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Isolation and Characterization of Fowl Adenoviruses Associated with Hydro-pericardium Syndrome from Broiler Chickens in Egypt

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ABSTRACT

One of the most prominent viral diseases affecting the poultry industry is hydropericardium syndrome caused by fowl adenoviruses. Hydropericardium syndrome has recently led to significant economic losses in the Egyptian poultry sector. Many outbreaks of hydropericardium syndrome have been documented across the country in the last few years. This research examined the epidemiology and molecular characterization of fowl adenoviruses in broiler chickens in Egypt. Samples were taken from 26 outbreaks of commercial broiler chicken farms in the Beheira and Menofia governorates, Egypt, from January 2021 to March 2022. Adenoviruses genomes were detected in cloacal swabs of 10 flocks using polymerase chain reaction. Clinically, infected broiler chickens (Cobb, Ross, Indian River, Modified-Avian, and Arbor Acres) showed depression, ruffled feathers, retarded growth, and ascites, with mortality rates of 10-28%. The most common postmortem lesions were hydropericardium, yellowish enlarged liver with ecchymotic hemorrhages, pancreatitis, and enteritis. Histopathologically, intranuclear inclusion bodies, commonly basophilic type, were scattered in the hepatocyte, proventriculus, duodenum, kidney, pancreas, and spleen. In addition, depletion of lymphocytes in the bursa of Fabricius and the thymus was observed. Seven samples were selected for gene sequencing of the loop 1 region of the hexon gene. The sequence analysis revealed that all samples were identical and similar to fowl adenoviruses species D serotype 2/11, suggesting that this serotype was the predominant fowl adenoviruses circulating in the study location in the last two years. Further studies are required to address the pathogenicity of isolated fowl adenoviruses and evaluate the vaccine used to control fowl adenoviruses in Egypt.

Keywords: Fowl adenoviruses, Hexon, Hydropericardium syndrome, Phylogenetic analysis, Polymerase chain reaction

INTRODUCTION

Fowl adenoviruses (FAdVs) belong to the genus *Aviadenovirus*in the family Adenoviridae. FAdVs are serologically classified into five species (FAdV-A to FAdV-E) according to restriction enzyme digestion pattern (Hess, 2000) and 12 serotypes (FAdV-1 to -8a and -8b to -11) based on serum cross-neutralization tests (Meulemans et al., 2004). The most common diseases caused by FAdVs-infection are inclusion body hepatitis (IBH), hydropericardium syndrome (HPS), and gizzard erosions (Hess, 2000). Hydropericardium syndrome is the most serious form, as it causes a high mortality rate (Kataria et al., 2005).

Hydropericardium syndrome was first observed in Angara Goth, Pakistan, in 1987 and has been named hydropericardium–hepatitis syndrome/Angara disease in Pakistan, Litchi heart disease in India, or inclusion body hepatitis-hydropericardium syndrome (IBH–HPS) in other places (Anjum et al., 1989). This disease affects broiler chickens at 3-6 weeks of age and can be transmitted vertically from infected chicken to young broiler, which becomes more susceptible to infection (Schachner et al.,

2021). The symptoms include sudden onset, anemia, retarded growth, depression, crouching position with ascites, which can lead to an increased mortality rate of up to 60% and immune depression (McFerran and Adair, 2003; Hafez, 2011). Due to their possible immunosuppressive properties, FAdVs can make chickens more susceptible to other microbes by decreasing humoral and cell-mediated immunity (Schonewille et al., 2008). The most prominent lesions are an enlarged, hemorrhagic, friable liver, hydropericardium with a flabby congested heart, swollen and hemorrhagic kidneys, and mottled spleen (Cizmecigil et al., 2020). Pathologically, HPS is characterized by massive degeneration and necrosis of the liver, heart, kidney, and lung with large basophilic intranuclear inclusion bodies in the liver and severe hyperemia and edema in kidney and lung (Maartens et al., 2014; Niu et al., 2018).

FAdVs Species D and E have mainly been associated with outbreaks of IBH (Mohamed et al., 2018; Kiss et al., 2021), while most HPS is caused by (species C) serotype (4, 9 and 10), especially the virulent serotype (FAdV-4; Schachner et al., 2014). However, Chen et al. (2017) reported that any of the twelve FAdV serotypes could cause IBH or HPS in broiler chickens, with a mortality rate ranging from 10% to 60%.

Conventional and molecular methods are employed to diagnose IBH/HPS, followed by sequencing to differentiate FadVsserotypes (Mittal et al., 2014). The serotype-specific gene sequence of the adenovirus is the hexon gene. It is a major adenovirus protein that possesses the neutralizing epitope used for serotyping FAdV (Niczyporuk, 2018). Since the FadVs have not gained much attention in Egypt, no vaccinations are currently available for use in the poultry sector. However, many studies have reported the spread of different serotypes in different Egyptian poultry farms, including FAdVs species D serotype2-11 (El-Tholoth and Abou El-Azm, 2019; Elbestawy et al., 2020), FAdV species E serotype 8a (Radwan et al., 2019), and FAdV serotypes 1, 3 and 8b (Adel et al., 2021).

The present study aimed to identify the current circulating FAdVs in Egypt by focusing on its pathological and molecular characterization using partial hexon gene DNA sequencing and phylogenetic analysis of obtained sequences. The results were then compared with other previously reported sequences in Egypt and other countries, which may be helpful in developing an efficient vaccination program in Egypt.

MATERIALS AND METHODS

Ethical approval

Infected broiler chickens were euthanized humanely then samples were collected following the regulations of the General Organization for Veterinary Services and Animal Health Research Institute, Giza, Egypt (AHRI 191221).

History of investigated broiler chicken flocks

From January 2021 to March 2022, a total of 26 broiler chicken flocks with a history of hydropericardium and variable mortality rates were examined for diagnosis of possible causes. These flocks representing different broiler chicken breeds (5 Cobb, 5 Ross, 4 IR, 7 Modified-Avian, and 5 Arbor Acres) were localized in Beheira (n = 15) and Menofia (n = 11) governorates in Egypt. All of them were unvaccinated against FAdV and negative for chicken anaemia virus, Infectious bursal disease virus, and reovirus using Polymerase chain reaction (PCR). Farms capacity ranged from 5000 to 19000 chicks. The chickens under examination were within the age range of 6-38 days. Infected broiler chickens showed depression, retarded growth, ascites, watery diarrhea, and variable mortality rates (Table 1). The main observed gross lesions in the examined flocks were pale, swollen livers with sub-capsular ecchymotic hemorrhages and hydropericardium.

