

Seroprevalence of bovine respiratory viruses in North-Western Turkey

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Abstract Bovine respiratory disease complex is a very important health problem around the world. Present study describes serological distribution of bovine major respiratory viruses in non-vaccinated cattle population of Marmara region in north-western Turkey. Neutralising antibodies specific to bovine viral diarrhoea virus (BVDV), bovine herpesvirus 1 (BHV-1), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (PI-3), bovine adenovirus serotype 1 (BAV-1) and serotype 3 (BAV-3) were investigated. Among 584 serum samples collected from 39 establishments in 7 provinces, 41.4% were positive for BVDV, 17.1% for BHV-1, 73.0% for BRSV, 43.0% for PI-3, 89.5% for BAV-1 and 92.3% for BAV-3. There were significant differences observed between seroprevalence rates detected in neighbouring provinces. Serological prevalence of BVDV, BHV-1 and BRSV were extremely higher in large capacity dairy farms than of small capacity farms ($p < 0.0001$). This study demonstrates that herd capacity is a very important risk factor for respiratory viruses and, on the other hand bovine adenoviruses and BRSV are the common reason of respiratory diseases in the region.

Keywords Bovine respiratory viruses · BRSV · PI-3 · BVDV · BHV-1 · Adenoviruses · Serological screening

Abbreviations

BAV	Bovine adenoviruses
BHV-1	Bovine herpesvirus 1
BRD	Bovine respiratory disease
BRSV	Bovine respiratory syncytial virus
BVDV	Bovine viral diarrhoea virus
FCS	Foetal calf serum
PI-3	Bovine parainfluenza virus 3
TCID ₅₀	Tissue culture infective dose
VNT	Virus Neutralization Test

Introduction

Bovine respiratory disease (BRD) complex is a very important health problem for cattle industry world wide. BRD impairs animal welfare, causes excessive use of antibiotics and increases the rate of death or obligatory culling in calves as well as young stock. Hence, an important economical impact on cattle production is generated. Viruses that commonly involved in BRD cases are bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (PI-3), bovine herpesvirus 1 (BHV-1), bovine viral diarrhoea virus (BVDV) and bovine adenoviruses (BAV). Although respiratory and faecal shedding was demonstrated (Hasöksüz et al. 2002) the role of bovine

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coronavirus (BCV) in clinical respiratory diseases is not clear and only few cases were reported to be associated with BCV (Heckert et al. 1990). Some other viruses like rhinoviruses, enteroviruses and reoviruses may also play a secondary role in the etiology of respiratory diseases in cattle (Stott et al. 1980). Management and environmental conditions are highly related to bovine respiratory diseases, moreover the other infectious agents like bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, *Haemophilus somnus* and *Salmonella dublin*) and *Mycoplasma* spp. (e.g. *M. bovis* and *M. dispar*) are frequently contribute to respiratory viruses for generation of BRD (Valarcher and Haaglund 2006).

In Turkey where approximately 30% of inhabitants are employed in agricultural production facilities, cattle production is distributed onto different geographical regions whereas a great population of culture breed dairy cattle are allocated in the Marmara region in north-west of the country. The presence of infections caused by different respiratory viruses in Turkey has been previously demonstrated (Alkan et al. 2000; Yeşilbağ et al. in press). The aim of this study is to define seroprevalence and distribution of bovine major respiratory viruses in 7 provinces in the Marmara region of Turkey.

Materials and methods

Animals and sample collection

For serological screening, a total of 584 cattle sera from dairy establishments located in Marmara region were used. There are 11 provinces in the region, of which 7 provinces namely Bursa, Balıkesir, Çanakkale, Yalova, Bilecik, Tekirdağ and Edirne were included in this study. Bursa and Balıkesir provinces are the main locations for dairy cattle production. Distributions of samples into locations are in Fig. 1. Sampled animals were randomly selected from the herds which voluntarily joined to this survey. Both small capacity family farms (n=31) and intensively managed dairy herds (n=8) were included. Small capacity farms where some of them also contained sheep and goats included less than 20 cattle, however, intensively managed herds consisted of more than 50 cows. There was no vaccination program in those of establishments applied against the viruses examined in this study. Sampled animals were Holstein-Frisian and older than 1 year old. There was no clinical disorder in the animals recorded at the sampling time conducted between May 2004 and December 2005. Blood samples were collected into vacutainer tubes and serum was separat-

