Serological data of bovine herpes virus type-1 and bovine viral diarrhea virus infections in various ruminants in small-scale farms in the Central and Eastern Black Sea Region, Turkey

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ABSTRACT
Bovine viral diarrhea virus (BVDV) and Bovine herpes virus type-1 (BHV-1) are economically important pathogens leading to critical health problems for widespread ruminant populations worldwide. This study was conducted in order to update the seroprevalence of both viruses in non-vaccinated ruminant breeding enterprises in the Black Sea Region of Turkey. Blood samples (n=1,025) were collected from 192 small-scale farms and were screened using a virus neutralization test. Overall percentages of BVDV and BHV-1 were 19.90 % and 13.56 %, respectively. All goat and cattle enterprises were seropositive for BVDV. Single and dual virus infections rates were 24.87 % and 5.26 % respectively. The Black Sea Region of Turkey has a great number of small-sized ruminant farms and the results confirmed that BVDV and BHV-1 viruses were still in circulation and a wide range of large and small ruminants were exposed to both viruses.

Key words: Bovine viral diarrhea virus, Bovine herpes virus-1, Neutralization, Serology.

INTRODUCTION
Bovine viral diarrhea virus (BVDV) and Bovine herpes virus type-1 (BHV-1) are two critical pathogens that affect domestic and wild ruminants. Both viruses have a widespread distribution in most parts of the world and are the cause of significant financial loss for the livestock industry with multi-systemic disorders (Shirvani et al., 2012). BVDV belongs to the Pestivirus genus within the Flaviviridae virus family (Yilmaz, 2016). This genus constitutes four species namely, bovine viral diarrhea virus 1 and 2(BVDV), border disease virus (BDV) and classical swine fever virus (CSFV) (Yesilbag et al., 2008; Aslan et al., 2015). It causes a serious acute, transient and intrauterine infection in ruminants with a variety of symptoms including severe diarrhea, congenital malformation, neurologic disorders, abortions, etc. (Okur-Gunusova et al., 2007; Tutuncu and Yazici, 2016). Intrauterine infections with BVDV during the first trimester of pregnancy result in the birth of persistently infected animals (PI). Furthermore, another highly fatal form of infection is known as mucosal disease (Raizman et al., 2011).

BHV-1 is known to cause several diseases in cattle, including rhinotracheitis, conjunctivitis, vaginitis, balanoposthitis, pneumonia and abortions in ruminants. It is classified under the Varicellovirus genus of the Alphaherpesvirinae subfamily belonging to the Herpesviridae family (Okur-Gunusova et al., 2007; Shirvani et al., 2012; Yazici et al., 2015; Yilmaz et al., 2016). Similar to other herpesviruses, BHV-1 leads to lifelong latent infections and intermittent shedding of the virus (Patil et al., 2017).

Both viruses are also involved in the bovine respiratory disease complex with other viral and bacterial pathogens and lead to respiratory diseases with major economic loss. Although respiratory symptoms are common, BVDV and BHV-1 also cause multi-sytemic disorders which have significant influence on milk and meat production as well as reproductivity. Accordingly, it is explicit that updated information is needed regarding the prevalence of BVDV and BHV-1 for screening and controlling of diseases. The aim of this study was to investigate and update seroconversions for two critical viruses in large and small ruminants throughout small scale enterprises in the Black Sea Region of Turkey.

MATERIALS AND METHODS
Samples: In this study, six governorates from the Black Sea Region of Turkey were selected for sampling (Figure 1). Blood samples (5ml) were randomly collected from cattle (n=482), sheep (n=402) and goats (n=140) from 192 enterprises having between 1 to 20 heads. All animals were apparently healthy, ≥ 1 year old, and non-vaccinated. Blood samples were centrifuged at 2000 rpm for 10 minutes and sera were aliquoted into sterile tubes and stored under -20 °C until used after inactivation at 56 °C for 30 minutes.
A comparison of differences in seroprevalence values detected for BVDV and BHV-1 in given provinces was evaluated for statistical significance using the Chi-square analysis method. The critical value of “p” for statistical significance was p < 0.05.

RESULTS AND DISCUSSION

This survey showed that 29.17% (299/1025) of sampled animals were carrying the neutralizing antibody with 29.25%, 29.28% and 28.27% distribution parameters for cattle, sheep, and goat, respectively (Table 1).

Overall individual seroprevalence of BVDV and BHV-1 was determined as 19.90% and 13.56%. While BHV-1 seropositivity in cattle, sheep and goat was 17.01%, 10.17% and 11.42%, BVDV seropositivity for cattle, sheep and goats was 18.25%, 22.08%, 19.28%, respectively (Table 1).

A total of 24.87% of sampled animals were seropositive for one virus, BVDV (15.60%) or BHV-1 (9.27%). Seroprevalence of BVDV and BHV-1 as one virus infection was 12.24% and 10.99% for cattle; 19.10% and 7.19% for sheep; and 17.14% and 9.28% for goat, respectively. Dual seropositivity for both viruses occurred in 4.26% of the sampled animals. Of dual seropositive samples, 6.01%, 2.97% and 2.14% were cattle, sheep, and goat, respectively and were statistically significant (p < 0.05 and p < 0.01)(Table 2). The results were also statistically significant (p < 0.01 and p < 0.05).

