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Molecular Diagnosis of Avihepatovirus a in Naturally Infected Duck Flocks in Egypt

A. El-Shemy[1*], Hoda M. Mekky[2], M. A. Bosila[2], A. M. Allam[1], Kh. M. Elbayoumi[2], M. M. Amer[3]

[1]Department of Parasitology and Animal Diseases, Veterinary Research Institute, National Research Centre, P.O. 12622, Giza, Egypt; [2]Poultry Diseases Department, Veterinary Research Institute, National Research Centre, P.O. 12622, Giza, Egypt; [3]Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt.

Abstract | Duck virus hepatitis (DVH) is one of the most significant viral diseases in young ducklings; it results inhigh potential mortality and great economic losses. We collected liver samples from three duck farms representing Muscovy and Pekin breeds at ages ranging from 2 to 4 weeks and located in two Egyptian governorates between August and October 2018. The affected ducks were suffering from a short course of nervous signs, and mortality was high, reaching 75% within 3–4 days. Implementation of one-step reverse transcription polymerase chain reaction (RT-PCR) for processed livers using specific primers aiming the 3D gene of duck hepatitis A virus-1 (DHAV-1) showed the output of the amplified band in all examined specimens at the certain supposed size of the 3D encoding gene (467 bp). The successfully amplified 3D gene of DHAV-1 isolates, namely DHV/Duck/Egypt/Monofia-Shemy-1/2018, DHV/Duck/Egypt/Al-Gharbia-Shemy-3/2018, and DHV/Duck/Egypt/Monofia-Shemy-2/2018, was subjected to sequencing and then submitted to NCBI GenBank, and the obtained accession numbers are MT157210, MT157211,and MT157212, respectively. Nucleotide sequences alignment analysis of the 3D gene exhibited 92.5%–98.6% homology between our sequenced isolates. The three examined isolates had 77.4%–80.2% nucleotide similarities with the most frequently used DHAV-1 vaccine in Egypt. However, the phylogenetic relationship using the partial 3D protein revealed that the isolated Egyptian strains in this study were distant from the vaccine strain used in Egypt. In conclusion, three isolates of DHAV-1 were characterized from the examined duck flocks. Phylogenetic analysis of these isolates revealed the occurrence of differentiation between our field DHAV-1 isolates and the vaccine strain. Therefore, applying an appropriate vaccination program for breeder duck flocks is significant in controlling DHAV-1 infection. Thus, we recommend the application of further studies to detect the efficacy of the used vaccine against the current field strains of DHAV-1

Keywords | Nervous signs, DHAV-1, RT-PCR, Nucleotide sequences, Phylogenetic analysis

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***Correspondence** | A El-Shemy, Department of Parasitology and Animal Diseases, Veterinary Research Institute, National Research Centre, P.O. 12622, Giza, Egypt; **Email:** dr.ahmedelshemy@gmail.com
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INTRODUCTION

Duck virus hepatitis (DVH) is an acute viral disease that commonly affects flock of ducklings that are up to 3 weeks old. It is characterized by a sudden onset causing severe losses because of its high mortality, ranging from 90% to 100% within 3–4 days. The disease is caused by three distinct serotypes of duck hepatitis virus (DHV-

1–3), which is a member of the genus Avihepatovirus of the Picornaviridae family (Fu et al., 2008, ICTV, 2017). Serotype 1 (DHV-1) is renamed as duck hepatitis A virus-1 (DHAV-1) and represents the most virulent serotype with an important effect on the worldwide poultry industry (Ding and Zhang, 2007; Tseng et al., 2007). The disease was first reported in 1945 inthe USA (Levine and Fabricant, 1950). After that, the spread of the disease was recorded in several countries (Guerin et al., 2007; Sandhu et al., 1992; Woolcock, 2008). The clinical manifestations are characterized by systemic convulsions, locomotor disorders, feet spasm, and opisthotonos. In addition, the liver appears enlarged with petechial and ecchymotic hemorrhages on the surface (Liu et al., 2008; Tsai, 2020). Therefore, both prevention and rapid diagnosis are the logical and most effective strategies for controlling the disease (Woolcock, 2003).

The DHAV-1 viral genome is formed from a nonenveloped, positive-sense, single-stranded RNA of approximately 7.8 kb. It comprises the open reading frame encoding leader protein (L), structural proteins (VP0, VP1, and VP3), and nonstructural proteins (2A1, 2A2, 2A3, 2B, 2C, 3A, 3B, 3C, and 3D) (Wei et al., 2012). The 3D gene is located in the P3 segment of the picornavirus genome. VP1 capsid protein is considered the main antigenic determinant, as in other picornaviruses. Therefore, it shared its viral pathogenicity and virulence with DHAV (Gao et al., 2012). In addition, the 3D gene encodes a protein that implicates a conserved domain known as RNA-dependent-RNA polymerase (RdRp) that contributes toviral RNA synthesis throughout virus replication (Kim et al., 2006; Kerkvliet et al., 2010).

Conventional diagnoses are based on clinical signs, such as gross lesions confirmed using virus isolation and serological tests, which are laborious, time-consuming, and exhibit low sensitivity. To overcome these drawbacks, various bioassays and techniques of polymerase chain reactionhave been developedfor the rapid, simple, and potent diagnosis and detection of the persistent evolution of DHAV-1. Moreover, the application of the PCR assay offered a great understanding of the pathogenesis and diagnosis of DHAV-1. The successful detection of DHAV-1 in cell culture and tissues from infected ducks via PCR using species-specific primer sets against viral DNA polymerase enzymes has been reported (Kim et al., 2007; Liu et al., 2007; Anchun et al., 2009; Wen et al., 2014; El-Samadony et al., 2018).

