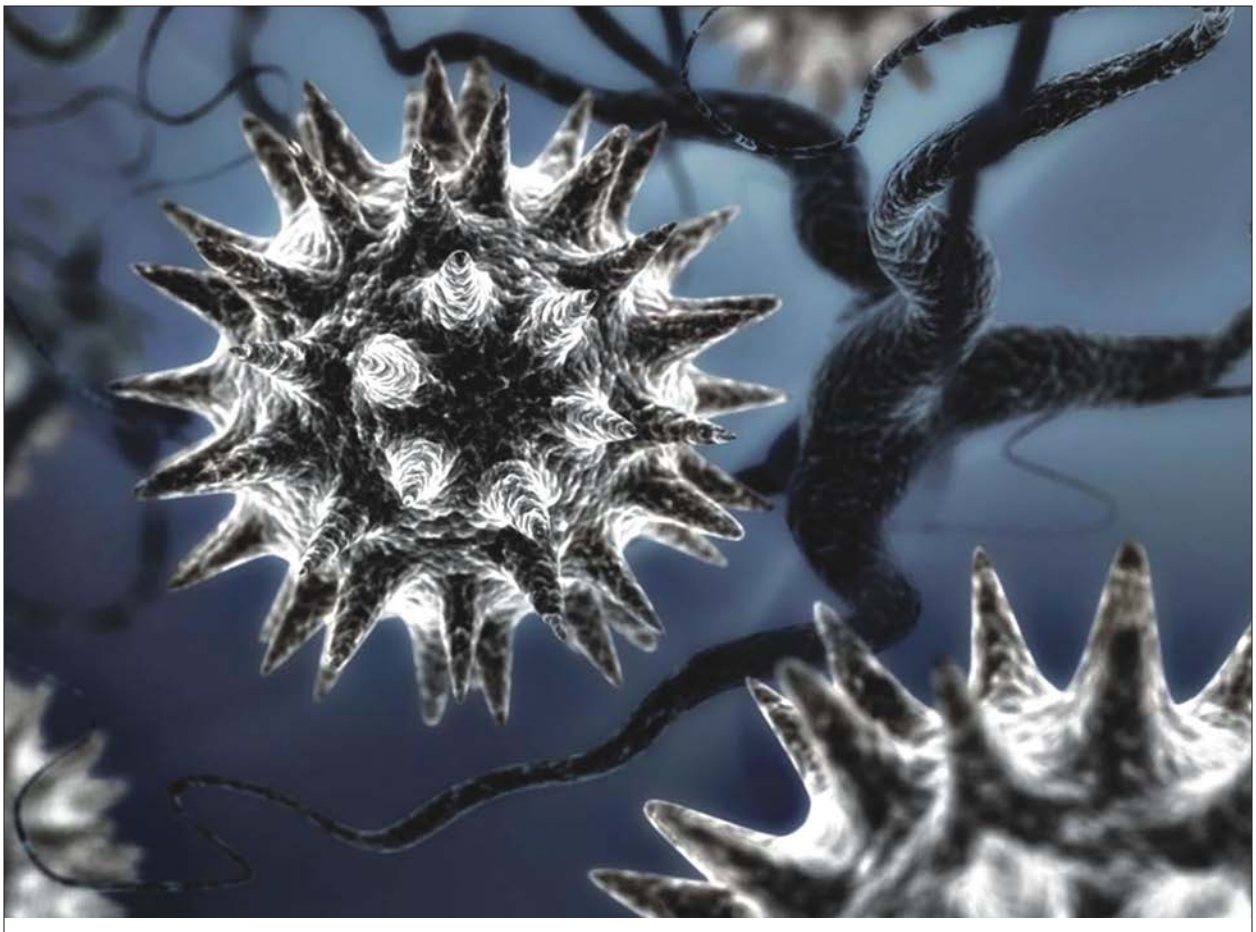

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Sequence Analysis of *Camelpox virus* Isolated in Egypt

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ABSTRACT

In this study the collected skin sample from camel suffered from *Camelpox virus* (CPV) from different regions in Egypt (South Sina Governorate and Maruite camel Farm of the Dessert Research Center) and the propagated isolated virus on Vero cell line were characterized by employing Polymerase Chain Reaction (PCR) and sequencing. The causative agent was identified as CPV, based on A-type inclusion and C18L genes-specific PCRs and partial sequencing of the C18L gene, which clearly confirmed that the outbreaks were caused by CPV. Further, phylogenic analysis of partial C18L gene of the isolated CPV and the Vaccinal strain (Jouf-78) of CPV sequence have showed that the isolated CPV clustered together with other reported isolates of CPV on contrast the vaccinal strain clustered with other *vaccinai* virus.

