



Molecular Characterization and Sequence Analysis of VP1 Gene of Duckling Beak and Dwarfism Syndrome Isolated In Egypt During 2023



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Abstract

GOOSE PARVOVIRUS (GPV) is a highly infectious disease of water fowls causing short beak and dwarfism syndrome (SBDS). The importance of the GPV resides in its ability to transmit horizontal, vertical and also through contamination of eggs hatchery. The appearance of the virus in Egypt and all over the world is still mysterious and not well understood. The current study aims at assessment of the current epidemic situation of GPV through isolation and molecular characterization of the capsid protein VP1 gene and comparative computational assessment of the antigenic structure between the recently isolated strains in 2023 and the strain isolated during 2018 epidemics that used for preparation of the local monovalent vaccine. Molecular analysis revealed that there 100 % homology between the local isolates of 2023 while the isolate of 2018 shares 98.1% homology with those isolated in 2023. Comparative sequence analysis of VP1 gene between 2023 and 2018 isolates revealed that there were 16 SNPs, 3 of them are nonsynonymous SNPs that lead to codon shift in the significantly important predicted antigenic domains of VP1 protein. The first codon shift occurs at residue number 28 from (alanine) in 2018 isolate to (serine) in 2023 isolates this lead to change in the antigenic structure at this portion, the second change occurs at residue number 217 to be aspartate in the local isolate of 2018 while being serine in the isolates of 2023, the 3rd codon difference is at residue 265 which is serine in the local isolate of 2018 and is aspartate in the local isolates of 2023. On the other hand the isolates of 2023 epidemics are typically the same at the molecular level and antigenic structures even though they have been isolated from different locations ;Gharbia governorates in the north of Egypt and Beni Suef in the upper Egypt which indicates that the isolate of 2023 is the most prevailing GPV during this period, so it is recommended to update the current prepared monovalent vaccine with bivalent vaccine that include both the isolates of 2018 and 2023 for better protection against GPV in Egypt and to make continuous monitoring of the current circulating viruses to make better assessment of the current epidemic status in Egypt for continuous vaccine update to cope with the existing field epidemic status.

Keywords: Goose parvovirus, Duck parvovirus, Molecular, Mutation, nGPV, SBDS.

Introduction

Parvovirus is a highly infectious disease of water fowls, classified as *Dependoparvovirus*, *anseriform dependoparvovirus 1*, Parvoviridae family. In 2019 the International Committee on Taxonomy of Viruses categorized the family Parvoviridae to main important groups; Muscovy duck parvovirus (MDPV) and goose parvovirus (GPV) groups [1].

GPV or Derzsy's Disease as originally named in the honor of Domokos Derzsy [2] who firstly characterized the disease as an infectious disease of waterfowl producing watery diarrhea 'as the virus replicate in the intestinal wall after the primary infection', oculo-nasal exudates, stunted growth and feather abnormalities in older goslings. The infection in young goslings and Muscovy ducks is considered fatal and leads to high mortality rate as much as

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70%–100% [3]. MDPV infect Muscovy ducks characterized by stunning, high mortality due to watery diarrhea and locomotor dysfunction [4]. Recently a new genetic variant of goose parvovirus named as novel goose parvovirus (nGPV) was known to infect mule ducks only [5]. This nGPV is mainly characterized by developing a short beak, hence it named short beak and dwarfism syndrome (SBDS) and was recently recorded in Peking ducks in China and Egypt. [6].

Parvovirus is a non-enveloped single-strand DNA virus with genome about 5100 bp the virus consists of non-structural protein and capsid proteins VP1, VP2, and VP3 [7]. The molecular characterization of a new MDPV from Muscovy ducks in the USA was studied and showed that GPV and MDPV are antigenically related and sharing about 85% protein sequence [8]. Scientists tried to understand the cause behind the emerging of the novel goose parvovirus (nGPV) and the appearance of SBDS in Pekin ducks in China and Egypt, and they suggested the presence of recombination events [9], and this is was emphasized after the appearance of novel recombinant MDPV (rMDPV) in many country side in china [10].

During 2017 many Muscovy duck flocks at Behira governorate showed symptoms of Angel Wing Syndrome, the cause was expected to be environmental or even hereditary, till 2022 where GPV was found to be associated with the appearance of such new syndrome, and the phylogenetic analysis of the isolated strains showed that the nucleotide identities were 95.7%–96.6%, and 96.8%–97.4% with goose parvovirus of Derzsy's disease and with the strain causing SBDS [11]. It is clear from the previous studies about the SBDS and GPV that the molecular aspects behind the disease needs more study and this evidenced by the emerging of the new syndrome, so this study aim to analyze the VP1 gene sequence of GPV of the field isolated strain from 2018 till 2023 to have a clear insight about the variation of the VP1 sequence analysis and recognize if there is a need to update the strain that used in the recent monovalent vaccine.

Material and Methods

Sample collection and preparation:

Samples were collected from two local unvaccinated mule duck farms located in Al Gharbia and Beni Suef governorate during March 2023, ducks showed watery diarrhea, stunted growth with protruded tongue and short beak, 25: 35% mortalities have been recorded in the investigated farms. From each farm, 10 freshly dead ducklings were collected and labeled then transferred rapidly on ice box to the lab. Ten liver samples were collected per farm, pooled, and kept in a -20°C for further study. Liver samples were homogenized manually followed by three successive times freezing and thawing process,

and clarified by centrifugation at 10,000 rpm for 10 min to collect the supernatant.

Control positive virus strain:

Novel Goose parvo virus (nGPV) strain AM/KH//Goose parvovirus/VSVRI/Perma/Egypt/2018 accession number # OR854265 this strain is used in the preparation of local vaccine that commercially available as inactivated Derzsy disease vaccine prepared at veterinary serum and vaccine research institute (VSVRI), was used as a positive control.

