OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Seroprevalence of Peste des Petits Ruminants and Infectious Bovine Rhinotracheitis Viruses in Dromedary Camels, Al-Ahsa, Saudi Arabia

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Abstract | The present study aimed at the seroprevalence analysis of Peste des Petits Ruminants virus and Infectious Bovine Rhinotracheitis virus in dromedary camels from Al-Ahsa region in Saudi Arabia. Serum samples collected from 225 dromedary camels including 175 female and 50 male camels kept in 13 local herds in Al-Ahsa region of Saudi Arabia were tested using ELISA. The overall camel-level seroprevalence was 2.7 % for PPR (95% CI = 1.2 – 5.7) and 0.4 % (95% CI = 0 – 2.5) for IBR. At herd-level, the seropositivity was 38.5 % and 7.7 % for PPR and IBR, respectively. In the present study, although the PPR seroprevalence is within the range reported in the literature for the seroprevalence of PPR in camels in Saudi Arabia, the very low seroprevalence of IBR found here contrasts with the previously reported prevalence of 13% in camels from the same area. For neither virus was there an association between herd size and the seroprevalence. Although it was statistically not significant, higher seroprevalence for PPR was found in Mojaheem (3.8 %) breed camels, in camels from the age group II (> 5 years, 3.6 %), in male camels (4.0 %), and in pregnant animals (3.7 %). Antibodies to IBR were detected in only one camel of the Mojaheem breed. In conclusion, the present study determined a relatively low seroprevalence rates of PPR and IBR among camel populations in the Al-Ahsa region in Saudi Arabia. Further studies are required with a larger sample size and covering multiple geographical areas in Saudi Arabia to characterize the epidemiological status of the two pathogens and to identify potential risk factors in camels.

Keywords | Camel, Antibody, ELISA, PPR, IBR

Received | November 25, 2025; **Accepted** | December 19, 2025; **Published** | January 10, 2026
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Citation | Falemban B, Aleid MS, Alamer AA, Hussen J (2026). Seroprevalence of peste des petits ruminants and infectious bovine rhinotracheitis viruses in dromedary camels, Al-Ahsa, Saudi Arabia. Adv. Anim. Vet. Sci., 14(1):72-77.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.1.72.77
ISSN (Online) | 2307-8316

INTRODUCTION

The dromedary camel is one of the important livestock species, especially in arid and semi-arid regions, due to its ability to survive and reproduce under harsh conditions and limited food and water resources (Faye, 2020; Birhanu *et al.*, 2016).

Peste des petits ruminants virus (PPRV), a member of the morbillivirus family that predominantly affects ovine and caprine species, has increasingly been documented in non-traditional hosts, including camels (Rahman *et al.*, 2020; Kinne *et al.*, 2010; Sowjanya-Kumari *et al.*, 2021). This evidence is mainly derived from serological studies, antigen/genomic detection methodologies, experimental inoculations, and epidemiological outbreak reports in different countries (Rahman *et al.*, 2020; Woma *et al.*, 2015). Camels can be at special risk if housed in close contact with infected small ruminants. Evidence also suggests that camels may shed virus and transmit the disease to small ruminants. While the majority of seropositive camelids exhibit subclinical form of the disease, sporadic clinical

outbreaks characterized by severe pathology have been also reported, and experimental studies indicate that camels are susceptible to infection and have the potential to transmit PPRV to small ruminants (Khalafalla *et al.*, 2010). Clinical presentations in camels range between asymptomatic or mild symptoms to severe disease with fever, ocular/nasal discharge, stomatitis, diarrhea, respiratory distress, skin lesions and fatalities (Zakian *et al.*, 2016). Numerous serological surveys conducted in Africa and the Middle East have identified PPRV-specific antibodies in dromedary camels, with variable seroprevalence ranges depending on geographic region, sampling techniques, and diagnostic assays (Hemida and Al-Ghadeer, 2019; Omani *et al.*, 2019). Although live attenuated PPR vaccines are used to immunize small ruminants, limited information is available on the performance of these vaccines in camels, and vaccination is not routinely practiced.