Key words: *Camelpox virus*, PCR, CPV genome, C18L gene, *vaccinei*, verocell line, farm, skin losions

INTRODUCTION

The CPV genome contains 211 putative genes of a central highly conserved region and identical inverted terminal repeats (Afonso *et al*., 2002). Polymerase chain reaction (PCR) based on the amplification of the "A" Type Inclusion Body Protein (ATIP) gene is a fast and sensitive method for diagnosis of CPV directly from scab material without the need for virus isolation (Meyer *et al*., 1994) also other genes can be used for amplification by PCR as heamagglutinin (HA) gene (Al-Ziabi *et al*., 2007), amplification of ankyrin repeat protein (C18L) gene, amplification of DNA polymerase (DNA pol) gene (Balamurugan *et al*., 2009) and amplification of B5R gene (Balamurugan *et al*., 2008).

Bhanuprakash *et al*. (2009) used the sequence analysis for ankyrin repeats protein gene (C18L) after gene amplification by PCR to characterize the isolates strains of CPV in India. The aim of the present study is characterization of the isolated CPV in Egypt from South Sina Government and Maruit Camel Farm of Desert Research Center by amplification for the ATIP gene and C18L gene with sequence analysis for the C18L in order to make a compression with other isolates of CPV and the vaccinal PCV strain.

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.

Isolated propagated *Camelpox virus* on Vero: The isolated propagated strain of CPV were used after virus adaptation on Vero cell line and identification by SNT and DASE using specific hyper immune sera against jouf -78 strain.

CPV DNA extraction from crust sample and propagated virus on Vero cell line: Innuprep DNA extraction mini kit (Analytic Jena AG, AJ Innuscreen GMBH) is used for DNA extraction from crust samples collected from affected camels the extraction was done according to life science unlimited manual Analytic Jena.

Polymerase chain reaction (PCR): Innuprep DNA extraction mini kit (Analytic Jena AG, AJ Innuscreen GMBH) is used for DNA extraction from crust samples, propagated isolated virus and vaccinal strain. Polymrase Chaine Reaction (PCR) assay was applied on the DNA extracted from crust samples and inoculated Vero cell line with isolated CPV according to OIE Terrestrial Manual (2008) as shown in Table 1 using ATIP gene specific primer pairs 5'-AAT-ACA-AGG-AGG-ATC-T-3' and 5'-CTT-AAC-TTT-TTC-TTT-CTC-3' for identifying the isolated virus, also PCR assay was applied on previously DNA extracted according to Balamurugan *et al.* (2009) as shown in Table 2 using the primer pair: C18L F 5'-GCG-TTA-ACG-CGA-CGT-CGT-G-3'and C18LR 5'GAT-CGG-AGA-TAT-CAT-ACT-TTA-CTT-TAG-3' for further sequence analysis and phylogenic tree constriction.

Nucleotide sequence analysis of C18L gene coding region: The purified PCR products were submitted to (Big Dye terminator Cycle Sequencing Kit, 3730 xl DNA sequence, Macrogen, Korea)

Table 1: Cycling protocol for amplification of ATIP gene

Steps	Temprature (°C)	Time (min)	No. of cycles
Initial denaturation	94	5.0	1
Denaturation	94	1.0	29
Annealing	45	1.0	
Extension	72	2.5	
Final extension	72	10.0	1
Preservation	4	∞	

Table 2: Cycling protocol for amplification of C18L gene

Steps	Temprature (°C)	Time	No. of cycles
Initial denaturation	94	3 min	1
Denaturation	94	30 sec	24
Annealing	45	30 sec	
Extension	72	30 sec	
Final extension	72	8 min	1
Preservation	4	∞	

for DNA sequencing of the C18L amplicon using florescent dye-labeled dideoxinucleotides as chain terminator. Analysis of the sequence identity, divergence and phylogenic relationship will be performed using Meg 4.0.2 program.

