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Molecular Characterization of Lumpy Skin Disease Virus in Comparison with Sheep Pox Vaccine.

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ABSTRACT

Fifty samples [scabs] were collected from cows suspected with lumpy skin disease virus in different areas of Iraq. The extractednucleic acid was amplified using Real-time PCR technique by amplification of attachment gene. Seven samples were positive out of fifty samples by using Real-time PCR. Sheeppox virus (vaccinal strain) was obtained from AL-Kindy company for production of veterinary vaccines and drugs. The extracted nucleic acid was amplified by Real-time PCR by amplification of attachment gene. The positive extracted nucleic acid from both viruses (lumpy skin disease and sheeppox virus)were amplified by using conventional PCR by amplification of P32 gene. The product of amplification of both viruses was sent by Iraqi Company for Biotechnology to Korea for sequencing. As a result of sequencing, the origin of Iraqi lumpy skin disease virus was similar to LSD strain [ksgp0] in Kenya. The results of sequencing confirmed a similarity between the lumpy skin disease virus and sheeppox vaccine up to 97-98%.

Keywords: Sheeppox virus PCR skin disease Iraq

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INTRODUCTION

Lumpy skin disease is a contagious disease caused by a virus in the family Poxviridae and genus Capripoxvirus[1]. The virus is one of the largest viruses, the brick shaped 170 to 260 by 300 to 450-nm-diameter capsid contains a linear, nonsegmented, double-stranded DNA genome of approximately 150 kilobases that is surrounded by a layer of lipid that is not a true envelope [2,3,4]. It mainly affects cattle and zebus, but has also been seen in giraffes, African buffalo, and impalas [5].Two to 5 weeks postinfection cattle will develop a fever [which can last 2 to 4 weeks], followed by the rash of painful nodules that cover the face and abdomen, eventually become necrotic then scab over, depression lack of appetite, swollen glands, salivation, nasal discharge, lameness due to tendon swelling, temporary and permanent sterility in bulls, and abortion of fetuses in pregnant females[6]. Mortality is usually low [1 to 3%] and the disease usually resolves in 2 to 6 months although nodules may be present for 1 to 2 years [4,6,7].
The disease is economically important in Africa due to the long recovery period and decrease in fecundity. Vaccines are available [3].Two vaccines are available, one a live attenuated version of the Neethling virus, the other a live attenuated version of the sheeppox virus [8].

The objective of this study is molecular detection and sequencing of Lumpy skin disease virus in compare with sheeppox vaccine, to elucidate the protective role of sheeppox vaccine against outbreaks of Lumpy skin disease virus in Iraq.

MATERIAL AND METHODS:

Collection of samples:

Fifty [50] samples [scabs] were collected from cows with suspected lumpy skin disease from different areas of Iraq

1. Eleven samples were collected from Al-Mothana governorate.
2. Ten samples were collected from Al-Hashemaiacity.
3. Four sampleswere collected from Abo-ghreb.
4. Five sampleswere collected from Al-Taje.
5. Seven samples were collected from Al-Soaera.
6. One sample was collected from Al-Dora.
7. One sample was collected from Al-Rrashedia.
8. One sample was collected from Dyala.
9. Ten samples were collected from Al-Yusufiyah.

Sheeppox vaccine was obtained by al-Kindy Co. for production of vaccine and veterinary drugs.

DNA Extraction kit:

The kit was used for DNA purification from scabesproduced by QIAmp DNA MINI (Qiagen).

Real time PCR kit:

This amplification kit has been manufactured by Genekam Biotechnology AG, Germany to detect Capripox / Goatpox virus (Lumpy skin disease).

Primers:

Primers for lumpy skin disease virus sequences within the gene for viral attachment protein

LSDV-F: AAATTATATACGTAAATAAC
LSDV-R: ATAGTAAGAAAAATCAGGAAA

Primers sets were specific for the P32 gene of sheeppox virus

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forward primer: 5'-GCGGATCCTTTCTACAGGCT- 3'
reverse primer: 5'-GCGGATCCACTATATATACGT-3'

Methods:

preparation of samples:

The frozen samples were removed out deep freezer and were let it thaw at room temperature, the sample was ground in mortar and pestle with the help of small quality of sterile sand and PBS,then filtered withMillipore filter 0.22*Mm.*

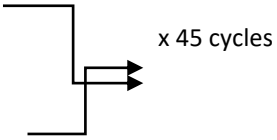
Extraction of nucleic acid from collected samples: The kit was used for DNA purification from scabes as instruction of Manufacture Company.

Detection of extracted nucleic acid by real-time PCR.