In Egypt, DVH was discovered in the1970s. Since the prevalence of duck farms in Egypt, DHAV-1 has posed destructive damages and constitutes a major menace for commercial duck farms (Ellakany et al., 2002; Erfan et al., 2015; Hisham et al., 2020). For influential domination on the DHAV-1, massive immunization was performed in Egypt (Ellakany et al., 2002; Abd-Elhakim et al., 2009). However, despite the attenuated Rispens strain E52 vaccine being commercially used to immunize breeder ducks against DHAV-1, severe mortalities were recorded in both vaccinated and non-vaccinated ducks and DHAV-1 still poses a continual significant threat in the duck sector in Egypt (Abd-Elhakim et al., 2009). Lately, research has been applied for the surveillance and molecular characterization of DHAV-1 in various studies (Erfan et al., 2015; Yehia et al., 2021; Rohaim et al., 2021).

This study aimed to perform a molecular diagnosis of the DHAV-1 circulating in naturally infected flock of ducks using reverse transcription PCR (RT-PCR) followed by obtaining a genetic sequence of the 3D gene to track the evolutional variations at the molecular level.

MATERIAL AND METHODS

Duck flocks

Three mixed-sexflock of ducks (two Muscovy and one Pekin) aged 2–4 weeks with flock capacity range of 4500–5000 birds were used in the study. These flocks showed signs and lesions of DVH, with a mortality rate of 8.9% on the firstday that increased till 75% in all flocks within 4 days. Flocks of duck were reared in commercial farms in Monofia and Al-Gharbia governorates, Egypt. As per the breed management manuals, the flocks were fed on a balanced ration (NRC, 1984).

Samples

The liver samples were aseptically collected from freshly dead ducklings or ducklings with clinical signs and lesions between August and October 2018. Each sample was classified into two groups. One group was sent for histopathological examination, and the other group was sent for RT-PCR application. First, each sample for RT-PCR was homogenized and suspended 20% W/V in sterile antibiotics saline and then subjected to three successive freeze–thaw cycles, and the suspension fluid was separated via centrifugation at 3000 rpm for 15 min. Finally, the clear supernatant was separated and kept at −20°C till further use in RT-PCR (Woolcok, 1998).

Histopathological examination

The liver specimens of the affected ducklings were fixed in 10% buffered neutral formalin solution, processed in paraffin sections of4–6 µm, stained with hematoxylin and eosin (H&E), and then microscopically examined (Suvarna et al., 2012).

Table 1: Published sequences of 3D of DHV used for multiple alignment analysis.

NO.	Accession No.	DHAV-1 strain name	Governorate	Breed	Collection date
1	MT157210	DHV/Duck/Egypt/Monofia-Shemy-1/2018*	Monofia	Muscovy	2018
2	MT157211	DHV/Duck/Egypt/Al-Gharbia-Shemy-3/2018*	Al-Gharbia	Pekin	2018
3	MT157212	DHV/Duck/Egypt/Monofia-Shemy-2/2018*	Monofia	Muscovy	2018
4	KP148263.1	DVH A virus strain vaccine			
5	KP148264.1	F340	Ismailia	Pekin	2012
6	KP148265.1	F953	Behara	Pekin	2013
7	KP148266.1	F86	Luxor	Pekin	2013
8	KP148267.1	F163	Luxor	Muscovy	2013
9	KP148268.1	F413	Qena	Muscovy	2013
10	KP148269.1	F729	Qaluibia	Mallard	2013
11	KP148270.1	F829	Giza	Green Winged	2013
12	KP148271.1	F10	Ismailia	Mallard	2014
13	KP148272.1	F243	Sharkia	Muscovy	2014
14	KP148273.1	F355	Sharkia	Pekin	2014
15	KP148274.1	F112	Ismailia	Pekin	2014
16	KP148275.1	F147	Ismailia	Pekin	2014
17	KP148276.1	F321	Behara	Pekin	2014
18	KP148277.1	F215	Beni-Suef	Green Winged	2014
19	KP148278.1	F387	Ismailia	Pekin	2014
20	KX639146.1	DHV-EGYPT/16160F-2016	Sharkia	Muscovy	2016
21	KX639141.1	DHV-EGYPT/141122F-2014	Behara	Pekin	2014
22	KX639142.1	DHV-EGYPT/151283F(3)-2015	Sharkia	Pekin	2015
23	KX639143.1	DHV-EGYPT/15298F-2015	Fayoum	Mallard	2015
24	KX639144.1	DHV-EGYPT/15P18-2015	Menoufiya	Muscovy	2015
25	KX639139.1	DHV-EGYPT/14921F-2014	Qaluibia	Pekin	2014
26	KX639145.1	DHV-EGYPT/151283F(4)-2015	Beni-Suef	Mallard	2015
27	KX639140.1	DHV-EGYPT/14923F-2014	Giza	Muscovy	2014
28	KX639147.1	DHV-EGYPT/151283F(2)-2015	Gharbia	Muscovy	2015
29	KX639148.1	DHV-EGYPT/151283F(1)-2015	Ismailia	Muscovy	2015
30	KP202874.1	DHV/Duck/Egypt/Al-Gharbia/2014	Al-Gharbia	Pekin	2014

(*) indicates DHV field isolates of the current study

Detection of RNA of DHAV-1 by one-step RT-PCR

RNA extraction methods

Viral RNA extraction from the liver tissue was performed using the Patho Gene-spin™ DNA/RNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea), following the manufacturer's instructions. RNA concentrations were measured using the NanoDrop ND-1000 (NanoDrop, Wilmington, DE). The samples were storedat -20°C for one-step RT-PCR (OIE, 2018).

