A serological investigation of Bovine enterovirus-1, Bovine herpesvirus-1, Bovine viral diarrhea virus, and Parainfluenza-3 infections in camels in Western Turkey

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Summary
Camels (*Camelus dromedarius*) are bred in Western Turkey, particularly in the province of Aydin, for touristic, social and cultural purposes. Bovine enterovirus-1 (BEV-1), Bovine herpesvirus type-1 (BHV-1), Bovine viral diarrhea virus (BVDV), and Parainfluenza-3 (PI-3) virus infections are significant causes of health and/or economic concerns in several animal species. These agents have not been investigated in the camel population in Turkey. The objective of this study was to serologically investigate the presence and infection rates of these viruses in camels in Aydin province, Western Turkey. Ninety-two serum samples were taken from clinically healthy camels that were kept in private farms or brought to the local slaughterhouses. Serum neutralization test was performed to assess the presence and the titers of specific antibodies against BEV-1, BHV-1, BVDV, and PI-3 virus in camel sera. Of the 92 camels tested, 30 (32.61%), 2 (2.17%), 54 (58.7%), and 20 (21.74%) were seropositive for BEV-1, BHV-1, BVDV, and PI-3, respectively. These results suggest that, except for BHV-1, these viral infections are common among camels in Western Turkey. To our knowledge, this the first comprehensive, large-scale study investigating these viral infections in camels in Turkey.

Introduction

Camels are bred in some provinces of Turkey for tourism and social and cultural purposes. Currently, there are approximately 2000 camels living in Turkey (Turkstat 2017, www.turkstat.gov.tr). Aydin province is the area with the largest camel population approximately 1,500 animals. Traditionally, camels have been brought to the Aydin province from several regions of Turkey including Mediterranean, Central Anatolia, and South and North regions for camel wrestling or tourism purposes.

Majority of camels are near other ruminants, and thus, frequently in contact with sheep, goats, and cattle in husbandry. There are four major ruminant viruses that can potentially infect camels: Bovine enterovirus-1 (BEV-1), Bovine herpesvirus type-1 (BHV-1), Bovine viral diarrhea virus (BVDV), and Bovine parainfluenza-3 (PI-3).

Bovine enterovirus-1 is a member of the genus *Enterovirus* within the family *Picornaviridae*, a group of small, non-enveloped, positive strand RNA viruses (Hyypia *et al.* 1997). Although it usually causes subclinical infection with mild symptoms, rare fatal cases with low morbidity have also been reported (Blas-Machado *et al.* 2007). Clinical symptoms associated with the infection include digestive and respiratory system disorders and abortion (Grooms 2004). The virus has a wide range of host spectrum including cattle, water buffaloes, sheep, and goats (Gur *et al.* 2008).

Bovine herpesvirus-1 classified in subfamily *Alphaherpesvirinae*, family *Herpesviridae*, is the cause

of infectious bovine rhinotracheitis characterized by reduced milk yield, abortions, and respiratory symptoms such as conjunctivitis and nasal mucopurulent discharge in cattle (Muylkens *et al.* 2007). It was isolated in different countries from aborted fetuses and lungs of camels with pneumonia (Ismail 2017).

Bovine viral diarrhea virus is a member of the *Pestivirus* genus, a group of serologically closely related single-stranded RNA viruses classified in the family *Flaviviridae*. BVDV infections are common world-wide. In cattle infection can cause diarrhea, mucosal disease, hemorrhagic lesions in respiratory and digestive system and reproductive disorders such as abortion, teratogenesis, embryonic resorption, fetal mummification, stillbirth, and congenital infections (Baker 1995, van Amstel and Kennedy 2010, Moening and Becher 2018).

Bovine parainfluenza-3 virus recently renamed as Bovine respirovirus 3 is a *Respirovirus* classified in the family *Paramyxoviridae* (Intisar *et al.* 2010b, Adams *et al.* 2017). It is the cause of acute respiratory tract disease and enzootic bronchopneumonia in calves (Oros *et al.* 1997). In adults, PI-3 substantially does not trigger a direct respiratory disease but, suppressing the respiratory system, leads to opportunistic infection agents infect these animals (Intisar *et al.* 2010b, Saeed *et al.* 2015).

