



MOLECULAR DETECTION OF BOVINE HERPES VIRUS-1 AMONG CATTLE IN MOSUL CITY, IRAQ

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Summary

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Bovine herpes virus-1 (BoHV-1) infects livestock and causes several forms of disease, the most prominent being bovine infectious rhinotracheitis, infectious balanoposthitis, and infectious postural vulvovaginitis. Infection can result in substantial loss, especially in feedlot cattle. The current study aimed to determine BoHV-1 in cows by using the conventional polymerase chain reaction. A total of 184 plasma and nasal swab samples were collected from cattle 6 months to 2 years of age of both sexes and of various breeds and from different regions in Mosul city between October 2020 and March 2022. The prevalence of BoHV-1 in cattle was 14/184 (7.6%). Animals infected with BoHV-1 had signs of fever, cough, mucoserous eye and nasal secretions, dyspnea, appearance of abnormal respiratory sounds, as well as congestion of the mucous membranes of the eyes. The prevalence of BoHV-1 was significantly higher in males (9.7%) ($P \leq 0.05$) than in females, in animals 6–8 months of age – 11/100 (10%) ($P \leq 0.05$) compared to those between 9 months and 2 years of age. Moreover, no significant difference between native and imported breeds, as well as between respiratory, ocular, and genital forms was found out. This study concluded that the prevalence of the BoHV-1 virus in Mosul city, Iraq was relatively high, particularly in terms of the age and sex of the animals.

Key words: BoHV-1, cattle, Mosul – Iraq, PCR, prevalence

INTRODUCTION

Bovine herpesvirus-1 of the genus *Varicellovirus*, subfamily *Herpesviridae*, Alphaherpesvirinae, is an important livestock pathogen that causes respiratory and reproductive diseases and highly contagious disease in cattle. There are four virus subtypes: (1.1, 1.2, 1.3, and 1.4), and 1.2a (related with infectious bovine rhinotracheitis), 1.2b (with infectious pustular vulvovaginitis and infectious balanoposthitis (Biswas *et al.*, 2013; Fulton *et*

al., 2016). The vast majority of studies point to the importance of serological testing as a result of the inability to differentiate between serotypes on the basis of common features (Ranganatha *et al.*, 2013). Animals that are latently infected are typically recognised by the presence of BoHV-1-specific antibodies in their serum. (Lemaire *et al.*, 2000). Bovine rhinotracheitis (IBR) causes drop in milk production, abortions, and respiratory

problems, all of which result in death and substantial financial losses for livestock producers (Lojkic *et al.*, 2011). In trigeminal ganglionic neurons, bovine herpesvirus type 1 (BoHV-1) establishes latency. Following natural or corticosteroid-induced stress, viruses might reactivate and disseminate to other susceptible animals (Winkler *et al.*, 2002). The prevalence of infection in the livestock population is determined by a number of different factors, both intrinsic and extrinsic influences (Constable *et al.*, 2017). There is no information available on BoHV-1 molecular diagnosis in Mosul, Iraq. So, the objective of this study was to assess the disease prevalence in cattle and to identify any relevant factors, such as age, sex, origin of animals, and clinical signs.

MATERIALS AND METHODS

Ethical approval

Ethical approval was issued by the Institutional Animal Care and Use Committee (UM.VET.2021.19) at the College of Veterinary Medicine, University of Mosul on September 6, 2021.

Animals and samples collection

The research was conducted on 184 cattle 6 months to 2 years of age from various farms in Mosul, Iraq, between October 2021 and March 2022 (184 blood samples and 184 nasal swabs for the same animals). Animal data including age, sex, and origin were collected. Blood was collected (with anticoagulant) from each animal, plasma was separated and stored at -20°C for subsequent tests (Hassan, 2020; Esmaeel *et al.*, 2021). In addition, nasal swab samples were taken from animals that showed respiratory signs.

DNA extraction from plasma samples and nasal swabs

Conventional polymerase chain reaction was performed for both plasma and nasal swabs (Mahmoud *et al.*, 2009; AL-Baroodi *et al.*, 2012). DNA was extracted using a commercially available DNA extraction kit (Favorgen Biotech Corp DNA extraction kits, Australia), as indicated in the manufacturer's protocol. To detect BoHV-1, all plasma and nasal samples from animals were used for *gC1* protein gene-based PCR. A Nano drop spectrophotometer was used to determine the concentration and purity of DNA (Analytik Jena, Germany). The isolated DNA was kept at -20°C for the consequent tests.