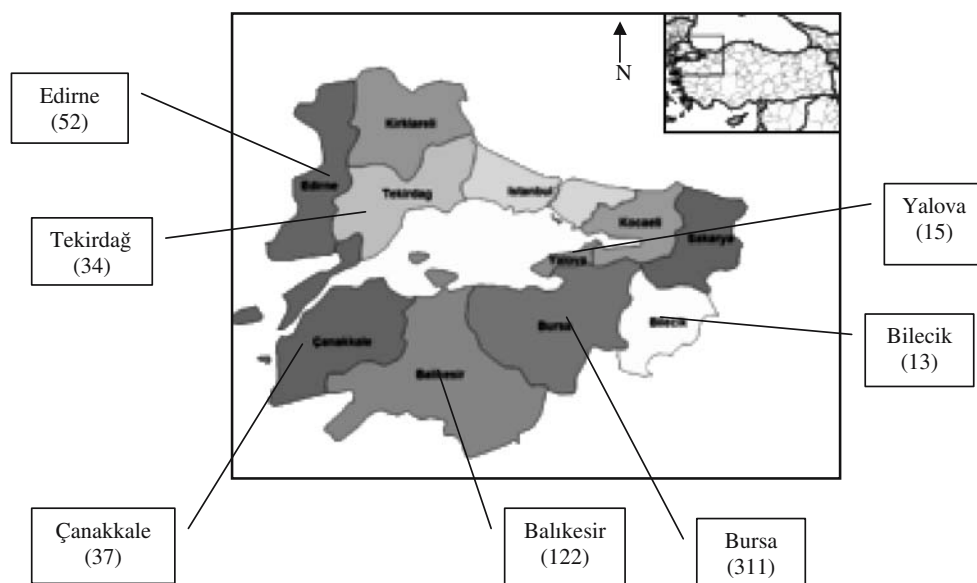


Fig. 1 Distribution of serum samples collected from seven provinces in Marmara region of Turkey

ed by centrifugation at 1800 \times g at 4°C for 10 min. After heat inactivation at 56°C for 30 min serum samples were stored at –20°C until testing.

Viruses and cell cultures

Major respiratory viruses causing infection in cattle were used in this study. NADL strain of BVDV, Cooper strain of BHV-1 and Atue strain of BRSV were used in virus neutralization test (VNT). BVDV, BHV-1, PI-3, BAV serotype 1 and BAV serotype 3 strains were originated from Department of Virology at Ankara University Faculty of Veterinary Medicine, Ankara- Turkey, while BRSV strain was supplied from Institute for Virology at Justus-Liebig University Faculty of Veterinary Medicine, Giessen-Germany. BRSV were propagated in Bel-26 diploid cell line, however MDBK cell line was employed for propagation and neutralization steps of other viruses. Cell cultures were grown in Dulbecco's MEM supplemented with 10% foetal calf serum (FCS). Cell lines and FCS were pre-tested to be free from indigenous BVDV contamination.

Serological examinations

Serological screening was performed using a Virus Neutralization Test (VNT) (Yeşilbaş et al. 2003) with some modifications. For that purpose serum samples were pre-diluted to be used in VNT in following dilutions: 1:2 for BHV-1 and BRSV; 1:5 for BVDV and PI-3; and 1:10 for BAV serotypes. Fifty micro-litres of diluted serum sample was mixed with an equal volume of virus suspension (100TCID₅₀) in 96-well microtitre plate wells as duplicates and conse-

quently incubated in a 5% CO₂ atmosphere at 37°C. Two hours incubation was applied for BHV-1 while it was 1 hour for the other viruses. Thereafter, MDBK cell suspension (3×10^5 cells /ml) was added into each well in a volume of 50 μ l. In the VNT performed for anti-BRSV antibodies, Bel-26 cell cultures were previously prepared in 96-well plates. After incubation of virus suspension with diluted serum samples in a transfer plate; cell culture medium in 96-well plates was removed and 100 μ l of virus-serum mixture was inoculated onto cells as duplicates. Test results were evaluated in an inverted light microscope after 5–7 days of incubation in 5% CO₂ atmosphere at 37°C. All the viruses used in present study are in cytopathogenic nature, thus, inhibition of virus growth indicated by non-destroyed monolayers of cell cultures was evaluated as indicator of virus neutralization.

Statistical analyses

Statistical significance of differences in seroprevalence values between locations were analysed using Chi-square analysis and Fischer's exact test where it is appropriate (GraphPad InStat V2.02). Same analyses were also applied to carry out the significance among the seroprevalence values detected against different viruses in a given location.

