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Camelpox virus

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ABSTRACT

Camelpox virus (CMLV) causes a smallpox-like illness in a unique host, the camel. The disease is enzootic in almost all regions where camel husbandry is practiced, and is responsible for severe economic losses. Although it is genetically the closest known virus to variola virus, the etiologic agent of smallpox, CMLV remains poorly studied. It is characterized by a narrow host range, the capacity to induce giant cells in culture and to counteract host immune defenses; however, the genetic bases associated with these features are not understood. Also, it still needs to be demonstrated whether CMLV strains of variable virulence circulate and how arthropod vectors might be involved in virus transmission. Current evidence indicates that, under certain circumstances, CMLV can be mildly pathogenic in humans. A reservoir host other than camels is unlikely to exist. We review here current knowledge of CMLV, including clinical and laboratory aspects of the disease. We also discuss prevention and therapy by use of vaccines and antiviral treatments, as well as the possibility of camelpox eradication.

1. Introduction

Camelpox is an infectious disease caused by camelpox virus (CMLV). CMLV belongs to the *Orthopoxvirus* (OPV) genus of the *Poxviridae* family (Damon, 2007). The disease, restricted to camels, is enzootic in almost every region where camel breeding is practiced with the exception of Australia. According to the UN Food and Agriculture Organization, the total world camel population is approximately 23 million animals (http://faostat.fao.org). CMLV is transmitted *via* the contaminated environment through skin abrasions and by aerosols, although a mechanical transmission might be considered (Wernery and Kaaden, 2002). Clinical manifestations range from local and mild (Alhendi et al., 1994) to severe systemic infections (Abu Elzein et al., 1999; Kinne et al., 1998). In general, young calves and pregnant females are more susceptible (Al Zi'abi et al., 2007; Kriz, 1982). The circulation of camelpox in herds has a strong economic impact as camels are valued for nomadic pastoralism, transportation, racing, and production of milk, wool and meat (Wernery and Kaaden, 2002; Bett et al., 2009). To date, protection against camelpox can be achieved using CMLV-based vaccines, but these practices are not widely used and only calves above 6 months of age are protected. Access to antivirals might also be beneficial for treating affected animals.

CMLV appears to share biological features with another OPV, variola virus (VARV). VARV is well known as the causative agent of smallpox, a dreadful disease eradicated in 1980. Both CMLV and VARV are (i) restricted to a single host (i.e., camels for CMLV and humans for VARV) and (ii) induce a similar disease course (Damon, 2007; Wernery and Kaaden, 2002). Interestingly, using phylogenetic analysis, CMLV has been shown to be the virus the most closely related to VARV (Afonso et al., 2002; Gubser and Smith, 2002). Also, CMLV has other strong similarities to VARV in terms of pock formation on chorioallantoic membrane (CAM), growth in cells and low or absence of pathogenicity in various animal models (Baxby, 1972, 1974; Baxby et al., 1975). For these reasons, learning more about the genomic and biological features of CMLV may enable a better understanding of VARV.

In this review, we will focus on camelpox disease. The etiologic agent will be presented, including its genomic, immunomodulatory and *in vitro* growth features. Further, the epidemiology and the modes of transmission of camelpox will be summarized. The *in vivo* growth properties of the virus will be detailed and the notion of human infection by CMLV will be explored. We will then describe the procedures suitable for its diagnosis, as well as the vaccination and antiviral strategies as countermeasures to combat this disease. Finally, we discuss forthcoming priorities for camelpox research.

2. Molecular characteristics of camelpox virus

2.1. Etiologic agent, classification and phylogeny

CMLV is a member of the OPV genus, which is one of eight genera of the subfamily *Chordopoxvirinae* of the *Poxviridae* family

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(Fig. 1) (Moss, 2007). The genus OPV is composed of four human pathogenic species, i.e., VARV, vaccinia virus (VACV, including the isolate buffalopox virus), monkeypox virus (MPXV) and cowpox virus (CPXV), and species of veterinary importance, i.e., CMLV, CPXV, buffalopox virus, and ectromelia virus, as well as taterapox virus, the North American OPVs (volepox virus, raccoonpox virus and skunkpox virus) and an unclassified OPV species, uasin gishu disease virus. There are numerous CMLV strains that have been isolated from different outbreaks, and they are reported in Table 1.

OPVs are large (250 nm × 350 nm), brick-shaped enveloped viruses (Fig. 2A) (Damon, 2007; Moss, 2007). They replicate in the cytoplasm of the host cell and their genome consists of a linear double-stranded DNA molecule terminated by an hairpin loop (Moss, 2007). The full-genome sequences of two CMLV strains from Iran and Kazakhstan, i.e., CMLV-CMS and CMLV-M96 respectively, have been published (Afonso et al., 2002; Gubser and Smith, 2002).

The CMLV genome is AT-rich (66.9%) and, in line with the genomic organization of other OPVs, it contains a central region of genes that are highly conserved. Among these, 87 are conserved in all sequenced members of the subfamily *Chordopoxvirinae* (Afonso et al., 2002; Gubser and Smith, 2002). The central region encodes proteins required for RNA transcription, DNA replication, and virion assembly. In contrast, genes located within the terminal regions are non-essential and encode proteins involved in host range, virulence and immunomodulation. For these reasons, the sequences of the terminal regions and the organization of the open reading frames (ORFs) are more variable between OPVs. However, the arrangement of ORFs close to and within the inverted terminal repeat (ITR) of CMLV and VARV showed a higher degree of similarity in comparison with other OPVs (Afonso et al., 2002; Gubser and Smith, 2002). Phylogenetic analyses have thus revealed that CMLV is distinct but closely related to VARV (Fig. 1).

It is interesting to note that CMLV was first regarded as an own OPV species in the mid seventies, some times before phylogenetic analyses were conducted (Baxby, 1972). At that time, CMLV was shown to share strong similarities with VARV as they both had a narrow host range and were undistinguishable in some cell cultures as well as on chorioallantoic membranes (CAMs) (Baxby,

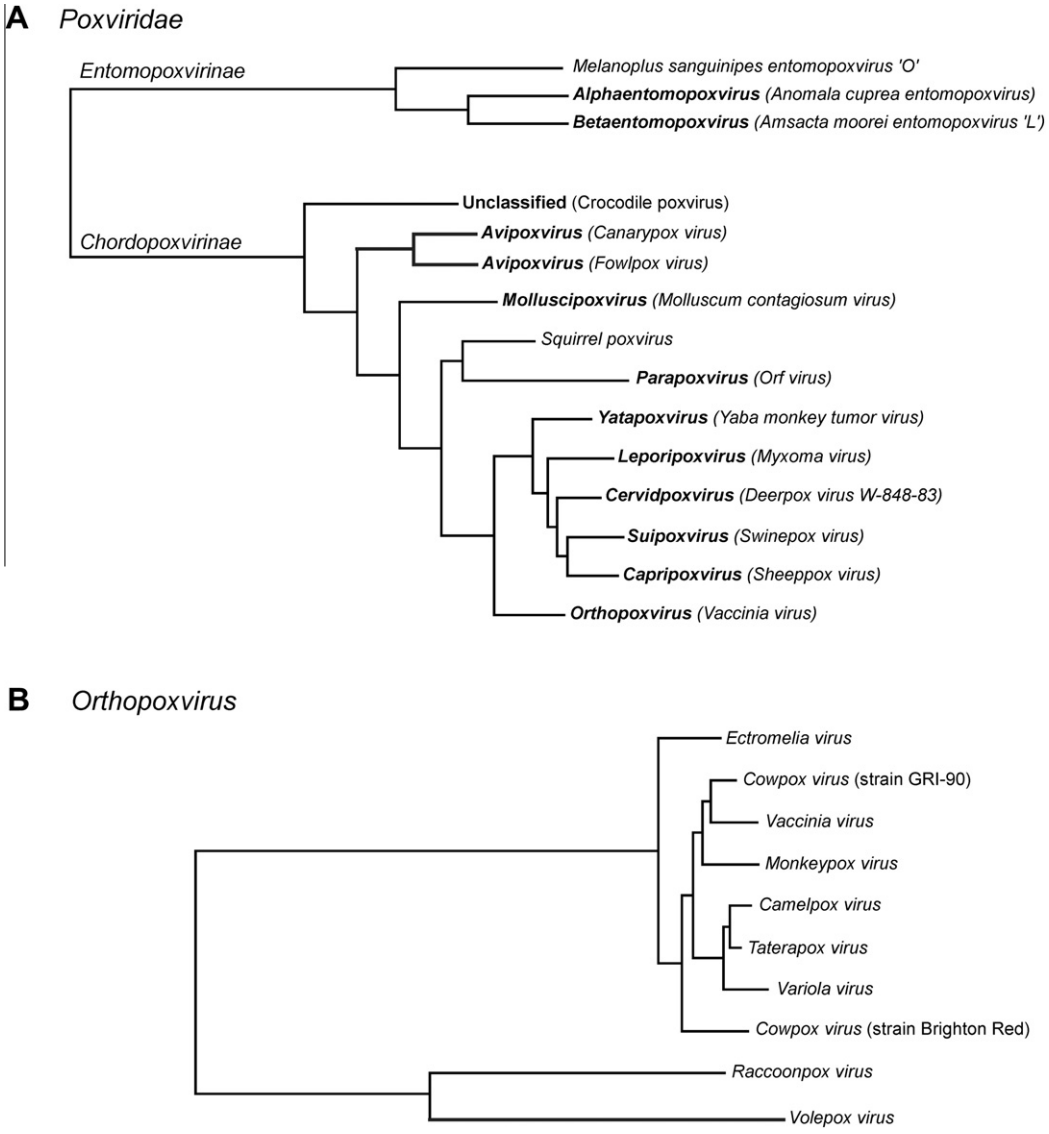

Fig. 1. Phylogenetic trees of (A) the poxviruses and (B) the orthopoxviruses (kindly provided by Prof. E. Lefkowitz, University of Alabama at Birmingham, AL, USA). (A) The phylogenetic prediction is based upon aligned amino acid sequences from 19 conserved genes of representative virus strains for each genus. (B) Codon-aligned nucleic acid sequences from nine conserved genes of representative strains were used. The phylogenetic predictions were inferred using Bayesian analysis, as implemented by the program MrBayes.

Table 1
Names and origins of CMLV strains described in the scientific literature since 1972.

