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Detection and Characterization of Parvovirus Associated with Retarded Growth in Ducks in Al-Beheira Governorate, Egypt

Eid G.S. Hussein[*]; Hossam Sh. Elsebaey[*]; Ahlam E. Yonis[*]; Gomaa Y. Elhaddad[*]; Marwa A. Abdelmagid[**]; Moataz M. Elsayed[**]; Ayman H. Abd El-Aziz[***]

*Reference Laboratory for Veterinary Quality Control on Poultry Production, Damanhour Branch, Animal Health Research Institute, Agricultural Research Center, Damanhour, Egypt.
**Reference Laboratory for Veterinary Quality control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Giza. 12618, Egypt
***Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, Damanhour University, Damanhour22511, Egypt.

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ABSTRACT

Parvoviruses cause significant economic losses for domestic waterfowl producers. From June 2020 to January 2022, eight mule and four pekin duck farms were investigated for parvovirus in Al -Beheira governorate, Egypt. The affected ducks exhibited severe growth retardation with atrophied and deformed beak, a protruding tongue, profuse white diarrhea, locomotor dysfunction and lost their feathers, especially on the back, neck, and wing. The mortality rate was 10 %–18% and the morbidity rate was 60%–80% in affected flocks. Histopathological analysis of samples from mule and pekin ducks at different ages revealed multiple pathological alterations in various organs including; heart, liver, spleen, lung, trachea, intestine, kidneys, even tongue and brain and consistent with clinical picture. Six out of twelve duck flocks were positive for parvovirus genome by PCR. These viruses were isolated from pooled fecal swabs and caused a cytopathic effect in duck embryo liver (DEL) cells. Phylogenetic analysis of the partial VP1 gene indicated that five of current Egyptian parvovirus strains belonged to the Novel goose parvovirus (NGPV) group and clustered with both Chinese NGPV strains and previously identified Egyptian NGPV strains and one strain belonged to the duck adeno-associated virus and clustered with the Australian duck adeno-associated virus DA. To the best of our knowledge, this can be considered the first record of duck adeno-associated virus infection in mule duck in Al-Beheira governorate.

Corresponding author: Eid G.S. Hussein, Avian Viral Diseases, Reference Laboratory for Veterinary Quality Control on Poultry Production, Damanhour, Animal Health Research Institute, ARC.
E-mail address: dreid.hussein@gmail.com
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INTRODUCTION:

Goose parvovirus (GPV) and muscovy duck parvovirus (MDPV) are the two types of waterfowl parvovirus infections. Derzsy's disease caused by GPV that infects both goslings and Muscovy ducks (Derzsy et al. 1970) with common clinical manifestations of growth retardation, watery diarrhea and high mortalities (Jansson et al. 2007; Woźniakowski et al. 2012).While Muscovy duck parvovirus (MDPV) was recorded in Muscovy ducks with similar symptoms to those of GPV (Chang et al. 2000; Zádori et al. 1995), with up to 85% nucleotide identity to GPV (Takehara et al. 1994). Both GPV and MDPV usually show 70 –100% morbidity and mortality during the first 3 to 4 weeks of age (Glávits et al. 2005; Jansson et al. 2007). Waterfowl parvovirus is a non -enveloped virus. The viral genome consists of a single-strand DNA with around 5100 base pairs.Two open reading frames are present in the translated regions (ORFs). The nonstructural protein is encoded by the left ORF, and the capsid proteins VP1, VP2 and VP3 are by the remaining ORF. (Zadori et al., 1994; Zádori et al. 1995).

As GPV and MDPV have 85% of the same protein sequence, they are both antigenically linked to one another (Poonia et al. 2006).Also,they exhibit nucleotide changes in VP1 of roughly 20:24%, in contrast to the GP V and MDPV groups, where these differences in VP1 are just 0.1-7% and 0.1–1.9%, respectively. Short beak and dwarfism syndrome (SBDS) was firstly identified in mule ducks in French in 1970s, and subsequently followed by epidemics in Taiwan in 1989, Poland in 1995 (Palya, 2013; Woźniakowski et al. 2009), Hungary in 2009 (Palya et al. 2009) and China in 2014 (Chen et al. 2016) and recently in Cherry Valley ducks in China (Yu et al. 2016). Affected ducks showed atrophied beak, retarded growth and protruded tongue (Palya et al. 2009).

New variant strains can be created by genomic recombination between different waterfowl parvoviruses and result in new epidemics (Li et al. 2021). Novel goose parvovirus (NGPV) is the causative agent of SBDS which shared the highest homology with goose parvovirus (GPV) and was considered as a variant of GPV (Li et al. 2016; Ning et al. 2017; Palya et al. 2009). In Egypt, SBDS was reported in 2017 in Sharqya and Gharbya governments (Saleh and Khoudeir, 2020). Moreover, Adeno -associated virus (AAV; genus *Dependoparvovirus*, family *Parvoviridae*) was first discovered in 1965 as a contaminant in adenovirus preparations (Muzyczka and Berns, 2001). The AAV group and the waterfowl parvovirus group belonged to the genus Dependoparvovirus (Zhu et al. 2014). Only 4 AAV strains were found to be of avian origin (avian adeno-associated virus; AAAV); one of these viruses, VR-865, was recovered from quail, while the other three were from chickens (DA-1, YZ-1, and ZN1). AAV is a non-enveloped virus with a diameter of 20 to 25 nm and a 4.7 kb ss DNA genome with equal plus and minus polarity. (Berns and Adler, 1972; Mayor et al. 1969) Muscovy duck parvovirus caused multiple pathological lesions included necrotic hepatitis, enteritis , epicarditis and intranuclear inclusion bodies within myocardial tissue (Mahardika et al. 2015; Poonia et al. 2006), and similar pathological alterations in addition to skeletal muscle fiber degeneration were observed in duckling infected with GPV (Ning et al., 2017).Spleen, lung, cecum and brain necrosis, as well as, erosion of the villus tips of the intestine were recorded in cherry valley ducks infected with duck-origin parvovirus (Chen et al. 2018). From June 2020 to January 2022, numerous mules and pekin duck flocks in different duck-producing regions in Al-Beheira governorate, Egypt have shown marked signs of retarded growth, short beak and protruded tongue. Therefore, the aim of present study is molecular and pathological analysis of parvovirus.

MATERIALS and METHODS

Ethical approval:

Ethical approval was approved by the (ARC-IACUC) committee : ARC-AHRI-22-36.

