Molecular Investigation of Fowl Aviadenovirus in Broiler Flocks in North Sinai Province

Hasan M. Abouzied[1], Mohamed A. Soliman[2], Eman kamal El-Sayed[3], Norhan M. Ali[3], Mokhtar M. El-Tarabilli[3]

*[1]Poultry vaccination center El Arish, Directorate of Veterinary Medicine, North Sinai Governorate; ** hasanabozid@yahoo.com,*[2]Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute;* mohahm@hotmail.com, *3Department of Virology, Faculty of Veterinary Medicine- Suez Canal University.*

Abstract:

Fowl aviadenoviruses (FAdV) have gained increasing attention recently in Egypt and worldwide. They are the primary etiology of many widespread diseases. Among these diseases, inclusion body hepatitis (IBH). Many outbreaks of IBH have been documented in the last few years across the country, causing significant economic losses to the Egyptian poultry sector. This study was established to identify FAdV from field outbreaks of IBH in commercial broiler farms in North Sinai province during the period from May 2016 till December 2017. Affected birds lie on their sides with reduced feed intake, depression, ruffled feathers, pale comb and facial skin. While the most common postmortem lesions were hydropericardium, yellowish enlarged liver with ecchymotic hemorrhages, pancreatitis, and enteritis. Histopathological examination of liver samples collected at different stages of infection showed multifocal necrosis, extensive congestion with inflammatory cellular infiltration. The presence of intranuclear inclusion bodies (INIB) was variable among samples. FAdV were detected in liver samples from eleven flocks out of the fourteen examined by real-time polymerase chain reaction (real-time PCR) using commercial kits with differences in viral load among samples. DNA samples from four flocks that exhibited the highest fluorescence curve altitudes were subjected to conventional PCR targeting the hexon loop-1 gene for genetic characterization purpose. Sequencing of the four specific PCR products and phylogenetic analysis of the sequences showed that the four isolates (Sin-1, Sin-2, Sin-3, and Sin-4) were clustered into serotype-2, FAdV-D species. The strains in this study are sharing 99.82%, 99.63%,99.60%, and 98.2% nucleotide identity with FADV-2 (species D) strains from

Israel, Japan, Egypt, and Canada, respectively. This study provides epidemiological data that could be useful in the design of an appropriate prevention strategy.

Keywords: Fowl aviadenoviruses, hexon, inclusion body hepatitis, phylogenetic analysis, real-time polymerase chain reaction.

Introduction

Fowl aviadenoviruses (FAdV) play a major role as primary pathogens affecting the poultry industry since they are involved in a variety of clinical diseases that are becoming increasingly important across the world. ***(Schachner et al., 2018)***. Serological and virological surveys indicate that FAdVs are widely distributed in avian species, infection is demonstrated in both sick and healthy birds ***(Adair and Fitzgerald, 2008).*** FAdVs are 5different species (A, B, C, D, and E) within the genus *Aviadenovirus* of the family *Adenoviridae*. FAdVs species are subdivided into 12 distinct serotypes(FAdV-1 to FAdV-8a and FAdV-8b to FAdV-11) based on serum neutralization tests ***(Hess, 2000).*** Adenoviruses are non-enveloped, icosahedral in symmetry, with precisely hexagonal outlines, measuring 70-90 nm in diameter. The genome involves a linear, double-stranded DNA molecule, 26-45 kbp in size **(*Hess et al., 1995)***. The viral capsid is made up from 252 capsomers (240hexons and 12pentons). Hexons occupy the facets of the capsid while, pentons cap the vertices ***(Russell, 2009).*** The hexon comprises conserved regions (pedestals, P1 and P2) that are situated deeper within the virion in addition to variable loops (L1 to L4) that are more superficial. The variable loops hold type-specific neutralizing epitopes that provide the basis for the classification of adenoviruses into serotypes that are currently known ***(Adam et al., 1998; Ebner et al., 2005; Toogood et al., 1992)***. PCR for molecular detection of hexon genes is the principal technique of detection of FAdVs nowadays, while earlier approaches such as viral isolation on cell cultures or embryonated eggs, serotyping using virus neutralization test, in addition to tissue staining using immunohistochemistry are still used in certain laboratories ***(Fitzgerald et al., 2020).*** In Egypt, tangible efforts were exerted to investigate FAdVs throughout the country, especially after the increased prevalence in the last few years ***(El-Basrey et al., 2020; El-Tholoth and Abou El-Azm, 2019; Elbestawy et al., 2020;***

Radwan et al., 2019; Sultan et al., 2021). In this study, 14 broiler flocks during the period from May 2016 to December 2017 from different regions in North Sinai province were investigated clinically and grossly. Seven flocks aged between 4-7 days, with a mortality rate ranging between 8-13%, while the other 7 aged between 23-26 days of age, with a mortality rate ranging between 3-7%) Samples for histopathology and molecular examinations were collected in different stages of infection. Molecular studies were included real-time PCR, conventional PCR and sequence analysis for the suspected FAdV that caused IBH. Real-time PCR using commercial kits was used for screening then, conventional PCR was done for selective samples. Sequencing followed by phylogenetic analysis of the specific PCR products were performed to identify the strain(s). The partial hexon gene sequences of the 4 strains in this study (Sin-1, Sin-2, Sin-3, and Sin-4) were submitted to the NCBI GenBank under accession numbers (OK634389, OK634390, OK634391, and OK634392) respectively.

Materials and methods

Samples

Liver samples from 14 different commercial broiler flocks (5 pooled samples from each flock) suspected to have IBH infection from different areas of North Sinai province during the period from May 2016 till December 2017 were collected at the 2^{nd} to 5^{th} days of infection (4 samples), on the 7^{th} day of clinical signs (5 samples), and at the end of the broiler production cycle (5 samples). Samples were transported in 50% buffered glycerin on ice and stored at -20 ^{o}C until processing for PCR assays. Moreover, samples from 9 flocks out of the 14 investigated were also collected at different stages of clinical signs (3 samples at the beginning of signs at 2nd, 3rd, and 4th day; 4 samples on the 7^{th} day of clinical signs; and 2 samples at the end of production cycle after recovery) and fixed in 10% formalin solution for histopathological investigations. The age of birds at the onset of clinical symptoms varied from 4-26 days old with mortality rates ranging from 3-13%. Clinical signs and postmortem (PM) lesions were suggestive of IBH.

