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Research Article

Properties of Pathogenic & Cell Culture Adapted Strains of *Camelpox* Virus

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ABSTRACT

The aim of the present study was to find changes in the biological properties of *camelpox* virus upon serial passages in Vero culture. Pathogenic *camelpox* virus (CMLV/WT) was serially propagated for hundred passages in confluent monolayers of Vero cells. Viruses from passages fifty (CMLV/50) and hundred (CMLV/100) were compared to CMLV/WT in terms of CPE, pock lesion and ability to induce lesions in rabbit's skin. When replicating in old Vero cells at 37˚C, CMLV/50 and CMLV/100 were distinguishable from CMLV/WT by appearance (24hrs), complete destruction of cell sheet (48hrs) and disappearance of syncytia and comets. Cell adapted virus replication on Vero cells at 35˚C differed in appearance of CPE (24hrs), complete destruction within (48hrs), rounding of cells and syncytia. Inoculation of cell adapted strains on chick embryos induced larger (0.2-4mm), white opaque pock lesions without death. Mild local lesions were noted when rabbits were scarified by passaged viruses. Cell passaged strains displayed slight deviation in the properties of the virus.

Key words: *Camelpox* virus, Vero cells, CPE, Pock lesion, Rabbits

INTRODUCTION

Camelpox is an economically important, contagious and notifiable to Office Internationale des Epizootics skin disease of camelids (Elliot and Tuppurainen, 2008; Bhanuprakash *et al*., 2010a) *Camelpox* virus belongs to the family poxviridae, genus orthopoxvirus (Panning *et al*., 2004; Essbauer *et al*., 2010). It can be propagated in a large variety of cell cultures including Vero cells (Tantawi *et al*., 1974; Davies *et al*., 1975; Salem *et al*., 2008; Sheikh *et al*., 2009; Mahmoud *et al*., 2012; Abdoel Motalab and Ahmed, 2014). The virus was isolated on the chick embryo producing opaque white, round pock lesions (Tantawi *et al*., 1974; Munz *et al*., 1997; Khalafalla *et al*., 1998; Salem *et al*., 2008; Sheikh *et al*., 2009; Mahmoud *et al*., 2012; Abdo el Motalab and Ahmed., 2014). It is host specific and does not infect other animal species (Ramyar and Hessami, 1972; Tantawi, 1974; Davies *et al*., 1975; Alfalluji *et al*., 1979). The mechanisms leading to attenuation by was not yet understood, but its assumed that properties of Poxviridae host range, virulence and genome composition have been shown to change upon many passages in tissue culture cells (Perkus *et al*., 1991; Massung *et al*., 1993; Steven *et al*., 2001; Monath, 2005).

No data were so far available concerning properties of cell culture adapted *camelpox* virus invivo and invitro. The study aimed to search for any difference between the pathogenic and passaged strains.

MATERIALS AND METHODS

Virus strain

A pathogenic Camelpoxvirus (Khalafalla *et al*., 1998) was used in this study.

Virus propagation

The virus was serially propagated for hundred times in confluent monolayers of *Vero cells* kindly provided by the Central Veterinary Research Laboratories (CVRL), Soba. Sudan. The supernatant of each passage was harvested, examined for freedom from bacterial and fungal contaminants and preserved at 4˚C until used. The third (CMLV/WT), fifty (CMLV/50) and hundred (CMLV/ 100) passages were selected.

Clone purification

During passage the virus was clone purified at passage 30 and 90 by three times limiting dilution

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technique according to the method described by Downie and Haddock. (1952), the procedure was repeated for two more successive times.

Inoculation on Old Vero cells at 37˚C

Viral preparations (CMLV/WT, CMLV/50and CMLV/100) were inoculated onto five days old *Vero* cultures. For each passage, two flasks were used, the growth medium was discarded and each of the flasks was inoculated with 0.2ml of the virus suspension ($100TCID_{50}$). After an adsorption time of 60min at 37˚C, the monolayers were washed three successive times using PBS (pH 7.2). Then 7ml maintenance medium were added to each of the infected flasks and to a similarly treated non-inoculated flask, it were inspected daily for CPE.

Inoculation on new *Vero cells*at 35˚C

Viral preparations were inoculated onto 18hrs old cells, incubated at 35˚C treated and examined dailyfor CPE.