Conventional polymerase chain reaction technique

Amplification of BoHV-1 was performed using a PCR assay targeting the *gC1* protein gene (527 bp) (Fuchs *et al.*, 1999) using the following forward and reverse primer nucleotide sequences (size 527 bp): F: 5'AGGAGCGCAAGTGGATGCTCTG3' and R: 5'GTAGCCGTTGCGGAACCAAGTGC3'. PCR amplification of the *gC1* gene was set up in a 25 μL reaction. The reaction mix included 3.0 μL of template DNA, 12.5 μL of $2\times$ master mix consisting of 10 mM dNTPS mix and Taq DNA polymerase, and 1.0 μL (10 pmol) of forward and reverse primers, respectively. The volume was adjusted to 25 μL with nuclease-free water (7.5 μL). Positive controls for BoHV-1 DNA from infected cattle were clinically seen and positive results were obtained with the sandwich antigen immunosorbent assay test (Abbexa BoHV-1 ELISA kit, USA). The set conditions were as followed: 35 cycles of initial denaturation at 96°C for 10 min, followed by denaturation at 95°C for 45 s, annealing at 60°C for 45 s, and exten-

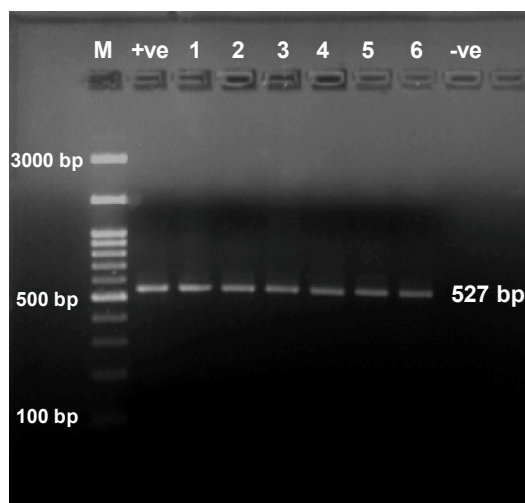


Fig. 1. The polymerase chain reaction of the *gC1* protein gene of the BoHV-1 using a primer pair with expected size of 527 bp. M: marker index, size of 100 bp; +ve: positive control; lanes 1–3: positive plasma samples, lanes 4–6: positive nasal swabs; –ve: negative control.

sion at 72 °C for 1 min. The final extension was conducted at 72 °C for 5 min. The product of PCR (5 µL) was loaded into the appropriate wells with a molecular weight ladder (100 bp). Electrophoresis was done for 45 min at 100 V. The gel was examined under an ultraviolet transilluminator, and the results were confirmed in the Bio-Rad gel system.

Statistical analysis

Statistical analysis was performed using SPSS (v.18.0; IBM, New York, USA). The interactions between prevalence and some factors such as sex and age were assessed by the chi-square test at $P \leq 0.05$.

RESULTS

The amplification of the DNA of the BoHV-1 (Fig. 1) extracted from 184 samples (110 plasma and 74 nasal swab samples) demonstrated an overall prevalence rate of 7.6 % (Fig. 2).

In this study, the animals infected with BoHV-1 showed signs of fever, mucoserous ocular and nasal secretions, dullness, loss of appetite, anorexia, coughing and congestion in the mucous membranes of the eyes, as well as rough hair, dyspnea, and appearance of abnormal respiratory sounds (Fig. 3).

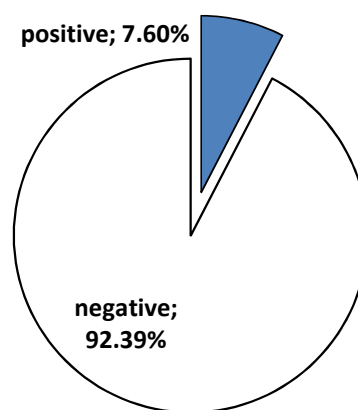


Fig. 2. Prevalence of BoHV-1 among cattle in Mosul city.