Isolation and adaptation of viruses in embryonated Muscovy duck eggs (EDE):

The age, 9-11 day old EDE purchased from local farm were disinfected and cleaned carefully to avoid the presence of any egg shell contaminants before using in isolation, propagation and adaptation of the virus isolate. In brief 0.2 ml of homogenized and filtered liver homogenate was inoculated via allantoic sac, eggs sealed and kept at 37°C and checked daily for vitality. Eggs were chilled at the day 5 post inoculation (PI) and the collected allantoic fluid was used for successive five blind passages and the collected allantoic fluids were tested by PCR.

Virus isolation on Primary Duck Embryo Fibroblasts cells (DEF):

The suspected liver homogenate supernatants was filtered using 0.2 µm syringe filter, then inoculated onto Primary DEF cell culture monolayer and incubated at 37°C for 7 days and checked daily for appearance of cytopathic effect of the virus which in the form of cell clumping and/or detaching when compared with control non infected cells [12, 5]. For confirmation the whole culture cells were taken and centrifuged at 500 rpm and the supernatant was tested by PCR to confirm the presence of the virus.

Molecular identification of parvovirus from infected duck liver homogenate, allantoic fluid of EDE and DEF:

Viral nucleic acid extraction and Polymerase Chain Reaction (PCR):

DNA was extracted employing 300 µl of supernatant recovered from liver tissue homogenate, allantoic fluid and DEF propagated virus, then DNA was extracted using genomic DNA Mini Kit (Geneaid) following the manufacture instruction, with some modification during lysis step, where 100 µl of 10% SDS and 20 µl of proteinase K were added. The collected DNA was eluted in 50 µl TE buffer and kept in -20°C till use. PCR was performed using Thermo Scientific DreamTaq Green PCR Master Mix 2X Cat# K1081 (# K1081), utilizing forward GTGGGTAATGCCTCGGGAA and reverse primers GACACAGGTCCGGGTTGTAG targeting VP1 gene and amplify 885 bp (This study). Using Gene Amp PCR system 9700 applied Biosystem

thermal cycler The PCR cycling started with an initial denature step at 95 °C for 5 min, followed by 35 cycles (95 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s), and a final extension step at 72 °C for 10 min. 1% agarose was used for inoculation of PCR products together with a gene ruler 100 bp ladder (Fermentas, Thermo scientific, MA, USA).

Preparation of DNA for sequencing:

The nucleic acid of the two identified parvovirus strain from Gharbia (Perma) and Beni-Suef governorate, were subjected to PCR amplification using high-fidelity nPfu-Forte Enzymomics (Cat. # P410). Pfu DNA polymerase of superior proof reading capacity used during preparation of the PCR fragment of interest for sequencing due to the superiority of its proofreading and thermostability compared with other Taq DNA polymerases [13]. Following the manufacture instructions a fragment of about 898 bp of the VP1 gene was amplified using the previous mentioned conditions. The PCR product was then loaded on a 1% low melting agarose, and the correct amplicon size was sliced and purified (Quiaquick gel extraction kit cat #.28704–sequencing accomplished by GATC Company, Germany by using ABI 3730xl DNA sequencer. Nucleotide sequence analyses using the Laser Gene sequence analysis software package (Laser Gene, version 10; DNASTar, Inc.) and alignments were performed using Clustal W module.

Results

Post mortem (PM) and virus isolation findings:

Dead mule ducks showed clear signs of short beaks and protruded tongue (Fig. 1). During virus isolation on EDE at the five blind egg passages, deaths of the embryos were detected 72 hr. PI, the infected duck embryos showed abnormalities of peak, dwarfism with petechial hemorrhage when compared with the control non infected embryo. (Fig. 2). During isolation on DEF, infected DEF cells showed rounding and shrank at 72 hr. PI while detachment of the entire cells sheet at 96-120 hr. PI (Fig. 3).

Multiple nucleotide and amino acid sequence alignments between 2023 and 2018 isolates:

Sequence analysis revealed the presence of 16 site of differences between the local isolate of 2018 namely AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 accession number # OR854265 and the local isolates of 2023 namely AME/Gooseparvovirus/VSVRI/Tanta/Egypt/2023 accession number # PP058119 and AME/Gooseparvovirus/VSVRI/ Beni Suef/Egypt/2023 accession number # OR878549 which seems that the isolates of 2023 typically the same genetically and antigenically although they have been isolated from two different locations between them more than 500 Kilometer distance, as shown in figure 5 from the first base till base number 330 there are 7 difference

between the local isolate of 2018 and the isolates of 2023 which are located at base number (39,82,147,216,231,276,309) only one substitution in the local isolate of 2018 at the base number 82 which is corresponding to the base number 781 of the whole Vp1 gene result in codon shift from **serine** in the local isolates of 2023 into **alanine** in the local isolate of 2018 which may lead to significant antigenic change as it is located in one of the predicted antigenic regions and lead to change in the secondary structure in the outer coat of Vp1 protein GPV, while the rest 13 base difference between them will not lead to codon shift as they all code for the same amino acid residues and considered as silent change which are all shown precisely in Table 1, the rest changes as shown in figure 6 from base number 331 till base number 660 showing 6 differences between the vaccine local isolate AM/KH//Gooseparvovirus/VSVRI/ Perma/Egypt/2018 and the other 2 local isolates of 2023 at nucleotide number (333,351, 468,612, 624,650) which are considered silent mutations except that change at base number 650 where the is codon shift from (aspartate) in the local isolate of 2018 into (serine) in the isolates of 2023 which is true mutation which is very characteristic for the local isolate AM/KH//Gooseparvovirus/ VSVRI/ Perma/ Egypt/2018 than the two other isolates as shown precisely in table 1, the last three difference are shown in figure 7 from base number 661 till base number 855 where the difference are precisely located at base number (717, 744,794) only one difference in local isolate of 2018 at base number 794 which is corresponding to base number 1493 of the whole Vp1 gene lead to codon shift from serine in the isolate of 2018 into aspartate in the isolates of 2023 such change may lead to change in the secondary structure at this site which may play a precise role in changing the antigenicity between the isolate of 2018 and those of 2023 as shown in figures (8,9,10)

Discussion

Recently GPV has gained a great attention due to its association with severe problems in waterfowl. However, the appearance of the virus all over the world and in Egypt is still mysterious and not well understanding.[5] The importance of the GPV reside in its ability to transmit horizontal, vertical and also through contamination of hatchery by egg shell pollution. Since 2018 where Saleh and Khodier [5] isolated and identified the 2018 GPV isolate, there was a great demand to follow up the emerging new cases of GPV infection especially at the un vaccinated farms and yards, in a way trying to understand the newly isolated virus from the molecular point of view depending on partial VP1 gene analysis.