Histopathology

Histopathological examination was performed at the pathology unit at the Reference Laboratory for Veterinary Quality Control on Poultry Production Animal Health Research Institute, by using routine stain: a Hematoxylin and Eosin (H&E) staining as described

by ***(Feldman and Wolfe, 2014).*** The stained slide was then examined under a light microscope using X10, X20, X40, and X63 magnification powers to observe the pathological changes in tissue samples. Histological photos were taken by using Leica EC3 digital camera.

Extraction of nucleic acid

The entire DNA was extracted directly from all samples. DNA extraction kit (QIAamp DNA Mini Kit, QIAGEN, Hilden, Germany) was used following the manufacturer's directions. Then, measurement of DNA concentration was done using Thermo Scientific NanoDrop 2000, Applied Biosystems. The extracted DNA was stored at -20°C until used in PCR studies.

Real-time PCR

The real-time PCR was performed using the Applied Biosystem 7500 thermal cycler machine (Applied Biosystem Thermo Scientific, USA) and by using Kylt® kits for fowl Adenovirus qPCR (Batch no.19 qFAdV:02 - Cat.No.31745) by adding 16 µl of the ready-to-use Reaction-Mix, 4 µl of the Negative Control, 4 µl of DNA preparation, and 4 µl of the Positive Control. The reaction starts at 95 ºC for 10 minutes for polymerase enzyme activation. Afterward, 42 cycles of (denaturation at 95 ºC for15 seconds then annealing & extension at 60 ºC for 1min) were carried out. Samples with a Ct value ≤39 was considered positive.

Conventional PCR

The conventional PCR assay was carried out through amplification of a portion of theloop-1 hexon gene using the primer pairs: Forward (5′-CAA RTT CAG RCA GAC GGT-3′) at the position 144–161 nt. And Reverse (5′-TAG TGA TGM CGS GAC ATC AT-3′) at the position 1041–1021 nt. produced specially by SIGMA-ALDRICH®, generating PCR product of the size of approximately 900 bp as described by ***(Mittal et al., 2014; Xia et al., 2017).*** The whole volume was 50 µl containing 10 ng of DNA, 200 µM dNTPs mix, 20 pmol of each primer, 2.5U Taq polymerase, and 1.5 mM MgCl2. The reaction has been performed in a thermal cycler (Biometra, UK). The initial denaturation temperature was 94°C for 5 minutes. After that,35 repeated cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 1 min were achieved then a final extension step at 72 °C for 10 min. Agar gel (1.5 %) was stained by ethidium bromide in which, the PCR products were electrophoresed in the presence of 250bp plus ladder and photographed by gel documentation system (Biometra, Germany).

DNA purification and Partial hexon gene sequencing

Purification of the amplified PCR products was performed by the use of the QIAquick Gel Extraction kit (QIAGEN) according to manufacture instructions then the purified PCR products were sequenced in both directions by using genetic analyzer machine 3130 (Applied Biosystems, USA) at Colors Medical Lab, El-Maadi, Cairo, Egypt.

Genotyping and phylogenetic analysis

The phylogenetic analysis was applied with Mega X. version-10 software, for alignment of the nucleotide sequences of the partial hexon loop-1 gene of the 4 samples obtained in this study, and the phylogenetic tree was created for the 4 samples in our study in comparison to the available strains in the GenBank database via neighbor-joining method; bootstrapping at 1000 repeats.

Results

Clinical and postmortem examination

Suspected flocks show sick birds lie on their sides with reduced feed intake, depression, ruffled feathers, pale comb and facial skin and die within 48 hours. The mortality curve takes about one week then recovered birds may show severe growth retardation and immune suppression.PM examination revealed hydropericardium, liver enlargement with yellow-tan discoloration and ecchymotic hemorrhages, Pancreatitis, Pneumonia, marbled spleen, Pale enlarged kidneys, enteritis, and ascites in severe cases (figure 1).

Histopathology

All samples collected during the course of infection revealed varying degrees of histological liver lesions mostly, multifocal necrosis, and extensive congestion with inflammatory cellular infiltration. The presence of intranuclear inclusion bodies was variable among samples. Samples collected early on the 2^{nd} and 3^{rd} days of clinical signs did not show any inclusions, but severe vacuolar fatty degeneration, hemorrhages, and congestions are the most predominant findings figure (2). The one sample collected on the 4^{th} day of clinical signs showed few INIB figure (3). The 4 samples collected at the sub-acute stage of infection on the 7^{th} day of clinical signs showed a large Basophilic INIB figure (4). The last 2 samples collected after recovery at the end of the production cycles showed normal liver tissues Figure (5).

Real-time PCR

FAdVs were detected in liver samples from 11 flocks out of the 14 examined by real-time PCR using commercial kits. The results of fluorescence curve altitudes

were variable indicating the differences in viral load among samples. The 4 liver samples collected in per-acute and acute stages of infection (2^{nd}-5^{th} days of infection) revealed the highest fluorescence curve altitudes indicating the highest viral load Figure (6). Of these 4 samples, 3 samples (75%) were from flocks that get infected at 4, 6, and 7 days old while, 1 sample (25%) was from a flock that exhibited clinical signs at 26 days old. The remaining 7 positive real-time PCR samples were collected on the 7^{th} day of clinical signs (5 samples) and at the end of production cycles (2 samples) were expressing lower fluorescence curve altitudes Figure (7). The cycle threshold (Ct) values of the eleven positive samples are presented in table (1). Of the 14 tested, 3 samples collected at the end of production cycles after recovery were negative by real-time PCR.

Conventional PCR

A band of approximately 900 bp was detected in the 4 samples amplified in conventional PCR as shown in figure (8).

