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# Molecular study for bovine herpes virus type 1 detection in Iranian cattle

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## ABSTRACT

Bovine herpes virus type 1 (BHV-1), the causative agent of infectious bovine rhinotracheitis, is a DNA virus. This pathogen is represents the most common viral pathogen found in cattle semen. The aim of the present study was to set up a of BHV-1 detection assay in bovine blood in Lorestan province using PCR assay. The blood samples of 285 cattle in Khoramabad, Azna, Aligoodarz, Borujerd and Poldokhtar were collected, total DNA was extracted and the region encoded the gI glycoprotein was amplified by PCR using specific primers. Out of 285 blood samples, 56 (19.64%) were positive for BHV-1 (468 bp). The highest and lowest frequencies of the bacterial infection were observed in Khoramabad and Borujerd cities with 21 and 12%, respectively. The results of this study demonstrated that PCR assay represent an excellent (suitable) alternative or additional tool for BHV-1 isolates detection. Finally the study revealed a high incidence of BHV-1 in the blood of Iranian cattle. Thus all cattle must be tested periodically for BHV-1 infection and antimicrobial drugs, to prevent BHV-1 occurrence in cattle must be used. The cattle must be free BHV-1 infection prior to use.

**Keywords:** Bovine herpes virus type 1, gI gene, PCR, Iran

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## Introduction

Bovine herpes virus type 1 (BHV-1) is a member of the genus Varicellovirus in the subfamily Alpha herpesvirinae, which belongs to the Herpesviridae family (1). The viral genome consists of double-stranded DNA that code for about 70 proteins, of which 33 are known to be structural and up to 15 are non-structural proteins (2). The viral glycoproteins are located in the envelope on the surface of the virion and plays an important role in pathogenesis and immunity. BHV-1 can be differentiated into subtypes 1.1, 1.2a, 1.2b and 1.3 (1). BHV-1.3, which is a neuropathogenic agent, has been re-classified as BHV-5 (3). The BHV-1.2 subtypes may be less virulent than subtype 1.1. BHV-1 is a cause of several infectious disease syndromes in cattle and buffaloes and occurs throughout the world (2). BHV-1 subtypes 1 and 2a mainly cause the respiratory form of the disease, with fever, drop in milk production and abortion. The infection with these subtypes have a mild outcome (4). Isolates of BHV-1.2a cause abortion, whereas BHV-1.2b isolates are not abortifacient. Isolates of BHV-1.1 are more virulent than are isolates of BHV- 1.2b. BHV-1.3 or BHV-5 has been isolated from calves that died of encephalitis and from aborted fetus (5).

BHV-1 is primarily associated with clinical syndromes such as rhinotracheitis, pustular, vulvovaginitis and balanoposthitis, abortion, infertility, conjunctivitis and encephalitis in bovine species. The main sources of infection are the nasal exudates and the respiratory droplets, genital secretions, semen, fetal fluids and tissues (2). The BHV-1 virus infections in cattle and buffaloes are mostly mild and non-life threatening. However, the introduction of IBR into a cattle farm can cause severe economic losses due to weight loss, decrease in milk production and restrictions in the international livestock trade (6). BHV-1 is associated with three major clinical syndromes namely, infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IPB). IBR, caused by BHV-1, is a disease of domestic and wild cattle. Cattle with typical IBR show conjunctivitis, which is either unilateral or bilateral and associated with profuse lacrimation (7). BHV-1 infections can be diagnosed by antibody detection directed against virus or virus components by serological tests or by detection of genomic DNA by polymerase chain reaction (PCR), nucleic acid hybridization or sequencing (8).

It is important to control the disease in the cattle population by imposing regulations to ensure BHV-1 negativity for livestock trade and their derivatives such as semen. The aim of this study was to determination of BHV-1 in cattle from Lorestan province in west of Iran using molecular technique.

## Materials and Methods

### Samples collection and DNA extraction

285 blood samples from four catlle herds were collected from five townships of Lorestan province located in west Iran. In these cattle herds 90, 60, 75, 25 and 35 specimens were obtained from Khoramabad, Azna, Aligoodarz, Borujerd and Poldokhtar townships, respectively. All animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. For ethical approval, the protocol and informed consent forms were approved by the Islamic Azad University, Shahrekord Branch, Shahrekord, Iran with 17621105 grant number. The blood samples were collected into tubes that contained EDTA (manufacturer, town and country). BHV-1 genomic DNA was extracted using DNA extraction kit (QIAGEN Ltd., Crawley, UK) according to the manufacturer's recommendation. The extracted genomic DNA concentration was quantified by spectrophotometric measurement at a wavelength of 260 nm (apparatus).

