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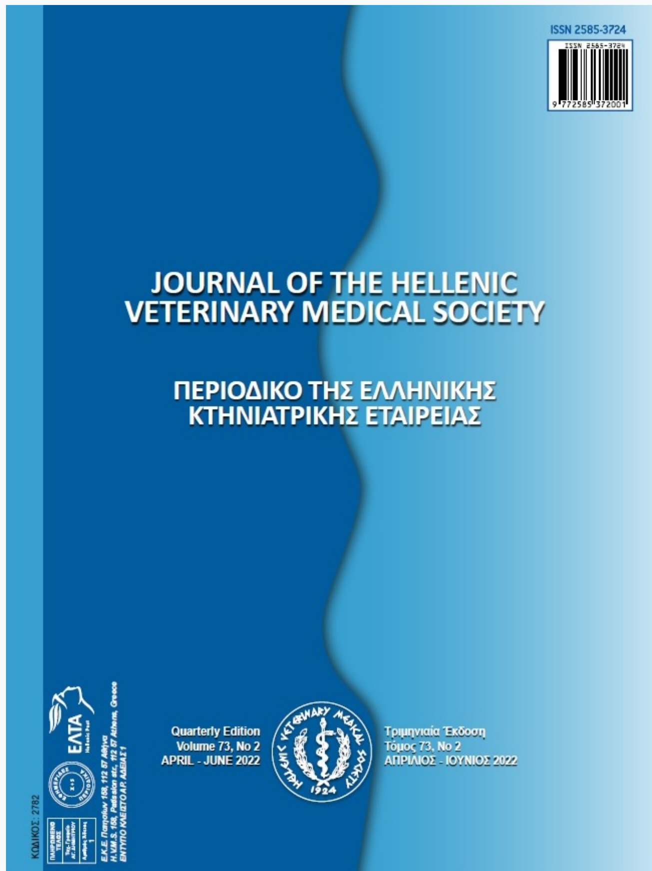

A Cross-sectional study on the transmission dynamics of Bovine Herpesvirus-1 infection in the farms located in the Thrace Region of Turkey

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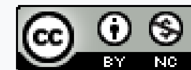

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Research article
Ερευνητικό άρθρο

A Cross-sectional study on the transmission dynamics of Bovine Herpesvirus-1 infection in the farms located in the Thrace District of Turkey

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ABSTRACT: In this study, detection of BHV-1 infections in cattle by using polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) and investigation of the risk factors associated with the spread of the virus within the herd were aimed. Three dairy farms were selected in the Thrace district, Marmara region, Turkey, and two visits to these farms were made. A questionnaire was prepared for the farmers. On the first visit, 4 (14.81%) of 27 animals in Farm 1, 6 (28.57%) of 21 animals in Farm 2, and 3 (7.31%) of 41 animals in Farm 3 were found to be seropositive for BHV-1. On the second visit, 6 (25%) of 24 animals in Farm 1, 6 (35.29%) of 17 animals in Farm 2, and 6 (20%) of 30 animals in Farm 3 were found to be seropositive for BHV-1.Thirteen (14.6%) of 89 blood samples were seropositive at the first sampling and 18 (25.35%) of 71 blood samples in the second sampling. Two calves belonging to 7 cows that were found to be seropositive at the first visit, and the calves of four of 10 mothers found to be seropositive at the second visit were also found seropositive.BHV-1 DNA was detected in only 3 of the 43 milk samples taken during the first and second visits (2 on the first visit, and 1on the second visit), and 13 of 160 nasal swabs (8 on the first visit, 5 on the second visit). All the cows with three milk samples whose BHV-1 DNA was detected by PCR were determined as seropositive. Three of the eight cattle with BHV-1 DNA detected in nasal swabs on the first visit, and 4 of the five cattle with BHV-1 DNA detected in nasal swabs on the second visit were detected seropositive. The statistical analysis has shown that the association between age, sex, and BHV-1 seropositivity was statistically significant. The BHV-1 seroprevalence was increased in animals on the second visit in all farms.The preventive measurements need to be applied in Turkey to control BHV-1 infections in cattle.

