Comparative Phylogenetic Analysis of the Camelpox Virus Isolated in the Mangistau Region of the Republic of Kazakhstan

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Abstract: The article presents the results of phylogenetic analysis of nucleotide sequences of Camelpox virus strain M-96 isolated in the Mangistau region, Republic of Kazakhstan. The nucleotide sequences of 7 genes of twenty-six CPV strains / isolates were selected from the international GenBank database to determine the degree of M-96 strain nucleotide sequences homology with the other CMLV strains using phylogenetic analysis. As a result of the conducted comparative phylogenetic analysis we determined genetic differences and the degree of Kazakhstan strain homology with other CMLV strains, isolated at different times and in different geographical regions and the ways of possible CMLV introduction on the territory of the Republic of Kazakhstan. Kazakhstan strain M-96 of Camelpox virus showed 100% homology with five genes of CMS strain (Iran, 1970), two genes of SP-1_Iran strain and one gene of Bikaner strain, isolated in 2008 in India. The obtained data analysis suggests that Camelpox virus can be transmitted to the territory of the Republic of Kazakhstan from Iran via Turkmenistan.
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1. Introduction

Camelpox virus (CMLV) is the causative agent of pox that infects camels. CMLV epizootic outbreaks have been registered in India, Iran, Iraq, Afghanistan, Pakistan, Saudi Arabia and the north-eastern Africa, Russia, Turkmenistan and Kazakhstan (Leese, 1909, Borisovich et al, 1966, Petunin, 1958, Baxby, 1972, Al-Falluji et al, 1979, Kritz, 1982, Hafez, 1986, Bhanuprakash et al, 2010).

CMLV, along with variola (VARV), vaccinia (VACV), cowpox virus (CPXV), monkeypox virus, ectromelia virus, and taterapox virus, comprise the African–Eurasian group of orthopoxviruses (OPVs), included in a wide family Poxviridae, consisting of the viruses of humans, animals, birds and insects (Wernery et al, 2002, Afonso et al, 2002). CMLV is one of the latest studied representatives of the Orthopoxvirus genus and its DNA sequence data is very limited.

In Kazakhstan, the last outbreak was observed Camelpox in Mangistau region in 1996. Wherein got sick 830 camels and including 43 camels have fallen. Of the pathological material to isolate the CMLV. This isolate was completely investigated and the sequence of this strain incorporated in genebank (Bulatov et al, 2001, 2004, 2009, 2010, Afonso et al, 2002, Sultankulova et al, 2008). CMLV M-96 strain genome consists of the nucleotide sequence of 205719 base pairs (bp). In the nucleotide composition of the virus genome there are 66,8% of A + T bases distributed evenly along the whole genome length. Like other poxviruses, the genome of the CMLV has a central region flanked by terminal inverted replications (TIR). It's length is 7736 bp and TIR contains direct replications and coded sections. There are 27 copies of the 71 pairs of perfect nucleotide direct replicates, a 70 bp inaccurate replicate and one partial replicate, distributed between 1 and 2039 nucleotides and 197984 and 205719 nucleotides. CMLV contains 211 genes encoding proteins of 53-1869 amino acid residues having a great similarity with the previously described by poxvirus genes (Afonso et al, 2002). The DNA site of 2.9 kb size containing the open reading frame (ORF) CMLV185 to CMLV187 is absent in Orthopoxviruses and homologous B22R to similar proteins of chordopoxviruses. Conservative central region (OCR CMLV017 to CMLV184) is collinear with the OPC from C9L to B8R and has 172 genes encoding in both directions. In the terminal sites of the genome genes are mostly oriented towards terminal direction (Afonso et al, 2002).

CMLV genes CMLV146, CMLV177, CMLV201, CMLV210, CMLV211, CMLV111 CMLV099 are similar to the genes of VARV, VACV, and goatpox virus (GTPV). Gene CMV210 (№ U87837) is homologous to B28R protein of VACV Copenhagen strain and to G2R of VARV Bangladesh strain, which are responsible for virulence of poxviruses (Upton et al, 1991, Alcami et al, 1999). Gene CMLV146 (№ X75156) is similar to A27L protein of Copenhagen strain and A31L protein of Bangladesh strain. It is localized on the virion surface and plays an important role in the virus