History of investigated duck flocks:

From June 2020 to January 2022, eight mule and four pekin duck farms in different duck-producing regions in Al-Beheira gov-

ernorate were investigated for parvovirus. The affected ducks in these farms exhibited severe growth retardation with atrophied and deformed beak, a protruding tongue, profuse white diarrhea, locomotor dysfunction and lost their feathers, especially on the back, neck, and wing. The mortality rate was 10 % –18% and the morbidity rate was 60%–80%. Farms capacity ranged from 1000-3000 ducks/farm and not vaccinated against parvoviruses. The ducks under examination ranged in age from 20 to 105 days.

Samples collection.

A total of 240 samples (fecal swabs and multiple organs) were collected and subjected to molecular and histiopathological analysis for parvovirus detection.

Prepration of samples:

Molecular detection:

Fecal swabs were collected from 10 diseased ducks from each flock then suspended in phosphate buffered saline containing 5,000 IU / ml penicillin G, 5 µg/ml amphotericin B, and 5 mg/ml streptomycin (Sigma Chemical Co., St. Louis, MO), pooled, and centrifuged at 3000 rpm for 20 minutes. The supernatant was filtered through a 0.22 µm filter and kept in -80° C until used to detect viral DNA by Polymerase chain reaction (PCR) according to **(Vibin et al. 2018)**

Viral DNA Purification:

Viral DNA was purified from 200 µl of pooled fecal swabs from each infected farm by the QIAamp DNA mini kit (Qiagen, Gmbh, Hilden, Germany) following the manufacturer's recommendation.

PCR Amplification:

PCR reactions were performed in 25 µl reaction tube using Emerald Amp Max PCR Master Mix (Takara, Japan) in a Biometra T3000 thermocycler (Biometra, Gmbh, Germany). PCR targeted a 593 bp fragment of the VP1 gene using specific primers. (forward: 50-CCTGGCTATAAGTATCTTGG 30;reverse:50GTAGATGTGGTTGTTGTAGC -30) **(Poonia et al. 2006).** The PCR assay started with a primary denaturation step at 95 ^{O}C for 5 min, followed by 35 cycles (95 ^{O}C for 30 s, 55 ^{O}C for 40 s, 72 ^{O}C for 45 s), and a final extension step at 72 ^{O}C for 10 min. PCR products were inoculated into 1.5% agarose, and a gene ruler 100 bp ladder (Fermentas, Thermo scientific, MA, USA) was used to determine PCR assay specificity. The gel photo was captured using a gel documentation system (Alpha Innotech, Biometra, Gmbh, Germany) and the obtained photos were analyzed using Automatic Image Capture Software (Cell bioscience, USA). Synthetic oligonucleotides fragment was synthesized by Bio Basic, Markham, Canada, to serve as a PCR positive control (based on NGPV representative strain JS1603, GenBank accession MF441226).

DNA Sequence:

A QIAquick PCR Product extraction kit (Qiagen, Gmbh, Hilden, Germany) was used to purify the 593 bp PCR products of the positive samples. The BigDye Terminator V3.1 cycle sequencing kit (Applied Bio-systems, Life Technologies, Thermofisher, MA, USA) was used to perform sequence reaction. The sequence reaction was then purified using Centrisep spin columns (Thermofisher, MA, USA). Sequence chromatograms were retrieved from a 3130 genetic analyzer (Applied Bio-systems, Life Technologies, Thermofisher, MA, USA). To determine sequence identity to GenBank published parvoviruses, a BLAST® search (Basic Local Alignment Search Tool) was performed (**Altschul et al. 1990).** A MegAlign module of Lasergene DNAStar was used to determine sequence identities among analyzed strains (**Thompson et al. 1994**). A neighbor-joining phylogenetic tree was constructed using the maximum composite likelihood model with 1000 bootstrap in MEGA6 **(Tamura et al. 2013).**

Virus isolation:

A. Preparation of duck embryo liver (DEL) cells:

DEL cells were prepared from 14-16 day old duck embryos **(Chen et al. 2016**). In brief, the livers were removed and minced into small pieces, then washed with phosphate-buffered saline (PBS) with constant stirring for three

times (five minutes per each wash). This was followed by three successive trypsinization cycles (five minutes each). The supernatant was collected following each cycle on complete growth medium, and then eventually centrifuged at 1500 rpm for 10 minutes at 4°C. Cells were seeded at initial cell count of 1 x $10^6$for T25 flask and cultured with DMEM supplemented by 10% fetal bovine serum and 1% penicillin-streptomycin antibiotic. The flasks were incubated at 37.5°C with 5% CO_2.

B. PCR positive samples were inoculated into DEL cells and monitored daily for cytopathic effects. The culture supernatants were harvested at 5 days post-inoculation and the virus was detected using PCR then stored at −80°C as a viral stock.

Histopathology:

Multiple organs were collected and fixed in 10% neutral buffered formalin and processed by paraffin embedding techniques (Rhodes, 2012).

RESULTS:

Epidemiological data:

From June 2020 - January 2022, A total number of twelve suspected Parvoviruses affected commercial duck farms (8 Mule and 4 pekin) located in different duck-producing regions of Al-Beheira governorate, Egypt were investigated and the obtained data of positive samples were presented in **Table (1)**.The owners complaints were mostly limited in retarded growth, locomotors dysfunction and mortality rates varied from 10 to 18 %.

Table 1. Epidemiological data of positive samples:

Serial. No	ID	Accession No	Identified agent	Date	District	Duck breed	Age (day)
2	BH-21-1	OM817452	NGPV	Oct.21	El Mahmoudiya	Pekin	105
7	BH-20	OM817457	DAAV	Jun.20	Edco	Mule	65
9	BH-21-2	OM817453	NGPV	Dec.21	Itay Elbaroud	Mule	25
10	BH-22-1	OM817454	NGPV	Jan.22	Damanhour	Mule	35
11	BH-22-2	OM817455	NGPV	Jan.22	Abu El Matamir	Mule	49
12	BH-22-3	OM817456	NGPV	Jan.22	Dillingat	Mule	20

NGPV: Novel goose parvo virus**.**
DAAV: Duck adeno-assocated virus

Clinical symptoms and Postmortem Findings:

Most obvious symptoms of the affected pekin and mule ducks were strong growth retardation, lost their feathers, especially on the back, neck, and wing (Fig.1.A), atrophied and deformed beaks with a protruding tongue and edematous sinuses (Fig.1.B), profuse white diarrhea, locomotor dysfunction including weakness, lateral recumbency, and inability to walk. while most characteristic post mortem findings were myocarditis with hemorrhagic patches (Fig.1.C), enteritis (Fig.1.D), enlarged and congested liver with fibrinous membrane on the surface (Fig.1.E).