RESULTS

Application of polymerase chain reaction (PCR) on the isolated propagated CPV and crust sample for ATIP gene: The two tested samples showed the characteristic PCR positive bands of 881 bp size fragment of *Camelpox virus* ATIP gene as shown in Fig. 1.

Application of polymerase chain reaction (PCR) for C18L on the isolated propagated CPV, CPV crust sample and jouf-78 strain: The isolated propagated CPV and the crust showed the characteristic PCR positive bands of 243 bp size fragment of *Camelpox virus* C18L gene as shown in Fig. 2 and 3 while the vaccinal strain (jouf-78) give PCR band at 1000 bp size fragment which not characteristic for CPV as shown in Fig. 4.

Amplification for CPV vaccinal strain (jouf-78).

Multiple alignment of nucleotide and deduced amino acid sequence analysis of C18L gene of the CPV extracted from crust sample and isolated propagated CPV on Vero cell line: The result revealed that there is 100% similarity on the level of nucleotide and deduced amino acid sequence for C18L gene between the CPV extracted from crust sample, propagated isolated CPV on Vero cell line and the different CPV isolates in GeneBank sequence for the C18L of the other CPV isolates present in the gene bank as no nucleotide deletion or insertion where seen in the sequence when compared to the reported CPV isolates in the multiple alignment as shown in Fig. 5 and 6.

Phylogenic relationship for Egyptian CPV isolates to other CPV isolates in GeneBank: Cluster analysis of the Egyptian isolates (Eg/Sina/2008-Eg/Alex/2009) and other published CPV isolates in gene bank illustrated in Fig. 7.

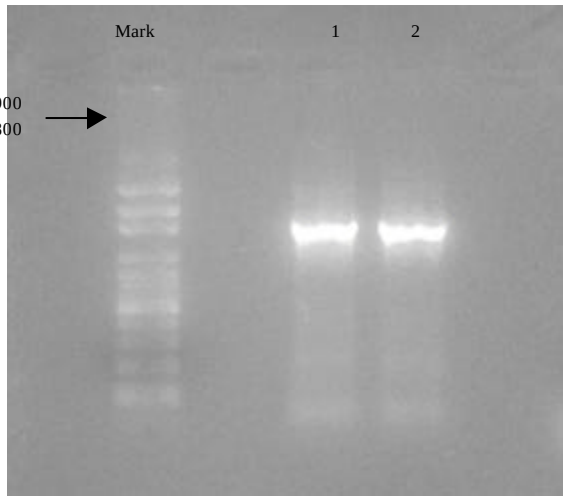

Fig. 1: Agrose gel electrophoresis of PCR product for ATIP gene, Lane 1: Isolated propagated CP and Lane 2: Crust sample

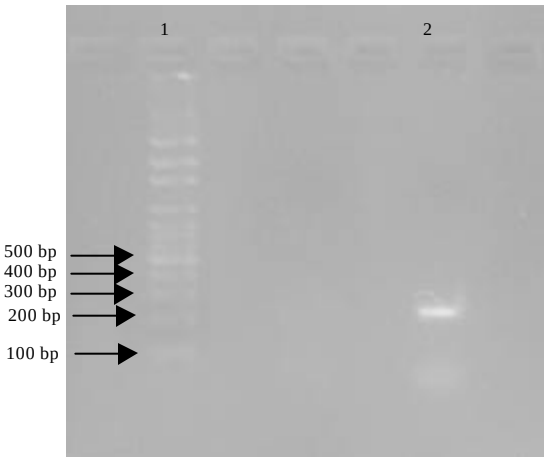

Fig. 2: Agrose gel electrophoresis of PCR product for C18L gene, Lane 1: Ladder and Lane 2: Isolated propagated CPV

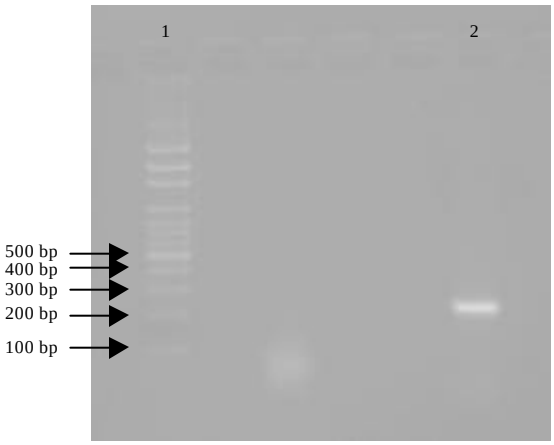

Fig. 3: Agrose gel electrophoresis of PCR product for C18L gene, Lane 1: Ladder and lane 2: Crust sample

