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SHORT COMMUNICATIONS

The genetic identification of camel contagious ecthyma virus as the causative agent of contagious ecthyma in dromedary camels (*Camelus dromedarius*) in Qatar

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Abstract
Camel contagious ecthyma is a contagious viral disease of camels caused by either Orf virus (ORFV) or camel contagious ecthyma virus (CCEV). It has been previously reported and shown to cause economic losses in some camel-rearing countries in Asia and Africa, but has not been detected in Qatar. The purpose of this study was to identify and genetically characterize the contagious ecthyma causative agent in Qatari dromedary camels between 2017 and 2018. Accordingly, we made diagnoses of camel contagious ecthyma based on the clinical signs and genetic analysis of the entire major envelop protein (B2L) gene. The sequence analysis showed that CCEV was the infecting virus, and the B2L gene sequences were highly conserved between the locally infected camels with 100% similarity with isolates from Bahrain. This is the first study reporting the detection of CCEV in Qatar. We suggest that sequencing of the CCEV genome is necessary to determine the origin and relationship of this virus with other members of the parapoxvirus genus.

Keywords B2L gene · Contagious ecthyma · Dromedary camels · Parapoxvirus · Qatar

Introduction

Camel contagious ecthyma (CCE) is a contagious viral skin disease affecting camels and is characterized by the presence of pox-like lesions mainly on the facial region of infected camels (Abubakr et al. 2007; Nagarajan et al. 2010). The disease begins with papules that grow into pustules on the lips, muzzle, nose, and eyelids (Abubakr et al. 2007; Khalafalla et al. 2015b). The pustules on the lips rupture and ulcerate, and those on the muzzle develop into dry gray or brown scabs (Abubakr et al. 2007). The disease is usually transmitted by direct interaction between infected and healthy camels and indirect exposure to tainted feed troughs, pasture, and through skin abrasions (Khalafalla 2000). In most cases, the disease causes high morbidity but not mortality (Abubakr et al. 2007). However, in severe cases, especially in young calves, it can be fatal (Khalafalla et al. 2015b, 2020).

The disease is caused by a *Parapoxvirus* within the family *Poxviridae* either the Orf virus (ORFV) or camel contagious ecthyma virus (CCEV) (Abubakr et al. 2007; Khalafalla et al. 2015b). The Parapoxvirus genus consists of four species: Orf virus (ORFV) which usually infects sheep and goats, pseudocowpox virus (PCPV) and bovine papular stomatitis virus (BPSV) which infect cattle, and parapoxvirus of red deer in New Zealand (PVNZ) found in red deer in New Zealand (King et al. 2012). Three other related viruses have not yet been established as members of the parapoxvirus genus: camel contagious ecthyma virus (CCEV), seal parapoxvirus (SePPV), and chamois contagious ecthyma virus (King et al. 2012).

CCE was previously reported in Kenya, Somalia, Kazakhstan and Turkmenistan, Libya, Saudi Arabia and Bahrain, Sudan, India (Khalafalla et al. 2015b; Khalafalla et al. 2020), Israel (van Straten et al. 2001), Ethiopia (Gelaye et al. 2016), Iran (Gharib Mombeni et al. 2014; Oryan et al.

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2017), and Nigeria (Adedeji et al. 2018). Camels are used for different purposes as production (meat, milk, leather, wool), trade, racing, and beauty competitions (Faye 2016). In Qatar, camels are used mainly for racing and camel beauty shows and to a lesser extent for milk and meat production. From an economic standpoint, CCE causes losses in milk and meat production and skin quality (Gharib Mombeni et al. 2014). Moreover, the disease's clinical signs affect the performance and appearance of camels which consequently affects the racing camel trade and beauty competitions. The present study aimed to characterize the causative agent of CCE in Qatar by amplifying and sequencing the B2L gene which encodes major envelope protein of parapoxviruses.

Material and methods

Ten dromedary camels of 10 months to 3 years and both sexes showed pox-like lesions in Leawaina, Qatar, in 2017 and 2018 (Table 1). Tissue samples were collected by skin scraping of scab lesions and prepared for DNA extraction as described previously (Gelaye et al. 2016); then, the extraction was performed using the QiaAmp DNA Mini Kit (Qiagen, Germany) as directed by the manufacturer.