Fig. (1) Mule ducks showing growth retardation and loss of feathers on the back and wing (A) ,short beaks and protruded tongue (B),myocarditis with hemorrhagic patches (C), enteritis (D), enlarged and congested liver with fibrinous membrane on the surface (E).

Identification of causative agent by PCR:

Six out of 12 samples were tested by polymerase chain reaction samples showed positive amplification of a 593 bp fragment of the VP1 gene for the DNA purified from Fecal swabs collected from diseased duck flocks

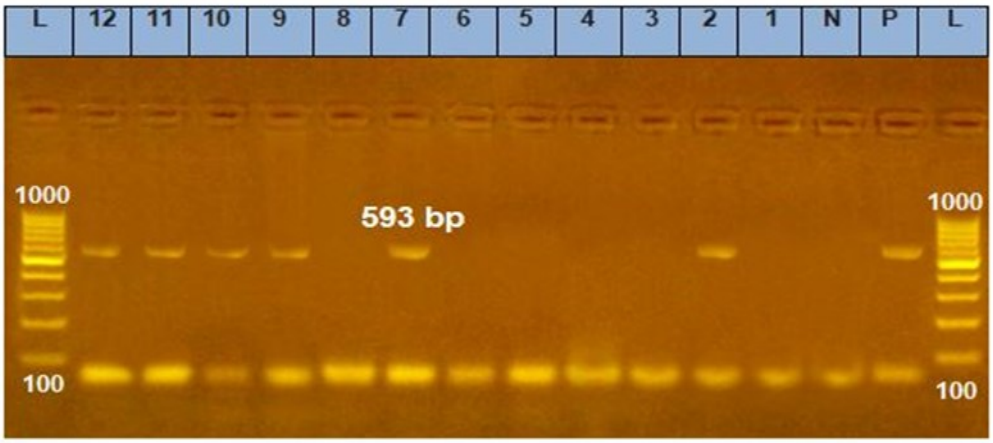

Fig. (2): shows PCR of parvovirus-VP1 gene. Lane L: 100bp DNA ladder, Lane P: positive control, Lane N: negative control, Lane 2, 7,9,10,11 and 12: samples (positive samples).

Nuclic acid Sequence and Phylogenetic Analysis:

DNA sequencing for 593 bp of VP1 gene from 6 positive isolates were performed. Sequences were assigned (Gen Bank accessions OM817452-OM817457).As shown in (Figure 4), results of phylogenetic Analysis revealed that five of the sequenced isolates which assigned (Gen Bank accessions OM817452-OM817456) were clustered with the novel goose parvovirus (NGPV) strains. Whereas, the sequence distances tool of the Laser gene DNA Star software revealed 95.5–100% nucleotide sequence identity among this five samples. while they showed nucleotide sequence identity ranged from 99.7-100% with NGPV representative strain RLQP1-4 (Gen Bank accession MT646477-MT646480), however they showed 81.6% identities with the MDPV representative strain (Gen Bank accession KU844281 and KT 865605).Also, this five samples showed nucleotide sequence divergence ranged from 8.1-8.3% with representative GPV strain (HQ891825GPV GDa GPV). While, one of the sequenced isolates which assigned GenBank accession OM817457 was clustered with the duck adeno -associated virus group. This sequenced strain showed nucleotide sequence identity 42.8 % with the other five samples grouped within NGPV. But it showed nucleotide sequence identity 96.5% with representative strain duck adeno-associated virus DA (GenBank accession MW380871) (Fig.3).

On amino acid level, the nucleotide alignment report showed a close amino acid patterns of the five NGPV isolated strains (Gen Bank accessions OM817452-OM817456) with compared samples on Gen bank data base NGPV strains (MT646477and MT646478). The alignment of this five in comparison with the reference GPV strain showed four constant amino acid substitutions at positions Q34L, A38S, D87E and V89I. whereas, the detected duck adeno-associated strain (Gen bank accession number OM817457) has multiple mutation sites in compared with reference GP.V strain and NGPV strains, but showed a close amino acid patterns with the reference duck adeno-associated strain (Gen bank accession MW380871).(Fig.5) .

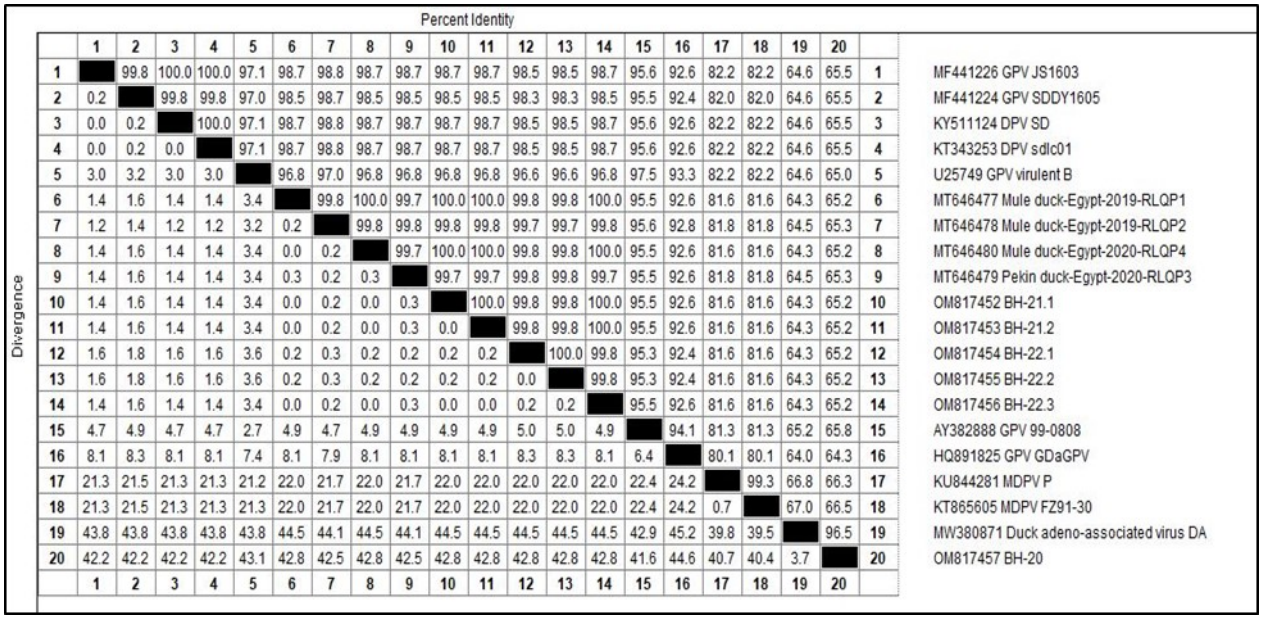

Fig.(3): Nucleotide identities of the VP1 gene for the current strains (GenBank accessions OM817452-OM817457) (with selected reference strains).