Real-time PCR was performed according to imstructions of manufacturer company as a followings:

1-Denaturation step at 95°C for 15 seconds

2-Annealing / Extension step at 57°C for 60 seconds



Extraction of nucleic acid from sheeppox vaccine: The kit was used for DNA purification from **sheeppox virus** as instruction of Manufacture Company.

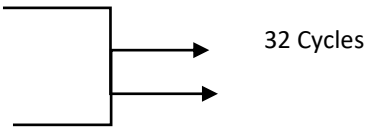
Detection of extracted nucleic acid of sheeppox vaccine by real-time PCR.

Conventional PCR was used for positive samples of lumpy skin disease and sheeppox vaccine.

The conventional PCR was conducted as the followings:

PCR condition:

Initial Denaturation	3min.	94°C
Denaturation	20sec.	94°C
Annealing [Gradient]	0.30 min.	40 to 43°C
Extention	0.30 min.	72°C
Final Extension	10min.	72°C



Sequencing of lumpy skin disease virus in compare with sheeppox vaccine:

The product of amplification of both viruses [lumpy skin disease virus and sheeppox virus] by conventional PCR technique was sent by [Iraqi company for biotechnology] to Korea for sequencing.

RESULTS

Results of amplification of extracted nucleic acid by Real- Time polymerase chain reaction for collected samples

Seven samples out of 50 samples were positive by real-time PCR and one sample was suspected as the followings:

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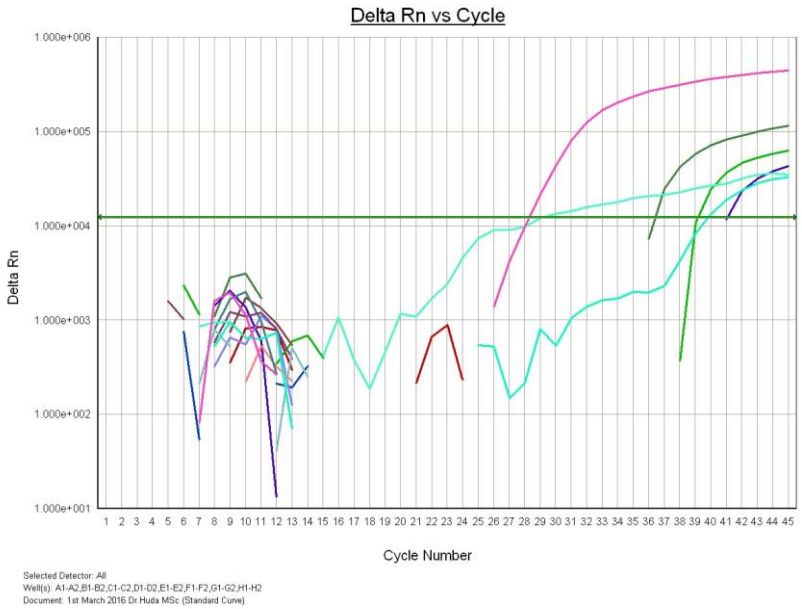

Figure 1: plot of real-time amplification of attachment gene of extracted nucleic acid from samples with suspected lumpy skin disease virus

Control +ve
Sample from Al-Hashemia +ve
Sample from Al-Muthana +ve
Sample from Al-Muthana +ve
Sample from Al-Muthana+ve
Sample from Al-Muthana Suspected

Results of amplification of extracted nucleic acid by real –time PCR for sheeppox vaccine as the followings:

Three samples of lumpy skin disease virus were positive by real-time PCR and extracted nucleic acid of sheeppox vaccine.

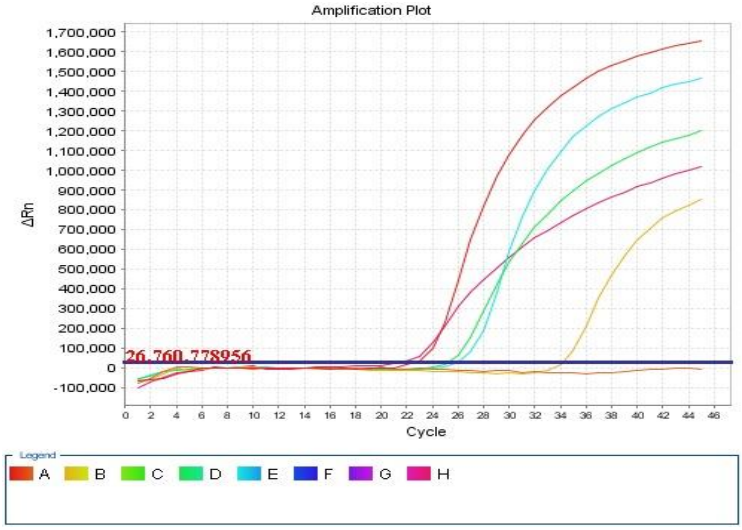

Figure 2: plot of real-time amplification of attachment gene of extracted nucleic acid from samples with suspected lumpy skin disease virus

