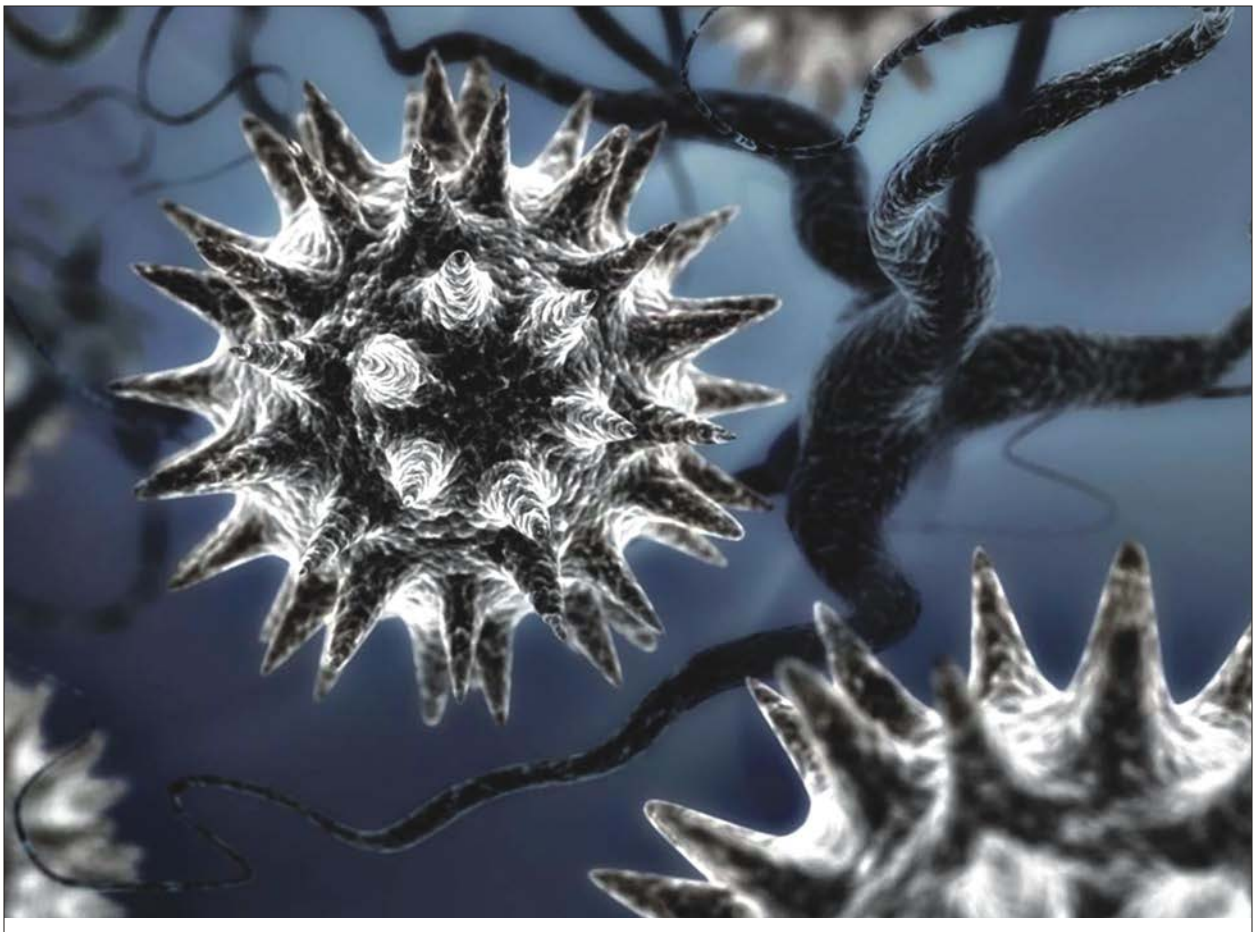

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Isolation of *Camelpox* Virus in Egypt

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ABSTRACT

In this study skin lesions were collected from camel suffered from *Camelpox* Virus (CPV) infection from different region of South Sina Governorate and Maruit camel farm of the Desert Research Center. CPV was isolated and propagated on CAM of 9-11 days SPF-ECE for 10 passages resulting in characteristic pock lesion of the CPV and the highest titer was (4.2 $\log_{10}EID_{50}$ mL^{-1}) at the 7th passage, also CPV was isolated and propagated on Vero cell line for 20 passages resulting in the characteristic CPE in the passage No. 3 after 5 days of inoculation and the virus titer increased gradually till reach (4.7 $\log_{10}TICD_{50}$ mL^{-1}) at the 17th passage. The isolated virus was identified as CPV using Virus Neutrization Test (VNT) with Neutrization Index (NI) equal to 2 and the identification confirmed by using the double antibody sandwich ELISA (DAS-Elisa) on antigen prepared from different virus passages on Vero cell line.