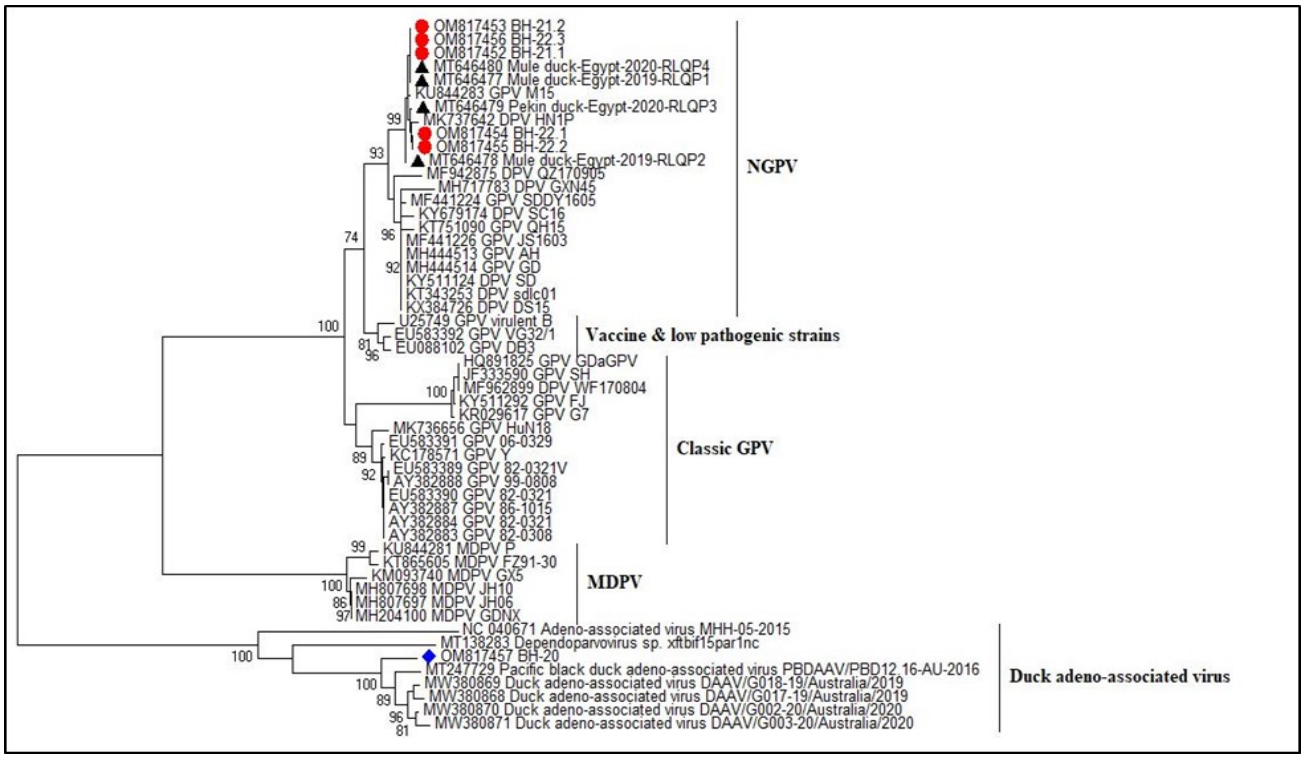

Fig. (4): Phylogentic tree of the VP1 gene for five current NGPVs strains (Gen Bank accessions OM817452 -OM817456) and one duck adeno –associated virus strain (Gen Bank accessions OM817457) with sequences available in the Gen Bank database. The phylogenetic tree was constructed using the neighbor-joining algorithm with1000 bootstrap replicates. Bootstrap values are shown on the tree. Note: The current NGPVs strains were marked with a red circle and one duck adeno –associated virus strain was marked with a blue square.

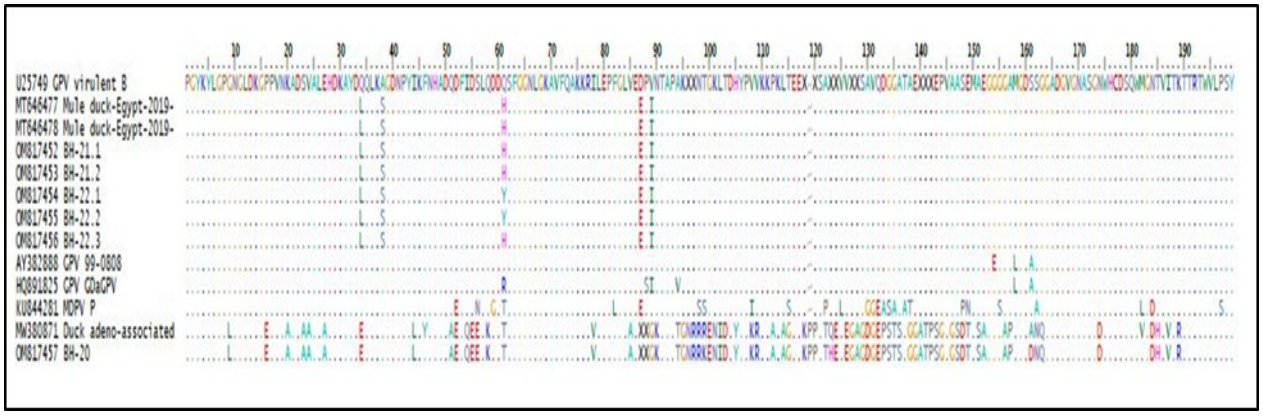

Fig.(5): Amino acid alignment report created by Bioedit software showing close amino acids patterns of five representative strains (Gen Bank accessions OM817452-OM817456) with both NGPV strains (MT646477and MT646478).while the recorded other strain (Gen Bank accessions OM817457) showing close amino acids patterns with duck adeno-associated strain (Gen Bank accessions MW380871).

