The seroprevalence of BHV-1 infection on selected dairy cattle herds in Turkey

F. ALKAN1*, I. BURGU1, S. BILGE-DAGALP1, Y. YILDIRIM3, A. GENCAY2, B. GÜNGÖR4, V.S. ATASEVEN5 and Y. AKÇA1

1 Ankara Univ, Faculty of Veterinary Medicine, 06110 Diskapi Ankara, Turkey
2 Kafkas Univ, Faculty of Veterinary Medicine, Kars, Turkey
3 Erciyes Univ, Faculty of Veterinary Medicine, Kayseri, Turkey
4 Uludağ Univ, Faculty of Veterinary Medicine, Bursa, Turkey
5 Yüzüncü Yıl Univ, Faculty of Veterinary Medicine, Van, Turkey
* Corresponding author: e-mail: falkan@veterinary.ankara.edu.tr

SUMMARY
In this study, serum samples of 13011 cattle from 31 dairy herds located in various regions of Turkey were tested for presence of neutralising antibodies against BHV1 using the serum neutralisation (SN) technique. It was found that 97% (30/31) of the herds selected had seropositive animals and the frequencies of seropositivity were between 0.5-79.5% in the surveyed herds. Furthermore, recommendations to the control and eradication programme of the BHV1 infection were presented.

Keywords : BHV1 - cattle - seroprevalence - Turkey.

RÉSUMÉ
Séroprévalence de l’infection par le BHV1 dans l’élevage bovin laitier en Turquie. Par F. ALKAN, I. BURGU, S. BILGE-DAGALP, Y. YILDIRIM, A. GENCAY, B. GÜNGÖR, V.S. ATASEVEN et Y. AKÇA.

Dans cette étude, la présence d’anticorps neutralisants anti-BHV1 a été recherchée par une technique de séroneutralisation dans les sérums de 13011 bovins issus de 31 fermes laitières localisées dans différentes régions de Turquie. 97% des troupeaux testés (30 sur 31) ont comporté des animaux séropositifs, et au sein des élevages suivis, les fréquences de séropositivité ont varié de 0,5 à 79,5%. De plus, un programme de contrôle et d’éradication de l’infection par le BHV1 est proposé.

Mots-clés : BHV1 - bovin - séroprévalence - Turquie.

Introduction
Bovine Herpes Virus type 1 (BHV1), the etiologic agent of infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV), is an important pathogen of cattle and cause significant economic losses to livestock around the world. Other signs of disease, like drop in milk production, conjunctivitis, systemic disease in young calves, enteritis and mastitis are also reported [12, 17].

BHV1 belongs to the alphaherpesvirinae. The most important and characteristic property of BHV1, among this group, is latency. This means that the virus genome persists in sensory ganglions that innervate the original site of infection. Following the stress and/or application of immunosuppressive compounds such as dexamethasone, infectious virus is possibly reactivated [1, 8]. Herd prevalence of BHV1 virus infection varies from country to country and herds to herds. Serological evidence of BHV1 infection is widespread in Turkey [3, 4], and has been reported with various rates of occurrence in Italy, Austria and Netherlands [9, 11, 16]. Several countries in the European Union, such as Denmark, Sweden and Finland, have eradicated BHV1 by prohibiting vaccination, removing seropositive animals and by additional preventive measures. Besides, some countries, like Austria and Netherlands, have an European Union-approved national compulsory eradication program [9, 15].

The purpose of this study was to determine the seroprevalence of BHV1 infection in 31 selected dairy herds and to suggest a control and/ or eradication program for the BHV1 in these herds in Turkey.

Materials and methods
SAMPLED HERDS
This study was carried out in 31 dairy herds belong to Ministry of Agriculture and Rural Affairs located in 7 different regions of Turkey (Figure 1). All of the cattle over 6 months of age in these herds were sampled. It was known that these animals were not vaccinated against BHV1. These state farms are chosen since they have good sources of management, have potency for BHV1 negative cattle stock and may be a model for subsequent control programmes in private breeding farms.

CELL CULTURE AND VIRUS
BHV1 was propagated and titrated in Madin Darby Bovine Kidney (MDBK) cells. MDBK cells were also used in a microtiter serum-neutralisation test. Cells were grown in Dulbecco’s Minimal Essensial Medium (DMEM) containing 10% heat-inactivated fútal calf serum, 50 IU/mL penicillín and 50 IU/mL streptomycine.
BHV1 Cooper strain was used in the microtiter serum neutralisation test. Stock virus contained $10^6$ TCID$_{50}$ of virus/mL.

**SERUM SAMPLES**

Blood samples were collected directly into caolin coated polystyrene tubes and centrifuged at low speed for separating the serum which was inactivated at $56^\circ$C for 30 minutes and stored at $-20^\circ$C until used. Serum samples obtained from 13,011 dairy cows were tested for the antibodies against BHV1 using the micro-neutralisation test.

**SERUM NEUTRALISATION TECHNIQUE**

Undiluted serum samples were examined for antibodies to BHV1 using serum neutralisation test as previously described [6]. Briefly, every serum samples were added to two wells of microtiter plates with flat bottom. BHV1 (0.05 mL) in DMEM containing 100 TCID$_{50}$ was added to each well used and the plates were incubated at $37^\circ$C for 2 h. Aliquots of 0.05 mL of MDBK cells (3X10$^5$ cells/mL) were added to each well, then the plates were incubated at $37^\circ$C in 5% CO$_2$ humidified atmosphere for 5 days and were evaluated for the presence of cytopathogen effect characterised as rounding of cells and lysis by daily microscopic examination.

**STATISTICAL ANALYSIS**

Chi - square test was employed to analyse the statistical significance (P<0.05) of seroprevalence differences between the herds belonging to different geographic regions or not.

