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Bovine Herpesvirus-1 in Khouzestan province in Iran: seroprevalence and risk factors

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Abstract

Bovine herpesvirus type-1 (BoHV-1) is a DNA virus that is classified in the family Herpesviridae, subfamily Alphaherpesvirinae and genus Varicellovirus. This virus causes the respiratory disease, abortion, conjunctivitis, infectious pustular vulvovaginitis or balanoposthitis and other clinical forms of the complex diseases. The aim of this study was to evaluate the seroprevalence and risk factors of BoHV-1 infection in cattle in Khuzestan province in Iran. Blood samples were randomly collected from 534 cattle in 9 districts of Khuzestan province including Ahvaz, Baghmalek, Shooshtar, Dezful, Shadegan, Hendijan, Behbahan, Ramhormoz and Susangerd. The sera were examined by a commercial ELISA kite. The association between age, sex, breed, history of abortion, husbandry type and geographic location was analyzed by chi-square test and logistic regression. Seroprevalence rate of BoHV-1 infection was 48.69 % (95% CI: 44.49-52.89). Statistical analysis showed that age, geographic location and type of husbandry are significantly associated with infection (*P*<0.001). This study highlights the significant prevalence of BoHV-1 in the cattle in Khuzestan province, suggesting that appropriate prevention and control programs should be considered to reduce prevalence and economic losses by health authorities and animal owners.

Keywords: Bovine herpesvirus type-1, Epidemiology, Prevalence, Serology, Khouzestan, Iran.

Introduction

Bovine herpesvirus type 1 (BoHV-1) is an enveloped DNA virus that is classified in the order Herpesvirales, family Herpesviridae, subfamily Alphaherpesvirinae and genus Varicellovirus (Smith 2015, OIE 2017). The first description of the disease case was probably in Germany dating back to 1841 (Rychner, 1841).

Following its apparent emergence in the USA, IBR has been diagnosed worldwide with the exception of the BoHV-1-free countries, paralleling the distribution of domestic cattle, and is listed as a notifiable disease by the World Organisation for Animal Health (OIE, 2017). In the early 1950s, BoHV-1 appeared as infectious pustular vulvovaginitis in cows and infectious pustular balanoposthitis in bulls but nowadays is recognized to cause a range of other clinical conditions in cattle, including abortion, infertility, conjunctivitis, encephalitis, mastitis, enteritis and dermatitis (Raaperi *et al*., 2014). Genetic analyses of various clinical isolates have found at least four distinct BoHV-l subtypes: a respiratory subtype, two genital subtypes, and an encephalitic subtype designated as BoHV-1.1, BoHV-1.2a, BoHV-1.2b, and BoHV-1.3, respectively. BoHV-1.3 as a neuropathic subtype has been renamed as the following genotypes: BoHV-5a, BoHV-5b, and BoHV-5non-a/non-b (Constable *et al.,* 2017).

The BoHV-1 infection is mainly transmitted by respiratory, ocular or genital secretions through direct contact between animals. However, the infection may also spread via fresh or frozen semen from infected bulls as well as contaminated equipment (Muylkens *et al.,* 2007). Following a primary infection with a field isolate or vaccination with an attenuated strain, BoHV-1 can become latent which in turn becomes reactivated and the animal may shed the virus following stimuli, for example, transport, parturition and glucocorticoid therapy (Pastoret *et al.*, 1982; Seyfi Abad Shapouri *et al.,* 2016). BoHV-1 infection can be diagnosed on the basis of clinical, pathological and epidemiological findings in cattle. However, in order to make a definite diagnosis, laboratory examinations (serological test or virus detection) are required. Serological tests detect the specific antibody against virus; but, viral antigen is detected by nucleic acid-based tests that detect genomic DNA, nucleic acid hybridization and sequencing. Infected cows are mainly detected by the presence of specific antibodies to the virus after acute phase and during latency. Both virus neutralization (VN) test and ELISA have been employed for detecting antibodies against BoHV-1. The ELISA is a specific, sensitive and practical test for detection of antibodies and has advantages over the VN test (Kaashoek *et al.,* 1994; OIE, 2017; Van Oirschot *et al.,* 1997). Moreover, several types of BoHV-1 ELISA including indirect and blocking are commercially available and some of them can be used in conjugation with marker vaccines to detect infected cattle in vaccinated populations (Mars *et al.,* 2001; Van Oirschot *et al.,* 1997).