Oligonucleotide primers design

The one-step RT-PCR was performed using the specific primers, DHAV-1 Com F (5'-AAG-AAG-GAG-AAA-ATY-[C or T]- AAG-GAA-GG-3') and DHAV-1 Com R (5'-TTG-ATG-TCA-TAG CCC-AAS- [C or G]-ACA-GC-3'). The primers were flanked by an expected 467 bp DNA sequence in the 3D gene (OIE, 2018).'

One-Step RT-PCR: The one-step RT-PCR was conducted using 20µL of reaction mixtures DHV-1 ComF and DHV-1 ComR, a T-gradient thermal cycler (Biometra, Gottingen, Germany) was used to perform one-step RT-PCR. First, reverse transcription was performed at 45°C for 30 min, after which the enzyme was inactivated at 94°C for 5 min.

PCR amplification was conducted using the following steps:an initial denaturation for 20 s at 94˚C, followed by 40 cycles of annealing for 30 s at 52˚C, extension for 30 s at 72˚C, denaturation for 20 s at 94˚C, final extension for 5 min at 72˚C, and reactions were stored at 4˚C (OIE, 2018).

DNA Molecular weight marker

The amplified products were read using a 100 bp ladder (NEW ENGLAND *BioLabs*).

Agarose gel electrophoreses

Electrophoresis for amplicon separation was applied on 1.5% agarose gel prepared according to Sambrook et al. (1989).

The gel was visualized and captured using a gel documentation system, and the data was analyzed using acomputer software.

Sequencing and phylogenetic analyses of the amplified part of the 3D gene

Sequencing of the PCR amplified products was conducted using the GATC company ABI 3730xl DNA sequencer and forward/reverse primers. The traditional Sanger sequencing technique was combined with the new 454 technology for sequencing 467 bp PCR product of the DHAV-1 3D gene. To establish the sequence identity for GenBank accessions, a BLAST® analysis (Basic Local Alignment Search Tool) (Altschul et al., 1990) was initially performed. The published data of 26 Egyptian viruses and one reference vaccine strain from the GenBank were available from the National Center for Biotechnology Information (NCBI) Duck hepatitis A virus resource (https://www.ncbi.nlm.nih.gov/nuccore/?term=duck+hepatitis+a+virus+3D+protein) (Table 1). The alignment of the viruses in this study was performedusing the MegAlign module of Lasergene DNAStar (Thompson et al., 1994). Then, the identity percent and divergence amongall viruses were determined. Finally, phylogenetic analysis was conducted using the MEGA v6.0 software (Tamura et al., 2013). The three reported partial sequences of the 3D gene were submitted to GenBank under the following accession numbers: MT157210, MT157211,and MT157212.

RESULTS AND DISCUSSION

DVH is one of the most significant viral diseases in young ducklings, causing high potential mortality and great economic losses. Shalaby et al. (1978) reported the first occurrence of this disease in Egypt in the 1970s. Because of the immature immune system in young ducklings, which is incapable of protecting them from virus infection and propagation, the seriousness of infection with DHAV-1 is chiefly age-dependent (Song et al., 2014). Earlier research recorded that acute infections caused by DHAV-1 was correlated with Pekin, Mallard, and hybrid breeds, whereas Muscovy ducklings appeared to be the stable carriers of DHAV-1 infections. However, pancreatitis and encephalitis have been reported to be correlated to Muscovy ducklings' infection (Zhu et al., 2019). Conventional methods used for diagnoses are based on clinical signs,such as gross lesions in ducklings confirmed usingvirus isolation and serological tests. Lately, a number ofquick and specific RT-PCR techniques have been designed to identify the presence of DHAV-1 RNA (Kim et al., 2007; Liu et al., 2007; Anchun et al., 2009; Wen et al., 2014; OIE, 2018).

In the current investigation, the liver samples were collected from three duck farms representing Muscovy and Pekin breeds aged 2–4 weeks, and located in two Egyptian governorates (Monofia and Al- Gharbia) between August and October 2018. The commonly recorded clinical signs among examined ducklings were nervousrelated,such as ataxia, imbalance, lateral recumbency, keel over, kick spasmodically, and opisthotonos followed by death (Figure 1). The mortality rate reported in affected flocks reached 75% within 3–4 days. The results were consistent with those previously recorded in DHV infection studies (OIE, 2010; Erfan et al., 2015).

Figure 1: Lateral, keel recumbency, keel over, and retracted head on back.

The liver of the examined ducklings was enlarged with hemorrhages. In addition, enlarged spleen and kidneys with congested renal blood vessels were also observed (Figure 2). Similarly, variable lesions were recorded in the liver and kidneys (OIE, 2010; Hisham et al., 2020).