Gene sequence alignment and phylogenetic analysis

The Purified PCR products of the four positive samples were sequenced in both directions. Phylogenetic analysis according to the acquired sequences was used to categorize the four FAdVs isolates into serotype 2 (species D). The partial hexon gene sequences of the 4 strains in this study were submitted to the NCBI GenBank under accession numbers (OK634389, OK634390, OK634391, and OK634392) respectively. The relationships, as well as similarities of the partial hexon gene sequences attained in this research, were compared with the previously published strains available in the GenBank shown in table (2).

The strains in this study are sharing 99.82%, 99.63%,99.60 and 98.2% base identity with loop-1 hexon gene sequences of FADV-2 (species D) of (MT759842.1), (MK572870.1), (MH782426.1) and (MG832884.1) of Israel, Japan, Egypt, and Canada respectively. The phylogenetic tree was created as offered in figure (9).

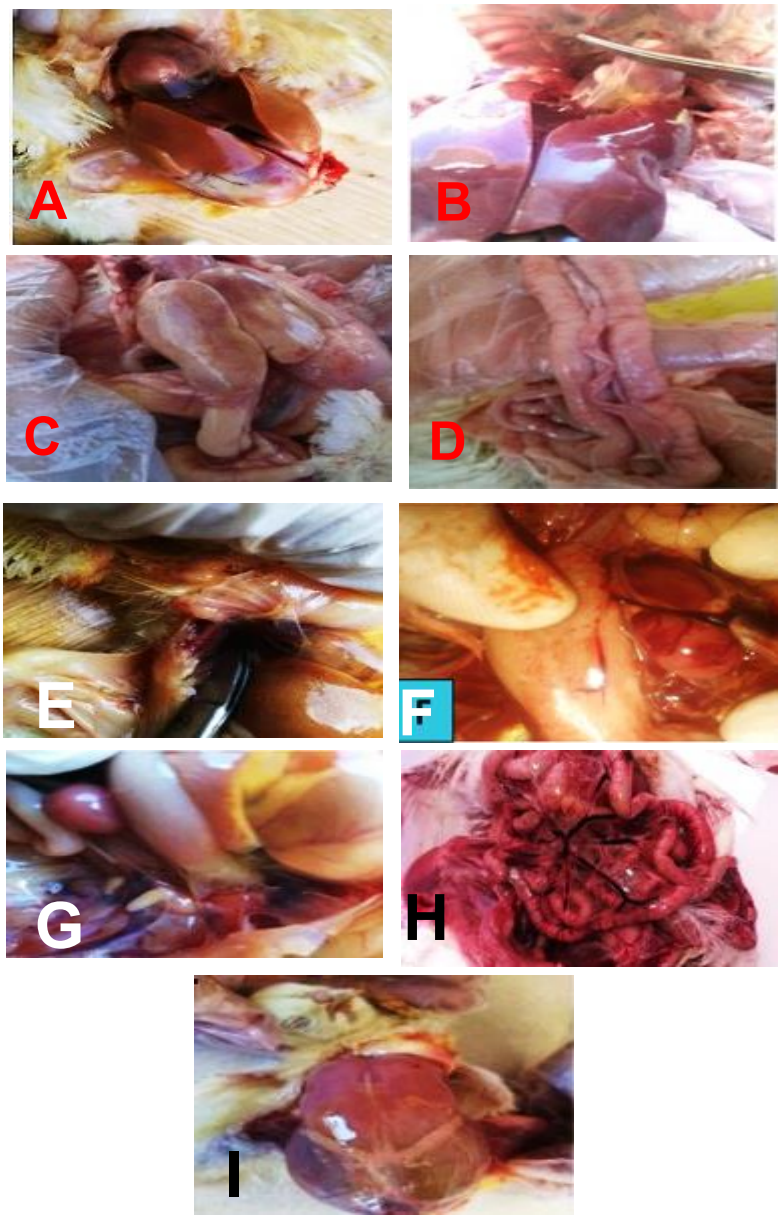

Figure (1): A: Hydropericardium with yellowish enlarged liver of 4 days old chick. **B:** hydropericardium with an enlarged liver of 25 days old broiler. **C:** Swollen yellow-tan discolored liver with ecchymotic hemorrhages. **D:** Pancreatitis with mottling surface. **E:** Pneumonia. **F:** Spleen with faint marbling appearance. **G**: Pale, enlarged kidneys, and pale spleen. **H:** Enteritis, ramified blood vessels are seen from the serosal surface. **I:** Ascites in a severe case.

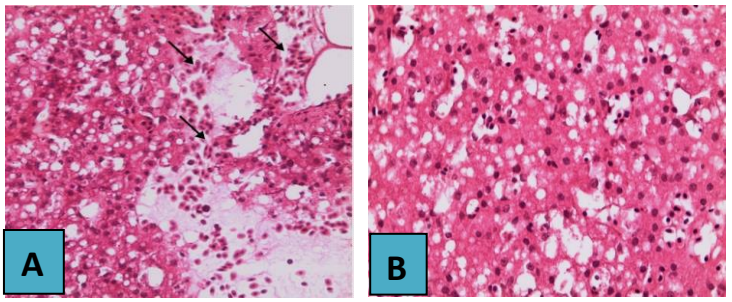

Figure (2): A: X10 H&E liver tissue on 2nd day of clinical signs showing severe vacuolar fatty degeneration, hemorrhages, congestion of hepatic sinusoids (upper right arrow), and leukocytic infiltration (2 left arrows). Note absence of intranuclear inclusions. **B:** X20 H&E liver tissue on the 3rd day of clinical signs showing fatty change, congestion of hepatic sinusoids, and leukocytic infiltration also with the absence of INIB.