Samples for histopathological examination

Tissues for histopathological examination from positive broiler flocks were collected from the liver, lung, spleen, kidney, heart, pancreas, proventriculus, bursa of Fabricious, thymus, and intestine of 10 freshly dead or euthanized infected broiler chickens aged 6 to 38 days. The samples were placed immediately in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin for pathological examination (Bancroft and Layton, 2013) using 10-20-40 lenses of the microscope (Micros Austria, Austria).

Samples for molecular detection of fowl adenoviruses

About 270 cloacal swabs from 26 broiler chicken flocks (10 from each flock) were obtained for FAdV molecular detection. Every10 different cloacal swab was pooled and dissolved in 1 mL of 7.4 pH phosphate-buffered saline.

Table 1. Epidemiological data of FAdV positive from broiler chicken flocks

Serial farm Number	NCBI Accession Number	Location in Egypt	Date	Broiler breed	Age (day)	Clinical signs	Mortality percentage
1		Menofia	Jan. 2021	Arbor Acres	12	Ascites	10%
2 *	OP297554	Menofia	Oct. 2021	Ross	36	Ascites	13%
13*	OP297555	Beheira, Shubrakhit	Jan. 2021	Cobb	6	Retarded growth and ascites	28%
16	--	Beheira, Edco	Oct. 2021	Ross	27	Ascites	23%
17*	OP297556	Beheira, Badr	Dec.2021	Indian River (IR)	27	Retarded growth and ascites	27%
21	--	Beheira,Abu El Matamir	Dec.2021	Arbor Acres	22	Retarded growth and ascites	28%
23*	OP297557	Beheira, Mahmoudiyah, Dayrut	Jan. 2022	Modified avian	32	Retarded growth and ascites	25%
24*	OP297558	Beheira, Mahmoudiyah, Fazara	Feb.2022	Modified avian	30	Retarded growth and ascites	20 %
25*	OP297559	Beheira, Mahmoudiyah, Monia Al-Saeed.	Mar. 2022	Modified avian	32	Retarded growth and ascites	12 %
26*	OP297560	Menofia	Feb.2022	Cobb	35	Retarded growth and ascites	22 %

*: Chosen samples for sequence.

Isolation and propagation of fowl adenoviruses in liver chicken embryo

The cloacal swabs supernatant was inoculated in the confluent monolayers of liver chicken embryo cells (CEL) obtained from 14 to 16-day-old specific pathogen-free embryos and incubated for 3-4 days until an intensive cytopathogenic effect was noticed using an inverted microscope (Olympus, Japan) as described by Mohamed Sohaimi et al. (2019). Virus isolation was performed using PCR to detect FAdVs in the collected supernatant of harvested tissue culture fluids.

Fowl adenoviruses molecular detection

Nucleic acid extraction

Total viral nucleic acid was extracted from 200 µL of the cloacal swabs supernatant using QIAampDNAMinikit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol.

PCR detection

The PCR was conducted using the EmeraldAmp Max PCR Master Mix (Takara, Japan) with hexon gene-specific primers (F: ATGGGAGCSACCTAYTTCGACAT, R: AAATTGTCCCKRAANCCGATGTA) according to Raue et al. (2005). As the initial denaturation step, the PCR reaction was heated at 95◦C for 5 minutes. Then, 40 cycles of; denaturation at 94˚C for 30 seconds, primer annealing at 58°C for 30 seconds, and elongation at 72°C for 45 seconds, followed by a final elongation step of 7 minutes at 72°C. The PCR product was analyzed by agarose gel electrophoresis to visualize the specific band at 590 bp by UV Trans illuminator.

Partial hexon gene nucleotide sequencing

Seven FAdVs samples were selected for partial hexon gene sequencing as they gave clear PCR bands indicating a higher viral load. Specific DNA bands with 590 bp size were excised from the gel, and the PCR products were extracted using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The purified PCR products were subjected to sequencing reactions with the forward and reverse primers in two reactions using a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's specifications. The obtained sequences were submitted to NCBI Gene bank (https://www.ncbi.nlm.nih.gov/WebSub/).

Similarity percent between strain sequences in this study and other published sequences in the NCBI database was done using Lasergene software. The nucleotide sequences were analyzed by the BIOEDIT program (Hall, 1999) using the Clustal W alignment algorithm. The obtained sequences in this study were aligned with the adenovirus field and reference strain

sequences from different countries accessible in the NCBI GenBank database. Phylogenetic trees were constructed by the maximum likelihood method using MEGA 6 software (Tamura et al., 2013).

RESULTS

Epidemiological findings

A total of 26 suspected FAdVs-affected broiler chicken flocks were investigated from January 2021 to March 2022 in Egypt provinces (Beheira and Menofia). The obtained data are presented in Table 1. The owner's complaints were generally about ascites, depression, retarded growth, increased susceptibility to various diseases, vaccination failure, morbidity rates ranging 20-38%, and mortality of 10 to 28%

Clinical signs and postmortem lesions

The infected broiler in investigated flocks showed retarded growth, depression, ruffled feathers, and a crouching position with ascites (Figure1 A and B). All infected chickens had hydropericardium, which is an accumulation of extra straw or amber-colored fluid in the pericardial sac (Figure 1 C). The pericardial sac contained a flabby, floating heart having congested blood vessels with hemorrhagic pericardial fat (Figure 1 D). The liver was pale, enlarged, and friable, with variable areas of necrosis and some petechial hemorrhages (Figure 1 C, D, and E). The duodenum was hemorrhagic (Figure1 F). Other lesions included congested and edematous lungs, slightly enlarged spleen, hemorrhagic and swollen kidneys with necrotic foci, and atrophy of the bursa of Fabricius and the thymus.