The major envelope protein (B2L) gene was amplified as described by Gelaye et al. (2016). The specific gel bands were excised and purified by GeneJET Gel Extraction Kit (Thermo Scientific). The DNA concentration of purified PCR products was measured by Qubit 2.0 Fluorometer (Life Technologies, USA). Two overlapping primers (Gelaye et al. 2016) were used for sequencing the full length of the B2L gene in both directions using the Big Dye terminator kit (Applied Biosystems, USA) in an ABI 3500 XL (Applied Biosystems, Foster City, CA, USA).

The sequences were assembled and aligned using BioEdit version (Hall 1999) with B2L gene sequences of CCEVs and other members of *Parapoxvirus* genus retrieved from GenBank (Supplementary Table 1). Multiple sequence alignment was performed by Clustal W in BioEdit. The phylogenetic tree of the B2L gene nucleotide sequences was constructed using the neighbor-joining method with the

Table 1 List of the samples characterized in this study including the strain name, date of collection, and the GenBank accession numbers for the nucleotide sequences of the B2L gene of CCEV

No	Strain name	Date of collection	Accession number
1	CCEV/01/2017	10/7/2017	MN178142
2	CCEV/02/2017	29/8/2017	MN178143
3	CCEV/03/2017	29/8/2017	MN178144
4	CCEV/04/2017	29/8/2017	MN178145
5	CCEV/05/2017	29/8/2017	MN178146
6	CCEV/06/2018	25/2/2018	MN178147
7	CCEV/07/2018	25/2/2018	MN178148
8	CCEV/09/2018	3/3/2018	MN178149
9	CCEV/10/2018	3/3/2018	MN178150
10	CCEV/11/2018	3/3/2018	MN178151

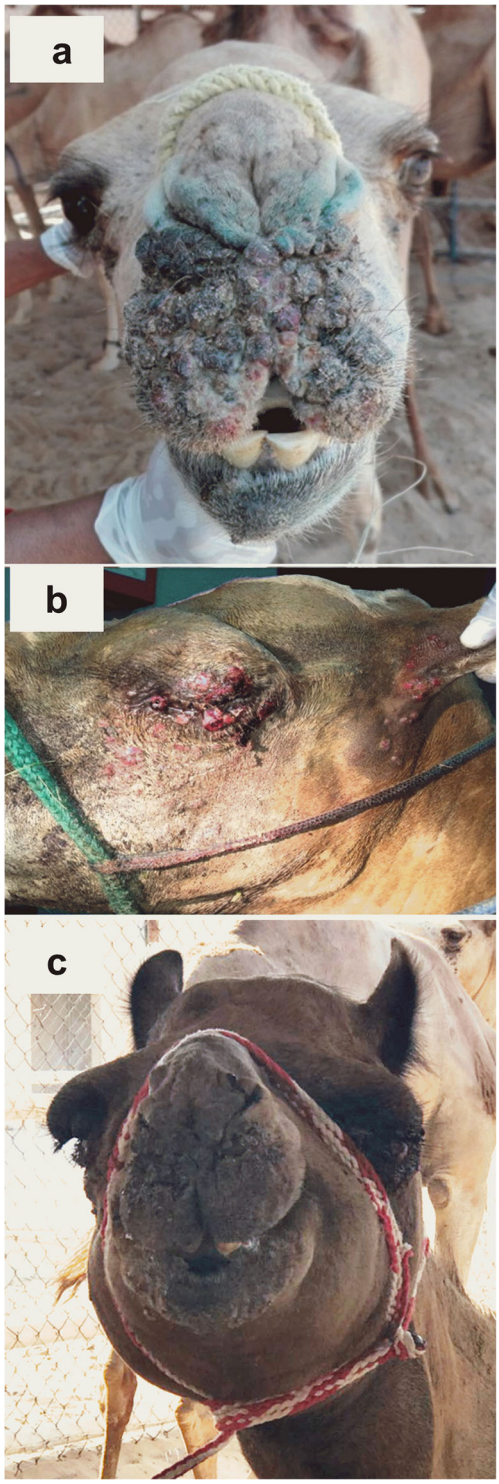

Fig. 1 Clinical signs of CCE pox-like lesions in infected camels. **A** Severe and nodular lesions on the upper lip and nostril. **B** Skin lesions on eyelids and under the ear. **C** Facial edema and blindness