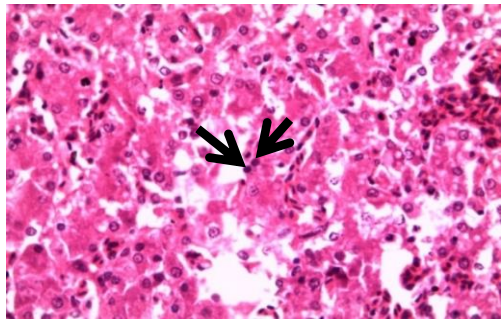

Figure (3): X63 H&E liver tissue on the 4th day of the start of the high mortality rate showing s vacuolar degeneration, hemorrhages, with few basophilic INIB (black arrows).

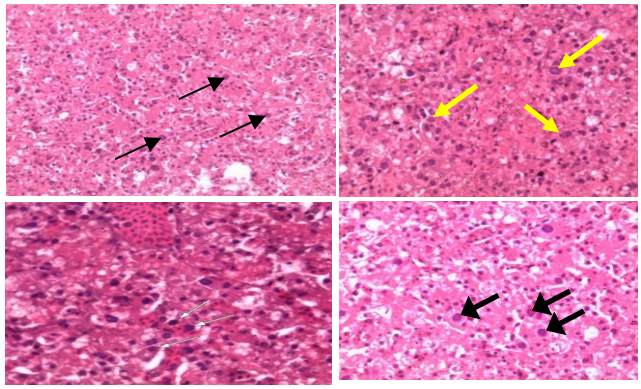

Figure (4): X10, X20, and X40 H&E liver tissue samples on the 7th day of clinical signs showing large multiple basophilic INIB.

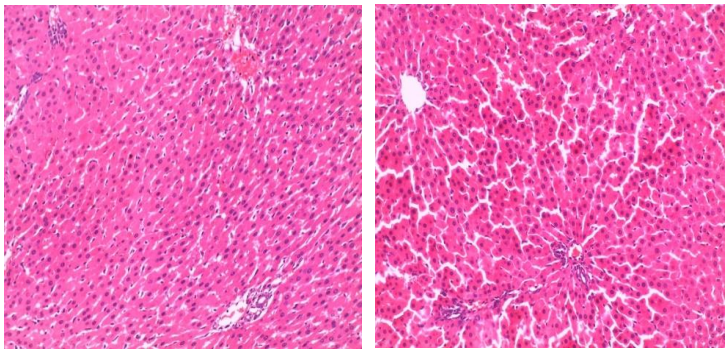

Figure (5): 2 samples 10x H&E collected after recovery showing normal liver tissue.

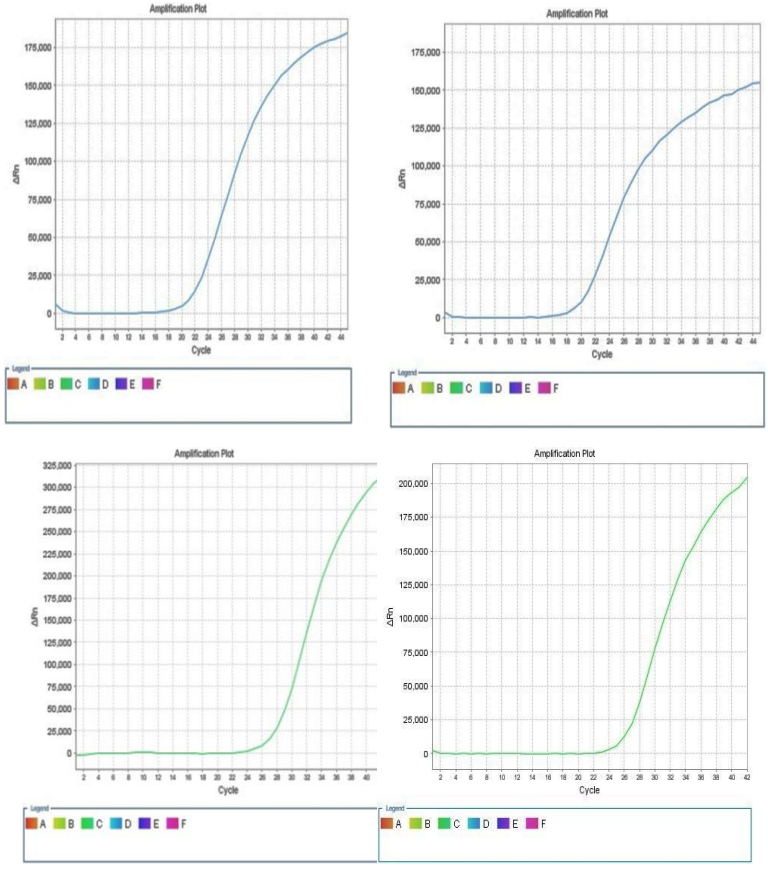

Figure (6): Amplification plots of the highest four fluorescence curve altitudes samples

