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Polymerase Chain Reaction and Sequence Analysis of P32 Gene of Lumpy Skin Disease Viruses Isolated During 2019 in Egypt.

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LUMPY skin disease (LSD) is one of the important viral diseases affecting cattle herds Not only in Egypt but all over the MENA region, Africa and Asia as well, the current Molecular study based on molecular detection and sequence analysis of Lumpy skin Disease virus using the P32 gene which is very conservative gene not only for LSDV But for the whole Capri pox group viruses including the sheep and goat poxviruses, Skin lesion from cattle showing the typical clinical picture of the disease from Sharqia governorate during summer 2019 gives positive PCR band, at the expected size. Which were about 1185 bp where the selected primers were up stream of the P32 gene open reading frame By 213 bp till the end of the coding sequence which spans bout 969 bp which is the Coding sequence of the gene of interest, the PCR product of the target product is 1185 bp Including the coding region of the P32 gene sequenced and submitted to gene bank accession number#OL423259, this primer could be used as universal primer for all Capripox group, the current study used PCR-RFLP to differentiate between the sheep poxvirus and Lumpy Skin Disease virus by Specific characteristic pattern for each virus and could be used as a tool of Differentiation between these two viruses, the used bioinformatics tools revealed Presence of two different signature residues very characteristic for either the local Isolate VSVRI/Sharqia/2019 and Lumpy Skin Disease virus in general and the Romanian sheep pox reference strain used in this study which assures the slight Difference in the antigenicity of the two viruses even though the great homology of The two viruses at the nucleotide sequence level while at the amino acid level residues vary Slightly in length in sheep pox virus is about 323 residues while in lumpy skin disease The amino acid residues of the P32 is only 322 residues due to insertion of extra aspartate residue at position 55 which is not present in Lumpy Skin Disease virus and even goat pox virus Which is related to the same Capri group which reveals slight differences between the sheep Poxvirus and lumpy skin Disease virus in antigensity so this insertion in SPV make the slight difference in length of the coding nucleotide sequence between the two viruses using HinfI restriction enzyme is very unique in the current study and could be used as a sensitive rapid tool to differentiate between the two viruses as it has two recognition sites of digestion in SPV while in LSDV has only one recognition site this may be due to the difference in the arrangement of the nucleotide along the length of the coding sequence of P32 due to the insertion of extra 3 nucleotide in SPV which is not present in LSDV.

Keywords: P32, LSDV, SPV, RFLP, MDBK, HinfI.

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Introduction

The disease mainly affects cattle, caused by lumpy skin disease virus (LSDV) which belongs to the genus Capri pox virus of the Poxviridae family [1].

The disease varies from mild to severe symptoms represented as fever, generalized skin nodules, lymphadenitis, edema, and death in rare cases according to the general health condition of the animal. The skin nodules are Varying in size from 1 to 7 cm in diameter and may coalesce together forming, a core of necrotic tissue called sit fasts [2]. LSDV is transmitted mainly by mechanical routes by a wide variety of vectors such as mosquitoes (Culexmirificens and Aedes natrionus), biting flies (Stomoxys calcitrans), and even hard ticks (Rhipicephalus decoloratus, Amblyomma hebraeum, and Rhipicephalus appendiculatus) [3], [4] , [5] , Moreover, the Stomoxys calcitrans (stable fly) is identified as most probably vector for transmission of LSDV because it is associated with outbreaks [6]. LSD causes serious economic losses in cattle following outbreaks which have high morbidity; the disease is associated with a reduction in milk yield, weight losses, abortion, skin damage, and pneumonia in animals suffering from nodules in the upper respiratory tract (Abera et al. 2015; Ocaido et al. 2009) [5].

In addition, the mosquito species Aedesa egypti (Diptera: Culicidae) is approved to transmit the virus from donor to receptor animals [7].

The first report of LSDV in Egypt was in Ismailia and Suez governorates during the summer of 1988 [8]. Upper Egypt recorded epidemics during summer 2018 especially in Beni Suef and Sohag Governorates [9] ,Sever outbreaks have been recorded by OIE during the period of 2012 and 2019, several out breaks been recorded in Egypt during 2017-2018 epidemics for LSDV in cattle herds vaccinated with the heterologous sheep pox vaccine showing severe clinical signs and recorded case fatalities which a assures slight differences in antigenicity between sheep pox and Lumpy skin disease virus may play a role in the protection when the homologous vaccine prepared from LSDV gives higher protection rate than the sheep pox vaccine that used in the previous decades as vaccination policy against LSDV [10] , this may be due to slight difference in the antigenic structure between Lumpy skin disease virus and sheep pox virus, the current study carried out on P32 gene of Lumpy kin Disease as the gene of interest coding for one of the most important immunogenic proteins and has big role in the virus pathogenicity for the Capripox group that used either in diagnosis and detection of the genetic relatedness between the different Capripox , and reveals the most crucial differences between the lumpy skin disease virus and the sheep pox virus through comprehensive bioinformatics studies that showed differences in the antigenic structure and the length of the coding protein for both of them which assures the antigenic difference between them The slight antigenic difference between both LSDV and SPV may be related to insertion of extra aspartate residue at position 55 in sheep pox virus but not present in Lumpy skin disease virus [11].

The current study also documents the lengths and the names of the P32 coding homologous protein which is one of the conserved protein domain families PH02688 of the putative IMV envelope protein in different pox viruses PHA2688 ORF059IMVproteinVP55 (ID2515675)-Protein Clusters-NCBI (nih.gov)

Material and Methods

Virus and cells

The Neethling strain of LSDV was used as a positive control virus in this study. Viral stock was prepared by infecting Madin Darby Bovine Kidney (MDBK) cells at a multiplicity of infection (MOI) of 0.1 from plaque-purified virus and was subsequently titrated on MDBK cell cultures. The MDBK cells were grown and maintained in minimum essential medium with Earle's salts (MEME) supplemented with heat-inactivated 10% bovine calf serum (BCS),100U/ml penicillin, and 100µg/ml streptomycin. Prior to experimental work, both MDBK cells and BCS were tested free of LSDV by indirect immunofluorescence [12] and the Romanian sheep pox virus used in VSVRI for producing the sheep pox vaccine used as a control positive, so the positive control viruses used in the current study kindly supplied by Pox virus vaccines department, VSVRI.

Sample preparation

Preparation of the sample from skin nodules of infected cattle showing the characteristic skin nodules distributed all over the animal skin surface was made in a 50% solution (w/v) by grinding the skin and crusted scabs and adding Eagle's medium with extra antibiotics (penicillin 1000 U/ml, streptomycin 1000 mg/ml, kanamycin

500 mg/ml and amphotericin B 25 mg/ml). The suspension was frozen at −70°C and thawed three times. After centrifugation at 2000×g for 30 min, 2 ml of the supernatant was collected and stored at −70°C until used for DNA isolation and PCR [13].

