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SEROPREVALENCE OF BOVINE VIRAL RESPIRATORY DISEASES

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In this study, sera from 188 unvaccinated cattle from Middle Black Sea Region of Turkey were investigated. Serum samples were tested for antibodies against five viruses which cause respiratory diseases in cattle, including bovine herpesvirus type 1 (BHV-1), bovine viral diarrhoea virus (BVDV), parainfluenzavirus type 3 (PIV-3), bovine adenovirus type 1 (BAdV-1) and bovine adenovirus type 3 (BAdV-3) by using a conventional method, i.e. the serum neutralization (SN) test.

The antibody seroprevalence found in cattle against 5 viruses (BHV-1, BVDV, PIV-3, BAdV-1 and BAdV-3) were found to be: 61.17%, 53.19%, 88.82%, 72.34% and 81.38%, respectively.

Key words: antibodies, cattle, respiratory viruses, sera neutralization test

INTRODUCTION

Five viruses including bovine herpesvirus type 1 (BHV-1), bovine viral diarrhoea virus (BVDV), parainfluenzavirus type 3 (PIV-3), bovine adenovirus type 1 (BAdV-1) and bovine adenovirus type 3 (BAdV-3) are important agents which cause respiratory tract infections (Alkan *et al.*, 1997; Akca *et al*., 2004; Peters *et al*., 2004). Beef and dairy cattle breeding is of economical importance for many regions in Turkey, and these viral infections are responsible for considerable economical losses in the cattle industry.

BVDV is widespread throughout the world and infections with BVDV have a wide clinical spectrum from mild to moderate subclinical forms to the highly fatal form known as mucosal disease (Stahl *et al*., 2002; Aly *et al*., 2003). Transplacental infection occurs during pregnancy and early infected foetuses may become immunotolerant to and persistently infected (PI) with BVDV (Obando *et al*., 1999; Stahl *et al*., 2002).

Bovine herpesvirus type 1 (BHV-1) causes infectious bovine rhinotracheitis (IBR), characterized by clinical symptoms of the upper respiratory tract such as conjunctivitis and nasal mucopurulent discharge, appetite loss, reduced milk yield and abortions (Alkan *et al.*, 1997; Aly *et al*., 2003; Mweene *et al*., 2003; Yesilbag *et al*., 2003; Rajkhowa *et al*., 2004).

Parainfluenzavirus 3 virus (PIV-3) causes subclinical infections and clinical symptoms arise when secondary pathogens are present (Radotitis *et al*., 2000).

Bovine adenovirus type 1 and type 3 (BAdV-1 and BAdV-3) are also important respiratory pathogens and form acute or subacute viral diseases in cattle characterized by pyrexia, nasoocular discharge and pneumonia (Akca *et al*., 2004). In this study, we report the serological profile of five viruses which result with respiratory disease in unvaccinated cattle in Samsun Province of Northern Turkey.

MATERIAL AND METHODS

Serum samples: Serum samples were obtained from 188 unvaccinated cattle of both gender, between 1-3 years of age, from herds with a history of respiratory disease in three different rural areas of the Samsun Region in Turkey. The blood samples were transported to the laboratory immediately upon venipuncture, centrifuged at 3000 rpm, inactivated at 56°C for 30 min. and stored at -20°C for subsequent analysis.

Cell cultures: Madin Darby Bovine Kidney cells (MDBK) were used for propagation, titration, and serum neutralization (SN) test. Cells were maintained at 37°C in Dulbecco's minimal essential medium (DMEM, PAA, Inc, Austria) containing 10% heat inactivated foetal bovine sera (FCS, PAA Inc, Austria).

Viruses and infectivity assays: Five viruses including BHV-1 (Cooper Strain), BVDV (NADL strain), PIV-3 (SF4, German strain), BAdV-1 (strain 11/66) and BAdV-3 (strain 13/66) were used in this study. The test was performed as previously described by Frey and Liess (1971). Titer of the virus was calculated on the basis of CPE determination, as 50% tissue culture infective dose ($TCID_{50}$/0.1 μL) described by Kaerber (1964).

Seraneutralization test (SN): To investigate the antibody profile against BHV-1, BVDV, PIV-3, BAV-1, and BAV-3 in serum samples, the SN method was carried out as previously described by Frey and Liess (1971). Serum samples, excluding BHV-1 were diluted; BAV-1 and BAV-2 to 1/10 and BVDV and PI-3 to 1/5. The results were evaluated according to the cytopathologic effects (CPE).