**Results**

Serological screening of the herds revealed that 30 out of 31 herds (97%) presented animals with neutralising antibodies against BHV1. The overall percentage of the seroconversion was found to be as 53.2% among the animals tested (n = 13011). Detected seroprevalence values varied between 0.5-79.5% among herds (Figure 1, Table I).

BHV1 seroprevalence was the highest in the Southeast Anatolia region, and was estimated as 73.8%. Following seropositivity rates were detected as 69.2%, 62.9%, 55.5%, 48.3%, 40.4 % for Aegean, Mediterranean, Central Anatolia, Blacksea and Marmara regions, respectively. Seropositivity rate was the lowest in the Eastern Anatolia region, detected as 23.8%. Seropositivity among 7 regions was found to be significant (P<0.001).

Also, BHV1 seroprevalence greatly varied from herd to herd in the same region (Table I). Differences among seroprevalence values of herds were detected to be significant (P<0.05) either for the herds in the same or in the different 7 geographic regions. Overall situation of the statistical analysis was as follows ; confidence intervals for herds in Central Anatolia, Blacksea, Eastern Anatolia and Marmara regions were P<0.001. On the other hand, these intervals were detected as p<0.01 for Mediterranean and Southeast Anatolia. Finally, confidence interval for Aegean Region was P< 0.05.

**Discussion**

BHV-1 outbreaks can lead to considerable economic losses due to emaciation of affected cattle or deaths, reductions in milk yield, mastitis, metritis, abortions and calf losses in cattle herd differentiated for beef units, dairy units or breeding [12, 17].

In Turkey, prevalence and etiological role of BHV1 in certain kinds of clinical features as respiratory tract infection and mastitis detected in closed dairy herds have been reported previously [2, 3, 4]. ALKAN et al. [2] reported that BHV1 was detected as an aetiological agent from cattle with respiratory disease symptoms. In this research, BHV1 positivity rate was detected as 13.6%. BILGE [4] reported that...
BHV1 isolation was obtained from one of the milk samples of 96 cows with mastitis. Results of this survey indicated once more that the prevalence of the infection in cattle herds monitored is as high as previously reported. Seropositivity rates of BHV1 infection were reported as 59.7% and 74% by ALKAN et al. \[3\] and BILGE \[4\], respectively. ALKAN et al. \[3\] have detected that BHV1 seropositivity rates were to be found as 59.7% on 480 sampled animals housed in 10 different herds. BILGE \[4\] also reported that seroprevalence value was 74% on 486 sampled animals housed in 10 different herds. Seropositivity rates were between 4% - 98% and 27%- 96% among sampled herds in these respective studies \[3, 4\].

Our study has showed that BHV1 seroprevalence greatly varied from herds to herds and also from region to region. It is known that some factors such as type of breeding, herd size, age of animals, geographic area, and disease control measures can act upon seroprevalence values \[10, 14, 18\]. In this study, seropositivity rates were found to be 23.8-73.8% according to the regions. These rates were statistically significant (P<0.001). The lowest seropositivity rate (23.8%) was detected in Eastern Anatolia region in which the weather is very cold at least half of the year. It is possible that it can be an effective factor for limiting the spreading of infection, leading to a low seropositivity value.

Our study showed that seropositivity rates varied from herd to herd. BHV1 seroprevalence was particularly low in 9 herds (≥ 20). One of herds (N° XIX, Central Anatolia Region) was negative for infection and 8 of them (N° VIII, XXVI, in Eastern Anatolia region ; X, XI, XXI, XXIV, in Marmara region; XVI, XVII, in Blacksea) had low seroprevalence levels (between 0.5-17.3%). Rest of the herds have seropositive animals in rate of 43.5-74.3%, except one herd (N° XXI) (Table I). Herd managers noticed that the specific control program for BHV1 infection had not been used before this project in any of these herds. Results of statistical analysis have shown statistically significant differences among seroprevalence values about herds either in the same region (P<0.05) or in different regions (P<0.001). However, more information is needed to understand the effect of some factors (management conditions, etc.) on the BHV1 seroprevalence into herd. Additionally, it was observed that in smaller herds (between 99 and 256 animals), the rates of BHV1 prevalence were lower (0.5- 4%) than in larger herds (Table I). This agrees with some previous reports \[10, 14\]. Low number of animals housed in a herd has positive effect on management, and consequently on seroprevalence detected in these herds.

It is known that the use of inactivated BHV1 vaccine is an useful tool to protect cattle against BHV1 induced disease by keeping up a high level of protection and by reducing the spread of field virus \[13\]. But, for the BHV1 infection eradication, two different programs will be developed : one of them is the elimination of all seropositive cattle from herds. This program was particularly indicated in herds or in countries with low seropositivity. The other one depends upon the use of marker vaccines, which allow to serologically differentiate vaccinated from field virus infected animals, and secondary the elimination of infected cattle by wild BHV1 \[5, 7, 13\].

**Table I.** — The BHV1 seroprevalence of BHV-1 infection obtained from serum neutralisation technique in herds surveyed.
According to the serological results observed in this study, we suggest the use of marker vaccines in these herds, and the vaccination program using marker vaccines has started. It plans that in following years, the status of infection would be monitored with the use of companion diagnostic technique and that cattle seropositive for wild virus strains would be eliminated from herds. Additionally, farm managers would be informed about the importance of sanitary measures, which allow to prevent the introduction of BHV1 into herds and to control and eradicate BHV1. According to this plan, seropositivity for wild virus will decrease in these herds.

In this study, the prevalence of BHV1 infection was investigated in sampled herds and the procedure for infection control/eradication was suggested. When collected data will be sufficient, the influence of this vaccination program of cattle on wild BHV1 prevalence will be discussed later.

References