Histopathological examination of the liver sections of the affected ducklings with clinical signs showed portal vein and bile duct congestion accompanied byhepatic cord disorganization. Focal areas of coagulative necrosis characterized by inflammatory cells infiltration and vacuolar degeneration in the cytoplasm of the hepatocyte were also

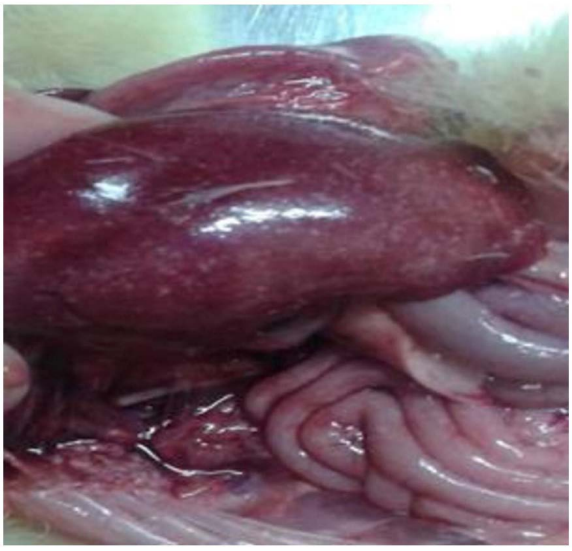

Figure 2: Enlarged liver with hemorrhages.

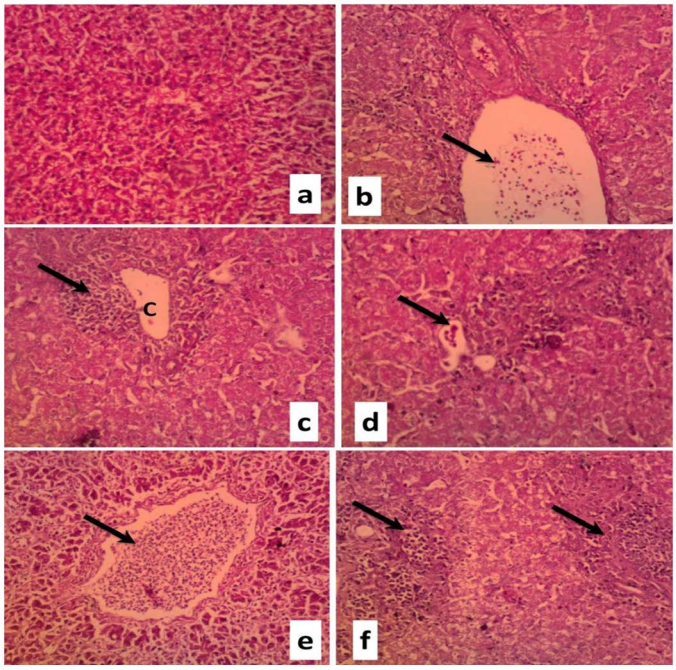

Figure 3: Liver sections of duckling naturally infected with DVH (H&E, X100) showing
a. Normal liver tissue
b. Portal vein and bile duct congestion (arrow) accompanied by hepatic cord disorganization
c. Focal area of coagulative necrosis (C) characterized by inflammatory cells infiltration (arrow) accompanied byhepatic cord disorganization and vacuolar degeneration in the cytoplasm of the hepatocytes
d. Focal area of coagulative necrosis characterized by inflammatory cells infiltration (arrow) accompanied byhepatic cord disorganization and vacuolar degeneration in the cytoplasm of the hepatocytes
e. Diffuse vacuolar degeneration and severe necrosis of hepatocytes and lymphocytic infiltration of the central vein
f. Multifocal area of coagulative necrosis characterized by inflammatory cells infiltration (arrow) accompanied byhepatic cord disorganization and vacuolar degeneration in the cytoplasm of the hepatocytes

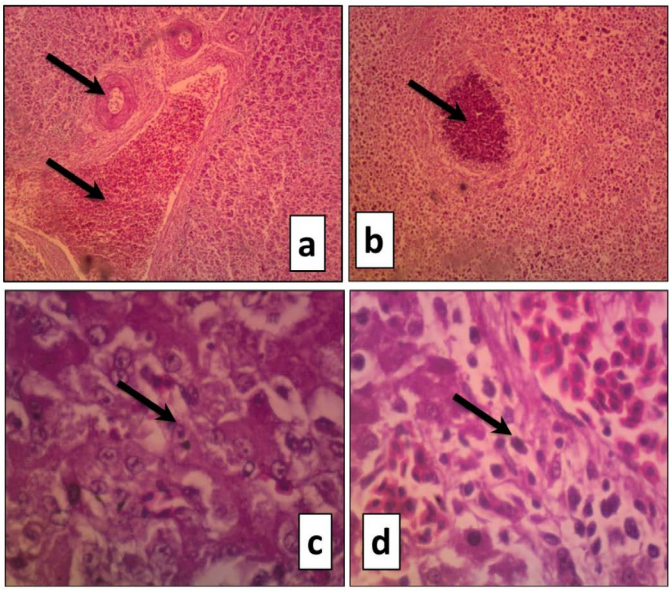

Figure 4: Liver sections of duckling naturally infected with DVH (H&E) showing
a. Severe portal vein congestion (arrow). The bile duct suffered from narrowing because of epithelial lining hypertrophy accompanied by severe hepatocyte necrosis (X100)
b. Focal area of coagulative necrosis characterized by inflammatory cells infiltration (arrow) accompanied byhepatic cord disorganization and severe hepatocytes necrosis (X200)
c. Large eosinophilic intranuclear inclusion surrounded by a clear halo in the hepatocytes (X400)
d. Large eosinophilic intranuclear inclusion surrounded by a clear halo in the hepatocytes (X400)

observed. Further lesions, including narrowing of the bile duct due to epithelial lining hypertrophy and the existence of large eosinophilic intranuclear inclusion bodies surrounded by a clear halo in the hepatocytes, were markedly seen (Figures 3 and 4). These results were analogous to those reported by Fitzgerald et al. (1969), Sheng et al. (2014), Hassaan et al. (2018), and Mansour et al. (2019).

