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Molecular Characterization of Hydropericardium Hepatitis Syndrome in Broiler Chickens

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ABSTRACT

Infection with fowl adenoviruses (FAdVs) can lead to a number of syndromes in chickens. These syndromes, which include inclusion body hepatitis (IBH) and hepatitis-hydropericardium syndrome (HHS), as well as others, are responsible for tremendous economic losses all over the world. FAdVs can be broken down into a total of 12 different serotypes and 5 different species (A–E; 1–8a and 8b–11). As a result of the extensive distribution of FAdV strains, the vast majority of bird species are susceptible to infection. IBH and HHS are both caused by viruses belonging to the genus aviadenovirus, which is a family within the adenoviridae viral family. In our study samples from IBH and HHS suspected outbreaks were collected from Alexandria, Mars Matroh and Behera governorates. The affected broilers showed enlarged liver and kidney, straw-colored fluid accumulates in the pericardial sac around the heart. The FAdVs infection confirmed by conventional PCR infection using Hexon gene specific primers then sequencing of 3 selected FAdv the isolates. Phylogentic analysis of the obtained sequences in comparing with reference strains from gene bank; the three isolates were highly similar to the circulating FAdV type D in Egypt with nucleotide identity ranged 99 -100% and amino acid identity ranged 97.2-100%. While they are of low similarity with the other FAdV serotypes located in group D FAdV. Interestingly we found that FAdV species D was predominant in Egypt now specially FAdVs- 2/11.

Key words: FAdV, Hexon, HHS and IBH.

INTRODUCTION

Members of the Adenoviridae family have only been isolated from vertebrates, including fish and humans. A bioinformatics investigation of the family genomic sequences has revealed five clades that correspond to the previously classified genera Atadenovirus, Ichtadenovirus, Mastadenovirus, Aviadenovirus, and Siadenovirus. Important chicken adenoviruses, which have been categorized into five species (A–E) and twelve serotypes (fowl adenovirus (FAdV) 1–8a and 8b–11) based on Hexon gene sequence, are included in group 1 of the avian adenovirus genus Aviadenovirus (Smyth and McNulty, 2008). The fowl adenovirus hexon gene L1 region refers to a specific part of the hexon gene found in fowl adenoviruses. A crucial structural protein of adenoviruses, hexon is

responsible for the formation of the capsid, also known as the outer shell of the viral particle. Within the hexon gene is a stretch that displays a significant degree of variability between the various adenovirus serotypes and strains. This region is referred to as the hyper variable loop 1, and it is also known as the L1 region (Smyth and McNulty, 2008).The hexon gene is commonly used in molecular studies and classification of adenoviruses due to its genetic diversity. There are sequences within the L1 region that considerably differ between different adenovirus strains. This enables researchers to discriminate between different serotypes of the virus and investigate the genetic links between them. The genetic information obtained from the L1 region can be utilized for a variety of purposes, including molecular type, epidemiological research, and the development of diagnostic tools, such as PCR assays and procedures for gene sequencing. Moreover, understanding the genetic variety and classification of fowl adenoviruses is critical for disease diagnosis, control, and prevention in chicken populations (Hess, 2013).