Amplification of the P32 gene using polymerase chain reaction (PCR)

DNA extraction and Gene amplification

DNA extraction and purification of LSDV were extracted from frozen supernatants of the skin lesions and infected cell lines carrying the reference strain (Neethling strain of LSDV) and the lyophilized virus of the reference Romanian sheep pox virus used in VSVRI for vaccine production carried out by using Qiagen DNeasy Blood & Tissue extraction (Qiagen, Germany) and according to the manufacturer's protocol. Purified DNA was recovered in 100- µl elution buffer and stored at -20°C for further testing.

Polymerase chain reaction (PCR)

Identification of the isolate by Polymerase Chain Reaction (PCR) DNA extraction and purification carried on tissue homogenate of skin nodule using Qiagen DNeasy Blood & Tissue extraction (Qiagen, Germany) and according to the manufacturer's protocol. Purified DNA was recovered in100-µl elution buffer and stored at -20°C for further testing. PCR primer pairs flanking about 1185 bp of the P32 gene of Lumpy skin disease(LSD) according to Zhou et al. [14] Upstream primer (5'ATGGCAGATATTCCATT3') and downstream primer (5'TTACCACAGGCTATTAGAAG 3') (Biosearch Technologies South McDowell Boulevard Petaluma, CA, USA).PCR was carried out using a thermostable, proofreading DNA polymerase 4x1.25ml Dream Taq Green PCR Master Mix (2X), which Includes Dream Taq DNA Polymerase, 2X Dream Taq Green buffer, dNTPs, and 4mM MgCl2 catNo#K1081 and the PCR reaction was performed as following protocol:(25µl2×Dream Taq Green PCR Master Mix (Thermo Fisher Scientific Inc., MA, USA),100pmol represented in1µl for each forward and reverse primer,5µl template DNA, and nuclease-free water up to 50 µl. The thermal cycler was adjusted to an initial 94°C for 5 min. cDNA was amplified with 40 cycles of 94°C for 30 seconds, annealing at 44°C for 30 seconds, and extension at 72°C for 80 seconds, followed by a final extension step at 72°C for 10 min. The amplified products were analyzed by electrophoresis using 1% agarose gel and visualized by ultraviolet transilluminator after staining the gel with ethidium bromide stain (Fisher) as shown in (Figure 2). The product size was measured using Gene Ruler 100 bp Plus DNA Ladder (Thermo Scientific) cat No # SM0311. The amplification reactions were performed using the thermal cycler Perkin Elmer Gene Amp PCR system 9700, and then the amplified product at the correct size was excised and purified from the gel using a Quiaquick purification kit (Qiagen) according to the manufacturer's instruction and sent for sequence analysis by GATC Company, Germany by using ABI 3730xl DNA sequencer.

Restriction Fragment Length Polymorphism (RFLP)

HinfI (10 U/µL) restriction enzyme, thermo scientific # Cat NO FD0804 5'G↓ANTC3' at Concentration10 U/µl, using 10X Buffer R (10 mM Tris-HCl (pH 8.5), 10 mM MgCl2, 100 mM KCl, 0.1 mg/mL BSAas) Recommended Protocol for Digestion of PCR Products is carried out Directly after Amplification according the manufacturer protocol by adding: (PCR reaction mixture 10 µl (~0.1-0.5 µg of DNA), nuclease-free water 17 µl ,10X Buffer R 2 µl and HinfI 1-2 µl) then Mix gently and spin down for a few seconds and Incubate at37°C for 3 hours. Then inactivation was carried out at 65°C for 1 hour, then the digested PCR product was electrophoresed after adding the loading dye to achieve 20 mM final concentration using 1% agarose gel the product size was measured using Gene Ruler 100bp Plus DNA Ladder (Thermo Scientific) cat No#SM0311 and visualized using a UV transilluminator.

Results:

*PCR using the primers flanking the whole fragment including the P32 coding region of the Capripox group where the length of the fragment is 1185 as in sheep pox virus and 1182 in Lumpy skin disease virus this difference in length between both viruses is due to presence of insertion of extra amino acid (Aspartate) at the 55th residue of the coding sequence of P32 in sheep pox which increase the length of the coding region of the P32 to be 972 bp while in Lumpy skin Disease is 969bp so the PCR product using the primers as mentioned by Zhou et al.[14] is at the correct size which is nearly the same in the Reference Neethling strain of LSDV, the local isolate the VSVRI/ Sharqia 2019 and Romanian sheep pox reference strain as in shown Figure 1. So PCR using these primers assures that the

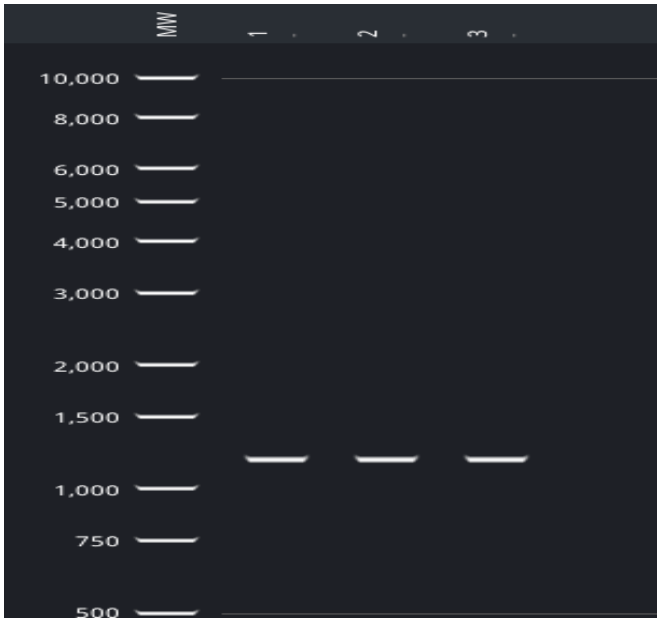

Fig. 1. PCR of the whole region1182amplified by the primer of interest including the P32 gene where the first Lane M is the Gene ruler 1KB DNA ladder thermos scientific cat No# kb Cat. No. SM0311, Lane 1 is the Neethling reference strain of LSDV, Lane 2 is the VSVRI/ Sharqia 2019 field isolate and lane 3 is the Romanian sheep pox reference strain.

product is related to the Capripox group but does not differentiate between them while PCR-RFLP used in this study can differentiate between the two viruses as each one has a different pattern after restriction enzyme digestion using HinfI restriction enzyme 5'G↓ANTC3'.