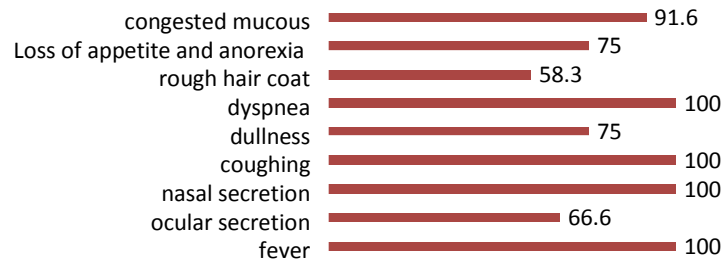


Fig. 3. Percentage of clinical signs associated with bovine herpes virus-1.

Table 1. Number and prevalence of bovine herpes virus-1 by age, sex, breed of cattle and form of the disease

Variables		Number of examined cattle	Number of infected cattle	Infected cattle %	Chi-square	P value
Age	6–8 months	100	11	11.0 ^a	3.5	0.05
	9–24 months	84	3	3.5 ^b		
Sex	male	144	14	9.7 ^a	4.20	0.04
	female	40	0	0 ^b		
Breed	native	50	3	6.0 ^a	0.25	0.61
	imported	134	11	8.2 ^a		
Disease form	respiratory and ocular	100	9	9.0 ^a	0.60	0.43
	respiratory and genital	84	5	5.9 ^a		

Different letters in each variable means statistically significant difference at $P \leq 0.05$.

This study found highly significant differences in BoHV-1 prevalence in 6-8 month-old animals compared to those between 9 months and 2 years of age and in males compared to females ($P \leq 0.05$; Table 1). The study revealed no significant difference in BoHV-1 prevalence between the respiratory and ocular form compared to respiratory and genital form neither significant difference between imported and native breed animals (Table 1).

DISCUSSION

Bovine herpes virus-1 infects cattle and is spread worldwide, causing a variety of respiratory and reproductive problems, as

well as weight loss and substantial economic losses (Tan *et al.*, 2006; Yan *et al.*, 2008). The overall prevalence in Mosul city was 7.6%. Previous studies in other countries have reported a higher and/or nearly equal prevalence of BoHV-1. In India, the prevalence in cows using PCR was 7.5% and 9.1% (Ranganatha *et al.*, 2013; Singh *et al.*, 2013) and 3.0% in nasal swab samples in bovines by using PCR (Gangil *et al.*, 2019). In West Bengal, Saha *et al.* (2010) have detected the BoHV-1 virus in only one (1.5%) out of 65 nasal swab samples tested by PCR. The prevalence of BoHV-1 may vary even between countries due to a variety of factors such as sample diversity numbers,

climate, management performance, the effectiveness of control initiatives, livestock trading industry size, population size, and biosecurity, and uncontrolled animal movement (Mani *et al.*, 2016). In this study, observed clinical signs were consistent with previously reported data (Holliman *et al.*, 2005; Graham, 2013). The current study showed a greater prevalence in calves aged 6–8 months which was in agreement with the results of Johannes *et al.* (2004). This is likely related to the fact that older animals build up an immune response as a result of repeated viral contact during their lives, which may avoid the onset of disease symptoms (Nandi *et al.*, 2009). The high incidence of the disease among young animals may be due to their higher susceptibility to infection compared to adults (Mahmoud *et al.*, 2009). The prevalence of BoHV-1 was higher in males in this study, which could be attributed to the fact that the majority of the samples were taken from beef fattening farm. This study also showed no significantly different prevalence between imported and native animals, which could be due to the lack of vaccination protection for this disease. According to the results of this study, there was no significant difference in BoHV-1 prevalence for disease form: 9% of respiratory and ocular form, and 5.9% of the respiratory and genital form, which agreed with previous findings (Benoit *et al.*, 2007; Nandi *et al.*, 2009).

CONCLUSION

The current study revealed that the prevalence of BoHV-1 among cattle in Mosul, Iraq examined by conventional PCR was relatively high. Many parameters related to the disease's prevalence such as age, sex, and origins, have been discussed. As a result, these variables should be evaluated for disease practical control in Mosul and throughout the country.

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