Keywords: Bovine herpesvirus-1; PCR; ELISA; risk factors; Thrace, Turkey

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INTRODUCTION

Bovine herpesvirus-1 (BHV-1) is the etiologic agent of infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis and balanoposthitis (IPB) in cattle. IBR is an economically important disease of cattle and has been reported in many countries affecting cattle health and livestock production by causing respiratory disorders, early embryonic deaths, abortus, infertility, mastitis, decreased milk production and weight loss.

The BHV-1 is an enveloped DNA virus and classified in the family *Herpesviridae* within the subfamily *Alphaherpesvirinae* (Murphy et al., 1999).The genome consists of UL and US regions, and the genes code 70 proteins. Virus has 10 glycoproteins (gB, gC, gD, gE, gG, gH, gI, gK, gM ve gL) and 3 of those, gB, gC and gC are in the envelope. BHV-1 has one serotype, but antigenic differences were found among isolates and those subtypes differentiated as BHV-1.1, BHV-1.2 (BHV1.2a and BHV-1.2b) (Murphy et al., 1999).

BHV-1.1 is generally responsible for respiratory disorders and abortionsin cattle, while BHV-1.2 causes balanoposthitis and pustular vulvovaginitis. Infected cattle are the primary source of infection; those cattle spread the virus *via* all secrets and excretes, including milk, semen, and vaginal fluid (Murphy et al., 1999; Wentink et al., 1993). Transmission occurs via respiratory and venereal routes by direct and indirect contact. Therefore, in closed breeding systems, the virus spreads very quickly among the nearest animals (Murphy et al., 1999; Wentink et al., 1993).

The incubation period for the respiratory and genital forms of BHV-1is between 2-6 days (Jones and Chowdhury, 2007).The highest virus titer was found 4-6 days after viral entry (Wentink et al., 1993). Abortions may co-occur as a respiratory disease but may occur up to 100 days after infection (Jones and Chowdhury, 2007).The maternally derived antibodies protect calves from BHV-1 infection for about 4 to 6 months. As a result, natural infections are generally seen after four months (Bilge-Dagalp et al., 2001). The genome of the BHV-1 in latency was found in trigeminal and/or sacral ganglions and tonsils (Winkler et al., 2000).The virus reactivates when the animals are exposed to stress and cortisol given parenterally. As a result of reactivation, viral spread occurs, and transmission to other cattle may occur (Wentink et al., 1993; Winkler et al., 2000).

The disease was first reported in Turkey in 1971 by Erhan and others (1971). The BHV-1 seropositivity rates among cattle were reported to be between 0 and 100 % in different regions of Turkey (Bilal et al., 1995; Cabalar and Can-Sahna, 2000; Gur et al., 2016; Ozgunluk and Yildirim, 2017; Yilmaz et al., 2018). These studies focused on the serological prevalence of the disease rather than risk factors associated with the spread of BHV-1 infections. This study aimed to investigate factors affecting the spread and transmission of the BHV-1 among animals in the farms. It was also aimed to compare the ELISA and PCR results to detect the infected animals indeed since finding the positive animals correctly is vital in controlling the BHV-1 infections.

MATERIALS AND METHODS

Study population and collection of samples

The farms analyzed in this study were selected according to the farmer's willingness. Also, for the ethical issue, international, national, and institutional guidelines (University of Istanbul Ethical Committee instructions) for the care and use of animals were followed. Two visits with six months intervals were made to 3 dairy farms located in the Thrace district (European border) of Turkey. In each visit, all the animals were examined clinically. Nasal swabs, blood to collect sera, and milk samples were taken on each visit (Table 1). Nasal swabs and milk samples were analyzed by PCR for the presence of BHV-1 DNA

Table 1. Number and type of samples collected from farms

Farm No	Visits	Sample Type and Number			Number of Pregnant Animals	Number of Animals Sold	Number of Newly Attending Cattle
		Sera	Nasal Swabs	Milk			
1	First	27	27	3	9		
	Second	24	24	10	1	12	**9**
2	First	21	21	4	7		
	Second	17	17	6	4	11	**7**
3	First	41	41	9	7		
	Second	30	30	11	4	18	7
	TOTAL	**160**	**160**	**43**	**32**	**41**	**23**

Farm 1: Kesan Kilic Village. Farm 2: Kesan Musellim Village Farm 3: Catalca Nakkas Village

and sera were analyzed for the presence of antibodies to BHV-1 by ELISA.