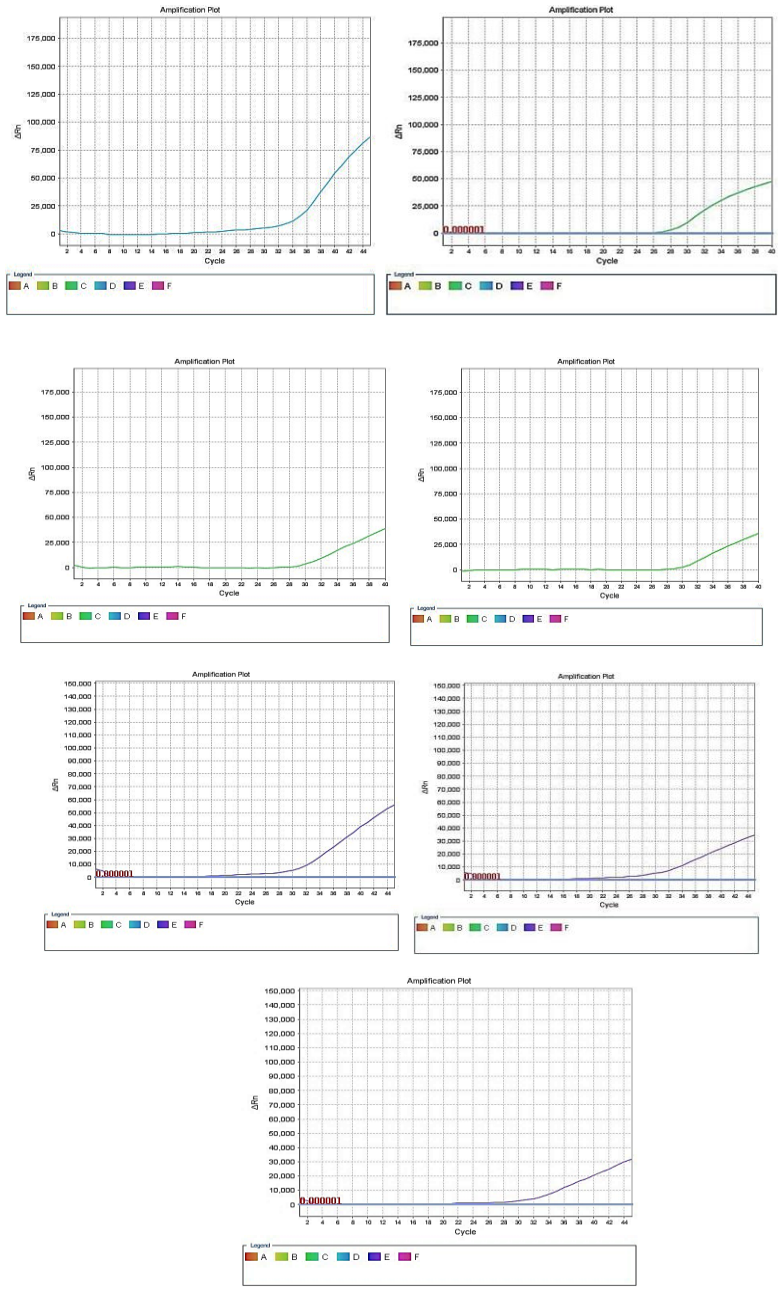

Figure (7): Amplification plots of seven real-time PCR positive samples with low curve altitudes.

Table (1): *Cycle threshold (Ct) values of the 11 real-time positive samples*

Sample ID	Cycle threshold (Ct)
B_1	20
B_2	22
Z_1	26
A_4	28
B_3	30
B_4	30
B_5	30
B_6	32
A_1	32
A_2	34
A_3	34

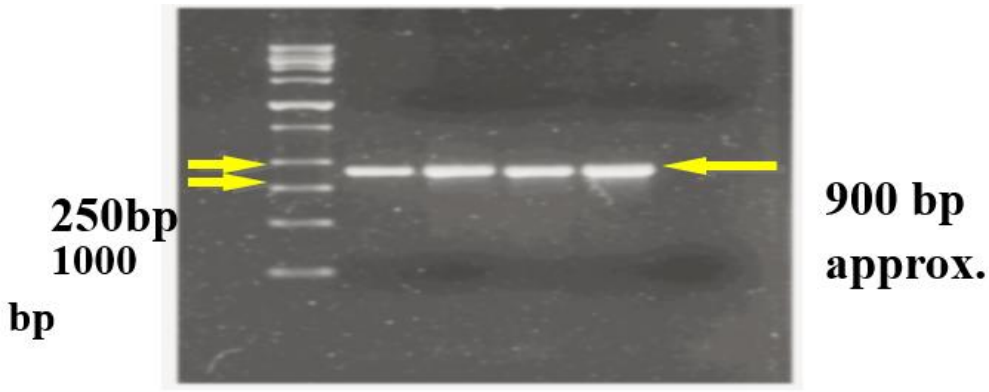

Figure (8): Agar gel electrophoresis of the amplified products of the partial hexon gene.

Table (2): *FAdVs strains with GenBank accession numbers used in sequence analysis of partial hexon gene.*

Genotype	Serotype	Isolate (Strain)	GenBank accession number	Country
FAdV- C	FAdV- 4	SCdl1602	**KY927937.1**	China
FAdV- C	FAdV- 4	4158	**HM592284.1**	Italy
FAdV- A	FAdV- 1	CELO	**EU979367.1**	Australia
FAdV- B	FAdV- 5	340	**EU979371.1**	Australia
FAdV- B	FAdV- 5	340	**AF508952.2**	Belgium
FAdV- D	FAdV- 9	764	**AF508958.2**	Belgium
FAdV- E	-----	USP401-4B/Brazil/2010	**JF288950.1**	Brazil
FAdV- E	-----	USP401-6B/Brazil/2010	**JF288951.1**	Brazil
FAdV- D	FAdV- 9	A02	**EU979376.1**	Australia
FAdV- D	FAdV- 3	75	**AF508949.1**	Belgium
FAdV- D	FAdV- 3	23548/t/263/2010 Debrecen	**KC750797.1**	Hungary
FAdV- D	FAdV- 11	Indovax_FadvD_RP18	**MN540445.1**	India
FAdV- D	FAdV- 11	Indovax_FadvD_KHR_17	**MN540444.1**	India
FAdV- D	FAdV- 11	Indovax_FADV_mhr_17	**MN537891.1**	India
FAdV- D	FAdV- 11	Indovax_FADVD_LT-BL_18	**MN537890.1**	India
FAdV- D	**FAdV- 2**	------	**MG832884.1**	**Canada**
FAdV- D	FAdV- 2	08-9513	**MK572870.1**	Japan
FAdV- D	FAdV- 2	GB591	**MK572868.1**	Japan
FAdV- D	FAdV- 2	--------	**AM407391.2**	Austria
FAdV- D	FAdV- 2	dmn2	**MH782424.1**	Egypt
FAdV- D	FAdV- 2	kfr4	**MH782426.1**	Egypt
FAdV- D	FAdV- 2	bst11	**MH782423.1**	Egypt
FAdV- D	FAdV- 2	IS/3346/2020	**MT759842.1**	Israel