Infectious bovine rhinotracheitis, caused by the alpha-herpesvirus bovine herpes virus 1 (BoHV-1) is a respiratory disease mainly affecting cattle. Although camels were thought to be relatively resistant, multiple studies reported natural exposure of camels and occasional isolation of virus or viral genome from camel tissues (Rimayanti *et al.*, 2024; Intisar *et al.*, 2009). Multiple serosurveys reported BoHV-1- specific antibody prevalence in dromedary populations across Africa and parts of the Middle East/North Africa with seroprevalences that range from low-to-moderate up to very high based on study location and diagnostic test. Few studies investigated the seroprevalence of Infectious Bovine Rhinotracheitis (IBR) in camels in Saudi Arabia. A local serosurvey in 2007 study showed a 13% prevalence of IBR in camels from the Eastern region of Saudi Arabia (Al-Afaleq *et al.*, 2007). The lag of recent prevalence studies on IBR in Saudi camels since the 2007 study represents an epidemiological gap concerning the disease. Studies on ruminants reported high seroprevalence of BHV-1 among cattle in the Western and Eastern regions of Saudi Arabia (50 %), presenting an infection risk for camels in these regions (Ali and Hemida, 2014). Considering the common practice of co-herding camels alongside various ruminant species in the Eastern Region of Saudi Arabia, it is anticipated that cattle could serve as a potential source for infections in camels through shared resources (water, feed) within the herds. In addition, the transition from extensive to semi-intensive and intensive management systems has expanded the interface between camels and other domestic ruminants, particularly cattle and small ruminants. Within this complex management framework, the transmission of respiratory diseases from cattle to camels has emerged as a significant veterinary health concern. The present study aimed to analyze the seroprevalence PPR and IBR in dromedary camels from Al-Ahsa region in Saudi Arabia.

MATERIALS AND METHODS

Study area and population

The present study analyzed serum samples collected from 225 dromedary camels with no vaccination history for PPR or IBR kept in local camel herds in Al-Ahsa region located in the Eastern Province of Saudi Arabia. The study population included 175 female (77.8%) and 50 male (22.2%) camels of the Mojaheem (n = 106, 47.1%), Magateer (n = 91, 40.4%) and Hamra (n = 28, 12.4%) camel breeds. Within the female camel group, there were 53 pregnant (30.3%) and 122 non-pregnant (69.7 %) camels. Animals age ranged between 0.5 and 15 years with a mean age of 6 years (SD = 3.0). All the animals were clinically healthy based on clinical examination by a trained veterinarian. In this study, samples were collected from herds located in different parts of the Al-Ahsa region to ensure broad geographical coverage (Figure 1). To be representative for general camel husbandry in Al-Ahsa region, the sampled herds were of small to moderate sizes with camels being co-herded with cattle and small ruminants. However, the 13 herds included in the study were selected based on owner permission and field accessibility, and within each herd, all camels that were available and manageable for sampling during the visit were included.

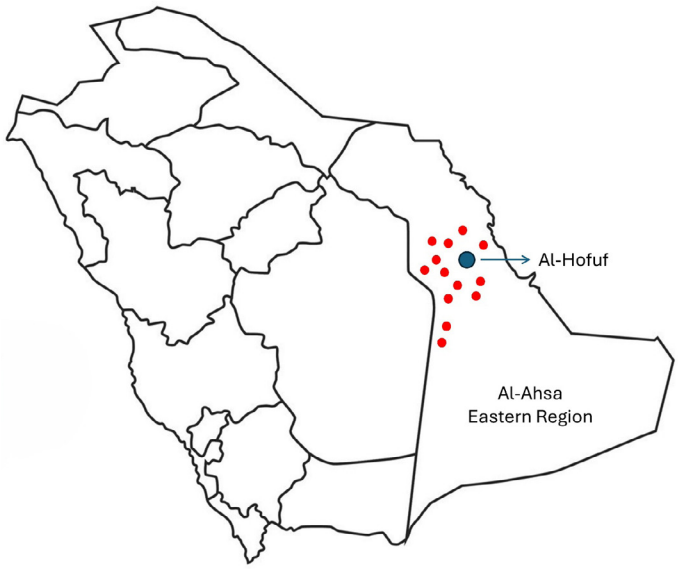

Figure 1: Saudi Arabia map adopted from "Vecteezy.com" and modified to show the location of the thirteen sampled camel herds within Al-Ahsa region.