ELISA

The sera were separated and analyzed by ELISA (HerdChek, IBR g (B) antibody test kit) to detect antibodies to BHV-1. The method was followed as described by the manufacturer (IBR-g (B) AntibodyTest Kit, IDEXX Herd Check Laboratories, Westbrook, ME, USA).

DNA extraction

The nasal swabs were placed into sterile tubes containing 1 ml sterile PBS. They were squeezed, and the cotton swabs were taken out. The fluid was kept at -20 °C for PCR. From each cow, 25-30 ml milk was taken into a sterile container and centrifuged at 4000 g for 10 minutes. The precipitate and supernatant were kept separately at -20 °C till analyzed.

DNA was extracted from controls and test samples using a commercial DNA extraction kit (NucleoSpin C+T Kit)described by the manufacturer (Macherey-Nagel, Germany). The fluid from nasal swab samples and supernatant from milk samples was briefly centrifuged at 12 000 g for 5 minutes. 20 µl was taken from both precipitate and supernatant into the sterile Eppendorf tube. 25 µl proteinase K was added and mixed by vortexing. They were then kept in a thermoblock at 56 °C for 1.5 hours by vortexing every 10-15 minutes. 200 µl buffer-3 was then added by vortexing. 210 µl ethanol was then added after incubating at 70 °C for 10 minutes. The procedure was completed following the steps indicated in the kit. DNA was also extracted from the 10 µl of positive control reference strains, Cooper strain (2 x $10^{7.5}$ pfu) and Turkish field isolate (4 x $10^{6.2}$ pfu) (kindly supplied by Professor Aykut Ozkul, The University of Ankara, Veterinary Faculty, Department of Virology, Ankara, Turkey) by using the similar protocol as described above. All DNA extracts were kept -20 °C till required.

Polymerase chain reaction (PCR)

Test samples were tested for the presence of BHV-1 DNA by PCR. The nucleotide sequence of primer pairs specific for BHV-1 was the same as in the study published by Van Engelenburg and others (1993). The primers (Invitrogen Corporation, UK) targeted the gC gene on UL of BHV-1. The nucleotide sequences of the primers wereas follows:F-5'-CTGCTGTTCGTAGCCCACAACG-3' and R-5'-TGTGACTTGGTGCCCATGTCGC-3' with an expected product size of 173 bp. Briefly, different amounts of primers (0.1, 0.2, 0.3, 0.4, and 0.5 µm), DNA, and Tag Master Mix (Qiagen, UK,Catalog No:1007544) containing dNTPs, Mg, and tag polymerase were used in different temperatures to optimize PCR conditions. A total volume of 50 µL standardized PCR mixture consisted of 25 µL of Tag Master Mix, 10 µL of template DNA and 1 µL of each primer (0.3 µM) and 13 µL of nuclease -free water. The mixture was then put into the thermocycler (Biometra GmbH) for amplification. Distilled water without DNA as negative control and Turkish field isolate and Cooper strain of BHV-1 were used as a positive control. The amplification was performed using the conditions as follows: 1 cycle of 95 °C for 4 min followed by 38 cycles of 95 °C for 1 minute, 60 °C for 1 minute, 72 °C for 1 minute and, finally, 1 cycle of 72 °C for 5 minutes.

Products were analyzed by 1% agarose gel electrophoresis and visualized using ethidium bromide under UV light.

The sensitivity of PCR was assessed using DNA extracted from positive controls. Serial 10-fold dilutions of DNA (BHV-1) were made in TE buffer, and 2 µL of aliquots was tested in PCR as described above. The DNA extracted from positive controls and negative control were used for specificity.