** Restriction Fragment Length Polymorphism (RFLP)*

carried out using HinfI restriction enzyme 5'G↓ANTC3' which has very characteristic cutting pattern as the enzyme has two cutting sites in SPV and only one cutting site in LSDV as shown in Table 1 that could be used to differentiate between the two viruses there is a common site of digestion between the two viruses which is at bp 490, agarose gel electrophoresis of the PCR products as shown in figure4 showing the different pattern of the digested products for both LSDV and SPV giving only two bands as in LSDV which are nearly about 500bp and 700 bp respectively in both the reference Neethling strain of LSDV and the local isolate VSVRI/ Sharqia2019 as shown intable1 and figure3 while in the Romanian sheep pox virus reference strain due to presence of 2sites there are 3 bands the size of each band is 300bp,394 and 490 respectively as shown in in tTable 1 and Figure 2

*Multiple sequence alignment as shown in figure (5) of the deduced amino acid residues of the local isolate VSVRI/ Sharqia 2019, LSDV, SPV and GPV deposited in gene bank showing that there is insertion of one amino acid residue at position 55 [14] which is aspartic acid this insertion residue is unique and very characteristic signature residue for SPV which is not present in both LSDV and GPV this indicate there is slight antigenic difference between the SPV and LSDV that makes the length of the p32 coding residue is 323 in SPV and is 322 in LSDV even there was great similarity between them either at the nucleotide or amino acid level of the P32 coding region which has significant antigenic domain for the family poxviridae infecting several animal species which is considered as a member of the conserved protein domain family PH02688 of the putative IMV envelope protein in different pox viruses and have different lengths starting from 278 residue as Nile crocodile pox virus till 354 residue in Pseudo cowpox virus, which assures that the necessity of using the homologous vaccines produced to protect against for the specific viral infection than using the heterologous antigen to protect against certain infection as that carried out using the SPV vaccines to protect against LSDV.

TABLE 1. Shows the pattern of HinfI restriction sites along the PCR products of both LSDV&SPV and the length of each fragment after digestion

Strain	HinfI				
Sheep pox	**Length**	**5'Enzyme**	**5'Base**	**3'Enzyme**	**3'Base**
	490	none	1	HinfI	491
	394	HinfI	791	none	1184
	300	HinfI	491	HinfI	790
VSVRI/Sharqia/2019/LSDV					
	490	none	1	HinfI	490
	692	HinfI	491	none	1182

*Genetic Map of both LSDV and SPV showing the unique and precise digestion sites of HinfI on the P32 coding sequence.

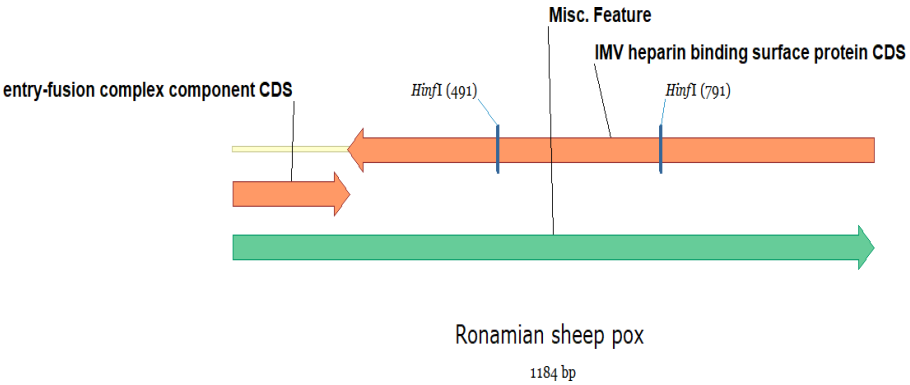

Fig. 2. Schematic representation and frame orientation of the PCR product which is 1185 bp in sheep pox virus showing the two restriction sites position one at 491bp and the other one at 791 bp which is unique site for SPV and does not exist in LSDV.

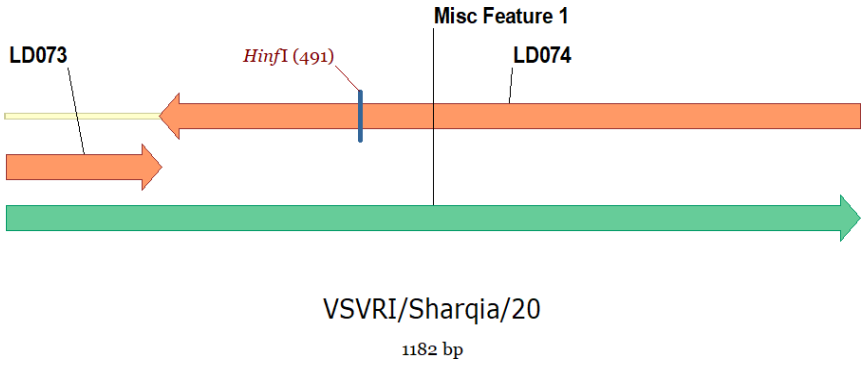

Fig. 3. Schematic representation and frame orientation of the PCR product which is 1182 bp LSDV (VSVRI/ Sharqia 2019) showing the there is only one restriction site position one at 491 bp

*Agarose gel electrophoresis shows pattern of the digested products of both LSDV and SPV using HinfI restriction enzyme.

