

Original Article

Serological Detection of SRMV, BVDV, BHV-1 and BEFV in Camels (*Camelus dromedarius*) in Southwest Iran

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ABSTRACT

Background: Small ruminant morbillivirus (SRMV), bovine viral diarrhea virus (BVDV), bovine herpes virus-1 (BHV-1), and bovine ephemeral fever virus (BEFV) are among the most important viruses of farm animals.

Objectives: This study aimed at the serological detection of SRMV, BVDV, BHV-1, and BEFV in the camel population of Khuzestan Province, located in southwest Iran.

Methods: A total of 150 camel blood samples were randomly collected from free-ranging, seemingly healthy camels of both sexes and various ages in 8 different regions of Khuzestan Province, Iran. The sera were tested for SRMV, BVDV, BHV-1, and BEFV with serum neutralization test.

Results: The seropositive samples for SRMV, BVDV, and BHV-1 were 1(0.67%), 7(4.67%), and 11(7.33%), respectively. There was no seroconversion against BEFV in the serum samples.

Conclusion: The camel population of Khuzestan Province is subclinically infected with SRMV, BVDV, and BHV-1 and could play a significant role in the epizootiology of these viral diseases in this region, which is very important for the control and eradication programs.

Keywords: Camel, BHV-1, BVDV, PPRV

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1. Introduction

Peste des petits ruminants (PPR) is an economically important and highly contagious viral disease of small ruminants and some wildlife species with high morbidity and sometimes high mortality rates (Fallahi, 2017; Asil et al., 2019; Pruvot et al., 2020). This disease is characterized by high fever, ocular and nasal discharge, depression, pneumonia, oral erosions, and severe diarrhea (Constable et al., 2017). PPR is caused by a small ruminant morbillivirus (SRMV), previously known as the PPR virus. SRMV belongs to the Morbillivirus genus in the Paramyxoviridae family and is antigenically related to the rinderpest virus, which infects cattle and other large ruminants (Fallahi, 2017). Firstly, PPR was documented in goats and sheep in west Africa in the early 1940s (Idoga et al., 2020). In Iran, the prevalence of PPR in small ruminants firstly was documented clinically, pathologically, and serologically in Ilam Province in 1995 (cited by Bazarghani et al., 2006). Recently, this disease has been reported in different parts of Iran (Zakian et al., 2016; Mokhtari et al., 2017; Rasooli et al., 2018; Allahverdizadeh et al., 2020; Alidadi et al., 2021). Seroprevalence of SRMV in dromedary camels has also been reported in different countries (Intisar et al., 2017; Chemweno et al., 2019; Hemida, & Al-Ghadeer, 2019; Rahman et al., 2020).

Bovine viral diarrhea virus (BVDV), a single-stranded RNA virus, is a pestivirus in the family Flaviviridae. This virus is closely related to the border disease virus (BDV) and classical swine fever virus, which is the cause of bovine viral diarrhea (BVD) in different mammalian species, with worldwide distribution and different prevalence between geographic regions and countries (Tsfaye et al., 2021; Dabiri et al., 2021; Constable et al., 2017). Infection among various animals such as cattle, sheep, and camels (Keyvanfar et al., 1999; Raoofi et al., 2010; Hashemi et al., 2022) and free-ranging wild ruminants (Hemmatzadeh et al., 2016) were showed serologically in Iran.

Infectious bovine rhinotracheitis (IBR), characterized by respiratory disease, abortion, conjunctivitis, and other clinical forms of the disease complex in cattle, is caused by bovine herpesvirus-1 (BHV-1), belonging to the genus Varicellovirus in the subfamily Alphaherpesvirinae under the family Herpesviridae (Constable et al., 2017). Two genetically distinct subtypes of BHV-1 are designated as BHV-1-1 and BHV-1-2 (Dagalp et al., 2020). This disease is one of the most economically important infectious diseases of farm animals with worldwide dis-

tribution (Intisar et al., 2009), but eradication has occurred in some countries (Constable et al., 2017). The prevalence of BHV-1 in old world camelidae (OWC) and new world camelidae (NWC) was documented in some countries (Nawal et al., 2003; Rivera et al., 1987). Raoofi et al. (2012) could not find positive cases of the disease in camels using serological tests in Iran.