Control +Ve

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Sheep pox vaccine
Sample from Baghdad aldora
Sample from Al rashedia
Sample from Dyala

Results of conventional PCR for detection of lumpy skin disease virus and sheeppox vaccine

electrophoreses for detection of extracted nucleic acid of sheeppox vaccine and lumpy skin disease virus

The gel electrophoreses reveled 2 bands ofextracted nucleic acidofsheeppox vaccine and lumpy skin disease virus

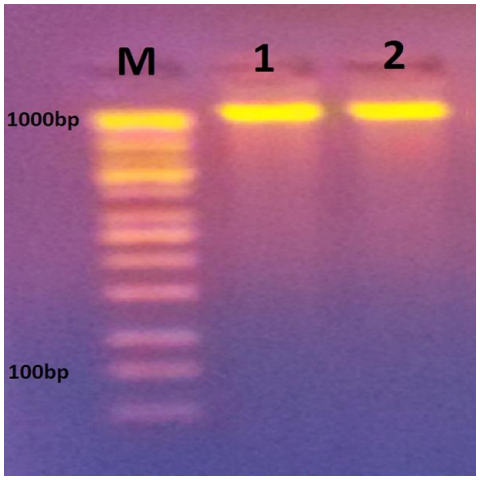

Figure 3 :Agarose gel electrophoresis pattern shows DNA extraction of sheep pox vaccine and lumpy skin disease virus

[M: ladder DNA kappa[1kb
1: Sample of sheep pox virus
2: Sample of lumpy skin virus

Results of amplification of extracted nucleic acid ofsheeppox vaccine and lumpy skin disease virus by conventional PCR

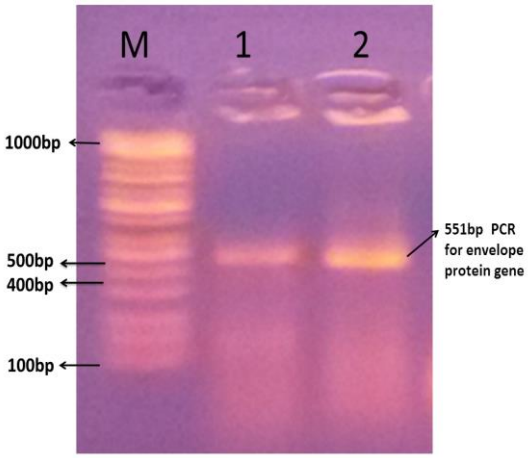

Figure 4: Agarose gel electrophoresis pattern shows PCR amplification of (551bp) for Envelope protein gene of Romanian strain of sheep pox vaccine and Iraqi lumpy skin disease virus

M: ladder DNA kappa 1kb

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1: PCR product of sheep pox virus
2: PCR product of lumpy skin virus

Results of sequencing of lumpy skin disease virus:

As a result of sequencing, the origin of Iraqi lumpy skin disease virus was similar to LSD strain (ksgp0) in Kenya**.**The sequencing of lumpy skin disease virus of the sample from[Al-Muthana] was sent to Korea can be explained as in figure [5 , 6] :