Strain*	Origin*	Comments	References
CMLV-Gurevski	Russia/Kasakhstan (1967)	Not mentioned	Tantawi et al. (1978)
CMLV-Turkmenski	Russia/Turkmenia (1967)	Not mentioned	
CMLV-T72	Russia/Turkmenia (1972)	Not mentioned	
CMLV-M96	Kazakhstan (Mangistauskiy area)	Isolated from a sick camels	Afonso et al. (2002)
CMLV-Teheran or CP-1 or CML1	Iran (Teheran, 1970)	Obtained from vesicles and crusts harvested from natural outbreaks of camelpox	Ramyar and Hessami (1972), Baxby et al. (1975), Tantawi et al. (1978), Renner-Muller et al. (1995), Pfeffer et al. (1996), Duraffour et al. (2007a,b)
CM-G1	Iran (Gorgan area, 1970)	Obtained from vesicles and crusts harvested from natural outbreaks of camelpox	Baxby (1972), Ramyar and Hessami (1972), Baxby (1974), Baxby et al. (1975)
CM-G2	Iran (Gorgan area, 1970)	Obtained from vesicles and crusts harvested from natural outbreaks of camelpox	
CM-S	Iran (Shiraz area, 1970)	Obtained from vesicles and crusts harvested from natural outbreaks of camelpox	
CMLV-Etha78	Iraqi-Iranian border (1978)	Isolated from camel skin lesions at different stages of development	Falluji et al. (1979)
CP-Syria	Syria (Duma and Hama regions, 2005)	Isolated from specimens of vesicles, pustules, crusts and scabs from affected camels	Al Zi'Abi et al. (2007)
CMLV1	India, Rajasthan, Bikaner (1997)	Isolated from an outbreak with considerable morbidity	Chauhan and Kaushik (1987), Bhanuprakash et al. (2010a,b)
CMLV2	India, Rajasthan, Bikaner (1997)	Isolated from an outbreak with considerable morbidity	
CMLV-Hyd 06	India, Rajasthan, Bikaner (2002)	Isolated from a male camel aged 10 years with eruptions on cheeks, nostrils, limbs, scrotum and sheath	
CP-SA	Saudi Arabia (1986)	Not mentioned	Renner-Muller et al. (1995), Pfeffer et al. (1996)
CMLV-Jouf	Saudi Arabia, Northern region, Al-Jouf	Pathogenic field strain isolated from skin scabs collected during an outbreak of camelpox at the Experimental Camel Farm of the Range and Animal Development Research Center	Hafez et al. (1992)
Jouf-78 (vaccinal strain)	Derived from CMLV-Jouf, Saudi Arabia, and passaged 80 times in CKCC	Vaccine strain used in Field testing	
CMLV-Gub/C_3	Saudi Arabia (Eastern region, 1989)	Isolated from a camel with a slow-spreading mild form of camelpox	Alhendi et al. (1994)
202-95 (or 202-Skin and 202-Lung)	Saudi Arabia (1997)	Field strain isolated from the lung of a dromedary which died from generalized and internal camelpox	Wernery and Zachariah (1999), Pfeffer et al. (1996)
CMLV-Al-Ahsa	Saudi Arabia (Al-Ahsa region, 1998)	Isolated from skin biopsies from camels with a moderate form of camelpox	Abu Elzein et al. (1999)
CP-5	United Arab Emirates (Dubai, December 1993–March 1994)	Not mentioned	Renner-Muller et al. (1995), Pfeffer et al. (1996)
CP-14 (CML14) to CP-29 (various CP strains)	United Arab Emirates (Dubai, December 1993–March 1994)	Isolated from scabs of camels with localized and generalized lesions	Pfeffer et al. (1996), Duraffour et al. (2007b)
Various strains (813–93, 864–93,...)	United Arab Emirates (winter 1993–1994)	Field strains isolated during camelpox outbreaks in winter 1993–1994 from camels that developed localized or generalized lesions.	Wernery et al. (1997a, b)
Various strains (1230–95, 1251–95,...)	United Arab Emirates (winter 1995–1996)	Field strains isolated during camelpox outbreaks in winter 1995–1996 from camels that developed localized or generalized lesions.	
O. Cameli	United Arab Emirates (Dubai)	Isolated from severe pox-lesions in a young camel	Azwai et al. (1996)
Ducapox 298/89 (vaccinal strain)	Derived from *O. Cameli* strain passaged 96 times in Vero	Used for camelpox-vaccine production (Dubai camelpox vaccine)	
CMLV-H520	Kenya	Isolated from pox-like vesicular eruptions of camels	Davies et al. (1975)
Various strains not named	Kenya (1992)	Isolated from sick camels	Gitao (1997)
CP/Mg/92/1	Sudan (Butana area, 1992–1994)	Isolated from skin samples of sick camels	Khalafalla and Mohamed (1998); Khalafalla and El Dirdiri (2003); Sheikh Ali et al. (2009)
CP/Nw/92/2	Sudan (Butana area, 1992–1994)	Isolated from skin samples of a two year old male dromedary in 1992	
CP/Dbg/92/3	Sudan (Butana area, 1992–1994)	Isolated from skin samples of sick camels	
CP/Nh/92/4	Sudan (Butana area, 1992–1994)	Isolated from skin samples of sick camels	
CP/Ab/93/5	Sudan (Butana area, 1992–1994)	Isolated from skin samples of sick camels	
CP/Tm/93/6	Sudan (Butana area, 1992–1994)	Isolated from skin samples of sick camels	
CMLV-Fayoum 71	Egypt (1970)	Isolated from a skin pox-like disease in a camel	Tantawi et al. (1978)
CP-NIG (or VD49)	Niger	Isolated from camels with generalized skin lesions	Nguyen et al. (1989); Renner-Muller et al., 1995; Otterbein et al. (1996)
CP-NIG #114	Derived from CP-NIG, Niger, and passaged 114 times in Vero	Selection of an attenuated CMLV strain	Otterbein et al. (1996)
VD47; VD45	Niger (1981)	Isolated from camels with generalized skin lesions and this strain gives strong cytopathic effect in cell cultures	Nguyen et al. (1989), Sheikh Ali et al. (2009)
VD47/25 (vaccinal strain)	Derived from VD47, Niger, and passaged 90 times in two cell types	Used for camelpox-vaccine production	Nguyen et al. (1996)

(*continued on next page*)

Table 1 (*continued*)

Strain*	Origin*	Comments	References
CP-MAU	Mauritania (1984)	Isolated from camels with generalized skin lesions	Nguyen et al. (1989); Renner-Muller et al., 1995, Otterbein et al. (1996), Pfeffer et al. (1998a)
CP-MAU #114	Derived from CP-MAU, Mauritania, and passaged 114 times in Vero	Selection of an attenuated CMLV strain	Otterbein et al. (1996)
VDR-A2	Mauritanie (1987)	Isolated from camels with camelpox	Nguyen et al. (1996)
T8 (vaccinal strain)	Morocco (1984)	Passaged on Vero cells and inactivated with formol: vaccine strain	El Harrak and Loutfi (2000)
CMLV-München	Not mentioned (1980)	Obtained from München, West Germany	Sehgal and Ray (1980)
CMLV-66	Not mentioned	Not mentioned	Klopries et al. (1995)
CMLV-298/2	Not mentioned	Not mentioned	

* Classification of the strains was performed per country.

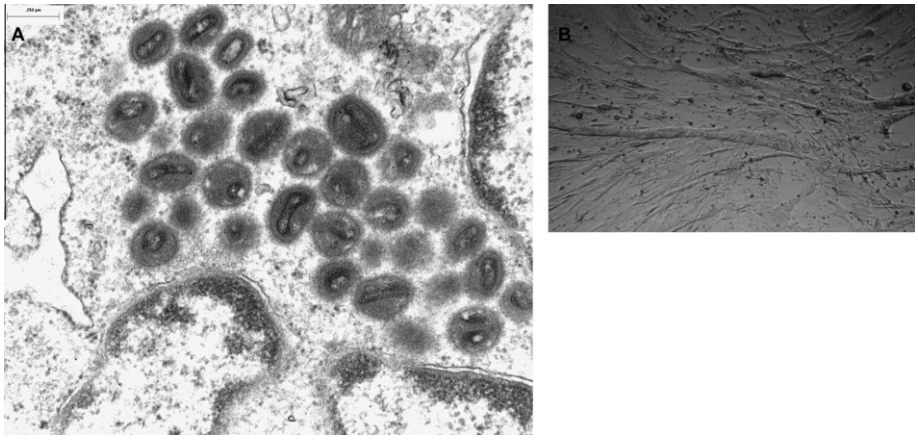

Fig. 2. (A) Electron microscopic view of CMLV (strain CML1)-infected human embryonic lung (HEL) fibroblast cells; (B) multinucleated giant cells in CMLV (strain CML1)-infected HEL cells as seen at day 4 post-infection by direct light microscopy.

1972; Baxby et al., 1975). Serological studies demonstrated the cross-antigenicity between VACV, VARV, CPXV and CMLV, but not with viruses belonging to the *Parapoxvirus* and *Avipoxvirus* genera (Baxby, 1972; Davies et al., 1975). *In vivo* experiments performed in the seventies in camels additionally supported these findings: infection of camels with VARV strain EA8 protected against subsequent challenge with a pathogenic dose of CMLV (Baxby et al., 1975). Twenty years later, *Hind*III restriction fragment length polymorphisms further sustained that CMLV was clearly a separate OPV species (Pfeffer et al., 1996; Renner-Muller et al., 1995). Similar methods using *Hind*III and *Xho*I restriction endonuclease analysis additionally allowed the partial differentiation between CMLV clinical isolates from different geographic regions (Kinne et al., 1998; Renner-Muller et al., 1995).

2.2. Physicochemical properties

It is well recognized that poxvirus virions show high environmental stability and can remain contagious over several months (Rheinbaden et al., 2007). This feature is enhanced by the materials in which the virus is released, such as crusts, serum, blood and other excretions. Poxvirus virions can also show strong tolerance to high temperatures, pH and chemicals (for review (Rheinbaden et al., 2007)). Table 2 depicts the physicochemical properties of

Table 2
Overview of the physicochemical properties of various CMLV strains.

Virus strain*	Chemical sensitivity		Heat sensitivity: 56 °C			References
	Chloroform	Ether	10–20 min	30 min	60 min	
CMLV-Teheran, -Shiraz, -Gorgan	Sensitive	Sensitive	Inactivated	–	–	Ramyar and Hessami (1972)
CM-G1, CM-G2, CM-S	–	–	Titer reduced by 5 logs	–	–	Baxby (1972)
CMLV-H520	Sensitive	Resistant	–	–	–	Davies et al. (1975)
CMLV-Fayoum 71, -T72, -Gurevski, -Turkmenski	–	–	Fayoum 71: resistant Titer reduced by 1–2 logs	–	–	Tantawi et al. (1978)
CMLV-Etha-78	Resistant	Resistant	–	–	Resistant	Falluji et al. (1979)
München	–	–	Titer reduced by 3 logs	Titer reduced by 5 logs	–	Sehgal and Ray (1980)
VD47 (Niger strain)	Sensitive	Resistant	Inactivated	–	–	Nguyen et al. (1989)
CP/Nw/92/2	Sensitive	Sensitive	Inactivated	–	–	Khalafalla and Mohamed (1998)

* See Table 1.
– not done or not mentioned.

CMLV strains and highlights the differences existing between them. It is of interest to note that the strain Etha-78 is resistant to heat and chemical treatments while other strains exhibit phenotypes of sensitivity. These discrepancies may warrant further laboratory testing including the comparison of different viruses at the same time. Whether this high degree of resistance to some treatments might predict for a higher virulence is not known, but for instance strain Etha-78, in contrast to others, was shown to induce a generalized rash in monkeys (Falluji et al., 1979). It could be also asked whether CMLV strains with a high tolerance to heat might predict for a higher environmental resistance, as CMLV circulates in arid countries.

2.3. Growth of camelpox virus on CAMs and in cell culture

Since the discovery of CMLV, several cell types have been evaluated for their capacity to propagate the virus. Furthermore, considering the possible existence of CMLV strains of different virulence, it was hypothesized that they could exhibit different growth properties on chorioallantoic membranes or in cell cultures.

At the time of smallpox eradication campaign, CAMs of embryonated eggs were used for the growth of VARV, VACV and CPXV for diagnostic and research purposes because the features of the pocks enabled their differentiation. In this context, the growth properties of CMLV strains inoculated on CAMs have been studied and are summarized in Table 3. A temperature of 37 °C allowed the growth of all CMLV strains tested which produce white pocks, flat in shape, with sizes varying between 0.5 and 1.5 mm diameters. Most of the CMLV strains did not induce pock proliferation, necrosis, or hemorrhage, in contrast to what was observed with VACV or CPXV (Davies et al., 1975; Renner-Muller et al., 1995; Sheikh Ali et al., 2009; Tantawi et al., 1978). It is also important to note that the pocks produced by VARV and CMLV were undistinguishable (Baxby, 1972). At 39 °C, only CMLV-Fayoum 71, -Etha-78 and -München were able to form pocks on CAMs. The lethality was variable between the strains and could not be predicted from the presence or absence of other features such as hemorrhage, proliferation or growth at 39 °C (Table 3).

Many authors have compared the behavior of numerous CMLV strains in cell cultures, and these results are summarized in Table 4. In general, cells derived from camel, lamb, calf, pig, monkey, chicken, hamster and mouse enable the propagation of CMLV strains. Both, transformed and primary human cells are permissive for CMLV replication. In contrast, cell monolayers derived from horse, rabbit and dog lead to a poor replication of CMLV for most of the strains tested and repeated sub-cultivations were required (Renner-Muller et al., 1995).

Usually, CMLV will produce typical large syncytia or multinucleated giant cells, which will then detach from the culture flask and form plaques (Fig. 2B). Such giant cell formation has not been reported with VACV or CPXV, although it could be seen with VARV (Baxby, 1974; Renner-Muller et al., 1995). In some cell lines, CMLV can lead to a cytopathogenic effect without production of giant cells which is similar to what is observed with VACV or CPXV (Table 4). The feature of CMLV to produce giant cells or syncytia may be correlated with the characterization, by cesium chloride gradients, of the different type of infectious particles produced by CMLV in human embryonic lung (HEL) cells (Duraffour et al., 2008b). Briefly, OPVs produce two types of infectious particles: intracellular mature viruses (IMV) with a single membrane and extracellular enveloped viruses (EEV) with a double membrane (Roberts and Smith, 2008). EEV virions that are still associated to the cell membrane (called CEV for cell-associated enveloped virus) have been shown to be abundantly produced by CMLV and it could be hypothesized that CEVs, promoting cell to cell spread, may be

Table 3
Growth of CMLV strains on the chorioallantoic membrane (CAM) of embryonated eggs.