FAdVs have been isolated from individuals that appeared to be healthy as well as those that showed a variety of clinical symptoms, such as respiratory problems, decreased egg production and even enteritis with varied degrees of mortality (Adair and McFerran, 2008). Due to this variation in sickness linkage, it is difficult to assess the FAdV's economic relevance or even their direct function as primary pathogens (Hess, 2013). In the pathogenesis of HHS, which has a high mortality rate of 20% to 80%, Asthana et al. (2013) found that the particularly (species C) virulent FAdV-4 suspected to play a substantial role than other viruses. While some FAdV-1 strains (species A) have the potential to seriously harm the liver and contribute significantly to the disease state known as inclusion body hepatitis (IBH), other strains have the potential to cause gizzard erosion and growth retardation. All available evidence supports the designation of some FAdV strains as major pathogens (Hess, 2013), resulting in a 5% to 10% increase in mortality that occurs suddenly, peaks after 3–4 days, and often lasts until day 5 (Hess, 2013; Adair and McFerran, 2008). A pale, enlarged liver with hemorrhages and intra-nuclear inclusion bodies in the hepatocytes are further symptoms of the illness. According to genetic research on FAdV, species D and serotype 8a/E have supposedly predominated in Egypt ever since it was first introduced (Radwan et al. 2019, El Bestawy et al. 2020, Adel et al. 2021). Also recently discovered in Egypt are new FAdV serotypes 1, 3, and 8b (Adel et al., 2021). In 2021, genotype 4 (FAdV-4) the pathogenic strain of was first identified in Egypt. It was later determined that this strain shared 98% of its genetic makeup with the Israeli strain IS/1905/2019. Comparative studies of the hyper-variable regions of the hexon gene L1 region showed specific features to each viral serotype. Generally, Hydropericardium hepatitis syndrome (HHS) is a disease syndrome that affects broiler chickens, which are chickens raised for meat production (Smyth and McNulty, 2008). It is caused by a variety of fowl adenovirus serotypes and typically affects young birds, specifically those that are between 3 and 5 weeks of age on average (Hess, 2013). The increasing accumulation of fluid places pressure on the heart, which can lead to malfunction in the cardiac muscle. Because the liver is involved in a number of important metabolic processes, any dysfunction in this organ can have a domino impact on the chicken's overall health as well as its rate of growth (Hess, 2013). HHS

is highly contagious among broiler chickens and can spread rapidly within a flock (Smyth and McNulty, 2008).

The virus is typically spread through the droppings of infected birds, through contact with contaminated equipment, or through direct interaction with infected birds. Implementing stringent biosecurity measures in order to decrease the likelihood of exposure to the HHS virus is necessary in order to prevent disease from occurring in grill chickens (Hess, 2013). This involves reducing the amount of time the flocks spend in contact with one another, making sure the equipment is properly sterilized, and reducing the number of wild birds that congregate near the farm (Smyth and McNulty, 2008).Because there is currently no cure for HHS, prevention is of the utmost importance. Vaccines have been developed and made accessible in certain areas to assist in protecting birds from the disease. This research primarily focuses on the detection of FAdV in commercial poultry farms located in Alex, Behera, and Marsa Matroh in Egypt. Additionally, molecular characterization of 3 selected isolates performed to identify the chicken adenovirus serotype by hexon gene L1 region sequencing.

MATERIAL AND METHODS

Sampling

Samples were collected during 2021 and 2022 from a total of 15 FAdV suspected flocks fromgovernorates of Alex, Mars-Matroh and Behera in Egypt. Samples included liver, heart, kidney and cloacal swabs obtained from different poultry farms.

Molecular detection of FAdVs

1. ***Viral nucleic acid extraction***

Viral nucleic acid extractionwas carried by using the QIAamp-MinElute Spin Kits (Qiagen, GmbH, Germany), the instructions of the manufacturer were as follows: a 200 µL volume of swab sample fluid or tissuehomogenate supernatant were incubated at 56 ^{0}C for 15 min with 200 µL of AL lysis buffer and 25 µL of Qiagen protease. Thereafter, absolute ethanol (250 µL) was added to the content, which was then washed to be centrifuged. By using 100 µL of elution buffer Nucleic acid was finally eluted. For further examination, DNA extracts were kept at 20^{0}C.

2. ***Viral nucleic acid amplification using conventional PCR.***

The amplification of the hexon gene L1 loop of the different adenovirus carried by serotypes specific oligonucleotide primers, which were synthesized by metabion (Munich, Germany). By using an Emerald Amp Max PCR Master Mix (Japan) PCR amplification was carried out.

The nucleotide sequences of the primers were as follows:

adeno-F- 5′-ACATGGGAGCGACCTACTTCGACA-3′

adeno-R- 5′-TCGGCGAGCATGTACTGGTAAC-3′.

The expected product size was 590bp.