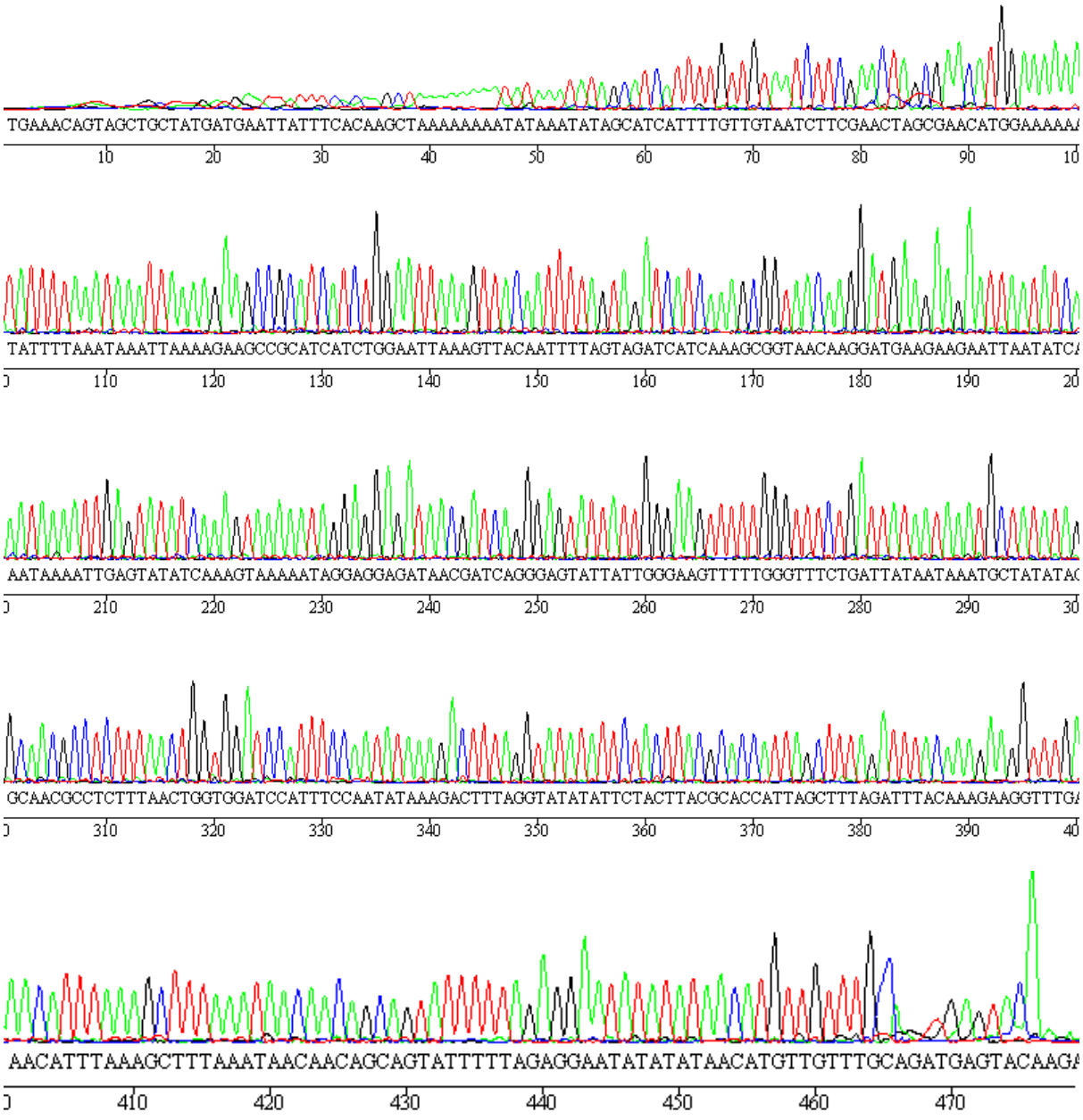

Figure 5: show the result of sequencing [forward]oflumpy skin disease virus of the sample from (Al-Muthana).

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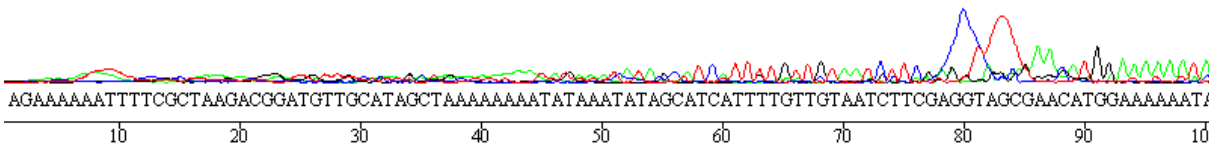
Figure 6: show the result of sequencing[reveres] of lumpy skin disease virus of the sample from (Al-Muthana).

Results of sequencing of sheeppox vaccine (Romanian strain) as in a figure [7] and [8]:



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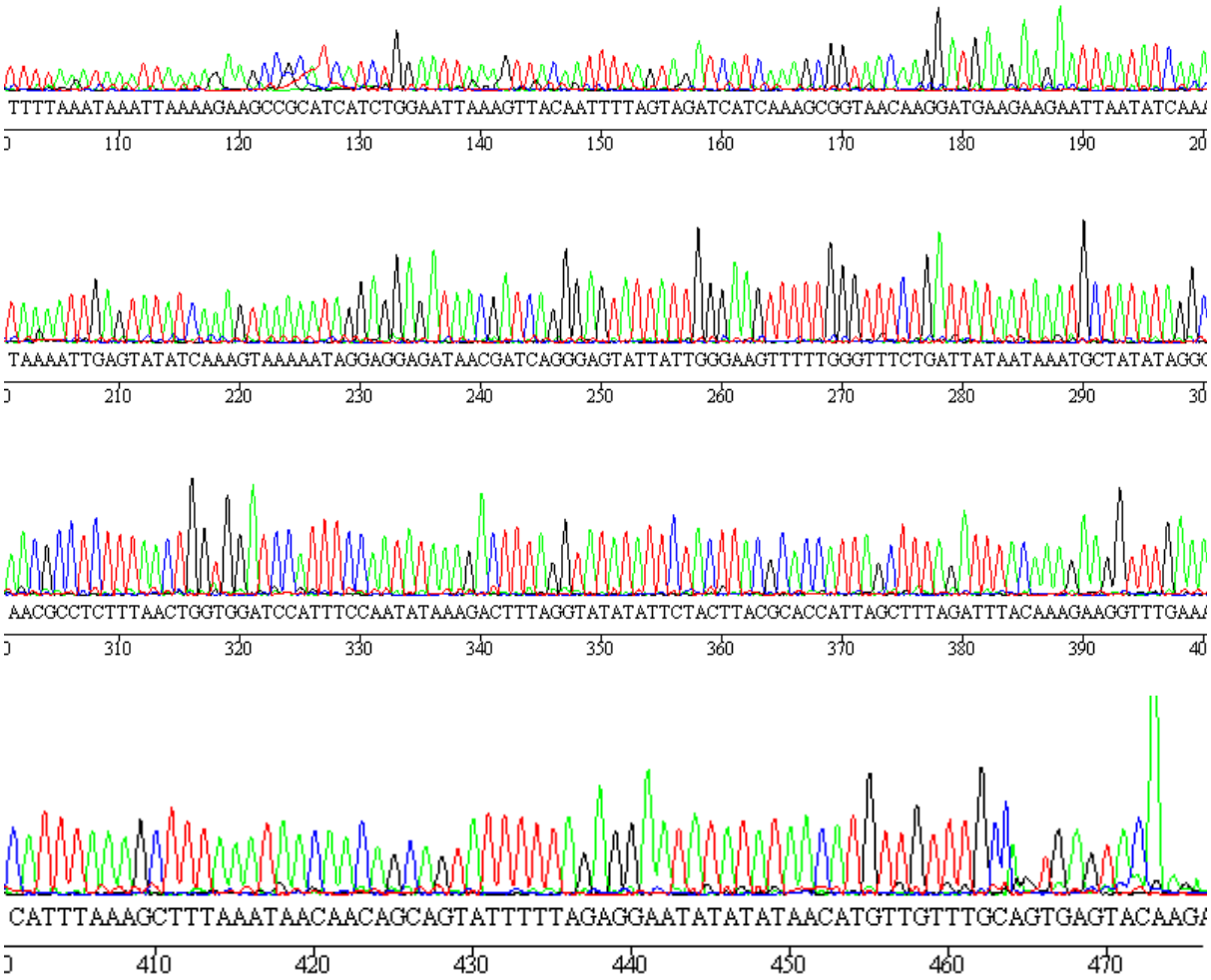
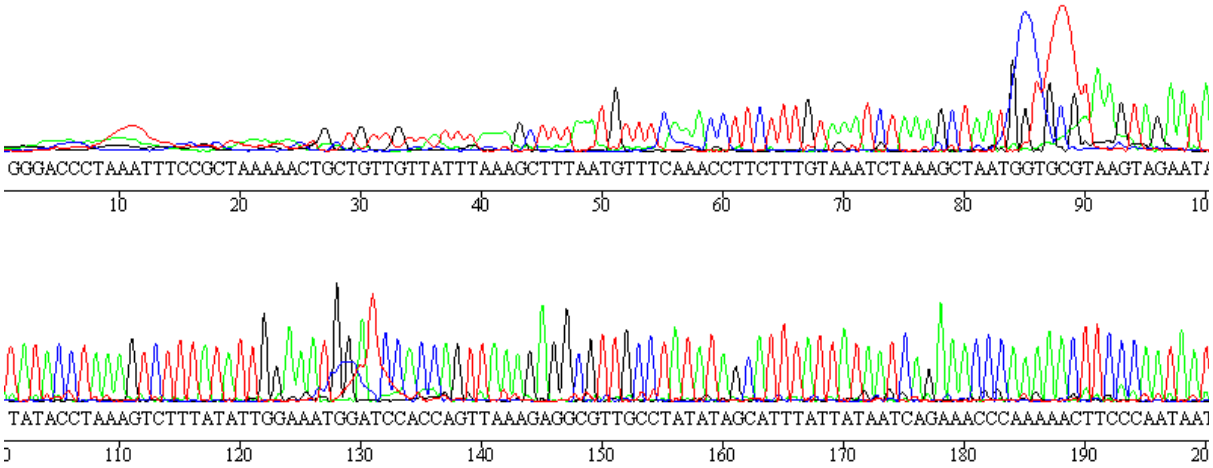