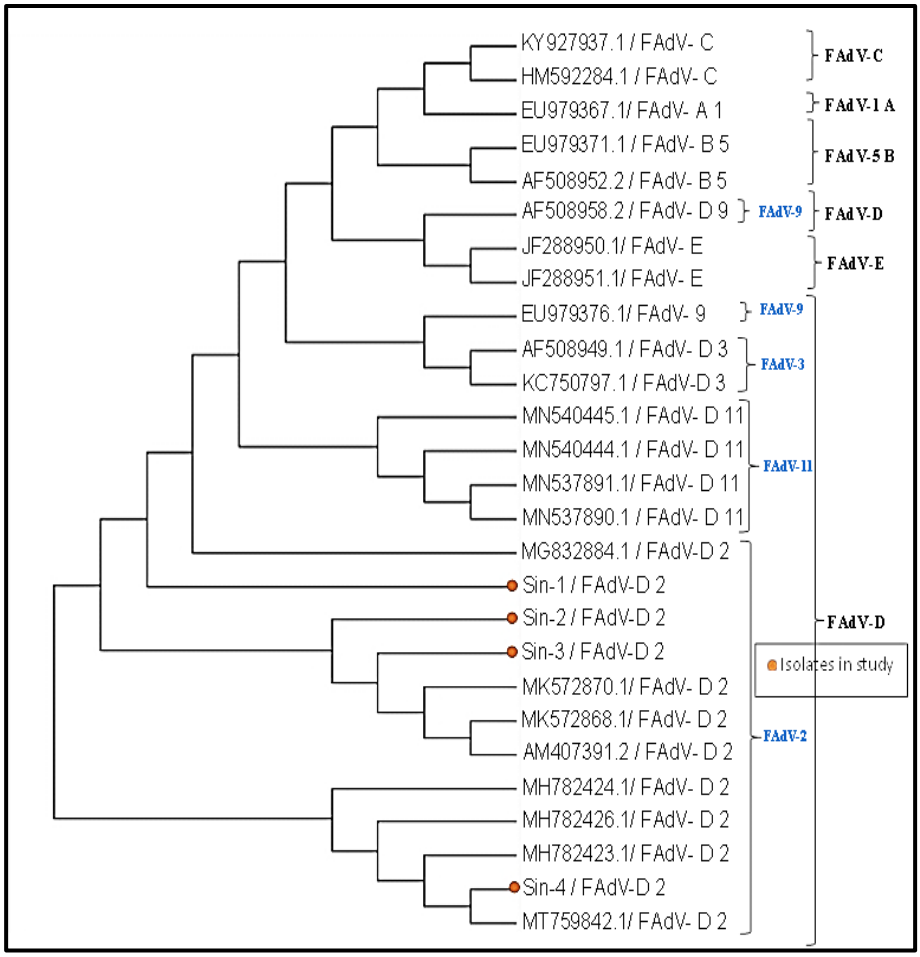

Figure (9): Phylogenetic tree for the nucleotide sequence of partial hexon gene sequences, using Mega X software at 1000 bootstrap.

Discussion

Fowl aviadenoviruses (FAdV) are widespread all over the world. Domesticated fowls of all ages may acquire infection, while other bird species appear to be vulnerable to infection with FAdV besides their adenovirus serotypes. ***(Hess, 2020)***. During the last two decades, IBH epidemics have been recorded in different geographic localities with increasing numbers proposing ubiquity spread of the disease worldwide, the main cause were FAdVs of the serotypes FAdV-2, FAdV-11 (species D), and FAdV-8a and FAdV-8b (species E) ***(Schachner et al., 2018).*** Vertical transmission is involved in the spreading of FAdV infection and FAdVs themselves can result in compromised

immune response in young broilers which are more susceptible to infection especially in the absence of maternal antibodies **(Schachner *et al.*, 2021)**. In Egypt, interest in the molecular characterization of the virus has increased in the past few years from field outbreaks in many regions of the country ***(El-Basrey et al., 2020; El-Tholoth and Abou El-Azm, 2019; Elbestawy et al., 2020; Radwan et al., 2019; Sultan et al., 2021).*** In our research, mortality rates and clinical signs were similar to that of IBH described previously by ***Ismahane et al. (2015)***. PM lesions were typical lesions of IBH infections in chickens ***(Grimes et al., 1978; Wibowo et al., 2019).*** histopathologically, although intranuclear inclusion bodies INIB are considered pathognomonic for adenoviral infections ***(McFerran and Smyth, 2000),*** the collection of samples at different stages of infection showed a marked variation in their presence, which made its reliability to confirm infection was not accurate enough. INIB were not present at very early stages in per-acute infections, samples collected at 2nd and 3rd day of mortality raise revealed severe vacuolar fatty degeneration, hemorrhages, congestions, and inflammatory cells infiltrations but did not show any inclusions, although both samples were positive for FAdVs in real-time PCR with high viral load. Dense basophilic inclusions are observed clear and abundant in samples collected on the 7th day of clinical signs. No inclusions were observed in liver samples collected at the end of the production cycle after recovery from infection. These findings were identical to the results reported earlier in the field study and confirmed by experimental infection by ***El-Meligy et al. (2002)***. Real-time PCR was proven for the detection and quantification of all FAdV species with high efficiency and sensitivity even in low viral load in cloacal swabs ***(Günes et al. (2012).*** In this study, FAdVs were detected in liver samples from 11 flocks out of the 14 examined by real-time PCR using commercial kits. The results of fluorescence curve altitudes were variable indicating the differences in viral load among samples. Since the real-time PCR technique used in the study is highly sensitive for all FAdV serotypes, It is doubtful that the three negative results were caused by poor test performance. The most likely probability is that after recovery of the birds, viral load decreases dramatically in the liver together with tissue regeneration ***(Matos et al., 2016; Steer et al., 2015).*** The gene most commonly used for the detection