Bovine ephemeral fever (BEF), or 3 days sickness, is an economically important arthropod-borne disease that affects cattle and water buffalo (Lee, 2019). The condition is caused by the bovine ephemeral fever virus (BEFV) of the genus Ephemerovirus belonging to the Rhabdoviridae family. It is characterized by fever, inappetence, depression, ocular and nasal discharge, muscle stiffness, reluctance to move, lameness, recumbency, and a drop in milk production (Walker & Klement, 2015). The morbidity rate is usually 25%-45% but can be as high as 100% in a highly susceptible population. The case fatality rate is commonly as low as 1% but may reach 10% (Constable et al., 2017). It occurs in tropical and subtropical regions with wide distribution across Africa, Asia, the Middle East, and Australia (Walker & Klement, 2015). The asymptomatic form of infection with seroconversion has also been found in camels, sheep, goats, pigs, and a wide range of wild ungulates (Walker & Klement, 2015). There are no reports of the prevalence of this disease in camels in Iran.

This study aimed to investigate the extent of camels' exposure to SRMV, BVDV, BHV-1, and BEFV by determining the presence of antibodies against these viruses in camels in Khuzestan Province, located in southwest Iran. It can also provide information on the distribution of diseases in the region and determine the role of camels in the epizootiology of these viral diseases, which are very important for control and eradication programs.

2. Materials and Methods

The study was performed on the camel population of Khuzestan Province, located in southwest Iran. The study protocol was approved by the Department of Clinical Sciences, Shahid Chamran University of Ahvaz, Ahvaz City, Iran. The total land area of Khuzestan Province is 64236 km², and it borders the Persian Gulf from the south and Iraq country from the west. The mean annual temperature of the province is 25.3°C with a mean minimum and maximum of 18.2°C and 32.4°C, respectively, and the mean annual rainfall is 284.3 mm. In rural areas of the province, livestock breeding is the main way of livelihood. The livestock population of the province includes 340200 cattle, 88000 buffaloes, 2246600 sheep, 1094400 goats, and 6200 camels.

A total of 150 camel blood samples were randomly collected from free-ranging, seemingly healthy camels of both sexes and various ages in 8 different regions of the province. The sera were separated and stored at -20°C until tested by virus neutralization test (VNT) for SRMV, BVDV, BHV-1, and BEFV.

The sera were heat inactivated at 56°C for 30 min before virus neutralization. In brief, for SRMV neutralization, 100 µL of 1:20 dilution of sera in RPMI (Roswell Park Memorial Institute) medium (with 2% FBS) were added into wells of 96-well cell culture microplates in duplicate. Then, 100 µL of virus suspension (SRMV vaccine strain, Razi institute) containing 1000 TCID₅₀ (the median tissue culture infectious dose)/mL was added to each well. Plates were incubated at 37°C for 1 h. Then, 1×10⁴ Vero cells in 50 µL RPMI medium containing 2% FBS were added to each well. Finally, the microplates were incubated at 37°C for 5-7 days and observed daily for cytopathic effects and compared to cell and virus controls.

For BVDV neutralization, 50 µL of sera diluted 1:4 in RPMI were mixed with 50 µL of virus suspension (NADL strain) containing 100 TCID₅₀ in duplicate wells of 96-well cell culture microplates. After incubation for 1 h at 37°C, 100 µL of MDBK (the Madin-Darby bovine kidney) cells (1×10⁴ cells) were added to each well, and plates were incubated in a CO₂ incubator at 37°C for 5 days.

For BHV-1 neutralization, 50 µL of undiluted sera were added into wells of 96-well cell culture microplates, then 50 µL (150 TCID₅₀) of a local isolate of BHV-1 (Seyfi Abad Shapouri et al., 2016) was added to each well, and the plates were incubated at 37°C for 18-24 h. Finally, 1×10⁴ RBK (Razi bovine kidney) cells in 100 µL RPMI medium containing 2% FBS were added to each well, and microplates were incubated in a CO₂ incubator at 37°C for 5 days.

For BEFV neutralization, 50 µL of 1:4 diluted sera in RPMI were added into wells of 96-well cell culture microplates and mixed with 50 µL of BEFV (laboratory strain, Razi institute) suspension containing 100 TCID₅₀. Plates were incubated at 37°C for 1 h, and then 1×10⁴ HmLu-1 cells in 100 µL RPMI medium containing 2% FBS were added to each well, and microplates were incubated in a CO₂ incubator at 37°C for 5 days.