Due to the direct and indirect role of BoHV-1in causing disease in the cattle and lack of epidemiological data, this study was performed to evaluate the seroprevalence and risk factors of this virus in cattle in some districts of Khouzestan province, southwest of Iran.

Materials and Methods

Sample Collection

The current cross-sectional study was carried out in Khuzestan province located in the southwest of Iran. This tropical province is 64,057 Km^2 and is located between latitude 29˚58´ to 32˚58´ N and longitude 47˚42´ to 50˚39˚ E. To create regional differences in the epidemiological determinants such as environment and management, Khuzestan province was divided into four different regions and from each region, two or three districts were randomly selected. Sera samples were randomly collected from 534 non-vaccinated cattle reared in nine districts of Khuzestan province including Ahvaz, Baghmalek, Shooshtar, Dezful, Shadegan, Hendijan, Behbahan, Ramhormoz and Susangerd during October to December 2015 (figure 1). In a total number of 534 samples, 492 (92.13%) were female and 42 (7.87%) were male. The selected animals were divided into three age groups (≤2, 2-4, and >4 years old) according to dental formula (Dyce *et al*., 2012). The variables of breed (native, Holstein or crossbreed), history of abortion (yes or no) and husbandry (semindustrial or traditional) were collected from all cattle according to observation and interview.

Serological analysis

The blood samples were centrifuged at 3000 rpm for 10 minutes to separate sera. Sera samples were stored at -20ºC till the time of the main experiment.

Sera samples were tested for the presence of BoHV-1 antibodies using indirect ELISA kit (ID vet, Grabels, France) according to manufacturer's instructions. The optical density (OD) of the samples was measured at 450 nm.

The S/P percentage of BoHV-1 antibody was calculated for each sample as follows:

$$\frac{S}{P}\% = \frac{\text{OD Sample} - OD\ negative\ control}{\text{OD}\ positive\ \text{Control} - OD\ negative\ control} \times 100$$

According to manual, the samples would be considered negative if the value of S/P% was less than 50%. The samples with the S/P% more than or equal to 50% and less than 60% were considered doubtful, and the samples with S/P% more than or equal to 60% were considered positive.

Statistical analysis

Statistical analysis of data was performed using SPSS (Version 16.0; SPSS Inc., Chicago, USA). The association between age, sex, breed, history of abortion, type of husbandry and geographic location was analyzed by chi-square test and logistic regression (calculation of odds ratio). Bivariate logistic regression models were fit to the data for each potential risk factor. Risk factors associated with BoHV-1 (P≤0.2) in bivariate regression were further analyzed in a multivariate logistic regression model, using a backward, stepwise algorithm. The goodness of fit of the model was determined using the Hosmer & Lemeshow test. Differences were considered statistically significant (P ≤ 0.05).

Results

Seroprevalence rate of BoHV-1 was 48.69% (95% CI: 44.49-52.89). Statistical analysis showed that the infection is associated with age and increases with aging ($\chi2$= 39.51, df = 2, P<0.001) (Table 1). Univariate logistic regression showed that the odds of infection between the age based on year and disease is 1.25 (95% CI: 1.16-1.36) implying that the odds of infection increased 25% with rising one year of age. Moreover, 9.1% of fluctuation in infection was justified by age (Table 1). In terms of sex, the prevalence of BoHV-1 in female and male cattle was 49.19% and 42.86%, respectively (Table 1). There was no significant difference between these sex groups ($\chi2$ =0.39, df=1, P=0.53). Obviously, the odds of infection in female cattle was 1.29 (95% CI: 0.68–2.44) compared to that in males. Furthermore, 0.2% of fluctuation in infection was justified by sexuality (Table 1). The prevalence of infection was significantly higher in Holstein and crossbreed breeds than native breeds ($\chi2$ =14.97, df=2, P<0.001) and thus 3.8% of fluctuation in infection was justified by breed (Table 1).