CMLV strains*	Growth at 34,5 °C: presence or size (mm)	Growth at 37 °C: pock features									Growth at 39 °C: presence or size (mm)	References
		Presence or size (mm)	Color	Shape	Proliferation	Necrosis	Hemorrhage	Generalization	Time of lesion formation (day post-infection)	Lethality		
CMLV-Teheran, -Shiraz, -Gorgan	-	Pocks	-	-	-	-	-	-	-	-	-	Ramyar and Hessami (1972)
CM-G1, CM-G2, CM-S	-	Pocks	White	-	-	-	-	-	-	Yes	-	Baxby (1972)
CMLV-H520	0.2–0.4	0.5–1	Gray–white	Flat	-	No	No	-	-	Yes	No	Davies et al. (1975)
CMLV-Fayoum 71, -T72, -Gurevski, -Turkmenski	Pocks	0.3–1	-	Flat	Yes	No	No, except strain T72	no	5	No	Pocks: Fayoum 71	Tantawi et al. (1978)
CMLV-Etha-78	Pocks	0.4–0.6	White	Flat	No	No	No	no	5	no	Pocks	Falluji et al. (1979)
München	0.5	0.5	White	Prominent	-	-	Yes	-	3	Yes	0.5	Sehgal and Ray (1980)
VD47 (Niger strain)	-	0.5–1	-	-	-	No	Some pocks	-	3	-	No	Nguyen et al. (1989)
CP/Nw/92/2	0.3–0.5	0.5–1.5	White	Round	-	No	No	-	5	-	No	Khalafalla and Mohamed (1998)

* see Table 1.
- not done or not mentioned.

Table 4
Growth of CMLV strains in various cell types.

CMLV strains*	Cell types derived from																					
	Camel		**Lamb**		**Calf**		**Pig**	**Horse**	**Monkey**			**Human**						**Chicken**		**Rabbit**	**Hamster**	**Mouse**	**Dog**
	Dubca	CKCC	Lamb kidney	Lamb testis	Calf kidney	Calf testis	Pig kidney	E-Derm	BSC-1	Vero	MA-104	Hela	WISH	HEL	HEK	PHK	HEP-2	CEC	CEF	RK-13	BHK-21	L929	MDCK
CMLV-M96			+															+					
CMLV-H520				RC	RC					RC											RC		
CMLV-Gurevski, -Turkmenski, -T72, Fayoum 71		+	+	+	+	+	+			+								+		+	+		
CMLV-Teheran or CP-1 or CML1	GC	+	+	+	RC	+	+	RC/RS		RC/GC	GC			GC			GC	+		0	RC	RC	RS/RC
CMLV-Etha78																			RC/GC				
CM-G1, -G2, -S		+	+	+	+	+	+		GC	SC/RC		GC	GC	SC (GC)	SC (GC)			+		+/0	+		
CMLV-66, -298/2, -202-95	+																						
CP-SA, -MAU, -5	GC				RC			RC/RS		RC/GC	GC			GC			GC			0	RC	RC	RS/RC
CP-NIG (or VD49)	GC				RC			RC/RS		RC/GC	RC (GC)						RC			0	RC	RC	RS/RC
CP-MAU #114, -NIG #114	0							RC		RC/GC	GC comet										0	0	0
Various strains not named			GC																				
CMLV-Jouf, -Jouf-78		RC/GC																					
CP-Syria										RC/GC													
VD47; VD45			+	+	GC	GC				RC (GC)	+								RC/GC		+		
VD47/25		+																					
VDR-A2	+		+							+													
CP-14 (CML14)	GC													GC									
CP/Mg/92/1; CP/ Nw/92/2; CP/Dbg/ 92/3; CP/Nh/92/4; CP/Ab/93/5; CP/ Tm/93/6; CMLV1, CMLV2, CMLV-Hyd 06, CP-15 to -29			GC	GC	GC	GC				RC/GC									RC/GC		RC/GC		
CMLV-Al-Asha										+													
O. Cameli; Ducapox 298/89										+													

Dubca, transformed camel skin fibroblasts; CKCC, camel kidney; E-derm, equine dermal fibroblsasts; BSC-1 and Vero, transformed green monkey kidney; MA-104, African green monkey kidney; Hela, human cervical carcinoma; WISH, transformed human amnion; HEL, human embryonic lung; HEK, human embryonic kidney; PHK, primary human keratinocytes; HEP-2, human epidermoid carcinoma cells; CEC, primary chicken embryo cells; CEF, chick embryo fibroblasts; RK-13, rabbit kidney; BHK-21, baby hamster kidney; L929, mouse fibroblasts; MDCK, Madin Darby canine kidney.

* for References, see Table 1.

+ CMLV replicates in the cell type; 0: no replication; SC: strand cells; RC: round cells; GC: giant cells or syncytium; RS: replication only after subcultures (bold), comets: indicate the formation of comets, +/0: poor replication.

involved in syncytia formation. To explore this, it might be interesting to compare, at the genome level, strains of CMLV with different cell-growth properties, such as CMLV-Teheran, CP-NIG, CP-MAU #114 and CP-NIG #114 (Table 4), and correlate with the production of CEVs.

In addition to cell monolayers, three-dimensional epithelial cultures or organotypic cultures can be used to study epitheliotropic viruses, such as poxviruses which replicate in a differentiated epithelium (Andrei et al., 2010). Interestingly, it has been demonstrated that human skin equivalent cultures are permissive for CMLV replication, even though the virus is not recognized as pathogenic for humans. Histopathological examination of infected tissues revealed changes resembling those observed in skin biopsies of CMLV-infected animals, including cytoplasmic swelling and ballooning of the keratinocytes of the *stratum spinosum* of the epidermis (Fig. 3) (Duraffour et al., 2007a, b). In the search for models permitting the study of CMLV pathogenicity, raft cultures represent an effective tool which, in the absence of animal models, were successfully used to demonstrate the antiviral potency of anti-CMLV molecules (Duraffour et al., 2007a, b).

2.4. Host-virus interactions that might regulate camelpox pathogenicity

Cell culture systems were not solely restricted to study the growth of CMLV, but also enabled the characterization of certain strategies used by CMLV to circumvent the immune response of the host. Indeed, poxviruses, including CMLV, encode multiple genes that antagonize or affect the antiviral host immune response by interfering with the interferon (IFN) response, key proinflammatory cytokines (IL-1β, IL-18 and tumor necrosis factors [TNFs]), chemokines and the complement system. The numbers of strategies used by poxviruses became increasingly complex and have been extensively reviewed elsewhere (Nazarian and McFadden, 2007; Perdiguero and Esteban, 2009).

While several immune-modulations mechanisms have been identified in CPXV and VACV, only few articles reported those of CMLV (Table 5A) (Alcami and Smith, 1995, 2002; Alcami et al., 1998, 1999; Gubser et al., 2007a, b). We can assume that the lack of small animal models of CMLV infection has greatly hampered the characterization of CMLV immune-escape pathways, but the sequencing of two CMLV strains has brought additional knowledge on potential immunomodulatory proteins encoded by CMLV. As depicted in Table 5A, CMLV has been shown to encode secreted proteins that bind to and subsequently inhibit the biological activity of IFN-γ, CC chemokines and tumor necrosis factor (Alcami and Smith, 1995, 2002; Alcami et al., 1998, 1999). Recently, it has been demonstrated that CMLV expressed a novel protein inhibiting apoptosis (v-GAAP) and a novel virulence factor, the schlafen-like protein 176R (Gubser et al., 2007a, b). In the case of IFN-α/β inhibition, CMLV secrete a protein with IFN-α binding activity (CMLV-CMS-252 with similarity to VACV B19R), but its inhibitory potency might be low as shown by Symons and colleagues (Symons et al., 1995; Alcami et al., 2000; Montanuy et al., 2011).

CMLV uses most probably other proteins to modulate interactions with the host, but whether or not these identified ORFs produce a functional protein is not known. Some of these ORFs with potential immunomodulatory, virulence or host range functions are shown in Table 5B and some of them are briefly discussed here. From bioinformatics analysis, CMLV-resistance to IFN could be also mediated by the *CMLV097* gene, homolog of the VACV *H1L* gene, which has been shown to encode an intracellular protein that dephosphorylates STAT1 and thus prevents the action of IFN-γ on infected cells (Najarro et al., 2001). Also, the *32L* and *55L* genes may encode proteins which, in VACV, have been shown to prevent the activation of PKR (dsRNA-dependent protein kinase) and the production of IFN (Perdiguero and Esteban, 2009).

IL-1β is a potent pro-inflammatory cytokine involved in inflammation. To inhibit it, CMLV could produce a viral soluble receptor encoded by three genes *CMLV193*, *194* and *196* which are seen as separate parts of the VACV B16R IL-1 binding protein (Afonso et al., 2002). Also, whether or not CMLV produces an active caspase 1 inhibitor (SPI-2, encoded by *CMLV191* gene homolog to VACV *B13R* gene) to block the cleavage of proIL-1β and proIL-18 into IL-1β and IL-18, respectively, is still unknown. However, Symons and colleagues demonstrated that CMLV inhibits IL-12-induced production of IFN-γ by mouse splenocytes (Symons et al., 2002). In this assay, splenocytes will produce IFN-γ if stimulated with IL-12, and IL-12 acts synergistically with IL-18, a key pro-inflammatory cytokine originally identified as an IFN-γ-inducing factor (Nakanishi et al., 2001). As a consequence, if IL-18 is inhibited, IL-12 will then be inactive and IFN-γ will not be produced. This assay enabled the mapping of an IL-18 binding protein (IL-18BP) of VACV (*C12L* gene). It is interesting to note that, in contrast to VACV, VARV or ectromelia virus, CMLV does not encode an IL-18BP. Therefore, the results of Symons et al. might suggest that CMLV produces an active caspase 1 inhibitor, which, by inhibiting the cleavage of IL-18, significantly contributes to reduce IFN-γ production by IL-12 (Symons et al., 2002; Fantuzzi et al., 1999).

As described here, CMLV may utilize several ways to alter or shut down the host immune response and, though these mechanisms have been described *in vitro*, they may reflect the *in vivo* situation and explain the pathogenicity of CMLV in its host, the camel.

3. General features of camelpox

3.1. Spectrum of illness in camels

Currently, CMLV is considered to solely naturally infect old world camelids, including *Camelus dromedarius* (dromedary camel)

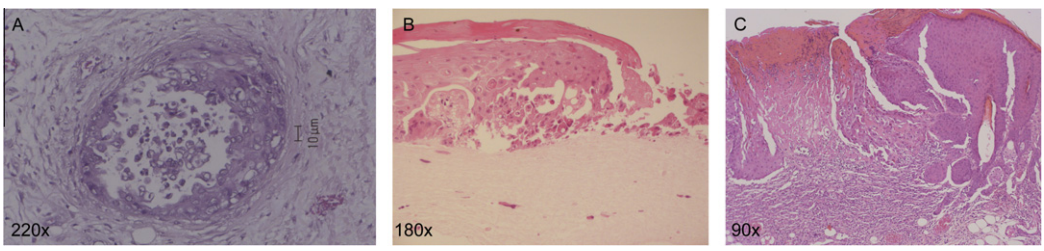

Fig. 3. Histopathology of skin biopsies. (A) Skin biopsy collected during a camelpox outbreak in Dubai in 1994–1995 on a dromedary exhibiting camelpox dermatitis with epithelial proliferation. Note the intracellular edema of keratinocytes and cellular debris in the center. This picture was kindly provided by Prof. Wernery U., Central Veterinary Research Laboratory, Dubai, United Arab Emirates. (B) Histology of human skin-equivalent cultures infected with CMLV (strain CML1), as observed at day 12 post-infection. Note the cytoplasmic swelling and the ballooning of the keratinocytes, as well as destruction of the epithelium. (C) Histology of back skin of an athymic nude mouse infected with CMLV (strain CML1) by scarification at the lumbosacral region. Note the exophytic lesion covered with hyperplastic squamous epithelium, which centrally invaginates to form a crater filled with parakeratin and inflammatory cells. (B) and (C), our data. All images: hematoxylin and eosin staining.

