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# The Seroprevalence of Bovine Herpes Virus type 1 (BHV-1) and Bovine Leukemia Virus (BLV) in Selected Dairy Cattle Herds in Aydın Province, Turkey

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**Abstract:** The prevalence of BHV-1 and BLV antibodies was studied in 313 serum samples collected from 5 dairy cattle herds. Serum samples were collected from both apparently healthy animals and animals with respiratory symptoms. The samples were tested for the presence of antibodies against BHV-1 and BLV using virus neutralization and agar gel immunodiffusion test, respectively. The results indicated that the prevalence of BHV-1 and BLV infections was 19.5% and 0.3%, respectively.

**Key Words:** BLV, BHV-1, seroprevalence, cattle

## Aydın Yöresindeki Süt Sığırcılığı İşletmelerinde Bovine Herpes Virus 1 (BHV-1) ve Bovine Leukemia Virus (BLV) Enfeksiyonlarının Serolojik Olarak Araştırılması

**Özet:** Bu araştırmada, Aydın İli ve çevresinde özel sektöre ait 5 adet kapalı süt sığırcılığı işletmesinde bulunan sağlıklı görünümlü ile solunum sistemi semptomları gösteren 313 adet inekten kan örneği alınarak, mikronötralizasyon testi ile Bovine Herpesvirus tip 1 (BHV-1) ve Agar Gel Immunodiffusion Testi (AGID) ile Enzootik Bovine Leukosis (EBL) antikorlarının varlığı araştırılmıştır. Test edilen 313 adet kan serumunun 61 (% 19,5) adedi BHV1 antikorları, 1 (% 0,3) adedi EBL antikorları yönünden pozitif bulunmuştur.

**Anahtar Sözcükler:** BLV, BHV-1, seroprevalans, sığır

## Introduction

Infectious Bovine Rhinotracheitis (IBR) and Enzootic Bovine Leukosis (EBL) are economically important infections of dairy cattle.

The causative agent of IBR is classified by Bovine herpesvirus type 1 (BHV-1) of the subfamily Alphaherpesvirinae, Herpesviridae (1). The most important epidemiological feature of the herpesviruses is the ability to establish latency in the regional ganglia after the primary infection. Latently infected animals are thought to be lifelong carriers of the virus. Reactivation and spread of the virus may occur as a result of stress

factors such as transport, vaccination, birth, extreme climatic changes, and corticosteroid administration for medical purposes (2-5).

BHV-1 infections cause great economic losses due to a variety of pregnancy pathologies including abortions, and early embryonic and fetal deaths, which result in infertility, decreased milk production and weight losses in dairy herds (6). The disease is worldwide and has been reported in some European countries with high prevalence, such as Belgium (7), Holland (8), Germany (9), Italy (6) and France (9). On the other hand, Sweden, Austria, Switzerland and Denmark have reported

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successful control strategies of the disease by achieving the detection and ultimately eliminating the seropositive animals from the herd (10).

The disease was first reported in Turkey in 1971 with detectable neutralizing antibodies against BHV-1 in cattle (11). Following this, numerous scientists detected the disease both serologically and virologically. The seroepidemiological characteristics of the disease have been studied by various investigators all over the country so far, and some of them have reported that significantly high prevalence (more than 50% of the animals) of the virus has been detected in closed-type dairy cattle herds (12-14).

The most validated approach to control the infection is the use of inactivated BHV-1 vaccine in herds. The eradication of BHV-1 infection is adopted from 2 different programs. The first is based on the elimination of animals detected to be positive for BHV-1 antibodies by sensitive test systems such as ELISA and virus neutralization (5,7,10,15). This strategy is mostly acceptable for the herds and/or countries with low seropositivity. The second strategy depends on the use of marker vaccines, which allow one to serologically differentiate vaccinated from field virus infected animals, thus enabling the elimination of infected cattle by wild BHV-1 from the herd (15).

Enzootic Bovine Leucosis (EBL) is an economically important viral infection of cattle characterized by malign neoplastic lymphatic disease including tumors in the lymph nodes, changes in blood content and increasing circulating leukocytes, and is common worldwide in dairy herds as a persistent infection of adult cattle (16).