Table 1. Prevalence of BoHV-1 antibodies in cattle in southwest of Iran based on host determinants

Category	Groups	Prevalence	Odds Ratio	95% CI for OR	P-Value
Age	Young[c]	25%(23/92)			
	Sub-adult[b]	43.96%(91/207)			
	Adult[a]	62.13%(146/235)	1.25	1.16-1.36	<0.001
Sex	Male[a]	42.86%(18/42)	-	-	-
	Female[a]	49.19%(242/492)	1.29	0.68-2.44	0.43
Breed	Native[b]	30%(24/80)	-	-	-
	Holstein[a]	55.93%(99/177)	2.96	1.69-5.2	<0.001
	Crossbreed[a]	49.46%(137/277)	2.28	1.34-3.89	0.002
Abortion	History of recently aborted[a]	45%(9/20)	-	-	-
	Delivered normally[a]	49.36%(233/472)	1.19	0.49-2.93	0.7

The different lowercase letters in each variable represent a significant difference.

Table 2. Prevalence of BoHV-1 antibodies in cattle in southwest of Iran based on husbandry + and geographical location determinants

Category	Groups	Prevalence	Odds Ratio	95% CI for OR	P-Value
Husbandry	Traditional [b]	42.68%(172/403)	-	-	-
	Semiindustrial[a]	67.18%(88/131)	2.75	1.82-4.16	<0.001
Geographical location	Ramhormoz[c]	15.25%(9/59)	-	-	-
	Dezful[c]	24.53%(13/53)	1.81	0.7-4.65	0.22
	Hendijan[c]	20.69%(12/58)	1.45	0.56-3.76	0.45
	Shushtar[b]	77.27%(34/44)	18.89	6.95-51.37	<0.001
	Susangerd[b]	56.67%(34/60)	7.27	3.03-17.42	<0.001
	Ahvaz[a]	93.48%(43/46)	79.63	20.26-312.98	<0.001
	Behbahan[b]	55.56%(45/81)	6.94	3.02-15.99	<0.001
	Shadegan[a]	27.12%(16/59)	2.07	0.83-5.15	0.12
	Baq-Malek[b]	72.97%(54/74)	15.00	6.25-36.01	<0.001

The different lowercase letters in each variable represent a significant difference.

In this regard, statistical analysis showed no statistically significant difference between BoHV-1 infection and history of abortion ($\chi2$ =0.02, df=1, P=0.88) (Table 1). In comparison to cattle with history of abortion, the odds of infection in cattle without a history of abortion was 1.19. Furthermore, 0.3% of fluctuation in infection was justified by this factor (Table 1). On the other hand, the prevalence rates in semi-industrial and traditional husbandry were 67.18% and 42.68%, respectively ($\chi2$ =22.78, df=1, P<0.001) (Table 2). As can be found from Table 2, univariate logistic regression showed that the odds of infection in semindustrial husbandry was 2.75 in comparison with traditional husbandry. Moreover, 5.9% of fluctuation in infection was justified by the type of husbandry.

As shown in Table 2, infection rates varied among different districts. Ahvaz with 93.48% and Ramhormoz with 15.25% prevalence rates were the highest and lowest infected places. Evidently, significant differences were found between infection and location ($\chi2$ = 139.8, df = 8, P< 0.001) and 33.3% of fluctuation in infection was justified by geographical location (Table 2).