These four listed above viral infections are significant causes of health and/or economic concerns in ruminants, especially cattle. Data on the prevalence of these infections in camels in Turkey are very limited because of low numbers and minor economic value compared to the other ruminants.

As of February 2018, entering the key words 'Bovine enterovirus', 'Bovine herpesvirus', 'Bovine viral diarrhea', or 'Parainfluenza' along with 'Camel' and 'Turkey' into the Pubmed database (http://www/pubmed.gov) produced only the following three publications. In these few studies which involved few animals, no BEV-1, BVDV or BHV-1 antibodies were detected in camels (Gur *et al.* 2008, Albayrak *et al.* 2010, Yesilbağ *et al.* 2011).

The objective of this study was to investigate the seroprevalence of these infections in dromedary camels (*Camelus dromedarius*) in Western Turkey.

Materials and methods

Samples

Ninety-two samples were taken from camels that were brought from various regions of Turkey to Incirliova Municipal Slaughterhouse for slaughter, to the Faculty of Veterinary Medicine clinics at the Adnan Menderes University for treatment, or to local farms in the Aydin province for breeding. All camels were apparently healthy at the time of blood collection. The animals that were brought to the clinics were suffering from long teeth or lameness that required surgical intervention and did not show any signs of systemic disease. Individual features of animals were recorded according to owners' and/or veterinarians' declaration. One of the most important evidence was that camels brought to clinics had been raised with ruminants (cattle, sheep, and goats). The blood samples were collected in serum separator tubes. Serum was obtained by centrifugation and stored at - 20 °C until testing. The camels brought to the clinic and the camels from local farms were housed with cattle. No housing history was instead available on animals brought to slaughterhouses. Therefore, it was not known whether they have had any contact with cattle.

Cell culture

Madin Darby bovine kidney (MDBK) cells were used for virus propagation, titration, and serum neutralisation test (SNT). Dulbecco's Modified Eagle Medium (DMEM) (Biochrom- Germany) containing 10% inactivated fetal calf serum (Biochrom, Germany) was used as the cell culture medium.

Viruses

In this study, BEV serotype 1, Colorado strain of BHV-1, Cytopathic NADL strain of BVDV, and SF-4 strain of PI-3 were used as test viruses. For each virus suspension 50% Tissue culture infection dose ($TCID_{50}$) was calculated using Spearman and Kaerber method (Ramarkrishnan 2016).

Serum neutralization test

Serum samples were examined for the presence of antibodies against BEV-1, BHV-1, BVDV and PI-3 virus using standard SNT according to the procedure by Frey and Liess (Frey and Liess 1971). In total, 368 serum neutralization tests were performed to investigate the four viruses in the 92 samples. Microneutralization test was used after serum samples were diluted 1:1 with medium to test for BHV-1, BEV-1, BVDV and 1:5 to test for PI-3. Fifty microliters of the diluted samples were added in duplicates into tissue culture microplates with the same volume of virus suspension containing 100 $TCID_{50}$ virus. After an hour of incubation at 37 °C in 5% CO_2, 50 µL cell suspension (300,000 cells/mL) was added and the plates were further incubated for 72 hours. Test results were evaluated based on the cytopathic effects on cells observed under an inverted microscope.

Determination of SN_{50} value

All positive sera were serially diluted in a two-fold series. This assay is different from first serum neutralization assay. First serum neutraliztion assay was performed to investigate sera positivity in which samples has been diluted 1:1 or 1:5. Second neutralization assay were performed to determine titration of antibody in positive-sera which has provided to detect SN_{50} value. The SNT was used to determine 50% serum neutralisation (SN_{50}) antibody titer values.

Statistical analysis

Chi-squared statistical test was used in order to estimate potential association between recorded data and study outcomes. All tests were performed at the 0.05 level of significance.