of FAdVs in PCR studies is the hexon gene ***(Raue & Hess, 1998; Xie et al., 1999).*** In our research, a 900 bp band was noticed for the 4 samples amplified in conventional PCR using primers targeting the hexon loop L1 gene as described by ***Mittal et al. (2014) and Xia et al. (2017)***. Moreover, sequencing of the specific PCR products and phylogenetic analysis using Mega-X software were carried out. Then, a neighbor-joining-based phylogenetic tree was constructed using the sequences previously mentioned. The results elucidated that the four FAdVs isolates in the study were clustered into serotype-2, FAdV-D species. The results revealed that our 4 strains were sharing 99.82%, 99.63%,99.60, and 98.2% base identity with loop-1 hexon gene sequences of FADV-2 (species D) of (MT759842.1), (MK572870.1), (MH782426.1), and (MG832884.1) of Israel, Japan, Egypt, and Canada respectively. Serotype FAdV-2 and species FAdV-D was also isolated from commercial poultry flocks in Egypt indicating its continuous circulation in different geographic areas of the country causing significant losses ***(El-Basrey et al., 2020; El-Tholoth and Abou El-Azm, 2019; Elbestawy et al., 2020).***

Conclusion

The FAdV-2 (species D) was the causative agent of IBH in broiler chickens in North Sinai province during the period from May 2016 till December 2017 with the opportunity to cause several outbreaks. Real-time PCR is a highly sensitive, specific, and rapid diagnostic aid for the identification and quantification of FAdVs. Further investigations are essential to determine the epidemiology of the FAdVs in different geographic areas in Egypt which could be important to give an integrated picture that helps in prevention efforts.

References

Adair, B., Fitzgerald, S.D. (2008). Group 1 adenovirus infections. Diseases of poultry 12, 251-266.

Adam, E., Nász, I., Hudecz, F., Lengyel, A., Mezö, G., Dobay, O. (1998). Characterization of intertype-specific epitopes on adenovirus hexons. Archives of virology 143, 1669-1682.

Ebner, K., Pinsker, W., Lion, T. (2005). Comparative sequence analysis of the hexon gene in the entire spectrum of human adenovirus serotypes: phylogenetic, taxonomic, and clinical implications. J Virol 79, 12635-12642.

El-Basrey, Y., Hamed, R., Mohamed, M., Lebdah, M. (2020). Detection of Inclusion Body Hepatitis Virus in broilers at Sharkia Province, Egypt. Journal of Animal Health and Production 9.

El-Meligy, A., Fetaih, H., Dessouki, A. (2002). Pathological investigations on naturally and experimentally adenovirus infected chicks and comparative study on the reliability of some diagnostic techniques. Suez Canal Veterinary Medicine Journal (Egypt).

El-Tholoth, M., Abou El-Azm, K.I. (2019). Molecular detection and characterization of fowl adenovirus associated with inclusion body hepatitis from broiler chickens in Egypt. Trop Anim Health Prod 51, 1065-1071.

Elbestawy, A.R., Ibrahim, M., Hammam, H., Noreldin, A., El-Bahrawy, A., Ellakany, H. (2020). Molecular Characterization of Fowl Adenovirus D Species in Broiler Chickens with Inclusion Body Hepatitis in Egypt. Alexandria Journal of Veterinary Sciences 64, 110.

Feldman, A. T., & Wolfe, D. (2014). Tissue processing and hematoxylin and eosin staining. Methods in molecular biology (Clifton, N.J.), 1180, 31–43. https://doi.org/10.1007/978-1-4939-1050-2_3

Fitzgerald, S.D., Rautenschlein, S., Mahsoub, H.M., Pierson, F.W., Reed, W.M., Jack, S.W. (2020). Adenovirus infections. Diseases of poultry, 321-363.

Grimes, T.M., Fletcher, O.J., Munnell, J.F. (1978). Comparative study of experimental inclusion body hepatitis of chickens caused by two serotypes of avian adenovirus. Vet Pathol 15, 249-263.

Günes, A., Marek, A., Grafl, B., Berger, E., Hess, M. (2012). Real-time PCR assay for universal detection and quantitation of all five species of fowl adenoviruses (FAdV-A to FAdV-E). J Virol Methods 183, 147-153.

Hess, M. (2000). Detection and differentiation of avian adenoviruses: a review. Avian Pathol 29, 195-206.

Hess, M. (2020). Aviadenovirus Infections, in David E. Swayne, M.B., Catherine M. Logue, Larry R. McDougald, Venugopal Nair, David L. Suarez (Ed.), Diseases of Poultry, Fourteenth Edition. John Wiley & Sons, Inc., pp. 322-332.

Hess, M., Cuzange, A., Ruigrok, R.W., Chroboczek, J., Jacrot, B. (1995). The avian adenovirus penton: two fibers and one base. Elsevier.

Ismahane, L., Alloui, N., Bennoune, O., Laabaci, W., Ammar, A., Benterki, M. (2015). Clinical and Histopathological Investigations on Inclusion Body Hepatitis in Chickens in the Ain Touta Area (Algeria). Global Journal of Animal Scientific Research 30, 72-76.

Matos, M., Grafl, B., Liebhart, D., Schwendenwein, I., Hess, M. (2016). Selected clinical chemistry analytes correlate with the pathogenesis of inclusion body hepatitis experimentally induced by fowl aviadenoviruses. Avian Pathology 45, 520-529.

McFerran, J.B., Smyth, J.A. (2000). Avian adenoviruses. Rev Sci Tech 19, 589-601.

Mittal, D., Jindal, N., Tiwari, A.K., Khokhar, R.S. (2014). Characterization of fowl adenoviruses associated with hydropericardium syndrome and inclusion body hepatitis in broiler chickens. Virus disease 25, 114-119.

Radwan, M.M., El-Deeb, A.H., Mousa, M.R., El-Sanousi, A.A., Shalaby, M.A. (2019). First report of fowl adenovirus 8a from commercial broiler chickens in Egypt: molecular characterization and pathogenicity. Poult Sci 98, 97-104.

Raue, R., Hess, M. (1998). Hexon-based PCRs combined with restriction enzyme analysis for rapid detection and differentiation of fowl adenoviruses and egg drop syndrome virus. J. Virol. Methods 73(2): 211-217.

