

**Short Communication**

**SCREENING FOR PERSISTENTLY INFECTED CATTLE WITH BOVINE VIRAL  
DIARRHEA VIRUS IN SMALL-HOLDER CATTLE FARMS LOCATED IN SAMSUN  
PROVINCE, NORTHERN TURKEY**

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**ABSTRACT**

Blood samples were collected from 651 cattle of 119 small scale family farms from four different regions in Samsun Province, Northern Turkey. Bovine Viral Diarrhea Virus (BVDV) antigens and antibodies in samples were investigated with Antigen Capture ELISA (ACE) and Serum Neutralization Test (SNT), respectively. Two out of 651 (0.03%) animals were found positive for BVDV antigens by ACE and antibody negative by SNT in the first sampling. The blood samples from antigen positive animals were re-taken after 28 days from the first sampling. These two positive animals were found antigen negative and antibody positive. 211 of the 651 (32.41%) animals were found seropositive by SNT. Antibody titers were calculated between 1:8 and 1:512.

**Key words:** BVDV, Cattle, Persistently Infected, ELISA, Neutralization.

**INTRODUCTION**

Bovine viral diarrhea virus (BVDV) is classified in the genus *Pestivirus* within the family of *Flaviviridae* together with other important animal pathogens such as Border disease virus (BDV) and Classical swine fever virus (CSFV) (Stahl and Alenius, 2012). Two genotypes of BVDV have been identified as BVDV-1 and BVDV-2 (Brodersen, 2014). There are two biotypes defined as cytopathic (cp) or non-cytopathic (ncp) according whether or not they produce cytopathic effects in cell cultures (Brodersen, 2014).

Acute infection with BVDV in cattle is often subclinical, or result in a variety of mild clinical symptoms including high fever, diarrhea, ulceration of the muzzle and oral cavity and leucopenia (Brodersen, 2014). Highly virulent strains of BVDV can produce severe symptoms including haemorrhagic diarrhea, lethargy, inappetance and reduced milk yield (Brodersen, 2014). BVDV has the ability to pass through the placenta and infect the foetus resulting in several outcomes such as abortion, reproduction failure, stillbirth, abnormal body conformation depending upon the point in gestation in which the foetus was infected as well as the strain of virus. Infection with ncp strain in the first trimester of gestation may generate persistently infected (PI) calves. PI animals are unable to produce an antibody response against the virus. They shed the virus throughout their lives and are the most important reservoir of the BVDV (Lee *et al.*, 2008, Yazici *et al.*, 2012). If PI animals become infected with a second cp strain of BVDV, a mucosal disease (MD), characterized by high mortality and low morbidity, occurs.

The aims of this study are as follows: (1) to investigate the cattle persistently infected with BVDV in small scale family farms and (2) to estimate the current seroprevalence of BVDV in Samsun Province.

**MATERIALS AND METHODS**

**Sample:** 119 small scale family farms having between 1 and 12 cattle were randomly selected from four different location in Samsun Province, Northern Turkey. Blood samples were collected from 651 cattle housed on these farms. All animals were unvaccinated and 1 year old. The samples were taken into tubes with and without EDTA. Blood samples in tubes without EDTA were centrifuged at 2000 rpm for 10 min at 4°C. The sera were separated into vials (Eppendorf, Germany), then inactivated at 56 °C for 30 min and stored at -20 °C until used. The whole blood samples in tubes with EDTA were stored at 4°C and used freshly in Antigen Capture ELISA (ACE).

**Cell, Virus, Infectivity and Neutralisation Tests:** Madin Darby Bovine Kidney (MDBK) cells were grown in Dulbecco's Modified Essential Medium (DMEM, Gibco) supplemented with 10% foetal calf serum (FCS, Sigma) and incubated at 37°C, 5% CO<sub>2</sub>. Cells, cell culture media and FCS were screened for the absence of BVDV. The NADL strain of BVDV was propagated on MDBK cells. The median tissue culture infectious dose 50% (TCID<sub>50</sub>) was determined by microtitration test as described by Frey and Liess (1971). Antibodies against BVDV were investigated using the serum neutralization test (SNT).

**Antigen Capture ELISA (ACE):** A commercial ELISA kit (BVDV Antigen/Serum plus, IDEXX Laboratories; USA) was used for the detection of BVDV antigens. The test was carried out according to the manufacturer's instructions. Plates were read with an ELISA plate reader at 450 nm absorbance and results calculated.

**Statistical Analysis:** Data was analyzed statistically by using Chi-square test. The Results were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Two of 651 (0.03 %) cattle were found BVDV antigen positive by ACE. They were also found to be BVDV antibody negative by SNT. For investigating persistence, the two positive cattle were screened a second time after 28 days from the first sampling. After the second sampling, the two cattle were determined to be BVDV antigen negative and antibody positive.

211 of 651 (32.41%) sera tested by SNT were found to seropositive (Table 1). According to location, seropositivity rates were between 13.60 % and 52.17%.

Antibody titres of seropositive animals were between 1:8 and 1:512 (Table 2).

There was a statistically significant difference in BVDV seroprevalence in Ladik location (52.17%) compared with the other three locations (Table 1). There was no significant difference in the prevalence of antibodies against BVDV between Alacam, Kavak and Terme locations.