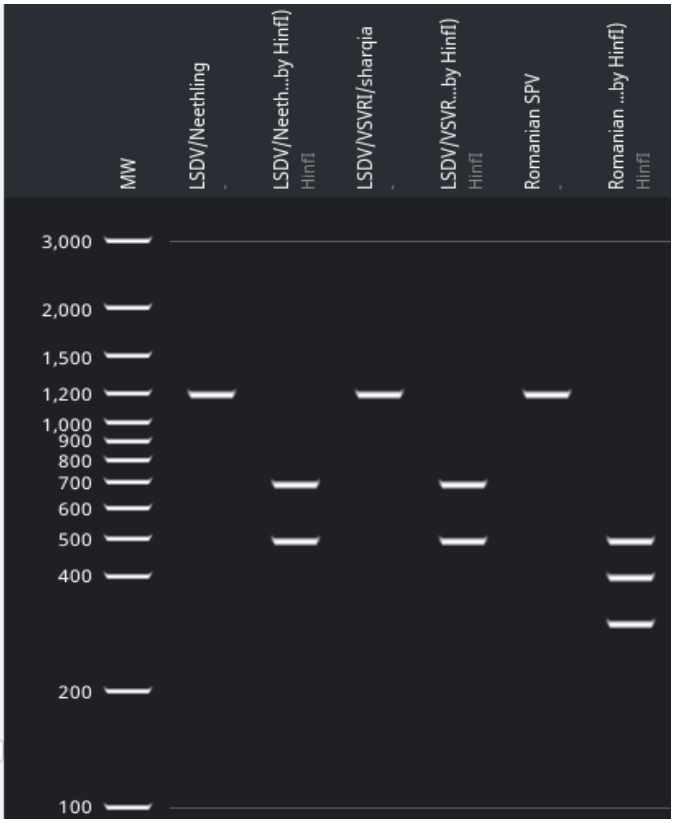

Fig. 4. RFLP of the PCR product which is 1181 bp including the P32 full coding region of the Capripox group which is 969 bp using HinfI restriction enzyme, first Lane is 100 bp DNA ladder cat No #SM0323, Lane 1 is the Neethling reference strain of LSDV, Lane 2 Neethling strain digested by HinfI, Lane3 is the Sharqia 2019 field isolate, lane 4 VSVRI/ Sharqia 2019 digested by HinfI Lane 5 Romanian sheep pox reference strain, lane 6 Romanian sheep pox reference strain digested by HinfI, restriction site digestion is 5'G↓ANTC3'

*phylogenetic analysis of the deduced amino acid sequence of p32 coding sequence of VSVRI/ Sharqia/2019 local against SPV and GPV isolate revealed the presence of 3 distinct clusters each cluster grouped for each specific virus as the cluster that includes the local isolate VSVRI/ Sharqia/2019 is related to the cluster group of LSDV and very close to the local isolates (LSDV-EGY-BSU/2012-R1p32, LSDV-EGY-BSU/2012-R2p32, LSDV-EGY-BSU/2018p32) accession no # MN418201.1, MN418202.1 and MN4182001.1 respectively which indicate that the local isolate VSVRI/Sharqia/2019 is closely related to the LSDV isolates circulating in the Egyptian field, so it's very recommended to prepare the vaccine produced in Egypt to be prepared from the local isolates of LSDV rather than using other stains of LSDV or even SPV which was used in Egypt for decades for vaccination against LSDV.

*Comparison between The conserved protein domain family PH02688 of the putative IMV envelope protein in different pox viruses affecting different species which has different synonymous according to host affected by this type of pox and the position of the open reading frame of the coding sequence inside the genome organization, but the most common names are (IMV envelope protein p35, ORF059 IMV protein VP55, immunodominant envelope protein, Virion envelope protein p35, LSDV074 putative IMV envelope protein or P32, m71L, ORF059 putative IMV protein VP55, IMV heparin binding surface protein) as listed in table 2 in full detail with their Accession numbers, length, organism and taxid.

*phylogenetic analysis of the deduced amino acid sequence of the conserved protein domain family PH02688 using the accessions in Table 2 using neighbor joining method which assures that the Capripox group is closely related to each other antigenically based on the amino acid sequence of the P32 coding region.

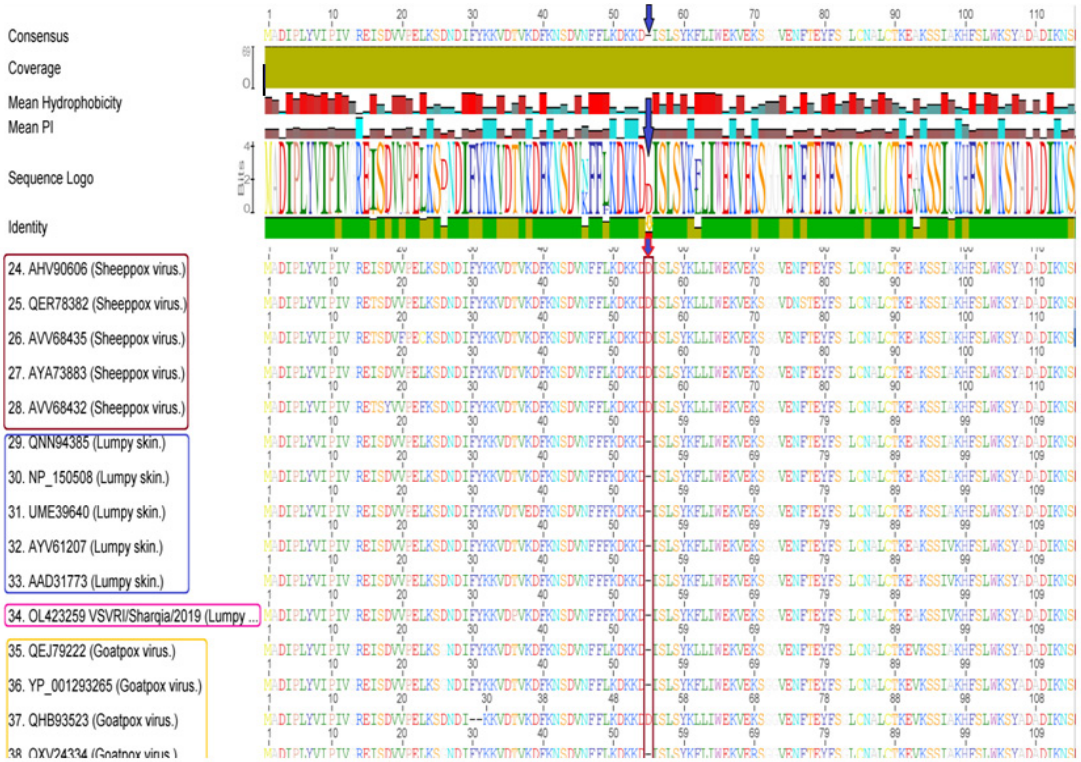

Fig. 5. Multiple alignment of the deduced amino acid residues Showing the insertion of extra residue at the position 55 of SPV which is not present either in LSDV in general including the local isolate (VSVRI/Sharqia/2019) and even the GPV which makes the length of the P32 residue 323 aa which is considered as signature residue of SPV while the length is 322 in both LSDV and GPV.