Russell, W. (2009). Adenoviruses: update on structure and function. Journal of General Virology 90, 1-20.

Schachner, A., Grafl, B., Hess, M. (2021). Spotlight on avian pathology: fowl adenovirus (FAdV) in chickens and beyond – an unresolved host-pathogen interplay. Avian Pathology 50, 2-5.

Schachner, A., Matos, M., Grafl, B., Hess, M. (2018). Fowl adenovirus-induced diseases and strategies for their control–a review on the current global situation. Avian Pathology 47, 111-126.

Steer, P.A., Sandy, J.R., O'Rourke, D., Scott, P.C., Browning, G.F., Noormohammadi, A.H. (2015). Chronological analysis of gross and histological lesions induced by field strains of fowl adenovirus serotypes 1, 8b, and 11 in one-day-old chickens. Avian pathology 44, 106-113.

Sultan, H., Arafa, A.-E., Adel, A., Selim, K., Hossiny, M., Talaat, S. (2021). Molecular Detection of a Novel Fowl Adenovirus Serotype-4

(FadV-4) from an Outbreak of Hepatitis Hydropericardium Syndrome in Commercial Broiler Chickens in Egypt. Avian diseases 65, 385-390.

Toogood, C.I., Crompton, J., Hay, R.T. (1992). Antipeptide antisera define neutralizing epitopes on the adenovirus hexon. Journal of General Virology 73, 1429-1435.

Wibowo, M., Sahesty, A., Mahardika, B., Purwanto, B., Lestariningsih, C., Suryawan, I., Suardana, K., Winaya, O., Irine, I., Suryanggono, J., Jonas, M., Murwijati, T., Mahardika, G., Cdf, M. (2019). Epizootiology, Clinical Signs, and Phylogenetic Analysis of Fowl Adenovirus in Chicken Farms in Indonesia from 2018 to 2019. Avian diseases 63, 619-624.

Xia, J., Yao, K.-C., Liu, Y.-Y., You, G.-J., Li, S.-Y., Liu, P., Zhao, Q., Wen Rui Wu, Y.-P., Huang, X.-B., Cao, S.-J., Han, X.-F., Huang, Y. (2017). Isolation and molecular characterization of prevalent Fowl adenovirus strains in southwestern China during 2015–2016 for the development of a control strategy. Emerg Microbes Infect 6, 1-9.

Xie, Z., Fadl, A.A., Girshick, T., Khan, M. I. (1999). Detection of avian adenovirus by polymerase chain reaction. Avian Dis. 43: 98-105.

الاكتشاف الجزيئي لفيروس أدينوالطيور المصاحب لإلتهاب الكبد المتضمن في دجاج اللحم بمحافظة شمال سيناء

حسن محمد أبوزيد[1] ، محمد أحمد سليمان[2] ، إيمان كمال السيد[3] ، نورهان محمد علي[3] ، مختار محمد الطرابيلي[3]

[1]مركز تحصين الدواجن بالعريش مديرية الطب البيطري بمحافظة شمال سيناء. [2] المعمل المرجعي للرقابة البيطرية على جودةالإنتاج الدواجني ، معهد بحوث الصحة الحيوانية ؛[3] قسم الفيروسات بكلية الطب البيطري جامعة قناة السويس

الملخص

تحظى فيروسات أدينو الطيور (FAdV) بمزيد من الاهتمام في مصر وفي جميع أنحاء العالم هذه الأيام ، لأنها السبب الرئيسي للعديد من الأمراض الشائعة. من بين هذه الأمراض ، التهاب الكبد المتضمن (IBH). في السنوات العديدة الماضية ، كان هناك العديد من حالات تفشي مرض IBH في جميع أنحاء البلاد ، مما أدى إلى خسائر مالية كبيرة لقطاع الدواجن في مصر. تم إنشاء هذه الدراسة للكشف عن FAdV من التفشي الميداني لـ IBH في الدجاج اللاحم في محافظة شمال سيناء خلال الفترة من مايو 2016 حتى ديسمبر 2017. الطيور المصابة تستلقي على جانبها مع انخفاض تناول العلف ، والخمول ، والريش المنتفش ، وبهوت العرف وجلد الوجه . فيما يتعلق

بالتشريح ، كانت الآفات الأكثر شيوعًا بعد الوفاة هي استسقاء القلب ، وتضخم الكبد المصفر مع كدمات نزيف ، والتهاب البنكرياس ، والتهاب الأمعاء. أظهر فحص الأنسجة المرضية لعينات الكبد التي تم جمعها في مراحل مختلفة من العدوى نخرًا متعدد البؤر ، واحتقانًا واسعًا مع ارتشاح خلوي التهابي. كان وجود هيئات الإدماج النووي (INIB) متغيرًا بين العينات. تم اكتشاف FAdV في عينات الكبد من 11 قطيعًا من أصل أربعة عشر تم فحصها بواسطة تفاعل البلمرة المتسلسل في الوقت الحقيقي باستخدام أدوات تجارية مع وجود اختلافات في الحمل الفيروسي بين العينات. تم تضخيم عينات الحمض النووي من أربعة قطعان أظهرت أعلى ارتفاعات منحنى الإزهار باستخدام PCR التقليدي الذي يستهدف جين hexon متبوعًا بتسلسل منتجات PCR المحددة. أظهر التحليل الوراثي المعتمد على تسلسل جين حلقة هيكسون -1 أن المعزولات الأربع (Sin-1 و Sin-2 و Sin-3 و Sin-4) تم تجميعها في النوع المصلي 2 و FAdV-D. تشترك السلالات في هذه الدراسة في 99.82٪ ، و 99.63٪ ، و 99.60٪ ، و 98.2٪ في هوية النوكليوتيدات مع تسلسل الجين Hxon loop-1 لـ FADV-2 (الأنواع D) في إسرائيل واليابان ومصر وكندا ، على التوالي. توفر هذه الدراسة معلومات وبائية يمكن أن تساعد في صياغة استراتيجية وقائية فعالة.