Virus isolation in duck embryo liver cells (DEL):

PCR positive samples were successful isolated on primary duck embryo liver (DEL) cell culture. The cytopathic effects were rounding, clumping and detachment of cells (Fig. 6). Positive PCR results for the harvested tissue culture fluids confirmed the successful isolation of the virus on DEL.

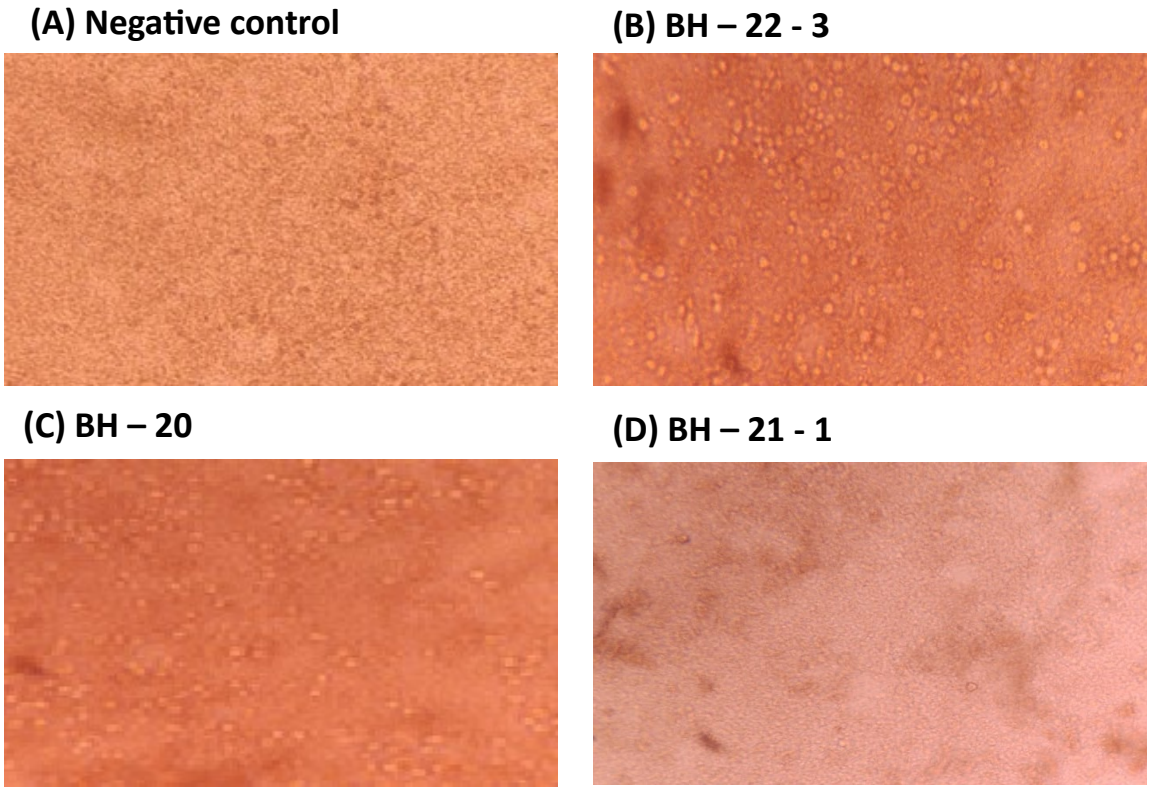

Fig. (6): Viral inoculation onto duck embryo liver (DEL) cell culture: (A) non-infected DEL cell monolayer (negative control). (B, C & D) Infected cells with Novel goose parvo virus (BH-22-3), duck adeno-associated virus (BH-20) and Novel goose parvo virus (BH-21-1), respectively, showing significant cytopathic effect (CPE) rounding, clumping and detachment at 72 HPI

Pathology results:

Most examined organs from mule ducks at different ages showed variable pathological alteration (Fig. 7); the intestine showed necrosed villi and marked lymphocytic cells infiltrations (Fig. 7-1). The pancreas characterized by edema, acinar degeneration and necrosis (Fig.7-2) with obvious mononuclear inflammatory cells infiltration. The liver revealed different severities of hepatocytic degeneration and lymphocytic cells infiltration with noticeable apoptotic figure in liver of ducks at 65 days (Fig. 7-3). Kidneys were showed severe tubular degeneration and necrosis which replaced by mononuclear cells infiltration (Fig. 7-4). Tongue of ducks at 65 days was more distinctive with severe edematous connective tissue layer with diffuse lymphocytic cells infiltration and congested blood vessels (Fig. 7-5). The heart revealed myocarditis and focal necrosis with obvious edema and mononuclear cells infiltration (Fig. 7-6). Brain of ducks at age of 65 days didn't showed significant changes except dilatation of blood vessels and perineuronal edema (Fig. 7-7). Respiratory organs revealed mild to moderate pathological alteration graded from tracheitis with submucosal edema with lymphocytic cells infiltration (Fig. 7-8) to severe necrosis (Fig. 7-9); while the lung showed moderate inflammatory reaction with congested blood vessels and mononuclear cells infiltration inside secondary and tertiary bronchi (Fig. 7-10).

In case of pekin ducks (Fig. 8); most of organs showed pathological alterations; the kidneys were the most affected organ showed severe necrosis of renal tubules replaced by lymphocytic cells infiltration (Fig. 8-11), other distinctive figure was observed in heart that showed obvious myocardidtis in addition to coagulative necrosis of the skeletal muscle (Fig. 8-12), while liver revealed hepatic degeneration and lymphocytic cells infiltration (Fig. 8-13).the tongue showed severe inflammatory reaction with necrosis of parakeratinized layer and edema of lamina propria which infiltrated with obvious lymphocytic cells (Fig. 8-14). The brain was characteristic with gliosis and perivascular edema (Fig. 8-15). The intestine revealed severe enteritis with marked lymphocytic cells infiltration and loss of villi (Fig. 8-16). Other microscopic alterations included spleenitis with lyphocytic depletion (Fig. 8-17), tracheitis (Fig. 8-18) and pneumonia in the lung (Fig. 8-19); as well as, pancreatic necrosis (Fig. 8-20).