Table 5
CMLV immune evasion genes, including (A) CMLV immunomodulatory proteins that have been studied and (B) CMLV open reading frames with potential immunomodulatory, virulence or host range functions.*

A

Gene name as reported in the manuscript (current official name)	Protein name	Localization	Notes	Role	References
181R (CMLV-CMS-233)	Soluble virus Interferon-γ receptor (vIFN-γR)	Secreted	Similarity to VACV-WR B8R	Binds host IFN-γ and prevents its interaction with cellular receptor. *In vitro*, CMLV-IFN-γR binds and inhibits the activity of human, bovine and rat IFN-γ but not of mouse. vIFN-γR functions as an homodimer in contrast to cellular IFN-γR which forms dimers	Alcami and Smith (1995, 2002)
2L and 205R (CMLV-CMS-002 and -265)	Soluble virus tumor necrosis factor receptor II CrmB (vTNFR)	Secreted	Similarity to VACV-Cop B28R/C22L (CPXV-GRI/CrmB)	Soluble vTNFR activity observed with CMLV, but not further studied	Alcami et al. (1999)
1L and 206R (CMLV-CMS-001 and -266)	Soluble virus chemokine binding protein (vCKBP)	Secreted	Similarity to VACV-Lis 35 kDa protein (B29R/C23L)	Binds to CC, but not CXC or C, chemokines, and blocks their interaction with cellular receptors	Alcami et al. (1998)
6L (CMLV-CMS-007)	Virus Golgi anti-apoptotic protein (v-GAAP)	Resident in the Golgi	Similarity to CPXV-GRI S1R protein, and protein 6L also found in VACV. V-GAAP and human GAAP have 73% amino-acid identity	v-GAAP is a virus-encoded apoptosis inhibitor. VACV deleted in v-GAAP: virulence decreased *in vivo*, but replication not affected. Protein v-GAAP expressed early during infection	Gubser et al. (2007a)
176R (CMLV-CMS-226)	Schlafen-like	Cytoplasmic	Similarity to murine schlafen-like, and ORF fragmented in VACV and VARV	Virulence factor affecting the host immune response to infection: accelerates virus clearance? Recombinant VACV with wild type 176R: virulence decreased *in vivo* in intranasal model but not in intradermal model. N-terminal extremity of the protein important for its function	Gubser et al. (2007b)
196R (CMLV-CMS-252)	Interferon α/β binding proteins (IFNα/β-bp)	Secreted	Similarity to VACV-Cop B19R	Low soluble IFNα binding activity compared to other OPVs. Higher activity at the cell surface. The IFNα/β-bp is secreted and targeted primarly to the cell surface. Glycosaminoglycans mediate the retention of IFNα/β-bp of VACV, MPXV and VARV at the cell surface to locally block IFN antiviral responses	Symons et al. (1995); Alcami et al. (2000); Montanuy et al. (2011)

B

Gene name as reported in the manuscript (current official name)	Protein name	Notes	Potential function: as determined in OPVs other than CMLV
CMLV013 (CMLV-CMS-013)	Ubiquitin Ligase	Similarity to VARV D6R	Host defense modulator protein: putative N1R/p28 -like host range RING finger protein
CMLV019 (CMLV-CMS-019)	Host range virulence factor	Similarity to VACV C7L	Putative host range protein
CMLV023 (CMLV-CMS-025)	Complement binding protein/secreted	Similarity to VACV C3L, known as complement control protein (VCP)	Virulence factor. *In vitro*: inhibit complement-mediated neutralization and lysis. *In vivo*: dampen both antibody and T cell responses
CMLV026 (CMLV-CMS-	Virokine/NFkB inhibitor	Similarity to VACV N1L	Virulence factor with a Bcl-2-like structure that inhibits apoptosis
CMLV031 (CMLV-CMS-033)	Serine proteinase inhibitor (SPI-3)	Similarity to VACV-K2L	Fusion, anti-inflammatory
32L (CMLV-CMS-035)	eIF2α-like PKR inhibitor/IFN resistance	Similarity to VACV-Cop K3L	Inhibition of translation and blockage of IFN-induced apoptosis
55L (CMLV-CMS-063)	dsRNA binding protein	Similarity to VACV E3L	Mediates resistance to interferon and inhibitor of PKR (Z-DNA binding)
CMLV097 (CMLV-CMS-115)	Tyrosine/serine phosphatase	Similarity to VACV H1L	Mediates STAT1 dephosphorylation which inhibits IFN-γ production
CMLV132 (CMLV-CMS-167)	IMV virulence factor (membrane protein)	Similarity to VACV A14.5	Virulence factor: not essential for VACV replication *in vitro*, but enhances virulence *in vivo*
163R (CMLV-CMS-209)	IL-1/Toll-like receptor inhibitor	Similarity to VACV A46R	Signalling inhibition
CMLV191 (CMLV-CMS-241)	Serine proteinase inhibitor (SPI-2 or CrmA)	Similarity to VACV B13R/B14R	Antiapoptotic, blocks IL-1β and IL-18 processing
CMLV193, 194, 196 (CMLV-CMS-243, -244, -246)	IL-1β receptor/secreted	Similarity to VACV B16R	Fragmented ORF, functional? Role: blocks IL-1β
CMLV205 (CMLV-CMS-258)	Serine proteinase inhibitor 1 (SPI-1)	Similarity to C12L	Host range

* Adapted from Gubser and Smith (2002) and Afonso et al. (2002).

and *Camelus bactrianus* (Bactrian camel) (Wernery and Kaaden, 2002). Camelpox is recognized as one of the most important viral disease in camels. The clinical features of camelpox have been extensively described by several groups (Bhanuprakash et al., 2010b; Wernery and Kaaden, 2002), thus, we will briefly summarize them. The course and the outcome of camelpox may vary depending on age, sex and the circulating CMLV strains, which may differ in virulence (Al Zi'abi et al., 2007; Gitao, 1997; Jezek et al., 1983; Kriz, 1982). The disease usually manifests in a localized form in adult camels, but under certain circumstances, generalized or fatal internal forms may be seen (Fig. 4). The disease is characterized by an incubation period of 9–13 days, followed by fever, enlarged lymph nodes, skin lesions and prostration. The typical skin rash will pass through all the stages of pock lesions progression, i.e., macules, papules, pustules, vesicles and scabs. Eruptions are mainly localized on the head, nostrils and eyelids, as well as on the mucous membranes of the lips and the nose and also in the oral cavity. Later, lesions may extend to the limbs, mammary glands or scrotum. It takes 4–6 weeks for the lesions to heal. In contrast, lesions seen in the generalized forms may spread over the body, particularly on the head and the limbs, and swellings on the neck and abdomen can be observed. In such cases, pock lesions may be found in the respiratory and digestive tracts, and the outcome of the disease is more likely fatal (Pfeffer et al., 1998a).

3.2. Geographic distribution and epidemiology

Camelpox was initially described in Punjab, India, in 1909 (Wernery and Kaaden, 2002). Subsequently, outbreaks have been reported in many countries of the Middle East, Asia, Africa and southern Russia, where the disease is enzootic (Fig. 5 and Table 1). Of note, camelpox has never been reported in Australia, even though camel farming is practiced (Wernery and Kaaden, 2002).

Table 6 shows an overview of camelpox outbreaks reported since 1976. As a general pattern, young camels under the age of four years and pregnant females appear more susceptible to camelpox. Abortion rates can reach 87%, as observed in Syria (Al Zi'abi et al., 2007), albeit this high percentage might be explained by the absence of immunity as CMLV circulation had never been reported in this country before. Furthermore, mean morbidity rates can be as high as 92%, while the mean mortality rates may vary from 0% to 15% and the case fatality rates may range from 0% to 25% (Table 6). The circulation of CMLV infections in herds has also been confirmed by sero-epidemiologic studies which showed the presence of neutralizing antibodies in 9.8% of the animals in Libya (Azwai et al., 1996) and in 9.14% in Saudi Arabia (Housawi, 2007). In these two studies, it was stressed that camelpox vaccinations were not practiced. In contrast, a prevalence of neutralizing antibodies of 72.5% was measured in Sudan in an unvaccinated population following the outbreak of 1992–1994 (Khalafalla et al., 1998).

In arid regions, camels provide transport and subsistence to both nomadic and non-nomadic populations. They are also used for racing and as a source of wool, milk and meat. Therefore, camelpox outbreaks have serious economic consequences in herds as affected camels may suffer from loss of condition and reduction in milk production and weight. In addition, the appearance of camelpox in herds may favor secondary infections from other circulating diseases from which camels might die. In this context, countermeasures are needed and they will be further discussed.

3.3. Modes of transmission

Risk factors associated with higher incidence of camelpox have been defined and include the average age of the animals (less than four years old), the rainy season of the year, the introduction of new camels in a herd and the common watering (Khalafalla and Ali, 2007). Transmission of camelpox occurs by direct contacts with sick animals through skin abrasions or *via* aerosols (Wernery and Kaaden, 2002). Scab materials, saliva and secretions of affected camels may shed virus in the environment, such as in water which becomes then the source of infection (Khalafalla and Ali, 2007). Various studies have demonstrated that the incidence of camelpox outbreaks increased during rainy seasons with the appearance of more severe forms of the disease, while during the dry season milder forms are seen (Table 6) (Wernery et al., 1997a, b; Khalafalla and Ali, 2007). It is hypothesized that CMLV strains of different virulence may explain the differences in pathogenicity seen between dry and wet seasons, but this has never been assessed.

Another possibility could be the involvement of arthropod populations which, abundant during rainy seasons, may exert a greater virus pressure onto camel populations. This idea is supported by the isolation of CMLV from *Hyalomma dromedarii* ticks (Wernery et al., 1997a, b). During an outbreak of camelpox in United Arab Emirates in 1995–1996, twenty ticks were collected from five camels with generalized camelpox (Wernery et al., 1997a). Ticks, processed for electron microscopic and cell culture analyses, were found to contain CMLV (Wernery et al., 1997a). However, the question remains whether ticks might transmit CMLV mechanically or whether they might be a true reservoir of the virus. In the last case, the maintenance and spread of camelpox would be explained by transstadial transmission (the pathogen is maintained in the vector from one developmental stage to subsequent stages) or transovarial transmission (the female vector passes the infectious agent through her eggs to the next generation).

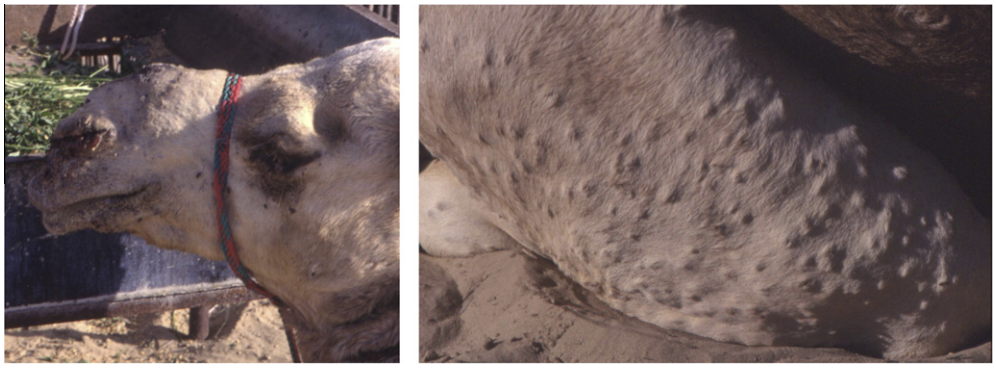

Fig. 4. Left: camelpox in a dromedary with pustular lesions around the nostrils. Right: generalized camelpox in a dromedary with nodular lesions (papules) distributed over the right hind leg of a sitting camel. These cases occurred during an outbreak in Dubai in 1994–1995. Pictures were kindly provided by Prof. Wernery U, Central Veterinary Research Laboratory, Dubai, United Arab Emirates.

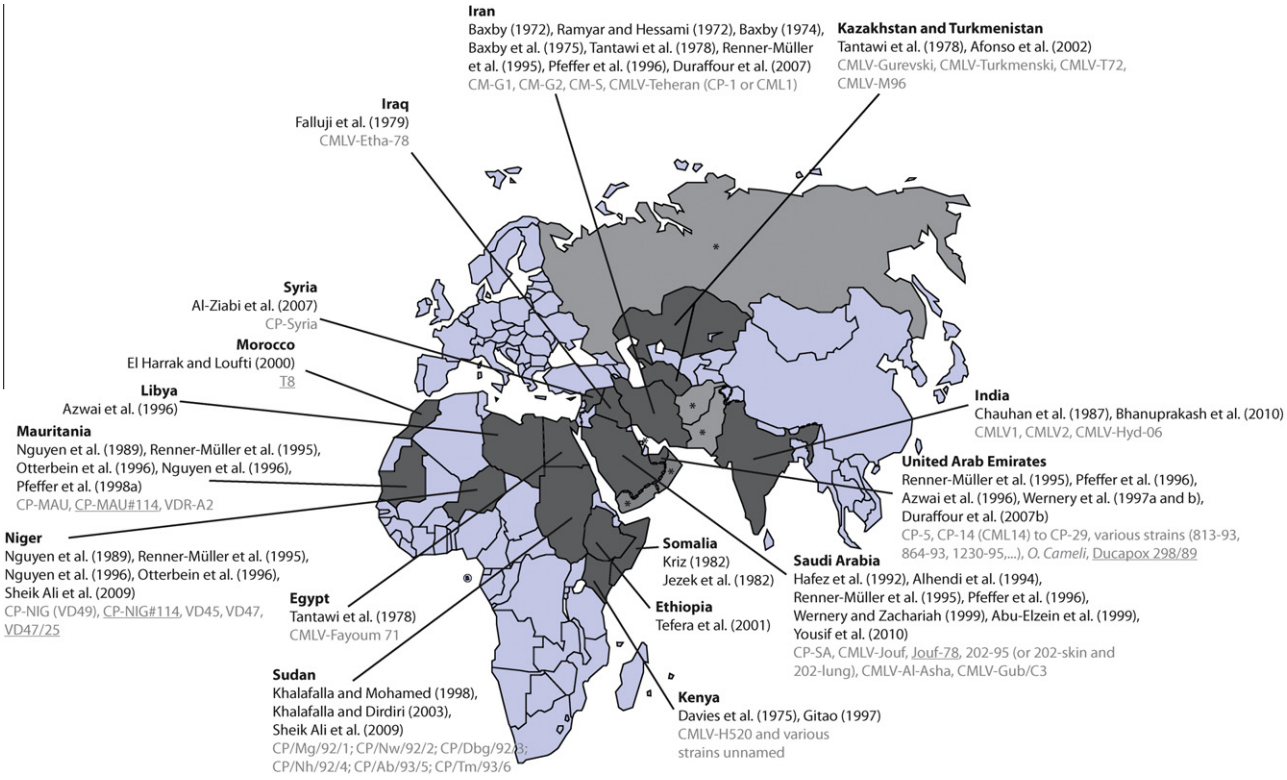

Fig. 5. Map of countries affected by camelpox. Those for which specific references were found (see Tables 1 and 2) are filled in dark gray. Those for which outbreaks were reported by Wernery and Kaaden (2002) are colored in light gray with the symbol ∗. The various CMLV strains isolated and identified since 1972 are stated in gray, while those used for vaccine production and/or have been highly passaged in cell culture are underlined.