Multivariate logistic regression showed that 40.8% of fluctuation in infection was justified by age, sex, breed, type of husbandry, history of abortion and geographical location. Table 3 shows the odds ratio in multivariate logistic regression. As can be observed, it is obvious that in backward stepwise logistic regression only geographical location, age, and type of husbandry had a significant effect on infection (Hosmer & Lemeshow test: $\chi2$ = 6.82, df = 8, P= 0.56).

Discussion

BoHV-1 is a worldwide disseminated pathogen displaying significant differences in regional incidence. Based on serological surveys, several studies have aimed at identifying the risk factors for BoHV-1 seropositivity. Some of them are well characterized such as age, sex and herd size. Direct animal contact, such as the purchase of cattle and participation in cattle shows was also found to be important risk factors for the BoHV-1 infection. Other factors such as livestock density or cattle density may increase the risk of BoHV-1 infection (Benoit et al., 2007). The results of this study showed that overall prevalence rate of BoHV-1 is 48.69%, indicating that BoHV-1 infection was widely distributed among the bovine population in Khuzestan province.

There are differences between the reports of the seroprevalence rate from various countries and areas. In this regard, Haji Hajikolaei and Seyfi Abad Shapouri (2006) reported a prevalence of 31.48% from 572 cattle in Ahvaz district by ELISA. In the study fulfilled by Kargar Moakhar *et al*. (2001), 33.97% of 9968 sera samples of cattle from different parts of Iran had antibodies to BoHV-1 by virus neutralization test. According to other studies, BoHV-1 was reported 7.1% from Qazvin (Ezzi *et al.,* 2013), and 35.6% from Arak (Ghaemmaghami *et al.,* 2013).

In England, the prevalence of BoHV-1 seropositivity in cattle and herds has increased since the 1970s (Woodbine *et al.,* 2009). BoHV-1 infection rate was determined 59% in cattle herds in the state of Parana´, Brazil (Dias *et al.,* 2013).

Table 3. Odds ratio in multivariate logistic regression

Category	Groups	Odds Ratio	95% CI for OR	P-Value
Age	-	1.29	1.16-1.44	<0.001
Husbandry	Traditional	-	-	
	Semindustrial	7.27	2.26-23.42	<0.001
Geographical location	Ramhormoz	-	-	-
	Dezful	2.15	0.6-6.42	0.17
	Hendijan	1.16	0.39-3.48	0.79
	Shushtar	13.79	4.64-41.01	<0.001
	Susangerd	8.77	3.33-23.11	<0.001
	Ahvaz	60.63	14.32-256.69	<0.001
	Behbahan	1.45	0.33-6.5	0.63
	Shadegan	2.17	0.77-6.1	0.14
	Baq-Malek	6.82	2.1-22.22	0.001

