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SEROLOGICAL SURVEY IN CAMELS (*Camelus dromedarius*) TO DETECT ANTIBODIES AGAINST BOVINE HERPESVIRUS TYPE-1 AND *Mycobacterium avium paratuberculosis* IN IRAN

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ABSTRACT

Serological survey was carried out to detect antibodies in dromedary camels against bovine herpesvirus type-1 (BHV-1) and *Mycobacterium avium paratuberculosis* (Map) in Iran. Serum samples were collected from camels at Khorein abattoir in suburbs of Tehran. Using the serum neutralisation test (SNT), 137 samples were examined and no antibodies to BHV-1 were detected. A modified camelid-specific serum antibody assay was used and 2 of 90 camels (2.2%) were found positive for Map antibodies. It is concluded that camels probably may play a role in the persistence and transmission of Map infection among ruminants in Iran. This is the first report for the detection of antibodies in dromedary camels against BHV-1 and Map in Iran.

Key words: Antibody, bovine herpesvirus type 1, camel, *mycobacterium avium paratuberculosis*, serology

Infection of ruminants with herpesviruses may lead to severe illness and death or, in contrast, as well pass completely without clinical signs (Engels and Ackermann, 1996). Seroprevalence surveys have found that 10-50%, or even higher number of cattle are serologically positive to the virus depending on vaccination practices in individual herds, and the frequency of contact between infected and non-infected animals (Davison, 2002). The role of bovine herpesvirus type-1 (BHV-1), or the infectious bovine rhinotracheitis (IBR) virus in diseases of camels is not well established and it seems that New World Camels are more susceptible to BHV-1 than Old World Camels (Wernery and Kaaden, 2002).

Paratuberculosis (Johne's disease) occurs worldwide most commonly in cattle and to a lesser extent in sheep and goats (Harris and Barletta, 2001). *Mycobacterium avium paratuberculosis* (Map) has a very broad host range and infection may occur in many different wildlife and exotic species. Water buffalo, captive and free living wild ruminants including deer, bighorn sheep, rocky mountain goats, aoudads, mouflon sheep, camels, mountain goats, reindeer, antelopes, llamas, alpacas and yaks are all susceptible (Radostits *et al*, 2007). There is considerable variation in the prevalence of infected herds in different countries and within specific geographic areas (Radostits *et al*, 2007). Reports suggest that 7 to 18% of cattle from slaughterhouse surveys are infected (Whitlock, 2009). Paratuberculosis is seen in dromedaries, but is less prevalent than in bactrian camels due to the conditions in which the dromedaries are kept (Wernery and Kaaden, 2002).

The population of camels in Iran is about 145,600 (Banabazi and Javanrouh, 2009), these were maintained on pasture and widely scattered in the great desert and central region of Iran. Camels frequently come in contact with domestic animals, such as sheep and goats, and to a lesser extent with cattle. The aim of this study was to detect antibody against BHV-1 and Map in dromedary camels in Iran and to examine the extent of exposure of the camels to these agents.

Materials and Methods

The study was carried out on 137 camels of both sexes and various ages at Khorein abattoir in suburbs of Tehran. Blood samples were collected from camels and serum samples were frozen at -20°C until tested.

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All serum samples were heat treated for 30 min at 56°C, for the serum neutralisation test (SNT) with IBR virus, then twofold serial dilutions from 1/2 to 1/128 were prepared in 100 µl amount in RPMI 1640 (Sigma) supplemented with 2% foetal bovine serum (Gibco), 100 U of penicillin, and 100 µg of streptomycin per ml. Each dilution of antisera was then mixed with 100 µl a suspension of the IBR virus containing 100 $TCID_{50}$ then incubated at 37°C for one hour. After incubation, 50 µl of a suspension of approximately 200000 MD-BK cells were placed into each wells of a 96-well cell culture plates and mixed with 100 µl of each dilution of virus–serum mixture from previous step. The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 3 days. The cells monitored daily for the development of CPE up to 5 days. The presence of any specific CPE indicated a failure of complete neutralisation and considered as negative. The highest serum dilution that neutralised virus infectivity was considered as serum titre. Serum samples with titre of 1/64 or higher was considered as positive (Murphy *et al,* 1999; Kargar Moakhar *et al,* 2001).

A commercially available HerdChek® *Mycobacterium paratuberculosis* Antibody Test Kit (IDEXX Laboratories, Westbrook, Maine) was modified by the addition of a Rabbit anti-llama HRP conjugate (Capralogics Inc, Hardwick, MA, USA) to determine camelid antibodies (Kramsky *et al,* 2000).

All samples were tested according to the manufacturer's instructions and following modifications by Kramsky *et al* (2000). In brief, 15 µl of sample were mixed with 135 µl of adsorbent solution and incubated for 60 min at RT. One hundred microlitres of each pre-incubated samples were transferred into a well of an antigen coated microtitre plate and incubated for 60 min at RT. The wells were then rinsed 5 times with diluted wash solution. One hundred microlitres of HRP conjugate were added to each well and incubated for 30 min at RT. The wells were washed 5 times with diluted wash solution. One hundred microlitres of substrate solution were added and the plates incubated for 10 min at RT in the dark. The reaction was stopped with 100 µl of stop solution. The absorbance was measured at 450 nm by ELISA plate reader (Start Fax 2100, Awarness Technology Inc. USA).