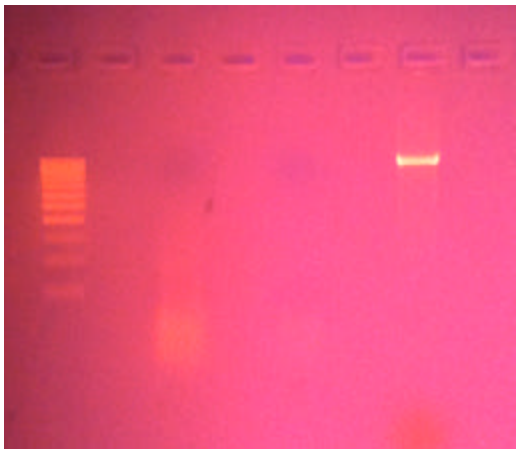

Fig. 4: PCR product of C18L am

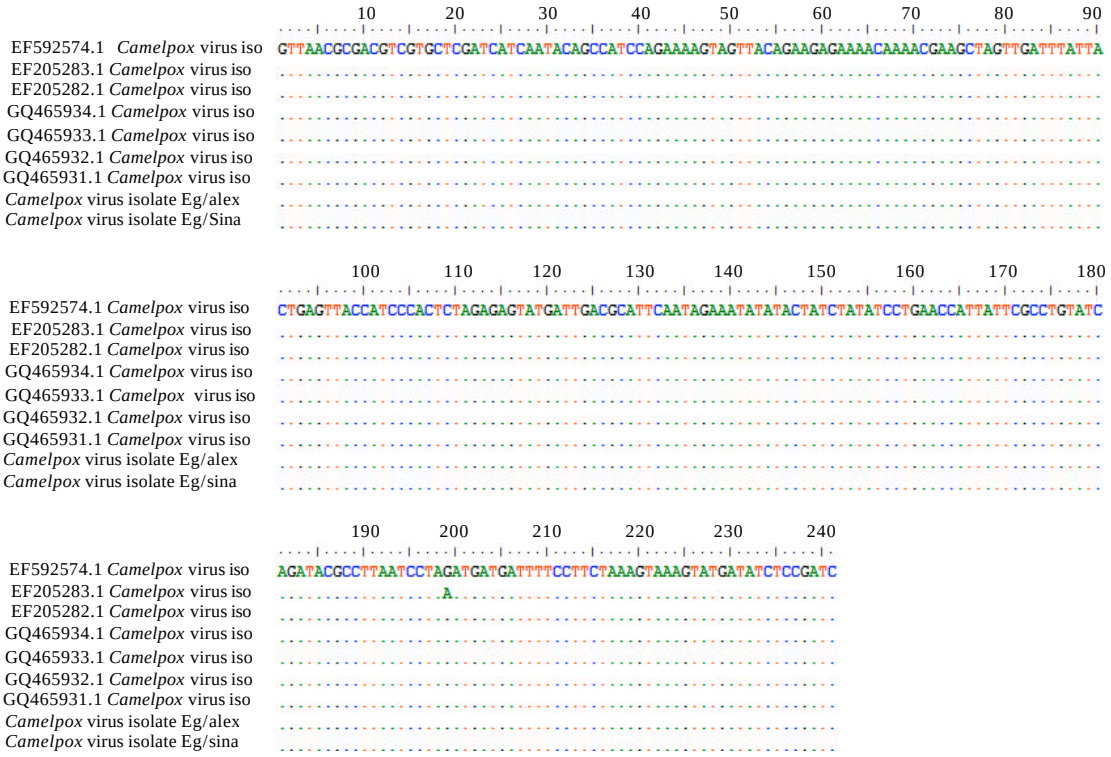

Fig. 5: Comparison of the deduced amino acid sequences of the ankyrin like (C18L) polypeptide as predicted from the nucleotide sequence of the C18L gene for isolated propagated CPV (EG/Alex/2009), crust sample (Eg/Sina/2008) and the other CPV isolates on the GeneBank

