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"Development of Duplex Real-Time PCR assay for rapid diagnosis and differentiation of Camel Pox and pox-like viral diseases in Camels"

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List of Abbreviations

ATIP	A-type inclusion protein
CMLV	Camelpox virus
BLAST	Basic Local Aligment Search Tool
Fig.	Figure
ELISA	Enzyme Linked Immunosorbent assay
%	Percentage
BPXV	Buffalopox virus
BHQ1	Black Hole Quencher 1
bp	Base pair
CCE	Camel contagious ecthyma
CCEV	camel contagious ecthyma virus
CdPV	Camelus dromedarius papilloma virus
CPPV	Camel parapox virus (usually referes to orf virus and CCEV)
CPXV	Cowpox virus
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
DNA pol	DNA polymerase
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme linked immunosorbent assay
FAM	6-carboxyfluorescein
ICTV	International committee for the taxonomy of viruses
Kb	Kilo base
Kbp	Kilo base pair
kDa	Kilo Dalton
L	Liter
LOD	Limit of Detection
MEM	Minimal Essential Medium
min	Minute
ml	Milliliter

mRNA	Messenger ribonucleic acid
NF-κB	Nuclear factor Kappa B
ng	nanogram
nm	Nanometer
nt	Nucleotide
NTC	No Template Control
µg	Microgram
µl	Microliter
µm	Micrometer
µM	Micromole
OIE	Office International des Epizootic
OPXV	Orthopoxvirus
ORF	Open reading frame
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
pg	picogram
PH	Hydrogen ion concentration
PPV	Parapoxvirus
PVs	Papillomaviruses
RNA	Ribonucleic acid
rpm	Round per minute
rtPCR	Real time PCR
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
Temp	Temperature
U	Unit
UK	United Kingdom
USA	United states of America
UV	Ultra-violet
VARV	Variola virus
VI	Virus Isolation

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VI. SUMMARY

Pox and pox like viral diseases are group of proliferative cutaneous lesions in dromedary camels. These diseases include camel pox, camel contagious ecthyma and camel papillomatosis which are difficult to be differentiated based on clinical signs only.

In the present study, two duplex real-time PCR assays were developed for rapid diagnosis and differentiation of camelpox and camel contagious ecthyma.

1. **Development of duplex real time PCR assay based on C18L and DNA pol genes for rapid diagnosis of camel pox in dromedary camels.**
   - The assay was designed by combination between two primer/probe sets; the first was for detection of pan-orthopoxvirus gene (DNApol gene). The second set was for C18L gene for specific detection of camelpox virus (CMLV).

   - The assay was specific and both genes were detected only in the CMLV reference strain. No amplification in Orf virus and camel contagious ecthyma virus. in sheeppox and goatpox viruses. Due to the unavailability of clinical cases of camelpox, Samples from healthy animals were collected and divided into two parts. The second part of each sample was spiked by CMLV reference strain. Different viral concentrations were used in spiking for efficient evaluation of the assay.

   - Both spiked and non-spiked samples were extracted and tested by the duplex probe based real-time PCR. The DNA pol gene and C18L genes were detected in spiked samples.

- The limit of detection of C18L gene was 0.4 pg. while for DNA pol gene, it was 4 pg. The efficiency of the duplex real time PCR assay for C18L gene was 100 % and 96.9 % for DNA pol gene.

2. Molecular and clinical diagnosis of camel contagious ecthyma in dromedary camels.

- Dromedary camels showed pox lesions in Qatar in 2017-2018, it was suspected camel contagious ecthyma. Twenty samples were collected and extracted then tested for C18L of CMLV and B2L gene of parapoxviruses by conventional PCR.
- Ten samples were positive for camel parapoxvirus. Sequencing and phylogenetic analysis revealed that the causative virus was more related to pseudocowpox virus than Orf virus and formed one cluster with camel contagious ecthyma viruses from Bahrain and Saudi Arabia and highly related to isolates from Sudan and Ethiopia.

- Presence of these well characterized samples of camel contagious ecthyma virus (CCEV) provides the chance for development of duplex real time assay for 2 diseases.

3. A SYBR Green based duplex real-time PCR for detection of camelpox virus and camel parapoxvirus

- Camelpox virus belongs to orthopoxvirus genus, while camel contagious ecthyma virus (CCEV) and orf virus (ORFV) belong to parapoxvirus genus of poxviridae family. The former two viruses were reported as causative agents for camel contagious ecthyma so we refere to them by camel parapox virus (CPPV).
- The assay was designed to detect CMLV C18L gene at melting point 76 ˚C while CPPV B2L gene was detected at 85.8 ˚C.

- The limit of detection was 20 copies per reaction for both CMLV and CPPV. The assay was evaluated in comparison with conventional PCR using 50 clinical samples from dromedary camels for detection of CMLV and CPPV.

- The positive detection rate of conventional PCR was 66% and 86% for conventional PCR and the duplex SYBR green real-time PCR assay, respectively. One case of coinfection with CMLV and CPPV was detected by both conventional PCR and the duplex SYBR green real-time PCR assay.

- These findings prove the higher sensitivity of the duplex SYBR green real time PCR assay than the conventional PCR methods with ability to avoid the false negative results which can happen with conventional PCR.

- The diagnosis of camelpox and camel contagious ecthyma will be accelerated by the use of these assays as they are in the duplex format so will increase the diagnostic capacity and save not only time but cost and effort as well.