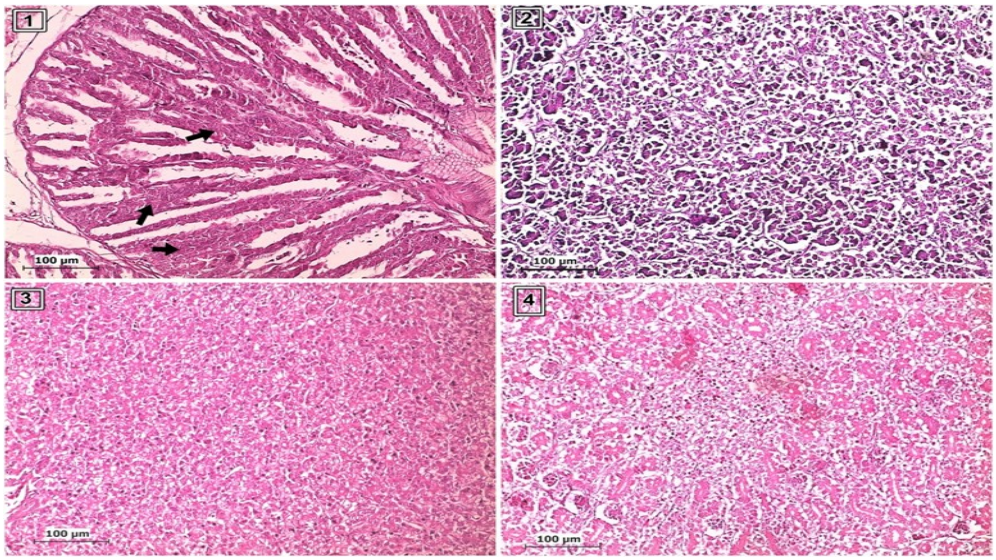

Fig. (7): Pathological changes in mule ducks: Necrosis of intestinal villi with massive lymphocytic cells infiltration (arrow) (1), pancreatitis with acinar degeneration (2), hepatic degeneration with marked apoptosis (3), kidney with tubular degeneration and necrosis with massive mononuclear cells infiltration (4).

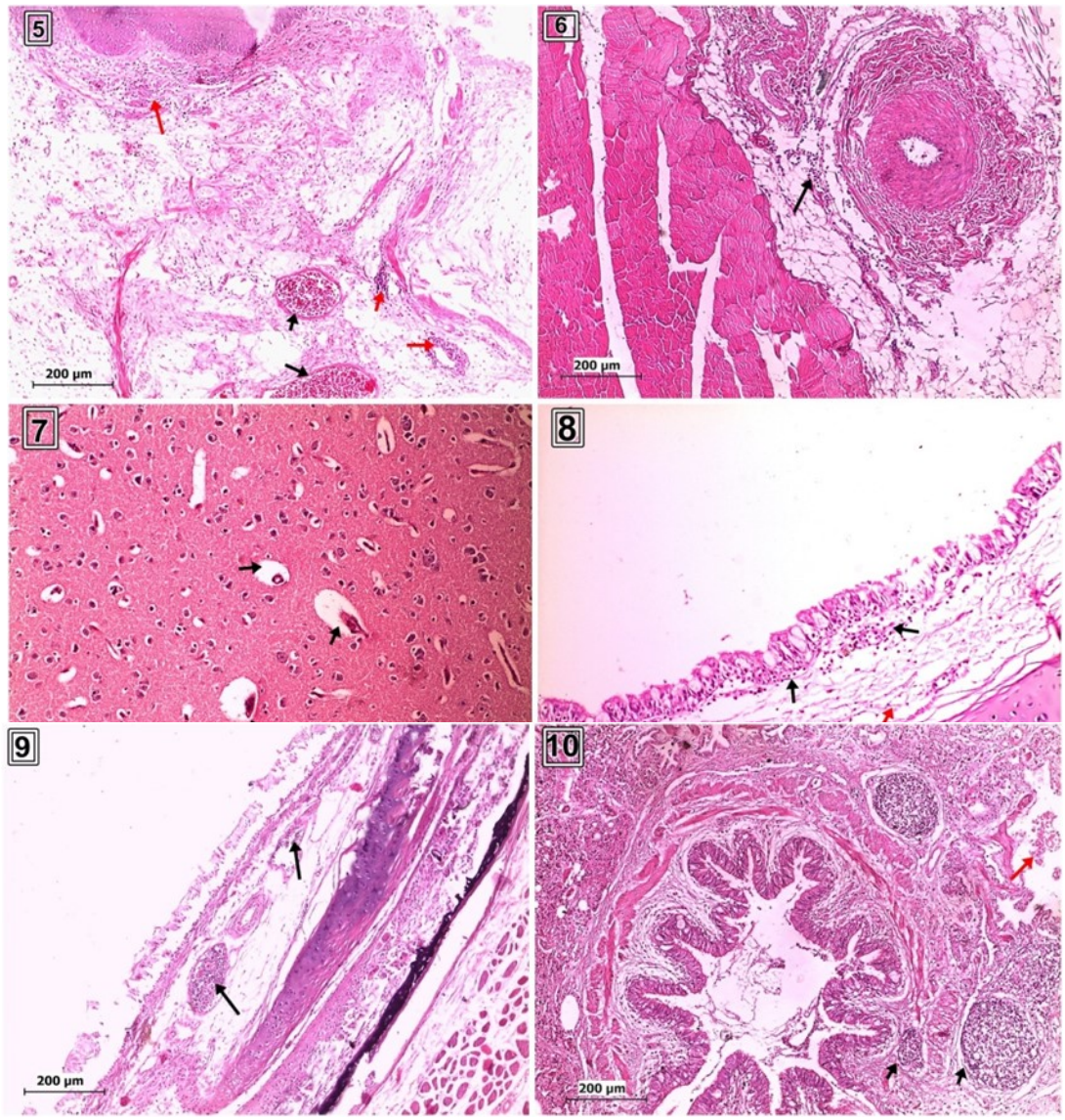

Fig. (7) Continue: Tongue showed severe edematous connective tissue layer with diffuse lymphocytic cells infiltration (red arrow) and congested blood vessels (black arrow) (5), myocarditis with massive lymphocytic cells infiltration (arrow) and vasculitis (6), brain with perineuronal edema (arrow) and neurophagia (7), tracheitis with massive submucosal edema (red arrow) and lymphocytic cells infiltration (black arrow) (8), severe tracheitis with necrosis of lining epithelium and massive lymphocytic cells infiltration (arrow) (9), lung showed pneumonia with abundant mononuclear cells infiltration (black arrow) and inflammatory exudates inside secondary bronchi (red arrow) (10).