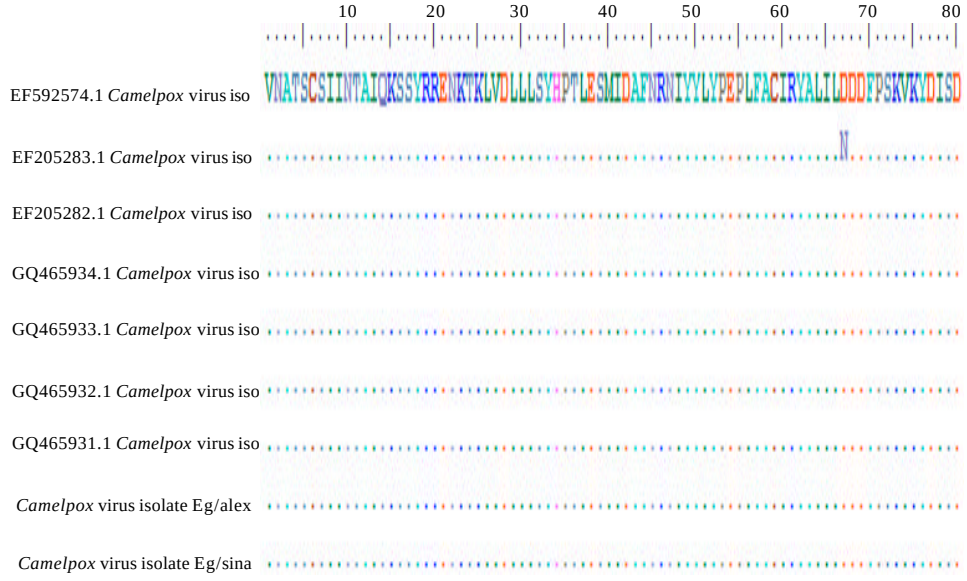

Fig. 6: Comparison of the deduced amino acid sequences of the ankyrin like (C18L) polypeptide as predicted from the nucleotide sequence of the C18L gene for isolated propagated CPV(EG/Alex/2009), crust sample (Eg/Sina/2008) and the other CPV isolates on the GeneBank

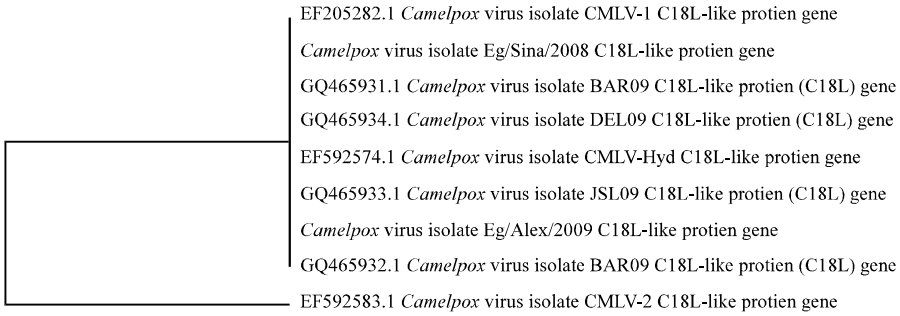

Fig. 7: Phylogenetic tree showing relationship among Egyptian CPV isolates (Eg/Sina/2008-Eg/Alex/2009) and other CPV isolates in GeneBank depending on C18L gene sequence of the viruses