Statistical analysis

The chi-square ($\chi 2$) test was used to compare the association between seropositivity and sex, age, and race. p-Values<0.001 were considered statistically significant. Data analyses were carried out by IBM SPSS statistics version 21.

RESULTS

Clinical signs

On the first visit, mastitis was detected in one animal in Farm 1, and on the second visit, cachexia was detected in one animal in Farm 2, and no other disease symptoms were found.

ELISA

Thirteen (14.6%) of 89 blood samples were seropositive at the first sampling and 18 (25.35%) of 71 blood samples in the second sampling. The BHV-1 seroprevalence was increased in animals on the second visit (Figure 1).When evaluated on a farm basis, antibodies, on the first visit 4 of 27 (14.81 %) animals, 6 of 21 (28.7 %) animals, and 3 of 41 (7.31 %) animals were found to be seropositive in Farm1, 2 and 3, respectively (Figure 2).Antibodies to BHV-1 were

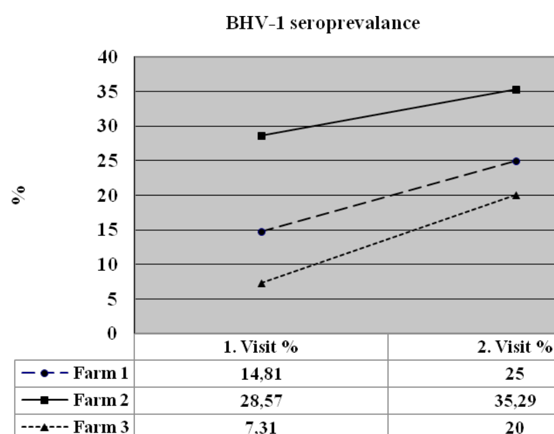

Figure 1. Comparison of BHV-1 seroprevalence on first and second visit in Farm1 Farm 2, Farm 3