The current study aims to make assessment of the current epidemic situation of the circulating GPV in Egypt which make devastating economic losses in

young ducklings and goslings, three isolates have been fully characterized through sequence analysis and further bioinformatics studies to analyze the differences between these isolates which have been isolated from different districts either in the lower or upper Egypt at the molecular or the antigenic level.

The characteristic clinical signs of SBDS considered a strong guide for possible diagnosis together with the polymerase chain reaction (PCR) that considered the preferred method for identifying GPV in any suspected sample [14,15]. Isolation of the nGPV was successively achieved on DEF cell culture showing clear clumping and detachment of infected cells. Similar results were recorded [16, 6]

Phylogenetic analysis using Maximum likelihood method based on the amino acid residues showed that parvo virus affecting water fowl has been grouped into distinct clusters as the Muscovy duck parvovirus (MDPV) is grouped in very distinct cluster that is totally different from either classical GPV (C-GPV), novel GPV and the classical duck parvovirus (DPV) group which are grouped in separate cluster showing that the classical duck parvovirus (DPV) and the *Dependoparvovirus anseriform* are very closely related to each other antigenically, while the 2018 and 2023 Egyptian local isolates were in separate clusters, However the local isolate AM/KH// Gooseparvovirus/VSVRI/Perma/Egypt/2018 accession number # OR854265 is very closely related to the Chinese isolate ANX99769 which indicate that one of the circulating field isolate may be introduced to Egypt through international trade, imported birds or even through migratory birds from china, as china is considered the main source of the disease as all the viruses that cause short peak and dwarfism syndrome has been emerged from china [17].

Sequence analysis of the two isolates from Gharbia Governorate Namely AM/KH// Gooseparvovirus/VSVRI/Perma/Egypt/2018 accession number # OR854265 which isolated during 2018 from Perma district that used in local vaccine preparation and AME/Gooseparvovirus/VSVRI/Tanta/Egypt/2023 accession number # PP058119 which isolated during 2023 from Tanta district are genetically and antigenically (computational analysis) different as the homology percent at the nucleotide level is 98.13%, where there are 16 differences at genomic level between them 13 of them does not lead to codon shift only 3 changes lead to codon shift which is very characteristic for the isolate of 2018 first change is located at nucleotide number 82 in the local isolates which is corresponding to base number 781 of the whole coding sequence of the VP1 gene of GPV, where the codon shifts from alanine in 2018 isolate to (serine) in the isolates of 2023 which has very significant importance due to its location in one of the antigenic regions of the Vp1 protein of GPV which result in changing the secondary structure

which represented by continuous coil which spans 25 SGTAQD 30 which is very characteristic for the isolate of 2018 while being in the form of coil followed by β -turn25 SGTSQD 30 in the other isolates of 2023 where one of them located in the same governorate (Gharbia Governorate) namely AME/Gooseparvovirus/VSVRI/Tanta/Egypt/2023 accession number # PP058119 which indicates that there is diverse antigenic circulating viruses in this area although there is a time span between the periods of isolation, while the other isolate of upper Egypt that has been isolated during 2023 namely AME/Gooseparvovirus/VSVRI/Beni Suef/Egypt/2023 accession number # OR878549 is typically the same like the isolate of 2023 namely AME/Gooseparvovirus/VSVRI/Tanta/Egypt/2023 accession number # PP058119 which isolated from Gharbia governorate, since serine metabolism antagonize the innate immunity by preventing ATP6V0d2-mediated YAP lysosomal degradation [18] so, this can explain why the newly isolated strains are highly epidemic and rapidly transmit causing severe losses.

the second change that characterize the isolate of 2018 is located at nucleotide number 650 in the local isolate which is corresponding to base number 1349 of the whole coding sequence of the VP1 gene of GPV which lead to shift in the corresponding codon number 217 of aspartate in the local isolate of 2018 which is corresponding to codon number 450 of the whole of the Vp1 protein of GPV while being serine in the isolates of 2023 but the antigenic sites and secondary structure at this site retained and remains the same in the three isolates, the 3rd change is located at the nucleotide number 794 in the local isolates which is corresponding to base number 1493 of the whole coding sequence of the VP1 gene of GPV lead to change in amino acid residue 265 which is corresponding to residue number 498 of the whole of the Vp1 protein of GPV to be serine in the isolate of 2018 while the two isolates of 2023 represented as aspartate which lead to change in the secondary structure at this area to be in the form of Beta strand 262 NIWS 265 in AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 to be β -turn 262 NIWN 265 in the 2 isolates of 2023 as shown in table (1). The conversion of serine to aspartate is known to be a very helpful to imitate phosphorylation and induce changes in the structure of the protein that makes the virus cell entry easy [19].

Conclusions

The two newly isolated 2023 strains AME/Gooseparvovirus/VSVRI/BeniSuef/Egypt/2023 and AME/Gooseparvovirus/VSVRI/Tanta/Egypt/2023 from the two different localities in upper and Lower Egypt are antigenically and genetically diverse from 2018 strain AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 accession number # OR854265 that used in local vaccine preparation,

hence it is recommended to update the current prepared monovalent vaccine with bivalent vaccine that include both the isolates of 2018 and 2023 for better protection against GPV in Egypt and to make continuous monitoring of the current circulating viruses to make better assessment of the current epidemic status in Egypt for continuous vaccine update to cope with the existing field epidemic status.

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Conflicts of interest

The authors declared no competing interests.

Funding statement

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Ethical statement

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

Author contributions

Amani design the study and isolate the virus and adapted it on DEF and DEE.

Eman molecular identification by PCR, sample preparation for sequencing and writing the manuscript.

