

## Phylogenetic tree analysis study of Lumpy skin disease virus based envelope protein P32 gene in Al-Qadisiyah Province- Iraq

Khalefa Ali Mansour

Coll. of Vet. Med./Univ. of Al-Qadisiyah

E-mail: Khalefa.mansour@qu.edu.iq

(Received 14/6/2017, Accepted 16/8/2017)

### Abstract

In this study, fifty samples from nodules of skin tissue collected from cattle were clinically supposed to be infected with lumpy skin disease (LSD) from deferent area in Al-Qadisiyah province .These specimens were provided to PCR assay. The endpoint of conventional PCR assay was sent to sequence analysis. The results revealed that thirty-nine samples out of fifty were positive to lumpy skin disease virus at 752bp PCR product of p32 gene, the sequence analysis of five positive samples were done as a confirmative diagnosis to PCR assay. The sequences of these five samples can be found under the accession numbers (KR066462.1,KR066463.1,KR066464.1,KR066465.1 and KR066466.1) at NCBI-Gen Bank submission. In conclusion; the sequence analysis of five local isolate of lumpy skin disease virus was close related to NCBI-Blast reference lumpy skin disease virus isolate (KU720359.1) and Kurdistan isolates, whereas others "Turkish and Egyptian isolates" were different.

**Key words:** LSDV, P32 gene, PCR, sequence analysis, Phylogeny.

### Introduction:

Lumpy skin disease is a viral disease that effect any age and gender of cattle with a high economic important (1,2& 3).Itis one of the economically important diseases of cattle industry caused by *capripoxvirus* within family *Poxviridae* causing nodules in the skin and internal lesion, decreased hide quality , fall in milk production and causing death in some sick animals. It was recognized as an infectious disease in 1943 when an outbreak occurred in Ngami land in the northern part of Botswana (4). Towards the end of 1944 it was reported for the first time in South Africa and spread rapidly throughout the country, despite enforced control measures(4,5). In Iraq, LSD was recorded for the first time in the autumn of 2013, and it continues to spread to all of the Iraqi governments (6).There are two form of Lumpy skin disease :acute and in apparent form characterized by high rise temperature , decrease milk production , enlargement of superficial lymph nodes, generalized distribution multiple hard, circumscribed nodules ( skin lesions) which are randomly spread in all part of the body ,in fatal cases the lesion ( necrotic plaques )may spread to the mucous membranes of the oral and upper respiratory tract (7,8,10).The etiologic agent

of lumpy skin disease, "*Neethling* strain in the genus of *Capripoxvirus* together with goat pox virus (GPV) and Sheep Poxvirus (SPPV) with in family *Poxviridae* in the subfamily *chordo-poxvirinae*(11,12).The DNA matrix of *Capripoxvirus* is consist of double strand DNA that covered large envelope protein. The length of genomic sequence of (LSDV) is nearly151-kbp.the central coding area Comprise of 156 putative genes and restricted by identical 2.4 kbp-inverted terminal recurrences .(13,14).LSD can be diagnosed upon the clinical signs. However, inapparent and mild forms require available and rapid laboratory assay to endorse diagnosis. Laboratory detection of LSD can be done using the identification of the virus using PCR. Several PCR tests were developed recently for more exact and fast detection of LSDV in appropriate specimens (15).The objective of this study was to detect emergence of LSD outbreaks from clinically suspected cattle that infected with LSD based on clinical and molecular basis. This study intends to record five strains (LSD virus – IQ1 to LSD virus –IQ5)in gene bank under submission number (KR066462.1, KR066463.1, KR066464.1, KR066465.1

And KR066466.1) and study the sequence analysis of nucleotide sequence.

## Materials and Methods:

### Samples collection:

Fifty skin biopsies of nodules were collected from different sites of body from cattle were clinically supposed to be affected by LSD from different fields in Al-Qadisiyah province. The samples were collected by incision of nodules using surgical blade and the incisions were sutured and sterilized around the area. The nodules placed in sterile container and transported to the laboratory as soon as possible in cooled boxes and store at -20°C for further investigations using PCR assay and sequencing analysis were done.

### Extraction of DNA:

DNA of LSDV can be extracted from iced specimens of the skin lesions (nodules) by (Geneaid USA). The extraction was carried out based on the company directive using tissue extraction rules using proteinase K. Checking the extracted DNA was done using Nanodrop, after that the DNA was stored at -20C.

