Acta Tropica 171 (2017) 1–5

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Short communication

Molecular characterization of Camelpox virus isolates from Bikaner, India: Evidence of its endemicity

Shyam Singh Dahiya[a,*], Sachin Kumar[b], Sharat Chandra Mehta[a], Raghvendar Singh[a], Kashi Nath[a], Shirish D. Narnaware[a], Fateh Chand Tuteja[a]

[a] National Research Center on Camel, Jorbeer, Bikaner, Rajasthan, 334001, India
[b] Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Assam, 781039, India

ARTICLE INFO

Article history:
Received 16 January 2017
Received in revised form 11 March 2017
Accepted 11 March 2017
Available online 14 March 2017

Keywords:
Camelpox
Isolation
PCR
Histopathology
Phylogenetic

ABSTRACT

Camelpox is an important viral disease of camels, which may produce mild skin lesions or severe systemic infections. Camelpox virus (CMLV) isolates retrieved from an incidence of camelpox in camels at Bikaner, India were characterized on the basis of genotype and pathotype. Histopathological examination of the CMLV scab revealed intracytoplasmic-eosinophilic inclusion bodies. The phylogenetic analysis of all eight CMLV isolates for C18L gene nucleotide sequence revealed its clustering with its strains M-96 from Kazakhstan and CMS from Iran. The study will help to understand the transmission chain, pathobiology, and epidemiology of circulating CMLV strains. The full genome sequencing of some of the exemplary samples of CMLV is recommended in order to plan and implement a suitable control strategy.

Short Communication

Camelpox has been recognized as one of the very important skin disease of camels (Salem et al., 2008). Except the introduced dromedary camel in Australia and tylopods (llama and related species) in South America (Mosadeghhesari et al., 2014), camelpox affects both dromedary as well as Bactrian camels (Kaaden, 2002). Camelpox is of significant concern since it may cause high morbidity and relatively high mortality in the young camels. In addition, loss of condition and drop in the milk production are other serious concerns affecting the economy and livelihood of the camel owners (Azwai et al., 1996). The disease is marked by fever, enlarged lymph nodes and skin lesions. Camelpox infection may lead to abortion in pregnant females (Anonymous, 2008). The disease can manifest itself either in the form of mild skin infection or the moderate to severe systemic infections depending upon the virus strain and the immune status of the animal (Kaaden, 2002). Camelpox has been reported from many countries where camel are reared (Al-Zi'abi et al., 2007). Camelpox outbreaks have been reported in the Middle East, in Asia and in the southern parts of Russia and India. The disease is endemic in these countries and sporadic outbreaks occur usually during the rainy season (Anonymous, 2008). There are incidences (unplublished) of camelpox both within as well as in the adjacent areas of Bikaner district of Rajasthan, India. Recently, camelpox has also been reported from Ethiopia and Iran (Gelaye et al., 2016; Mosadeghhesari et al., 2014).

Camelpox is caused by *Orthopoxvirus cameli* virus (CMLV), which belongs to the genus *Orthopoxvirus* within the family *Poxviridae*. CMLV is most closely related to the variola virus, the aetiological agent of smallpox (Anonymous, 2008). CMLV may get transmitted directly from the infected camels or indirectly via the contaminated secretion and excretion (Ramyar and Hessami, 1972). The arthropods have been reported to transmit CMLV among camels (Pfeffer et al., 1996). In general, Camelpox can be diagnosed based upon the clinical lesion (Kaaden, 2002). However, it may be misdiagnosed with other pox like diseases. Among available techniques, polymerase chain reaction (PCR) offers a rapid way of diagnostics for CMLV (Khalafalla et al., 2015). At present, CMLV infection is diagnosed based on the amplification of various genes viz. A-type inclusion body protein (ATIP), the hemagglutinin (HA), the ankyrin repeat protein (C18L) or the DNA polymerase (DNA pol) genes (Meyer et al., 1994; Meyer et al., 1997; Ropp et al., 1995). Antigenically related ATIPs have been denoted to several orthopoxviruses (OPV) and its sequence comparison revealed the conserved N terminus of ATIPs among cowpox, vaccinia, variola, camelpox and mousepox virus. The restriction enzyme analyses and blot hybridizations disclosed the existence of defined deletions and thus its proficiency for diagnostic differentiation among

* Corresponding author.
E-mail address: dshsing04@gmail.com (S.S. Dahiya).