3. ***Hexon gene- L1 region Sequencing***

A QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) was used to purify the amplified PCR products of the proper size. The purified PCR products were used for sequencing using Biosystems Big Dye Terminator v3.1 Cycle Sequencing Ki then purified by exclusion chromatography using a DyeEX 2.0 Spin Kit. Using a 3500 XL DNA Analyzer (Applied Biosystems, Foster City, USA).

4. ***Hexon geneL1 region sequence analyses***

The ClustalW alignment algorithm was used to achieve multiple nucleotide sequence alignment, and the % identity matrices between various virus sequences were calculated. The distance-based strategy was applied to create neighbor-joining

phylogenetic trees in MEGA software version 11. The trees comprised the sequences produced in this investigation as well as other strains of FAdV serotypes that could be found by downloading them from GenBank.

RESULTS

Clinical signs and gross lesions.

The 32-day-old broiler flock experienced sudden onset and rising death rates that reached 15%. Depression, loss of appetite, agitation, greenish diarrhea and unwillingness to move were among the clinical signs. Chicken livers from recently deceased animals had necrotic foci and appeared yellow-brown at necropsy. The pericardial sac contained a straw-colored fluid, leaving the hearts saggy (Fig. 1). The kidneys were engorged with uric acid buildup and enlarged (Fig. 1).

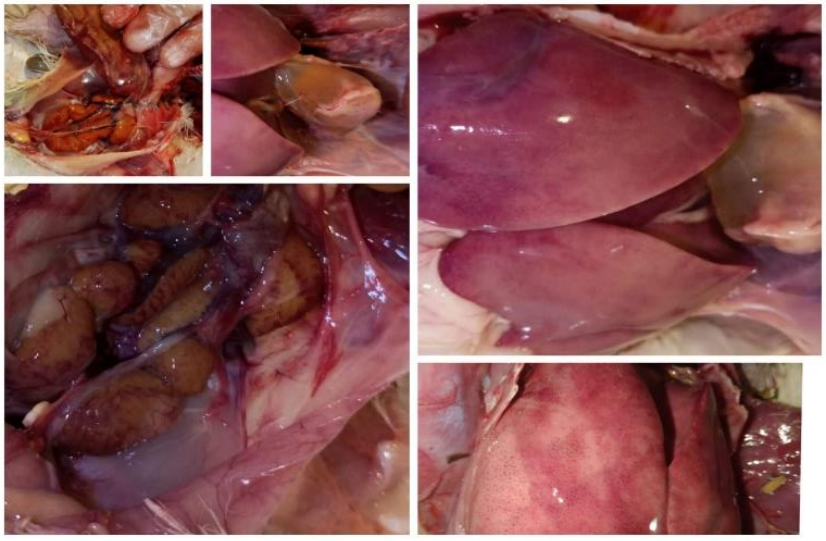

Figure 1: Post mortem lesions in liver, heart and kidney.

FAdV detection by conventional PCR.

Three flocks out off 15 flocks were tested positive for FAdV by PCR using hexon gene specific primers .The PCR positive product was about 590 bp.

Molecular characterization of FAdV:

Identity matrix of partial nucleotide and amino acid sequence of the hexon gene.

The partial nucleotide sequences of the hexon gene of the 3 samples under investigation were highly similar to the circulating FAdV type D in Egypt with nucleotide identity % of 99 -100% and amino acid the identity % of 97.2-100%. While they are of low similarity with the other FAdV serotypes (Table 1).

Table 1: Nucleotide identity percent and amino acid the identity percent of the newly detected FAdV strains with other reference strains.