### Amplification of DNA :

PCR test was carried out using specific primer for p32 gene in lumpy skin disease virus:

(Forward/ CGCGAAATTCAGATGTAGTTCCA) and (Reverse/ TGAGCCATCCATTTTCCA ACTC). This primers were designed according to (16). The master mix was set by (PCR Pre Mix kit. Company ,Bioneer). The premix tub of PCR

contains dried freeze pellet of (dNTPs 250µM ,Tag DNA polymerase IU, Tris-HCL (pH 9.0)10 M,MgC12 1.5 M, KCL 30mM, stabilizer, and tracking dye) and the master mix of PCR was prepared according to protocol instructions. The total volume ( 20 µl)prepared by adding 5 µl of DNA and reverse primer (1.5 µl) and forward primer (1.5 µl ),then complete the PCR water to 20 µl and mixed by vortex.

### Thermocycler conditions:

Thermocycler was used in the reaction (Techne TC-3000) by fixing up the following thermocycler conditions; the temperature of initial denaturation was 95 °C for 5 min.; tracked by 30 cycles at 95°C of denaturation for 30s, annealing at 58°C for 30s, step of extension at 72°C for 30s and the final step of extension at 72 °C for 5 min. The products of PCR was examined by gel electrophoresis in a 1.5 % agarose, stained with ethidium bromide ,and pictured under UV illumination

### DNA Sequencing

The sequencing of DNA was done to confirmative detection of the virus of (LSD) by using phylogenetic distance tree analysis depend on envelope protein p32 gene. The product of PCR(five positive samples) was sent to Korea( Bioneer Company) for performed the sequencing of DNA which done using AB-DNA sequencing system based on gpB forward primer. The DNA Sequencing method was done by Phylogenetic analysis by using Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version) with Unweight Pair Group method.

## Result

The result of conventional PCR assay revealed that thirty nine samples out of fifty were positive to lumpy skin disease at 752bp of p32 gene as shown in fig.1. The sequence analysis of five positive samples was done as a confirmative diagnosis to PCR assay. The nucleotides sequence set was examined and confirmed using NCBI- BLAST, analysis was done depending on the nucleotide query program online .The accession numbers of

five positive samples (at NCBI-Gen Bank) (KR066462.1,KR066463.1,KR066464.1,KR066465.1 AND KR066466.1).The phylogenetic tree showed that The local Iraqi LSD virus (IQ1 to -IQ5) was related to NCBI-Blast reference isolate (KU720359.1), while other NCBI-Blast(Sheep and goat pox virus) were different and out of tree as in Fig.(2).

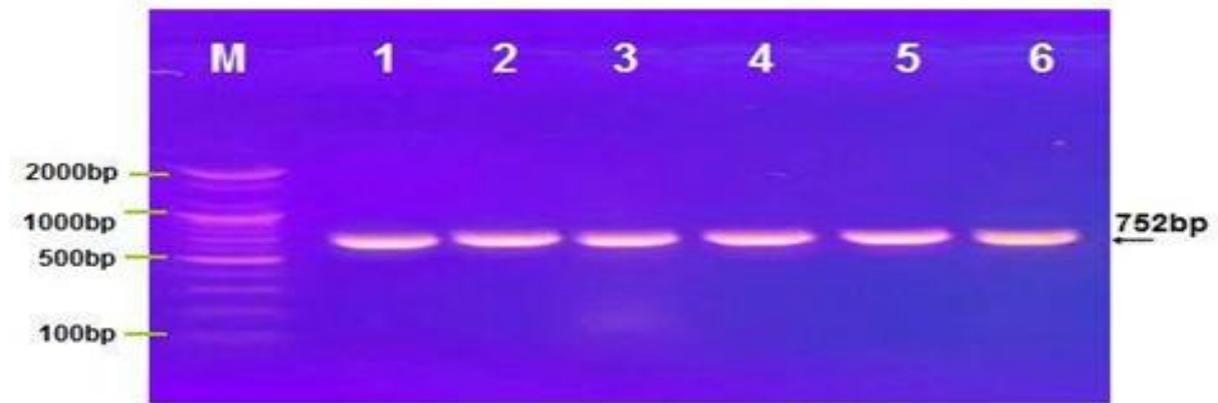


Fig. (1): Electrophoresis picture showing the product of PCR analysis of P32 gene in LSD. (M): marker (2000-100bp), (lane, 1-6) positive samples at 752bp.