The agent belongs to the subfamily Deltaretrovirinae of the family Retroviridae and is called Bovine Leukemia Virus (BLV). In natural conditions transmission of the infection is horizontal through milk of infected females and iatrogenic factors such as dehorning, and rectal palpation. The results of epidemiological and experimental studies have revealed that the possibility of vertical transmission is negligible (16,17).

It is well known that, like other slow virus infections, EBL infection also spreads slowly over long periods. Once the cattle population is infected by this virus, the achievement of eradication is thought to be difficult. Various control and eradication programs based on detection and follow-up culling of seropositive cattle and

development of proper sanitation regulations to prevent the transmission of BLV have been in use at some State farms in Turkey over the last 5 years (18).

The aim of this study was to investigate the seroprevalences of BHV-1 and BLV not only in cattle with respiratory symptoms but also in apparently healthy cattle from 5 private dairy herds in Aydın province, using Virus Neutralization (VN) and Agar Gel Immunodiffusion (AGID) tests.

## Materials and Methods

### Animals Used

A total of 313 cattle reared on 5 private intensive dairy herds in Aydın province were used in the study. Of these animals, 71 (22.6%) were less than 6 months old. Herd questionnaires revealed that BHV-1 vaccine administration was not performed in the herds investigated. Distribution of the sample animals is shown in Table 1.

Table 1. The number of animals used for the study in herds.

Herd Code	Number of		
	Cows	Calves	Total
I	27	14	41
II	43	18	61
III	53	16	69
IV	94	23	117
V	25	-	25
Total	242	71	313

### Virus and Cell Culture

The Cooper strain of BHV-1 was used in the VN test for detecting the BHV-1 antibodies in serum samples. Madin Darby Bovine Kidney (MDBK) cell culture was used for propagation and titration of the reference virus and for VN.

### Serologic Tests

VN was performed in order to detect BHV-1 specific antibodies in undiluted serum samples as described elsewhere (19). The mean antibody titers (Serum Neutralization 50; SN<sub>50</sub>) of positive samples were then

calculated using the same method. AGID was carried out as described by Frenzel and Kaaden (20). Serum samples were screened against the BLV gp51 (Seromed D7503) in reference to anti-gp51 antiserum (Seromed D-7104).

## Results

Out of 313 serum samples, 61 (19.5%) were positive for BHV-1 antibodies, while only 1 (0.3%) was positive for BLV antibodies in herd II (Table 2).

The seroconversion rates of BHV-1 in herds were 54.7% for herd I, 14.8% for herd II, 8.7% for herd III, 18.8% for herd IV and 8% herd V (Table 2). Among the group of animals less than 6 months old, 22.5% (16/71) of them were antibody carriers for BHV-1. The mean neutralizing titers of the positive samples ranged from 1:1 to 1:128, and the overall geometric mean of the neutralizing antibody was 1:20.7. Meanwhile, 12 of the seropositive calves had seroconverted dams, indicating the maternal origin of antibodies. Since the remaining dams (n: 4) of the seropositive calves were negative, either postpartum infection or tank milk feeding were postulated to be the likely reason for the seroconversion in calves (Table 3).

## Discussion

Symptoms of BHV-1 may be associated with respiratory and genital tract symptoms, conjunctivitis, enteritis, encephalomyelitis, dermatitis and mastitis, and may lead to a decrease in the use of dietary nutrients and an increase in mortality in young animals or economic losses due to weight loss, decrease in milk production, fetal lethality or abortions (3,9,21).

Table 2. The seropositivity rates of BHV1 and BLV infections according to the farms.

Herd Code	Number of sampled animals	Number (%) of seropositive animals for	
		BHV1	BLV
I	41	22 (54.7)	-
II	61	9 (14.8)	1 (1.6)
III	69	6 (8.7)	-
IV	117	22 (18.8)	-
V	25	2 (8)	-
Total	313	61(19.5)	1(0.3)

There are many studies (12-14,22) on the prevalence of IBR-IPV infection in Turkey. Akça (12) screened BHV-1 antibodies in 487 blood sera taken from different regions of Turkey using neutralization and reported that 233 (54.46%) of the serum samples were positive for BHV-1 specific neutralizing antibodies. Burgu and Akça (13) reported 55.73% prevalence for BHV-1 antibodies on a State farm. More recently, Çabalar (14) examined blood sera collected from cows with fertility problems in 20 different dairy herds located in various regions of Turkey and reported that 68.1% of the samples (425 out of 624) were seropositive. Moreover, that study also pointed out the presence of IBR-IPV infection in all herds and the prevalence of the infection ranged from 6.66% to 100% on a herd basis. Bilge (22) reported that 74% of the serum samples from 10 dairy cattle herds were positive for BHV-1 specific neutralizing antibodies.