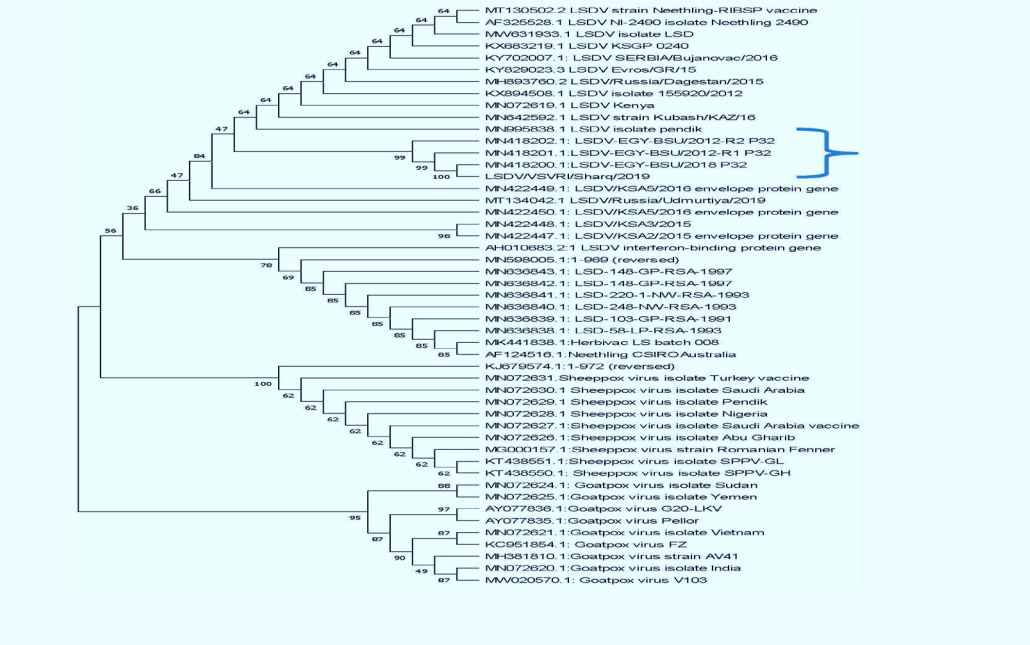

Fig.6. Showing the phylogenetic analysis using maximum like hood method of the deduced amino acid sequence of VSVRI/Sharqia/2019local isolate against the Capripox group using only the coding sequence of the LSDV074 putative IMV envelope protein (p32) which is about 322 residues.

TABLE 2. Shows the conserved protein domain familyPH02688 showing the variable lengths of the P32 homologous coding protein in different pox viruses affecting different species with variable lengths varying from 278 to 354 amino acid residues ORF 059 IMV proteinVP55; PHA2688ORF059IMVproteinVP55 (ID2515675)-ProteinClusters-NCBI(nih.gov).

#Accession	Definition	Organism	Taxid	Length
NP_957968	ORF059IMVproteinVP55	Bovine popular stomatitis virus	129727	340
NP_570489	CMLV099	Camel poxvirus	28873	325
NP_955209	CNPV186 putative IMV envelope protein	Canary poxvirus	44088	330
NP_619897	CPXV112protein	Cow poxvirus	10243	325
YP_002302423	IMVenvelopeproteinp35	DeerpoxvirusW-1170-84	305676	326
YP_227458	IMVenvelopeproteinp35	DeerpoxvirusW-848-83	305674	326
NP_671603	EVM085	Ectromelia virus	12643	324
NP_039103	Virion envelope protein ,p35	Fowl poxvirus	10261	327
YP_001293265	Hypothetical protein	Goat poxvirus Pellor	376852	322
NP_150508	**LSDV074putativeIMVenvelope proteinorP32**	**Lumpy skin disease virus NI-2490**	**376849**	**322**
NP_044035	MC084L	Molluscum contagiosum virus subtype1	10280	298
NP_536520	H3L	MonkeypoxvirusZaire-96-I-16	619591	324
NP_051785	m71L	Myxoma virus	10273	324
YP_784293	IMV envelope protein	Nile crocodile pox virus	1285600	278
NP_957836	ORF059putativeIMVproteinVP55	Orfvirus	10258	342
YP_003457364	Immune dominant envelope protein	Pseudo cow pox virus	129726	354
NP_051960	gp071L	Rabbit fibroma virus	10271	324
NP_659646	putative IMVenvelopeproteinp35 SPV0 71	Sheep pox virus	10266	323
NP_570231	p35	Swine pox virus	10276	324
YP_001497069	IMVenvelopeproteinp35	Tana pox virus	99000	323
YP_717410	IMV heparin binding surface protein	Tatera pox virus	28871	325
YP_232983	IMV heparin binding surface protein	Vaccinia virus	10245	324
NP_042130	Hypothetical protein	Variola virus	10255	325
NP_938329	74L	Yaba monkey tumor virus	38804	320
NP_073459	74Lprotein	Yaba-like disease virus	132475	323

Analysis and comparison of the antigenic structure of both LSDV & SPV using EMBOSS protein structure prediction method:

The antigenic structure of both Lumpy skin disease virus and sheep pox virus has slight difference between them at two portions along the amino acid residues this may be related to the difference in the length of the amino acid residues between the two viruses which is 322 residue in LSDV and 323 residue in SPV this due to insertion of extra aspartate residue at position 55 which is (DD) in SPV as shown in figure (8) which is not present in LSDV, the bioinformatics prediction tool using EMBOSS protein structure prediction method has detected two sites of antigenic difference between the two viruses,

*The first antigenic difference as shown in table (3) showing that the first antigenic region in LSDV is 20 residues with interval 4 -> 23 and the signature residue of this antigenic site is (IPLYVIPIVGREISDVVPEL), while this signature residues has some what difference in SPV as it may be divided into two portions where first one spans 11 residue with intervals 4 -> 14 and the signature residues (IPLYVIPIVGR) , while the second portion is 6 residues with intervals 18 -23 and the signature residues (SDVVPEL) , the area spanning about 20 residue in the first part of the p32 which begins from the residue number 4 from the first methionine which is the first residue of the gene of interest till the residue number 23 represented as one portion or one domain in LSDV while the same area in SPV

differs as it is divided into two portions unlikely that of LSDV in the same sequence of the protein of interest which represent about 3 predicted turns in this area for sheep pox which these turns are missing in LSDV as shown in fig () this is due to variation in the amino acid number 16 which is T (Threonine) In sheep pox virus and I (Isoleucine) in Lumpy skin disease virus which shows some sort of linearity and has no turns like that of SPV as indicated by red arrows in the upper sequence of SPV as shown in Figure (8)

*The second antigenic difference as shown in table (3) shows that the antigenic region in LSDV is 54 residues with interval 266 -> 319 and the signature residue of this antigenic site is PDFSYYVSHPLVSFFGIFDISIIGALIIL F I I I M I I F D L N S K L L W F L A G M L F T) While this signature residue has somewhat difference in SPV as it may be divided into two portions where first one spans39residue with intervals267->305 with signature residues (SFFGIFDISIIGALIILFIIIMIIFN), while the second portion is 14 residues with intervals 307 -> 320 and the signature residues (NSKLLWFLAGMLFT), the difference at the position 305 attributed to the antigenic difference between the two viruses at this residue which is N (Asparagine) in SPV and is D (Aspartic acid) in LSDV as shown in figure (9) which shows differences in the antigenic structure along the 54 residues covering this area in the two viruses which shows different turns and coils between the two viruses.