Abd El Hamid, VP1 gene computational analysis and gene bank submission and data interpretation.



Fig. 1. Mule ducks showed clear signs of short beak and protruded tongue



Fig. 2. Death of duck embryos after 72 hrs. PI showing abnormalities of peak, dwarfism with petechial haemorrhage compared with control one (C).

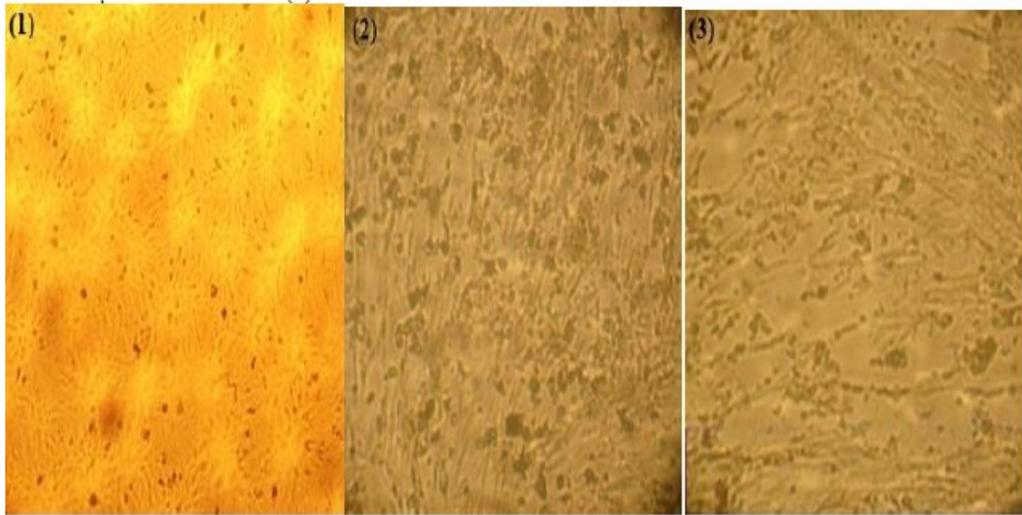


Fig. 3. Normal duck embryo fibroblast cells DEF (1); Infected DEF cells 72 hr. PI showing rounding and shrinkage of cells; (2): Infected DEF cells 96-120 hr. PI; (3) showed detachment of the entire cells sheet.

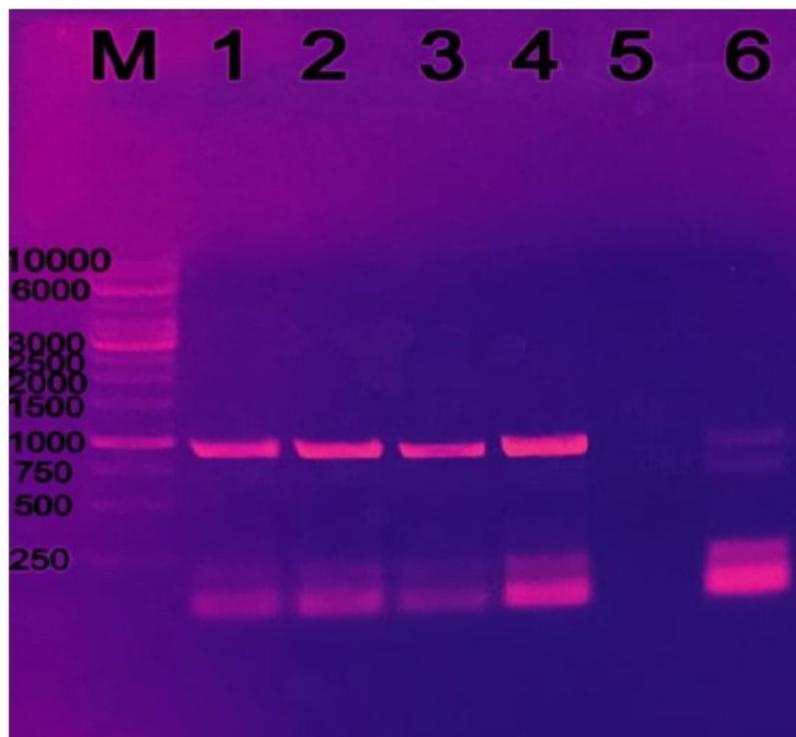


Fig. 4. Representative PCR amplification of the isolated virus strains showing clear band at 898 bp where M (100 bp DNA ladder); Lane 1, virus vaccine strain as control positive; Lane 2 and 3 the isolated virus strain from DEF; Lane 4, from allantoic fluid of DEE; Lane 5 negative control and lane 6 from field infected liver tissue homogenate.

TABLE 1. Showing comparison between the three local isolates showing 16 sites of nucleotide differences between AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 and the other 2 isolates of 2023 namely AME//GooseParvoVirus/VSVRI/Beni Suef/Egypt/2023 and AME//GooseParvoVirus/VSVRI/Tanta/Egypt/2023, 13 of that differences showing silent mutations while only three (red labeled) changes in AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 result in residue changes most of these changes result in significant antigenic structure differences which is very characteristic for the isolate of 2018 while the two isolated of 2023 typically the same at the nucleotide and amino acid residue levels.

