Seroprevalence of Bovine Herpes Virus 1 (BoHV-1) in breeding bulls in Northeastern Anatolian Region of Turkey

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ABSTRACT

Bovine herpesvirus-1 (BoHV-1) is well recognized as a pathogen that infects the respiratory and reproductive tracts. This study is a serological investigation of BoHV-1 in breeding bulls at 1–5 years of age from small scale family operations in three Northeastern Anatolian provinces (Kars, Ardahan and Igdir) in Turkey. For this purpose, blood was collected from 250 breeding bulls and was tested for antibodies against BoHV-1 using the virus neutralization technique. Out of 250 blood sera samples tested, 110 (44.00%) were detected as positive against to BoHV-1 and antibody titres were found to be varied between 1/2- 1/64. Among the controlled regions, the highest seroprevalance of BoHV-1 infection was found in Kars (64.81%) followed by Ardahan (30.00%) and Igdir (26.82%) provinces. The results suggested that the infection was spreading in breeding bulls in small scale family operations. This study is the first serological study to determine seroprevalence of BoHV-1 infection in breeding bulls in North-eastern Anatolia region.

Key words: BoHV-1, Breeding bulls, Seroprevalence, Virus neutralization technique.

BoHV-1 (Bovine herpes virus 1) infections 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). BoHV-1 is classified in the Varicellovirus genus in the subfamily Alphaherpesvirinae of the family Herpesviridae. The BoHV-1 virion is enveloped, has a diameter of 150-200 nm and an icosahedral capsid consisting of 162 capsomers. It has a 136 kbp, linear double-stranded infectious genome with at least 65 genes. 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 Balanoposthitis (IPV-IPB) characterized by genital lesions (Muylkens et al., 2007). Subtype 3 causes encephalitis. Like other Herpesviruses, BHV-1 can remain latent by entering the trigeminal and sacral ganglia after the primary infection (Roizman et al., 1992). Latent virus can be reactivated by different stressful conditions such as infections, corticosteroid applications or transportation (Muylkens et al., 2007).

The aim of this study was to survey serum samples obtained from breeding bulls in three provinces in North-eastern Anatolia for the presence antibodies against BoHV-1. The data that was obtained showed that the percentage of seropositive bulls in small scale family farms needs to be evaluated as an epidemiological indicator. Furthermore, sanitary programs for preventing and controlling the BoHV-1 infection may be based on vaccination programs and control of animal movement (entrance of contraband animals or transfers between operations or from other countries via importation).

Clinical samples: This study took random blood samples from 250 unvaccinated, healthy-looking breeding bulls between the ages of 1-5 found in small-scale family operations in three Northern Anatolia provinces in Turkey (Kars, Ardahan and Igdir) that in the past had raised animals that had respiratory problems, metritis, mastitis, spontaneous abortion and failed to conceive (Fig.1). Blood samples were collected directly into blood tubes with silicon and centrifuged at 1500 g for 10 minutes to separate the serum. Before testing the serum in virus neutralisation test, serum samples were heat-inactivated at 56°C for 30 min. This research was conducted after the approval of Kafkas
Virus: The Colorado reference strain of BoHV-1 was used on the micro virus neutralization test. 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 MDBK cell culture.

Cell culture: MDBK cell culture was used on the micro virus neutralization tests and for BoHV-1 growth and titration. EMEM containing inactivated fetal calf serum (10%) was used as a cell growth medium.

Virus neutralization technique (VNT): A total of 250 bull blood samples collected to identify neutralizing antibodies specific to the BoHV-1 Colorado strain were tested in accordance with the micro virus neutralization method reported by Frey and Liess (1971).

Determination of the serum neutralization 50 (SN\(_{50}\)) values for positive sera: At the conclusion of micro virus neutralization test, serum samples in which BoHV-1 antibodies were detected had VNT applied to two-fold dilutions (1/2, 1/4, 1/8………1/256) to determine the antibody titer.

Statistical analysis: The statistical evaluation of the seropositivity rates determined for the breeding bulls with respect to the study sites (foci) was made using the Minitab 14.0 Inc. (State College, PA, USA) and with the chi-square (\(\chi^2\)) test. P values smaller than 0.05 (P<0.05) were considered to be statistically significant.