In this study, implementation of one-step RT-PCR for processed livers using specific primers aiming the 3D gene of DHAV-1 showed the output of the amplified band in all examined specimens at acertain supposed size of the 3D encoding gene (467 bp). These results were analogous to those obtained by El-Samadony et al. (2016) and Zanaty et al. (2017).

In our study, the successfully amplified 3D gene of DHAV-1 isolates named DHV/Duck/Egypt/Monofia-Shemy-1/2018, DHV/Duck/Egypt/Al-Ghar-

bia-Shemy-3/2018, and DHV/Duck/Egypt/Monofia-Shemy-2/2018 was subjected to sequencing and then submitted to the NCBI GenBank; the accession numbers were MT157210, MT157211,and MT157212, respectively.

In Egypt, live attenuated vaccine prepared from the E52 Rispens strain is used for vaccination of breeder duck intramuscularly, which is administered 2–3 weeks before laying to yield high titers of passively transferred maternal antibody in newly hatched ducklings, thereby maintaining them for the first two weeks of their lives (Ellakany et al., 2002; Abd-Elhakim et al., 2009). Occasionally, there is an infection in non-vaccinated ducklings with low immunity because of vaccinal shedding from vaccinated ducklings. Further, the occurrence of the DHAV-1 disease in immunized ducklings was recorded (Mahdy, 2005; Jin et al., 2008; Li et al., 2013). However, the actual reason for the appearance of DHAV-1 infection in immunized flock of ducks in Egypt wasnot identified. This may bebecause of the antigenic variation that occurred in the field virus. Thus, the accustomed vaccine cannot provide enough protection (Zanaty et al., 2017).

The alignment of the sequenced amplified 3D gene of three examined field isolates with the published 3D sequence of some Egyptian DHV isolates and vaccine strain was performed (Figure 5). Sequence analysis of nucleotide alignment for the 3D gene revealed 92.5%–98.6% homology between our sequenced isolates.

Our isolate, DHV/Duck/Egypt/Monofia-Shemy-1/2018, showed 79.9%–97.3% nucleotide identity with the previous isolates from Egyptian governorates. Further, the field isolate, DHV/Duck/Egypt/Al-Gharbia-Shemy-3/2018,-showed 77.4%–93.1% similarity with the previous field isolates fromEgyptian governorates. Our isolate, DHV/Duck/Egypt/Monofia-Shemy-2/2018, showed a nucleotide identity of 80.2%–98.8% withtheprevious isolates from Egyptian governorates. The three examined isolates (DHV/Duck/Egypt/Monofia-Shemy-1/2018, DHV/Duck/Egypt/Al-Gharbia-Shemy-3/2018, and DHV/Duck/Egypt/Monofia-Shemy-2/2018) showed nucleotide similarities of 77.4%–80.2% with the most frequently used DHAV-1 vaccine in Egypt. The phylogenetic relationship using the partial 3D protein revealed that the isolated Egyptian strains used in this study were distant from the vaccine strain used in Egypt (Figure 6). The deduced amino acid substitutions of the 3D gene of our investigated strains was studied (Figure 7) to discover the DHAV-1 genome evolution. The area of analysis constituted 115 amino acid residues. Substitution mutations for DHV/Duck/Egypt/Monofia-Shemy-3/2018 strain were noted at several amino acid residues in comparison to amino acid

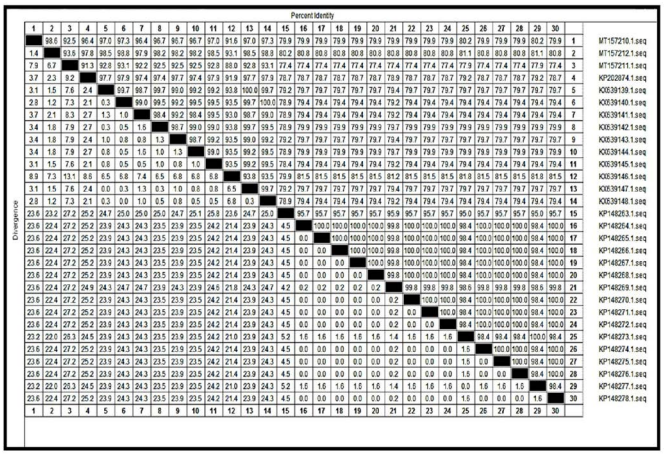

Figure 5: Nucleotide identities of Egyptian isolates with selected published field Egyptian strain references and vaccinal DHAV-1 sequences of different serotype.