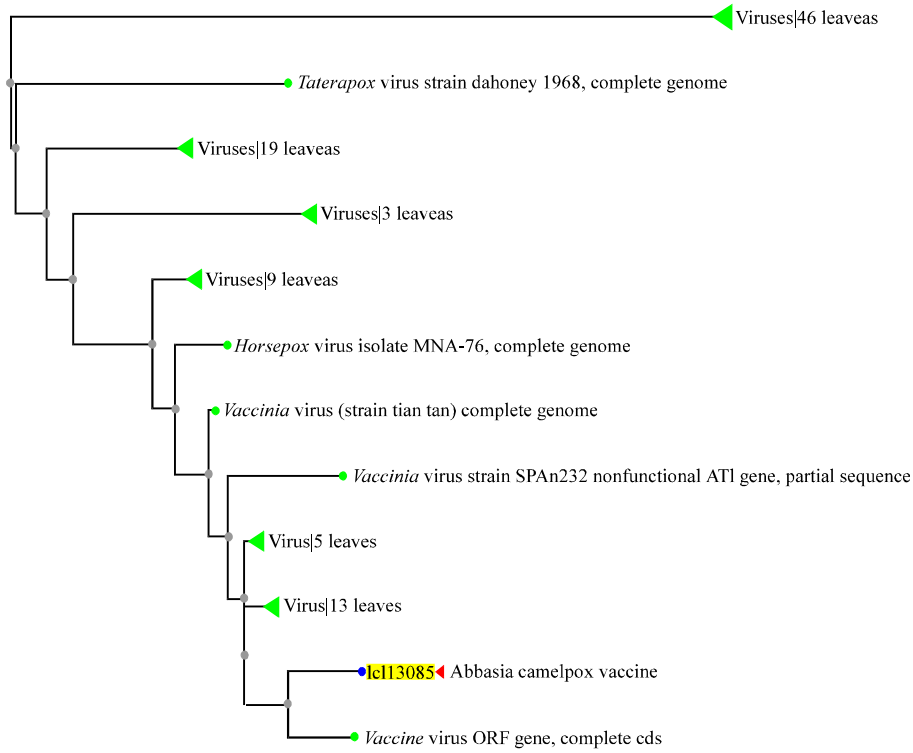

Fig. 8: Phylogenic tree showing relationship between the CPV abassia vaccinal strain (jouf-78) and the other *vaccinia* viruses

The result showed that the Egyptians CPV clustered in the same group with the other published CPV isolates.

Nucleotide sequence and phylogenitic analysis of the C18L gene of the vaccinal strain (jouf -78 strain-Abassia *Camelpox* vaccine): The nucleotide sequence of the tested sample were completely differ from all CPV isolates present in the GeneBank as the result indicate that the obtained sequence belong to *vaccinia virus*. As CPV vaccinal strain (Jouf-78) clustered with the *vaccinia virus* orf gene. As shown in Fig. 8.

DISCUSSION

Two PCR assays were done on the extracted DNA from crust samples and isolated propagated CPV on Vero cell line, the first PCR assay was for amplification of the ATIP gene and the PCR products were 881pb which is specific for CPV. These results agree with those obtained by Meyer *et al.* (1994) who amplified the ATIP gene of CPV isolated from scab material from affected camels and the size of amplified product were 881 bp. Also, similar results recorded by Gubser and Smith (2002) and OIE Terrestrial Manual (2008), while the 2nd PCR assay was for amplification of the C18l gene and the PCR products were 243 bp which is specific for CPV similar results obtained by Balamurugan *et al.* (2009) who mentioned that the amplification of the C18L gene is a fast and effective method for CPV diagnosis in compared to the usual PCR strategy which depend on the amplification of the ATIP and hemagglutinin (HA) genes. The Egyptian CPV isolates were designed as Eg/Alex/2009 for the isolated propagated CPV on Vero cell line isolated from Maruite camel farm-Desert Research Center and Eg/Sina/2008 for the CPV extracted from the crust samples obtained from South Sina Governorate.

The sequence analysis and comparison of the C18L of the tested isolated samples with other *Orthopox* (OPXV) viruses and CPV isolates showed high similarity between the Egyptian isolates with the reported isolates. Phylogenic analysis showed that CPV isolates clustered in separated group differ from other OPXV member While the vaccinal strain (jouf-78) clustered with other vaccinai viruses. similar result obtained by Bhanuprakash *et al.* (2009) who compared between different Indian isolates of CPV and other member of *Orthopox* virus using the C18L gene and found high similarity among the Indian isolates.

These study report the high genetically relationship between the CPV isolates in Egypt (Eg/Alex/2009 and Eg/Sina/2008) on the level of the C18L gene sequence analysis which considered one of the important virulence gence responsible for ankyrin like protein which is very important for the formation of viroplasma from infected cell and control the interaction between the integral membrane proteins and element of the cell cytoskeleton with the invading pox viruses as reported by Kochneva *et al.* (2005) also these study reporting the genetic deviation on the level of C18L gene sequence analysis between the isolated CPV and the used vaccine (jouf-78 strian) so these study recommended the production of local CPV vaccine from the local Egyptian strain instead of the used Jouf -78 stain vaccine which is not related genetically to CPV.

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