Chorioallantoic membrane (CAM)

Nine to eleven day-old chicken eggs were obtained from the poultry farm of Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum. A suspension (0.2ml) of each viral preparation was inoculated into 6 embryos. It were checked daily for viability and examined 6 days later.

Rabbits

Two months old rabbits were chosen and purchased from a local farm. They were reared for one month acclimatization period.

Inoculation of rabbits

Twelve rabbits were divided into four groups of three animals each, scarified on the inner part of the left thigh. Rabbits were treated as showed in Table 1, monitored daily for appearance of pox lesions.

Scab pieces were collected from skin lesions and homogenized using sterile mortars and pestles with the aid of sterile sand and normal saline. 10% of suspensions were centrifuged at 1000rpm for 10min. Supernatant fluids were collected into sterile bottles and kept in –20˚C till tested by AGDT.

Production of hyper immune Serum (HIS)

Hyper immune Serum was done following the procedure described by Davies *et al*. (1975). Two rabbits of two months old were used. One ml of infected cell culture fluid was used to inoculate rabbits through intramuscular injections. Two further doses were given 14 and 21 days later. Rabbits were bled 14days after the last dose for serum.

Agar gel diffusion test (AGDT)

Equal volume of scab suspension (20µl) and sodium deoxycolate was mixed, then 20µl of the mixture wastested against hyperimmune serum. Plates were incubated in humidified chamber at room temperature for 24-48hrsbefore the test was read.

Table 1: Treatment and dose used for Scarification of rabbits

Group	A	B	C	D
Treatment ($TCID_{50}$)	CMLV/WT $10^{5.5}$	CMLV/50 $10^{5.5}$	CMLV/100 $10^{5.5}$	GMEM -

TCID: Tissue Culture Infective Dose; GMEM: Glasgow Minimum Essential Medium

Table 2: Characteristics of CPE induced by viral passages on old *Vero* cells

CPE	CMLV/WT	CMLV/50	CMLV/100
Appearance (hours)	72	24	24
Rounding & Agglomeration of cells	+	+	+
Complete destruction (70 - 90%) (days)	9	3	2
Syncytia formation	+	-	-
Comets formation	+	-	-

Table 3: Characteristics of CPE induced by viral passages on new *Vero* cells

CPE	CMLV/WT	CMLV/50	CMLV/100
Appearance (hours)	48	24	24
Rounding& Agglomeration of cells	-	+	+
Complete destruction (70 – 90%) (days)	7	2	2
Syncytia formation	+	-	-
Comets formation	-	-	-

Table 4: Pock lesion and viability of the embryos inoculated with viral preparations

Character	CMLV/WT	CMLV/50 - CMLV/100
Appearance	White-opaque	White-opaque
Size (mm)	0.2 – 1	0.2 – 4
Death of the embryo % (6 day pi)	20	0

RESULTS

Inoculation on *Vero* cells

Direct inoculation of old *Vero* cells at 37˚C with viral passages resulted in formation of CPE started 1-3 days post inoculation (pi) (Fig 1). Inoculation of 18hrs old *Vero* cells at 35˚C monolayer with viral preparations resulted in the formation of CPE started 1-2 days pi (Fig 2). Differences in CPE were noted in Table 2-3.

Chorioallantoic membrane (CAM)

Virus inoculation on CAMS produced circular white - opaque pocks 6 days pi (Fig 3). Lesions induced by viral passages were compared (Table 3).

Inoculation of Rabbits

Rabbits scarified with CMLV/WT showed local lesions four days pi confined to the line of scarification. Mild local lesions were noted in rabbits inoculated with CMLV/50 and CMLV/100on six and eight days pi respectively, no lesions were observed in the control (Fig 4).

Lesions appeared as infiltrates of low density which later turned into scabs and fissured crusts within ten days up to 2wks. Later, crusts fallen down and healing occurred within 3 weeks pi.

AGDT

Clear lines of identity were formed when Scab suspensions were tested against HIS.