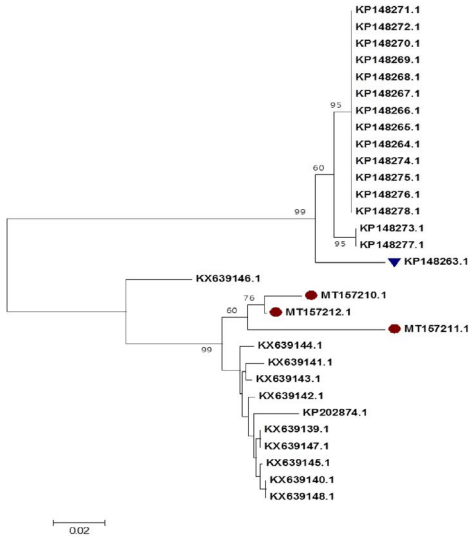

Figure 6: Phylogenetic tree based on a partial sequence of the DHAV-1 3D gene. The dots refer to viruses isolated in the current study, and the triangle refers to the vaccine strain. The robustness of individual nodes of the tree was assessed using 1,000 replications of bootstrap resampling of the originally aligned nucleotide sequences

of other examined Egyptian strains during this study or those previously isolated from Egyptian governorates. Deduced amino acid substitutions analysis of the investigated strains, DHV/Duck/Egypt/Monofia-Shemy-1/2018 and DHV/Duck/Egypt/Monofia-Shemy-2/2018, revealed the same amino acids (except at position I4T and Y5I). Both strains showedmany amino acids changes compared with some previously isolated Egyptian strains (F340, F953, F86, F163, F413, F729, F829, F10, F243, F355, F112, F147, F321, F215, and F387), and it also showed low amino acids changesin relation to other previously isolated Egyptian strains (DHV-EGYPT/16160F-2016, DHV-EGYPT/141122F-2014,

DHV-EGYPT/151283F(3)-2015, DHV-EGYPT/15298F-2015, DHVEGYPT/15P18-2015, DHV-EGYPT/14921F-2014, DHV-EGYPT/151283F(4)-2015, DHV-EGYPT/14923F-2014, and DHV-EGYPT/151283F(2)-2015). All isolated Egyptian strains in this study were also completely unrelated to the Egyptian DHAV-1-based vaccine and was further confirmed by the low amino acid similarities to this vaccine.

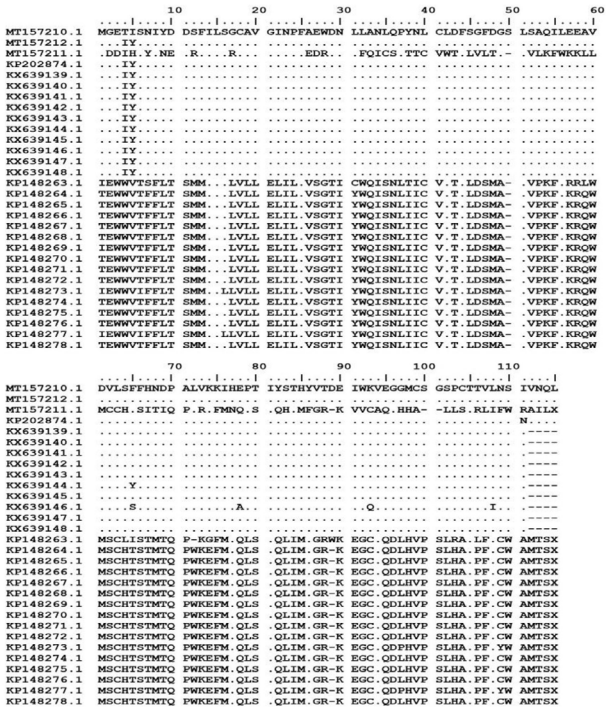

Figure 7: Deduced amino acid sequence of the 3D gene of the isolated strainscompared with the 3D gene of thepublished vaccinal and field Egyptian strains

Phylogenetic analysis in addition to nucleotide and amino acid similarities between our isolated strains with other Egyptian DHAV-1 strains and vaccinal strain corresponded to those obtained by Zanaty et al. (2017), who stated that Egyptian strains are different from the vaccine.

CONCLUSION AND RECOMMENDATIONS

Three isolates of DHAV-1 were identified from the examined flocks of duck. A phylogenetic analysis of these isolates revealed differentiation between our field DHAV-1 isolates and the vaccine strain.

For correct controlling of the DHAV-1 infection in flocksof duck, avaccination program for breeder duck flocks against DHAV-1 is important. Thus, further studies are neededto detect the efficacy of the used vaccine against the current field strains of DHAV-1.

ETHICAL APPROVAL

All work procedures were reviewed and approved by Medical Research Ethics Committee of the National Research Centre, Egypt (Approval no. 7417082021).

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CONFLICT OF INTEREST

The authors have no conflict of interests to declare regarding the publication of this paper. Also, the authors declare that the work was self-funded.

AUTHORS' CONTRIBUTIONS

M.M. Amer and Kh. M. Elbayoumi designed and planned this study. A. El-Shemy, Hoda M. Mekky and A. Allam collected samples, and performed all laboratory work. M. Bosila carried out pathological examination. All authors shared sample collection, performing the tests, collecting research data for writing, manuscript writing, drafted, revised the manuscript, and approved the final manuscript.

REFERENCES

Abd-Elhakim MA, Thieme O, Schwabenbauer K, Ahmed ZA (2009). Mapping traditional poultry hatcheries in Egypt. In: AHBL–Promoting Strategies for Prevention and Control of HPAI, FAO, ed. Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy.

Altschul SF, Gish W, Miller W, Myers EW,Lipmanl DJ (1990). Basic Local Alignment Search Tool. J. Mol. Biol., 215: 403-410.