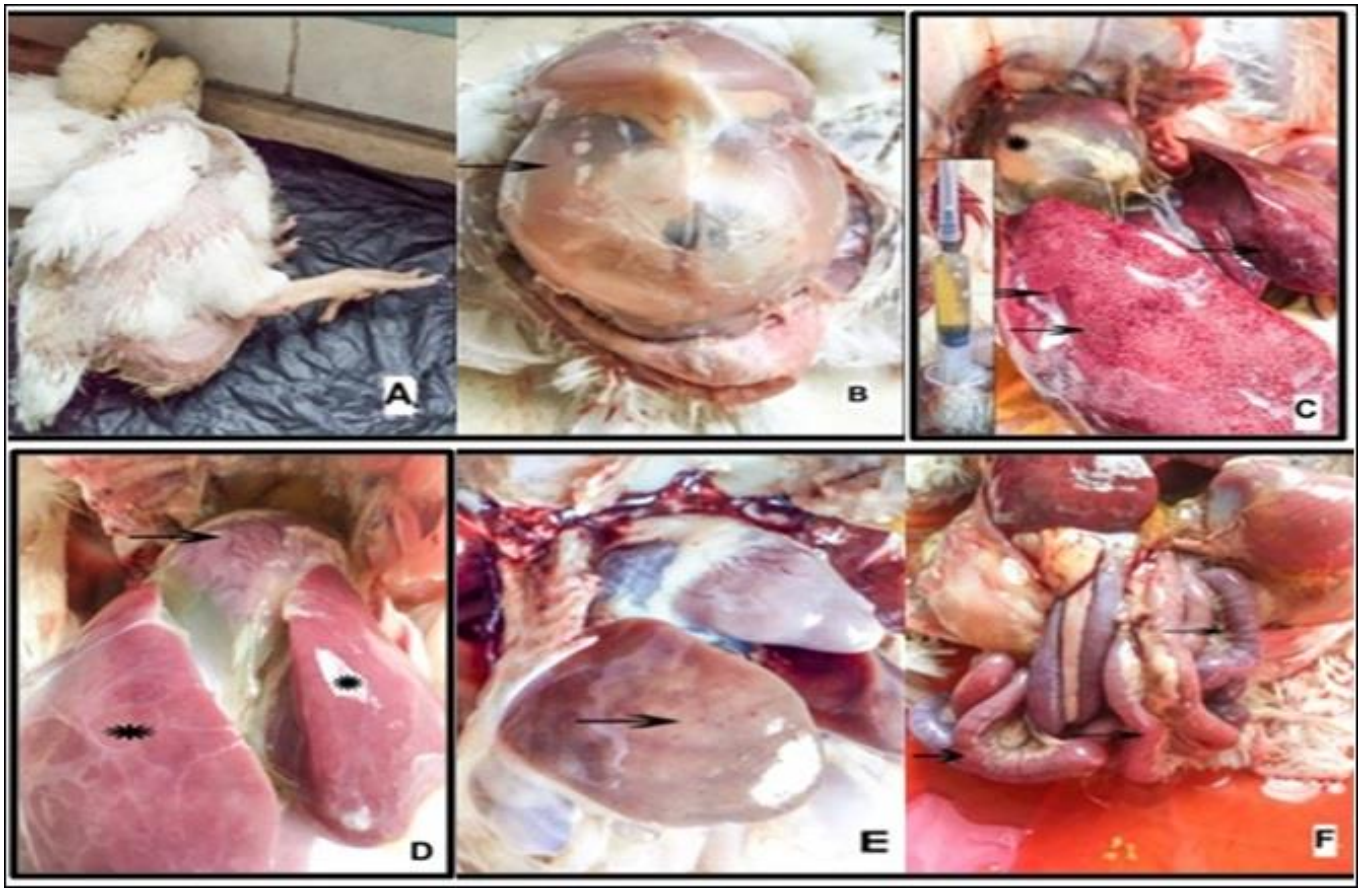

Figure 1. Clinical signs and postmortem lesions of broiler chickens infected by Hydropericardium syndrome. **A:** Broiler chicken looks listless, with ruffled feathers and in a crouching position. **B:** Severe ascites (Black arrow). **C:** The pericardial sac was distended with clear straw-colored fluid (Asterisk) and the liver was enlarged and mottled, with extensive necrosis (Black arrow). **D:** The heart was seen within the dilated pericardial sac engorged with blood (black arrow) and the liver was swollen and friable (Asterisk). **E:** Necrotized liver with some petechial hemorrhages (Black arrow). **F:** Hemorrhagic duodenitis (Black arrow).