The data were analyzed using SPSS software, version 24 (Illinois, USA). The association of the seroconversion against SRMV, BVDV, and BHV-1 with age and region was evaluated using the Chi-square test.

3. Results

Serological detection of SRMV, BVDV, BHV-1, and BEFV in camel populations of different regions of Khuzestan Province is shown in Table 1. The seropositive samples for PPRV, BVDV, BHV-1, and BEFV were 1(0.67%), 7(4.67%), 11(7.33%), and 0(0%), respectively. The statistical analysis showed no differences between different age groups and regions in seroconversion against SRMV, BVDV, and BHV-1 (Table 2).

4. Discussion

Till 1992, there was no report that camels could be PPR hosts. Firstly, Ismail et al. (1992) showed serological infection in Sudanese camels in Egypt. Then, various reports from endemic areas of Africa and Asia have shown PPR seroconversion and susceptibility of camel populations (Intisar et al., 2017; Abraham et al., 2005; Ismail et al., 1992; Chemweno et al., 2019). The clinical form of the disease in camels has also been reported (Roger et al., 2001; Zakian et al., 2016; Khalafalla et al., 2010; Saeed et al., 2015; Omani et al., 2019). Roger et al. (2001) first

Table 1. Serological detection of SRMV, BVDV, BHV-1, and BEFV in camel populations of different regions in Khuzestan Province

Region	Number	No. (%)			
		SRMV	BVDV	BHV-1	BEFV
North	57	0(0)	3(5.3)	3(5.26)	0(0)
West	55	1(1.82)	3(5.5)	7(12.73)	0(0)
East	21	0(0)	0(0)	1(4.76)	0(0)
South	17	0(0)	1(5.9)	0(0)	0(0)
Total	150	1(0.67)	7(4.67)	11(7.33)	0(0)

Abbreviations: SRMV: Small ruminant morbillivirus; BVDV: Bovine viral diarrhea virus; BHV-1: Bovine herpes virus-1; BEFV: Bovine ephemeral fever virus.

Table 2. Serological detection of SRMV, BVDV, BHV-1, and BEFV in different age groups of camel populations in Khuzestan Province

Age (y)	No.	No. (%)			
		SRMV	BVDV	BHV-1	BEFV
≤5	32	0(0)	0(0)	1(3.1)	0(0)
5-≤10	60	1(1.7)	2(3.3)	7(11.7)	0(0)
10-≤15	34	0(0)	2(5.9)	1(2.9)	0(0)
>15	24	0(0)	3(12.5)	2(8.3)	0(0)
Total	150	1(0.67)	7(4.67)	11(7.3)	0(0)

Abbreviations: SRMV: Small ruminant morbillivirus; BVDV: Bovine viral diarrhea virus; BHV-1: Bovine herpes virus-1; BEFV: Bovine ephemeral fever virus.

documented the PPR virus's suspected role in an epizootic disease that infected 100 camels in Ethiopia during 1995-1996. [Khalafalla et al. \(2010\)](#) reported an outbreak of PPR in camels in Sudan with a mortality rate of 7.4%. In the present study, 0.67% of dromedary camels showed serological infection for SRMV in the southwest of Iran. This finding resulted from a natural infection because the camels were not vaccinated against the SRMV. In the same area, [Zakian et al. \(2016\)](#) reported for the first time an outbreak of PPR in a herd of camels, and [Rasooli et al. \(2018\)](#) documented a PPR seroprevalence of 58% and 23% in sheep and cattle, respectively. Accordingly, the camels showed a lower prevalence of SRMV in comparison with cattle and sheep in this region. The recorded serological infection of PPR in this study is lower than the results obtained by [Abraham et al. \(2005\)](#) in Ethiopia (3%) and [Rahman et al. \(2020\)](#) in Pakistan (8.5%). The susceptibility of camels to SRMV has been well documented. The SRMV may rarely overcome the innate resistance of large ruminants and cause clinical symptoms because the SRMV, like the other morbilliviruses, has an immunosuppressive effect. However, there is currently limited information about their role in maintaining the disease in the animal population, especially small ruminants ([Rahman et al., 2020](#)). In a recent study, the ocular secretions of one of the camels were PCR-positive for the SRMV ([Omani et al., 2019](#)). However, there is still disagreement about the role of camels in the epidemiology of the disease. In this regard, in the study of [Fakri et al. \(2019\)](#), experimental exposure of camels to SRMV did not cause disease transmission.