Hyalomma dromedarii have been found to be the predominant (90%) tick species infesting camels. Ticks seasonality or periods with the highest infestations have been seen in Egypt (March–November), Sudan (March–October), and Saudi Arabia (Mai, August, October and December) (Al-Khalifa et al., 2007b; Elghali and Hassan, 2009). However, these exhaustive reports did not reveal any correlations between tick's infestations with temperature, relative humidity or rainfall (Al-Khalifa et al., 2007b; Elghali and Hassan, 2009). Further studies are needed to ensure the involvement of arthropods in the transmission of CMLV, but if confirmed, CMLV would be the first OPV transmitted via arthropods. Transmission of poxviruses through arthropods is not surprising. This has been reported with yatapoxviruses (biting insects), capripoxviruses (mosquitoes and stable flies), leporipoxviruses (mosquitoes, fleas, black flies, ticks and mites), suipoxvirus (louse) and avipoxvirus (mosquitoes) (Smith, 2007b; Diallo and Viljoen, 2007; Barrett and McFadden, 2007; Delhon et al., 2007; Boyle, 2007).

4. Host range

4.1. Experimental infections of camels and guanacos

CMLV is considered to solely naturally infect old world camelids. Therefore, camels have been used as animal models of camelpox infection, mainly for evaluating the efficacy of camelpox vaccines under development (Hafez et al., 1992; Wernery and Zachariah, 1999; Nguyen et al., 1996). Of importance, such studies require camels that are seronegative for camelpox neutralizing antibodies. However, camelpox is endemic in camels breeding areas and the average seropositivity rates for CMLV neutralizing antibodies may range from 9.14% to 72.5% (Azwai et al., 1996; Khalafalla et al., 1998; Housawi, 2007). Animals might thus be imported from Australia, a camelpox-free country. For the challenge experiments, two routes of infection have been investigated, subcutaneous and intradermal. Also, the impact of the age of the camels on disease development has been explored, but most of the studies in camels have been done in young animals of 6–10 month-old (Baxby et al., 1975; Hafez et al., 1992; Ramyar and Hessami, 1972; Wernery and Zachariah, 1999).

As shown in Table 7, intradermal inoculations of the pathogenic CMLV strains Teheran, Jouf and CP/Nw/92/2 induced a generalized rash at a virus dose as low as 0.18 $TCID_{50}$ and as high as $10^{5.5}$ $TCID_{50}$ with complete healing four weeks after the onset of the disease (Baxby et al., 1975; Hafez et al., 1992; Khalafalla and Mohamed, 1998; Ramyar and Hessami, 1972). These experiments led to the establishment of a model of moderate camelpox infection. Importantly, easy and rapid transmission of camelpox to non-infected contact camels has been demonstrated (Baxby et al., 1975). A model of fatal camelpox has also been developed by using the CMLV strain 202-95: two-year-old camels exposed to the virus died of generalized camelpox with internal lesions by day 13 post-virus contact (Wernery and Zachariah, 1999).

It is of interest to note that the pathogenicity of VACV strain Elstree and VARV strain EA8 (isolated in Tanzania) was also evaluated in camels in the seventies. Both strains, given at a dose higher than 10^6 PFU, did not produce lesions in camels, although small transient vesicules were seen with VARV (Baxby et al., 1975; Hafez et al., 1992).

The two models of camelpox, i.e., moderate and fatal, have been successfully used to evaluate camelpox vaccine pathogenicity and efficacy (see Vaccines). In terms of pathogenicity, the vaccine strains Jouf-78, Ducapox®, T8 and VD47/25, administrated subcutaneously or intradermally to camels, did not lead to lesion development or disease (Hafez et al., 1992; Nguyen et al., 1996).

Experimental infections of guanacos with CMLV have been successful, while natural infection of new world camelids has never

Table 6
Overview of camelpox outbreaks.

Country	Districts (number)	Year	Period (months)	Number of animals (number of herds)	Number of camelpox cases	Number of deaths	Mean age of cases with camelpox (years)	Mean morbidity rate (%)	Mean mortality rate (%)	Case fatality rate (%)	Comments	References
Somalia	-(4)	1978	May to September	1052 (30)	295	16	2,9	28	1,52	5,4	Co-circulation of a parapoxvirus and CMLV	Kriz (1982); Jezek et al. (1983)
	Badade	-	-	- (5)	-	-	-	-	-	10–50		
	Kismayo	1976	April to June	-	20	0	-	-	-	0		
	-	1977	April to June	-	10	7	New born	-	-	70		
Iraq	Al-Etha	1977	December	-	450	-	-	-	-	-	Used for laboratory testing	Falluji et al. (1979)
Saudi Arabia	Eastern region	1989	October	100	10	0	>2	10	0	0		Alhendi et al. (1994)
Niger	-	1981	-	-	-	-	-	-	-	-		Nguyen et al. (1989)
Kenya	Turkana	1992	September	600 (25)	36	0	0,8	6	0	0		Gitao (1997)
	Samburu	1992	October	500 (20)	135	2	4,1	27	0,4	1,5		
Sudan	Butana	1992–1994	January to December	2560 (35)	230	32	2,7	9	1,2	14		Khalafalla, 1998; Khalafalla and Mohamed (1996); Khalafalla and Ali (2007)
United Arab Emirates	Dubai	1993–1994	October to March	-	- [20 investigated]	-	-	-	-	-	-	Pfeffer et al. (1996)
		1993–1994	October to March	- (16)	15	1	0,6	-	-	6,7	First time: identification of CMLV in camel ticks: arthropod vector?	Wernery et al. (1997a, b)
		1995–1996	November to April	- (22)	22	3	-	-	-	13,6		
Libya	Numerous	-	-	520	0	0	-	0	0	0	Average seropositivity rate (CMLV antibodies) of 9.8%	Azwai et al. (1996)
India	Bikaner	1997	-	-	-	-	-	Considerable	-	-		Bhanuprakash et al. (2010a, b)
Saudi Arabia	-	1998	March to April	-	4	-	3–4	-	-			Abu Elzein et al. (1999)
Ethiopia	Dire Dawa, Hara Zuria, Jijiga and Gewane	-	May–June	350 (100)	11	-	>0,25	3	-	-	Tick populations of camels identified	Tefera and Gebreah (2001)
Syria	Duma and Hama	2005	Summer and Spring	875 (7)	489	-	-	28–92	1–15	25	Mean abortion rate: 87%	Al Zi'Abi et al. (2007)
Saudi Arabia	Eastern province	2010?	-	15 (1)	3	0	-	20	0	-		Yousif et al. (2010)

- unkown.

been reported (Wernery et al., 2000). CMLV strain 202/95 induced a generalized pox disease of the skin 14 days after exposure which led to euthanasia of the animal due to severe illness. In contrast, a female guanaco vaccinated with the vaccine strain Ducapox did not exhibit signs of lesions.

4.2. Other large animals

Since 1972, there have been several attempts to infect animals other than camels with CMLV in order to define its host range and to develop animal models of camelpox (Tables 7 and 8). Horses appeared refractory to CMLV strain CP/Nw/92/2 infection (Khalafalla and Mohamed, 1998). Inoculations of CMLV strains H520, Etha-78 and CP/Nw/92/2 to sheep by the intradermal route or by scarification have been unsuccessful, while the vaccine strain VACV Elstree was pathogenic (Davies et al., 1975; Khalafalla and Mohamed, 1998; Ramyar and Hessami, 1972). Similarly, no lesions or signs of sickness were observed following intradermal inoculations of CMLV to goat and cattle (Khalafalla and Mohamed, 1998; Ramyar and Hessami, 1972). However, it is of interest to note that a study demonstrated that sheep and goats had a prevalence rate of anti-CMLV neutralizing antibodies of 6% and 10%, respectively, while it was 0% in cattle (Housawi, 2007). These results, obtained in Saudi Arabia, might suggest the potential adaptation of CMLV to hosts other than camels, in countries where the disease is enzootic.

Notably, CMLV strain Etha-78 administered intradermally to rhesus monkeys at a dose of 10^3 PFU (pock forming unit) induced typical pox lesions (Falluji et al., 1979). CMLV strain CM-G2 has also been shown to be pathogenic in monkeys at a dose of 10^4 PFU, although no generalized rash was observed; in the same study, infection with VARV resulted in severe generalized disease (Baxby, 1972). In contrast, CMLV strain CP/Nw/92/2 did not induce any reaction when administered intradermally to monkeys, but the dose of virus used was not stated (Table 7) (Khalafalla and Mohamed, 1998). The fact that CMLV strain Etha-78 can provoke in monkeys a disease resembling camelpox or smallpox might be of interest for the development of an additional animal model of OPV infection, considering that (i) the infectious dose of CMLV-Etha-78 was low, (ii) the route of infection mimicked the natural pathway of infection and (iii) the pathogenicity of CMLV for humans is considered to be low or not existent. However, only two studies described the susceptibility of monkeys to CMLV infection, and further experiments are required to evaluate whether the results can be reproduced.

4.3. Chicks, rabbits and rodents

Camelpox pathogenicity has been assessed in chick embryos (Table 8), in which lethality was observed with CMLV strains München, CM-G1, CM-G2, CM-S and CP-NIG #114 (Baxby, 1972; Otterbein et al., 1996; Sehgal and Ray, 1980), while no embryo deaths were reported with other strains, such as Teheran, CP-MAU or CP-NIG (Davies et al., 1975; Falluji et al., 1979; Otterbein et al., 1996; Renner-Muller et al., 1995; Sehgal and Ray, 1980;

Table 7
Outcome of the experimental infection of large animals with CMLV.

	Animal species	Route of inoculation	CMLV strains* evaluated	Observations	References
Experimental infections of camels and guanacos	Camels (9–10 month-old)	Subcutaneous	CMLV-Jouf-78; VD47/25; T8	No reaction observed, and they all conferred immunity from CMLV challenge	Hafez et al. (1992); Nguyen et al. (1996); El Harrak and Loutfi (2000)
		Intradermal	CMLV-Teheran; Jouf; -Jouf-78; CP/Nw/92/2; VDR-A2	Generalized rash with complete healing 4 weeks after the onset of the rash at a dose of 0.18 $TCID_{50}$ with CMLV-Teheran, or of $10^{5.5}$ $TCID_{50}$ with CMLV-Jouf. Generalized rash seen with CP/Nw/92/2. Death and edemas reported with VDR-A2 at day 13 pi.No reaction seen with CMLV-Jouf-78, but conferred immunity from CMLV challenge	Ramyar and Hessami (1972); Baxby et al. (1975); Hafez et al. (1992); Nguyen et al. (1996); Khalafalla and Mohamed (1998)
	Camels (above 12 month-old)	Subcutaneous	CMLV-202-95; Ducapox; T8	Generalized and lethal infection with CMLV-202-95 (death by day 13 pi, internal lesions). No reaction reported with Ducapox and T8: they conferred immunity from CMLV challenge	Wernery and Zachariah (1999); El Harrak and Loutfi (2000)
		Intradermal	CMLV-202-95; CP/Nw/92/2	Severe generalized camelpox disease with CMLV-202-95 (euthanasia), while CP/Nw/92/2 induced camelpox lesions around the eyes, nose, limbs, genital and anal areas, and complete healing after several weeks	Wernery and Zachariah (1999); Khalafalla and El Dirdiri (2003)
	Guanacos (adult male and pregnant female)	Subcutaneous	Ducapox	No reaction observed, and it conferred immunity from CMLV challenge	Wernery et al. (2000)
		Intradermal	CMLV-202-95	Severe generalized camelpox disease at day 14 pi leading to euthanasia at day 21 pi	
Other large animals	Horses	Intradermal	CP/Nw/92/2	No reaction	Khalafalla and Mohamed (1998)
	Sheep	Intradermal	CMLV-H520; -Teheran; -Shiraz; -Gorgan; CP/Nw/92/2	No reaction	Ramyar and Hessami (1972); Davies et al. (1975); Khalafalla and Mohamed (1998)
		Scarification	CMLV-H520	No reaction	Davies et al. (1975)
	Goats and cattle	Intradermal	CMLV-Teheran; -Shiraz; -Gorgan; CP/Nw/92/2	No reaction	Ramyar and Hessami (1972); Khalafalla and Mohamed (1998)
	Monkeys (1 year-old)	Intradermal	CMLV-Etha-78; -CM-G2; CP/Nw/92/2	Typical pox lesions passing all stages as observed with Etha-78 (appearance day 8 pi/disappearance day 18 pi). Localized rash seen with CMLV-CM-G2. No reaction reported with CP/Nw/92/2	Baxby (1972); Falluji et al. (1979); Khalafalla and Mohamed (1998)

* See Table 1 for additional references.