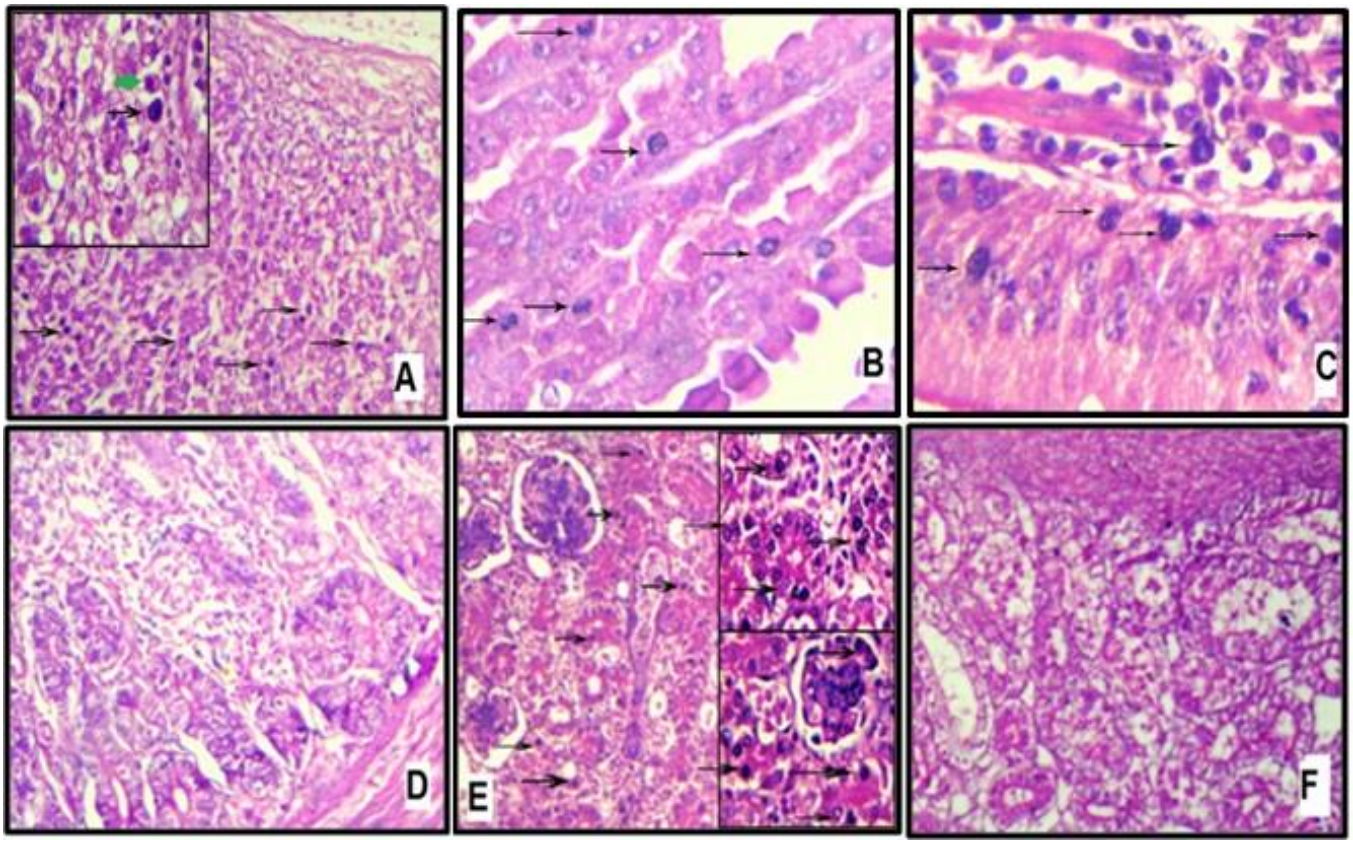

Figure 2. Pathological lesions of liver, proventriculus, duodenum and kidney of infected broiler chickens with FAdVs. **A:** Liver tissue, hepatic vacuolar degeneration, and necrosis with large basophilic (black arrows) and eosinophilic (green arrow) intranuclear inclusion bodies. **B:** Proventriculus tissue, the presence of intranuclear inclusion bodies in the epithelial cells of glandular lobules. **C** and **D:** Duodenumtissue, intranuclear inclusion bodies (Black arrow) with necrosis of intestinal villi with lymphocytic infiltration, edema, and hemorrhage in the submucosa. **E** and **F:** Kidney tissue, interstitial edema, and severe degeneration and necrosis of renal tubules and intranuclear inclusion bodies in the glomerular and tubular epithelium (Black arrow, H&EX200).

Histopathological lesions

Different organs of 10 dead or euthanized chickens from each positive flock were selected for histopathological examination and showed similar histopathological lesions. Massive intranuclear basophilic and eosinophilic inclusion bodies were the obvious feature of the liver which was widely distributed but frequently observed near the areas of necrosis (Figure 2 A). Other than the liver, intranuclear inclusion bodies were detected in a variety of organs, as in the epithelial cells of glandular lobules of the proventriculus (Figure 2 B), duodenum (Figure 2 C), renal glomeruli and tubules (Figure 2E), glandular cells of the pancreas (Figure 3 C) and inside the lymphocytes of the spleen (Figure 3 D). In addition, the intestines of infected chickens showed enteritis and necrosis with sloughing of the intestinal mucosa by lymphocytic infiltration of the submucosa (Figure 3 D). The kidneys displayed vacuolar degeneration and necrosis of renal tubules with interstitial edema and hemorrhage (Figure 3 F). Heart revealed pericardial edema with noticeable infiltration of heterophils and monocytes between myocytes (Figure 3 A). Lung indicated pneumonia with perivascular edema, hemorrhages, and alveolar infiltration of inflammatory cells (Figure 3 B). Diffuse focal necrosis of glandular cells of the pancreas (Figure 3 C), as well as depletion of lymphocytes in the bursa of Fabricius and thymus, were observed (Figures 3 E and F).

Fowl adenoviruses detection in commercial broiler farms

Of 26 tested samples, 10 (38.46%) were positive for FAdV by PCR. Seven gave intense PCR bands, so they were chosen for DNA sequencing, while the other three samples gave faint bands (Figure 4). Data of positive samples are shown in Table 1.

Isolation of fowl adenoviruses in chicken embryo liver cells

The positive samples of FAdVs species D serotype 2/11 were cultured in CEL cells. The cytopathic effect

appeared after 4 days including cell clumping and sloughing (Figure 5). Successful isolation of the virus on CEL cells was confirmed by positive PCR results for the harvested tissue culture fluids.

DNA sequencing of the partial hexon gene

Blast analysis of seven sequenced FAdVs in this study revealed that the nucleotide identity percent of the detected FAdVs sequences with the available Egyptian strains on the NCBI GenBank database ranged from 53.8% to 100% (Figure 6). The highest identity percent was with FAdV species D strains (93.3%-100%) while with FAdV serotype 8a was 70.6% followed byFAdV serotype 8b (67.8%),FAdVspecies B (63.5%,FAdV species A (56.1%), and lowest identity was forFAdV serotype 4 (53.8%; (Figure6). Sequence alignment of the obtained sequences revealed that the 7 FAdVs strains detected in this study were identical. This suggests that FAdVs species D is an important cause of HPS in Egypt between 2021 and 2022.

The partial hexon gene sequences of seven representative FAdV-D strains (Men1, Beh1, Beh2, Beh3, Beh4, Beh5, and Men2) detected in this study were submitted to the NCBI GenBank under accession numbers (OP297554, OP297555, OP297556, OP297557, OP297558, OP297559 and OP297560), respectively.Phylogenetic analysis of FAdVs indicated that these strains clustered with Egyptian FAdVs species D serotype 2/11strains, such as dmn2, kfr4, bst11, sin1, 2, 3, 4, and AD1, 4, 7, 9 (Figure 6). Moreover, FAdVs strains detected in the current study are closely related to those isolated from other countries, such as Germany (isolate 08-8872), Sweden (strain GB 1340-11), Spain(strain 12-2014), Japan (strain Gunma 2009) and Israel (strains 3346, 3114, 1917, Figure 7).