Sample collection and preparation

The samples were collected from September 2023 to June 2024. Blood samples (4 mL) were collected from the jugular vein into serum collection tubes, kept in a cool box, and transported to the lab within 6 hours. Serum samples were collected after centrifugation of the tubes for 15 min at x1000 g and collected serum was stored at -80°C until serological analysis.

Detection of PPR antibodies in camel serum using enzyme-linked immunosorbent assay (ELISA)

Camel antibodies to PPR virus were detected in serum samples using a commercially available ELISA kit (ID Screen PPR competitive) according to the manufacturer instructions. The kit is based on a competitive ELISA test to detect antibodies to the viral protein antigen. Test and control sera (25 µl) were added to the wells of antigen-coated microplate prefilled with 25 µl of dilution buffer. After incubating for 18h at room temperature, the plate was washed three times with washing buffer and 100 µl of a 1:10 dilution of the conjugate (Anti-nucleoprotein antibody conjugated with horseradish peroxidase) was added to each well. After 30 min incubation at room temperature the plate was washed three times and substrate solution (100 µl) was added for 15 min in the dark followed by the addition of 100 µl stop solution and reading the plate at 450 nm on an ELISA reader (MR 5000, Dynatech, Denkendorf, Germany). The competition percentage (S/N %) was calculated for all samples as the percentage of OD value of sample to the OD value of the negative control. Samples with an S/N % ratio ≤ 50 % were considered positive.

Detection of IBR antibodies in camel serum using enzyme-linked immunosorbent assay (ELISA)

Antibodies to BoHV1 were detected in camel serum based on a commercially available competitive ELISA kit (IDEXX IBR gB x3) according to the manufacturer instructions. Test and control sera (50 µl) were added to the wells of antigen-coated microplate prefilled with 50 µl of wash solution. After incubating for 18h at 2–8 C, the plate was washed five times with washing buffer and 100 µl of a the conjugate was added to each well. After 1h incubation at room temperature the plate was washed three times and substrate solution (100 µl) was added for 10 min in the dark followed by the addition of 100 µl stop solution and reading the plate at 450 nm on an ELISA reader (MR 5000, Dynatech, Denkendorf, Germany). The blocking percentage (100 x (N-S/N)) was calculated for all samples after calculating the difference between OD value of the negative control (N) and the OD value of the sample (S) and multiplication of the ratio (N-S)/N by 100. Samples with blocking value (100 x (N-S/N)) a S/N % ratio ≥ 50 % were considered positive.

Statistical analysis

Statistical analysis was performed using the statistical program Prism (GraphPad Software V10, California, USA). Overall seroprevalence and the 95 % confidence interval (CI) were calculated using the Fraction of Total analysis function of the Prism program. The contingency analysis tool of Prism was used to perform *Chi-square* test and Fishers exact test to compare the differences in prevalence between groups in relation to breed, sex, and age ($p<0.05$). The correlation coefficient (R-squared) was calculated to test the association between seropositivity and animals age or herd size.

RESULTS AND DISCUSSION

The present study included 225 dromedary camels kept in 13 local herds in Eastern Province of Saudi Arabia with no vaccination history for PPR and IBR. Most of the camels studied were female camels (77.8%), of the Mojaheem (47.1 %) and Magateer breeds (40.4 %), and aged > 5 years (60.4%). Within the female camels, there were 53 (30.3 %) pregnant and 122 (69.7 %) non-pregnant she-camels The number of animals within each herd ranged between 5 and 65 (mean and SD = 25.4 ± 14.3).