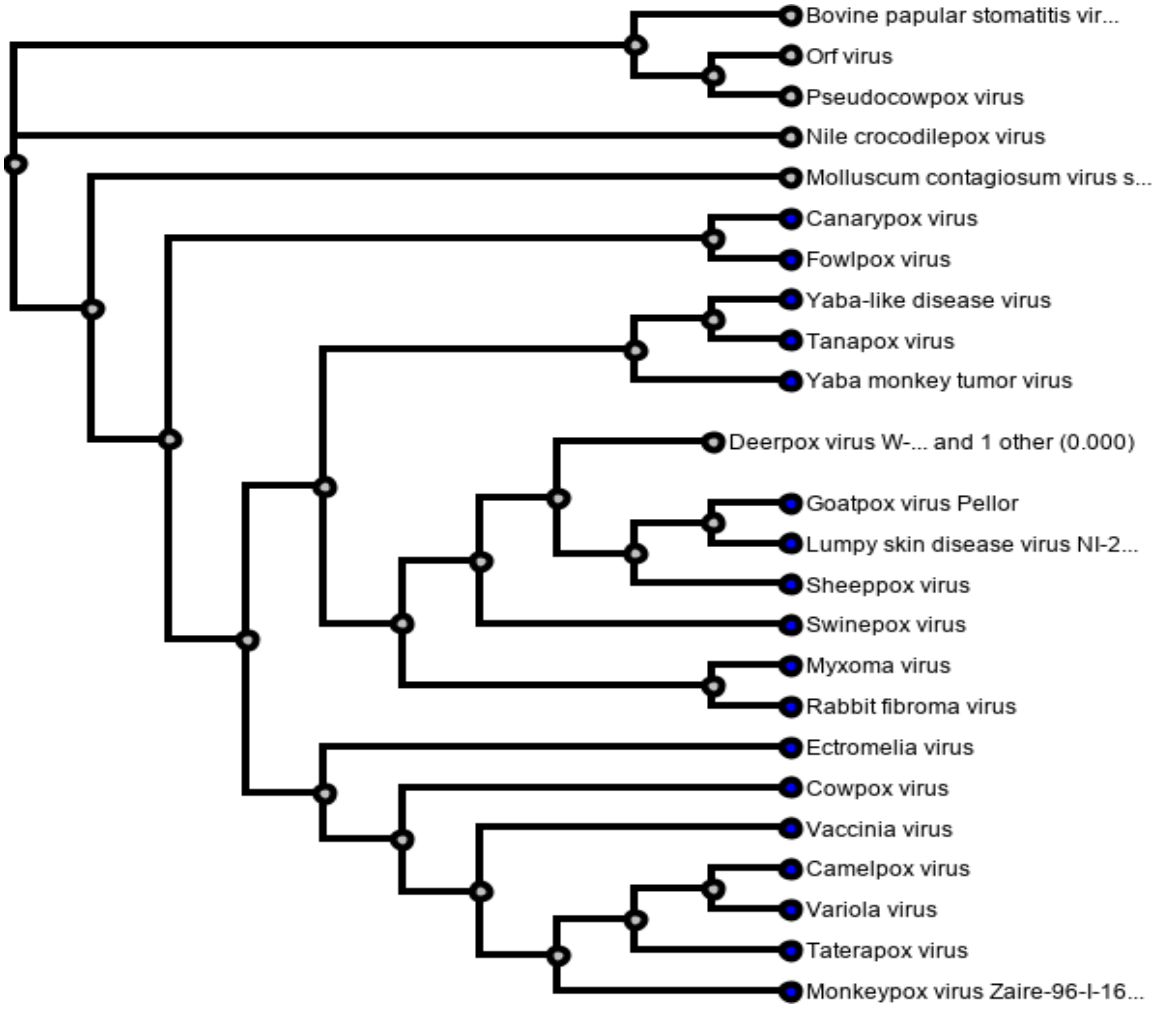

Fig. 7. Showing the phylogenetic analysis of the deduced amino acid sequence of the conserved protein domain family PH02688 of the putative IMV envelope protein in different poxviruses for different species where the length of the amino acids of the gene of interest varies from 287residue as in Nile crocodile pox virus till 354 residues in Pseudo cow pox virus.

TABLE 3. Shows Analysis and comparison of the antigenic structure of both LSDV &SPV using EMBOSS protein structure prediction method.

Antigenic differences (Predicted)	Lumpy skin disease virus			Sheep pox		
	Antigenic Length	Interval	Residues sequence	Antigenic Length	Interval	Residues sequence
1st difference	20 Residues	4->23	IPLYVIPIVGREISDV VPEL	11 Residue	4->14	IPLYVIPIVGR
				6 residues	18->23	SDVVPEL
2nd difference	54 Residues	266->319	FPDFSYYVSHPLVS FFGIFDISIIGALIILFII MIIFDLNSKLLWFLA GMLFT	39 Residue	267->305	FPDFSYYVSH PLV
						SFFGIFDISIIG ALIILFIIIMIIFN
				14 Residue	307->320	NSKLLWFLAG MLFT