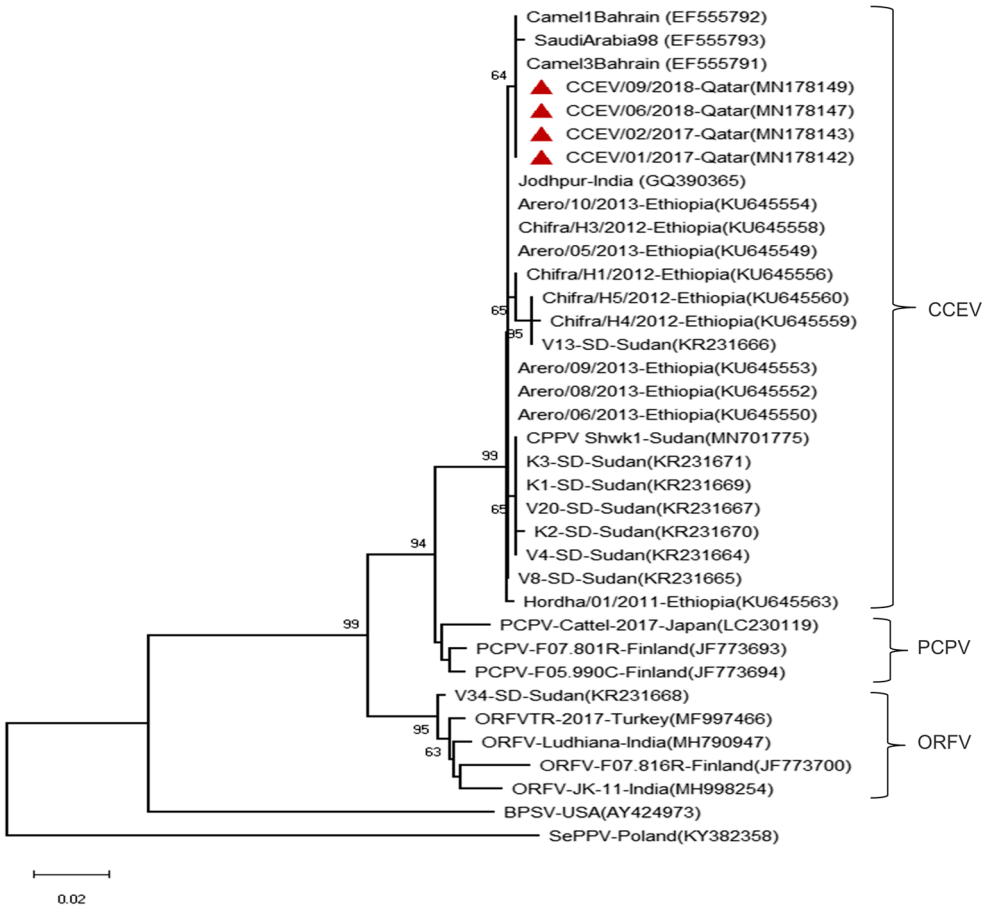

Fig. 2 Phylogenetic tree of parapoxviruses based on the partial nucleotide sequence of the B2L gene of four Qatari CCEV isolates represent different collection dates (marked with ▲) with 32 sequences retrieved from the GenBank. The neighbor-joining method with the maximum composite likelihood nucleotide substitution model was computed using MEGA X software. The homolog gene sequences from two PPV: BPSV and SePPV were used as the outgroup

maximum composite likelihood model by MEGA version X (Kumar et al. 2018). The robustness was tested by performing 1000 replicas of bootstrap.