Table 8
Outcome of the infection of small animals with CMLV.

Animal species	Route of inoculation	CMLV strains* evaluated	Observations	References
Chicks (3 day-old)	*Via* defoliated feather follicules	CMLV-H520; -Fayoum 71; -Teheran, -T72, -Gurevski, -Turkmenski, -München; -Etha-78	Mild inflammation seen with CMLV-T72. Few vesicles observed with CMLV-München, which dried up by day 6 pi (no virus detected in liver, lung and spleen tissues). No reaction reported with the other strains.	Davies et al. (1975) ; Tantawi et al. (1978); Falluji et al. (1979); Sehgal and Ray (1980)
Chicks (1 month-old)	*Via* defoliated feather follicules	CMLV-München	6–10 localized vesicles observed in 1 out of 4 chicks which dried up by day 5 pi (no virus detected in skin, liver, lung, and spleen tissues).	Sehgal and Ray (1980)
	Scarification	CP/Nw/92/2	Localized pox lesions.	Khalafalla and Mohamed (1998)
Rabbits (adults)	Intradermal	CMLV-H520; -Fayoum 71; -Teheran, -T72, -Gurevski, -Turkmenski, -München; -Etha-78; -Shiraz, -Gorgan, -CM-G1; -CM-G2; -CM-S; CMLV 1; CMLV 2; CMLV-Hyd; VD47; CP/Nw/92/2	Erythema only observed with CMLV-Fayoum 71, -Teheran, and -Etha-78: appearance 72 hpi, and disappearance on the 5th day. Single maculo-papular lesion observed with VD47, for 8 days (dose of 4×10^5 PFU). No reaction observed with the other strains.	Baxby (1972); Ramyar and Hessami (1972); Davies et al. (1975); Tantawi et al. (1978); Falluji et al. (1979); Sehgal and Ray (1980); Nguyen et al. (1989); Bhanuprakash et al. (2010a, b); Khalafalla and Mohamed (1998)
	Scarification	CMLV-H520; -Fayoum 71; -Teheran, -T72, -Gurevski, -Turkmenski, -Etha-78; VD47; CP/Nw/92/2	Erythema only observed with CMLV-Fayoum 71, -Teheran, and -Etha-78.	Davies et al. (1975); Tantawi et al. (1978); Falluji et al. (1979); Sehgal and Ray (1980); Nguyen et al. (1989); Khalafalla and Mohamed (1998)
	Intratesticularly	CMLV-Etha-78	No reaction	Falluji et al. (1979)
Rats	Intradermal	CMLV-Teheran; -Shiraz; -Gorgan	No reaction	Ramyar and Hessami (1972)
Guinea-pigs	Intradermal	CMLV-Etha-78; -München; Teheran; -Shiraz; -Gorgan; CP/Nw/92/2	No reaction	Ramyar and Hessami (1972); Falluji et al. (1979); Sehgal and Ray (1980); Khalafalla and Mohamed (1998)
	Metatarsal pad	CMLV-München	Localized reaction with vesicles (no virus detected in liver, lung and spleen tissues).	Sehgal and Ray (1980)
Suckling mice, immuno-competent	Intradermal	CMLV-Teheran; -Shiraz; -Gorgan	No reaction	Ramyar and Hessami (1972)
	Intracerebral	CMLV-H520; CP-MAU; CP-NIG; CP-MAU #114; CP-NIG #114, CMLV-CM-S	Lethality observed with LD_{50} values of 3 PFU for CP-MAU and of 225 PFU for CP-NIG and ranging from 10^3 to 10^5 PFU for CMLV-H520; CP-MAU #114; CP-NIG #114, and CMLV-CM-S.	Baxby (1972); Davies et al. (1975); Otterbein et al. (1996)
	Intraperitoneal	CP-MAU; CP-NIG; CP-MAU #114; CP-NIG #114	Lethality observed with mean LD_{50} value of 4 PFU.	Otterbein et al. (1996)
Mice of 2–3 week-old, immuno-competent	Intracerebral	CMLV-Fayoum 71; -Teheran, -T72, -Gurevski, -Turkmenski,	Lethality observed with LD_{50} values ranging from $10^{3,2}$ to $10^{4,5}$ PFU.	Tantawi et al. (1978)
	Intranasal	CML1	No reaction but production of CMLV neutralizing antibodies.	Duraffour et al. (2011)
Mice of 4–6 week-old, immuno-competent	Intradermal	CMLV-Teheran; -Shiraz; -Gorgan; CM-G2, -CM-S; CMLV 1, CMLV 2, CMLV-Hyd	Localized small lesions in three out of five mice with CMLV-CM-S in the ear pinnea model. No reaction observed with the other strains.	Ramyar and Hessami (1972); Baxby (1972); Tscharke et al. (2002); Bhanuprakash et al. (2010a, b)
	Intracerebral	CMLV-Etha-78; VD47	No death reported (dose of 10^5 PFU).	Falluji et al. (1979); Nguyen et al. (1989)
Athymic nude mice of 3–4 week-old, immuno-deficient	Scarification	CML1	Appearance of vesicles at the scarification site and development of disease with edemas of the joints (dose of 2.5×10^6 PFU). Death by euthanasia at day 80 pi due to disease severity.	Duraffour et al. (2011)
	Intranasal	CML1	Dose of 2.5×10^6 PFU: death by euthanasia by day 40–50 pi due disease severity and absence of weight gain.	
	Intraperitoneal	CML1	40% death reported between days 20 and 50 pi (dose of 10^7 PFU).	
SCID mice of 3–4 week-old, immuno-deficient	Intranasal	CML1	50–80% death reported between days 20 and 30 pi (dose of 10^4–10^5 PFU).	Duraffour et al. (2011)
	Intraperitoneal	CML1	80% death reported between days 20 and 30 pi (dose of 10^6 PFU).	

* See Table 1 for additional references.

Sheikh Ali et al., 2009; Tantawi et al., 1978). Only mild inflammations were seen in 3 day-old chicks upon exposure with CMLV-T72 and CMLV-München. In one month-old chicks, the strain CP/Nw/92/2 has been shown to induce localized vesicles (2–5 mm diameter) which fell off within 7–10 days (Khalafalla and Mohamed, 1998).

Seven studies evaluated CMLV pathogenicity in adult rabbits, and only the CMLV strains Fayoum 71, Teheran, Etha-78 and VD47 were shown to induce an erythema, which disappeared on the 5th to 8th day post-intradermal inoculation (Baxby, 1972; Bhanuprakash et al., 2010a; Davies et al., 1975; Falluji et al., 1979; Ramyar and Hessami, 1972; Sehgal and Ray, 1980; Tantawi et al., 1978). It should be noted that the VARV strains Harvey, EA8 or EA17 did not infect rabbits (*via* intradermal inoculation), while VACV strain Lister and CPXV strain Brighton caused nodular lesion development (Baxby, 1972; Tantawi et al., 1978). A localized reaction with vesicles has been described in guinea pigs after inoculation of CMLV strain München into the metatarsal pad, but not after intradermal infection or scarification (Falluji et al., 1979). In rats, no lesions or signs of sickness were observed following intradermal inoculations of CMLV (Khalafalla and Mohamed, 1998; Ramyar and Hessami, 1972).

Immunocompetent adult mice (4–6 week-old) have been reported to be resistant to CMLV challenge (intradermal, intracerebral or intranasal) (Bhanuprakash et al., 2010a; Falluji et al., 1979; Ramyar and Hessami, 1972; Tscharke et al., 2002). In contrast, suckling mice and 2–3-week-old mice were susceptible to CMLV intracerebral inoculation (average LD_{50} of 20,000 PFU) (Davies et al., 1975; Otterbein et al., 1996; Tantawi et al., 1978). Very recently, athymic nude mice (3–4 week-old) have been shown to be sensitive to a challenge with CMLV strain Teheran (CML1): intranasal exposure resulted in disease development and absence of gain of body weight leading to euthanasia of 100% of the infected animals and scarification led to the development of disseminated lesions. These two mouse models, although not mimicking the natural camelpox disease, represent small animal models available for evaluating CMLV pathogenicity and therapeutics. Moreover, histopathological examinations of skin biopsies after CMLV scarification proved to be characteristic of those seen in clinical lesions induced by CMLV (Fig. 3) (Duraffour et al., 2011).

4.4. Camelpox virus infection of humans

At the time of the smallpox eradication campaign, it was thought that camelpox might represent a non-human reservoir of VARV, because the two causative agents were indistinguishable under certain laboratory conditions. Therefore, it was also asked whether camelpox could be transmitted to humans, but various observations suggested that human infections were rare and Baxby hypothesized then that "camelpox virus is different from variola and is incapable of infecting man" (Baxby, 1972). It is interesting to note that centuries before Jenner developed vaccination, camelpox was circulating in Baluchistan (Southwest Asia), and it was described as being as effective as cowpox in protecting humans against smallpox (Tadjbakhsh, 1994). This suggests that CMLV was able to elicit immune responses in humans that were strong enough to cross-protect against VARV.

Among the few human cases of camelpox in the literature, people drinking milk from camelpox-affected animals have been reported to develop ulcers on the lips and in the mouth, but these observations could not be visually checked or laboratory confirmed (Davies et al., 1975). Also, Kriz and Jezek et al. assessed the risk of camelpox infections in humans. Some 286 camel herdsmen were investigated, and only three presented a rash; all three were positive for herpesvirus. One camel handler, in contact with sick animals and without a history of smallpox vaccination, developed four pock-like lesions on his arms and seroconverted for OPVs, but the involvement of CMLV could not be demonstrated (Jezek et al., 1983; Kriz, 1982). Further searches in Somalia did not lead to the identification of human cases of camelpox.

Moreover, from the understanding in 1975 that cross-immunity between VACV, VARV and CMLV existed, it was also questioned whether the rarity of human camelpox was not brought in part by the immunity induced *via* smallpox vaccination (Baxby et al., 1975; Falluji et al., 1979). As a consequence, the immunological status of the individuals (i.e., previous smallpox vaccination or history of smallpox) might have been a bias for estimating the possible human cases of camelpox. However, from the 1970s until recently, it has been well accepted that CMLV rarely infects humans. As stated by Baxby, "whether human camelpox will become more common as the immunity of the human population wanes will be a subject for long-term inquiries" (Baxby et al., 1975). Currently, 30 years after the end of smallpox vaccination, the statement of Baxby might find an answer. However, only three human cases of camelpox have been reported in India by Bera and colleagues (Bera et al., 2011). They were detected in animal handlers during an outbreak of camelpox of the Border Security Force in 2009. The lesions were confined to the hands and fingers of camel handlers, and passed through all the stages of pock lesions until the formation of scabs (Bera et al., 2011). Serum samples of the three suspected cases showed neutralizing antibodies against CMLV and, of importance, none of these patients had ever been vaccinated against smallpox. In addition, in one of the three human cases, viral DNA could be detected by conventional PCR for CMLV-specific genes (Bera et al., 2011). Further epidemiological studies in regions endemic for camelpox are necessary to assess the circulation of CMLV, but currently, infections of humans do not seem to be of public health importance.

