Investigation of Bovine Herpes Virus Type 1 Infection in Sheep in the Kars Province of Turkey

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Abstract: This study is a serological investigation of BHV-1 (Bovine herpes virus type 1) infection in indigenous bred sheep from 8 small scale family operations in the Kars province of Turkey. For this purpose, serum and leukocyte samples were collected from 220 healthy-looking sheep between the ages of 1-5. Serum samples were tested for antibodies against BHV-1 using the virus neutralization technique (VNT). Out of 220 sera samples tested, 5 (2.27%) were detected as positive against to BHV-1. The present study results reveal that seroprevalence of BHV-1 infection exists at low levels in indigenous bred sheep in the Kars province of Turkey. This study is the first serological study to investigate of BHV-1 infection in sheep in the Kars province of Turkey.

Keywords: *BHV-1, Virus neutralization, Sheep, Seroprevalence*

Kars İlinde Koyunlarda Bovine Herpes Virus Tip 1 Enfeksiyonunun Araştırılması

Özet: Bu çalışmada Kars ilinde 8 adet küçük ölçekli aile işletmelerinde yetiştirilen yerli ırk koyunlarda Bovine herpes virus tip 1 (BHV-1) infeksiyonu serolojik olarak araştırıldı. Bu amaçla, sağlıklı görünümlü, 1-5 yaş aralığında değişen yaşlardaki 220 adet koyundan serum örnekleri toplandı. Serum örnekleri BHV-1 antikorları yönünden virus nötralizasyon tekniği (VNT) ile test edildi. Test edilen 220 adet serum örneğinin 5 adedi (%2.27) BHV-1'e karşı pozitif olarak belirlendi. Araştırma verileri, Kars ilindeki yerli ırk koyunlarda BHV-1 enfeksiyonu seroprevalansının düşük düzeyde olduğunu ortaya koymuştur. Bu çalışma Kars ilindeki koyunlarda BHV-1 enfeksiyonunun araştırıldığı ilk serolojik çalışmadır.

Anahtar Kelimeler: *BHV-1, Koyun, Virus nötralizasyon, Seroprevalans*

Introduction

Infectious bovine rhinotracheitis (IBR) is highly contagious disease of domestic and some wild ruminants caused by bovine herpesvirus type 1 (BHV-1). IBR cause major economic losses in animal operations due to weight-loss, reduced milk production, infertility disorders, embryonic and fetal deaths, abortion, and respiratory and nervous system disorders in young animals (Raaperi et al., 2010). BHV-1 is classified in the Varicellovirus genus in the subfamily Alphaherpesvirinae of the family Herpesviridae. There are three sub-types of BHV-1. Subtypes 1 and 2a cause respiratory symptoms and abortions. Subtype 2b results in Infectious Pustular Vulvovaginitis/Infectious Pustular Balanopostitis (IPV-IPB) characterized by genital lesions (Muylkens et al., 2007). BoHV-5 (bovine encephalitic herpesvirus) causes encephalitis. Like other Herpesviruses, BHV-1 can remain latent by entering the trigeminal and sacral ganglions after the primary infection. Latent virus can be reactivated by different stressful conditions such as infections, corticosteroid applications or transportation (Roizman et al., 1992).

Sheep and goats are thought to be a possible source of infection for the cattle population because of the fact that inter-species virus transmission is possible between small ruminants and cattle, because various ruminant species are housed together in the Kars region, especially in small-scale operations, and because they share pasture lands. Some studies have reported that it is possible for BHV-1 to be transmitted between cattle, sheep and goats, and that sheep and goats may be the source of the infection in cattle (Hage et al., 1997). The aim of this study was to survey serum samples obtained from sheep in the Kars province of Turkey for the presence antibodies against BHV-1.

Materials and Methods

Clinical Samples

Blood samples collected from 220 healthy-looking sheep between the ages of 1-5 found in 8 small-scale family operations in the Kars province of Turkey that in the past had raised animals that had respiratory problems, metritis, mastitis, spontaneous abortion and failed to conceive. The sheep are kept together with cattle in this small-scale family herds. Blood samples (n=352) was collected from jugular vein in nonheparinised

vacutainer tubes. Blood samples were centrifuged for 15 min at 2000 rpm to separate the serum. Before testing the serum in virus neutralisation test, serum samples were heated at 56°C for 30 min.

Virus and Cell Culture

The Colorado reference strain of BHV-1 was used on the virus neutralization test. The Eagle's Minimum Essential Medium (EMEM) without serum was used as a virus growth medium. The infectious power of the viral strain was calculated as $TCID_{50}$ = $10^{-5.25}$/0.1 ml as a result of the microtitration test conducted on Madin-Darby bovine kidney (MDBK) cell culture. MDBK cell culture was used on the virus neutralization tests and for BHV-1 growth and titration. EMEM containing inactivated fetal calf serum (5%) was used as a cell growth medium.

