Betaretrovirus infections in dromedary camels in Saudi Arabia

Maged Gomaa Hemida1,2 | Abdelmohsen A. Alnaeem3

1 Department of Microbiology, College of Veterinary Medicine, King Faisal University, Al Ahsa, Saudi Arabia
2 Department of Virology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt
3 Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, Al Ahsa, Saudi Arabia

Correspondence
Maged Gomaa Hemida, Department of Microbiology and Parasitology, College of Veterinary Medicine, Office No: 2127, Building No: 13, Al-Hufuf, Al-Hasa, Po Box: 400, King Faisal University, Al Ahsa, Saudi Arabia.
Email: mhemida@kfup.edu.sa

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Abstract

Background: Retroviral infections have been reported in many species of animals, especially cattle, sheep and goats. However, there are no available reports about retrovirus infection in dromedary camels. Several dromedary camels showed visible tumor-like lesions on and around the nostrils as well as around the eyes.

Objectives: Following are the objectives: to identify the causative agents of these identified tumours in dromedary camels and to perform molecular characterization of the detected strains of the causative agent.

Methods: We extracted the nucleic acids from some fresh lesions out of these animals, and then amplified some key retrovirus genes. We amplified several regions of the rotavirus genome using the PCR technique. The obtained sequences were assembled and the phylogenetic trees were conducted per each target retrovirus gene.

Results: Our results revealed a high degree of identity to some retroviruses of sheep. Phylogenetic analysis based on some retrovirus genes revealed that the causative agents of these lesions are closely related to sheep retroviruses, particularly the Jaagsiekte sheep Retrovirus (JSRV) and the ENTV.

Conclusions: To the best of our knowledge, this is the first report of retrovirus infections in dromedary camels in the Arabian Peninsula. This highlights the possible species jump for the retrovirus from sheep and goats to the dromedary camels, which live in close proximity with these animals in many parts of the world, especially the Arabian Peninsula.

KEYWORDS
dromedary camel, genome, interspecies transmission, nasal lesions, phylogenetic analysis, retrovirus, sequencing

1 | INTRODUCTION

The family Retroviridae includes a large number of viruses affecting various species of animals, birds and humans. Retrovirus infection induces various forms of clinical diseases including inflammatory changes, immunosuppression and neoplasia. There are several methods for the classification of retroviruses. The older system was mainly dependent on the morphology of the virus under the electron microscope into four groups (A, B, C and D). However, the most recent classification of the virus was mainly based on the genetic sequences of the full-length genome of these viruses or the partial sequences of some key retrovirus genes (2018b, 2019). According to the recent classification, retroviruses are divided into two subfamilies (Orthoretrovirinae and Spumaretroviridae) (2018b, 2019). The retrovirus genome is mainly composed of two copies of single-stranded (+ve) sense RNA molecules, each of which is around 7 to 12 kb in length. The viral genomes are
flanked by 5′cap and poly(A) tails at their 5′ and 3′ ends, respectively. The typical genome organization of the retroviruses is as follows: 5′-R U5 gag pro pol env U3 R-3′. The three major proteins in the retrovirus structure and functions are Gag, Pol and Env. The Gag protein represents three layers of the retroviruses. This protein plays important role in the assembly and packaging of the new viruses. The Pol gene has the function of the RNA-dependent DNA polymerase enzyme. Any changes in the genetic materials of this gene are responsible for the diversity among retroviruses. The provirus is integrated into the host cells representing a continuous source of infection to other animals (Toma et al., 1990). Integration of the retroviral genes into the host cell genome especially during the early embryonic stages resulted in the development of many endogenous retroviruses (ERVs). The ERVs have been reported in many species of animals including pet animals (dogs and cats) as well as other domestic animals such as sheep, goats, cattle and horses (Garcia-Etxebarria et al., 2014). These ERVs have the vertical ability of transmission from one generation to another (Garcia-Etxebarria et al., 2014). There are several DNA viruses causing tumours in animals such as poxvirus, herpesviruses, adenoviruses and papillomaviruses. However, almost all tumours caused by RNA viruses belong to the family Retroviridae (Wahren, 2014). Retroviral infection in sheep was reported as in the case of the exogenous (Jaagsiekte sheep Retrovirus [JSRV]) and the endogenous retrovirus [enJSRV] (Chessa et al., 2009; Palmarini et al., 2004). We examined several dromedary camels showing skin lesions in different regions of the head including some lesions around the nostrils, some other lesions inside the nasal passage starting from the external nasal orifices, as well as some lesions around the eyes. The main objective of the current study was to identify these reported lesions in camels as well as to perform molecular characterization of their causative agent.