Nucleotide identity%

	Seq-ID	1	2	3	4	5	6	7	8	9	10	11	12
1	Z67970-FAV1 (CELO)		98.7%	60.1%	56.5%	55.3%	60.9%	52.3%	52.3%	52.3%	51.7%	52.3%	52.3%
2	AD17-2020- A	98.1%		59.8%	56.8%	55.3%	61.2%	52.0%	52.0%	52.0%	51.4%	52.0%	52.0%
3	AD19-2020- B	52.6%	51.7%		63.6%	61.6%	54.4%	58.9%	58.6%	58.6%	58.0%	58.6%	58.3%
4	Fowl adenovirus 8a isolate MMR-T1	50.0%	50.8%	60.7%		75.5%	54.4%	65.7%	66.0%	66.0%	65.4%	66.0%	65.7%
5	AD16-2020- 8b/E	49.1%	50.0%	59.8%	79.4%		49.7%	62.5%	62.7%	62.7%	62.2%	62.7%	62.5%
6	FAdV 4/C - Egypt/2021	58.1%	59.0%	48.2%	50.8%	44.6%		51.9%	51.9%	51.9%	51.3%	51.9%	51.6%
7	AD3-2020-D	44.6%	45.5%	57.1%	72.3%	67.8%	45.9%		99.6%	99.6%	99.0%	99.6%	99.3%
8	FAdV-D strain Beh5/Egypt/2022	44.6%	45.5%	57.1%	72.3%	67.8%	45.9%	99.0%		100.0%	99.3%	100.0%	99.6%
9	FAdV-D strain Men1/Egypt/2021	44.6%	45.5%	57.1%	72.3%	67.8%	45.9%	99.0%	100.0%		99.3%	100.0%	99.6%
10	FAdV-D strain-broiler-Alex-Egypt-14-2021	42.8%	43.7%	55.3%	70.5%	66.0%	45.0%	97.2%	98.1%	98.1%		99.3%	99.0%
11	FAdV-D strain-broiler-Alex-Egypt-47-2021	44.6%	45.5%	57.1%	72.3%	67.8%	45.9%	99.0%	100.0%	100.0%	98.1%		99.6%
12	FAdV-D strain-broiler-Alex-Egypt-49-2021	44.6%	45.5%	56.2%	71.4%	66.9%	45.0%	98.1%	99.0%	99.0%	97.2%	99.0%	

Amino acid identity%

Phylogenetic analysis of partial nucleotide sequence of hexon gene:

The tree includes the representative sequences for the different serotypes and species of Fowl adenovirus circulating in the world and Egypt. The sequences of the 3 FAdV strains under investigation are labeled with red color font and black circle. The tree shows that the 3 FAdV strains under investigation are genetically related to the FAdV species D and very close to the Egyptian circulating viruses (Fig. 2).