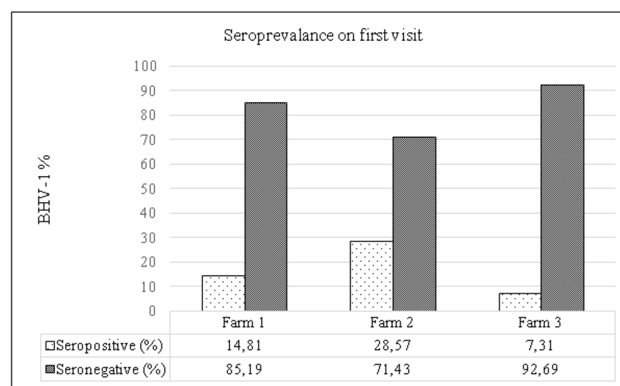

Figure 2. Seroprevalence distribution in farms on first visit

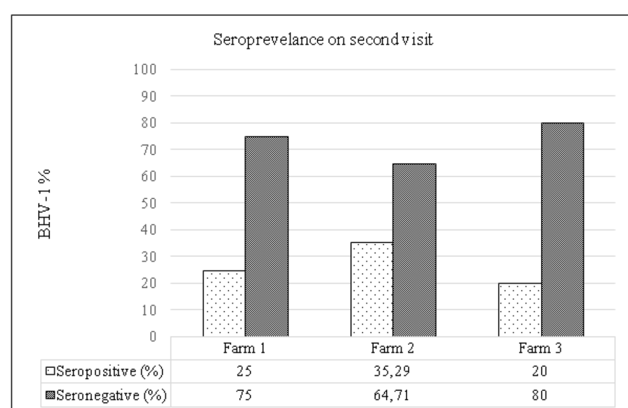

Figure 3. Seroprevalence distribution in farms on second visit

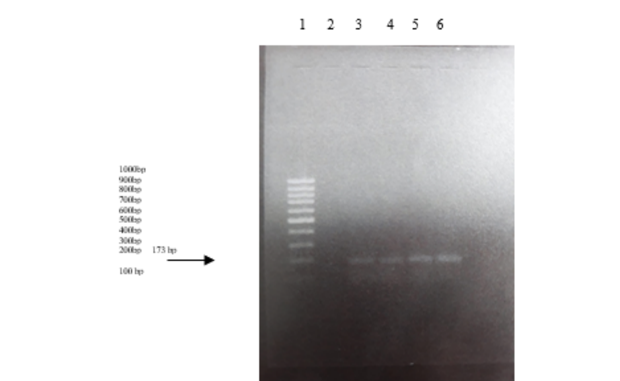

Figure 4: PCR with DNA samples extracted from field isolates and Cooper strains. 1. Molecular marker 2. Negative control 3-4. DNA extracted from field strain 5-6. DNA extracted from Cooper strain

detected in 18 (25.3 %) of 71 animals in 3 farms (Figure 3 3). On the second visit, 6 of 24 (25 %) animals, 6 of 17 (35.29 %) animals, and 6 of 30 (20 %) animals were found to be seropositive in Farm 1, 2 and 3, respectively (Figure 3).

Comparison of seropositivity at the first and second visit

When the farms were visited for the second sampling, some cattle were sold and removed from the herd, and second samples could not be taken from these. Since some calves were born and added to the herd in the second sampling, no sample was taken on the first visit. Apart from these, all animals that were seropositive in the first sampling were found to be also seropositive in the second sampling. These results are summarized in Table 2.

Seropositivity of mothers and their calves

During the sampling of the farms, information about the mother and the offspring was recorded.The presence of antibodies to BHV-1 in mothers and their calves on the first and second visit are given in Table 3.

Statistical evaluation of seropositivity

The association between sex and age BHV-1 seropositivity was statistically significant. ($p<0.001$). BHV-1 seropositivity of female cattle was higher than males. As the age got older, it was determined that the BHV-1 seropositivity increased. The association between breed and BHV-1 seropositivity was not statistically significant ($p>0.001$). The results regarding the relationship between sex, age, breed, and BHV-1 seropositivity are detailed in Tables 4, 5, and 6.

Polymerase chain reaction

PCR products of 173 bp for the BHV-1 gene were detected in the positive control strains in a standardized PCR reaction containing 2 µL of DNA and 0.3 µM of each primer. BHV-1 DNA was detected when 10 µL of test DNA was used in this standard PCR reaction. The PCR product was not seen in the negative samples or the negative controls (Figure 4).

BHV-1 DNA was detected in 8 of 89 nasal swabs

Table 2. Comparison of seropositivity on the first and second visits infarms

Farm No	Cattle No	First Visit	Second Visit	Farm No	Cattle No	First Visit	Second Visit	Farm No	Cattle No	First Visit	Second Visit
	1	+			1	+	+		1	+	+
	2	+	+		2	+	+		2	+	+
1	3	+		**2**	3	+	+	**3**	3	+	
	4	+	+		4	+			4	-	+
	5	-	+		5	+			5	-	+
	6	-	+		6	+	+		6	-	+
	7	-	+		7		+		7		+
	8		+		8		+				
Total number of seropositive cattle		4	6	**Total number of seropositive cattle**		6	6	**Total number of seropositive cattle**		3	6
Seropositivity (%)		14.81	25	**Seropositivity (%)**		28.75	35.29	**Seropositivity (%)**		7.31	20
Total number of cattle		27	24	**Total number ofcattle**		21	17	**Total number ofcattle**		41	30

Table 3. Seropositivity of mothers and their calves

	1. Visit			**2.Visit**		
	Total Mothers	Mother seropositivity	Calf seropositivity	**Total Mothers**	**Mother seropositivity**	**Calf seropositivity**
FARM1	3	1	0	**9**	**4**	**1**
FARM2	8	5	1	**8**	**4**	**2**
FARM3	6	1	1	**7**	**2**	**1**
	17	7	2	**24**	**10**	**4**

Table 4. The association between sex and BHV-1 seropositivity (p‹0.001)