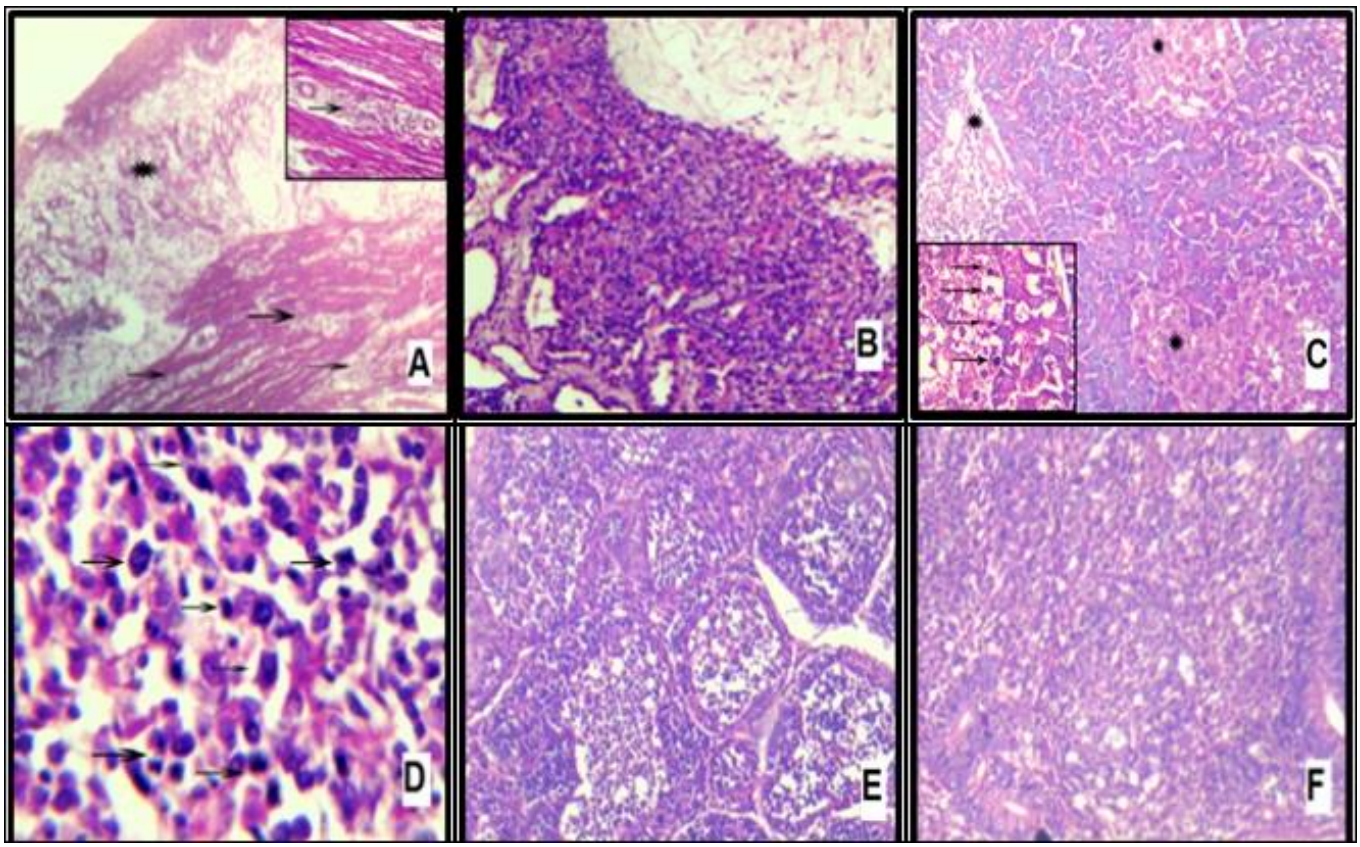

Figure 3. Pathological lesions of heart, lung, pancreas, spleen, bursa of Fabricius, and thymus of positive FAdVs infected broiler chickens**. A:** Heart, severe pericardial edema (asterisk) with myocytic necrosis and heterophilic and monocytic cell infiltration (Black arrow, H&E X 100, 200). **B:** Lung, pneumonia with massive edema and inflammatory cell infiltration (H&E X 200). **C:** Pancreas, diffuse glandular cell necrosis (asterisk) with intranuclear inclusion bodies (Black arrow, H&E X 200- 400). **D:** Spleen, intranuclear inclusion bodies in the splenic lymphocytes (Black arrow, H&E X 400). **E:** Bursa of Fabricius, and **F:** Thymus, showed lymphocytic depletion (H&E X200).

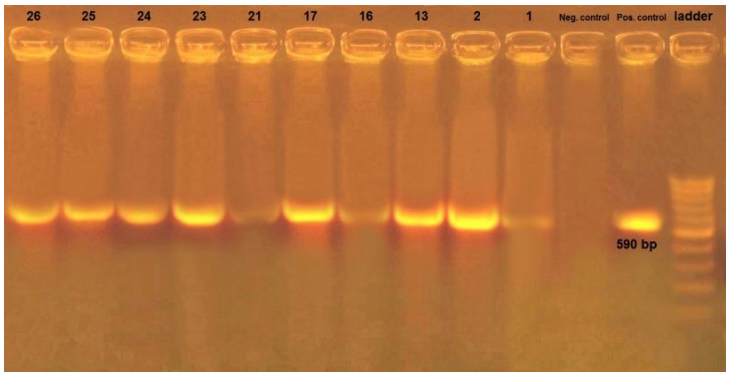

Figure 4. Agar gel electrophoresis of the amplified products of the partial hexon gene. Showing ladder: DNA marker, Pos: positive control with molecular weight 590bp, Neg: Negative control. Samples number 2, 13, 17, 23, 24, 25, and 26 showed clear bands and samples number 1, 16, and 21 showed faint bands.