Results

Results obtained by serological examinations are summarised in Table 1. Overall seroprevalence values for the viruses BVDV, BHV-1, BRSV, PI-3, BAV-1

Table 1 Seropositivity rates of viruses detected in different locations

Location	Number of enterprises	Number of sampled animals	Seropositivity rates (%)					
			BVDV	BHV-1	BRSV	PI-3	BAV-1	BAV-3
Bilecik	3	13	41.6	0.0	75.0	38.4	66.6	83.3
Yalova	3	15	13.3	13.3	78.5	53.3	85.7	92.6
Bursa	11	311	29.4	21.4	71.9	43.9	80.7	86.7
Balıkesir	8	122	62.2	10.0	53.7	31.3	89.3	93.4
Çanakkale	2	37	64.8	24.3	97.2	48.6	91.8	89.1
Edirne	10	52	34.6	5.8	65.7	41.1	91.4	87.7
Tekirdağ	2	34	75.0	21.2	76.6	20.5	86.4	97.0
Total	39	584	41.4	17.1	73.0	43.0	89.5	92.3

and BAV-3 were 41.4%, 17.1%, 73.0%, 43.0%, 89.5% and 92.3%, respectively. Antibodies against all of the tested viruses were detected in each province with one exception, Bilecik province, where no BHV-1 specific antibodies were detected. There were significant differences between seroprevalence values obtained in different locations. Extremely significant differences were observed for BVDV antibody prevalence in different provinces ($p < 0.0001$). The same situation existed in the two main dairy production areas Bursa and Balıkesir where the neighbouring provinces are. Seroprevalence to other viruses in these two provinces were also significantly different ($p < 0.05$). There was no significant difference between seroprevalence of BAV-1 and BAV-3 as well as between BVDV and PI-3, while the overall seroprevalence of the other viruses detected in Marmara region was significantly different from the each other ($p \leq 0.0001$). Antibody prevalence of BVDV, BHV-1 and BRSV was significantly higher in large capacity farms ($p < 0.0001$) where no significant difference was observed for PI-3 and BAV-3 ($p > 0.05$).

Among 584, twenty seven animals were detected to have antibodies against all the viruses investigated while only 6 animals were free of antibodies to those of viruses (Fig. 2). Most commonly, antiviral antibodies were detected against 4 viruses in the same animal ($n=188$) which was followed by triplet detection of antiviral antibodies in 164 animals. Only 25 animals were found seropositive against one virus. Three animals had antibodies only against BRSV and one animal was positive only for PI-3 antibodies.

Serological test results were further confirmed in selected samples using a commercially available ELISA kit (Pentakit Respiratory ELISA kit, Bio-X Diagnostics, Belgium). Similar results were obtained in the confirmatory analysis.

Discussion

In most cases respiratory viruses are the preliminary cause of respiratory distress in cattle establishments. Presence of BVDV, BHV-1, PI-3 and adenovirus infections in respiratory illness of calves in Turkey has previously been virologically investigated (Alkan et al. 2000). A serological screening conducted in closely managed high capacity state farms was also reported (Alkan et al. 1997). Present study was performed to demonstrate, for the first time, the infection status in small capacity family farms as well as in privately owned high capacity dairy herds in Marmara region where is the main dairy production area in Turkey.

Antibodies to bovine respiratory viruses were detected in all of 39 dairy establishments located in 7 provinces indicating bovine respiratory infections are distributed in the region. There were great variations in the prevalence values of different viruses, for example the overall prevalence of BHV-1 was 17.1% while it was 92.3% for BAV-3 (Table 1). Statistically important differences among prevalence of individual viruses may be affected by different factors e.g virus spread and type of breeding etc.

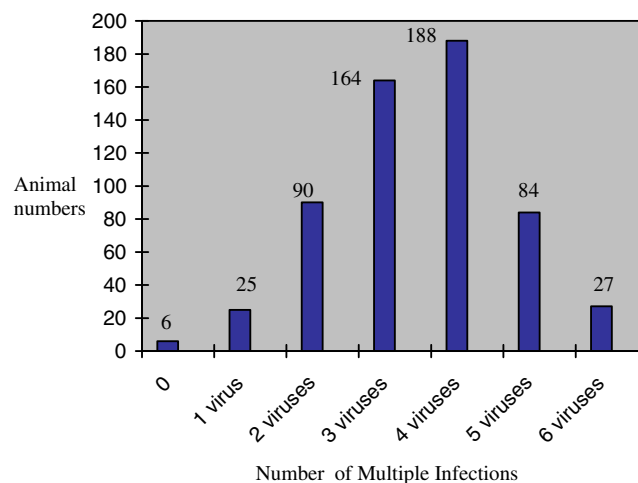


Fig. 2 Multiple seropositivity numbers among 584 animals against bovine respiratory viruses

Contrary to previous studies performed in different regions in Turkey (Alkan et al. 1997; Yavru et al. 2005; Gumusova et al. 2007), the most common respiratory viruses in north-western Turkey are adenoviruses which have a seroprevalence of 92.3% for BAV-3 and 89.5% for BAV-1. Similar results were also observed in Finland where among the evaluated herds 100% were infected with BAV-7 and 83% with BAV-1 (Hartel et al. 2004). The seroprevalence of BAV-3 was statistically higher than of other viruses except BAV-1 ($p < 0.0001$). The close relationship detected between the seropositivity rates of BAV-1 and BAV-3 is possibly due to belonging to the same serological group (group I) of bovine adenoviruses (Table 2).