In the present study, 19.5% seropositivity was detected among the sampled population. However, the prevalence of the infection was low when compared with data from previous studies in Turkey. The effective control mechanism in breeding bull stations, importation of high quality certificated sperm and practicing breeding techniques more intelligently in this area are thought to be the main causes of the lower prevalence rate. However, the rate of prevalence still suggests the importance of the disease for breeding in this area.

Seropositive individuals are considered potentially infected since latent persistence is a characteristic of BHV-1 infection (2,4,5). On the other hand, seropositivity observed in calves may occur as a result of passive transfer of the immunoglobulins via colostrum from the

Table 3. The seroprevalence of BHV1 in adults and in animals <6 months years old.

Herd Code	Number of		Number (%) of BHV-1 Seropositive	
	Cows	Calves	Cows	Calves
I	27	14	14 (51.85)	8 (57.14)*
II	43	18	9 (20.93)	0 (0)
III	53	16	3 (5.66)	3 (18.75)**
IV	94	23	17 (18.09)	5 (21.74)*
V	25	-	2 (8)	-
Total	242	71	45 (18.6)	16 (22.5)

\* One of which has a seronegative dam.

\*\* Two of which have seronegative dams

mother. Thus, it would be more meaningful to consider the seropositive animals and seropositive calves whose mothers are seronegative while expressing the prevalence. In this study, 49 animals (45 seropositive adults plus 4 seropositive calves with seronegative dams) out of 313 may be considered latently infected. Therefore, the prevalence may be redefined as 15.65%.

In this study, 16 animals less than 6 months old were positive. The retrospective analysis of mother-calf pairs revealed that 4 seropositive calves were from naive dams for BHV-1. This situation may suggest a postnatal natural infection in these 4 animals within 6 months of birth. When this high spread ratio of infection is considered, the need for more serious control and production measures (vaccination, hygiene, slaughtering etc.) becomes more obvious.

Sampled farms are in the Holstein-Friesian Breeding Association and keep imported breeds. Besides the sampled population, regular controls of the records have been done by the association. According to the data given by breeders, upper respiratory infections are frequent in these sampled herds. On the other hand, in spite of these high prevalence rates, examinations based on association recordings and breeder information revealed that genital tract problems (such as fetal-embryonal deaths, abortions or repeat breeder) did not exist or occurred rarely and this is in contrast with the literature data (21,23).

EBL infection was first detected in Turkey with its clinical and pathological symptoms in imported high productive, pedigree Holstein and Swedish milking cows on a State farm (24). Seroepidemiological studies showed

that EBL infection was present in dairy cattle herds in Turkey (18,25-27). Burgu et al. (28) performed a pilot project on BLV eradication from 31 State farms. In this project, firstly all 31 dairy herds were monitored and it was found that 48.3% (15/31) of herds selected had seropositive animals and positivity rates were 0.5%-34.4% in these herds. Afterwards, an EBL eradication program depending on identification and culling of the seropositive animals was used in the infected herds (28). In this study, one of the 313 animals serologically tested was positive for EBL antibody and the seroprevalence rate among sampling populations was 0.3%.

There are significant differences in prevalence rates between the results obtained from different studies. This may be due to differences in the cattle populations sampled and management practices. In addition, direct and indirect effects of herd size on BLV seropositivity were shown by some researchers (29,30). The results of this epidemiological survey provide additional evidence that in general BHV-1 infection is widespread in cattle herds; however, the presence of BLV infection is at lower rates in the population sampled in Aydın province.

It is considered that management factors in addition to breeding models and herd size may be the cause of the low seropositivity rate for BLV infection in the small herds sampled in this study.

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