Bovine viral diarrhea is a worldwide disease and has been reported in many countries. The prevalence of BVDV has been reported mainly based on the detection of antibodies against the BVDV. It is well known that new world camels (NWCs) and old world camels

(OWCs) are susceptible to BVDV infection and may develop serious illnesses ([Tesfaye et al., 2021](#)). The results of our study for the first time showed BVDV seroconversion of 4.7% (7/150) in dromedary camels in the southwest of Iran. Because of no vaccination against BVDV in Iran, detecting these antibodies indicates that camels are exposed to the virus naturally. The serological infection rate of BVDV in sheep, goats, and buffaloes in this region, respectively, were reported to be 46.62%, 32.87% ([Seyfi Abad Shapouri et al., 2007](#)), and 33.9% ([Haji Hajikolaie et al., 2010](#)). There are limited reports of BVDV prevalence in camels in different parts of Iran. [Raoofi et al. \(2010\)](#) showed a 19.7% seroprevalence of BVDV in camels slaughtered in Tehran Province. There is a wide variety in the seroprevalence of BVDV in dromedary camels of different countries. The prevalence achieved in the present study was lower than the results reported in Oman (6.7%) ([Hedger et al., 1980](#)).

One of the risk factors for BVDV infection is keeping different animal species together. In this regard, even if these species are not in direct contact, PI (persistently infected) animals spread BVDV by polluting the environment or using shared equipment ([Nelson et al., 2016](#)). Given that BVDV can cause abortion in camels, it may be necessary to implement control programs in some areas similar to those conducted in cattle.

BHV-1, one of the most important viral diseases of bovines, can also transmit to camels and cause respiratory infection ([Intisar et al., 2009](#)). In Iran, [Afshar & Tadjbakhsh \(1970\)](#), for the first time, showed precipitating antibodies against BHV-1 in cattle. [Nikbakht et al. \(2015\)](#) reported 31.9% IBR (infectious bovine rhinotracheitis) seroprevalence in cattle in Iran. In Fars and Khuzestan Provinces (south and southwest of Iran), [Hashemi et al. \(2022\)](#) and [Adeli et al. \(2017\)](#) reported

the seroprevalences of 39.76% and 48.69% for BHV-1 in cattle respectively, indicating the widespread of BHV-1 infection in cattle in these regions. This study showed serological infection of 7.33% (11/150) for BHV-1 in dromedaries in the southwest of Iran. In rural areas of Iran, different livestock species usually share housing areas, water sources, and pastures. Hence, the conditions for cross-contamination are provided. The previous serological studies in Iran could not detect BHV-1 antibodies in camels. [Raoofi et al. \(2012\)](#), in an abattoir study in Tehran (the Capital of Iran), examined 137 camels' serum samples using the serum neutralization test, and no antibodies to BHV-1 were detected. However, the pathogenic effect of BHV-1 in camels was confirmed by [Nawal et al. \(2003\)](#) and [Intisar et al. \(2009\)](#). [Intisar et al. \(2009\)](#) showed that 1.6% of 186 tested camel lungs in Sudan were positive for BHV-1 antigen by ELISA, PCR, and FAT. Using indirect ELISA, these researchers found antibodies to BHV-1 in 76.9% of 260 camels' sera. [Wernery & Wernery \(1990\)](#) did not detect antibodies against BHV-1 in camels in the Emirates. These authors explained that the camels tested in the study were kept for racing and rarely came in contact with other animals.

For the first time, a disease with a BEF-like appearance was reported in camels of Somalia and North East Kenya with prominent signs of high fever and lameness, known as "Lahaw Gaal" ([Dirie & Abdurahman, 2003](#)). The first evidence of antibodies to BEFV in camels came from a study by [Elbayoumy et al. \(2013\)](#), which found a seroprevalence of 12.72%. In the present study, there was no seroconversion against BEFV in camels. Neutralizing antibodies against BEFV have been detected in a wide range of wild ungulates ([Walker & Klement, 2015](#)). [Walker & Klement \(2015\)](#) believed that due to the low prevalence of BEFV antibodies in these species and their small population, these animal species are of little importance in the epidemiology of BEF outside Africa.