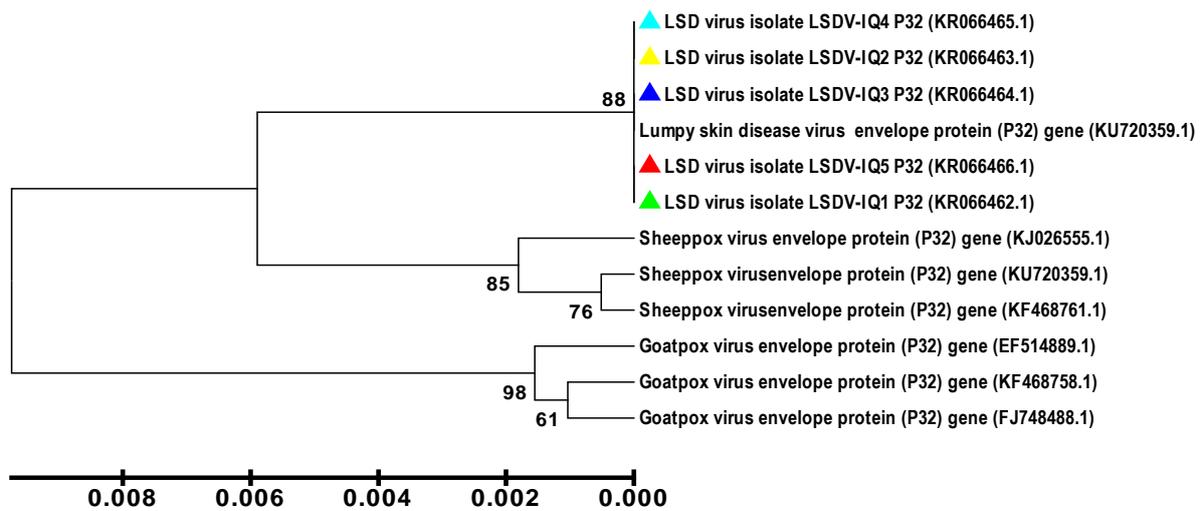


Fig. (2): Phylogenetic tree analysis showed the local LSD virus (IQ1–IQ5) isolates were close related to NCBI-Blast reference isolates (KU720359.1), Whereas other NCBI-Blast Sheep and goat pox virus were different and out of tree.

On the other hand, the sequence blast analysis revealed that the LSD virus was close related to Kurdistan isolates, whereas other NCBI-Blast LSD virus of Turkish and Egyptian isolates were different and out of tree as showed in figure. (3).

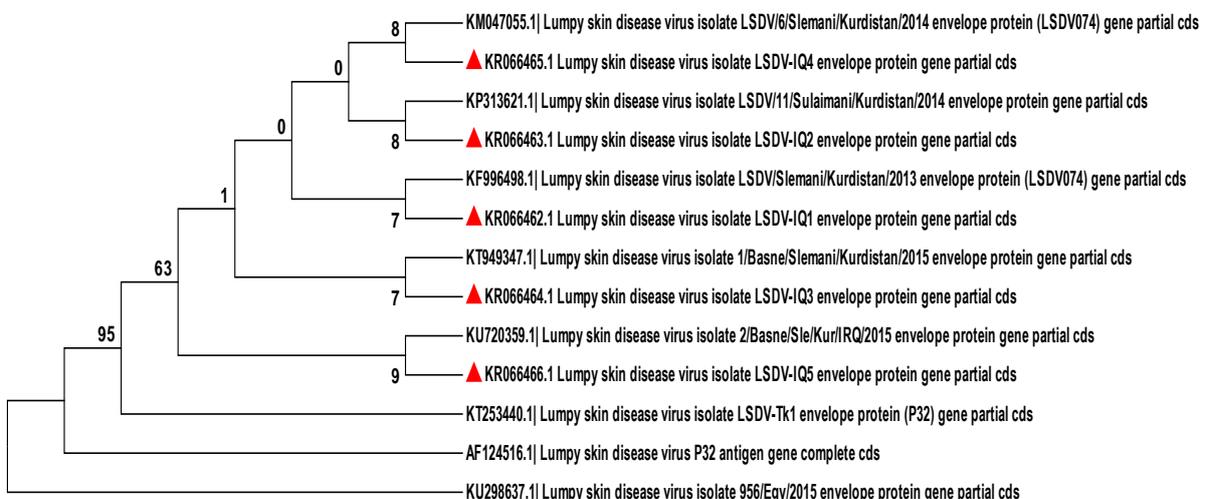


Fig. (3): The tree analysis of strain from Iraq versus the reference strains obtained from Gen Bank. Kurdistan isolates was close related with them. Whereas other NCBI-Blast viruses (Turkish and Egyptian) were different and out of tree.