Perma/Egypt/2018 Accession number # OR854265			Beni Suef/Egypt/2023 Accession number # OR878549			Tanta/Egypt/2023 Accession number # PP058119			Precise Site of nucleotide and amino acid residue change in whole VP1 gene and the corresponding's in the local 3 isolates		
Site of Nucleotide	Codon	Amino Acid Name	Site of Nucleotide	Codon	Amino Acid Name	Site of Nucleotide	Codon	Amino Acid Name	Site of nucleotide whole VP1 gene	Site of corresponding Amino acid residue in whole VP1	Site of corresponding Amino acid residue of VP1 in local isolates
39 C	CCC	Proline	39 A	CCA	Proline	39 A	CCA	Proline	738	246	13
82 G	GCT	Alanine	82 T	TCT	Serine	82 T	TCT	Serine	781	261	28
147 C	CGC	Arginine	147 A	CGA	Arginine	147 A	CGA	Arginine	846	282	49
216 G	AAG	Lysine	216 A	AAA	Lysine	216 A	AAA	Lysine	915	305	72
231 G	AAG	Lysine	231 A	AAA	Lysine	231 A	AAA	Lysine	930	310	77
276 G	ACG	Threonine	276 A	ACA	Threonine	276 A	ACA	Threonine	975	325	92
309 C	ATC	Isoleucine	309 T	ATT	Isoleucine	309 T	ATT	Isoleucine	1008	336	103
333 C	CAC	Histadine	333 T	CAT	Histadine	333 T	CAT	Histadine	1032	344	111
351 G	CTG	Leucine	351 C	CTC	Leucine	351 C	CTC	Leucine	1050	350	117
468 C	AGC	Serine	468 T	AGT	Serine	468 T	AGT	Serine	1167	389	156
612 G	GTG	Valine	612 A	GTA	Valine	612 A	GTA	Valine	1311	437	204
624 C	CTC	Leucine	624 G	CTG	Leucine	624 G	CTG	Leucine	1323	441	208
650 A	AAC	Aspartate	650 G	AGC	Serine	650 G	AGC	Serine	1349	450	217
717 G	CCG	Proline	717 A	CCA	Proline	717 A	CCA	Proline	1416	472	239
744 T	GTT	Valine	744 G	GTG	Valine	744 G	GTG	Valine	1433	481	248
794 G	AGT	Serine	794 A	AAT	Aspartate	794 A	AAT	Aspartate	1493	498	265



Fig. 5. Schematic representation of the both nucleotide and corresponding amino acid sequence alignment of the sequenced region of the Vp1 gene of Vp1 protein of GPV of the three local isolates from nucleotide number 1 which is corresponding to nucleotide number (700)of the whole Vp1 protein of GPV of the three local isolates showing 7 differences between the in the local isolate AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 and the other 2 local isolates at nucleotide number (39,82,147,216,231,276,309) which are considered silent mutations except that change at base number 82 where there is codon shift from alanine to (serine) which is true mutation which is very characteristic for the local isolate AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018.

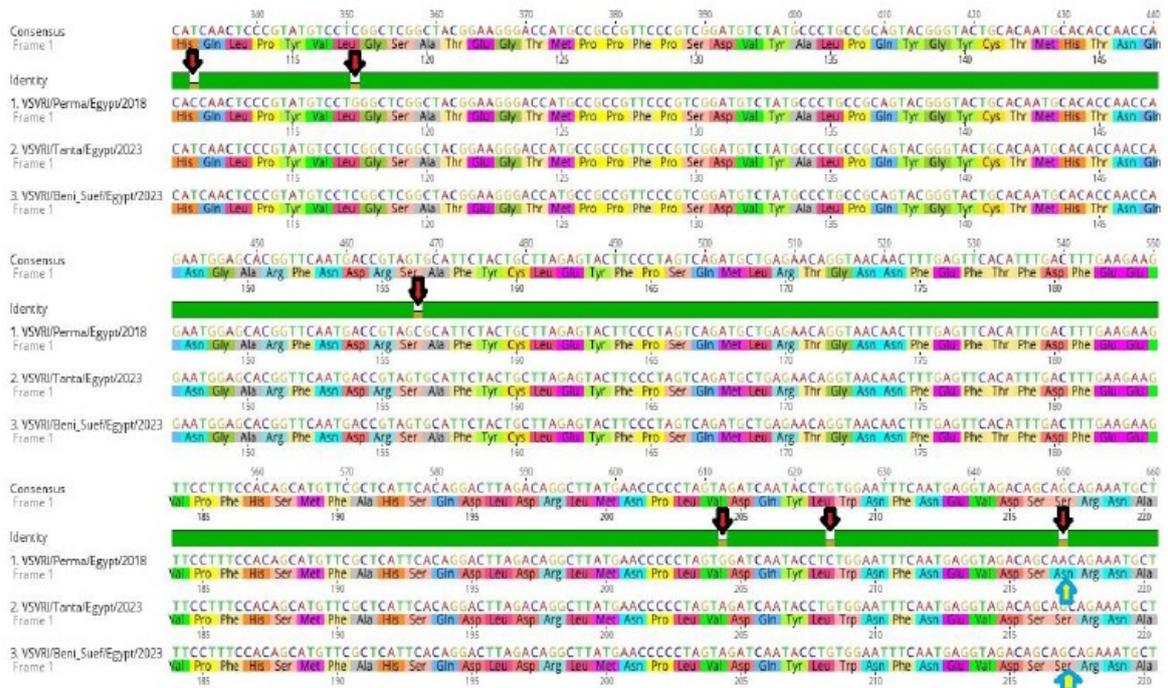


Fig. 6. Schematic representation of the both nucleotide and corresponding amino acid sequence alignment of the sequenced region of the Vp1 gene of Vp1 protein of GPV of the three local isolates from nucleotide number 331 which is corresponding to nucleotide number (1031) of the whole Vp1 protein of GPV of the three local isolates showing 6 differences between the in the local isolate AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 and the other 2 local isolates at nucleotide Number (333,351,468,612,624,650) which are considered silent mutations except that change at base number 650 where the is codon shift from (aspartate) to (serine) which is true mutation which is very characteristic for the local isolate AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 than the two other isolates