Table I. *Seropositive rates in camels from Western Turkey.*

Virus	N	Strain used	Positive samples	Seropositivity %
BEV-1	92	Serotype 1	30	32.61
BHV-1	92	Colorado	2	2.17
BVDV	92	Cytopathic NADL	54	58.7
PI-3	92	SF-4	20	21.74

N = Number of tests performed; BEV-1 = bovine enterovirus-1; BHV-1 = bovine herpesvirus-1; BVDV = bovine viral diarrhea virus; PI-3 = parainfluenza-3.

Results

The serological results are presented in Table I. The seropositive rates were 32.61%, 2.17%, 58.7%, and 21.74% for BEV-1, BHV-1, BVDV, and PI-3, respectively. The effect of sample source on the seropositive rates were presented in Table II. Multi-infection status were summarised in pie-chart (Figure 1). Antibody titers in camels were generally low (Figure 2). The highest titer (1/80) was detected in two samples against BEV-1. The highest titer against BVD and PI-3 was 1/40. The SN_{50} value of two BHV-1 positive samples was 1/2.

Discussion

To our knowledge, this is the first comprehensive study on seropositive rates of BEV-1, BHV-1, BVDV, and PI-3 in camels in Turkey. Due to their high prevalence and their capacity to cause economic losses, these viruses represent a major threat to livestock in Turkey (Erol *et al.* 2007, Gur *et al.* 2008,

Table II. *Effect on sample source on seropositive rates.*

Animal source	Animals tested	Animals tested seropositive				
		BVD (%)	BHV-1 (%)	BEV-1 (%)	PI-3 (%)	
Slaughterhouse	65	34 (52.3%)	1 (1.5%)	15 (23.1%)	7 (10.8%)	$p > 0.05$
Clinics	13	9 (69.2%)	1 (7.7%)	8 (61.5%)	6 (46.2%)	$p < 0.05$
Local Farms	14	11 (78.6%)	0 (0%)	7 (50%)	7 (50%)	$p > 0.05$
Total	92	54 (58.7%)	2 (2.17%)	30 (32.61%)	20 (21.74%)	

SNT = serum neutralization test; BEV-1 = bovine enterovirus-1; BHV-1 = bovine herpesvirus-1; BVDV = bovine viral diarrhea virus; PI-3 = parainfluenza-3.

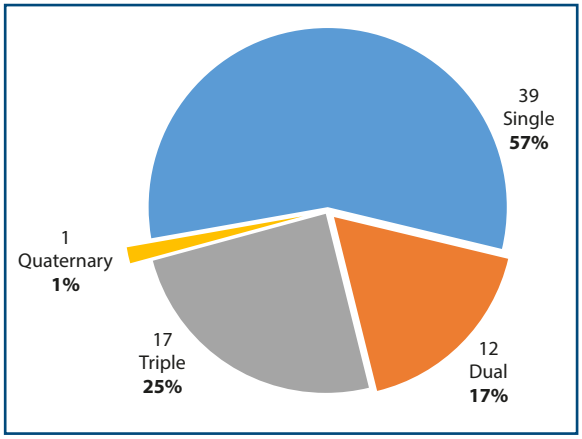

Figure 1. *Multi-infection status of Wetern Turkey camels according to neutralising antibodies against Bovine enterovirus-1 (BEV-1), Bovine herpesvirus type-1 (BHV-1) Bovine viral diarrhea virus (BVDV), and Bovine parainfluenza-3 (PI-3).*

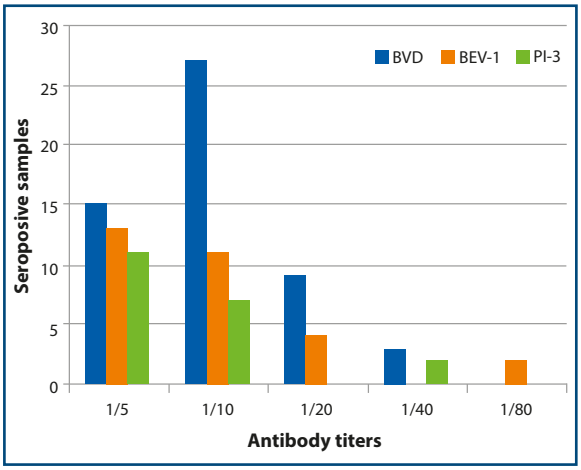

Figure 2. *Bovine enterovirus-1 (BEV-1), Bovine viral diarrhea virus (BVDV), and Bovine parainfluenza-3 (PI-3) neutralising titers in camels of Western Turkey.*