Key words: Skin lesions, deserts, *Comelpox* virus, vero cell line, antibody, antigen

INTRODUCTION

Camelpox (CP) is the most frequent infectious viral disease widely reported in camels in Africa and Asia Alhendi *et al.* (1994). In Egypt, *Camelpox* virus (Fayoum-71) was firstly isolated in Fayoum during 1971-1972. Tantawi *et al.* (1974a), in Sharkia Governorate (Kenawy *et al.*, 1989), in Assiut, Bni-Adi and neighboring village (El-Kom and EL-Hawkta) (Zaitoun *et al.*, 2000) and from three Egyptian governorates, Marsa Matroh, Aswan and Giza as (CPV/Aswan/07) (Salem *et al.*, 2008).

CPV could be isolated and propagated on CAM, giving typical pock lesions with a titer of $10^{5.3}$; Gabery *et al.* (1997). CPV also can be isolated and propagated on Vero cell line resulting in rounding, aggregation and plaques formation (Davies *et al.*, 1975; Abdel Baky *et al.*, 2006).

The aim of the present study is isolation and characterization of CPV in Egypt from South Sina government and Maruit camel farm of Desert Research Center by amplification and sequence analysis for the (C18L) in order to make a compression with other isolates of CPV.

MATERIALS AND METHODS

Skin lesions: A number of camels were reported to have skin lesions in Southern Sinai Governorate and Maruit camel farm of the Desert Research Center. Dried scabs were collected (at different stages of development) and kept in sterile vials contain transport medium to be used in this study. The collected scabs were ground in a sterile mortar according to Kenawy *et al.* (1989).

***Camelpox* virus:** Saudi strain of CPV (Jouf-87 strain) was kindly supplied from Pox Vaccine production and Research Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Rabbit and Hyperimmune serum against *Camelpox* virus: It was prepared at Pox Vaccine Production and Research Dept, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo using vaccinal strain of CPV (Jouf-78), according to the method described by Aboul Soud (2006).

Embroynated chicken eggs (ECE): Specific Pathogen Free (SPF) Embroynated Chicken Eggs (ECE) Fertile SPF- ECE (9-11 days old) were used for virus propagation via the Chorio-allanoic Membrane (CAM) route of inculcation it was obtained kindly from the new castle disease Department in the Veterinary Serum And Vaccine Research Institute.

African green monkey kidney cell line (vero): It was kindly supplied by Pox Vaccine production and Research department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, using Eagl's Minimum Essential Medium (MEM) with new born calf serum (10% for growth medium and 2% for maintenance medium).

Propagation and titration of the isolated *Camelpox* virus in embryonated chicken eggs (ECE): The isolated *Camelpox* virus was propagated and titrated on the CAM of SPF of 9-11 days old ECE according to Gabery *et al.* (1997).

Propagation and titeration of the isolated *Camelpox* virus in Vero cell line: Isolated *Camelpox* virus was adapted and titrated in Vero cells according to Abdel Baky *et al.* (2006).

Application of VNT test for identification for the propagated isolated CPV test in vero cell line: VNT was conducted as described by Abdel Baky *et al.* (2006) to specify the virus isolate as a *Camelpox* virus.

Application of double antibody sandwich elisa (DASE) for identification for the propagated isolated CPV: Double antibody sandwich elisa was used for identification for the antigen prepared from different passages of the isolated CPV on Vero cell line (passages No. 5, 10, 15).

RESULTS

Propagation and titration of the isolated *Camelpox* virus on CAM of ECE: Isolated *Camelpox* virus was inoculated on CAM of SPF- ECE 9-11day old for 10 successive passages resulting in the characteristic pock lesion of CPV as illustrated in Fig. 1.