Anchun C, Mingshu W, Hongyi X, Dekang Z, Xinran L, Haijuen C, Renyong J, Miao Y (2009). Development and application of a reverse transcriptase polymerase chain reaction to detect Chinese isolates of duck hepatitis virus type 1. J. Microbiol. Methods, 77: 332-336. https://doi.org/10.1016/j.mimet.2008.07.018

Ding C, Zhang D (2007). Molecular analysis of duck hepatitis virus type 1. Virology, 361(1): 9-17. https://doi.org/10.1016/j.virol.2007.01.007

Ellakany H, El Sebai AH, Sultan H, Sami AA (2002).Control of experimental DHV infection by amantadine. In: Proceedings of the 6thScientific Veterinary Medical Conference of Zagazig University, Zagazig University, Hurghada, Egypt, pp. 757-775.

El-Samadony HA, Tantawy LA, Mekky HM (2016). Isolation and molecular detection of duck viral hepatitis. Glob Vet., 16(4): 314-322. https://www.idosi.org/gv/gv16(4)16/1.pdf

El-Samadony HA, Mekky HM, Mahgoub KhM (2018). Amino

acid sequences of local isolates of Duck Hepatitis Virus A type 1 (DHAV-1) in Egypt. Biosci. Res., 15(3):1559-1567.

Erfan AM, Selim AA, Moursi MK, Nasef SA,Abdelwhab EM (2015).Epidemiology and molecular characterisation of duck hepatitis A virus from different duck breeds in Egypt. Vet. Microbiol., 177(3-4): 347-352. http://dx.doi.org/10.1016/j.vetmic.2015.03.020

Fitzgerald JE, Hanson LE, Simon J (1969). Histopathologic changes induced with duck hepatitis virus in the developing chicken embryo. Avian Dis., 13: 147-157.

Fu Y, Pan M, Wang X, Xu Y, Yang H, Zhang D (2008). Molecular detection and typing of duck hepatitis A virus directly from clinical specimens. Vet.Microbiol, 131(3-4): 247-257.

Gao J, Chen J, Si X, Xie Z, Zhu Y, Zhang X, Wang S, Jiang S (2012). Genetic variation of the VP1 gene of the virulent duck hepatitis A virus type 1 (DHAV-1) isolates in Shandong province of China. Virol.Sin., 27: 248-253. http://dx.doi.org/10.1007/s12250-012-3255-8

Guerin J-L, Albaric O, Noutary V,Boissieu C (2007). A duck hepatitis virus type I is agent of pancreatitis and encephalitis in Muscovy duckling. In: Proceedings of the 147th American Veterinary Medicine Association/50th American Association of Avian Pathologists Conference, 14–18 July 2007, Washington, DC, USA, Abs 4585.

Hassaan MN, ShahinAM, Eid AAM (2018). Isolation and molecular identificationof Duck Hepatitis A virus in Sharkia Governorate.Zagazig Vet. J., 46(1): 88-95. https://zenodo.org/record/1246280#.YgbPNtJBxdg

Hisham I, Ellakany HF, Selim AA, Abdalla MAM, Zain El-Abideen MA, Kilany WH, Ali A, Elbestawy AR (2020). Comparative pathogenicity of Duck Hepatitis A Virus-1 isolates in experimentally infected Pekin and Muscovy ducklings. Front. Vet. Sci., 7:234. https://doi.org/10.3389/fvets.2020.00234

ICTV (2017). Virus Taxonomy: The Classification and Nomenclature of Viruses. The Online (10th) Report of the ICTV, 2017. https://talk.ictvonline.org/ictv-reports/ictv_online_report/

Jin X, Zhang W, Zhang W, Gu C, Cheng G, Hu X (2008). Identification and molecular analysis of the highly pathogenic duck hepatitis virus type 1 in Hubei Province of China. Res. Vet. Sci., 85: 595-598.

Kerkvliet J, Edukulla R, Rodriguez M (2010). Novel roles of the picornaviral 3D polymerase in viral pathogenesis. Adv. Virol., 2010, 368068. https://doi.org/10.1155/2010/368068

Kim MC, Kwon YK, Joh SJ, Lindberg AM, Kwon JH, Kim JH, Kim SJ (2006). Molecular analysis of duck hepatitis virus type 1 reveals anovel lineage close to the genus Parechovirus in the family Picornaviridae. J. Gen.Virol., 87(Pt 11): 3307-3316. https://doi.org/10.1099/vir.0.81804-0

Kim MC, Kwon YK, Joh SJ, Kim SJ, Tolf C, Kim JH, Sung HW, Lindberg AM, Kwon JH (2007). Recent Korean isolates of duck hepatitis virus reveal the presence of a new geno-and serotype when compared to duck hepatitis virus type 1 type strains. Arch. Vrol., 152: 2059-2072.

Levine P, Fabricant J (1950). A hitherto-undescribed virus diseaseof ducks in North America. Cornell Vet, 40: 71-86.

Li J, Bi Y, Chen C, Yang L, Ding C, Liu W (2013). Genetic characterization of duck hepatitis A viruses isolated in China. Virus Res., 178: 211-216. https://doi.org/10.1016/j.virusres.2013.10.007

Liu G, Wang F, Ni Z, Yun T, Yu B. (2007). Complete genomic sequence of a Chinese isolate of duck hepatitis virus. Virol. Sin., 22: 353-359.

Liu G, Wang F, Ni Z, Yun T, Yu B, Huang J, Chen J (2008). Genetic diversity of the VP1 gene of duck hepatitis virus type I (DHV-I) isolates from southeast China is related to isolate attenuation. Virus Res., 137: 137-141.