There was no association between serological infection with SRMV, BVDV, and BHV-1, and different age groups. It has been shown that usually, in older age groups, the frequency of infection increases, which is due to more exposure to infectious agents over time ([Adeli et al., 2017](#)). This contradictory finding can be due to the low frequency of seroconversion against these infectious agents in the camel population. Also, the frequency of these infectious agents was not different in different geographical areas, which could be due to the same management and climate conditions or very low frequency of infection.

In the study area, camels are in contact with other domestic animals, especially sheep, and goats, and usually share pasture and water sources that facilitate the spread of infectious agents. However, the lower prevalence of under-studied infectious diseases in camels compared to cattle and small ruminants in this region could be due to factors such as population density and management practices. In this regard, the small population of camels in Khuzestan Province, which graze in small groups in wide rangelands, minimizes the spread of the infectious agents. It has been shown that increasing animal density in industrial husbandry systems increases the possibility of transmission of the infection between the animals ([Adeli et al., 2017](#); [Constable et al., 2017](#)).

This study showed subclinical infection with SRMV, BVDV, and BHV-1 in the camel population of Khuzestan Province, Iran. Because of no vaccination against these diseases in Iran, the findings indicate natural exposure. Therefore, this population may be considered reservoirs and transmit these viruses to the susceptible hosts and play a significant role in the epizootiology of these viral diseases.

Ethical Considerations

Compliance with ethical guidelines

All procedures were conducted according to the animal care guideline of the Research Committee of the Faculty of Veterinary Medicine, [Shahid Chamran University of Ahvaz](#).

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Authors' contributions

Conceptualization: Mohammad Nouri and Masoud Reza Seyfi Abad Shapouri; Methodology: Masoud Reza Seyfi Abad Shapouri, Mohsen Lotfi and Maryam Daghari; Investigation: Aria Rasooli, Hamid Reza Baghbanian and Sahar Mohseni-Parsa; Data collection: Hamid Reza Baghbanian and Sahar Mohseni-Parsa; Data curation, formal analysis: Aria Rasooli; Visualization, Masoud Reza Seyfi Abad Shapouri; Validation and Writing-original draft: Masoud Reza Seyfi Abad Shapouri and Aria Rasooli.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

ردیابی سرمی موربیلیوویروس نشخوارکنندگان کوچک، ویروس اسهال ویروسی گاو، هرپس ویروس تیپ ۱ گاوی و ویروس تب بی دوام گاو در شترهای یک کوهانه در جنوب غرب ایران

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چکیده



زمینه مطالعه: موربیلیو ویروس نشخوارکنندگان کوچک (SRMV)، ویروس اسهال ویروسی گاو (BVDV)، هرپس ویروس تیپ ۱ گاوی (BHV-1) و ویروس تب بی دوام گاو (BEFV) از مهم‌ترین ویروس‌های حیوانات اهلی هستند.

هدف: هدف از این مطالعه ردیابی سرمی SRMV، BVDV، BHV-1 و BEFV در شترهای یک کوهانه استان خوزستان واقع در جنوب غرب ایران بود.

روش کار: در مجموع از ۱۵۰ نفر شتر به ظاهر سالم که به صورت باز نگهداری می‌شدند، از هر دو جنس، در سنین مختلف و از ۸ منطقه مختلف استان خوزستان نمونه خون از ورید و داج تهیه شد. نمونه‌های سرم از نظر SRMV، BVDV، BHV-1 و BEFV به روش SNT بررسی شدند.

نتایج: نتایج نشان داد به ترتیب ۱ (۰/۶۷ درصد)، ۷ (۴/۶۷ درصد) و ۱۱ (۷/۳۳ درصد) نمونه از نظر سرمی برای SRMV، BVDV و BHV-1 مثبت بودند و هیچ‌گونه تغییر سرمی برای BEFV در نمونه‌های شتر مشاهده نشد.

نتیجه‌گیری نهایی: شترهای استان خوزستان به صورت تحت بالینی به SRMV، BVDV و BHV-1 آلوده هستند و می‌توانند نقش مهمی در اپیدمیولوژی این بیماری‌های ویروسی در منطقه داشته باشند، بنابراین در برنامه‌های کنترل و ریشه‌کنی این بیماری‌های ویروسی باید به نقش آن‌ها توجه کرد.

کلیدواژه‌ها: شتر، BEFV، BHV-1، BVDV، SRMV

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