Results and discussion

Infection of dromedary camels with numerous viruses causing pox lesions has been frequently reported such as camel pox, camel contagious ecthyma, and camel papillomatosis (Khalafalla et al. 2015a; Gelaye et al. 2016). Camel pox is usually characterized by generalized papules and eruptions on leg, groin, and the head, but lesions in CCE are localized in lips, nostrils, and eye (Khalafalla 1998). On the other side, lesions in camel papillomatosis appear as cauliflower-like masses on submandibular region skin and on the lips (Khalafalla et al. 1998). Ten dromedary camels were observed with pox-like lesions, which appeared as papules then pustules in lips, muzzles, eyelids, and under the ear (Fig. 1). Although there was no mortality, as reported previously in Bahrain (Abubakr et al. 2007), some severe cases showed facial edema and or blindness in the infected camels. Mortalities were also not mentioned in reports from India (Nagarajan et al. 2010; Narnaware et al. 2013), Ethiopia (Gelaye et al. 2016), and Iran (Oryan et al. 2017) while the mortality rate of CCE in Sudan was 1.5% (Khalafalla et al. 2015b) and 6.5% (Khalafalla et al. 2020) and 6% in Iran (Gharib Mombeni et al. 2014). Camels are normally raised in small numbers in closed systems (camel complexes) according to the country regulations and the camel workers are resident in the same complexes (Elford 2013) so this helps in continuous monitoring and isolation of infected camels. Unusually, the disease emerged in the summer (dry) season in 2017. It re-emerged during the rainy season of 2018, which is consistent with previous studies (Khalafalla et al. 2015b; Gelaye et al. 2016; Khalafalla et al. 2020).

The B2L gene from the 10 clinically infected camels was successfully amplified and the obtained nucleotide sequences (1137 nt) each were submitted to GenBank with the accession numbers provided in Table 1. Multiple sequence alignment of partial nucleotide sequences of the B2L gene revealed no nucleotide variation among the Qatari isolates, showing that they were identical. Thus, these cases may have been caused by a single strain.

The phylogenetic tree showed that the Qatari CCEVs formed one cluster together with isolates from Bahrain (EF555791 and EF555792) (Fig. 2) with 100% identity and were highly related to isolates from Saudi Arabia, Sudan, Ethiopia, and India with 99.7% and 99.3% for nucleotide

and amino acid identities, respectively (Supplementary Table 1), while the highest identity based on the available full length B2L gene sequences was 99.6% nucleotide identity with the Ethiopian isolate (KU645558) and 99.4% amino acid identity with the Sudanese isolate (KR231670) (Supplementary Table 2). This can be explained by the frequent movement of camel herds across the Arabian Peninsula for grazing and participation in camel races and camel shows (Hemida et al. 2017) as well as the live camel export from the African horn (Sudan, Ethiopia, Kenya, Somalia, and Djibouti) to gulf countries (Saudi Arabia, Qatar, Bahrain, Oman, Kuwait, and Emirates) as previously mentioned (Faye 2019).

The Qatari CCEVs were more related to pseudocowpox virus than ORF virus (Fig. 2). This is in agreement with previous reports (Abubakr et al. 2007; Nagarajan et al. 2010; Gelaye et al. 2016; Khalafalla et al. 2020) which concluded that CCEV is a variant of the pseudocowpox virus which had adapted to camels and was not the same as the virus infecting cattle. Notwithstanding, ORF virus was reported as a possible causative agent for CCE in a Sudanese dromedary camel (Khalafalla et al. 2015b).

To control the disease, infected camels were physically separated from healthy camels and treated. Recovery of the affected animals required less than 2 weeks to 1 month depending on the severity of the disease after supportive treatment with antiseptic mouthwashes, intramuscular injections of penicillin, streptomycin, and antihistaminic, as described previously (Abubakr et al. 2007; Khalafalla et al. 2020).

In conclusion, this is the first study to detect and identify CCEV as the causative agent of CCE in dromedary camels in Qatar. A standard identification system is needed to help in tracing the frequent movement of camels. Further studies of different viral genes and or complete-genome sequencing of CCEV isolates from Qatar and other countries where the disease has previously been reported are required for a better understanding of this virus, its origin, and phylodynamics.

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Author contribution A.A.S. and H.A.A. conceived and designed the study; A.A.S. performed the experiments; A.A.S., E.M.E, E.M.A., and S.S.S. analyzed the data and A.A.S., E.M.E., E.M.A., S.S.S., and H.A.A. wrote and corrected the paper. All authors read and approved the manuscript.

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Code availability Software application complies with field standard.

Declarations

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