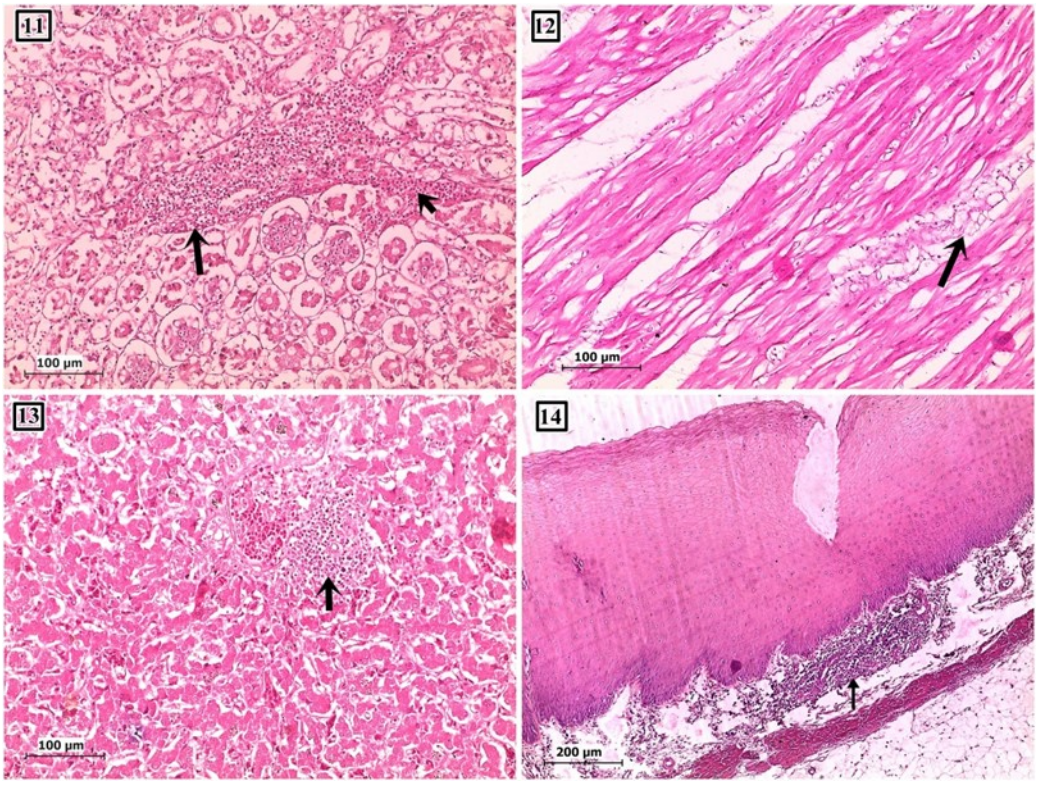

Fig. (8):Pathological changes in Pekin ducks: kidney with severe necrosis of renal tubules replaced by abundant lymphocytic cells infiltration (arrow) (11), heart with myocardial edema with focal necrosis (arrow) (12),liver showed hepatocytic degeneration with focal lyphocytic cells infiltration (arrow) (13), tongue with obvious lymphocytic cells infiltration in parakeratinized layer (arrow) with edema in lamina propria (14).