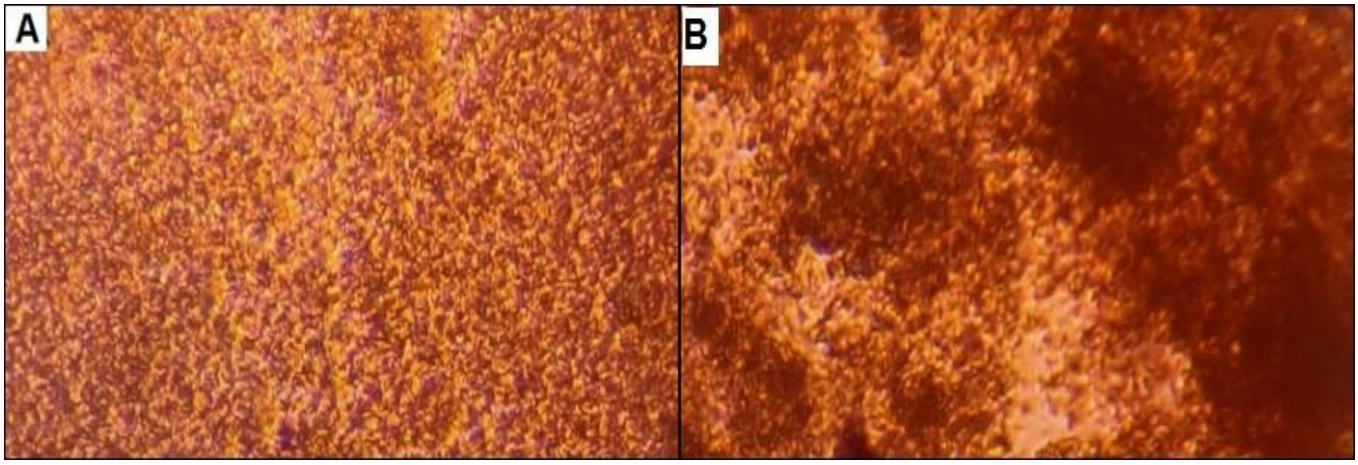

Figure 5. Cytopathic effect of FAdV on CEL cells in comparison with normal CEL cells**. A:** spindle shape of the liver cells (Control). **B:** Growth of FAdV on CEL cell culture in form of small and large areas of focal cell death with the beginning detachment of the cells at 4 days post-infection (X10).

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
1		100.0	100.0	100.0	100.0	100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	1	OP297554-FAdV-D-Men1-Egypt-2021
2	0.0		100.0	100.0	100.0	100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	2	OP297555-FAdV-D-Beh1-Egypt-2021
3	0.0	0.0		100.0	100.0	100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	3	OP297556-FAdV-D-Beh2-Egypt-2021
4	0.0	0.0	0.0		100.0	100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	4	OP297557-FAdV-D-Beh3-Egypt-2022
5	0.0	0.0	0.0	0.0		100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	5	OP297558-FAdV-D-Beh4-Egypt-2022
6	0.0	0.0	0.0	0.0	0.0		100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	6	OP297559-FAdV-D-Beh5-Egypt-2022
7	0.0	0.0	0.0	0.0	0.0	0.0		99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	7	OP297560-FAdV-D-Men2-Egypt-2022
8	0.2	0.2	0.2	0.2	0.2	0.2	0.2		99.8	100.0	100.0	100.0	99.8	99.4	99.6	99.6	99.8	99.6	99.4	93.5	97.0	55.9	63.7	70.4	67.8	53.8	8	OK634392.1-FAdV-D-Sin-4-Egypt-2017
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2		99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	9	MH782425.1-FAdV-D-kom3-Egypt-2018
10	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2		100.0	100.0	99.8	99.4	99.6	99.6	99.8	99.6	99.4	93.5	97.0	55.9	63.7	70.4	67.8	53.8	10	MH782424.1-FAdV-D-dmn2-Egypt-2018
11	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2	0.0		100.0	99.8	99.4	99.6	99.6	99.8	99.6	99.4	93.5	97.0	55.9	63.7	70.4	67.8	53.8	11	MH782423.1-FAdV-D-bst11-Egypt-2018
12	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2	0.0	0.0		99.8	99.4	99.6	99.6	99.8	99.6	99.4	93.5	97.0	55.9	63.7	70.4	67.8	53.8	12	MH782426.1-FAdV-D-kfr4-Egypt-2018
13	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.4	0.2	0.2	0.2		99.6	99.4	99.8	100.0	99.8	99.6	93.3	97.2	56.1	63.9	70.2	67.6	53.8	13	MW699421.1-FAdV-D-AD1-Egypt-2019
14	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.6	0.8	0.6	0.6	0.6	0.4		99.1	99.4	99.6	99.4	99.3	92.9	96.8	56.1	63.5	70.0	67.4	53.8	14	MW699424.1-FAdV-D-AD4-Egypt-2020
15	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.4	0.6	0.4	0.4	0.4	0.6	1.0		99.3	99.4	99.3	99.1	93.5	97.0	55.7	63.7	70.8	68.0	53.6	15	MW699427.1-FAdV-D-AD7-Egypt-2020
16	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.4	0.6	0.4	0.4	0.4	0.2	0.6	0.8		99.8	99.6	99.4	93.1	97.0	55.9	63.9	70.2	67.6	53.6	16	FN869962.1-FAdV-D-08-8872-Germany-2016
17	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.4	0.2	0.2	0.2	0.0	0.4	0.6	0.2		99.8	99.6	93.3	97.2	56.1	63.9	70.2	67.6	53.8	17	JX257176.1-FAdV-D-GB-1340-Sweden-2011
18	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.4	0.6	0.4	0.4	0.4	0.2	0.6	0.8	0.4	0.2		99.4	93.1	97.0	55.9	63.9	70.0	67.8	53.8	18	LN907534.1-FAdV-D-12-2014-Spain-2012
19	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.6	0.8	0.6	0.6	0.6	0.4	0.8	1.0	0.6	0.4	0.6		93.3	97.2	56.2	63.7	69.8	67.4	54.2	19	LC505993.1-FAdV-D-Gunma-Japan-2009
20	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.0	7.2	7.0	7.0	7.0	7.2	7.6	7.0	7.4	7.2	7.4	7.2		92.9	55.9	63.1	69.1	67.0	53.6	20	MG029109.1-FAdV-D-SA4-Saudi-Arabia-2016
21	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.1	3.3	3.1	3.1	3.1	2.9	3.3	3.1	3.1	2.9	3.1	2.9	7.6		56.1	63.7	69.3	67.8	53.8	21	MG832884.1-FAdV-D-685-Canada-2007
22	62.2	62.2	62.2	62.2	62.2	62.2	62.2	62.7	62.2	62.7	62.7	62.7	62.2	62.2	63.1	62.7	62.2	62.7	61.7	62.6	62.2		59.0	57.4	56.2	62.6	22	MW689188.1-FAdV-A-AD17-Egypt-2020
23	48.3	48.3	48.3	48.3	48.3	48.3	48.3	48.0	48.3	48.0	48.0	48.0	47.6	48.3	48.0	47.6	47.6	47.6	48.0	49.1	48.0	55.0		65.4	63.3	56.2	23	MW699419.1-FAdV-B-AD18-Egypt-2020
24	38.7	38.7	38.7	38.7	38.7	38.7	38.7	39.0	38.7	39.0	39.0	39.0	39.4	39.7	38.4	39.4	39.4	39.7	40.1	41.5	41.2	59.5	45.1		79.3	56.4	24	KT781516.1-FAdV-8a-MMR-B15-Egypt-2015
25	42.8	42.8	42.8	42.8	42.8	42.8	42.8	42.9	42.8	42.9	42.9	42.9	43.2	43.5	42.6	43.2	43.2	42.9	43.6	44.2	42.9	63.0	49.6	24.2		54.7	25	MW712888.1-FAdV-8b-AD15-Egypt-2020
26	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	69.1	69.1	68.6	68.6	67.6	69.0	68.6	49.2	60.3	62.2	64.5		26	MW660887.1-FAdV-C-Alex-1-Egypt-2021

Figure 6. Pairwise analysis for nucleotide sequences of seven targets Egyptian FAdVs strains isolated from broiler chickens and other related strains.