2 | METHODS

2.1 | Clinical examination, specimen’s collection and processing

Five dromedary camels showing skin lesions around the nostrils, mouth and eyes were identified in Saudi Arabia during 2016 (Table 1). These animals were subjected to the routine slaughtering protocol. Physical inspection of the animals was conducted. The body of each animal was examined carefully for the presence of any signs of abnormal discharges from the eyes and nostrils. The vital signs of each animal were assessed particularly the temperature and the colour of the mucous membranes of the eyes and vagina (in case of female animals). Tissue specimens from the lesions and some adjacent normal tissues were collected immediately after the slaughtering of these animals. Tissue specimens from each animal were divided into two halves. One half was placed on 10% tissue formalin for downstream histology processing while the other half was collected on the RNA later (QIAGEN, Catalog #76104). Samples collected on the RNA later were stored at (−80°C) for further processing. Meanwhile, we collected similar tissues from the exact locations from two healthy dromedary camels (one from Magaheem and one from Magateer camel) as negative controls. Samples from these animals were collected in the slaughterhouse during the necropsy examination of some of the animals showed the characteristic lesions under testing. Processing of the tissue specimens was done as previously described elsewhere (Khalafalla et al., 2017).

2.2 | Tissue processing and extraction of the viral DNAs and RNAs

2.2.1 | Extraction of viral DNA

Viral DNAs were extracted from the freshly excised tumour tissues as previously described (Yousif et al., 2010). Simply, 10 grams per lesion was collected and washed several times with sterile saline. These lesions were crushed using sterile sand in clean mortars with a pestle. We prepared 10% tissue suspensions per lesion by adding 90 mL of sterile saline. We centrifuged the prepared tissue suspensions at 5000 rpm for 10 min at 4°C. We collected the supernatants and stored them at (−80°C) until further DNA extraction.

2.2.2 | Extraction of viral RNA

We extracted the total viral RNAs from the prepared tissues using RNAzol-RT-RNA isolation reagent (GeneCopoela, Rockville, MD, USA) as per the instruction from the manufacturers. The RNA concentrations were carried out by the NanoDrop machine. The extracted RNAs were stored at (−80°C) until further testing.

| N | Breed | Age/Years | Sex | Place of collection | Type of lesion/size |
|---|-------|-----------|-----|---------------------|---------------------|
| 1 | Magaheem | 5 | Male | Al-Hasa | Cauliflower/large |
| 2 | Magaheem | 4 | Female | Dammam | Cauliflower/large |
| 3 | Magaheem | 4 | Male | Ibqaiq | Nodule/small |
| 4 | Magateer | 6 | male | Al-Hasa | Nodule/small |
| 5 | Magateer | 5 | female | Al-Hasa | Cauliflower/small |
| 6 | Magaheem | 5 | male | Al-Hasa | None/apparently healthy |
| 7 | Magateer | 4 | female | Al-Hasa | None/apparently healthy |
TABLE 2  List of oligonucleotides used in the current study

| N | Virus | Primer name | Target gene | Sequence (5′-3′) | Amplicon size (nt) | Refs. |
|---|-------|-------------|-------------|------------------|--------------------|-------|
| 1 | CPV   | CdPV1-F     | CdPV1-L1- ORF | CTACCTGCCGGATCATGTCCA | 480               | Bernard et al. (2010) |
| 2 | CPV   | CdPV1-R     | CdPV1-L1- ORF | ATCAGCTCCTGCACTAGTCTT | 480               | Bernard et al. (2010) |
| 3 | CPV   | CdPV2-F     | CdPV2-L1- ORF | CAATTAGAGTGTCAAAAGTCGAA | 480               | Bernard et al. (2010) |
| 4 | CPV   | CdPV2-R     | CdPV2-L1- ORF | ATGGGGGTACCTTTGGTATGT | 480               | Bernard et al. (2010) |
| 5 | BRTV  | BRTV-gag-F  | Gag         | AAACAGACAGCTAGGGCGT | 859               | He et al. (2017) |
| 6 | BRTV  | BRTV-gag-R  | Gag         | GCTCGACAGAGGTCTGCAAT | 479               | He et al. (2017) |
| 7 | BRTV  | BRTV-Env-F  | Env         | TGGAGGCACGAGATGGACTAC | 479               | He et al. (2017) |
| 8 | BRTV  | BRTV-ENV-R  | Env         | CGACATTCCGTTTTGCGACA | 653               | He et al. (2017) |
| 9 | BRTV  | BRTV-Pol-F  | Pol         | GACGTAGAGGCCCATCCAA | 445               | He et al. (2017) |
| 10 | BRTV  | BRTV-Pol-R  | Pol         | CAGCGCGAAGTCCCATGAT |                   |       |
| 11 | BRTV  | BRTV-Pro-F  | Pro         | AATGTTACCGGACACGACC | 445               | He et al. (2017) |
| 12 | BRTV  | BRTV-Pro-R  | Por         | AGATCGAAAAAGCTGGGGT |                   |       |

2.3 Oligonucleotides

We used the designed oligonucleotides for the papillomavirus full-length ORF-L1 as previously described (Bernard et al., 2010) and as listed in Table 2. Meanwhile, we used the primer design free online tools to design the primers of the betaretrovirus target genes (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Table 2 shows the primer sequences used for the amplification of various betaretrovirus genes including gag, env, pol and pro.