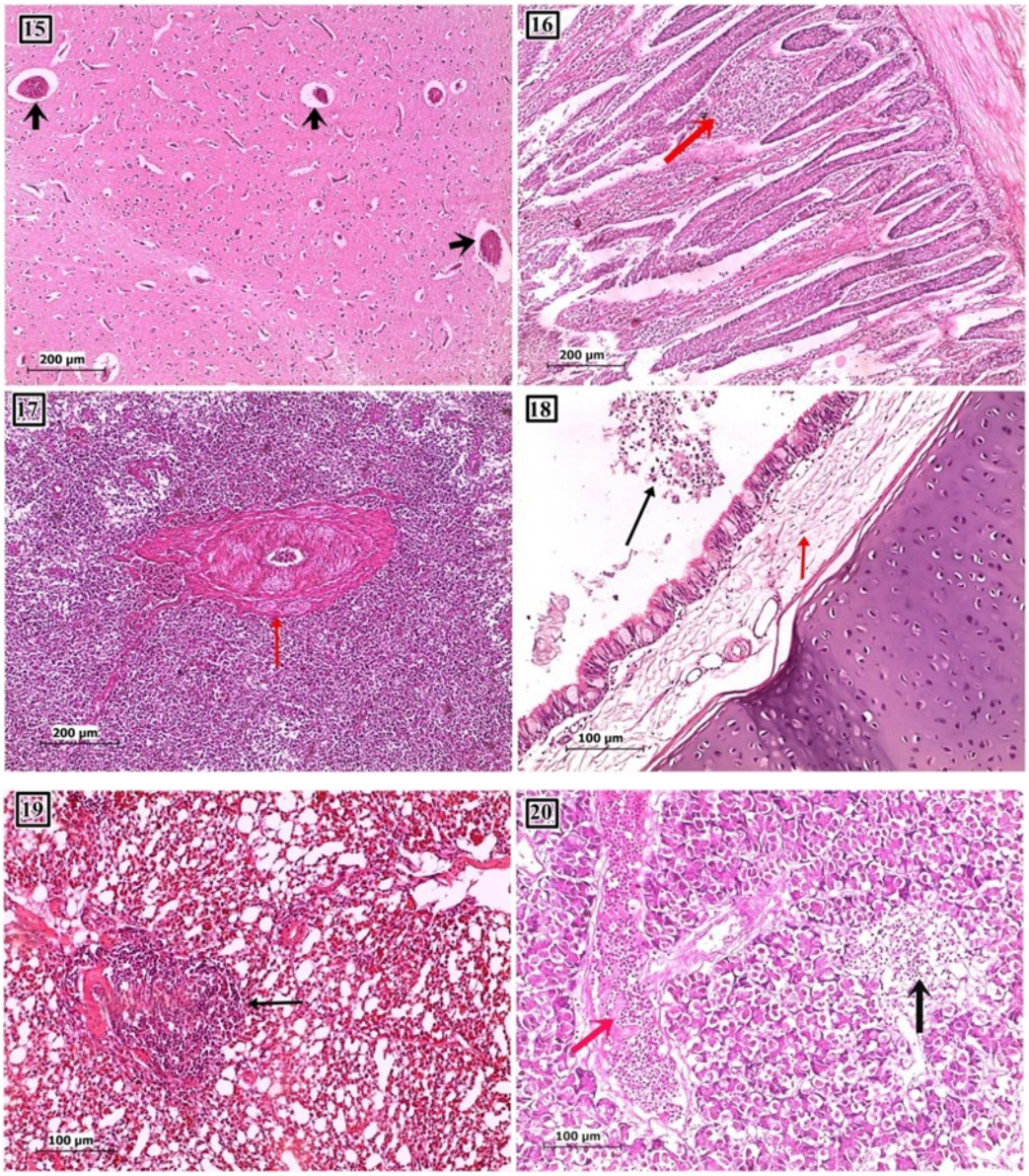

Fig. (8) Continue: Brain revealed moderate gliosis with perivascular edema (arrow) (15), intestine showed severe enteritis with massive lymphocytic cells infiltration (arrow) (16), spleen with spleenitis and vasculitis (arrow) (17), trachea showed tracheitis with submucosal edema (red arrow), mononuclear cells infiltration (black arrow) (18), lung showed pneumonia with massive lymphocytic cells infiltration (arrow) (19), pancreas with pancreatic necrosis infiltrated with lymphocytic cells (black arrow) and congested blood vessels filled with lymphocytic cells (red arrow) (20).

DISCUSSION:

Parvoviruses spread among duck farms in Egypt causing increase of mortalities and retarded growth so that it become necessary to focus on studying and control of parvovirus infection in Egyptian duck farms (**Soliman et al. 2020**). As the Egyptian Delta has the largest duck sector in Egypt, one large delta governorate (Al-Beheira) was selected in this work for detection of parvoviruses incidence, genetic, biochemical and pathological analysis of obtained viruses. In this study, from May 2020 to April 2022, a total of 12 diseased Mule and pekin duck farms presenting typical symptoms of retarded growth, short beak and protrouded tounge that indicated SBDS infection and similar to

those reported by **(Palya et al., 2009; Saleh and Khoudeir, 2020; Soliman et al., 2020).**

The variation in mortalities (from 10% to 18%) could depend on age, immune status and susceptibility of duck's breed, as well as virus pathogenicity. Infection of investigated duck farms were confirmed by PCR methods as six samples out of 12 showed positive amplification of a 593 bp fragment of the VP1 gene .Based on standard PCR and serological techniques, it is difficult to differentiate between GPV and MDPV due to the significant amino acid sequence similarity and antigenic cross-reactivity **(Zádori et al. 1995). As VP protein contains** viral antigenic sites, it is crucial in stimulating the body's immune response to the virus (Berns, 1990). Phylogenetic analysis based on nucleotide sequence of the partial VP1 gene was done to classify various strains under investigation, and it showed clear clustering of five strains (Gen Bank accessions OM817452-OM817456) within the NGPV group. Interestingly, and as a first record ,it is the first study that reports duck adeno-associated virus (AAV) infection in Egyptian mule ducks as one isolated sequenced sample (Gen Bank accessions OM817457) showing clear clustering with duck adeno-associated viruses (Gen Bank accessions MW380871) **(fig.3 - fig. 4)** . the current strain Gen Bank accessions OM817452) that clustered within NGPV were isolated from pekin duck and closely related to strain MT 646479 isolated from pekin ducks suffering from retarded growth, while the other four current strains Gen Bank accessions OM817453-OM817456) related to NGPV were isolated from mule ducks and were closely related to strains MT646477, MT646478 and MT646480 that were isolated from mule ducks (**Soliman et al. 2020).**

In the current study, there is no significant difference in sequence identity of the partial VP1 gene among NGPV isolated from mule duck and those isolated from pekin duck, as they share 95.5–100% identity. The same results mentioned in a previous study when comparison was carried out between full VP1 gene of NGPV from mule duck and Cherry Valley duck **(Bian et al. 2019).**

While the recorded duck adeno-associated virus strain GenBank accessions OM817457 showed nucleotide sequence divergence 42.8% with the isolated NGPV strains whereas it showed nucleotide sequence identity 96.5% with the Australian strain duck adeno-associated virus DA (Gen Bank accessions MW380871) and the chines isolate adeno-associated virus MHH-05-2015 (Gen Bank accessions NC 040671). However, it was not known exactly how those newly isolated strain was introduced into duckling farms in Egypt. The possible emergence of the identified duck adeno-associated virus strain from GPV strain previously infected this farm at young age especially the infected ducks exhibited typical clinical signs of SBDS with regarding the newly isolated adeno-associated virus strain showed nucleotide sequence divergence 42.8% with the isolated NGPV strains and multiple mutation sites in compared with reference GPV strain and NGPV strains. This supported by **Kailasan et al. (2015)** who reported that parvoviruses had high evolution rates similar to RNA viruses, and cell passages with very small numbers could induce the selection of new natural mutants .

Histopathological results of different field samples from mule and pekin ducks at different ages revealed multiple pathological alterations in various organs including; heart, liver, spleen, lung, trachea, intestine, kidneys, even tongue and brain. This finding comparable to previous records in experimental study on parvovirus in ducks (**Chen et al. 2018**) which explained the wide spread of the virus in most internal organs even the brain. Concerning to mule ducks; the most affected organs showed severe alterations rather than tongue were lung, trachea especially in older ducks. Also, brain and kidneys showed pathological lesions which similar to previous study (**Ning et al. 2017).** Our finding of pancreatic edema with acinar necrosis lead to obstruction of pancreatic ducts resulting in

atrophy of pancreas, and as the pancreas is the major source of digestive enzymes, an obstruction of the pancreatic ducts interferes with the production of digestive enzymes that results in the disturbance of the digestive process similar to explanation of **(Nili et al. 2007; Qamar et al. 2013).**

Pathological alteration of digestive organs believed to be the main cause of retarded growth and thus economic losses in case of parvovirus due to maldigestion and malabsorption of nutrients and electrolytes.

CONCLUSION:

This study has contributed better understanding of molecular and pathological analysis of NGPV and duck adeno-associated strains associated with retarded growth in mule and pekin ducks. Further studies are required to explore the effectiveness of currently available vaccines against these viruses and to develop a new vaccine against them. As well as investigation the possible role of duck adeno-associated in SBDS.

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