interaction with cell. This protein is involved in the virion formation and further development of infection, and is also involved in intercellular and intracellular metabolism (Gubser, 2002, Vazouez et al, 1998). Gene CMLV099 (№ X76264) is an analog H3L protein of Copenhagen strain and I3L protein of Bangladesh strain. The references data show (Davies et al, 2005) that protective pox antibodies are produced to this particular gene. This protein is an important immunogenic unit that is responsible for creating an immune response to poxviruses (Davies et al, 2005). Gene CMLV111 (№ X97857) is similar to D8L protein of Copenhagen strain and F8L protein of Bangladesh strain (Hsiao et al, 1999). Gene CMLV177 (№ Y15035) is also homologous to A56R protein of WR-181 and Copenhagen strains and it is a type 1 membrane glycoprotein preventing cell fusion. Based on the amino acid sequence of the protein, some researchers conducted PCR diagnostics of camel pox (Saad et al, 2005). Gene CMLV211 (№ P19063) is similar to the C23L/B29R protein of Copenhagen strain and G3R protein of Bangladesh strain and H5R protein of GTPV. It inhibits chemokines and reduces the immune response of the host cells (Alcami et al, 1998). Gene CMLV201 (№ 1.22579) is homologous to the B19R protein of Copenhagen strain and B17R protein of Bangladesh strain and GTPV strains. It blocks the production of interferon in host cells and suppresses the immune response (Alcami et al, 1995).

The search for alternative methods of rapid and accurate pathogens identification, the study of diversity at the species and intraspecies levels are implemented all over the world. Phylogenetic analysis of molecular data is one of the approaches to the theoretical study of the structure and function of genetic macromolecules (RNA, DNA, and protein) and their evolutionary changes. The main purpose of phylogenetic analysis is the study of the evolutionary divergence of the genes sequences and proteins, or parts of them, as well as recovery of evolutionary events (nucleotide substitutions, deletions and insertions) in the ancestral lines of these macromolecules.

The main instrument of a phylogenetic analysis is comparing similar structures or functions of genes or proteins, and especially comparison of their primary sequences.

The aim of our study was to determine the level of homology of the nucleotide sequences of the CMLV M-96 Kazakhstan strain with the nucleotide sequences of other CMLV strains available in the GenBank database and to identify ways of possible CMLV transmission to the territory of the Republic of Kazakhstan.

2. Materials And Methods

Nucleotide sequences of the CMLV strains isolated in different years and of different geographical origin, published in the international database GenBank were used as the object of this study. Two of these strains, M-96 (205719 bp) and CMS (202205 bp), have complete nucleotide sequence and the other strains have data for the individual genes.

Nucleotide sequences of CMLV146, CMLV177, CMLV201, CMLV210, CMLV211, CMLV111 and CMLV099 genes of twenty six CMLV strains / isolates were selected from the international database GenBank for a comparative analysis. African gerbil's virus Dahomey strain was taken as a standard of comparison for appropriate assessment of evolutionary changes within the CMLV group. According to many authors conducting phylogenetic analysis of the CMLV nucleotide sequence with other strains of viruses of the family Poxviridae it was established that all strains belong to one genetic group (Orthopoxvirus), which includes the well-known strains of VARV, CMLV, CPXV, MPXV and form one branch. The closest neighbor is a group formed by strains of VARV and African gerbils (Tateropox virus) (Douglass, 1996, Babkin, 2006, Austin, 2010).

Comparative phylogenetic analysis was performed with the help of MEGA 4.1 software and method of "nearest neighbors" using M. Kimura two-parameter method, followed by 1000-fold resampling (Kimura, 1980, Tamura, 2007).

3. Results

A comparative phylogenetic analysis of the nucleotide sequence of the above mentioned genes with similar sequences of other CMLV strains was performed to identify genetic differences and determine the degree of homology of the CMLV M-96 strain. The research results are presented in Figures 1-7.

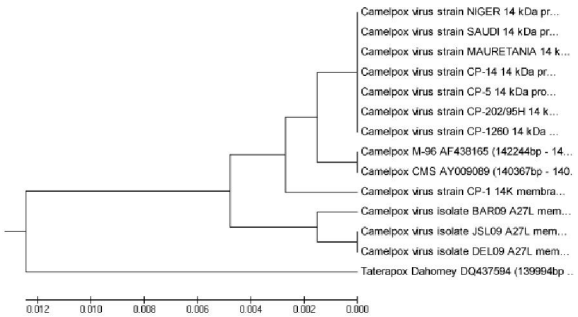

Figure 1 - Phylogenetic tree of CMLV strains constructed on the basis of CMLV146 gene analysis

From the analysis data (Fig. 1) it can be seen that according to the CMLV146 gene the studied strains and isolates form the six genetic groups corresponding to geographical locations of virus isolation. The highest percentage of affinity (100%) of the CMLV M-96 strain was with CMS strain, 99,7% was with CP-1_Iran M-96, CP-1260, CP-202/95H, Dubai-1992_CP-5, CP-14, Mauretania, Niger and Saudi-M3 strains isolated in Iran, Saudi Arabia and on the African continent and 99,4-99,1% of affinity was with JSL09, DEL09 and BAR09 strains isolated in India.