Sex	Total cattle number	Positive cattle number	(%)
	First Visit		
Female	60	12	20
Male	29	1	3.45
	Second Visit		
Female	49	16	32.65
Male	22		9

Table 5. The association between age and BHV-1 seropositivity (p‹0.001)

Age (Year)	Total cattle number	Positive cattle number	(%)
	First Visit		
0-3	63	4	6.34
4-6	21	6	28.57
7-9	5	3	60
	Second Visit		
0-3	50	10	20
4-6	11	3	27.27
7-9	10	5	50

Table 6. The association between breed and BHV-1 seropositivity (p›0.001)

Breed	Total cattle number	Positive cattle number	(%)
	First Visit		
H	73	11	15.06
M	10	2	20
CB	6	0	0.0
	Second Visit		
H	48	13	27.08
M	13	3	23.07
CB	10	2	20

H:Holstein. M:Montofon. CB:Cross-Bred.

Table 7. Sex, breed and age distribution of the animals found positive for BHV-1 DNA by PCR

Farm	Breed	Age	Sex	1.Visit		2.Visit		1.Visit	2.Visit
				Nasal Swabs	Sera	Nasal Swabs	Sera	Milk	Milk
				PCR	ELISA	PCR	ELISA	PCR	PCR
1	M	7 years	F	-	+	+	+	+	-
	CB	6 months	F	+	-	-	-		
	M	1 year	F	+	-				
	CB	4 months	M			+	+		
	CB	4 months	F			+	-		
Total number of BHV-1 positive cattle in farm 1				2		3		1	**0**
2	H	6 years	F	-	+	-	+	+	-
	H	3 years	F	-	-	+	-	-	
	H	6 years	F	-	+	-	+	-	+
	H	6 years	F	-	-	+	-	-	-
Total number of BHV-1 positive cattle in farm 2				0		2		1	**1**
3	H	2 years	F	+	-	-	-		-
	H	6 years	F	+	-	-	-	-	-
	H	2 years	F	+	-	-	-	-	-
	H	5 months	M	+	-	-	-		
	H	5 Months	F	+	-				
	H	8 Months	M	+	-	-			
Total number of BHV-1 positive cattle in farm 3				**6**		**0**		**0**	**0**
TOTAL				**8**	**3**	**5**	**4**	**2**	**1**

H: Holstein. M: Montofon CB: Cross-Bred F: Female. M: Male.

and 2 of 16 milk samples from 3 farms in the first visit, but none of these animals were seropositive by ELISA. BHV-1 DNA was detected in 2 nasal swab samples and one milk sample from Farm 1, 1 milk sample from Farm 2, and 6 nasal swab samples from Farm 3.

During the second visit, BHV-1 DNA was detected in 5 of 71 nasal swabs and 1 of 27 milk samples from 3 farms, but only two were seropositive. BHV-1 DNA was detected in 3 nasal swab samples from Farm 1, 2 nasal swabs, and one milk sample from Farm 2. No BHV-1 DNA was detected in samples taken from farm 3.In the second visit, no DNA was detected in the samples taken from the positive cows for BHV-1 DNA from the first visit (Table 7). BHV-1 DNA was detected by PCR in 3 milk samples from all milk samples taken at the first (16 milk samples) and second visit (26 milk samples), and all these animals were seropositive.

DISCUSSION

BHV-1 infections cause economic losses in cattle production and therefore need to be controlled by applying preventive measurements to control the disease spread and transmission of the virus, and the prevalence in the herd level needs to be investigated. BHV-1 infections were first reported in Turkey by Erhan and others (1971). Later studies have indicated

that the prevalence of BHV-1 in Turkey is high and needs to be controlled to prevent economic losses. It is essential to find the infected animals early before antibodies can be detected. Forthis, many investigators have widely used ELISA and PCR (Barrett et al., 2018; Boelaert et al., 2000; Fuchs et al., 1999; Lemaire et al., 2001; Kramps et al., 1996; Ros and Belak, 1999).In the present study, three farms were investigated in the Thrace district in the Marmara region of Turkey, which borders the European Union, and antibodies to BHV-1 and BHV-1 DNA were detected in all three farms, and an increase in seroprevalence was determined in all three farms.

