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Serological Detection of Small Ruminant Morbillivirus, Bovine Viral Diarrhea Virus, Bovine Herpes Virus-1 and Bovine Ephemeral Fever Virus in Camels *(Camelus dromedarius)* in South-West of Iran

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Abstract

BACKGROUNDS: Small Ruminant Morbillivirus (SRMV), bovine viral diarrhea virus (BVDV), bovine herpes virus-1 (BoHV-1) and bovine ephemeral fever virus (BEFV) are among the most important viruses of farm animals.

OBJECTIVES: The aim of this study was serological detection of SRMV, BVDV, BoHV-1 and BEFV in the camel population of Khuzestan province, located in south-west 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 eight different localities of Khuzestan province. The sera were tested by SNT for SRMV, BVDV, BoHV-1 and BEFV.

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.

CONCLUSIONS: 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: BEFV, BoHV-1, BVDV, Camel, SRMV

Introduction

Peste des petits ruminants (PPR) is an economically important and a 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 Small Ruminant Morbillivirus (SRMV), previously known as PPR Virus. SRMV belongs to *Morbillivirus* genus in *Paramyxoviridae* family and is antigenically related to 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, prevalence of PPR in small ruminants firstly was documented clinically, pathologically and serologically from Eilam province in 1995 (cited by Bazarghani *et al*., 2006). In recent years, this disease has been reported from different parts of Iran (Zakian et al., 2016; Mokhtari et al., 2017; Rasooli et al., 2018; Allahvirdizadeh et al., 2020; Alidadi et al., 2021). Seroprevalence of SRMV in dromedary camels has also been reported from different countries (Intisar *et al*., 2017; Chemweno *et al*., 2019; Hemida, and 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 border disease virus (BDV) and classical swine fever virus, that is the cause of Bovine Viral Diarrhea (BVD) in different mammalian species, with world-wide distribution and different prevalence between geographic regions and countries (Tesfaye *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 (BoHV-1), belonging to the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae* under the family *Herpesviridae* (Constable *et al*., 2017). There are two genetically distinct subtypes of BoHV-1 designated as BoHV-1-1 and BoHV-1-2, respectively (Dagalp *et al*., 2020). This disease is one of the most economically important infectious diseases of farm animals with worldwide distribution (Intisar *et al*., 2009), but eradication has occurred in some countries (Constable *et al*., 2017). Prevalence of BoHV-1 in Old World Camelidae (OWC) and New World Camelidae (NWC) was documented in some countries (Burgemeister *et al*., 1975; Nawal *et al*., 2003; Rivera *et al*., 1987). Raoofi *et al*. (2012) could not report 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 disease is caused by Bovine ephemeral fever virus (BEFV) of the genus *Ephemerovirus* belonging to *Rhabdoviridae* family and characterized by fever, inappetence, depression, ocular and nasal discharge, muscle stiffness, reluctance to move, lameness, recumbency and drop in milk production (Walker and Klement, 2015). The morbidity rate is usually 25-45% but can be as high as 100% in highly susceptible population. The case fatality rate commonly is 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, Middle-east and Australia (Walker and

Klement, 2015). The asymptomatic form of infection with seroconversion has also been found in camels, sheep, goats and pigs, and in a wide range of wild ungulates (Walker and Klement, 2015). There are no reports of the prevalence of this disease in camels in Iran.

The aim of this study was to investigate the extent of camels' exposure to SRMV, BVDV, BoHV-1 and BEFV by determining the presence of antibodies against these viruses in Khuzestan province located in south-west 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 in this region which are very important for control and eradication programs.

Materials and Methods

The study was performed on camel population of Khuzestan province located in south-west Iran. The study protocol was approved by the department of clinical sciences, Shahid Chamran University of Ahvaz. The total area of Khuzestan province is 64236 Km^2 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 mean minimum and maximum of 18.2 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 eight different localities of Khuzestan province. The sera were separated and stored at - 20 °C

until tested by Virus neutralization test (VNT) for SRMV, BVDV, BoHV-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 medium (with 2% FBS) were added into wells of 96-wells cell culture microplates, in duplicate. Then, 100 µl of virus suspension (SRMV vaccine strain, Razi institute) containing 1000 $TCID_{50}$/ml was added to each well. Plates were incubated at 37°C for 1 hour. Then, 1×10^4 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 the presence of 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 $TCID_{50}$ in duplicate wells of 96-wells cell culture microplates. After incubation for 1 h at 37°C, 100 µl of MDBK cells (1×10^4cells) were added to each well and plates were incubated in a CO2 incubator at 37°C for 5 days.

For BoHV-1 neutralization, 50 µl of undiluted sera were added into wells of 96-wells cell culture microplates, then 50 µl (150 $TCID_{50}$) of a local isolate of BoHV-1 (Seyfi Abad Shapouri *et al*., 2016) was added to each well and the plates were incubated at 37°C for 18-24 hours. Finally, 1×10^4 RBK (Razi Bovine Kidney) cells in 100 µl RPMI medium containing 2% FBS were added to each well and microplates were incubated in a CO_2 incubator at 37°C for 5 days.

For BEFV neutralization, 50µl of 1:4 diluted sera in RPMI were added into wells of 96-wells cell culture microplates and mixed with 50 µl of BEFV (laboratory

strain, Razi institute) suspension containing 100 $TCID_{50}$. Plates were incubated at 37°C for 1 hour and then 1×10^4 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). Association of the seroconversion against SRMV, BVDV and BoHV-1 with age and region were evaluated using chi-square test.

Results

Serological detection of SRMV, BVDV, BoHV-1 and BEFV in camel populations of different regions of Khuzestan province is shown in Table 1. The seropositive samples for PPRV, BVDV, BoHV-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 different regions in terms of seroconversion against SRMV, BVDV and BoHV-1.