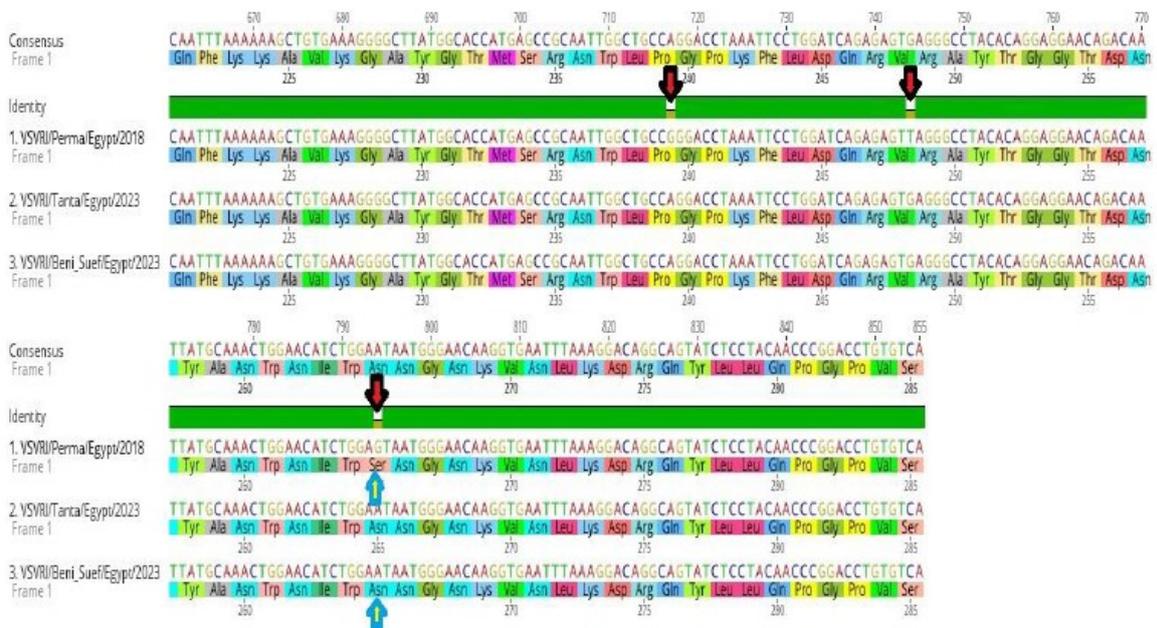


Fig. 7. Schematic representation of the both nucleotide and corresponding amino acid sequence alignment of the sequenced region of the Vp1 gene of Vp1 protein of GPV of the three local isolates from nucleotide number 661 which is corresponding to nucleotide number (1361) of the whole Vp1 protein of GPV of the three local isolates showing 3 differences between the in the local isolate AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 and the other 2 local isolates at nucleotide number (717,744, ,794) which are considered silent mutations except that change at base number 794 where the is codon shift from (serine) to (aspartate) which is true mutation which is very characteristic for the local isolate AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 than the two other isolates.

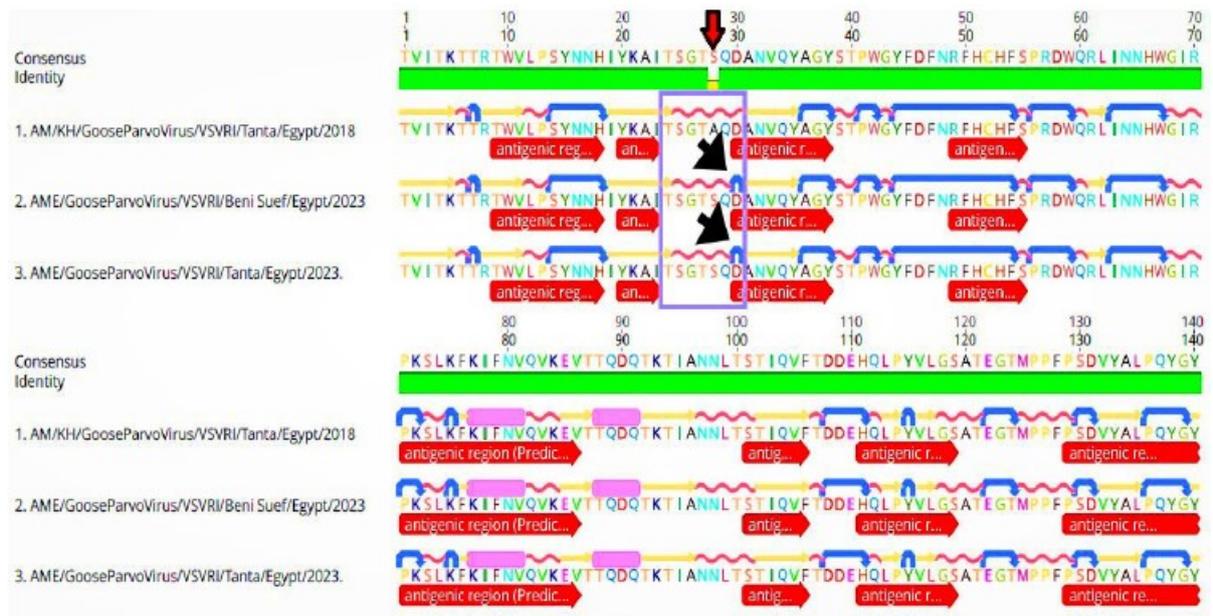


Fig. 8. Schematic representation of the amino acid sequence alignment from amino acid number 1 which is corresponding to residue number (234) of the whole Vp1 protein of GPV to the residue number 140 which is corresponding to the residue number (373) of the whole Vp1 protein of GPV of the three local isolates showing the predicted antigenic regions and the secondary structures (arrows) where there is significant difference at residue number 28 (alanine) which is corresponding to the residue number 261 of the whole Vp1 protein in the local isolate AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 while the residue 28 in the other 2 local isolates AME/GooseParvoVirus/VSVRI/Beni Suef/Egypt/2023 and AME/GooseParvoVirus/VSVRI/Tanta/Egypt/2023 is (serine) which lead to change in the secondary structure as shown by black arrows which may lead to change in the corresponding antigenic region as predicted by Emboss Method.