A total of 250 serum samples were examined for antibodies against BoHV-1. Out of these, 110 (44%) samples were found positive. Seropositivity rate ranged from 26.82 to 64.81%. Seroprevalence for BoHV-1 was the highest (64.81%) in Kars followed by Ardahan (30%) and Igdir (26.82%) (Table 1). As a result of the SN\(_{50}\) test applied to the serum of 110 bulls found to be seropositive for BoHV-1, it was determined that the sera had antibody levels that varied from 1/2 to \(\geq\)1/64. The antibody titer distributions and ratios of the bull sera are provided in Table 2.

There was a statistically significant difference in BoHV-1 seroprevalence in Kars province (64.81%) compared with other two provinces. There was no significant difference in the prevalence of antibodies against BHV-1 between Ardahan and Igdir provinces (Table 1).

BoHV-1 occurs around the globe and causes serious economic losses in the cattle industry. BHV-1 seroprevalence between 33.36% and 61% have been reported in different countries around the world (Cerqueira et al., 2000; Yan et al., 2008; Nandi et al., 2011; Rypula et al., 2012). Studies conducted in Turkey in previous years reported BoHV-1 seroprevalence of between 19.5% and 74% (Alkan et al., 2005; Tan et al., 2006; Yildirim et al., 2011). The first serological research conducted on BoHV-1 in bulls in Turkey was carried out by Burgu and Akça (1986). Researchers identified the presence of neutralizing antibodies for BoHV-1 in 30 (63.8%) of the blood samples taken from a total of 47 bulls used for artificial insemination, but said they were unable to isolate the virus. Yavru et al. (1998), on the other hand, isolated and identified, for the first time, BoHV-1 from semen samples taken from bulls used for breeding in Turkey, but they did not check the animals serologically.

This study is the first one to determine the seroprevalence of BoHV-1 in randomly sampled breeding
bulls in three North-eastern Anatolian provinces (Kars, Ardahan and Igdir). While conducting research in the provinces of Kars and Ardahan, it was obvious that care and shelter conditions were inadequate as was the uncontrolled movement of animals (animal entering/exiting the operations from outside). The fact that the locals still use natural insemination is thought to play a role in the rise of antibody prevalence. Sperm reportedly plays a significant role in the spread of infection and seropositive bulls are recognized epidemiologically as carriers and transmitters of the virus. Therefore, bulls used for breeding should be subjected to regular serological control, and virus isolation studies should be conducted on semen, preputial agitation discharge, leukocyte, nose and eye discharge. Bulls that are seropositive or from which the virus is isolated should not be used for breeding (Magana-Urbina et al., 2005).

A study conducted to determine the relationship between the seroprevalence of BoHV-1 infection and the age of the animals (Dagalp et al., 2001) reported higher seroprevalence in older animals. In this study, seropositivity was lower (26.82%) in the province of Igdir than it was in the other provinces, and this fact is thought to result from the young age (<2) of the animals that were sampled.

Even though it is impossible to speak of a protective antibody titer with BoHV-1 infection, the severity of the clinical symptoms of infection is lower in animals with high antibody levels, which therefore may prevent economic losses (Lemaire et al. 2000). Assessment of the results obtained in this study related to a titer level that could offer protection shows that the highest population was 18 bulls (16.36%) with titer values of 1/24, followed by 15 bulls (13.63%) with titer levels of 1/32 and 8 bulls (7.27%) with titer levels of 1/48.

The fact that the percentage of the bull population in small-scale family operation that have developed antibodies to the BoHV-1 infection has reached 44% in the provinces of Kars, Ardahan and Igdir in the Northeastern Anatolia Region indicates it is a risk factor for the bull population. In spite of the random sampling, the fact that infection was identified in unvaccinated bulls demonstrates that the operations which were sampled need to be periodically checked for the presence of BoHV-1. Furthermore, it is recommended that even if virus identification is not made in seropositive bulls, they be removed from the operation and especially that they not be used for breeding.

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REFERENCES