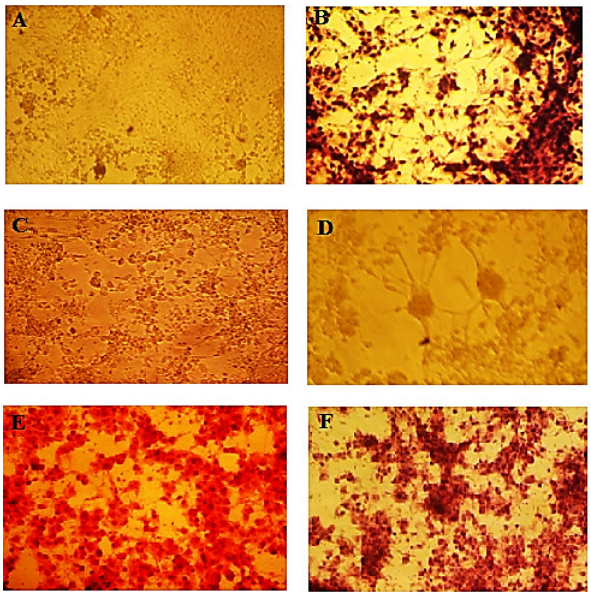

Fig. 1: Old *Vero* cells inoculated with viral passages. **A.** Focal rounding of cells and plaque formation induced by CMLV/WT (100X). **B.** Fusion of cells and plaque formation induced by CMLV/WT. H&E stain (200X). **C.** Accumulation of round cells and plaque formation induced by CMLV/WT. (100X). **D.** Plaque and giant cells induced by CMLV/WT. (200X). **E.** Focal rounding and plaque induced by CMLV/50. H&E stain (100X). **F.** Cell rounding and plaque induced by CMLV/100. H&E stain (100X).

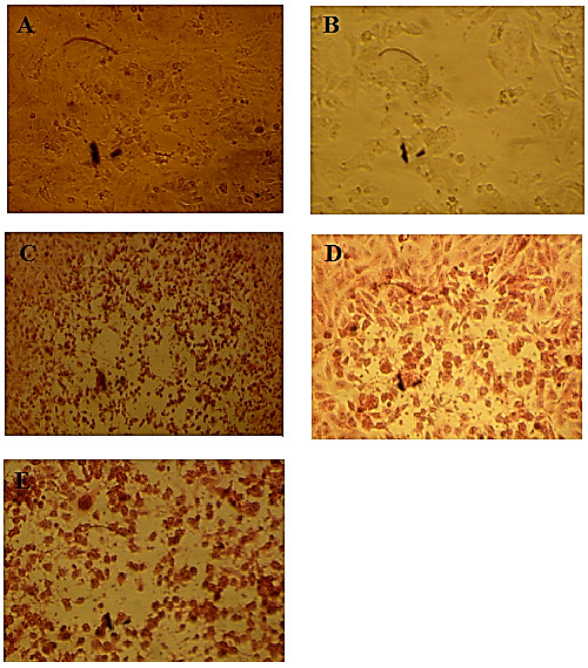

Fig 2: New *Vero* cells inoculated with viral passages. **A.** Cell rounding and plaque induced by CMLV/WT (100X). **B.** Syncytia and plaque induced by CMLV/WT (200X). **C.** Cell rounding and plaque induced by CMLV/50. H&E (100X). **D.** Accumulation of rounded cells and plaque induced by CMLV/100. H&E (200X). **E.** Cell rounding and plaque induced by CMLV/100. H&E (200X).

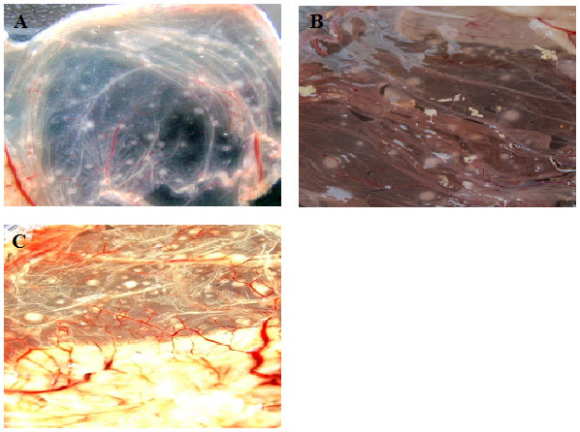

Fig. 3: Round white opaque pocks induced by **A.**CMLV/WT**B.** CMLV/50 **C.** CMLV/100.