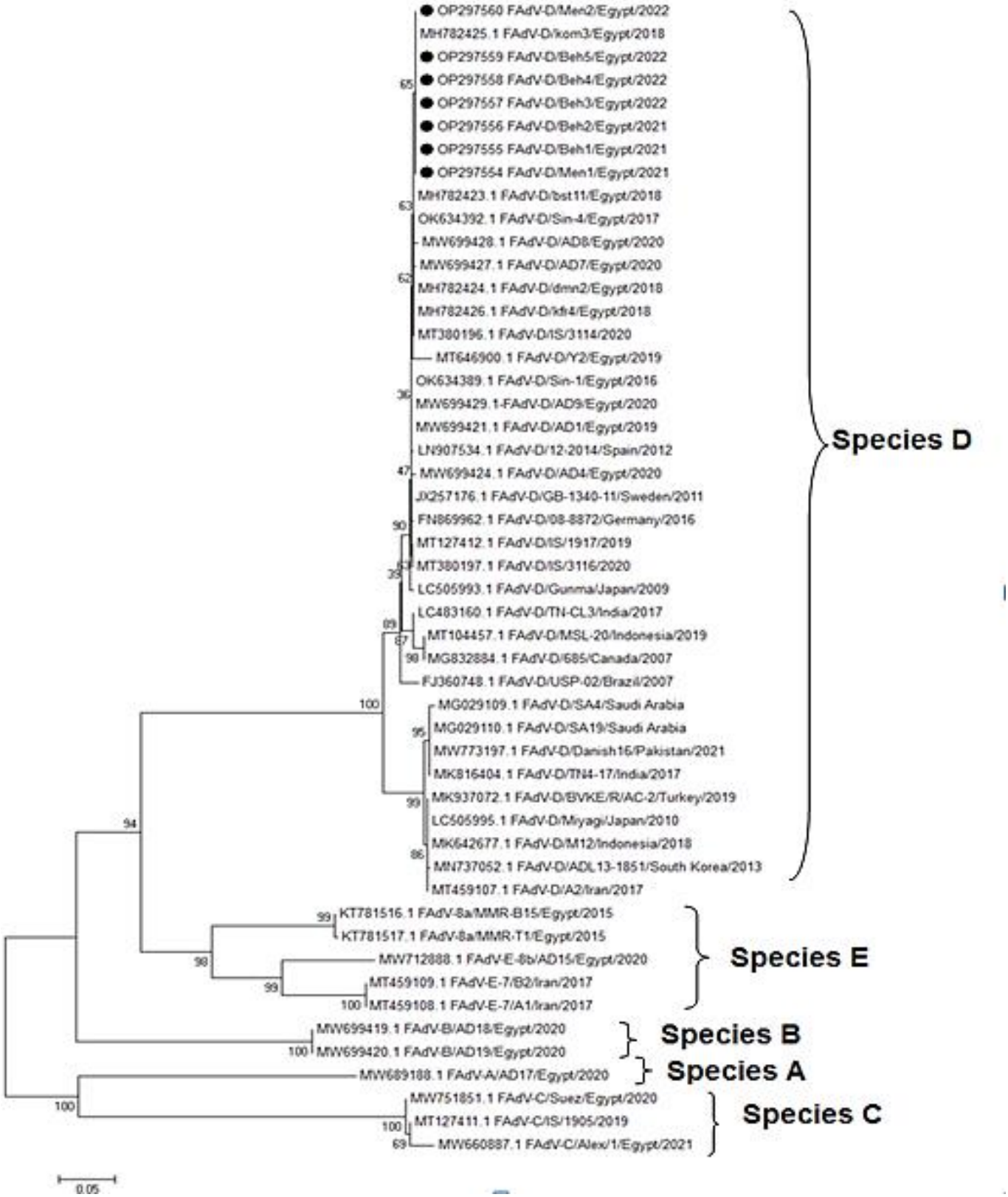

Figure 7. Phylogenetic tree for the nucleotide sequence of Loop1 region on hexon gene of FAdVs shows that the seven Egyptian strains of this study (labeled with black circle) which were isolated from broiler chickens are related genetically to FAdVs species D

DISCUSSION

Several avian species, including wild birds and domestic poultry, have recently been infected by fowl adenoviruses (Mohamed et al., 2018). Due to FAdVs spreading in the last years among chicken farms in Egypt and its effect on the elevation of mortalities and poor performance (El bestway et al., 2020; Adel et al., 2021), the current study was performed on 26 suspected chicken flocks to detect FAdVs incidence and genetic characterization of obtained viruses.

In the present study, clinical signs and postmortem lesions, especially hydropericardium, the most pathognomic postmortem lesions for HPS, were extensively detected, which is similar to previous studies (Chen et al., 2017; Schachner et al., 2018; Sultan et al., 2021). The variation in mortalities (from 10% to 28%) could depend on the age and susceptibility of the chicken's breed, the condition of the chicken's immune system, virus infection load, and/or concurrent infection with other infectious agents (Schachner et al., 2018).