5. Diagnosis of camelpox

Following the appearance of clinical signs of disease, tissue samples (skin or organ biopsies) are most useful to identify the infectious agent. Because camelpox in camels can be confused with other viral diseases, such as contagious ecthyma (parapoxvirus) and papillomatosis (papillomavirus), differential diagnosis may be needed (Wernery and Kaaden, 2002). Camelpox is routinely diagnosed based on clinical signs, pathological findings and cellular and molecular assays. Five complementary techniques might be advised for camelpox diagnosis: transmission electron microscopy (TEM), cell culture isolation, standard PCR assays, immunohistochemistry and demonstration of neutralizing antibodies. For each of these techniques, exhaustive descriptions of sample preparations and storage and of each test procedure have been provided elsewhere (Elliot and Tuppurainen, 2010; Pfeffer et al., 1998b). Here, we briefly describe the first three techniques, which are rapid and efficacious for routine identification of CMLV.

TEM is a reliable and rapid method to demonstrate the presence of OPVs in scabs or tissue samples, although a relative high concentration of the virus in the sample is required. This technique enables the differentiation between OPVs, which are brick-shaped, and parapoxviruses, which are ovoid-shaped (Damon, 2007). Tissue samples suitable for TEM should be processed as reported (Elliot and Tuppurainen, 2010).

In parallel to TEM, virus isolation in cell culture must be initiated. As shown in Table 4, many different cell lines can be used, but Vero, MA-104 or Dubca cells, in which the virus replicates easily, are preferred (Pfeffer et al., 1998b). Blood, serum and homogenized tissue samples can be used to infect cell cultures. Cultures should be monitored for 10–12 days. However, depending on the virus concentration, cytopathic effects, including the formation of

multinucleated syncytia, can already appear at one day post-infection. Chorioallantoic membranes (CAMs) can also be used for the growth of CMLV (Sheikh Ali et al., 2009), but it is important to consider that the pocks produced by VARV and CMLV in this system are indistinguishable (Baxby, 1972). The identity of the causative agent as CMLV must be confirmed by TEM, PCR and/or sequencing (Elliot and Tuppurainen, 2010).

DNA can be extracted from cell culture samples and clinical material using numerous commercial kits. Recently, a reliable and low-cost two-step extraction procedure has been developed for isolating CMLV DNA from skin samples (Yousif et al., 2010). The PCR assays available to identify CMLV are based on the detection of sequences encoding for the A-type inclusion body (*ATI*), the hemagglutinin (HA), the ankyrin repeat protein (*C18L*) or the DNA polymerase (*DNA pol*) (Meyer et al., 1994; Meyer et al., 1997; Ropp et al., 1995; Balamurugan et al., 2009). ATI gene-based PCR is performed with a single set of primer which enables the differentiation of OPV species by producing amplicons of different sizes. An extra step consisting of a *Bgl*II or *Xba*I restriction digestion allows then an unequivocal identification of the virus species (Meyer et al., 1994, 1997). The HA-PCR amplicon *Taq*I restriction fragment length polymorphism (RFLP) permits to differentiate between OPV species, but species-specific primers within the HA open reading frame of OPVs have also been described (Ropp et al., 1995). Recently, a single-plex C18L and a duplex C18L-DNA pol PCR have been developed to specifically identify CMLV and to differentiate it from other OPVs, capripoxviruses and parapoxviruses (Balamurugan et al., 2009). These assays have the advantage of avoiding an extra step of restriction analysis. The same authors reported the use of a SYBR Green quantitative PCR, but only for quantifying CMLV and evaluating the efficiency of the conventional single-plex or duplex PCRs mentioned above. To date, no real-time quantitative PCR has been described for the specific diagnosis of CMLV.

6. Vaccines

As a result of major economic losses from numerous camelpox outbreaks (Table 6), research has been oriented toward the development of prophylactic methods to contain the spread of camelpox in enzootic countries. In this context, camelpox vaccines have been generated. It is important to remember that the development of these vaccines has been initiated after the worldwide eradication of smallpox. At that time, the use of VACV as a prophylactic agent for other orthopoxviral diseases of animals was not recommended by health authorities, most probably due to the potential danger to non-vaccinated human contacts (Hafez et al., 1992). Therefore, CMLV itself, rather than VACV, has been used for vaccine development (Hafez et al., 1992; Nguyen et al., 1996; Pfeffer et al., 1996; Wernery and Zachariah, 1999). Nevertheless, it has been reported that a camelpox vaccine, developed in the former Soviet Union, was prepared from VACV (Hafez et al., 1992). To date, knowledge of camelpox vaccine efficacy originates from field investigations using the two commercialized CMLV-based vaccines, which might not warrant the use of VACV. Also, considering that camelpox is restricted to camels, any spread of the virus in the environment from body fluids of recently vaccinated animals should not endanger other species, while VACV might.

In the search for vaccines, it has been seen that camels infected intradermally or subcutaneously with high doses of VACV (strain Elstree) or VARV (strain EA8 at a dose of 3×10^6 PFU, as reported in 1975) (i) did not develop any signs of illness and (ii) were protected from subsequent challenge with pathogenic CMLV strains (Baxby et al., 1975; Hafez et al., 1992). Therefore, both viruses were able to confer immunity to CMLV. With regards to smallpox eradication campaign and the success of smallpox vaccination, it may have been helpful to use the already available smallpox vaccine to vaccinate camels. However, as reported by Hafez, it was prepared from virulent VACV strain Elstree, which could induce generalized infections among young susceptible or immunodeficient camels and was responsible for severe side effects in humans with atopic skin or immunodeficiencies (Hafez et al., 1992). The highly attenuated modified vaccinia virus Ankara (MVA) vaccine, which was passaged more than 500 times in cell culture, has been considered as a possible alternative, due to its safety profile in humans. However, a high dose of 10^8 $TCID_{50}$ of VACV-MVA, as well as a second injection 3 weeks after, was required for efficient protection in animals (Hafez et al., 1992).

Four camelpox vaccines, of which two are commercialized, have been evaluated. They contain the following CMLV strains: Jouf-78 (Hafez et al., 1992), $VD_{47/25}$ (Nguyen et al., 1996), Ducapox 298/89 (Wernery and Zachariah, 1999), CMLV-T8 (El Harrak and Loutfi, 2000) (Table 9). In Saudi Arabia, the CMLV strain Jouf-78, an attenuated CMLV strain passaged 80 times in cell culture, has been shown to offer full protection from CMLV challenge. In camels, this strain is not pathogenic when given intradermally or subcutaneously at a dose of $10^{6.8}$ $TCID_{50}$ (Hafez et al., 1992). From field studies, a single dose of vaccine ranging from 10^3 to 10^4 $TCID_{50}$ was advised for full protection.

The attenuated CMLV strain $VD_{47/25}$, passaged 80 times in cell culture, has also been evaluated as camelpox vaccine (Nguyen et al., 1996). As observed during experiments in Mauritania, this strain was innocuous in camels (dose of $10^{4.7}$ $TCID_{50}$, subcutaneous) and protected camels from severe to lethal CMLV infection. It has to be mentioned that a bias might have been brought in the results since it was difficult to guarantee the naïve status of animals enrolled due to the endemicity of the disease in the country at the time of the study (Nguyen et al., 1996).

In the United Arab Emirates, a modified live CMLV vaccine obtained from passaging the strain CaPV298-2 or *O. cameli* in Vero cells, has been used (Azwai et al., 1996; Pfeffer et al., 1996). This

Table 9
CMLV vaccines currently in commercial production.

CMLV vaccine strain/commercial name	Manufacturing company	Market places	Notes	References
Jouf-78	–	Saudi Arabia	Attenuated CMLV strain, passaged in cell cultures	Hafez et al. (1992)
$VD_{47/25}$	–	Mauritania	Attenuated CMLV strain, passaged in cell cultures	Nguyen et al. (1996)
Ducapox (298/89)/ Ducapox®	Highveld Biological, South Africa	United Arab Emirates and other countries	Attenuated CMLV strain, second injection for young calves, immunity lifelong	Azwai et al. (1996); Pfeffer et al. (1996); Wernery and Zachariah (1999); Khalafalla and El Dirdiri (2003)
CMLV-T8/CAMEL-POX®	Biopharma, Morocco	Morocco and other countries	Inactivated CMLV strain, second injection required, repeat injection annually	El Harrak and Loutfi (2000); Khalafalla and El Dirdiri (2003)

vaccine, so-called Ducapox®, standing for DUbai CAmelPOX vaccine, is produced by Highveld Biological, South Africa. It was used for field vaccination just before the onset of a large camelpox outbreak in Dubai in 1993–1994. Among 2000 vaccinated camels, seven developed the disease, but it was not known if these animals were infected before vaccination, or were true vaccination failures (Pfeffer et al., 1996). Further, Wernery and Zachariah showed that a single vaccination protected camels for 6 years, although this was only shown with two animals (Wernery and Zachariah, 1999). Vaccine efficacy has also been demonstrated in new world camelids against an otherwise lethal CMLV challenge (Table 7) (Wernery et al., 2000). The safety and potency of Ducapox® has also been explored in a study of Khalafalla and El Dirdiri. The vaccine requires subcutaneous injection, but a single dose is enough to sustain protection for at least one year; the starting age for vaccination is 6 months (Khalafalla and El Dirdiri, 2003).

In Morocco, an inactivated camelpox vaccine with an adjuvant is manufactured and distributed by Biopharma (El Harrak and Loutfi, 2000). The vaccine is derived from the CMLV strain T8, isolated from scabs during an outbreak in Morocco in 1984. It is safe for young and adult camels and it has been shown to induce CMLV-neutralizing antibodies (El Harrak and Loutfi, 2000). Khalafalla and El Dirdiri performed field experiments with this vaccine and demonstrated that a second injection after one month was needed for efficient protection against virulent CMLV challenge (Khalafalla and El Dirdiri, 2003). It is advised to administer it annually.

The appropriate age for vaccination with both commercial vaccines is 6 months; a second vaccination might be necessary for young calves. It has been shown that camels under the age of 6 months showed poor immune responses, possibly due to the immaturity of the immune system (Khalafalla and El Dirdiri, 2003). These observations may justify the use of antiviral therapies. Today, it is difficult to estimate the extent of vaccine coverage among camels, but El Harrak and Loutfi reported that it was of 50% in 1999 in Morocco (El Harrak and Loutfi, 2000), and Wernery et al. stated that it was 88% and of 66%, respectively, in 1997 and 2008 in the United Arab Emirates (Wernery et al., 2008; Pfeffer et al., 1998b).

7. Antiviral therapy

Over the last years, potent antiviral molecules active *in vitro* and *in vivo* against poxviruses, including OPVs, have been developed and could be envisaged for the treatment of camelpox disease (Smee, 2008; Snoeck et al., 2007). They include in particular (i) the molecules belonging to the acyclic nucleoside phosphonate (ANP) family, i.e., cidofovir (Gilead, CA, USA) and its lipid derivative CMX001 (Chimerix Inc, NC, USA) (De Clercq et al., 1987; Kern et al., 2002), and (ii) the compound ST-246 (SIGA Inc, OR, USA) (Fig. 6) (Yang et al., 2005). These three drugs have gained Investigational New Drug (IND) status, allowing their emergency use for the treatment of life-threatening VACV infections (2007; 2009a, b; Bristol, 2007; Kaiser, 2007; Marris, 2007; Vora et al., 2008).

Cidofovir and CMX001 are active against a broad range of DNA viruses including poxviruses. Both compounds target the viral DNA polymerase of OPVs (Andrei et al., 2006). Cidofovir is already marketed for the treatment of cytomegalovirus (CMV) retinitis in HIV-infected patients, given by intravenous injection. Also, because cidofovir is nephrotoxic in humans, a concomitant administration of probenecid and hydration is required. In contrast to cidofovir, CMX001 is orally bioavailable and the drug did not cause toxicity in mice as shown in studies employing CMX001 for up to 14 days (Ciesla et al., 2003). Phase I clinical trial in healthy volunteers demonstrated that the compound was well tolerated at all doses, and phase II studies are currently ongoing for prophylaxis of CMV infection (http://www.chimerix-Inc.com/). The molecule is also under development using the FDA "animal rule" efficacy for smallpox infection.