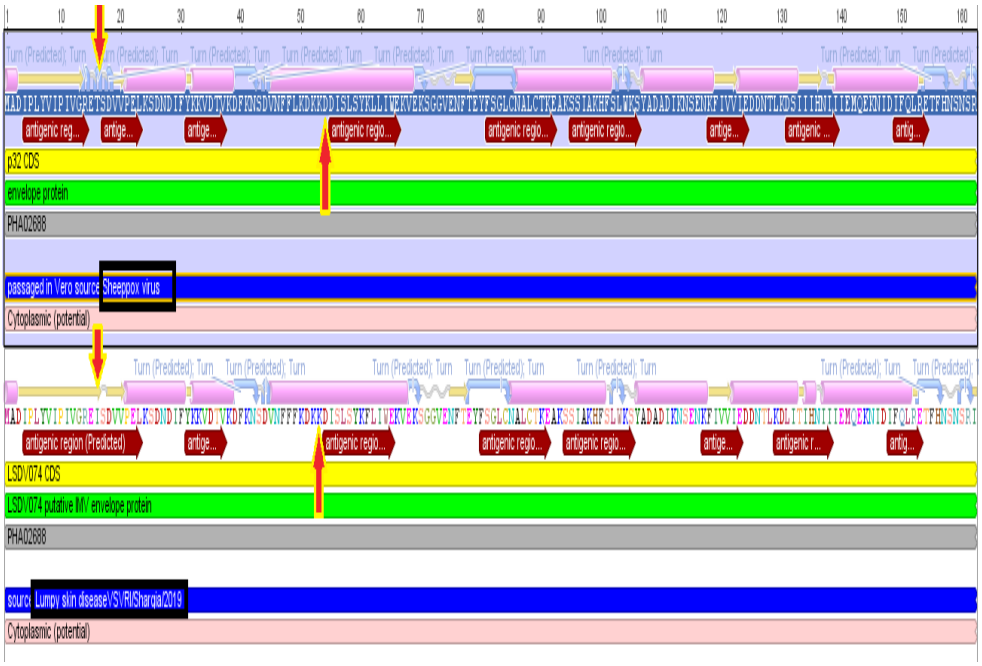

Fig.8. Schematic representation of the amino acid sequence from the first methionine till residue 152 the upper one represents the arrangement of amino acid residues and antigenic structure of SPV and the lower one represents LSDV.

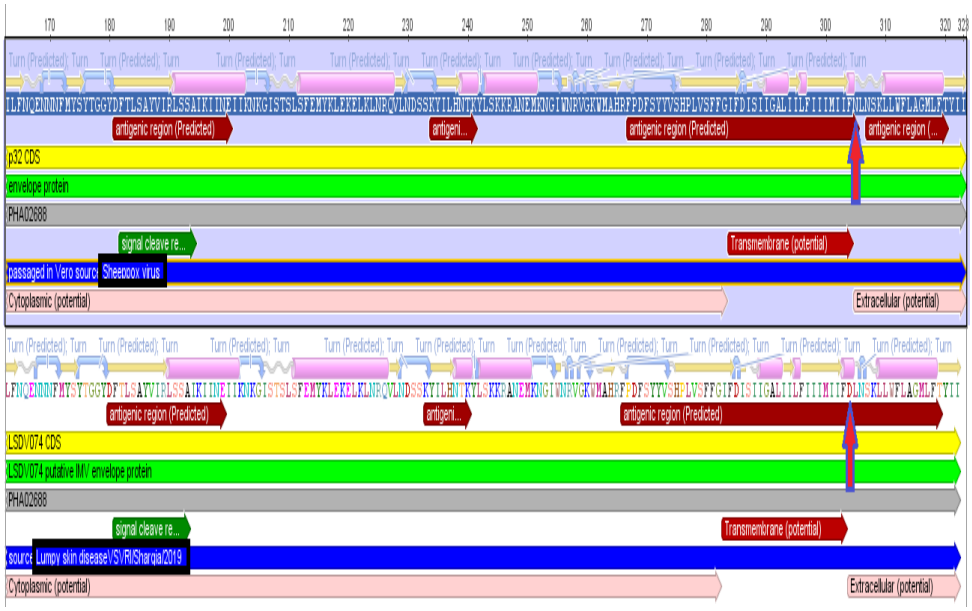

Fig. 9. Schematic representation of the amino acid sequence from160 till residue 323 the upper one represents the arrangement of amino acid residues and antigenic structure of SPV and the lower one represents LSDV.

Discussion

Sequence analysis and PCR-RFLP method in this study developed for unequivocal genomic differentiation of SPV and LSDV although presence of great homology on the nucleotide and amino acid level which indicate that they are antigenically closely related to each other, using the HinfI restriction enzyme digestion using the PCR product 1182 or 1185 in LSDV& SPV respectively confirms the slight differences between the two viruses at the nucleotide sequence level as it gives distinct banding profile between them as the HinfI restriction enzyme has two cutting sites in SPV resulting in 3 different bands and only one cutting site as in LSDV resulting in only two bands which assures the slight difference at the nucleotide sequence level which by its role reflects on the amino acid level and antigenic Structure a long side the whole length of the P32 protein , phylogenetic analysis of the deduced amino acid sequence of p32 coding sequence of VSVRI/Sharqia/2019 local against SPV and GPV isolate revealed presence of 3distinctclusters each cluster grouped for each specific virus . the cluster of LSDV that include the local isolate VSVRI/Sharqia/2019which is closely related to other Egyptian isolates (LSDV-EGY-BSU/2012-R1 p32, LSDV-EGY-BSU/2012-R2 p32, LSDV-EGY-BSU/2018 p32) accession no # MN418201.1, MN418202.1 and MN4182001.1 respectively which assures great conservation among the circulating strains on the Egyptian field so The genetic structure of p32 among Capripox group showing genetic stability and conservation [15]. using the Vaccine of Romanian sheep pox virus for decades to protect against LSDV in Egypt and other countries due to this antigenic relation between the both viruses, the current study used sequence analysis of the P32 local isolate that has been isolated during the outbreak during 2019 showing slight antigenic difference between the two viruses based on the bioinformatics studies carried out on P32 this either due to the difference in the length of the amino acid sequence due to insertion of one aspartate residue at 55 position which is considered as signature residue for SPV and is absent either in Lumpy skin Disease virus or Goat Pox Virus which makes the length of p32 of SPV is 323 residue and 322 residue in LSDV &GPV, variation in the amino acid number 16 which is T (Threonine) In sheep pox virus and being I (Isoleucine) in Lumpy skin disease which makes the antigenic structure in this position at the first coding portion of makes some sort of antigenic differences as shown in figure (8) as it represents several beta turns in SPV while showing linear structure in LSDV which is considered signature residue for both viruses and share in the difference of antigenic structure between both of them ,as well the difference at the position 305 attributed to

the antigenic difference between the two viruses at this residue which is N (Asparagine) in SPV and is D (Aspartic acid) in LSDV as shown in figure (9)the different bioinformatics prediction tools using EMBOSS protein structure prediction method used in this study assures presence of differences in the antigenic structure of P32 of both SPV &LSDV so due to these differences on P32 structure of both viruses it's better to use the homologous virus of LSDV as vaccine candidate to protect against LSDV infections than using the heterologous vaccine of Romanian sheep pox virus as recorded in turkey and Balkan region between 2013 and 2017makes insufficient protection [16] , the antigenic structure of the P32 is related to and grouped in the conserved protein domain family PH02688 which is immunodominant envelope protein and varies in length and structure according to the species affected by this type of pox virus which vary in size form 287residue as in Nile crocodile pox virus till 354 residue in Pseudo cow pox virus as shown in table (2), phylogenetic analysis of the deduced amino acid sequence of the conserved protein domain family PH02688 revealed that the Capripox are grouped in one cluster and being related to each other due to high conservation of the P32 among the Capripox group, the slight difference in the amino acid level not present in the protein of interest of our study P32 but also present among most of the proteins among the Capripox group which assures the necessity of changing the vaccination regimen to use the sheep pox virus vaccine against lumpy Skin disease to be substituted to use the homologous virus of lumpy Skin disease to protect cattle against lumpy Skin disease Virus.