According to the NCBI-Blast sequence alignment, all the local LSD (IQ1–IQ5) have a high identity (100%) with other isolates in Iraq (Kurdistan, Egypt) isolate is

(100%), whereas the percentage of identity between LSDV Iraq isolate and the Sheep & goat pox virus is 98% as in figure. (4).

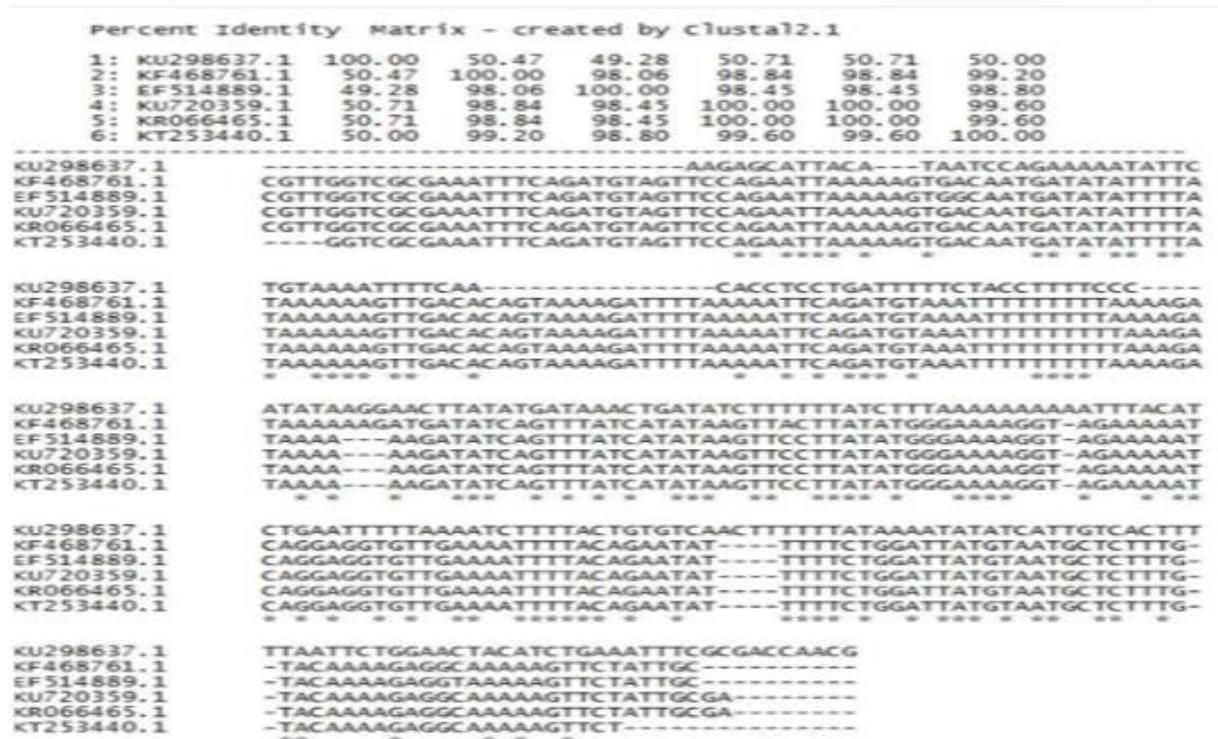


Fig. (4) Alignment of nucleotide sequence of LSDV isolates from Iraq versus other linked *capripoxvirus*, created from the most matching nucleotide sequences encoding from the viral attachment protein (P32 gene) of the analyzed viral genomes, using online program (CLUSTAL W) . (Stars) Indicate that nucleotides, which found in column, are matching in sequences in the alignment.

