-up tests for the controls in individual or bulk milk, and monitoring tests to confirm the infection free status (Houe et al 2006.) However the most important problem in the small enterprises is lack of veterinary consultancy and regular health controls. Clinical disorders of the BVD are non- specific, diagnosis is so not so easy in the absence of laboratory findings. Clinicians are not generally using the regional or national labs in the field and herd owner are not giving to adequate interest to the diseases that can be herds problem, prominently BVDV.

As conclusion, determined values (up to 95.2% proportions) were among highest values comparing to different part of the Turkey. BVDV is stand out as a main problem infections especially in dairy breeding. Considering cattle breeding understandings in all over the country, small-medium scale private enterprises still can be accepted as main bone and they deserve more interest and support from both local and general veterinary services.

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