Microscopically, the liver showed vacuolar degeneration and acute hepatic necrosis with intranuclear inclusion bodies in hepatocytes, which are signs of HPS and IBH infection. There were two types of intranuclear inclusions, namely basophilic and eosinophilic inclusions. The dense basophilic inclusions occupied most of the nucleus, which was enlarged with chromatin margination, and the eosinophilic inclusions with a halo around them. Presence of both basophilic and eosinophilic inclusions was similar to previous studies by Elbestway et al. (2020), Abouzied et al. (2021), and Ishag et al. (2022). The basophilic inclusions were found to be virus particle aggregation, whereas the eosinophilic inclusions were fibrillar-granular material and crystal aggregation (Itakura et al., 1977). In most cases, basophilic inclusions were formed first, followed by eosinophilic inclusions. Basophilic inclusions in the liver, kidney, proventriculus, intestine, pancreas, and spleen indicate a severe and rapid infection with FadVs (Nakamura et al., 2011). The cause of hydropericardium and ascites in chickens was acute hepatic necrosis leading to hepatic failure and consequently heart circulatory failure (Nakamura et al., 2002). Heart circulatory failure causes acute hydremia and hypoproteinemia resulting in the large immediate effusion of pericardial fluid through the blood capillaries of the epicardium into the pericardial sac, leading to hydropericardium that might impair heart function by decreasing sound, filling with blood and pulse pressure leading to death (Nakamura et al., 1999). The heart had severe edema and hyperemia in the pericardium, and cardiac myocytes were necrotized with moderate neutrophil and monocyte infiltration. These lesions were according to the findings of previous studies by Niu et al. (2018) and Ishag et al. (2022). There was also severe pneumonia, perivascular edema, and hemorrhage in infected chickens, which was reported in studies by Kataria et al. (2013) and Niu et al. (2018). In the kidneys, there were considerable edema and hemorrhage in the renal interstitium, as well as massive vacuolar degeneration and necrosis of renal tubules similar to that mentioned by Kataria et al. (2013), Zhao et al. (2015) and Niu et al. (2018). Zhao et al. (2015) also reported severe enteritis of the intestinal mucosa, edema, and hemorrhage in the submucosa of the duodenum. The current results agreed with previous studies regarding intranuclear inclusion bodies in epithelial cells of renal tubules (Schachner et al., 2018; Ishag et al., 2022), glandular cells of the pancreas (Nakamura et al., 2011), glandular lobules of proventriculus and duodenum (Nakamura et al., 1999), and inside the lymphocytes of the spleen (Ishag et al., 2022) as well as lymphocytic depletion of the bursa of Fabricius and thymus (Matos et al., 2016).

Of 26 samples, 10 (38.46%) from Beheira and Menofia were positive for FAdV using primers that target the L1 region of the hexon gene. Similarly, previous studies in Egypt indicated FAdV in 45% of flocks in Alexandria, Beheira, and Kafr El Sheikh governorates (Elbestawy et al., 2020), and 22% in Behira governorate (Radwan et al., 2019). However, Abouzied et al. (2021) reported 11 positive samples (out of 14) from the North Sinai governorate, Egypt. The difference in virus detection from suspected flocks may be due to the course of the virus, virus infection load, age of the chicken, and its immune status.

In the present study, genetic sequencing for the 590bp DNA fragment of loop 1 of the hexon gene of seven FAdVs was done using the same PCR primers since this gene is used for FAdVs serotyping and produces the main protein against which the neutralizing epitope was directed (Niczyporuk, 2018).

Neighbor-joining based phylogenetic tree was constructed using sequences of this study and obtained sequences from Egypt and other countries representing different FAdVs species and serotypes. The analysis showed that present sequences were clustered into FAdV species serotype 2/11. This serotype was previously recorded in Egypt in different governorates, such as

Alexandria, Beheira, Kafr El Sheikh, Sharkia, North Sinai, Assiut, Daqahlia, Sohag, and El Wadi El gedid (Elbestawy et al., 2020; Abouzied et al., 2021; Adel et al., 2021; Safwat et al., 2022) indicating the dominance and wide spread of this serotype in Egypt. Globally, 34% of FAdV isolates from 38 countries were classified as FAdV Species D (Kiss et al., 2021). Moreover, the sequences of the current study were close to other published sequences from countries, such as Germany, Spain, and Japan (Marek et al., 2010; Mase et al., 2012; Schachner et al., 2016, Figure 7).

Nucleotide identity between sequences in this study and other selected sequences from Egypt and other countries was performed using Lasergene software. The tested strains and the reference strain had a high nucleotide similarity of 99.3% to 100% with other FAdV-D strains previously detected in Egypt during the last four years (Elbestawy et al., 2020), which previously detected the same serotype from 17 different poultry farms. This study revealed that the virus is still present, creating significant losses in Egyptian poultry flocks.

The Beh1, 2, 3, 4, 5 and Men1, 2 showed high divergence from the FAdVs strains previously detected in Egypt that belong to FAdVs species A, B, C, and E (Figure 6) as the identity percent with them ranging 53.8-70.6%.

The studied strains showed high similarity with FAdVs species D detected in Germany, Spain, Sweden, and Japan (more than 99%), while the similarity percent was lower with FAdVs-D detected in Canada (96.8%) and Saudi Arabia (93.3%, Figure6) indicating its continuous circulation in different geographic areas of the world causing significant losses.

CONCLUSION

This study identified FAdV in 10 broiler flocks suffering from HPS and located in Beheira and Menofia provinces of Egypt using molecular and histopathological techniques during 2021-2022. Partial hexon gene DNA sequencing of detected FAdVs revealed that all of them are identical and classified as FAdV species D suggesting that this FAdV species is the most important cause of HPS in Egypt. Ascitis and hydropericardium are the main postmortem lesions, while intra-nuclear inclusion bodies in the liver are the main histopathological finding observed in infected flocks. Further investigations are essential to determine the epidemiology of the FAdVs subtypes in all geographic areas of Egypt and investigate its pathogenicity and full genome characterization to implement protective control measures, including the application of homogenous or multivalent vaccines to prevent further losses in poultry flocks.

DECLARATIONS

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Authors' contributions

Eid Hussein put the research protocol, collected the samples from chicken farms, made postmortem examinations, virus isolation, and participated in manuscript writing. Nessreen Fouad Anwar and Marwa Ali Abdelmagid made pathological and histopathological analyses and contributed to the manuscript writing. Osama Mahana and Hossam Shabaan Elsebaey made molecular tests, DNA sequence analyses and participated in manuscript writing. Mohammed Abo Elkhair revised the final draft of the manuscript. All authors have checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors claim that they have no conflicts of interest.

Ethical consideration

Authors considered ethical concerns, such as (plagiarism, misconduct, permission to publish, double publishing, data fabrication and/or falsification, and/or submission, and redundancy).

Availability of data and materials

The data described in this study are accessible from the relevant authors upon request.

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