### Discussion

The lumpy skin disease was first recorded as an outbreak in Iraq during 2013(5). In the present study we used PCR technique to detect the DNA of the Nettling strain of *capripoxvirus* at 752 bp that obtained from nodules (skin biopsies) which taken from cattle that clinically suspected with the infection. These results agreed with (15,16) who has explained that PCR was considered as the test of choice in the diagnosis of the causative agent of LSD. The DNA sequence samples were carried out to sequence trimming tool to remove the non-identical nucleotide sequence by align with NCBI blast LSD isolate to get final trimming sequence (260-261) So that ours submission is 260-261 and that different from original product. According to the NCBI-Blast; sequence alignment revealed that the local LSD virus (LSD virus –IQ1 to LSD virus –IQ5) has a high identity (100%) with other

isolates circulating in Iraq Kurdistan. Whereas the percentage of identity between IQ LSD and the GPV & SPV was 98%. This result justifies with other publications had done by (19,20,21 and 22) and coincides with the concept that the all *capripoxviruses* are originated from the same ancestor lineage and genetically closely related. In this work the distance of the phylogenetic tree analysis showed that the Iraqi isolate (IQ1 LSD) was clustered in lineage with NCBI-Blast reference isolate (KU720359.1) and in the other sub cluster the IQ1 LSD virus was substituted as compares to other sheep poxvirus (KJ026555, KU172035 and KF468761) and goat poxvirus (EF514889, KF468758 and FJ74888). This finding indicated that LSD and (GPV, SPV) had a close genetic relationship and it is similar to a phylogenetic analysis done by

(23and25). Other phylogenetic tree sequence analysis of LSDV of Iraq isolate versus reference strains of LSD virus isolate obtained from Gen Bank (KU720359.1) and Kurdistan isolates had showed close related with each other. In the same tree, other NCBI-Blast LSD virus envelope protein (P32) gene of Turkish and Egyptian isolates were different and out of tree, this may justify that the source of infection has moved from neighboring countries and this result agreed with(25,26,27 and28). In conclusion;

the LSDV that caused disease in Iraq had highly related with other *capripoxviruses* (Sheep and goat poxvirus); and LSDV appear close branch distances in the phylogenetic tree and high nucleotide sequence identity. This agrees with the theory that the disease may be transited from Kurdistan to the free disease area in the middle of Iraq .Methods of DNA extraction from skin lesion and using conventional PCR technique applied in this study has reliable laboratory diagnostic tool for LSDV.