Results

Using the serum neutralisation test, 137 samples were examined to detect antibodies in dromedary camels against BHV-1 and no antibodies were detected.

A modified bovine ELISA was used to detect Map antibodies and 2 of 90 camels (2.2%) were found positive. Both positive camels were female with 5 and 8 years age.

The camels in this study were older than 3 years, as the pastoralists and camel producers never want to slaughter younger camels, and information about the maternal antibody situation in the young camels was not available.

Discussion

In this survey 137 serum samples were examined and no antibodies to BHV-1 were detected. Hedger *et al* (1980), Bornstein and Musa (1987), Bohrmann *et al* (1988) and Wernery and Wernery (1990) were not able to detect any antibodies to BHV-1 in dromedaries. In a serological survey in Germany, BHV-1 antibody was recorded from 11 of 85 red deer and one of 71 roe deer but no antibody was detected in blood samples taken from horse, swine, sheep, goat, dog, hen, turkey-hen, goose, reindeer, elk, camel, lion, white-handed gibbon, hare, fallow deer, wild boar and man (Kokles, 1977). The prevalence of BHV-1 antibodies in Tunisian dromedaries tested was 5.8% (Burgemeister *et al,* 1975). In a serological survey conducted in Egypt, Moussa *et al* (1990) found BHV-1 antibodies in 13.5% of 171 dromedaries. In an experimental trial, Wernery and Kaaden (2002) infected 2 dromedaries intranasally with a BHV-1 strain and both camels failed to develop any clinical signs and failed to seroconvert. Results on the prevalence of IBR antibodies in a serological survey in Saudi Arabia indicated that 13% of the examined dromedary camels had serum antibodies against IBR virus and that the positive camels were from the eastern region only (Al-Afaleq *et al,* 2007). Natural exposure of dromedary camels to BHV-1 was studied in Sudan and detection of BHV-1 antigen, genome using polymerase chain reaction, isolation in cell culture and antibodies was reported (Intisar *et al,* 2009). Detection and isolation of parainfluenza 3 virus and BHV-1 as mixed infection among camels in Egypt have been recorded by Nawal *et al* (2003). They concluded that camels most probably can play a significant role in the epidemiology of these viral diseases. In a serological survey in Argentina, Puntel *et al* (1999) identified 0.77% BHV-1 positive llamas. The prevalence of BHV-1 antibodies in 118 alpacas tested was 5.08% in Peru (Rivera *et al,* 1987).

To determine the presence of neutralising antibodies against IBR, a total of 9968 bovine serum samples from different parts of Iran were examined. Antibodies against IBR were observed in 33.97% of

the samples. The results indicated that IBR virus infection is widely distributed among the bovine population in Iran (Kargar Moakhar *et al,* 2001). In a serological survey in Iran, Raoofi *et al* (2004) detected 10.7% BHV-1 positive sheep. Camels frequently come in contact with domestic animals, such as sheep and goats, and to a lesser extent with cattle but no cases of clinical IBR have been confirmed in camels in Iran.

In this survey 2 of 90 camels (2.2%) were found positive for Map antibodies. Since the serum samples examined in the present study were collected from camels older than 3 years of age, and camels are not vaccinated against paratuberculosis in Iran, the antibodies which were detected in the serum must have been due to exposure to the Map circulating in the environment. Cases of clinical paratuberculosis have been confirmed in dromedary camels in Iran (Taghipour Bazargani *et al,* 2009) and some other countries (Amand, 1974; Al-Hizab, 2010).

In a serological survey in Tunisia, Burgemeister *et al* (1975) detected 21.2% Map positive dromedaries. In a serological survey in mixed farming of domesticated wild herbivores and livestock in Kenya antibody titres to Map were found only in camels and goats. Mycobacteria were not detected in faeces of 2 serologically positive camels (Paling *et al,* 1988). The analysis of 95 serum samples from dromedary camels at different ages with commercial bovine ELISA indicated 8.4% positive samples. The positive samples were restricted to older animals (7-15 years old) (Alluwaimi, 2008). However, serological tests for paratuberculosis on individual animals are often inconclusive, but they are of value when entire herds are screened (Wernery and Kaaden, 2002).

Limited information is available regarding the epidemiology of Johne's disease in camelids (Stehman, 1996). As the camelid industry continues to gain in popularity, large economic losses would occur should paratuberculosis become established and be allowed to spread. Similar to cattle, camelid paratuberculosis infections may proceed undetected for a prolonged period of time. The subclinically infected animal may be contaminating the premises with the organism, thus exposing susceptible herd mates to infection (Kramsky *et al,* 2000). Ruminants paratuberculosis is difficult to be controlled due to the involvement in the disease epidemiology of ruminants and non-ruminants wild animals, birds and inferior living creating natural disease reservoirs maintaining and disseminating the causative agent (Papastergiu *et al,* 2009).

Although, the prevalence rate of antibodies in camels in Iran against Map in this study was low, they are at risk for infection. Authors feel that further studies are required to elucidate the exact role of camels in the epidemiology of paratuberculosis in Iran.

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