Tan *et al.* 2006 a, b). Although the viruses can infect camels, data on prevalence of these viruses in camels are very limited in the literature probably because camel populations have little economic impact in Turkey. Therefore, it is not known whether camels might play a role in the epidemiology and spread of these viruses in rumiinants in Turkey.

In this study, 30 of the 92 (32.6%) samples collected from camels were positive for BEV-1. Although new camel enteroviruses have been recently described (Woo *et al.* 2015), data on susceptibility of camels to this infection are very limited. There is only one study by Gur and colleagues (Gur *et al.* 2008) that didn't detect antibodies against BEV-1 in 18 camels in Turkey. Our study showed that 32.6% of the camels were seropositive to this infection. The reason for this discrepancy could be due to the difference in sample size between the two studies [i.e., 18 in the study by Gur and colleagues (Gur *et al.* 2008) vs 92 in our study].

The low seropositive rate to BHV-1 found in this survey is similar to those reported in some other studies. Yesilbağ and colleagues (Yesilbağ *et al.* 2011) didn't detect BHV-1 antibodies in two camels in Turkey. No antibodies against BHV-1 were detected in 111 sera collected from camels in Algeria (Saidi *et al.* 2018). However, 76.9% seropositivity was reported in Sudan (Intisar *et al.* 2009), suggesting that BHV-1 infection rates can vary greatly in different countries.

In our study, 58.7% of the camels were seropositive to BVDV. This is a common finding world-wide. High BVDV seroprevalence in cattle in different regions of Turkey include 41.4% (Yesilbag *et al.* 2008), 58.2% (Gur *et al.* 2011), and 70.8% (Aslan *et al.* 2015). Tan and colleagues (Tan *et al.* 2006a) detected antibodies against BVDV in 86% and BVDV antigen in 12.85% of cattle in Aydin province; 4.9% of the animals were persistently viremic. High seropositive rates in camels have been reported in several countries including Sudan (84.6%, Intisar *et al.* 2010a) and Egypt (52.5%, Zaghawa *et al.* 1998). In a recent comprehensive review, Wernery (Wernery 2012) suggested that BVDV infection in camels is common and that camels may play a role in epizootiology of the BVDV.

In our study, 21.74% of the camels were exposed PI-3 virus. PI-3 infection rates in camels can be very different in different countries. It was 82.2% in Sudan (Intisar *et al.* 2010b) but 5.6% in United Arab Emirates (Afzal *et al.* 1994). Ayelet and colleagues (Ayelet *et al.* 2013) found no PI-3 seropositive camels in Ethipoia.

In this study, the source from where samples were taken had a significant impact on seropositive rates. Camels brought to the clinics for treatment had higher infection rates than those brought to slaugtherhouse and local farms (Table II, $p < 0.05$). This is probably due to the fact that in the clinics animals were kept together with other ruminants, especially cattle.

Camels housed near or along with other ruminants may have been infected with viruses from other ruminants. This should be considered when drawing plans to control these infections.

In summary, our results showed that BEV-1, BVDV, and PI-3 infections are common in camels in Turkey. These infections are also common in cattle causing significant economic losses. The camels with high seropositive rates were those kept together with other ruminants, especially cattle. This finding might suggest a possible transmission between camels and other ruminants. Therefore, camels may play a critical role in the transmission cycle of these viruses under field conditions and should be considered in future potential prevention program against these infections.

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Ethical statements

This study was approved by the Animal Ethical Committee of the Adnan Menderes University (Approval No: B.30.2.ADU.0.06.00.00/124-H EK/2009/018).

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