## References:

- 1-Fenner F. Poxviruses. In B.N. Fields, D.M. Knipe, and P.M. Howley (ed.), *Fields virology*. Lippincott-Raven, Philadelphia, Pa, (1996); 2673-2702.
- 2-Esposito JJ, Fenner F. Poxviruses, In: *Fields Virology*. Field, B.N., D.M. Knipe, P.M. Howley, R.M. Chanock and J.L. Melnick et al., (Eds.). Lippincott, Williams and Wilkins, Philadelphia, Pa. (2001).
- 3-Davies FG. LSD- An African Capripox virus disease of cattle. *Br. Vet. J.*, (1991);147: 489-503.
- 4-JAW Coetzer, Dr. EevaTuppurainen. *Lumpy skin disease*. Oxford University Press Southern Africa, (2004); 2:1268-1276.
- 5-Tuppurainen E, Oura C. Review: Lumpy skin disease: An emerging threat to Europe, the Middle East and Asia. *Trans-boundary and Emerging Diseases*, (2012); 59, 40-48.
- 6-Al-Salihi K, Hassan I. Lumpy skin disease in Iraq: Study of the disease emergence. *Transboundary and Emerging Diseases*, ( 2015); 62 , 457-462.
- 7-Prozesky L, Barnard BJH. A study of the pathology of LSD in cattle. *Onderstepoort. J. Vet. Res*, (1982); 49: 167-175.
- 8-Hamoda FK, Aboul-Soud EA, Magda MS, Shahein MA, Michael A, Daoud AM. Field and laboratory studies on lumpy skin disease. *J. Egypt. Vet. Med. Ass.*, (2002); 625: 183-199.
- 9-Zangana, I, M Abdullah. Epidemiological, clinical and histopathological studies of lamb and kid pox in Duhok, Iraq. *Bulgarian Journal of Veterinary Medicine*,(2013).16,133-138.
- 10-Buller RM, Arif BM, Black DN, Dumbell KR, Esposito JJ, Lefkowitz EJ, McFadden G, Moss B, Mercer AA, Moyer RW, Skinner MA, Tripathy DN. Poxviridae. In: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), *Virus Taxonomy: Eight Report of the International Committee on the Taxonomy of Viruses*. Elsevier Academic Press, Oxford, (2005); pp. 117–133.
- 11-Fauquet CM, MA Mayo, J Maniloff, U Desselberger, LA Ball. *Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses*, Academic Press. (2005).
- 12-El-Kenawy AA, El-Tholoth MS. Isolation and identification of lumpy skin disease virus from cattle on chorioallantoic membranes (CAMs) of fertile eggs. *Proceedings of the 6<sup>th</sup> International Science Conference*, April, 22-23, Mansoura, (2009); PP: 115-133.
- 13-Rashid PMA, Baba Sheikh MO, Raheem ZH, Marouf AS. Molecular characterization of lumpy skin disease virus and sheeppox virus based on P32 gene. *Bulg. J. Vet. Med.* (online first). (2016).
- 14-Heine HG, Stevens MP, Foord A, Jand Boyle DB. A capripoxvirus detection PCR and antibody ELISA based on the major antigen P32, homologue of the vaccinia virus H3L gene. *Journal of Immunological Methods*, (1999); 227: 187-196.
- 15-Khalefa AM, Hassan HN, Asaad j. Molecular and Clinical investigation of lumpy skin disease in cattle in AL .Qadisiyya province. *Kufa Journal For Veterinary Medical Sciences*. (2015); 6 :8-14.
- 16-El-Bagoury GF, El-Nahas EM, El-Habbaa AS, Abdel-Nour ET. Isolation and Molecular Characterization of Lumpy Skin Disease Virus from Kalubia Governorate. *Benha Vet. Med. J.*, Vol. 20, No.2. (2009).
- 17-IRELAND DC, BINEPAL YS. Improved detection ofcapripoxvirus in biopsy samples by PCR. *Journal of VirologicalMethods*, (1998); 74:1-7.
- 18-Tulman E, Afonso C, Lu Z, Zsak L, Sur JH, Sandybaev N, Kerembekova U, Zaitsev V, Kutish G, Rock D. The genomes of sheeppox and goatpox viruses. *Journal of Virology*, (2002);76, 6054-6061.
- 19-Beard P, Sugar S, Bazarragchaa E, Gerelmaa U, Tserendorj S, Tuppurainen E, Sodnomdarjaa R. A description of two outbreaks of capripoxvirus disease in Mongolia. *Veterinary Microbiology*, (2010); 142, 427-431.
- 20-Orlova ES, Shcherbakov AV, Diev VI, et al. Differentiation of capripoxvirus species and strains by polymerase chain reaction.. *Molecular Biology*, (2006); 40 (1): 158-164.
- 21-Lamien CE, Goff CLe, Silber R, Wallace DB, Gulyaz V, E Tuppurainen, H Madani, P Caufour, T Adam, M El Harrak, AG Luckins, E Albina, Diallo A. Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and

- genotyping target: development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus. *Vet Microbiol*, (2011); 21, 149: 30-9.
- 22-Zhu X, Yang F, Li H, Dou Y, Meng X, H. Li, X. Luo & X. Cai. Identification and phylogenetic analysis of a sheep pox virus isolated from the Ningxia Hui Autonomous Region of China. *Genetics and Molecular Research*, (2013);12, 1670-1678.
- 23-Fulzele S, RK Singh, M Hosamani, B Mondal, MP Yadav. Evaluation of duplex PCR and PCR-RFLP for diagnosis of sheep pox and goat pox. *International Journal of Tropical Medicine*, (2006);1,66-70.
- 24-El-Nahas EM, El-Habbaa AS, El-bagoury GF, Mervat EI Radwan. Isolation and Identification of Lumpy Skin Disease Virus from Naturally Infected Buffaloes at Kaluobia, Egypt. *Global Veterinaria*. (2011);7 (3): 234-237,
- 25-Stram Y, Kuznetzova L, Friedgut O, Gelman B, Yadin H, Rubinstein-Guini M. The use of lumpy skin disease virus genome termini for detection and phylogenetic analysis. *Journal of Virological Methods*. (2008); 151: 225-229.
- 26-Black DN, Hammond JM, Kitching RP. Genomic relationship between capripoxviruses. *Virus Res.*, (1986). 5:277-292.
- 27-Tulman ER, Afonso CL, Lu Z, Zsak L, Kutish GF, Rock DL. Genome of Lumpy Skin Disease Virus. *Journal of Virology*, Vol. (2011); 75 (15): 7122-7130.