Conclusion

The antigenic structure of SPV and LSDV is closely related to each other but not identical as there are slight variations in the antigenic structure between the two viruses as detailed in the current study at the level of P32 which plays an important role in the virus antigenicity due to the extra amino acid insertion in SPV which reflects on the arrangement of the nucleotide sequence and leads to a different pattern of digestion when digested by the same enzyme HinfI and assured by the detailed bioinformatic analysis that detailed the very fine difference between the two viruses of the same family at the molecular and antigenic structure so using the LSDV as a candidate for vaccination against Lumpy skin disease confers better protection than using the SPV as vaccination strategy used for decades for protection against LSDV.

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Conflict of interests

There is no potential conflict of interest was reported by the authors.

Ethical statement: This research did not require any ethical issue because it does not deal with an animal

Authors' contributions

1-All the practical work and experimental design and writing the whole manuscript from A to Z made by the first author, Dr. Mohamed Ibrahim Abd El Hamid Sayed

2-Samples were introduced by Dr. Mohamed Hashim El Sayed from the Pox virus vaccine research department.

3-Editing of references has been carried out by Dr.Nadine Adel Mohamed.

4-Editing the scientific content has been made by Prof. Dr. Seham Abd El Rasheed Sayed El Zeedy.

5-Primers used in this study were purchased by Prof. Dr. Ashraf Mohamed Abbas.

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إستخدام تفاعل البلمرة المتسلسل وتحليل التتابع النيوكليوتيدى للجين المشفر للبروتين ٣٢ لأحد المعزولات المحلية لفيروس إلتهاب الجلد العقدى المعزول عام ٢٠١٩

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مرض الجلد العقدي (LSD) هو أحد الأمراض الفيروسية المهمة التي تصيب قطعان الماشية ليس فقط في مصر ولكن في جميع أنحاء منطقة الشرق الأوسط وشمال إفريقيا وآسيا أيضًا ، الدراسة الجزيئية الحالية تعتمد على الكشف الجزيئي والتحليل التسلسلي لفيروس مرض الجلد العقدي باستخدام الجين P32 وهو جين يتمتع بدرجة ثبات عالية ونادرا ما يحدث به تغيرات ليس فقط لـ مرض الجلد العقدى الذى يصيب الماشية ولكن بالنسبة لفيروسات مجموعة Capri pox بأكملها بما في ذلك فيروسات جدري الأغنام والماعز ، حيث أن الصورة المرضية فى الماشية والتى تظهرفى إيجابية باستخدام تفاعل البلمرة المتسلسل (PCR) ، بالحجم المتوقع. والتي كانت حوالي ١١٨٥ قاعدة أساس حيث كانت البادئات المختارة مصممة قبل البداية من إطار القراءة المفتوح للجين P32 بمقدار ٢١٣ قاعدة أساس حتى بداية التسلسل النيكليوتيدى المشفر للجين موضع الدراسة و الذي يمتد على ٩٦٩ قاعدة أساس ، حيث أن منتج PCR لـ المنتج المستهدف هو ١١٨٥ قاعدة أساس بما في ذلك منطقة التشفير الخاصة بتسلسل الجين P32 و وتم رفعه وتسجيله إلى رقم انضمام لبنك الجينات # OL423259 ، حيث يمكن استخدام هذه البوادىء كأساس عام للكشف عن جميع مجموعات Capripox ، استخدمت أيضا الدراسة الحالية تقنية PCR-RFLP للتمييز بين فيروس جدري الأغنام وفيروس مرض إلتهاب الجلد العقدي حسب نمط مميز محدد لكل فيروس ويمكن استخدامه كأداة للتمييز بين هذين الفيروسين ، كشفت أدوات المعلوماتية الحيوية المستخدمة عن وجود أختلافات بسيطة فى تسلسل الأحماض الأمينية والتى تعتبر مميزة جدًا لكلا من المعزلة المحلية (VSVRI/ المحلي / الشرقية / ٢٠١٩) و مرض الجلد العقدي بشكل عام والسلالة المرجعية لجدري الأغنام الرومانية المستخدمة في هذه الدراسة والتي تؤكد الاختلاف الطفيف في تركيب التتابع الأمينى للفيروسين على الرغم من التماثل الكبير للفيروسين على مستوى تسلسل النيوكليوتيدات بينما تتفاوت عل مستوى الأحماض الأمينية تفاوتا قليلاً من حيث الطول في فيروس جدري الأغنام والذى يبلغ حوالي ٣٢٣ حمضا أمينيا بينما في مرض الجلد العقدي يبلغ حوالى ٣٢٢ حمضا أمينيا فقط وذلك لوجود حمض أمينى زائد إضافي (أسبارتات) في الموقع رقم ٥٥ والتي لا توجد في فيروس مرض الجلد العقدي الذى يصيب الماشية أوحتى فيروس الجدري الذى يصيب الماعز والذي يرتبط بنفس مجموعة الكابري والذي يكشف عن اختلافات طفيفة بين فيروس جدري الأغنام وفيروس مرض الجلد االعقدى ، لذا فإن هذاه الإضافة للحمض الأمينى الزائد ا في مرض جدرى الأغنام SPV يحدث فرقًا طفيفًا في طول تسلسل النيوكليوتيدات المشفر بين الفيروسين, و باستخدام إنزيم القطع المحدد HinfI و الذى فريد جدًا في الدراسة الحالية ويمكن استخدامه كأداة سريعة حساسة للتمييز بين الفيروسين حيث أنه يحتوي على موقعين محددين للتقطيع بالنسبة لفيروس جدرى الأغنام (SPV) بينما في فى مرض ألتهاب الجلد العقبى الذى يصيب الماشية (LSDV) يحتوي على موقع للتقطيع المحدد واحد فقط قد يكون هذا بسبب الاختلاف في ترتيب النوكليوتيدات بطول تسلسل الترميز P32 بسبب إدخال ٣ نيوكليوتيدات إضافية في SPV غير الموجودة في LSDV والذى بدورة قد يؤثر على الأستجابة المناعية للتحصين بإستخدام مرض جدرى الأغنام ضد مرض الجلد العقدى.

P32, LSDV, SPV, RFLP, MDBK, HinfI

الكلمات الدالة: الفيروس المسبب لمرض إلتهاب الجلد العقدى ، البروتين رقم 32 ، إنزيمات القطع المحددة ، فيروس جدرى الأغنام.