This study shows that BHV-1 infection is quite common in all three farms in the Thrace district. As explained below, a similar risk has been emphasized by others in cattle in Turkey. Cabalar and Can-Sahna (2000) obtained 471 blood sera from dairy cattle in 12 private and five public farms in Eastern and South-eastern Anatolia; they found 16 of 17 farms had BHV-1 seropositive animals. The average seropositivity rate of BHV-1 in the farms was 52.4%. In a study conducted in 7 provinces in the Marmara region, 17.1% seropositivity was identified (Yesilbag and Gungor, 2008), while 35.98 % seropositivity was detected in another study in the same region (Bilal et al., 1995). In a study conducted in 5 provinces in the Aegean Region, 17.6% of the cattle were seropositive (Gur et al., 2016).BHV-1 seropositivity was reported to be 69.7% in Afyonkarahisar (Ozel and Gur, 2015). In another study, 718 blood serum samples from cattle in 9 provinces (Adiyaman, Batman, Diyarbakir, Gaziantep, Mardin, Kilis, Siirt, Sanliurfa and Sirnak) in the South-eastern Anatolia region were analysed and 40.11 of the cattle was found to be seropositive for BHV-1 (Ozgunluk and Yildirim, 2017).The results of the studies mentioned above indicate that BHV-1 seroprevalence is very high in Turkey. These findings are like those obtained in other countries. The prevalence rates were 67% for herds and 35.9 % for cows in Belgium (Boelaert et al., 2000), 50% in Germany (Teuffert, 2006), 80% in Hungary (Palfi and Földi, 2006), before the start of their eradication programs, and 61% in unvaccinated dairy herds in Italy (Cavinari, 2006).Prevalence of Bovine Herpes Virus 1 (BHV 1) in 161 Irish beef herds was detected 90% (Barrett et al., 2018). In the present study, an association was found between the seropositivity and sex of the animals. The seropositivity was higher in females than in males. Similarly, Bilal et al. (1995) could not detect BHV-1 in 13 bulls in 428 cattle. The reason for high seropositivity in females must be associated with the artificial insemination, and this point needs to be accounted for in the control of BHV-1 infections. Higher prevalence in females was also found in other studies, as explained below. In the northern province of India (Uttarakhand), females (19.02%) had a higher incidence of BHV-1 than males (16.22%) (Vipul et al., 2015). Jain and others (2006) reported in Uttarakh and that BoHV-1 antibodies were more prevalent in females (12.35%) than males (5.80%); this was evident even at the species level for both cattle and buffalo. Saravanajayam and others (2015) also observed that the prevalence of IBR antibodies in females (67.92%) was higher than in males (33.33%). Vipul and others. (2017), Krishnamoorthy and others (2015), and Sharma and others (2009), females have detected a higher prevalence than males in southern India.

The most frequently reported risk factor for BHV-1 seropositivity is the age group; older animals have been reported to have higher seroprevalences (Segura-Correa et al., 2016). In this study, there was an association between age and seropositive cow ratio following previous studies. The seropositivity increased with the age of the animals. Similar results were obtained in other studies (Bilge-Dagalp et al., 2001; Boelaert et al., 2000; Kaddour et al.,2019; Romero-Salas et al., 2013; Segura-Correa et al., 2016; Solis-Calderon et al., 2003). All bovine breeds of any age are susceptible. However, the disease occurs in animals older than six months, possibly due to further infective agent exposure (e.g., nasal exudate and cough droplets, genital secretions, semen, fetal fluids, and tissues, etc.) and decrease in maternal immunity (Kaddour et al., 2019).

The maternally derived antibodies remain in calves for about six months (Bilge-Dagalp et al., 2001).In the present study, two calves in the first visit and four calves in the second visit, and their mothers were seropositive. It was difficult to determine that these antibodies in the calves were derived either from mother or infection. Interestingly, the calves of 5 cows found to be seropositive at the first visit and six cows found to be seropositive at the second visit were seronegative (Table 3). The reason for the seronegativity of calves born from seropositive mothers might be that either the calves could not receive colostrum or failed to absorb colostrum.