RESULTS

Infectivity of the viruses were calculated $10^{7.0}$ for BHV-1, $10^{5.0}$ for BVDV, $10^{4.75}$ for PIV-3, $10^{5.0}$ for BAdV-1 and $10^{4.75}$ $TCID_{50}$/0.1 ml for BAdV-3. The results are presented in Table 1.

The results of neutralising antibody prevalence against 5 viruses in cattle are presented in Table 2. Overall percentages of BHV-1, BVDV, PIV-3, BAdV-1 and BAdV-3 antibodies in cattle were determined as 61.17%, 53.19%, 88.82%, 72.34% and 81.38%, respectively.

Table 1. The $TCID_{50}$/0.1 ml and 100 $TCID_{50}$ /0.1 ml values for BHV-1, BVDV, PIV-3, BAdV-1, BAdV-3

Viruses	$TCID_{50}$/0.1 ml	100 $DKID_{50}$/0.1 ml
BHV-1	10^7	10^5
BVDV	10^5	10^3
PIV-3	$10^{4.75}$	$10^{2.75}$
BAdV-1	10^5	10^3
BAdV-3	$10^{4.75}$	$10^{2.75}$

Table 2. Seropositivity results against five viruses in 188 unvaccinated cattle in the Middle Black Sea Region, Northern Turkey*

Viruses	No of Tested unvaccinated Cattle*	Serum Neutralisation		Prevalance (%)
		Positive	Negative	
BHV-1	188	115	73	61.17
BVDV	188	100	88	53.19
PIV-3	188	167	21	88.82
BAdV-1	188	136	52	72.34
BAdV-3	188	153	35	81.38

*($p<0.05$)

Only one cattle (0.53%) did not possess antibodies against any of the studied viruses, while 13 cattle (6.91%) carried antibodies related to a single infection with one of the viruses (Table 3).

Table 3. Single infections seropositivities (n=188)

Viruses	Single infections number	
	No of positive animals	Seropositive (%)
PI-3	7/188	3.72
BAdV-1	2/188	1.06
BAdV-3	3/188	1.59
BHV-1	1/188	0.53
BVDV	–	–
Total	13/188	6.91

Multiple (double, triple, quartet and five viruses) infection seropositivity obtained at the end of the study were 59.04%, 58.5%, 39.3% and 35.8%,

respectively. Highest multiple infection rates were determined against two and three agents (Figure 1).

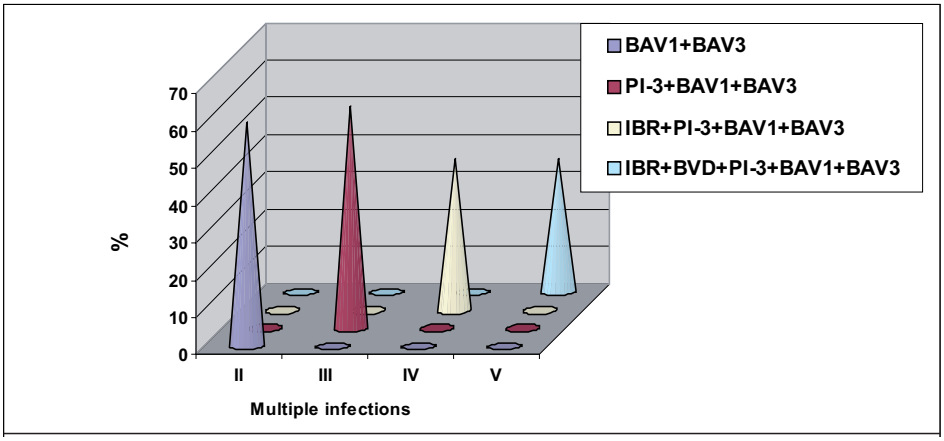

Figure 1. Multiple infection rates

DISCUSSION

In our study, results of the antibody profile against 5 viruses (BHV-1, BVDV, PIV-3, BAdV-1 and BAdV-3) in 188 unvaccinated cattle were 61.17%, 53.19%, 88.82%, 72.34% and 81.38%, respectively. These results indicate that these five viruses must be circulating amongst these animals, because, all of the 188 cattle were adults and no vaccine was used against these viruses.