2.4 Amplification of papillomavirus ORF-L1

We tried to amplify the papillomavirus ORF-L1 regions from the extracted DNAs of all samples. The PCR amplification procedure and conditions were carried out as previously described (Forslund et al., 1999).

2.5 RT-PCR and PCR

The cDNAs were synthesised by using random hexamer as previously described (He et al., 2017) which have been used as a template for the downstream PCR reactions. We used four pairs of primers to amplify the betaretrovirus target genes (Table 2). The PCR reactions were performed as described elsewhere (He et al., 2017).

2.6 Sequencing and phylogenetic analysis

The nucleotide sequences for each target genes were assembled into one contig using the Lasergene version 7.1 software sequencing analysis (DNASTAR Inc., Madison, WI). We used the GCG programs Clustal-W and the Cluster to calculate the nucleotide identity of the reported gag, env, pol and pro genes. The maximum likelihood, pairwise nucleotide identities were established by the Mega-7 software as previously described (Kumar et al., 2016).

3 RESULTS

3.1 Clinical signs and gross pathology of the retrovirus infection in dromedary camels

Five dromedary camels (three Magaheem and two Magateer) were examined during the ante-mortem inspection in the regional abattoir in eastern Saudi Arabia. Animals showed a decrease in body weight and loss of appetite. Animals showed opened nares with forced breathing with serous to mucopurulent discharges from the nose. Physical examination of animals revealed the presence of a cauliflower-like mass of tumours in the left nostrils (Figure 1). This tumour connected to the ethmoid bone and extended upwards toward the nasal turbinate bones resulting in their damage. These tumours partially blocked the left nasal meatuses (Figure 1). Examination of the excised tumour tissues revealed the presence of a large, dark solid area of necrosis. Meanwhile, the nasal septum was penetrated by these tumours (Figure 1).

3.2 Molecular characterization of retrovirus infection in dromedary camels

Our results show that all the tested tissue specimens were negative for the camel papillomavirus. Five out of the tested tissue specimens from animals were positive for the retrovirus using the gag, env, pol and pro genes of retroviruses. The bioinformatics and the phylogenetic analysis, the obtained sequences from these genes revealed a high degree of identity with other members of the betaretroviruses particularly the JSRV and the enzootic nasal tumour virus (ENTV) (Figures 2 and 3).
FIGURE 1 Gross lesions of the betaretrovirus infection in dromedary camels. The growth lesion of the betaretrovirus infection in some dromedary camels. (a) Some lesions appear as dry crust with fissures around the nostril of the affected animals. Other animals show small crusts around the eyes of the affected animals. (b) Some lesions appear on the nostrils as a large cauliflower-like mass of tumors in the left nostrils. (c) Large tissue crust lesion at the bifurcation of the nostrils of one retrovirus infected dromedary camel. (d) Cut section of the retrovirus induced tumor in dromedary camels, this tumor connected to the ethmoid bone and extended upwards towered the nasal turbinate. This tumor partially blocked the left nasal meatuses

3.3 Nucleotide sequencing and accession numbers

We deposited all the developed sequences from the current study in the Genbank under the accession numbers (MK205419, MK205420, MK205421 and MK205422) for the gag, env, pol and pro genes, respectively.