All goat and cattle enterprises were BVDV seropositive. The highest BVDV seroprevalence was detected in goat enterprises in Samsun (45%) followed by sheep enterprises in Trabzon (34.88%) and finally cattle enterprises in Ordu (28.39%).

The BHV-1 antibodies were detected in all cattle enterprises, with the exception of Ordu. Similar to BVDV, the highest seropositivity BHV-1 seroprevalence was obtained from goat enterprises in Rize (45%) followed by sheep and cattle enterprises in Trabzon (39.53% and 33.33%) (Table 2). Furthermore, no seropositivity for BHV-1 could be detected in goat enterprises in Samsun and Tokat.

Dual seropositivity was not detected in cattle enterprises in Rize and sheep enterprises in Ordu. In addition, dual seropositivity occurred in sheep enterprises in Samsun, Giresun and Trabzon as well as all cattle enterprises with the exception of Rize.

A present 22.7% of Turkey’s population is composed of rural residents who work in agriculture and

Table 1: Percentage distribution of seropositivity rates by animal species according to one virus and both viruses mixed. Pos/tot*: a ratio of positive species samples to total numbers of animal species.

<table>
<thead>
<tr>
<th>Seropositivity</th>
<th>Cattle Pos/tot</th>
<th>Sheep Pos/tot</th>
<th>Goat Pos/tot</th>
<th>Overall Pos/tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV only</td>
<td>59/482 (12.24%)</td>
<td>77/403 (19.10%)</td>
<td>24/140 (17.14%)</td>
<td>160/1025 (15.60%)</td>
</tr>
<tr>
<td>BHV-1 only</td>
<td>53/482 (10.99%)</td>
<td>29/403 (7.19%)</td>
<td>13/140 (9.28%)</td>
<td>95/1025 (9.26%)</td>
</tr>
<tr>
<td>Dual</td>
<td>29/482 (6.01%)</td>
<td>12/403 (2.97%)</td>
<td>3/140 (2.14%)</td>
<td>44/1025 (4.29%)</td>
</tr>
<tr>
<td>Total</td>
<td>141/482 (29.25%)</td>
<td>118/403 (29.28%)</td>
<td>40/140 (28.57%)</td>
<td>299/1025 (29.17%)</td>
</tr>
</tbody>
</table>

α (P<0.05) and β (P<0.01) .

Cell and virus strains: Cell lines and virus strains were provided from cells and virus collections from the Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Turkey. Before commencing work, medium, fetal serum (FCS) and cell lines were screened for non-cytopathogenic (ncp) pestivirus contamination risk.

Madin Darby Bovine Kidney (MDBK) cells were used in all stages of this study as previously described (Yazici et al., 2015). MDBK cells were grown in Dulbecco’s Modified Eagle medium (DMEM, Gibco) supplemented with 10% FBS, 1% antibiotics and 1% L-glutamine. BVDV type 1 NADL strain and BHV-1 Cooper strain were used in this study. All strains were grown on MDBK cells with DMEM supplemented with 2% FBS and the supernatant was collected when the obvious cytopathic effect (CPE) was observed. The supernatant was clarified by centrifugation at 3000 rpm for 5 min. Virus suspension was aliquoted and stored at – 80°C until used.

Infectivity assay: BVDV and BHV-1 were titrated as previously described (Yazici et al., 2015). Briefly both viruses were diluted ten-fold into dilutions in DMEM supplemented with 2% FBS. 100 µl of each dilution were put into quadruplicates in 96 well plates (TPP, Switzerland). Then 50 µl of 3.0 x 10⁵ MDBK cells were added to each well and plates were incubated at 37°C for 72 hours, and in a humidified incubator with 5% CO₂. After incubation 50% tissue culture infective dose (TCID₅₀) was calculated as TCID₅₀/ml.

Serology: Virus Neutralization (VN) was conducted on separate plates for BVDV and BHV-1 in order to detect neutralizing antibodies in sera as previously described (Yazici et al., 2015). Briefly, 1/2 and 1/5 dilutions of all serum samples for BVDV and BHV-1, respectively, were prepared in 96 well plates using 50 µl of each serum diluted in DMEM containing 2% FBS. 100 TCID₅₀ of both viruses was then added to the corresponding well and incubated at 37°C for 1 hour in a humidified incubator with 5% CO₂. Finally, 50 µl of 3.0 x 10⁵ MDBK cells were added to each well and plates were incubated at 37°C for 72 hours. The results were evaluated according to whether CPE was present or not.

Statistical analysis: A comparison of differences in seroprevalence values detected for BVDV and BHV-1 in
Table 2: Percent seropositivity distribution of BVDV and BHV-1 rates according to provinces and animal species.