In cell culture, cidofovir inhibits 50% of CMLV replication (EC_{50}), at a mean concentration of 2.5 μM, 8.2 μM and 5.3 μM, respectively, in Vero 76, HEL and PHK cells (Duraffour et al., 2007b; Smee et al., 2002). Although the antiviral activity of CMX001 has not been reported against CMLV, it is reasonable to assume that its EC_{50} value would be in the range of those reported for VACV and CPXV, which are of 0.8 μM and 0.6 μM in human foreskin fibroblasts (HFF), respectively (Kern et al., 2002). Other derivatives of cidofovir have been evaluated *in vitro* against two strains of CMLV, and among them, the analog HPMP-5-azacytozine demonstrated a potent anti-CMLV effect that might warrant further investigations. Until the recent development of two mouse models of camelpox infection, no data were available about the *in vivo* antiviral activity of cidofovir against CMLV. *In vivo*, intraperitoneal administration (once per day for 3 days at a concentration of 50 mg/kg) of cidofovir afforded 100% protection from morbidity in an intranasal model of CMLV infection in athymic nude mice. Also, topical administration of a 1% cidofovir cream (once per day for 5 days) protected 100% of nude mice from CMLV-induced disease following infection by scarification. An intermediate level of efficacy of a 2,6-diaminopurine derivative of cidofovir, i.e., HPMPDAP, has also been observed in these mouse models (Duraffour et al., 2011).

In terms of the appearance of resistance to cidofovir and its analog HPMPDAP, CMLV-resistant viruses could be obtained *in vitro*,

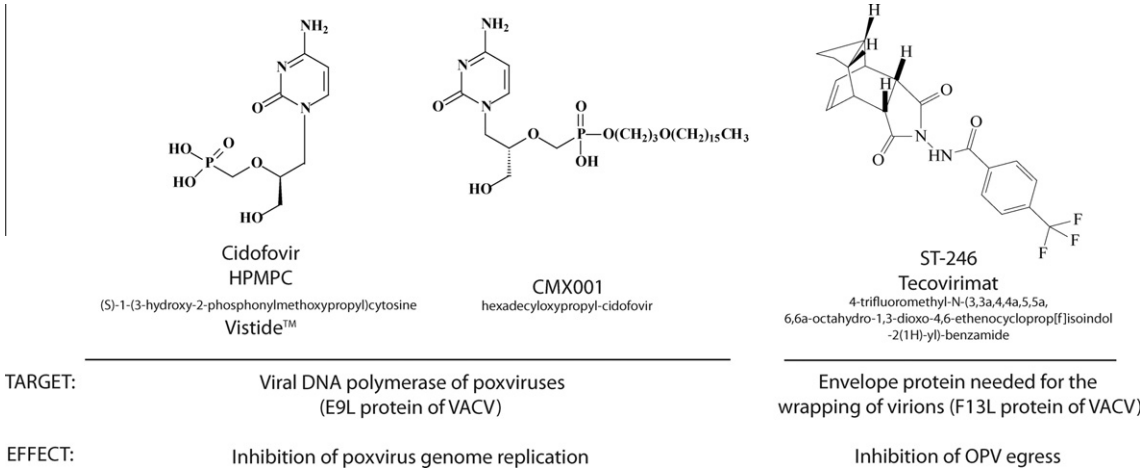

Fig. 6. Chemical structures and modes of action of cidofovir, CMX001 and ST-246.

but only after passaging the virus 20–40 times in increasing concentrations of the drug (Duraffour et al., 2009; Smee et al., 2002). Sequencing of the viral DNA polymerase gene led to the mapping of mutations conferring resistance (Duraffour et al., 2009). Some point mutations were similar to those described with VACV, while others were newly characterized (Andrei et al., 2006; Duraffour et al., 2009; Gammon et al., 2008).

ST-246 is a potent inhibitor only of orthopoxviruses. It targets the protein F13L of VACV, which is required for the wrapping of IMVs and the production of EEVs (Yang et al., 2005; Duraffour et al., 2008a). The drug is orally bioavailable. Following Phase I clinical trials, the molecule demonstrated a good safety profile in all tests conducted, and efficacy studies in monkey models of smallpox and monkeypox have been completed (Jordan et al., 2009, 2010; Huggins et al., 2009). Numerous studies have shown that ST-246 administered for 10–14 days at a dose of 100 mg/kg once per day protects OPV-infected animals from disease development (for review, (Duraffour et al., 2010)). In the case of CMLV, the activity of the molecule has only been evaluated *in vitro*. ST-246 exhibited EC_{50} values of 0.01 μM in Vero cells infected with CMLV, and of 0.08 μM and 0.05 μM in HEL and PHK, respectively (Duraffour et al., 2007a; Yang et al., 2005).

Cidofovir, CMX001 and ST-246 are potent inhibitors of CMLV replication *in vitro*. In mouse models of camelpox infection, cidofovir either formulated as cream or for systemic use protected animals from disease development and/or death. Nevertheless, CMX001 and ST-246 offer the advantage of being orally available which may render them more attractive for veterinary use.

8. Priorities for research

Our present limited knowledge of CMLV prompts a series of interesting questions that may drive further investigation. It is believed that strains of different virulence may exist, which could explain the two forms of the disease, moderate and generalized lethal, but this has never been demonstrated. Observations made during experimental infections of camels tend towards this idea as, depending on the CMLV strain, differences in pathogenicity have been observed (Table 7). This hypothesis would not be surprising since it evokes the features of other OPV strains, such as VARV and VACV, that also exhibit different virulence phenotypes (Damon, 2010; Smith, 2007a). In this context, further sequencing of CMLV strains isolated from moderate and generalized camelpox cases could emphasize discrepancies in their gene contents and allow the identification of genes associated with virulence or immune-evasion properties.

Furthermore, the sequencing of highly passaged strains with an attenuated phenotype, such as CMLV-NIG #114 and CMLV-MAU #114, could be of great value in the search for virulence and host-range genes. CMLV-MAU #114 harbors a loss of 22 kbp in genome size compared with its parent strain, i.e., 17.5 kbp in the right terminus and 4.5 kbp in the left terminus (Otterbein et al., 1996). This strain lost its ability to propagate in Dubca cells and exhibited an attenuated phenotype *in vivo*. CMLV-NIG #114 also showed an attenuated phenotype *in vivo* and *in vitro*, but with a loss of only 1 kbp compared to its parent virus (Otterbein et al., 1996). Deeper studies of these strains may reveal the determinants of the narrow host tropism of CMLV. Also, in contrast to cell culture systems that have been unfruitful, mouse models may be attractive to elucidate differences in pathogenicity between CMLV strains. It could be envisaged to evaluate their virulence in the intracranial model of infection in suckling mice or in the intranasal and subcutaneous models in athymic nude mice (Table 8). Furthermore, in the search for an immunocompetent mouse model, the sensitivity of wild-derived mice to CMLV challenge could be examined, as they have been shown to be sensitive to monkeypox virus, while laboratory-derived strains were not (Americo et al., 2010).

In contrast to VACV and CPXV, the pathways used by CMLV to counteract host immune responses have not been studied in detail, and it is still not known whether CMLV ORFs with putative immunoregulatory functions are active. To this end, the construction of recombinant viruses with fluorescently tagged viral proteins or with knock-out genes represent tools that need to be developed. In addition, it would be interesting to identify which cytokines, chemokines and interferons are deregulated *in vitro* and *in vivo* following CMLV exposure. It might be then expected that strains of different virulence may impact immune responses differently. Research driven towards the study of CMLV immunomodulators and immune pathways targeted after virus exposure will not only be relevant for CMLV, but also for other OPVs.

It would also be of interest to study more into details camelpox pathogenesis in camels in order to answer the following points: the duration of viral shedding, the spread from the initial site of infection and the tissue tropism of the virus. None of these questions has been addressed yet and the use of modern techniques of molecular biology, including real-time PCR, combined with virus isolation and immunohistochemistry would be of great value to understand the spread of CMLV in its natural host.

CMLV is considered to solely infect camels and, in addition, the virus appears restricted to arid and semi-arid regions. From this feature, it may be speculated that the virus co-evolved with its reservoir host, camels, and this could explain why CMLV is only found in camel housing territories. It may also be asked whether it is a CMLV-specific property to survive in hot temperatures. In addition, the isolation of CMLV from the *Hyalomma dromaderii* ticks, which are the major tick species found on camels, raised other questions: is there a continuous chain of transmission involving arthropod vectors? Can ticks mechanically transmit CMLV, or are they a true reservoir of the virus? Considering the last option, do ticks maintain CMLV via transstadial or transovarial transmission? Epidemiological studies should be carried out to further explore the circulation of CMLV among camels and possibly among sheep and goats, as previously reported (Housawi, 2007). Indeed, any adaptation of CMLV to other animal species should not be neglected, as it could spread to neighboring CMLV-free countries. If arthropod vectors should be implicated in the transmission of CMLV, it could predict other potential hosts. Indeed, ticks of the *Hyalomma* genus have been reported as parasites not only of camels, but of cattle, sheep and goats (Al-Khalifa et al., 2007a, b; Elghali and Hassan, 2009; Nabian et al., 2009). In order to explore any role of arthropod vectors in the transmission of camelpox, it would be beneficial to screen tick libraries collected from goats, sheep, cattle and camels in countries where the disease has been reported, for the presence of CMLV, using quantitative real-time camelpox-specific PCR accompanied by the isolation of the agent in cell culture. Other tick genera should also be examined, such as *Rhipicephalus* (Al-Khalifa et al., 2007b), and studies could be extended to other arthropods that infest camels, such as suckling lice, flies and fleas (Wernery and Kaaden, 2002). Such data would be of great value to identify a potential vector for CMLV, and might highlight animals other than camels that might be at risk of infection.

Based on the success of smallpox eradication and the fact that camels are the single reservoir host of camelpox, it may be envisaged, with a political will, to eradicate camelpox. This idea is additionally supported by the absence of a proven insect reservoir and by the access to an effective, thermostable, single-dose, live attenuated vaccine that can elicit long-term immunity. Indeed, one dose of the vaccine Ducapox® has been shown to protect camels from camelpox infection for six years, albeit only a few animals were included in the study (Wernery and Zachariah, 1999). However, because camels can live for forty to fifty years, a vaccine affording

long-term immunity might be needed. Further research should therefore be oriented toward determination of the long-term immunity conferred by CMLV-based vaccines. It is likely that, if existing, any cycle of vector transmission would be broken by a mass CMLV vaccination campaign. Also, the few reported cases in humans and the results of experimental infection of small animals indicate that CMLV may not infect species other than camels. We should note, however, that vaccination efforts may be significantly hindered by the difficulty of vaccinating camels in certain geographic regions where the disease circulates, including wild (non-domestic) Bactrian camels, a critically endangered species still found in China and Mongolia, which might serve as a reservoir for the disease.

In terms of antiviral treatments, they may be beneficial for the management of a camelpox outbreak and/or in the event of vaccination failure. Although no drugs are approved for the treatment of camelpox infections in camels, anti-OPV molecules might be regarded as promising for further veterinary development, i.e., cidofovir (and other derivatives), CMX001 and ST-246. Cidofovir was already shown to be efficacious against CMLV *in vivo*, but it would be of interest to evaluate the *in vivo* efficacy of CMX001 and ST-246. These studies could be considered not only in mouse models of camelpox infection but also in camels, as ST-246 and CMX001 are orally available. It is important to mention that CMX001 and ST-246 are lead compounds for the treatment of OPV infections in humans, and thus their pharmacokinetic and safety profiles have been well characterized in animals and in clinical trials (Duraffour et al., 2010; Hostetler, 2009).

Further, the benefit of an antiviral therapy based on acyclic nucleoside phosphonates, such as cidofovir and CMX001, is not limited to OPV infections. Their broad range antiviral activity towards DNA viruses means that other viral infections occurring in camels could be prevented, including camel contagious ecthyma, caused by a parapoxvirus (Wernery and Zachariah, 1999; Abubakr et al., 2007), and papillomatosis, caused by *Camelus dromedarius* papillomavirus type 1 and 2 (Wernery and Zachariah, 1999; Ure et al., 2011). Although not tested against these viruses, potent antiviral and antiproliferative activities of cidofovir and CMX001 have been demonstrated against the parapoxvirus orf and human papillomavirus positive cervical cancer cells, respectively (Dal Pozzo et al., 2005, 2007; Hostetler et al., 2006; Andrei et al., 1998). In Sudan, while papillomatosis can be seen as a mild disease in camels with a morbidity rate of 3.3% in animals less than two years, camel contagious ecthyma is enzootic, with mean morbidity and mortality rates of 60% and 8.8%, respectively, in calves less than 1 year of age (Khalafalla, 1998). Camel contagious ecthyma is a severe disease which requires strict measures to control its spread, and antivirals might represent a control option. It is interesting to note that the seasonal prevalence of this disease, with annual appearance during rainy season, suggests the existence of an arthropod vector, perhaps recalling the epidemiology of camelpox (Khalafalla, 1998).

In the context of increasing OPV infections, particular attention should be given to camelpox outbreaks, as well as to the identification of any human infections. Intensified surveillance should be implemented to detect and restrain the spread of the disease. In addition, further analysis of virus pathogenesis and immune modulation should be relevant to other aspects of poxvirus biology. Finally, if arthropod vectors prove to be involved in CMLV transmission, it would bring camelpox into a novel light, as the first vector-borne orthopoxvirus disease.

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