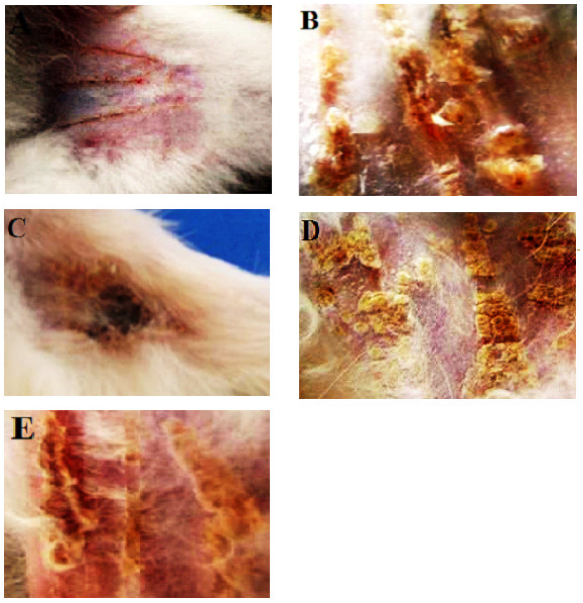

Fig 4: Inoculation of rabbits. **A**. Control rabbit seven days post scarification**, B.** Rabbit rubbed with CMLV/WT four days pi. Infiltrates on the line of scarification. **C.** Rabbit rubbed with CMLV/WT seven days post scarification. Formation of scab.**D.** Rabbit rubbed with CMLV/50 twelve days post scarification. Formation of fissured crusts.**E.** Rabbit rubbed with CMLV/100 2wks pi. Formation of localized fissured crusts.

DISCUSSION

Multiple passaging of the virus in tissue culture cells is one of the most commonly used attenuation technique (Monath, 2005). The mechanisms leading to attenuation by cell culture passages was not yet understood, but the properties of Poxviridae host range, virulence and genome composition have been shown to change upon many passages in tissue culture cells (Perkus *et al*., 1991; Massung *et al*., 1993; Monath., 2005).

In this study a pathogenic strain cultivated in old Vero cells displayed similar characteristics when compared to the data described in the literature (Baxby, 1972; Mahnel and Bartenbach, 1973; Tantawi, 1974; Marennikova *et al.,* 1974; Davies *et al*., 1975; Nguyen *et al*., 1989; Pfahler and

Munz, 1989; Renner-Muller *et al*., 1995; Munz *et al*., 1997; Salem *et al*., 2008; Sheikh *et al.*, 2009; Mahmoud *et al*., 2012; AbdoelMotalab and Ahmed, 2014).

Replication of the virus preparations invivo were assessed in CAM of 11 days old embryonated chicken eggs. The pathogenic virus induced pock lesions similar to those described by researcher (Davies *et al*., 1975; Al Falluji *et al*., 1979; Chauhan and Kaushik., 1987; Kaaden *et al.,* 1992; Salem *et al.,* 2008; Sheikh *et al*., 2009; Mahmoud *et al*., 2012; AbdoelMotalab and Ahmed, 2014).

Pathogenic camelpox virus was serially propagated for hundred passages in confluent monolayers of Vero cells. Low and high passages were compared to pathogenic strains in terms of CPE, pock lesion and ability to induce lesions in rabbit's skin. When replicating in old Vero cells at 37˚C, cell adapted strains were distinguishable from the wild type virus by appearance (24hrs), complete destruction of cell sheet (48hrs) and disappearance of syncytia and comets. Cell adapted virus replication on Vero cells at 35˚C differed in appearance of CPE (24hrs), complete destruction (48hrs), rounding of cells and syncytia. Inoculation of cell adapted strains on chick embryos induced larger (0.2-4mm), white opaque pock lesions without death. Mild local lesions were noted when rabbits were scarified by passaged strains.

No data were so far available concerning properties of cell culture adapted camelpox virus, with absence of a model sensitive animal to test the safety of the attenuated strain.

Conclusion

Vero adapted *camelpox* strains displayed slight deviation concerning biological properties of the wild virus. Observed differences may be due to genetic changes which have occurred during viral propagation in Vero cells. Sequence analysis is needed to find genetic alteration between pathogenic and attenuated strains.

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