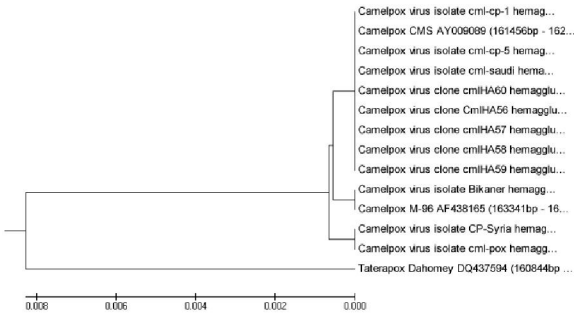

Figure 2 - Phylogenetic tree of CMLV177 gene nucleotide sequences

In the study of nucleotide sequences of CMLV177 gene of CMLV thirteen strains (Fig. 2) it was found that the investigated strains form four genetic groups on the tree diagram. M-96 strain has a great similarity to isolate Bikaner, and unlike other compared strains has 7-nucleotides deletion.

The presented data on the genetic distance show that M-96 strain has 100% homology with the isolate Bikaner and 99,9-99,8% with other CMLV strains in CMLV177 gene.

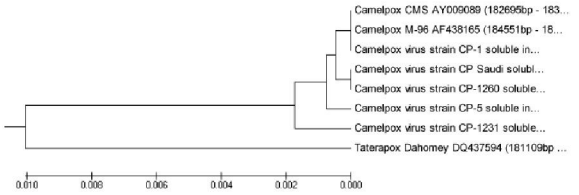

Figure 3 - Phylogenetic tree of CMLV201 gene nucleotide sequences

Comparison of the nucleotide sequence of the CMLV201 gene of M-96 strain with six CMLV strains showed that M-96 strain has 100% similarity with CMS and CP-1 strains isolated in Iran. The similarity of the strain with Saudi-M3 was 99.9%, with CP-1260 - 99,8%; Dubai-1992_CP-5 - 99.8%, CP-1231 - 99.6%.

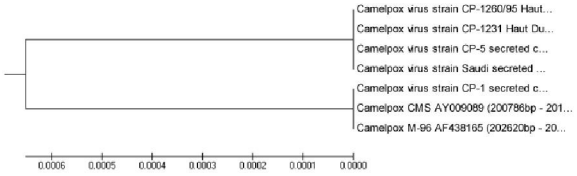

Figure 4 - Phylogenetic tree of CMLV211 gene nucleotide sequences

The conducted phylogenetic analysis of the nucleotide sequences of CMLV211 gene of M-96 strain with six CMLV strains (Figs. 4) showed that the studied isolates form two major genetic groups. The M-96 strain was included in one group together with CP-1_Iran and CMS strains with 100% identity and it had 99.9% homology with the strains CP-1260, Dubai-1992_CP-5, Saudi-M3 and CP-1231_Dubai.

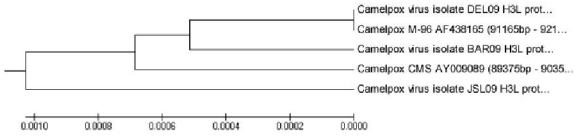

Figure 5 - Phylogenetic tree of CMLV099 gene nucleotide sequences

The data presented on pictures 9 and 10 shows that nucleotide sequence analysis of CMLV099 gene of M-96 strain with 4 CMLV strains revealed 100% homology of M-96 strain with DEL09, 99,9% with BAR09, CMS strains and 99,8% - with JSLO9 strain.

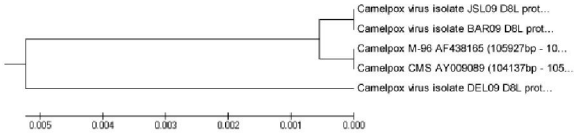

Figure 6 - Phylogenetic tree of CMLV111 gene nucleotide sequences

The M-96 strain has 100% homology with CMS strain in CMLV111 gene and DEL09 strain was significantly different from the other strains. Its homology is only 99%.