The study evaluated the prevalence of antibodies to PPR and IBR viruses in camels from Al-Ahsa region in Saudi Arabia. Collectively, the results showed very low number of seropositive camels for both diseases (6 for PPR, 1 for IBR). The overall seroprevalence was 2.7 % for PPR (95% CI = 1.2 – 5.7) and 0.4 % (95% CI = 0 – 2.5) for IBR. At herd-level, the seropositivity was 38.5 % and 7.7 % for PPR, and IBR, respectively. For none of the viruses studied there was an association between herd size and the seroprevalence. The correlation coefficient (r^2) ranged between 0.04 and 0.05 ($p > 0.05$).

Table 1: Seropositivity of PPR, and IBR in 225 camels from the Eastern Province of Saudi Arabia according to animal breed.

Breed	Animals tested	Animals positive n (%)	
		PPR	IBR
Mojaheem	106	4 (3.8 %)	1 (0.9 %)
Magateer	91	2 (2.2 %)	0 (0.0 %)
Hamra	28	0 (0.0 %)	0 (0.0 %)
Grand total	225	6 (2.7 %)	1 (0.4 %)

Descriptive analysis of seroprevalence results for the two viral diseases is presented in Tables 1 and 2. Camels of the Mojaheem (3.8 %) breed showed higher seroprevalence for PPR than those of the Magateer breed (2.2 %), while no PPR seropositive animals detected within the Hamra breed camels (0.0 %). Camels from the age group II (> 5 years, 3.6 %) showed higher seroprevalence for PPR as compared to the camels from age group I (≤ 5 years, 1.1 %). Male camels (4.0 %) showed a higher PPR seroprevalence than females (2.3 %). Within the female camel group, there was a higher percentage of pregnant seropositive animals (3.7 %) than non-pregnant (1.6 %) animals. Antibodies to IBR

Table 2: Seropositivity of PPR, and IBR in 225 camels from the Eastern Province of Saudi Arabia according to animal age and gender.

Animal variable	Group	Total (n)	PPR positive n (%)	95% CI	PPR statistics	IBR positive n (%)	95% CI	IBR statistics
Gender	Female	175	4 (2.3%)	0.9 – 5.7	P = 0.50	1 (0.6%)	0.0 – 3.2	P = 0.59
	Male	50	2 (4.0%)	0.7 – 13.5	(χ2: 0.44)	0 (0.0%)	0.0 – 7.1	(χ2: 0.28)
Age	Group I (≤ 5y)	89	1 (1.1%)	0.1 – 6.1	P = 0.24	1 (1.1%)	0.1 – 6.1	P = 0.21
	Group II (> 5y)	136	5 (3.6%)	1.6 – 8.3	(χ2: 1.40)	0 (0.0%)	0.0 – 2.7	(χ2: 1.50)

were detected in only one camel of the Mojaheem breed. The positive animal was a non-pregnant she-camel. This is reflected by the very wide confidence intervals observed for the studied whole camel population or the subgroups as shown in Table 2.

Several studies in different countries, including Saudi Arabia, reported the exposure of camels to the PPR virus. The results of the present study with the prevalence of anti-PPR antibodies in 2.7 % of the tested camels seems within the range reported in the literature for the seroprevalence of PPR in camels in Saudi Arabia showing values between 0 and 3 % according to the region. A seroprevalence study conducted between 2014 and 2016 in the eastern and southern regions of Saudi Arabia found an overall PPRV seroprevalence of approximately 2.97% in dromedary camels. The study included camels kept in herds that were in close contact with PPRV-positive small ruminants (Hemida and Al-Ghadeer, 2019). This low seropositivity at animal-level along with the high herd-level seropositivity of 38.5% suggests sporadic low-level exposure within herds. Given the high prevalence within small ruminants in the Eastern Province of Saudi Arabia (Boshra *et al*., 2015), this pattern seems in support of an occasional spillover of the PPR virus from small ruminants to camels rather than sustained camel-to-camel transmission. Although some differences in the percentages of PPR seropositive camels were found between the studied camel population according to breed, gender, age, and pregnancy status, none of the mentioned factors showed a statistically significant association with PPR seroprevalence.