http://dx.doi.org/10.1016/j.actatropica.2017.03.011
0001-706X/

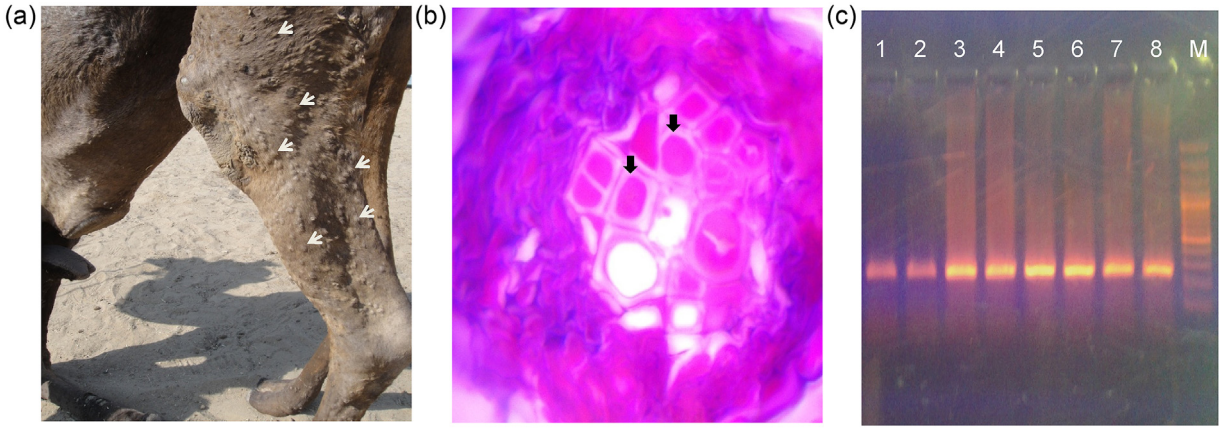

Fig 1. (a) Camelpox specific lesions over the body of dromedary camel. (b) Histopathological examination of camelpox scab: intracytoplasmic- eosinophilic inclusion bodies are marked with arrow. (c) PCR amplification for C18L gene; lane 1–8: CMLV/Bikaner isolates, M: DNA size marker-100 bp ladder.

Table 1a
Details of CMLV isolates from Bikaner, India.

S. No.	Age (Years)	Sex	Breed	Clinical history (Pregnancy/lactation/past diseases or concurrent infection with pox)
01	4–5	M	Mewari	Severly affected, scabs over complete body
02	9–10	M	Mewari	Mange, Severly affected, scabs over complete body
03	5–6	F	Jaisalmeri	No clinical history
04	5–6	F	Kachchhi	Mange, parturition once
05	5–6	F	Bikaneri	Parturition once
06	11–12	M	Mewari	Mange
07	9–10	M	Mewari	Mange
08	2–3	M	Kachchhi	No clinical history

OPVs (Meyer et al., 1994). Among poxviruses, only OPVs produce an HA which upon digestion with *Taq*I, *Hha*I, and *Rsa*I, provides sufficient differentiation to identify species, certain subspecies and strains (Ropp et al., 1995). Likewise, DNA pol gene is group specific and most conserved non-structural protein among OPV (Sahay, 2006). A single-plex C18L gene for CMLV and a duplex-PCR comprising of C18L along with DNA pol gene have been reported to diagnose OPV and CMLV simultaneously (Balamurugan et al., 2009). The immunomodulatory genes of CMLV like 6L gene (also known as Golgi anti-apoptotic protein-GAAP) has been utilized for camelpox characterization (Nagarajan et al., 2013). In addition, schlafen-like protein and open reading frame (ORF) 11R proteins of CMLV is very similar to the vaccinia virus epidermal growth factor have also been explored for its characterization (Nagarajan et al., 2013).

Camelpox can be controlled by vaccination. At present, Ducapox—a live attenuated vaccine produced in South Africa and an inactivated adjuvant vaccine produced in Morocco are commercially available to vaccinate camel calf as early as six months (Khalafalla and El Dirdiri, 2003). Both the vaccines have been tested satisfactorily for their safety, potency and immunogenicity for humoral as well as cellular immune response against challenge (Khalafalla and El Dirdiri, 2003). The inactivated adjuvant vaccine requires revaccination at eight weeks post primary immunization whereas live attenuated vaccine provides protection for at least one year (Khalafalla and El Dirdiri, 2003). However, a booster vaccination is recommended for young animals to avoid interference by maternal antibodies. The animals must be vaccinated annually in case of inactivated vaccines.

Although, different laboratories have characterized and confirmed CMLV in India, any report of its incidence/outbreak has not been reported recently. In the present study, a Camelpox incidence has been reported in dromedarian camels from the Rajasthan province in India. An organized camel farm in the Rajasthan/Bikaner province which lies in the Thar Desert of India recorded the outbreak of camelpox. The geographical location is characterized by high temperature and tropical climatic condition. The camels were maintained under a semi-intensive system of management. In December, 2014, camels of age ranging from 1 to 13 years of either sex were reported to the veterinary clinics with high fever and skin lesion in the form of pox-like scabs (Fig. 1a). The scabs were collected in 10% formal saline for histopathological examination. The formalin fixed tissue samples were embedded in paraffin, cut into 4–5-μm sections and stained with hematoxylin and eosin stain using the standard procedure. Total genomic DNA was extracted from collected scab samples (Table 1a) using PureLink® Genomic DNA Kit (Invitrogen, USA). The genomic DNA was used to amplify the C18L, ATIP, and HA genes of CMLV by specific primers designed from available gene sequences from GenBank (GenBank accession number: AF438165, AY009089). The 243 bp PCR amplified product of all the eight isolates were purified and sequenced by dideoxy method (ABI Biosciences, USA). The nucleotide sequences and the deduced amino acid sequences of the C18L gene were analyzed and compared with available GenBank sequences. The phylogenetic analysis of the nucleotide sequence of the CMLV isolated from Rajasthan/Bikaner was done by the maximum likelihood statistical method using molecular evolutionary genetics analysis software (MEGA 6) (Tamura et al., 2013). In the present study, histopathological examination of the collected scab samples after haematoxylin and eosin staining revealed intracytoplasmic-eosinophilic inclusion bodies (Fig. 1b). The histopathological examination showed characteristic cytoplasmic swelling, vacuolation and ballooning of the keratinocytes of the outer stratum spinosum of the epidermis (Duraffour et al., 2011). It has been proposed that intracytoplasmic eosinophilic inclusion bodies may be considered as characteristic for poxvirus infection (Anonymous, 2008). Our histopathological finding of intracytoplasmic inclusion bodies suggested the poxvirus infection in the Bikaner isolates.

All the eight isolates showed positive amplification for C18L, ATIP, and HA genes of CMLV. The topoisomerase gene specific