Virus Neutralization Technique

A total of 220 sheep serum samples collected to identify neutralizing antibodies specific to the BHV-1 were tested in accordance with the virus neutralization method reported by Frey and Liess (1971). For this purpose, 0.05 ml of blood serum sample diluted with DMEM to a ratio of 1:2 were placed in two wells side by side on the micro-neutralization plate. Later, the BHV-1 Colorado strain diluted to a ratio of 100$TCID_{50}$ was added in equal volume to the serum samples. The plates were left to incubate for 2 hours in a 5% CO_2 environment at 37°C, after which MDBK cell suspension (300,000 cells/ml) was added to all of the wells on the plate and they were again incubated with 5% CO_2 at 37°C. Virus and cell controls were conducted during the application of the test, which was assessed with invert microscope controls based on the cytopathological changes that occurred in the cells at the end of day 3.

Results

Neutralization Technique

A total of 220 serum samples were examined for antibodies against BHV-1. Out of these, 5 (2.27%) samples were found positive (Table 1).

Table 1. Serologic test results for BHV1 in sheep.

Herds No	No of Sampled Animals	BHV 1	
		Ab (+)	(%)
I	17	-	-
II	30	1	3.33
III	23	-	-
IV	37	2	5.40
V	31	-	-
VI	23	-	-
VII	32	2	6.25
VIII	27	-	-
Total	**220**	**5**	**2.27**

Discussion

IBR (Infectious Bovine Rhinotracheitis), which is formed by BHV-1, is an enzootic infection listed by the International Office of Epizootics as one of the most important diseases in international trade. The presence of the BHV-1 infection has been demonstrated through studies conducted in previous years on a variety of animal species both in Turkey and in countries around the world (Frölich et al., 2006; Yan et al., 2008; Nandi et al., 2011; Rypuła et al., 2012). A comprehensive serological study conducted by Alkan et al. (2005) identified the BHV-1 infection in 97% of herds. It is said that identifying seropositivity following natural BHV-1 infection in a cattle herds means that some of the animals in that herds may have a latent infection and that these animals should, from an epidemiological perspective, be viewed as carriers and transmitters of the virus (Cabalar and Ozgunluk, 2014). Studies of BHV-1 conducted on sheep in different geographical regions of Turkey have found that BHV-1 seroprevalence varies between 0% and 23% (Yavru et al., 1999; Yesilbag et al., 2003; Yesilbag and Dagalp, 2006; Albayrak et al., 2007; Yesilbag and Gungor, 2009; Alpay et al., 2014; Cabalar and Ozgunluk, 2014). There is not yet any information about the prevalence of the BHV-1 infection in sheep raised in the Kars area. Cabalar and Ozgunluk (2014) tested 564 blood serum samples they collected from six local sheep herds in the central and outlying areas of the province of Van using the virus neutralization technique, and found that BHV-1 seroprevalence was 1.4%. Even though BHV-1 causes infections

particularly in cattle, it has been reported to affect sheep and goats as well (Yesilbag et al., 2003). In a study conducted by Alpay et al. (2014) in Gökçeada, where cattle, sheep and goats are raised together and isolated in terms of animal movements, they found that seroprevalence for BHV-1 in cattle was 58.6% and 26.6% in goats, but that BHV-1 antibodies could not be detected in sheep. In this study, BHV-1 seroprevalence in sheep was studied for the first time in the Kars region, and the BHV-1 specific neutralizing antibody was identified in 2.27% of the sheep that were sampled, which is higher than that in the studies by Cabalar and Ozgunluk (2014). This finding might be due to the small number of samples and herds which were analyzed, the more limited geographical distribution of the samples and factors related to how they are raised.

In the operations where the study was conducted in the Kars region, sheep are kept together with cattle. Yıldırım et al. (2011) found that BHV-1 seroprevalence in dairy cows at herds in the same area where this study was conducted was 61.4%. The fact that BHV-1 infection has been found in cattle in this area suggests that the sheep may also have BHV-1 infections originating in cattle. In some European Union countries and in Turkey, glycoprotein E (gE) gene-deleted vaccines, known as marker vaccines, are being used to fight the BHV-1 infection in cattle (Muylkens et al., 2006). However, vaccinations are not expected in some countries, such as Germany and Switzerland. There are two reasons for this. First of all, the protection they provide are not satisfactory. The vaccine can protect against severe disease, but it is not effective against latent and field (wild) strains. The second reason is that vaccinated animals cannot be distinguished from infected animals in serological screening conducted as part of prevention programs (Muylkens et al., 2007).

In conclusion, this study demonstrates that the BHV-1 infection is not as widespread in small ruminants as it is in cattle, which is true in other geographical regions of Turkey as well. However, sheep and goats are thought to be a possible source of infection for the cattle population because of the fact that inter-species virus transmission is possible between small ruminants and cattle, because various ruminant species are housed together in the Kars region, especially in small-scale operations, and because they share pasture lands. This situation may have a negative effect on the implementation of BHV-1 control programs in cattle. More comprehensive epidemiological studies must also be conducted to better understand the potential for virus transmission.

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