### Gene amplification

Primers described by Vilcek for *gI glycoprotein* gene of BHV-1 (accession number: DQ006850.1) were used in the present study (Vilcek, 1993). The sequence of primers pairs

were as follows: forward primer BHV-1-F: 5'-CACGGACCTGGTGGACAAGAAG-3' and reverse primer BHV-1-R: 5'-CTACCGTCACGTGCTGTGTACG-3'. These primers amplified a 468 bp fragment after PCR reaction. PCR was carried out in a total of 25 µl mixture containing 1 µg of genomic DNA, 1 µM of each primer (BHV-1-F and BHV-1-R), 2 mM Mgcl2, 200 µM dNTP, 2.5 µl of 10X PCR buffer and 1 unit of *Taq* DNA polymerase (Fermentas, Germany). The procedure of the PCR reaction included 5 min of denaturation at 94°C; followed by 32 cycles of 1 min at 94°C, 1 min at 62°C, and 1 min at 72°C, and a final extension of 72°C for 5 min. The PCR amplification products (10 µl) were subjected to electrophoresis in a 1% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with ethidium bromide, and images were obtained in UVI doc gel documentation systems (apparatus, UK).

**Statistical analyses**

The frequency of re-isolation of BHV-1 from the blood samples were analyzed by the chi-square test using the SPSS 17.0 (SPSS Inc, Chicago IL, USA) software. P values <0.05 were considered significant.

## Results

The PCR product of the primer specific for *gI glycoprotein* gene (BHV-1-F and BHV-1-R) allowed to obtain a 468-bp DNA fragment. The results of electrophoresis for IBR amplification by the PCR are shown in Figure 1.

In Khoramabad region, 19 samples were found positive out of 90 and giving an apparent frequency rate of 21.11%. In Azna, 13 out of 60 samples were found to have BHV-1 infection. The apparent prevalence rate of BHV-1 was 15 out of 75 in Aligoodarz (20%), and only 3 out of 25 samples in Borujerd Township s were found positive (12%). In Poldokhtar region, 6 out of 35 samples (17.14%) were found positive for BHV-1 virus. These results were shown in table 1 completely. These findings showed a wide occurrence of BHV-1 infections in Iranian cattle. Positive and negative controls of known sequence were also run for each reaction. The positive control showed the excepted amplification product specific for BHV-1 (468 bp).

## Discussion

BHV-1 is the causative agent of the OIE notifiable B list disease that includes transmissible diseases considered to have a socio-economic importance within the countries and that are significant impact in the international trade of animals and animal products (7). The virus also causes a wide variety of other clinical syndromes such as abortion, infertility, conjunctivitis and encephalitis. BHV-1 is also one of the most important pathogens involved in the development of the respiratory disease syndrome called shipping fever (9). Three BHV-1 subtypes, BHV-1.1, BHV-1.2a (2a) and BHV-1.2b (2b), have been identified (10). Subtype 1 virus isolates are the causative agents of IBR and are frequently found in the respiratory tract as well as aborted fetuses. Subtype 1 strains are prevalent in Europe, North America and South America. Subtype 2a is frequently associated with a broad range of clinical manifestations in the respiratory and genital tracts such as IBR, IPV, balanopostitis (IPB) and abortions (11). Subtype 2a is prevalent in Brazil and was present in Europe prior to the 1970s. Subtype 2b strains are associated with respiratory disease and IPV/IPB, but not abortion (12). Subtype 2b strains are less pathogenic than subtype 1 and are frequently isolated in Australia or Europe (13). Cattle that recover from an acute IBR infection can be a source of contamination of disease-free herds because they are silent carriers of BHV-1. These animals remain a healthy carriers of BHV-1 for the rest of their life until immuno-suppressive treatments or other conditions reactivates virus replication, leading to the spread of the infection to the rest of the herd (6). Viruses antigenically related to BHV-1 have also been isolated from several ruminant species including sheep, goat, pronghorn, antelope and wildebeest. Buffalo, cattle and wildlife may play an important role in the maintenance of the infection (14).

Serological studies on BHV-1 in different parts of Iran showed a prevalence of 31.5% in Ahvaz (15), 30.4% in Kerman (16), 27.7% in Shiraz (17), 46.7% in Chaharmahal and Bakhtiari province (18) and 48.9% in Urmia (19). The virus diagnosis assay performed in our study showed that the BHV-1 frequency in the investigated regions was in the lower limit of this range. In a previous evaluation performed in 2003, Nahida Laiju *et al*. detected BHV-1 in Hordeum vulgare. Also their study showed that large type chromosomes were found in BHV-105, BTON-10 and conquest of Hordeum vulgare. Medium type and relatively short type chromosomes were absent in BEL-36, BHV-1 and BTON-10 of Hordeum vulgare, respectively. More metacentric chromosomes (7 pairs) were found in BHV-1 of Hordeum vulgare (20).