The titer increased from the 3rd passage reached its beak at the 8th passage (4.2 $\log_{10}$TICD$_{50}$ mL^{-1}) and still constant after that as shown in Table 1.

Propagation and titration of the isolated *Camelpox* virus in African green monkey cells (vero cell lines): Isolated CPV inoculatd on Vero cell line for 20 passages, the CPE of the isolated CPV in different passages illustrated in Fig. (2, 3, 4, 5), the titer of the virus was increased

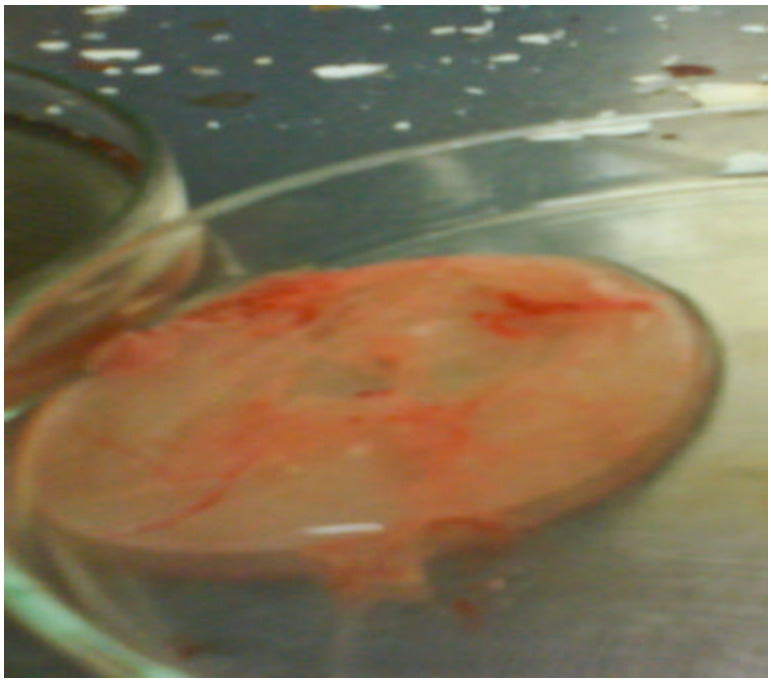

Fig. 1: Pock lesion of the inoculated isolated CPV

Table 1: Titration of the propagated isolated *Camelpox* virus on CAM of SPF-ECE

No. of passage	Titre as $\log_{10}$ $TICD_{50}$ mL^{-1}
1	-
2	-
3	3.5
4	3.5
5	3.7
6	4.0
7	4.0
8	4.2
9	4.2
10	4.2

(from 3.0 log $_{10}TCID_{50}$ mL^{-1} in the 3rd passage to highest virus titer 4.7 $\log_{10}TCID_{50}$ mL^{-1} at 17th passage) and the complete CPE of the virus appeared more earlier by the serial successive passages as shown in Table 2.

Application of virus neutralization test in vero cell line for the propagated isolated *Camelpox* virus (Alfa procedure): Vero cell line shows reduction of virus titer with $2\log_{10}TICD_{50}$ mL with neutralizing index of 2.0. For both isolated virus passages No. (5) and (10) (Table 3). Four Identification of the isolated CPV antigen by Double Antibody Sandwich ELISA (DASE).

The results were calculated on the basis of S/P ratio, the highest dilution of propagated isolated *Camelpox* virus antigen gave positive reaction was 1/40 at the passage 15, compared to the highest dilution of the Jouf-78 strain CPV antigen give positive reaction at 1/60.

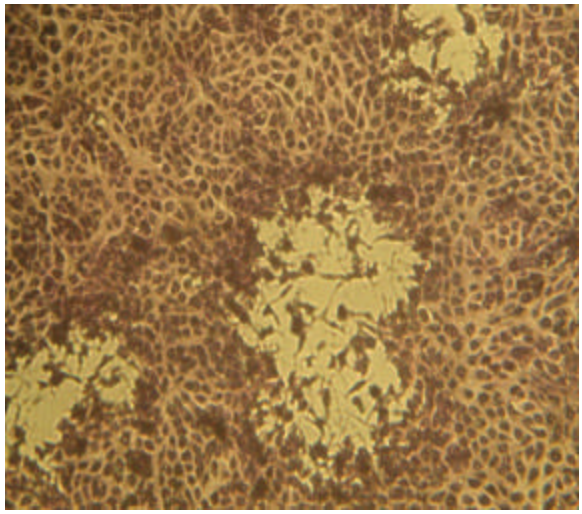

Fig. 2: CPE of isolated CPV in vero cells (low power)