Figure 7 : show the result of sequencing of[forward] of sheeppox vaccine[Romanian strain]



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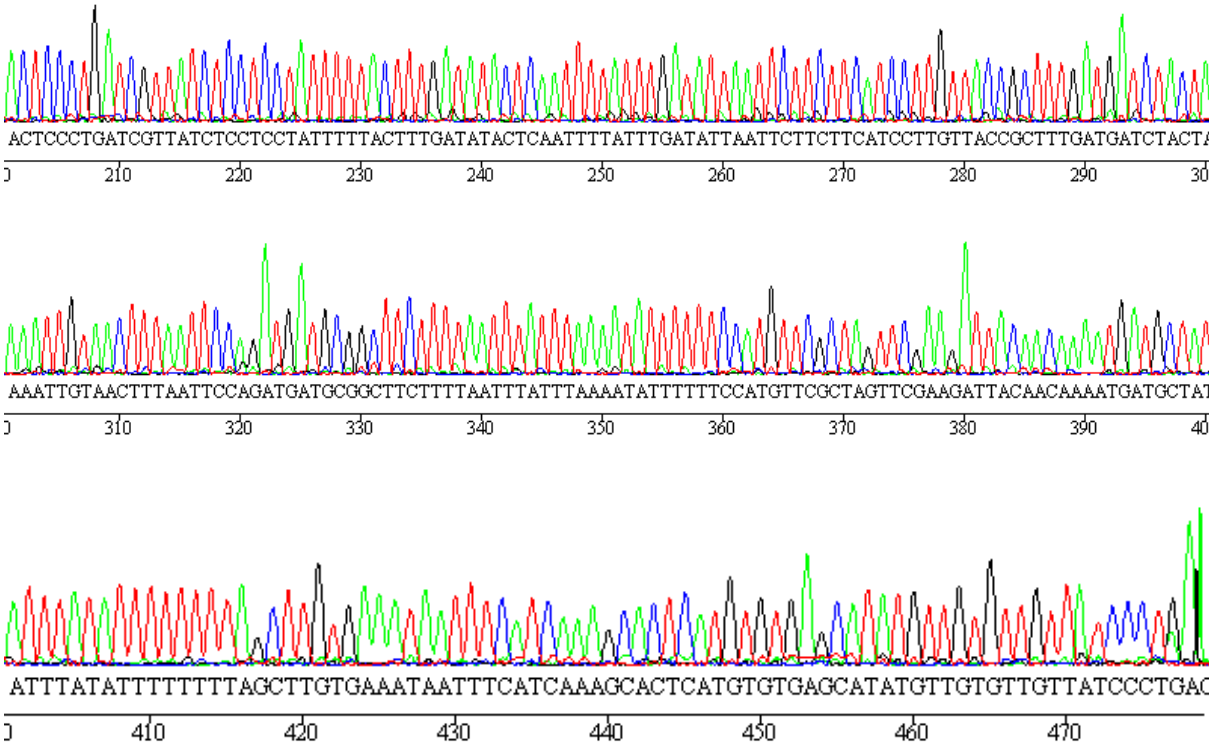

Figure 8 : show the results of sequencing of sheeppox vaccine[Romanian strain][reveres]:

Phylogenic tree of lumpy skin disease virus:

The attenuated KSGP O-240 vaccine virus present in the Kenyavac and KS-1 isolates were identified as LSDVs.