Many studies were performed about BHV-1 infection in cows and described its correlation with abortion, infertility, shipping fever, conjunctivitis and encephalitis in cattle. Anon in 2007 showed that the disease is endemic in India and during the period of 1986 to 2006, out of 7313 tested serum samples, 3152 were positive for BHV-1 by indirect and competition ELISA (c-ELISA) or micro-serum neutralization test (m-SNT). Also Nandi in 2008 reported during the period of 2000 to 2008, 26 of 953 semen samples were positive by polymerase chain reaction (PCR) or isolation in cell culture (2). In Europe countries, the BHV-1 infection prevalence was reported to be 47.2% in Portugal, 61.0% in Italy, 50.0% in Germany and 80% in Hungary (17). In a study performed in Egypt, Mahmoud *et al*. detected BHV-1 antibodies in serum samples of 1600 small ruminants (sheep and goats) using indirect ELISA technique and showed that the prevalence of BHV-1 reactors were 25.1% of the total examined animals, with higher incidence in goats (27.6%), than in sheep (23.8%) (21). Vilcek *et al*. in 1994 and Santurde *et al*. in 1996 reported detection of BHV-1 by a PCR assay in mucosal swabs and tissues from adult cattle. However, their experiments were performed on tissues from experimentally infected animals (22,23). The results of their study confirmed the findings of our current research. In another study in 1997, Bosch *et al*., carried out a comparative study to evaluate the ability of three BHV-1 marker vaccines to reduce the re-excretion of virus after reactivation of latent BHV-1 (24). Mweene *et al*. in 1996 also reported BHV-1 detection in tissues from experimentally infected animals by an immune-PCR/antigen procedure, although no positive virus isolation results were obtained from samples (25).

Based on our results it was concluded that BHV-1 infection was present noticeably in cattle in west of Iran. Furthermore, a high number of BHV-1 carriers in this area could not be excluded. Also the introduction of BHV-1 into a cattle farm can cause severe economic impact due to production losses and restrictions in the international trade of livestock. The control of this pathogen is useful to prevent end to reduce viral infection. Inactivated vaccines and modified live virus vaccines are useful for prevention of BHV-1 infections in cattle and can be used a prophylactic strategy.

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**Statement of animal rights**

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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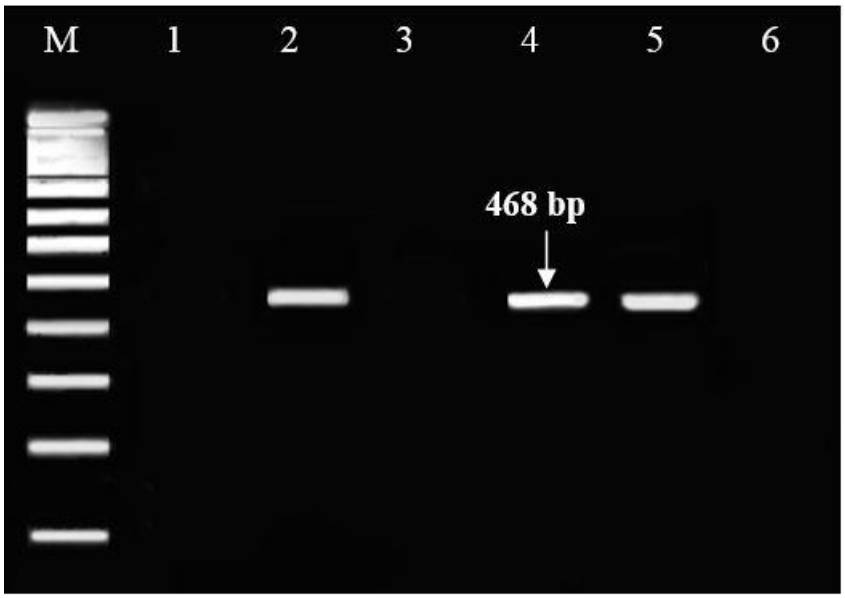


**Figure 1.** Identification of BHV-1 using PCR amplification of the *gI glycoprotein* gene. Lanes 1and 2 are negative and positive controls respectively. Lanes 3 and 6 are negative samples. Lanes 4 and 5 are positive samples of BHV-1. Lane M is 100 bp DNA ladder (Fermentas, Germany).

**Table 1.** Frequency of BHV-1 in Lorestan province located in west Iran.

| Township | Number of samples | Positive | | Negative | |
|---|---|---|---|---|---|
| | | Number | Percentage | Number | Percentage |
| **Khoramabad** | 90 | 19 | 21.11 | 71 | **78.89** |
| **Azna** | 60 | 13 | 21.66 | 47 | **78.34** |
| **Aligoodarz** | 75 | 15 | 20 | 60 | **80** |
| **Borujerd** | 25 | 3 | 12 | 22 | **88** |
| **Poldokhtar** | 35 | 6 | 17.14 | 29 | **82.86** |
| **Total** | **285** | **56** | **19.64** | **229** | **80.36** |