Seroprevalence of BoHV-1 infection was determined 64.4% and 64.5% in Mexico (Segura-Correa *et al.,* 2016; Romero-Salas *et al.,* 2013), 65.88% in 255 non-vaccinated cattle population in northern part of Tamil Nadu, India (Saravanajayam *et al.,* 2015), 79.5% in 380 cattle over 2 years and with no history of vaccination against BoHV-1 from Pernambuco Brazil (Silva *et al.,* 2015). In Belgian cattle, seroprevalence rate of BoHV-1 infection in the unvaccinated group was 67%, 35.9%, and 33% in the overall herd, individual-animal, and median within-herd, respectively (Boelaert *et al.,* 2000). The apparent prevalence of bulk milk antibody positive herds for BoHV-1 has been approximately 80% and 78% in unvaccinated Irish dairy herds (Sayers *et al.,* 2015). The survey of Stahl *et al.* (2002) showed that a high proportion of the selected herds (33/60) was free from BoHV-1, and that there was a clear distinction between negative and positive herds. No association between BoHV-1 ODs and herd size was demonstrated, and several of the median and large herds were free from the infection. The individual seroprevalence to BoHV-1 in Ecuador was 43.2%. The herd prevalence was 82.1%, and the intraherd prevalence ranged from 12.5 to 100% (mean = 64.1%) (Carbonero *et al.,* 2011). As it is observed, the prevalence rate of BoHV-1 infection differs not only between different countries but also between different areas within one country. It may be attributed to differences in management systems, climate and environmental conditions in the various countries and different areas. There is a positive association between BoHV-1 infection and the location of a herd. It may be related to cattle densities that explain some of the differences in the prevalence of BoHV-1 in herds (Paton *et* al., 1998). It is well known that farms located in colder areas at a higher altitude are more prone to BoHV-1 infections. However, the lower altitude (≤1800m over the sea level) acts as a risk factor (Carbonero *et al.,* 2011). In addition to other factors such as sampling and diagnostic methods, pollution and the use of sperm infected bulls, sample size, the age of cattle, farming system (industrial, semi-industrial and traditional), number of animals per farm (cows density), health management and control measures such as vaccination should also be considered.

Risk factors of BoHV-1 infection have been identified in several studies. The results of this study showed that prevalence of BoHV-1 increased with age. All ages and breeds of cattle are susceptible, but the disease occurs most commonly in animals over 6 months of age, probably because of their greater exposure (Constable *et al.*, 2017). The higher seroprevalence in older cattle was probably due to a greater exposure to the virus over time (Carbonero *et al.*, 2011).

The prevalence of BoHV-1 infection has been significantly affected by the farm cattle husbandry system so that semi-industrial examined farms had higher BoHV-1 prevalence compared to that in traditional farms. It could possibly be due to the fact that increased cattle density in industrial husbandry increases the possibility of the transfer of the BoHV-1 between the farmed animals. Variability in management systems, climate and environmental factors among cities may explain these differences of BoHV-1 infection. Several management factors have been associated with BHV-1 infection in a herd. Infected herds purchase cattle and participate in cattle shows more often than negative farms. The positive farms have more visitors and are situated closer to other cattle farms (Constable *et al.*, 2017). For example, all programs used to control brucellosis and tuberculosis by the veterinary organization are taken in industrial or semi-industrial farms. These operations result in commuting of person from farm to farm and introduction and distributing BoHV-1 to negative farm. Furthermore, increased stressful conditions could occur where animals are kept in high-density farming which in turn increases the incidence of BoHV-1 infection with a decrease in immune system response. It should be noted that climate variability could also affect husbandry system which in turn influences BoHV-1 infection. It is noteworthy that geographical situation drastically affected BoHV-1 prevalence in cattle studied. In this regard, Boelaert *et al.* (2005) showed that sex, origin and herd size are risk factors for BoHV-1 infection in Belgian cattle population. Dias *et al.* (2013) showed that variables such as beef herds, natural service, purchased animals, pasture rental, the presence of calving pens and records of abortion in the last 12 months have been associated with BoHV-1 in the multiple logistic regression. Carbonero *et al.* (2011) showed that animal age (>4 years), BRSV infection, altitude over the sea level (≤1800m), and average slope are risk factors associated with BoHV-1 infection, while a good cleaning of the facilities was shown to be a protective factor. Romero-Salas *et al.* (2013) showed that purpose of the herd, age of cattle, and reproductive stage of cattle are risk factors for BoHV-1 infection. Segura-Correa *et al.* (2016) showed that rural district and herd size are risk factors for BoHV-1 infection.

Comparing the results of this study with that of Haji Hajikolaei and Seyfi Abad Shapouri (2006), it is concluded that the prevalence of BoHV-1 in Khuzestan province has increased since 2006. Therefore prevention and control programs such as natural exposure, and biosecurity, should be considered by health authorities and cattle owners.

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Figure. 1. *Khuzestan province of Iran*

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