4 DISCUSSION

In many regions in the world, such as the Arabian Peninsula and the Horn of Africa, dromedary camels, sheep and goats are usually kept together especially in the open grazing area (Hemida et al., 2017). There are a large number of viral infections reported in dromedary camels and sheeps such as the bluetongue virus (BTV), the pest des petite ruminants and the foot and mouth disease virus (FMDV) (Larska et al., 2009; Touil et al., 2012; Zakian et al., 2016). Although these viral infections cause diseases of various severities in sheep and goats, some of them are found to cause no clinical signs of diseases in dromedary camels such as BTV and FMDV (Larska et al., 2009; Zakian et al., 2016). There is no available data about retrovirus infections in various species of animals, especially sheep, goats and dromedary camels in the Arabian Peninsula. We observed some dromedary camels showing some skin lesions and warts in different regions of the head (Figure 1). There are several viruses causing skin tumours in dromedary camels such as camel poxvirus, Orf [contagious pustular dermatitis] and camel papillomavirus (Alajlan & Alsubeeh, 2020; Duraffour et al., 2011; Sobhy et al., 2020). These viral infections induce various forms of skin lesions in camels. Camel poxivirus is endemic in many regions around the world that have dromedary camels especially the Arabian Peninsula and Africa (Yousif & Al-Naeem, 2012; Yousif et al., 2010). The camel poxvirus skin lesions vary from generalized skin rashes to the production of papules and vesicles of different sizes (Yousif & Al-Naeem, 2012; Yousif et al., 2010). While Orf virus infection develops lesions in the affected animals starting from papules, they progressed and accumulated to develop fissured crust in various regions of the head, especially around the external nares and the eyes (Azwai et al.,...
Camel papillomavirus causes wart-like skin lesions on the head of the affected animals, and also particularly around the lips, eyes and nostrils (Munz et al., 1990). However, retrovirus infection in dromedary camels was never reported. We observed several dromedary camels showing various forms of skin lesions particularly in the form of crusts and warts around the eyes and the external nares (Figure 1). Some animals showed big masses extending from the nasal orifices and continuing deeper into the ethmoid bone (Figure 1). Treatment of the wart-like or mass of tumors from various regions in the body of the dromedary camel’s particularity the face was done by surgical removal (Khalafalla et al., 2017). Based on the clinical examination of the reported lesions and the description and the distribution of the skin lesions, we suspected these tumors are of viral origins either due to papillomavirus or retrovirus infections. Initially, we tested these tissue specimens from the warts of these animals for the presence of camel papillomavirus by PCR, however; all the tested samples were negative. The obtained sequences from some lesions revealed the presence of on-going retrovirus infection. The reported sequences showed a high degree of similarity to some other members of retroviruses affecting sheep and goats especially the JSRV and the ENTV based on some key retrovirus genes (gag and env) as well as (pol and pro), (Figures 2 and 3) respectively and Table 3. Based on the high similarity between the reported retroviruses in dromedary camels in this study and that of sheep and goats, we assume there might be a species jump of these viruses from small ruminants to dromedary camels. However, these phenomena still require further study. To our knowledge, this is the first report about

**Figure 2** Phylogenetic analysis of the betaretrovirus detected in the dromedary camels in Saudi Arabia based on the gag and env genes sequences. Phylogenetic analysis based on the maximum likelihood of the betaretrovirus detected in dromedary camels in Saudi Arabia based on the partial sequences of the gag (a), env (b). The bootstrap (1000) is showing next to branches. The phylogenetic analysis was conducted using Mega-X software. Sequences reported in this study are marked with black triangles.

**Figure 3** Phylogenetic analysis of the retrovirus detected in the dromedary camels in Saudi Arabia based on the Pol and Pro genes sequences. Phylogenetic analysis based on the maximum likelihood of the betaretrovirus detected in dromedary camels in Saudi Arabia based on the partial sequences of the Pol (a), Pro (b) and bootstrap (1000) is showing next to branches. The phylogenetic analysis was conducted using Mega-X software. Sequences reported in this study are marked with black triangles.
the detection of retrovirus infection in dromedary camels not only in Saudi Arabia but also worldwide. Further studies are required for a better understanding of the retrovirus infection in domestic animals in the Arabian Peninsula and the Middle East regions.

5 | CONCLUSIONS

We detected retrovirus infection in some dromedary camels in Saudi Arabia for the first time. These viruses showed a high degree of similarities with other retroviruses of sheep. This study points out the importance of studying retroviruses in various species of animals in the Arabian Peninsula.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Maged Hemida: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing original draft; Writing review & editing. Abdelmohsen Alnaeem: Conceptualization; Formal analysis; Investigation; Methodology; Resources; Visualization; Writing original draft, Writing review & editing.

ETHICS STATEMENT

All the animal experiments carried out in this study were conducted as per the instructions of the Animal Ethics protocols and the National Committee of Bio-Ethics, King Abdul-Aziz City of Science and Technology. The animal experiments and protocols were reviewed and approved by the animal ethics committee of the deanship of scientific research, King Faisal University, Saudi Arabia (Approval No: KFU-REC/2020-09-21).

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| TABLE 3 | Percentage nucleotide identity between the betaretrovirus, the ENTV-2 of goats and the JSRV |
|---------|-----------------------------------|
| Gene/Virus | ENTV-2-Gt | JSRV |
| Gag | 94.3 | 87.5 |
| Env | 85.3 | 84.7 |
| Pol | 88.7 | 90.5 |
| Pro | 93.7 | 97.3 |

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.760.

ORCID

Maged Gomaa Hemida https://orcid.org/0000-0003-1663-5820

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