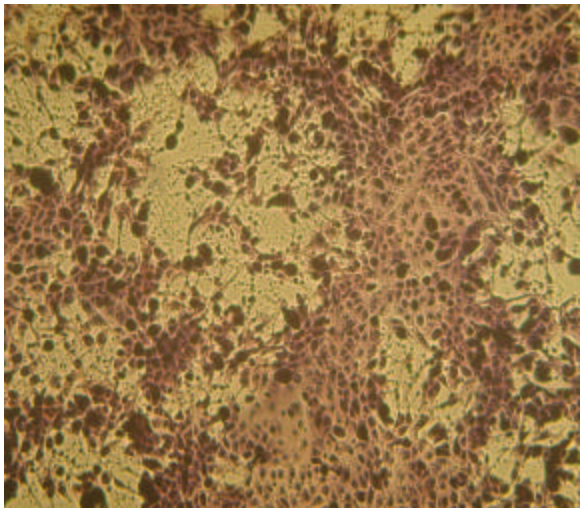

Fig. 3: CPE of isolated CPV in vero cells (low power)

DISCUSSION

CPV was isolated and propagated on CAM of SPF-ECE, the pock lesions increased in numbers and size by successive serial passages with survival of the inoculated chicken embryo post virus inoculation. It indicate the adaptation of the isolated CPV on the ECE, these results are in accordance with Alhendi *et al*. (1994) and Zaitoun *et al*. (2000).

The CPV virus titer on the ECE increased from 3rd passage till the 10th passage, the peak titter was reached by the 8th passage reached 4.2 $\log_{10}$ $TICD_{50}$ mL and then it became fixed at this level till the 10th passage. Similar result obtained by Tantawi *et al*. (1974b).

Local CPV isolate revealed characteristic CPE on the inoculated Vero cell line after three successive passages as small aggregates, formation of Grape like appearance and detachment of

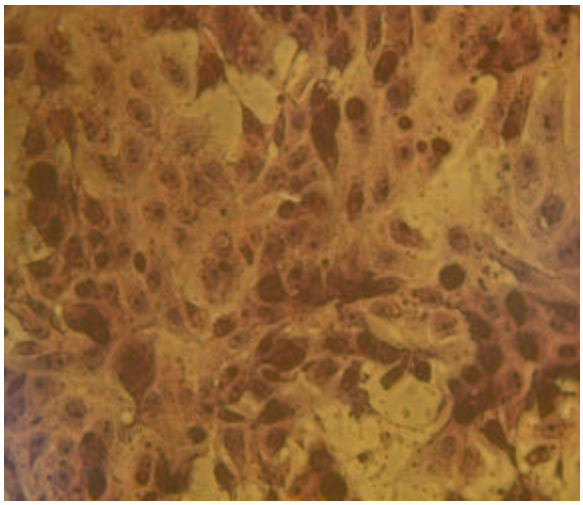

Fig. 4: CPE of isolated CPV in vero cells (high power)

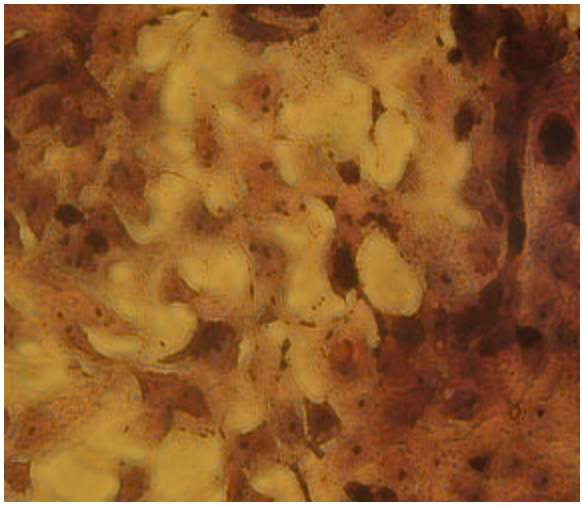

Fig. 5: CPE of isolated CPV in vero cells (high power)

some aggregates with forming of network sheet like, also Intra-cytoplasmic Inclusion Body (ICIB) were detected in intact infected Vero cells. Similar result obtained by Davies *et al.* (1975) and Abdel Baky *et al.* (2006).