PI animals are the major source of BVDV in the herd. The rates of PI cattle range from 0.5 to 2% in countries endemically infected with BVDV (Houe, 1999). In Turkey, PI cattle have been investigated by many researchers and the prevalence reported as 0.25%, 0.07% and 4.9%, respectively. (Burgu *et al.*, 2003; Tan *et al.*, 2006). In our study, PI animals were not found amongst the 651 cattle tested from 119 small scale family farms in Samsun Province. Two (0.03 %) cattle were BVDV antigen positive and antibody negative in the first sampling. These animals were BVDV antigen negative and antibody positive in second sampling after 28 days, and accepted as transiently viraemic due to the elimination of virus.

**Table 1. Distribution of BVDV antigens and BVDV antibodies in 119 small scale farms of four different locations in Samsun Province, Northern Turkey.**

Location	No of Small Scale Family Farms	No of Tested Sample	Antigen Capture ELISA		Neutralization Test	
			No of Positive	No of Negative	No of Positive	No of Negative
Alaçam <sup>c</sup>	25	167	-	167(100.0)	43 (25.74) <sup>b</sup>	124(74.26)
Kavak <sup>i</sup>	30	161	-	161(100.0)	49(30.34) <sup>b</sup>	112(69.66)
Ladik <sup>i</sup>	30	184	2 (1.08)	182(98.92)	96(52.17) <sup>a</sup>	88(47.83)
Terme <sup>c</sup>	34	169	-	169(100.0)	23(13.60) <sup>b</sup>	146(86.40)
<b>TOTAL</b>	<b>119</b>	<b>651<sup>n</sup></b>	<b>2 (0.3)</b>	<b>649 (99.97)</b>	<b>211(32.41)</b>	<b>440(67.59)</b>

i inland location, c:coastal location, n: Number of Animal tested, a and b: Values bearing different superscript in column differ significantly ( $P < 0.05$ ).

Serological investigations are important criteria for BVDV infection. In various countries, researchers have reported different seropositivity rates ranging between 50% and 90% (Obando *et al.*, 1999, Burgu *et al.*, 2003). Differences of seropositivity rate in these countries may depend on the age of animals, density of population, housing system, climate, vaccination, uncontrolled animal movement, biosecurity, and persistence of BVDV. Previous studies reported that seropositivity of BVDV was found between 62% and 80% in Turkey. (Burgu *et al.*, 2003, Tan *et al.* 2006, Yazici *et al.*, 2012). In our study, the seropositivity of BVDV was determined on all of the 119 farms (100%). The percentage of seropositive animals was 32.41% (211/651) and SN antibody titers were between 1:8 to 1:512. We determined that 178 of the 211 (84.36%) seropositive cattle had SN antibody titers between 1:8 and 32 while 33 of the 211 (15.64%) cattle

had 1:64 indicating that recent or current exposure to transient or persistently infected animals. Therefore, these low SN titers can indicate ongoing BVDV infection. The rates of seropositivity were 25.74%, 30.43%, 52.17%, and 3.60% in Alacam, Kavak, Ladik, and Terme locations, respectively. The differences may depend on the structure of herds, animal movements, and the close contact between animals in livestock markets. Thus, the highest rate (52.17%) was determined in Ladik Location where animal movements were more than other locations due to one of the largest livestock markets in Samsun Province. Consequently, climatic factors and geographical status can play a role in virus spread (Gumusova *et al.*, 2006). Geographically, Ladik and Kavak are inland zones while Alacam and Terme are coastal zones. The mean seroprevalence was significantly higher in inland zones with a colder climate than the coastal areas.

In Conclusion, despite the absence of PI animals detected in our study, overall seropositivity of this study was high (32.41%). This rate indicates an ongoing BVDV infection in farms and is always a risk for re-occurring persistent infection. For this reason, major sources of BVDV infection such as transmission routes, PI animals

and animal movement are most important. Considering the economic impacts of BVDV for the livestock industry, we recommend that serological investigations and the screening of BVDV antigen for PI cattle should be regularly performed and the control program of BVDV should be started.

**Table 2. Serum neutralizing antibody titers in BVDV seropositive cattle according to location in Samsun Province.**

Location	SN antibodies Titer of Positive Samples							Total
	1:8	1:16	1:32	1:64	1:128	1:256	1:512	
Alaçam <sup>c</sup>	13	11	15	1	3	-	-	43
Kavak <sup>i</sup>	7	14	21	3	3		1	49
Ladik <sup>i</sup>	20	17	40	5	9	3	2	96
Terme <sup>c</sup>	3	5	12	1		2	-	23
<b>Total</b>	<b>43</b>	<b>47</b>	<b>88</b>	<b>10</b>	<b>15</b>	<b>5</b>	<b>3</b>	<b>211</b>

I : inland location, c:coastal location.

**Acknowledgments:** This study was funded by the Scientific Research Council of Ondokuz Mayıs University, Samsun Turkey. (The Project Nr: PYO.VET.1904.13.001). The Language of this manuscript has been revised by Dr. Andrew Shaw from MRC Centre for Virus Research, University of Glasgow.

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