Previous studies performed in the various regions of our country reported 22.04-56.00%, 14.20-55.00%, 11.00-53.93%, 52.40-57.08% and 44.09-62.00% intervals for BAdV1, BAdV3, PI-3, BHV1 and BVDV, respectively (Alkan *et al*., 1997; Çabalar and Can Sahna, 2000; Yavru *et al*., 2005). Similar BVDV rates were observed when data of our study was compared with previous reports. Furthermore, BHV1 infection rates in and around the Samsun province reported in the present study were similar to the data reported by Yavru *et al*. (2005). Besides, we observed that in the region we investigated, PI-3, BAdV1 and BAdV-3 caused infections more frequently, compared to the regions described in other studies.

BHV1, BVDV, PI-3, BAdV1 and BAdV3 results reported by various researchers in other countries are between 14.3-67.0%, 36.0-76.2%, 94.0-94.4%, 33.9%, 87.4%, respectively (Ghirotti *et al*., 1991; Heally *et al*., 1993; Pernthaner *et al*., 1990). When these results are compared with the presented data, they indicate that BHV-1, BVDV, PI-3 and BAdV-3 infections occur in similar rates, but BAdV-1 infections are encountered more frequently in and around the Samsun province.

Especially, BHV-1 seropositive unvaccinated cattle may reflect BHV-1 carriers, because, following primary infection with BHV-1, the virus remains latent

and it may be reactivated and spread to susceptible cattle (Obando *et al*., 1999). Furthermore, seropositive unvaccinated cattle indicated a persistent infection with BVDV and PI in cattle is a major source of BVDV infection, thus being an infection risk for healthy unvaccinated animals (Aly *et al*., 2003). Damages in the genital system and pregnant animals, as well as infections in the respiratory system caused by these two viruses are of economic importance. Therefore, the prevalence of these two infections in healthy herds should be investigated and strategies to eradicate these infections should be developed.

Alkan *et al*. (1997), in their antibody surveillance study against 9 viruses (IBR, PI-3, BRSV, BVDV, BAdV1, BAdV2, BAdV3, Enterovirus1 and enterovirus 2), determined 9.38%, 11.46% and 72.012% infection rates against single, double and 3-8 viruses, respectively. Yavru *et al*. (2005) reported 14.7%, 36.22%, 29.92%, 14.56%, 3.93%, 1.57% and 0.39% seropositivity against one, two, three, four, five, six and seven viruses, respectively. Lauchli *et al*. (1989), determined 1/4 single infection rate and ¾ multipl infection rate in the seroprevalance study concerning BRSV, BAdV1, BAdV4, Coronavirus, BVDV and PI-3. In the present study, single, double, triple, quartet and five virus infection rates were 6.91%, 59.04%, 58.5%, 39.3% and 35.8%, respectively. The obtained data indicates the widespread presence of multiple infections.

Finally, the results obtained from our serological study reflected an antibody profile against 5 viruses causing respiratory infections. Like many other countries, it is necessary to practice effective eradication programs for the protection against BHV-1 and BVDV infections. Vaccination, which is an important step in these programs, should also be applied to animals in rural areas. Designation of the vaccination programs, application of the required hygene rules and informing the owners will be the first steps in the prevention from these diseases.

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SEROPREVALANCIJA VIRUSNIH RESPIRATORNIH BOLESTI GOVEDA

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SADRŽAJ

U ovom radu su prikazani rezultati seroloških ispitivanja sprovedenih kod 188 nevakcinisanih grla goveda iz srednjeg Cromorskog regiona u Turskoj. Serumi su ispitivani na prisustvo antitela protiv 5 virusa koji izazivaju respiratorna oboljenja goveda: goveđeg herpesvirusa tipa 1 (BHV-1), virusa goveđe dijareje (BVDV), parainfluenca virusa tipa 3 (PIV-3), goveđeg adenovirusa tipa 1 (BAdV-1) i goveđeg adenovirusa tipa 3 (BAdV-3). Za analize je korišćen standardni serum neutralizacioni (SN) test.

Seroprevalenca ispitivanih antitela protiv 5 virusa (BHV-1, BVDV, PIV-3, BAdV-1 i BAdV-3) je iznosila 61.17%, 53.19%, 88.82%, 72.34% i 81.38%, respektivno.