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Species</th>
<th>Tokat</th>
<th>Samsun</th>
<th>Ordu</th>
<th>Trabzon</th>
<th>Giresun</th>
<th>Rize</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV</td>
<td>Cattle</td>
<td>22.22</td>
<td>16.66</td>
<td>28.39</td>
<td>7.69</td>
<td>25.92</td>
<td>3.77</td>
<td>18.25</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>33.33</td>
<td>20.42</td>
<td>0.00</td>
<td>34.88</td>
<td>26.25</td>
<td></td>
<td>22.08</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>16.66</td>
<td>45.00</td>
<td></td>
<td>10.00</td>
<td></td>
<td>20.00</td>
<td>19.28</td>
</tr>
<tr>
<td>BHV-1</td>
<td>Cattle</td>
<td>6.17</td>
<td>15.74</td>
<td>8.64</td>
<td>33.33</td>
<td>32.09</td>
<td></td>
<td>17.01</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>13.33</td>
<td>6.25</td>
<td>3.33</td>
<td>39.53</td>
<td>5.00</td>
<td></td>
<td>10.17</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>1.66</td>
<td>0.00</td>
<td></td>
<td>15.00</td>
<td></td>
<td>45.00</td>
<td>11.42</td>
</tr>
</tbody>
</table>

α (P<0.05) and β (P<0.01), * Sample could not be taken.

Fig 1: A map of provinces where serum samples were collected. Sample size for each governorate are shown in parenthesis.

animal husbandary (Yilmaz, 2015). Around 94% of livestock breeding farms in Turkey are small-scale enterprises with a concentration between 1 and 20 heads (Koseman and Seker, 2015). BVDV and BHV-1-related disorders lead to negative impacts on these enterprises due to major economic loss (Chamorro et al., 2014).

In Turkey, while BVDV subtypes 1a, 1b, 1d, 1f, 1h, 1g, 1l and 2a have been previously detected in the circulation, BVDV 1a, 1b, and 1l have been reported as more prevalent than other subtypes (Yesilbag et al., 2008). In many countries as well as Turkey, the cp NADL strain of BVDV, known as BVDV 1a, is preferred for serological studies. In this context, the NADL strain is also used in our study.

The seroprevalence of both viruses in ruminants was previously reported in Turkey as 14.3-86% for BVDV and 9.25-74% for BHV-1 (Albayrak et al., 2007; Yesilbag and Gungor, 2009; Albayrak et al., 2016). Current research shows that the seroprevalence of both viruses, 19.90% for BVDV; 13.56% for BHV-1, was also similar to the aforementioned results. However, high seroprevalence rates regarding BVDV in small ruminants (22.08%) and goats (19.28%) were considerable as they were higher than that of cattle (18.25%). Furthermore, all goat enterprises were detected to be seropositive. High BVDV seroprevalence in small ruminants, in particular sheep, possibly indicates border disease (BD) among these animals. It is well-known that BVDV may also be a more common cause of BD in some parts of the world, although border disease virus (BDV) is the most common cause of BD in many parts of the world. Other interpretations for the high seroprevalence are thought to be inter-species transmission of BVDV between goats, sheep and cattle keeping together in the same barn as well as grazing in the same pasture (Broadus et al., 2007). Other reasons could be the import of biological products in particular, fetal bovine serum (FBS) that is used to propagate cell culture (Yesilbag et al., 2008) and the use of modified live vaccines that are produced by using primary ovine or bovine cell cultures having contamination risks with ncp pestiviruses to control other viral infections (e.g. peste des petits ruminants, sheep and goat pox etc.) (Aslan et al., 2015).

The seroprevalence of BHV-1 revealed that the highest seropositivity rate was obtained in cattle (17.01%) followed by goats (10.17%) and sheep (11.42%) as expected. It is concluded that BHV-1 circulates in screened enterprises. Current results for small ruminants were remarkably high compared to previously recorded results in Turkey (Albayrak et al., 2007, Yesilbag and Gungor, 2009). Like previous results, BHV-1 seroprevalence in goats (11.42%) was found to be slightly higher than that in sheep. Furthermore there was an increasing seroprevalence of BHV-1 in sheep compared to previous studies conducted by Albayrak et al. (2007) in the same regions. These results depend on a close antigenic relationship between BHV-1 and caprine herpesvirus type -1 (CpHV-1), keeping cattle, goats and sheep together, enviromental conditions, animal movement and an increasing number of the import cattle. These are the main factors that allow for the transmission of BHV-1 from cattle to sheep and goats and vice versa (Mahmoud and Ahmed, 2009).

BVDV and BHV-1 still pose a threat for small enterprises due to many factors e.g. uncontrolled animal movement, poor nutrition, immunosupression, biosecurity, environmental factors. It is important that improving our knowledge about the epidemiology of economically important diseases will improve our means to properly address them. As a result, we recommend that preventative and control measures should be taken for both viruses including readjustment of by-laws for animal imports, regular herd screening for detecting...
and removing BVDV persistent and BHV-1 latent animals, restrictions for uncontrolled animal movements and the establishment of national eradication or control programs.

**CONFLICT OF INTEREST**

The authors declare that there are not conflicts of interest in this work.

**REFERENCES**