The titer of the virus was increased from 3.0 $\log_{10}TCID_{50}$ mL in the 3rd passage till it reached its highest virus titer 4.7 $\log_{10}TCID_{50}$ mL at 17th passage and still constant after this passage as it indicate the complete adaptation of the isolated virus on Vero cell line similar result obtained by Abdel Baky *et al.* (2006).

Alpha procedure of neutralization test was carried on the isolated propagated virus on Vero cell line at the 5th and 10th passages showing reduction of virus titer with 2 $\log_{10}TICD_{50}$ mL with neutralizing index of 2.0 in the tow passages (No. 5th and No. 10th), showing cross reactivity between the prepared hyper immune sera against CPV (Jouf-78 strain) and the isolated CPV similar results was obtained by Abdel Baky *et al.* (2006). ELISA results reveled that antigen dilutions 1/20, 1/30 and 1/40 were the end points, respectively and in case of CPV vaccine

Table 2: Cytopathogenicity and titer of the of the isolated propagated CPV on vero cell line

	Vero cell								
Culture						Time of appearance			
No. of passages	1st day	2nd day	3rd day	4th day	5th day	CPE (h)	ICIB (d)	Harvestation time (d)	Titre
1	No CPE	No CPE	No CPE	±	±	-	-	5th	-
2	No CPE	No CPE	±	±	+	-	-	5th	-
3	No CPE	±	+	++	++	36	2nd	5th	3.0
4	No CPE	±	+	++	+++	36	2nd	5th	3.0
5	No CPE	±	+	++	+++	36	2nd	5th	3.5
6	No CPE	+	++	+++		48	2nd	4th	3.5
7	No CPE	+	++	+++		48	2nd	4th	3.5
8	No CPE	+	++	+++		48	2nd	4th	3.7
9	No CPE	+	++	+++		48	2nd	4th	3.7
10	±	+	++	+++		48	2nd	3rd	4.0
11	±	+	+++			24	1st	3rd	4.0
12	±	+	+++			24	1st	3rd	4.2
13	±	+	+++			24	1st	3rd	4.2
14	±	++	+++			24	1st	3rd	4.2
15	±	++	+++			24	1st	3rd	4.5
16	±	++	+++			24	1st	3rd	4.5
17	±	++	+++			24	1st	3rd	4.7
18	±	++	+++			24	1st	3rd	4.7
19	±	++	+++			24	1st	3rd	4.7
20	±	++	+++			24	1st	3rd	4.7

d: Day, ±: Cell rounding, highly retractile cells and ICIB, +: Small aggregates formation and foci of clear area, ++: Grape like appearance and detachment of some aggregates, +++: Network sheet like, complete CPE (70-80%) and sheet destruction (70-80%)

Table 3: S/P ratio of ELISA for the antigens obtained from propagated isolated *Camelpox* virus on vero cell line and jouf-78 strain

Dilutions	S/P ratio of CPV propagated on vero cell line passage No. 5	S/P ratio of CPV propagated on vero cell line passage No. 10	S/P ratio of CPV propagated on vero cell line passage No. 15	S/P ratio of (jouf 78- strain)
1/10	1.60	2.10	2.40	2.90
1/20	1.10	1.60	1.80	2.60
1/30	0.80	1.20	1.40	2.20
1/40	0.67	0.90	1.10	1.90
1/50	0.35	0.80	0.91	1.50
1/60	0.18	0.53	0.60	1.10
1/70	0.12	0.24	0.30	0.75
1/80	0.05	0.11	0.17	0.47
1/90	0.00	0.03	009.00	0.21
1/100	0.00	0.00	0.02	0.06

S/P ratio: Mean optical density of sample -mean optical density of negative sample mean optical density of positive sample- mean optical density of negative sample. S/P ratio>1 is considered as the positive result

(Jouf-78 strain) the end points for the antigen dilution which gave positive reactivity was 1/60, this result indicate the cross reactivity between the prepared hyper immune sera against antigen prepared from the CPV (Jouf-78 strain) and the isolated propagated CPV and these results were in accordance with the result of virus neutrization test of the isolated CPV.

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