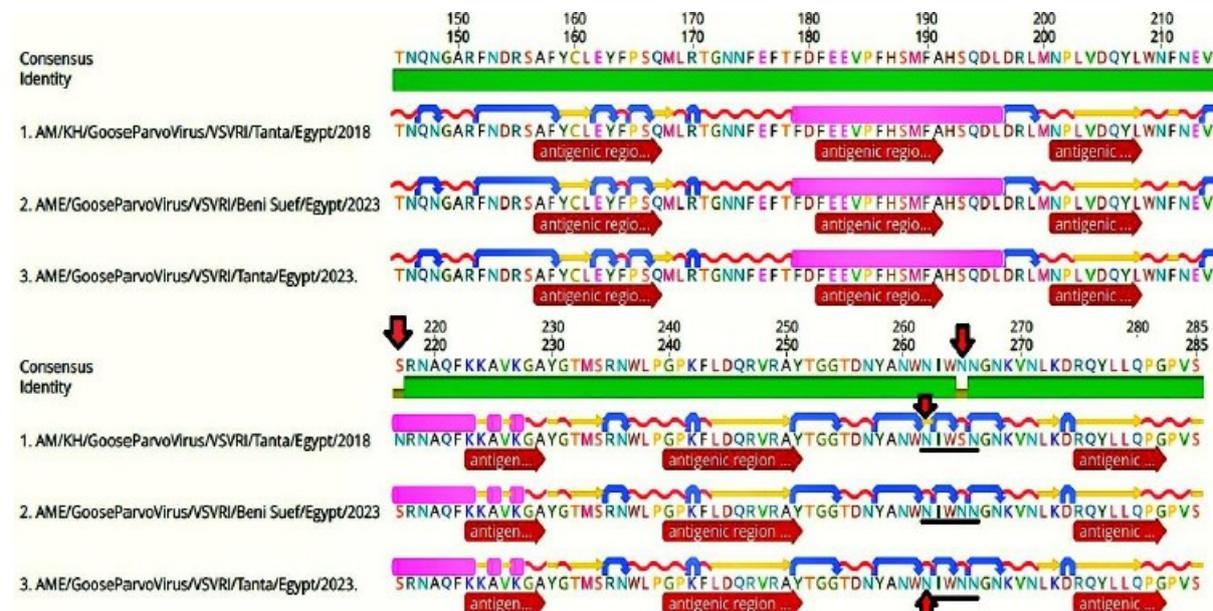


Fig. 9. Schematic representation of the amino acid sequence from amino acid number 141 which is corresponding to residue number (374) of the whole Vp1 protein of GPV to the residue number 285 which is corresponding to the residue number (518) of the whole Vp1 protein of GPV of the three local isolates showing the predicted antigenic regions and the secondary structures where there is significant difference at residue number 217 (aspartate) which is corresponding to the residue number 450 of the whole Vp1 protein in the local isolate AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 while the residue 217 in the other 2 local isolates AME/GooseParvoVirus/VSVRI/Beni Suef/Egypt/2023 and AME/GooseParvoVirus/VSVRI/Tanta/Egypt/2023 is (serine) , another change exist at residue 265 which is corresponding to the residue 498 in the whole genome ,in AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 which represented by (serine) while in the two other isolates is (aspartate) as predicted by Emboss Method.

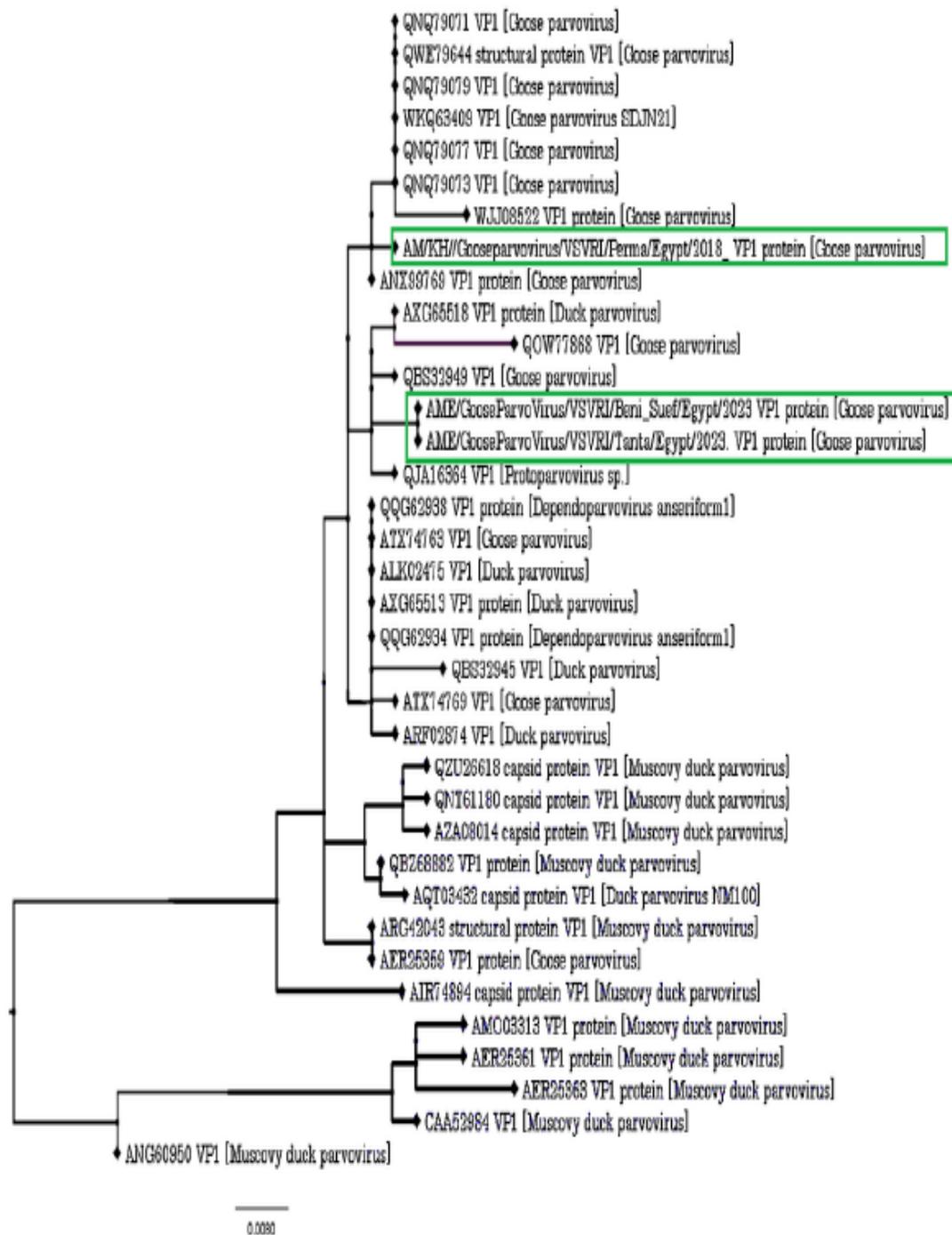


Fig.10. The phylogenetic analysis by maximum like hood method by bootstrap probabilities after 1,000 replicate trials and rooted with sequences of different genotypes using of the deduced amino acid sequence of 3 local isolates with other parvoviruses affecting *water fowl* where, the local isolate of 2018 AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 is located in different cluster rather than the other 2 local isolates of 2023 namely AME/GooseParvoVirus/VSVRI/Beni Suef/Egypt/2023 and AME/GooseParvoVirus/VSVRI/Tanta/Egypt/2023.