Mansour SMG, Mohamed FF, Elbakrey RM, Eid AM, Mor SK, Goyal SM (2019). Outbreaks of duck hepatitis A virus in Egyptian duckling flocks. Avian Dis., 63: 68-74. https://doi.org/10.1016/j.ijvsm.2018.07.004

Mahdy SA (2005). Clinicopathological studies on the effect of duck viral hepatitis in ducks. M.V.Sc Thesis (Clinical Pathology), Faculty of Veterinary Medicine, Zagazig University.

NRC (1984). National Research Council, National Requirement for Poultry. 9th ed. National Academy Press, Washington, DC.

OIE (2010). Duck Virus Hepatitis. OIE Terrestrial Manual 2010. (Chapter 2.3.8), available online at: http://www.oie.int/ fileadmin/Home/eng/Health_standards/tahm/2.03.08_DVH.pdf

OIE (2018). Duck Virus Hepatitis. OIE Terrestrial Manual 2018.(Chapter 3.3.8), pp 883- 894.available online at: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.08_DVH.pdf

Rohaim MA, Naggar RFE, AbdelSabour MA, Ahmed BA, Hamoud MM, Ahmed KA, Zahran OK,Munir M (2021). Insights into the genetic evolution of duck hepatitis Avirus in Egypt. Animals, 11: 2741. https://doi.org/10.3390/ani11092741

Sambrook J, Fritscgh EF,Mentiates T (1989). Molecular coloning.A laboratory manual.Edition 2, Cold spring Harbor Laboratotry press, New York.

Sandhu TS, Calnek BW,Zeman L (1992). Pathologic and serologic characterisation of a variant of duck hepatitis type I virus. Avian Dis., 36: 932-936.

Shalaby MA, Ayoub MNK, Reda IM (1978). A study on a new isolate of duck hepatitis virus and its relationship to other duck hepatitis virus strains. Vet. Med. J. Cairo Univ., 26: 215-221.

Sheng XD, Zhang WP, Zhang QR, Gu CQ, Hu XY, Cheng GF (2014). Apoptosis induction in duck tissues during duck hepatitis A virus type 1 infection. Poult. Sci., 93: 527-534. https://doi.org/10.3382/ps.2013-03510

Song C, Liao Y, Gao W, Yu S, Sun Y,Qiu X, Tan L, Cheng A, Wang M, Ma Z, Ding C (2014). Virulent and attenuated strains of duck hepatitis A virus elicit discordant innate immune responses in vivo. J. Gen. Virol., 95: 2716-2726. https://doi.org/10.1099/vir.0.070011-0

Suvarna KS, Layton C, Bancroft JD (2012).Bancroft's Theory and Practice of Histological Techniques E-Book.Elsevier Health Sciences.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725-2729.

Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22(22): 4673-4680.

Tsai HJ (2020). Duck hepatitis. In: Swayne DE, editors. Diseases of Poultry. Hoboken, NJ: Wiley-Blackwell. pp. 450-459. https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781119371199.ch13

Tseng CH, Knowles NJ, Tsai HJ (2007). Molecular analysis of duck hepatitis virus type 1 indicates that it should be assigned to a new genus. Virus Res., 123: 190-203.

Wei CY, Su S, Huang Z, Zhu WJ, Chen JD, Zhao FR, Wang YJ, Xie JX, Wang H, Zhang G (2012). Complete genome sequence of a novel duck hepatitis A virus discovered in Southern China. J.Virol., 86: 10247. https://doi.org/10.1128/JVI.01643-12

Wen XJ, Cheng AC, Wang MS, Jia RY, Zhu DK, Chen S, Liu MF, Liu F, Chen XY (2014). Detection, differentiation, and VP1 sequencing of duck hepatitis A virus type 1 and type 3 by a 1-step duplex reverse-transcription PCR assay. Poult. Sci., 93: 2184-2192. http://dx.doi.org/10.3382/ps.2014-04024

Woolcok PR (1998). Duck Hepatitis. Laboratory Manual for the Isolation and Identification of Avian pathogens, Fourth Edition, pp: 200-204.

Woolcock, PR (2003). Duck hepatitis. In: Saif YM, HJ Barnes, JR Glisson, AM Fadly, LR Mcdougald and DE Swayne, eds. Diseases of Poultry. Eleventh ed. Iowa State Press, Ames, IA., pp. 343-354.

Woolcock PR (2008). Duck hepatitis. In: A Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens, 5th Edition, Dufour-Zavala L, DE Swayne, JR Glisson, JE Pearson, WM Reed, MW Jackwood and PR Woolcock, eds. American Association of Avian Pathologists, Jacksonville, Florida, USA, 175-178.

Yehia N, Erfan AM, Omar SE, Soliman MA (2021). Dual circulation of duck hepatitis A virus genotypes 1 and 3 in Egypt. Avian Dis., 65(1): 1-9. https://doi.org/10.1637/aviandiseases-D-20-00075

Zanaty A, Hagag N, Samy M, Abdel-Halim A, Soliman MA, Arafa A,Nasef SA (2017). Molecular and pathological studies of duck hepatitis virus in Egypt. J. Vet. Med. Res., 24(1): 374-384.

Zhu T, Qi B, Liu R, Jiang X, Lu R, Xiao L, Fu G, Fu Q, Shi S, Wan C, Huang Y (2019). Comparative pathogenicity of two subtypes (hepatitis-type and pancreatitis-type) of duck hepatitis A virus type 1 in experimentally infected Muscovy ducklings. Avian Pathol., 48: 352-361. https://doi.org/10.1080/03079457.2019.1605146