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

Region	NO	SRMV	BVDV	BoHV-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 (4.7%)	11(7.33%)	0 (0%)

SRMV: Small Ruminant Morbillivirus; BVDV: Bovine Viral Diarrhea Virus; BoHV-1: Bovine Herpes Virus-1; BEFV: Bovine Ephemeral Fever Virus

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

Age	NO	SRMV	BVDV	BoHV-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 (4.7%)	11 (7.3%)	0 (0%)

SRMV: Small Ruminant Morbillivirus; BVDV: Bovine Viral Diarrhea Virus; BoHV-1: Bovine Herpes Virus-1; BEFV: Bovine Ephemeral Fever Virus

Discussion

Till 1992, there was no report that camels could be possible PPR host. 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) firstly documented PPR virus 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 mortality rate of 7.4%. In the present study 0.67% of dromedary camels showed serological infection for SRMV in southwest of Iran. This finding was 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 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. But, 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 from 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 presence of antibodies against the BVDV was first reported by Burgemeister *et al*. (1975) in OWC. The results of our study for the first time showed BVDV seroconversion of 4.7% (7/150) in dromedary camels in southwest of Iran. Due to the lack of vaccination against BVDV in Iran, the presence of these antibodies indicates that camels are exposed by the virus naturally. 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 Hajikolaei *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 some countries such as, 18% in Saudi Arabia (Al-Afaleq *et al*., 2006), 6.7% in Oman (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 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 conducts in cattle.

BoHV-1 that is 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 and Tadjbakhsh (1970) for the first-time showed precipitating antibodies against BoHV-1 in cattle. Nikbakht *et al*. (2015) reported 31.9% IBR seroprevalence in cattle in Iran. In Fars and Khuzestan provinces (south and southwest of Iran) respectively the seroprevalences of 39.76% and 48.69% for BoHV-1 in cattle were reported by Hashemi et al. (2022) and Adely et al. (2017), indicating the widespread of BoHV-1 infection in cattle in these regions. The results of this study showed serological infection of 7.33% (11/150) for BoHV-1 in dromedaries in southwest of Iran. In rural areas of Iran, different species of livestock usually share housing area, water sources and pasture. Hence, the conditions for cross-contamination are provided. The previous serological studies in Iran were not able to detect BoHV-1 antibodies in camels. Raoofi *et al*. (2012) in an abattoir study in Tehran (Capital of Iran) examined 137 camels' serum samples using the SNT and no antibodies to BoHV-1 were detected. However, the pathogenic effect of BoHV-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 BoHV-1 antigen by ELISA, PCR and FAT. Using indirect ELISA these researchers found antibodies to BoHV-1 in 76.9% of 260 camels' sera. Burgemeister *et al*. (1975) detected neutralizing antibodies against BoHV-1 in 5.8% of camels' sera in Tunisia. Wernery and Wernery (1990) did not detect antibodies against BoHV-1 in camels in 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 BEF-like appearance was reported in camels of Somalia and north-eastern Kenya with prominent signs of high fever and lameness, known as "Lahaw Gaal" (Dirie and Abdurahman, 2003). The first evidence of antibodies to BEFV in camels came from a study by Elbayoumy *et al.* (2013), which found 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 and Klement, 2015). Walker and Klement (2015) believe that due to the low prevalence of BEFV antibodies in these species as well as 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 BoHV-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 very 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 possibly be due to the same management and climate conditions or very low frequency of infection.

In the study area, camels are in contact with the other domestic animals, especially sheep and goats, and usually share pasture and water sources which facilitate the spread of infectious agents among them. However, the lower prevalence of under studied infectious diseases in camels in comparison to cattle and small ruminants in this region could be due to various 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).

The results of this study showed subclinical infection with SRMV, BVDV and BoHV-1 in camel population of Khuzestan province, Iran. Due to the lack of vaccination against these viruses in Iran, the findings indicate natural exposure. Therefore, this population may be considered as reservoirs and transmit these viruses to the susceptible hosts and play a significant role in the epizootiology of these viral diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

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ردیابی سرمی موربیلی‌ویروس نشخوارکنندگان کوچک، ویروس اسهال ویروسی گاو، هرپس ویروس تیپ 1 گاوی و ویروس تب بی‌دوام گاو در شترهای یک کوهانه در جنوب غرب ایران

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پیشینه: موربیلی ویروس نشخوارکنندگان کوچک (PPRV)، ویروس اسهال ویروسی گاو (BVDV)، هرپس ویروس تیپ 1 گاوی (BoHV-1) و ویروس تب بی دوام گاو (BEFV) از مهم‌ترین ویروس‌های حیوانات اهلی هستند.

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

روش‌ها: در مجموع از 150 نفر شتر به ظاهر سالم که در به صورت باز نگهداری می‌شدند، از هر دو جنس، در سنین مختلف و از 8 منطقه مختلف استان خوزستان نمونه خون از ورید وداج تهیه شد. نمونه‌های سرم از نظر PPRV، BVDV، BoHV-1 و BEFV به روش SNT مورد بررسی قرارگرفتند.

نتایج: نتایج نشان داد که به ترتیب 1 (0/67٪)، 7 (4/67٪) و 11 (7/33٪) نمونه از نظر سرمی برای PPRV، BVDV و BoHV-1 مثبت بودند و هیچگونه تغییر سرمی برای BEFV در نمونه‌های شتر مشاهده نشد.

آلوده هستند و BoHV-1 و BVDV، SRMV**نتیجه‌گیری:** شترهای استان خوزستان به صورت تحت بالینی به 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 نمود.

کلمات کلیدی: شتر، SRMV، BVDV، BoHV-1، BEFV