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التوصيف الجزيئي والتحليل الجيني لجين VP1 لمعزولة محلية للفيروس المسبب لقصر المنقار والتقرم في صغار البط المعزول في 2023 في مصر

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الملخص

فيروس بارفو الإوز (GPV) هو مرض شديد الضراوة يصيب الطيور المائية. تكمن أهمية GPV في قدرته على الانتقال الأفقي والرأسي وأيضاً من خلال من خلال تلوث قشرة البيض في المفقسات الملوثة. ولا يزال ظهور الفيروس في مصر وجميع أنحاء العالم غامضاً وغير مفهوم جيداً. لذا تهدف الدراسة الحالية إلى تقييم الوضع الوبائي الحالي لفيروس الإوز الصغير (GPV) الذي يسبب متلازمة المنقار القصير للبط ومتلازمة التقرم (SBDS) والتي تسبب خسائر اقتصادية فادحة في مصر في صغار البط والأوز من خلال العزل والتوصيف الجيني الكامل عن طريق تحليل التسلسل الجيني والتحليل المقارن للاختلافات الأنتيجينية بين السلالات المعزولة مؤخراً في عام 2023 والسلالة المعزولة خلال أوبئة 2018 والتي تم وصفها سابقاً على أنها Novel Goose parvovirus وتستخدم لتحضير اللقاح المحلي AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 رقم الدخول # OR854265 والسلالات المعزولة مؤخراً خلال أوبئة عام 2023 من مناطق مختلفة وهي AME/Gooseparvovirus/VSVRI/Tanta/Egypt/2023 رقم الدخول # PP058119 وAME/Gooseparvovirus/VSVRI/ بني سويف/مصر/2023 من فيروس بارفو الأوز المنتشر (GPV) رقم الدخول # OR878549، أظهر التحليل الجيني أن هناك تماثل 100% بين العزلات المحلية لعام 2023 بينما تشترك عزلة 2018 في التماثل بنسبة 98.1% مع تلك المعزولة عام 2023 ويوجد حوالي 16 موقع تغير في النوكليوتيدات بين عزلة 2018 المستخدمة في تحضير اللقاح المحلي وعزلات 2023 ثلاثة تغييرات بينهما فقط تؤدي إلى تحول الكودون بينما الباقي 13 اختلافات تؤدي إلى طفرات صامتة ولا تؤدي إلى تغيير الكودون مع عزلات أوبئة 2023 عادة ما تكون نفسها على المستوى الجزيئي والأنتيجيني على الرغم من أنهم تم عزلهم من مواقع مختلفة وهي متباعدة جداً عن بعضها البعض، أحدهما من طنطا (محافظة الغربية) شمال مصر في منطقة الدلتا والآخر من بني سويف وهي في صعيد مصر مما يدل على أن العزلة من عام 2023 هو فيروس بارفو الإوز الأكثر انتشاراً (GPV) خلال هذه الفترة، هذه التغييرات الثلاثة التي تؤدي إلى تحول الكودون تحدث في مواقع أنتيجينية مهمة جداً لبروتين السطح الخارجي VP1 الذي يحتوي على مواضع أنتيجينية مهمة مسؤولة عن الاستجابات المناعية ضد فيروس بارفو GPV. أولاً يوجد اختلاف في الحمض الأميني رقم 28 وهو ما يقابل الحمض الأميني رقم 261 الكامل لبروتين غلاف VP1 من (الانين) في المعزول عام 2018 إلى (سيرين) في المعزولات عام 2023 مما يؤدي إلى تغير في التركيب الأنتيجيني في هذا الجزء، ويحدث التغيير الثاني عند الحمض الأميني رقم 217 يكون aspartate في العزلة المحلية لعام 2018 والذي يقابل الحمض الأميني رقم 450 لكامل بروتين Vp1 لفيروس GPV بينما يكون سيرين في عزلات 2023، وفرق الكودون الثالث هو عند الحمض الأميني 265 وهو الموافق الحمض الأميني رقم 498 من كامل Vp1 وهو سيرين في العزلة المحلية لعام 2018 وهو حمض الأسبارتات في العزلات المحلية لعام 2023 وهذا التغيير له أهمية كبيرة لأنه يؤدي إلى تغير في التركيبات الخارجية الثانوية لبروتين Vp1 في هذا الموقع مما قد يؤدي إلى اختلاف في التركيب الأنتيجيني بين عزلات 2023 وعزلة AM/KH//Gooseparvovirus/VSVRI/Perma/Egypt/2018 المستخدمة لتحضير اللقاح محلياً، لذلك من الضروري تحديث اللقاح الحالي المعد محلياً بإضافة السلالات المعزولة حديثاً خلال أوبئة 2023 للقاح لتوفير حماية أفضل ضد فيروس GPV في مصر وإجراء مراقبة مستمرة للفيروسات المنتشرة الحالية لإجراء تقييم أفضل للحالة الوبائية الحالية في مصر لتحديث اللقاح بشكل مستمر للتعامل مع الوباء الميداني الحالي.

الكلمات الدالة: متلازمة المنقار القصير للبط ومتلازمة التقرم - بارفو الأوز - بارفو البط - جزيئه - طفرات - جديد.