The second common virus was BRSV to which a great importance is attributed in clinical respiratory disease and pneumonia cases (Uttenthal et al. 1996; Larsen et al. 2001). Comparing to adenoviruses and BRSV, the other viruses (BVDV, BHV-1 and PI-3) existed a lower degree of seroprevalence in the study area ($p < 0.0001$). PI-3 is the other common respiratory viral pathogen in many countries (Abondo et al. 1999; Fulton et al. 2000; Hartel et al. 2004; Autio et al. 2007). In present study, PI-3 had an antibody prevalence of 43.0% indicating a moderate distribution of this agent in Marmara region. A relationship between PI-3 and BVDV in respiratory infections was reported (Fulton et al. 2000). Accordingly not significant difference between seroprevalence rate of PI-3 and BVDV was detected in this study.

The results of this study showed that BHV-1 infections are not very common in this region. This data is not in accordance with the previous reports from different parts of the country (Alkan et al. 1997; Çabalar and Can Şahna 2003; Yavru et al. 2005; Gumusova et al. 2007). Because lots of our samples were obtained from privately owned and small

capacity farms, a detailed survey of BHV-1 comparing large and small capacity farms in this region may be valuable. On the other side it has to be taken account that some of animals having antibodies to BHV-1 and BVDV may be infected not by respiratory but via reproductive tract.

As causing digestive and reproductive infections as well as respiratory symptoms, pestivirus infections are very important for cattle and other ruminants. In a previous study (submitted to publication) we detected a seroprevalence of 45.8% against BVDV-1 NADL strain and 5.8% against BVDV-2 Gi-2 strain among cattle in this region. Results of various studies are consistent that pestivirus infections are common in Marmara region of Turkey (Alkan et al. 1997; Yeşilbaş et al. *in press*). Immunosuppressive effects of BVDV may accelerate the respiratory infections; moreover BVDV impairs pulmonary clearance mechanism which is a very important barrier for viral and bacterial infections (Lopez et al. 1982; Potgieter et al. 1984)

There were significant differences between seroprevalence of a given virus in different provinces. That kind of differences was detected especially for BVDV and BRSV. Interestingly Balıkesir and Bursa, the neighbouring provinces, showed statistically extremely significant differences in prevalence rate of five viruses (BVDV, BHV-1, BRSV, PI-3 and BAV-1). This heterogeneity may be related to regional density of dairy establishments as well as relatedness between individual herds.

Multiple detection rates of viral antibodies was also analysed in this study (Fig. 2). Quadruple detection of viral antibodies was very common which was followed by triple and double combinations. Only 6 animals were negative for antibodies to all the analysed viruses while 25 animals had antibody to one virus. That situation may be described by multi-agent nature of the etiology of respiratory diseases

Table 2 Comparison of seroprevalence of bovine respiratory viruses in small and large capacity dairy herds

Capacity of herd*	Number of enterprises	Number of sampled animals	Seropositivity rates (%)					
			BVDV	BHV-1	BRSV	PI-3	BAV-1	BAV-3
Large	8	384	72.8	27.4	87.9	43.3	94.3	95.4
Small	31	200	36.6	14.2	65.9	42.8	87.4	90.7
p**			<0.0001	<0.0001	<0.0001	>0.05	0.0195	>0.05

*: Small capacity herds included less than 20 cows while large capacity herds included more than 50 animals

** : Degree of significance between seroprevalence values detected in small and large capacity herds

(Autio et al. 2007). Multiple antibody detection in an animal may be due to contribution of these viruses in a BRD case (Hagglund et al. 2006) meanwhile those of antibodies might be generated by individual virus infections caused in different time intervals.

Detection of statistically significant differences in serological prevalence of BVDV, BHV-1 and BRSV in large capacity dairy herds indicates that herd size is an important risk factor for those of infections.

Present study shows that infections caused by bovine respiratory viruses are very common in Marmara region, north-west of Turkey, where is the most popular dairy cattle production area. Although respiratory diseases cause slight decrease in milk yield, as a herd health problem that kind of infections threat calves and young stock which is very important for continuation of the herd and profitability of production. Despite high prevalence of seropositive animals awaited to limit circulation of infections in the field condition, low protective efficiency of neutralizing antibodies and short duration of mucosal immunity allows repeated respiratory infections in cattle herds. Thus, protective measures like vaccination and good management practises may be beneficial in this region. This study also confirms that along with BRSV and PI-3, bovine adenoviruses are common reason of bovine respiratory diseases.

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