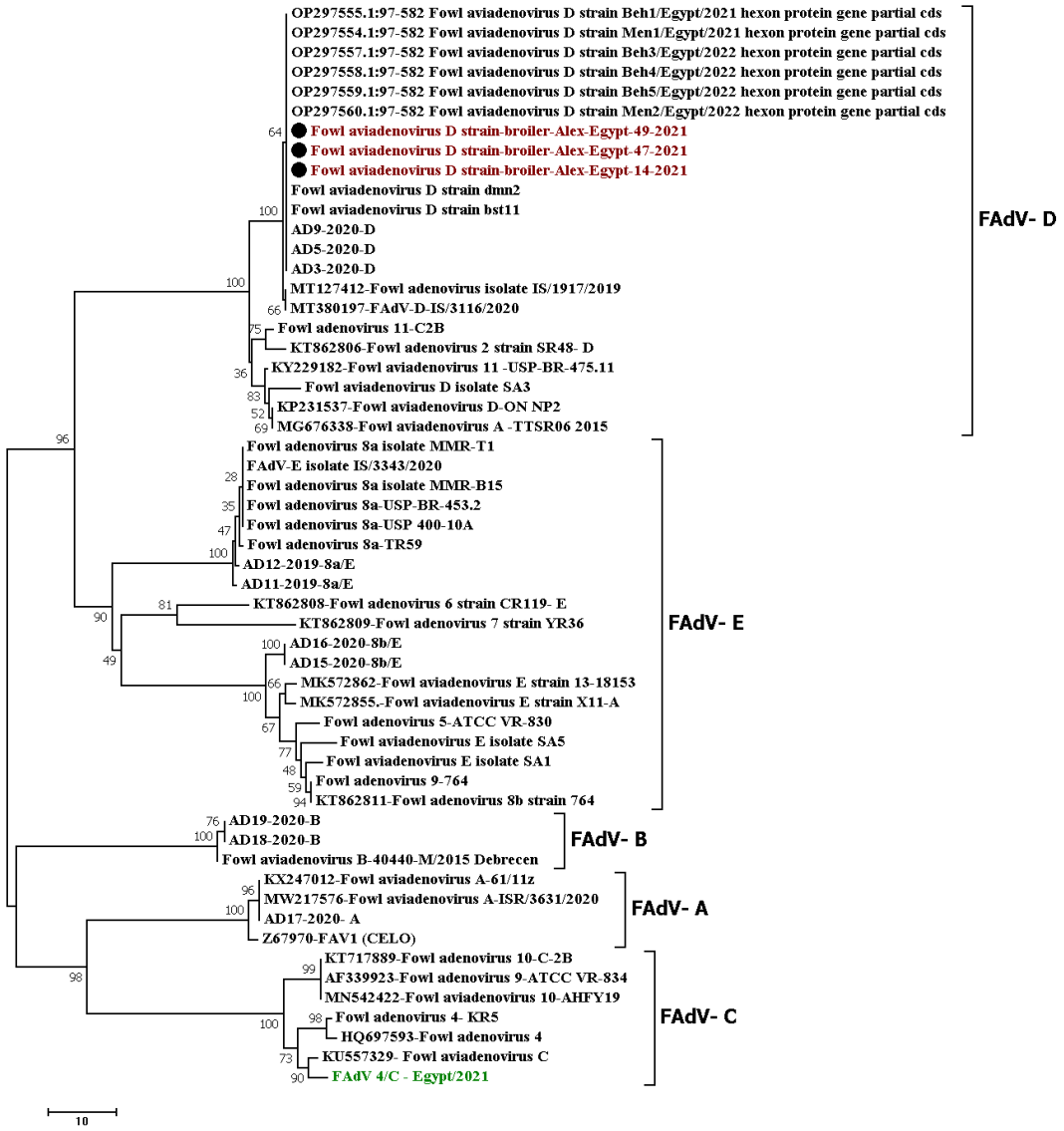

Figure 2. Phylogentic tree for the partial nucleotide sequence of hexon gene, the analysis has been applied by MEGA 7 software with Nieghbour joining statistical methods for 1000 replicates of the bootstrap.

Amino acid Alignment of different serotypes of FAdV in Egypt:

The multiple alignment of the amino acids sequence of the L1 region on hexon gene has been created by bioedit software, including the 3 FAdV strains sequences under investigation and some representative sequences of the circulating Egyptian strains of different FAdV species (Fig. 3). The sequences of interest show the same characteristic motifs of the FAdV species D including ^{33}GQMTT^{37}