The exposure of camels to BHV-1 has been reported in several serological surveys across Saudi Arabia. In the present study, a very low prevalence of IBR-specific antibodies was found in camels from Al-Ahsa region in Saudi Arabia, a result that is in contrast to the seroprevalence of 13% reported 2007 in camels from the same area (Al-Afaleq *et al*., 2007). Looking for an interpretation of this massive difference in seroprevalence ratios for the same geographical area one may discuss different potential reasons. Given the moderate to high seroprevalence of BHV-1 in cattle reported in several studies conducted in Saudi Arabia between 2007 and 2016 (Mahmoud and Allam, 2013; Al-Hammadi, 2016), indicating the virus is still endemic in the country, it is unlikely that the observed difference in seroprevalence is due to a decline in virus circulation among camel populations. On the other hand, a role of different analysis methodologies in the observed difference would be speculative as the exact ELISA method used in the study from 2007 was not described in the paper. Given the lack of vaccination programs against IBR for camels in Saudi Arabia, one might exclude the role of different vaccination histories for the camel populations included in the two studies. Whether the sampling approach employed in the two studies contributed to this discrepancy, is to be investigated in future epidemiological studies involving a higher number of camels with an unbiased random sampling strategy.

In the present study, although samples were collected from herds located in different parts of the Al-Ahsa region to ensure broad geographical coverage, seroprevalence results are still limited by the convenience sampling strategy with samples being collected from only accessible herds and camels. Such approach may introduce selection bias and limit the generalizability of the prevalence estimates to the entire camel population of Al-Ahsa region.

CONCLUSION

The present study determined relatively low seroprevalence rates of PPR and IBR among camel populations in the Al-Ahsa region in Saudi Arabia. Despite these low levels, the marked difference in IBR seropositivity compared with data reported in the study from 2007 suggests a potential shift in exposure that warrants follow-up studies to determine whether this discrepancy reflects true epidemiological shift or is due to differences in sampling approaches between the two studies. To better characterize virus circulation, future work should incorporate molecular diagnostics and include other susceptible ruminant species in the region to clarify interspecies transmission dynamics. The low seroprevalence found for the two pathogens makes it difficult to draw conclusions regarding potential risk factors. Therefore, future studies should include a larger sample size, employ random sampling approaches, and cover multiple geographical areas to characterize the epidemiological status of the two pathogens and guide veterinary control strategies in camels in Saudi Arabia.

ACKNOWLEDGEMENT

The authors thank he Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University for funding this project.

NOVELTY STATEMENT

The present study determined relatively low seroprevalence rates of PPR and IBR among camel populations in the Al-Ahsa region in Saudi Arabia. Especially the marked difference in IBR seropositivity compared with data reported in the study from 2007 suggests a potential shift in exposure that warrants follow-up confirmation studies.

AUTHOR'S CONTRIBUTION

JH supervised the project and wrote the first draft of the manuscript. BF performed the analysis. MSA collected and processed the samples. AAA collected and processed the samples. All authors read and approved the final manuscript.

Funding

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (KFU254507).

Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. This study was granted approval by the Ethics Committee of King Faisal University, Saudi Arabia (KFU-REC-2023-SEP-ETHICS1372). All measures were taken to minimize stress and discomfort to the animals during blood sampling.

Generative AI and AI-assisted technology statement

The authors declare that all scientific interpretations and conclusions are entirely the authors' own and no generative AI or AI-assisted technologies were used to generate, analyze, or interpret the research data. Generative AI tools were used only to assist in improving the grammar.

Conflict of interest

The authors have declared no conflict of interest.

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