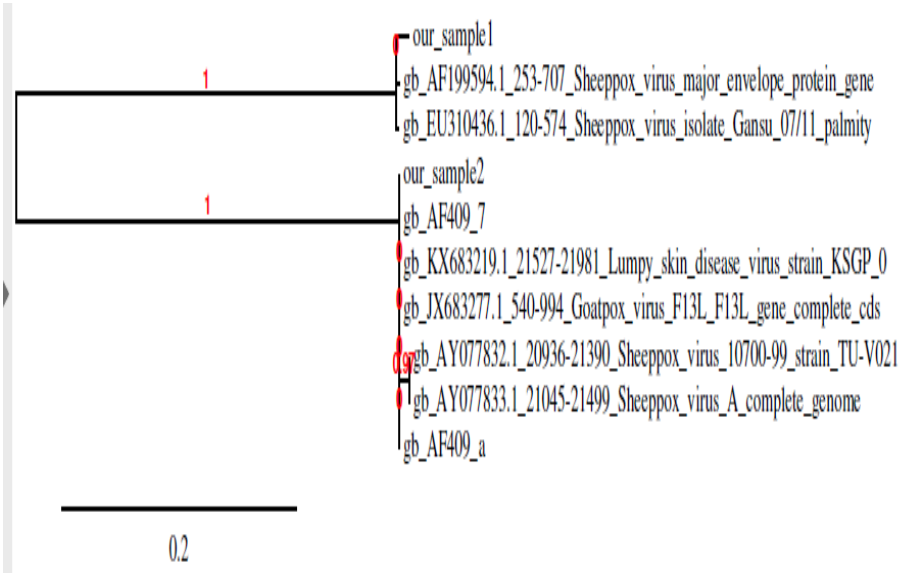

Figure 9: Phylogenetic tree of Iraqi lumpy skin disease virus

Comparison between Lumpy skin disease virus and sheep pox vaccine:

As a result of sequencing there is a similarity between the lumpy skin disease virus and sheep pox vaccine up to 97-98%.

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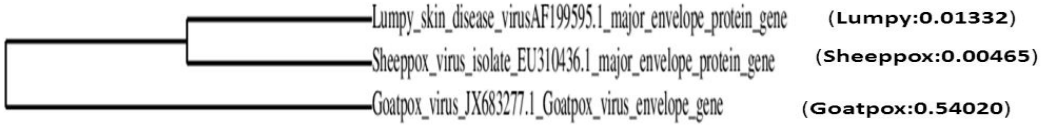

Figure 10: Phylogenetic tree of capripox viruses

Sheeppox vaccine [Romanian strain] major envelope protein gene, complete cds.

Gen Bank accession number :Ky348526

```
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DEFINITION  VERIFIED: Sheeppox virus major envelope protein gene, complete
            cds.
ACCESSION   gb1
VERSION
SOURCE      Virus
  ORGANISM  Virus
            Unclassified.
REFERENCE   1  (bases 1 to 455)
  AUTHORS   Mohsen,H.H. and Allawe,A.B.
  TITLE     gene
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 455)
  AUTHORS   Mohsen,H.H. and Allawe,A.B.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-DEC-2016) college of veterinary medicine, University
            of Baghdad, Al-Gadriah st.(Baghdad university, Baghdad 009641, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: TOTAL # OF SEQS:2.
            Bankit Comment: TOTAL # OF SETS:1.

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                     /organism="Sheeppox virus"
                     /mol_type="genomic DNA"
BASE COUNT      184 a     54 c     76 g    141 t
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       61 tagcgaacat ggaaaaaaaa ttttaaataa attaaaagaa gccgcatcat ctggaattaa
      121 agttacaatt ttagtagatc atcaaagcgg taacaaggat gaagaagaat taatatcaaa
      181 taaaattgag tatatcaaag taaaaatagg aggagataac gatccgggag tattattggg
      241 aagtttttgg gtttctgatt ataataaatg ctatataggc aacgcctctt taactggtgg
      301 atccatttcc aatataaaga ctttaggtat atattctact tacgcaccat tagctttaga
      361 tttacaaaga aggtttgaaa catttaaagc tttaaataac aacagcagta tttttagagg
      421 aatatatata acatgttgtt tgccagtgag tacaa
```

figure [11] : Sheeppox vaccine[Romanian strain] submitted in NCBI under the accession number Ky348526.

Lumpy skin virus major envelope protein gene, complete cds.

Gen Bank accession number :Ky348527.

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```
//
LOCUS       gb2                       455 bp    DNA     linear       07-DEC-2016
DEFINITION   Lumpy skin virus major envelope protein gene, complete
            cds.
ACCESSION   gb2
VERSION
KEYWORDS    VERIFIED.
SOURCE      Virus
  ORGANISM  Virus
            Unclassified.
REFERENCE   1  (bases 1 to 455)
  AUTHORS   Mohsen,H.H. and Allawe,A.B.
  TITLE     gene
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 455)
  AUTHORS   Mohsen,H.H. and Allawe,A.B.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-DEC-2016) college of veterinary medicine, University
            of Baghdad, Al-Gadriah st.(Baghdad university, Baghdad 009641, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: TOTAL # OF SEQS:2.
            Bankit Comment: TOTAL # OF SETS:1.

            ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..455
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                     /mol_type="genomic DNA"
BASE COUNT      184 a     54 c     75 g    142 t
ORIGIN
        1 aattatttca caagctaaaa aaaatataaa tatagcatca ttttgttgta atcttagaac
       61 tgctaaacat ggaaaaaaaa ttttaaataa attaaaagaa gccgcatcat ctggaattaa
      121 agttacaatt ttagtagatc atcaaagcgg taacaaggat gaagaagaat taatatcaaa
      181 taaaattgag tatatcaaag taaaaatagg aggagataac gatccgggag tattattggg
      241 aagtttttgg gtttctgatt ataataaatg ctatataggc aacgcctctt taactggtgg
      301 atccatttcc aatataaaga ctttaggtat atattctact tacgcaccat tagctttaga
      361 tttacaaaga aggtttgaaa catttaaagc tttaaataac aacagcagta tttttagagg
      421 aatatatata acatgttgtt tgccagtgag tacaa
//
```

figure[12] : lumpy skin disease virus of Iraqi isolate submitted in NCBI under the accession number Ky348527.

DISCUSSION

Lumpy skin disease [LSD] is a serious disease of cattle characterized by nodular cutaneous eruptions, lymphadenitis, and edema in one or more limbs [9]. In this study, LSD virus in Iraq was detected by real-time PCR, the collected sample were 50 samples.

The negative samples collected during 2016 proves that Iraq is free of the disease this year due to the use of sheeppox vaccine,The Positive samples were collected from areas of Iraq during the outbreak of LSD virus in 2013.

The real-time PCR technique was very sensitive, suitableand test of choice for rapid detection and identification of the LSD virus in agreement with[10,11,12] who explained PCR for the diagnosis of LSD is with a greater sensitivity and good specificity and it is most appropriate technique,The main advantage of real-time PCR over PCR is that real-time PCR allows you to determine the initial number of copies of template DNA [the amplification target sequence] with accuracy and high sensitivity over a wide dynamic range. Real-time PCR reveled that the sheeppox vaccine was positive and this technique was very sensitive , suitable for detection of sheeppox virus, by the amplification ofattachmentproteinin agreement with[13,14,15]who explained that ThePCR, which is specific ,rapid, sensitive and accurate assay for thedetection of Capripoxviruses. Recently, various PCR methods for detection and diagnosis ofsheeppoxvirus infection were developed.

The conventional PCR was used to amplified both viruses [LSD virus and sheeppox].The conventional PCR was suitable to detect both viruses , in agreement with [EFSA Panel on Animal Health and Welfare, 2014] who explain that the conventional PCR method is cheaper to perform than real-time PCR and is less vulnerable to technical problems, but it is not quantitative. However, both sensitivity and specificity are still superior to those of other methods available

Sheep pox vaccine was used to control outbreaks of LSD virus in Iraq therefore in this study sheeppox vaccine was compared with LSD virus and was sent for sequencing.

Phylogentic analysis of lumpy skin disease virus revealed that the Iraqi LSD similar to LSDstrain[ksgp0] in Kenya and this probably due to trading of infected animals with this strain .

The virulent KSGP O-240, the attenuated KSGP O-240 strain [Kenyavac] as well as the KS-1 isolate were identified as LSDV in Kenya . The real identity of the vaccine virus explains the easy attenuation of the virus for safe use in sheep and goat vaccines. It is however clear that the level of attenuation of the virus was insufficient for the use of KSGP O-240 for cattle, in which clinical disease was observed post-vaccination [16,17,18].As a result of sequencing of lumpy skin disease virus the phylogenetic analysis revealed that lumpy skin disease was similar to sheeppoxvaccineby 97-98% this agreement with [14,19].who explained that Primers for the viral attachment protein gene and the viral fusion protein gene are specific for all the strains within the genus Capripoxvirusby the use of sequencing and phylogenetic analysis; strains of virus can be identified.

Neverthe-less, in this study the P32 gene was used for identification of LSDV and sheeppox vaccine in agreement with [20]who determined the validity of the primers to detect LSDV in cattle and to provide P32 gene sequence information in LSDV. Several re-searches used the P32 gene to detect Sheeppox virus and Goatpox virus because it contains a most significant antigenic determinant present in all species of Capripoxvirus genus[13,21].

CONCLUSION

- As a result of sequencing of Iraqi LSDV , phylogenetic analyses revealed that Iraqi lumpy skin disease strain was similar to lumpy skin disease strain (KSGPO) in Kenya and this probably due to trading of infected animals with this strain.
- As a result of sequencing of Iraqi LSDV and sheeppox vaccine, the similarity between both viruses was [97 -98]% and this explained the protective role of sheeppox vaccine against outbreaks of lumpy skin disease in Iraq .
- Real-time PCR was sensitive to detect lumpy skin disease virus andsheeppox virus.
- Sheeppox virus (vaccine) was useful as protective measure in out breaks of lumpy skin disease virus .

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