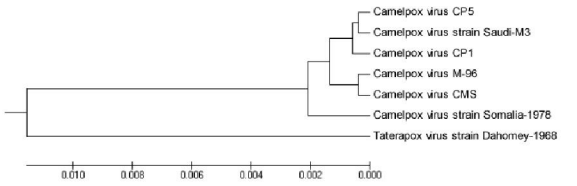

Figure 7 - Phylogenetic tree of CMLV210 gene nucleotide sequences

Results of the comparative analysis of nucleotide sequences of tumor necrosis factor gene (CMLV_TNF) of M-96 strain with other 5 CMLV strains showed that the studied strains can be divided into 5 genetic groups. CMLV CMS strain was the closest to M-96 strain. Their homology was 99,9%. At the same time M-96 strain's percentage of homology with the other strains Dubai-1992_CP-5, Saudi-M3, CP-1_Iran and Somalia-1978 was a bit lower, 99,8%, 99,7%, 99,6% respectively.

4. Discussion

Camel breeding is one of the unique agricultural industries giving leaser, fat, meat, milk and wool of high-quality. Currently, along with other agricultural sectors development of camel breeding becomes very important in farms located in desert and semi-desert areas of the Republic of Kazakhstan and which are the main sources to improve people's welfare in these regions.

Camelpox is one of the limiting factors in the development of this sector. The economic damage caused by this infection consists of the death and culling of young animals and costs of materials for eradication and control of the disease. Current lack of specific measures for the camelpox control, repeated epizooties, rapid development of this agricultural industry and its economic significance determine the relevance of this work and is a great scientific and practical interest for veterinary in the Republic of Kazakhstan.

Camelpox is registered in almost every country with camel breeding (Wernery et al, 2002, Amanscholow, 1930, Renner-Müller, 1995).

Repeated outbreaks of the diseases caused by new virulent strains of the pathogen are the basis for biological, physical and genetic characterization which is of great importance for the development of prophylactic means, identifying possible natural reservoirs of the virus and the ways of its evolutionary changes.

Usually, the infection is not transmitted to humans, but Bera et al. (Bhanuprakash et al, 2010) described three cases of the disease in humans caused by the Camelpox virus during an epizooty in India in 2009. This fact raises some concerns, as a slight change in the CMLV genome could lead to emergence of a new human pathogenic variant causing epidemic. The possibility of such event is based on a high degree of genetic identity in genes order, genome central site ORF length and amino acid composition of CMLV with the VARV (Totmenin et al, 2002, Shivaprasad et al, 2002, Jackson et al, 2005).

Study of the genetic characteristics of new CMLV virulent strains is important for determination of CMLV evolutionary changes and spread, as well as the development of effective prophylactic and diagnostic means.

The analysis of CMLV certain genes nucleotide sequences showed that M-96 strain is a typical representative of Camelpox type, as it has 99,1-100% homology with CMLV isolated in different geographical regions. CMS strain isolated in 1970 in Iran is the closest to the studied Kazakhstan strain in genes composition. Despite 100% identity of M-96 and CMS strains their complete genomes detailed comparison revealed at least 400 nucleotide substitutions in more than 20 genes, excluding terminal non-coding site.

M-96 strain has genetic differences with the CMLV strains isolated in India.

CMLV Kazakhstan strain M-96 showed 100% homology in the five genes (SMLV111_D8L, SMLV146_A27L, CMLV201_INF-alpha/beta binding protein, CMLV210_TNF and CMLV211_secreted chemokine binding) with the CMS strain (Iran, 1970), in two genes (CMLV201, CMLV211) with CP-1_Iran strain and in one SMLV177_hemagglutinin gene with Bikaner strain isolated in 2008 in India.

Phylogenetic analysis of CMLV M-96 strain isolated in Mangistau region in the Republic of Kazakhstan showed the uniqueness of the studied strain genome. Its high degree of homology with the well-known CMLV strains allows considering this strain as a candidate for the development of diagnostic and prophylactic means.

Analyzing the obtained data, it can be assumed that in 1996 CMLV can be introduced into the territory of the Republic of Kazakhstan from Iran via Turkmenistan, as five major genes of CMS strain isolated in 1970 in Iran is 100% identical to the same genes of Kazakhstan M-96 strain.

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