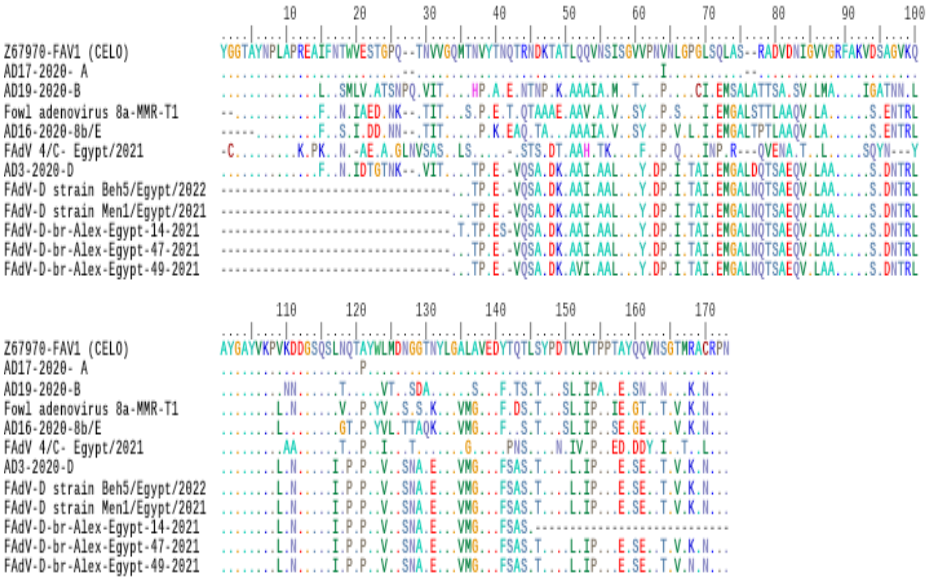

Figure 3: Amino acids alignment of the L1 region on hexon gene of the different Egyptian FAdV serotypes**.**

DISCUSSION

In the past, sporadic epidemics of hepatitis and the hydropericardium syndrome (HHS) as well as inclusion body hepatitis (IBH) in Egypt have not drawn much attention. We discovered the normal clinical manifestation of FAdV infection when examining this emergency case in a 32-day-old broiler flock (Hess et al. 2013). The suspected case showed signs of upset stomach, greenish diarrhea, weight loss, depression, and a 15% fatality rate. Additionally, the typical pathological signs were present, including hydropericardium (a condition where a clear, straw-colored fluid, an enlarged liver (Fig. 1A), swollen kidneys from edema, and uric acid buildup (Fig. 1B). These clinical findings are odd and resemble those that have been described in earlier investigations on FAdV infection (Cheema 1989, Abdul-Aziz TA & Hasan SY 1995). The most noticeable gross pathologic sign and largely accepted pathognomonic for HHS is fluid buildup in the pericardial sac (Schachner *et al* 2018). Although Rodr'guez*et al*. (2014) noted the absence of hydropericardium in some outbreaks, Cheema (1989) considered hepatic sings and petechial hemorrhages to be the hallmark symptoms of HHS.As a quick confirmatory diagnostic test for FAdV-4, PCR was used in this investigation (Hess 2000, Hess 2013, Hess 1999). Using particular primers that focused on 590 base pairs on the HVRs L1 region of the hexon gene, the extracted putative viral nucleic acid was amplified (Li et al. 2017, 2017). The FAdV species and serotypes can be distinguished by the four hypervariable regions (HVRs1-4) found in the L1 region of the hexon gene (Niczyporuk 2018). Additionally, it is utilized to examine the genetic evolutionary connections between various reference isolates. One FAdV serotype, comprising previously identified serotypes of species D (FAdVs-2/11) (El-Tholothand Abo El-Azm, 2019; El-bestawy et al., 2020), was found in the current investigation. Similar to FAdVs- 2/11 were found as was previously reproduced, FAdV species D was prominent in the present study (Niczyporuk, 2018). One of the 4 loop sections (L1, L2, L3, and L4) on the hexon gene surface that contain immunogenic HVRs is the L1 region (Crawford- Miksza

and Schnurr, 1996). Furthermore, because it contains four HVRs with distinctive compositions for each FAdV species and amino acid sequence lengths, the L1 region is regarded as a type/species-specific section (Niczyporuk, 2018; Raue et al., 2005). The FAdV strains alignment in current investigation as viewed in revealed 33GQMTN37 in FAdV-1 and 8b. Despite the fact that the amino acid sequence 33GQMTT37 was unique to FAdV-2/11/D, FAdV-3 and 8a both carried 33GQMTH37 and 33GQMSN37, respectively. Since the HVRs enclose this protected area, it has been hypothesized that it is essential to the virus' immunogenicity and antigenicity. Therefore, the surface structure may differ depending on the species.

CONCLUSIONS

In Egypt, studieson the clinical cases have highlighted in the past few years followed by more invistegation on pathogenicity and types of FAdVs. In the present study by genetic variable analysis of the HVRs in the L1 area of the hexon we reported the emergence of FAdV-2/11 of species D. In Egyptfor more different FAdV species pathogenicity and establish the genetic and antigenic constitutions determination, Further studies are required.

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