In the present study, nasal swabs and milk samples were analyzed by PCR in order to determine the virus spread. Mweene and others (1996) could detect BHV-

1 DNA in nasal andeye swabs after 19 days of experimental infection. They performed the study in acutely infected animals. In the field like in the present study, it is difficult to know the type of infection of animals (acute, chronic, or latent). Therefore, PCR was performed on nasal swabs and milk samples to determine the animals spreading virus and ELISA to determine the seropositivity. The primers targeting the gC gene were used in PCR since these primers could detect 18 field strains tested (Van Engelenburg et al., 1993). It is noteworthy that in the present study, most of the animals positive for BHV-1 DNA were seronegative by ELISA. In the first visit, BHV-1 DNA was detected by PCR in 8 of 89 nasal swabs, but none of them had antibodies to BHV-1. In the second visit, BHV-1 DNA was detected by PCR in 5 of 71 nasal swabs, but only two of these were seropositive.BHV-1 DNA was detected by PCR in 3 milk samples from all milk samples taken at the first (16 milk samples) and second visit (26 milk samples), and all of them were seropositive. This might be because of acute infection, which did not raise the antibody when the nasal and milk samples were taken for PCR. Alternatively, these animals might be latently infected, as similar results reported by others (Lemaire et al., 2001).These findings are important since seronegative carriers may spread the virus when the virus reactivated after stress. It is also important that antibodies may not be detected if antibody-based tests such as ELISA are used in the early stage of acute infections (Kramps et al., 1996). Fuchs and others (1999) detected BHV-1 DNA in the blood of 23 animals, but antibodies to BHV-1 in only 13 of those. 4 of 13 animals seroconverted two weeks later. At the end of the study, two seronegative PCR positive animals were indicating seronegative latently infected animals.

According to the survey results, animals are kept in separate compartments according to their age and sex only in Farm3. The statistical significance of age and sex indicates that these animals should be kept separately. The farmers in this study used artificial insemination for breeding. Venereal transmission of BHV1 can occur by artificial insemination. It is essential to use semen that does not contain BHV-1 (Queiroz-Castro et al., 2021).

According to the survey results within the scope of this study, it was determined that no BHV-1 vaccine was administered in any of the farms, and the farm owners did not know about BHV-1 infections and transmission routes. In the present study, farmers were asked about their grazing system, and they informed that all animals go outside for grazing during spring and get contact with other animals belongs to other farms. In Turkey, the open grazing system is used, and all the animals travel to different grazing places that means neighboring animals also graze in the other animals grazing. Also, there are sheep and buffalos in the same area. Sheep and goats are thought to be a possible source of BHV-1 infection for the cattle population (Yilmaz and Coskun, 2016). This might also be important in the spread of BHV-1 infection amongst animals.

Controlling BHV-1 has always been difficult because of easy transmission of the virus (Murphy et al., 1999; Wentink et al., 1993), latently infected animals (Wentink et al., 1993), seronegative latent carriers (Lemaire et al., 2001), failure of serological tests to detect antibodies in the early stage of acute infections (Kramps et al., 1996), presence of other herpesviruses which share determinants with BHV-1 (Ros and Belak, 1999). In addition, many reasons make it even more challenging to control BHV-1 in Turkey. These are, number of neighboring countries and difficulty in controlling the animals entering to Turkey, difficulty in controlling animal movement in the country, usage of same grazing area by different farmers, lack of information of the farmers about the disease, usage of same bulls in some areas for natural insemination (especially in the villages).

CONCLUSIONS

Integrated farms are applying preventive measurements in Turkey. However, like those analyzed in this study, the small farms are not considering the factors affecting the spread of the virus, such as separation, hygiene, grazing system, testing, culling, and vaccination, since they do not follow any control program. The preventive measurements mentioned above need to be applied in Turkey to control BHV-1 infections in cattle.

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CONFLICT OF INTEREST

None declared by the authors.

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