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*Brief Original Article*

# Occurrence and phylogenetic analysis of fowl adenovirus E in broiler flocks from Gaza Strip Palestine

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## Abstract

Introduction: Inclusion-body hepatitis (IBH) and hydropericardium syndrome (HPS) are highly infectious diseases caused by fowl adenoviruses (FAdVs). IBH and HPS cause major economic losses in poultry production. IBH is caused by multiple FAdV serotypes such as FAdV-11, FAdV8a, and FAdV8b; while HPS is mainly caused by the FAdV-4 serotype. In 2018, we detected FAdVs in West Bank - Palestine for the first time. This study aims to monitor the emergence of new FAdVs in broiler farms in Gaza Strip-Palestine in 2022.

Methodology: The clinical signs, necropsy, and histopathological findings associated with IBH in the suspected birds were recorded. Polymerase chain reaction (PCR) was performed using primers matching the virus-encoded L1 loop of the hexon gene. The sequences of the L1 loop were analyzed and a phylogenetic tree was constructed and compared with the related FAdV field isolates and reference strains available in GenBank.from different regions of the world.

Results: The infected broiler displayed FAdVs-induced clinical symptoms and pathological lesions with mortality rates ranging from 20-46%. The L1 loop sequences from the infected flocks were submitted to GenBank with accession numbers ON638995, ON872150, and ON872151. The identified L1 loop gene has high nucleotide homology (96.7-97.9%) to the highly pathogenic FAdV E serotype 8b strain FAdV_isolate_04-53357-122_Canada_2007 (GenBank: EF685489) and 94.5-94.6% to FAdV_10_Belgium_2010 isolate 11-15941 (GenBank: AF339924.1). Furthermore, the phylogenetic analysis indicated that they belong to FAdV-E serotype 8b.

Conclusions: Our study reports the emergence of FAdV-E causing IBH disease in broiler chickens for the first time in Gaza in Palestine.

**Key words:** *Aviadenovirus*; Loop-1; HS; IBH; Palestine.

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## Introduction

Adenoviruses are distributed worldwide and infect a wide range of vertebrates including humans and animals [1]. According to the International Committee on Taxonomy of Viruses (ICTV), the family Adenoviridae includes six genera: *Mastadenovirus*, *Aviadenovirus*, *Siadenovirus*, *Atadenovirus*, *Ichtadenovirus*, and *Testadenovirus* [2]. The genus *Aviadenovirus* includes 11 species that infect several avian species, and five *Fowl Aviadenovirus* species *(FAdV A to E*) are common infectious agents for chickens. FAdV is further divided into 12 serotypes based on cross-neutralization tests (1-8a, 8b-11) [3]. Inclusion body hepatitis (IBH) and hydropericardium syndrome (HPS) in chickens are characterized by lesions of the liver, heart, digestive and renal systems; causing major economic losses [4-7]. These diseases are primarily associated with pathogens that impair the immune system (i.e. chicken infectious anemia virus (CIAV) and infectious bursal disease virus (IBDV) [8]. Vertical transmission of FAdV is the most important variable associated with early-onset disease, which usually occurs within one to three weeks post-hatching [9]. The FAdV genome encodes several structural proteins; the hexon loop-1 protein is the most abundant capsid protein [10]. Variations of the L1 loop of the hexon gene are important for infectivity, diagnosis, phylogenetic evolution analysis, and classification of genotypes and serotypes of the different FAdV strains [11]. Molecular epidemiological analysis showed that FAdV-D and FAdV-E strains have been isolated mostly from IBH and HPS outbreaks in different localities [12-14]. The disease caused by FAdV-D serotype 10 was reported for the first time in Tubas city of the West

Bank - Palestine in 2018 [15]. The Gaza Strip and West Bank are geographically separated by Israeli territories. Until now, there has been no study on the occurrence of FAdVs outbreak in Gaza Strip. Therefore, the current study reports the first time FAdVs-causing IBH outbreaks in Gaza Strip in 2022 based on clinical findings and molecular detection and analysis of the L1 loop of the hexon gene sequence. Our sequencing results provide an update on circulating FAdV in a specified region.

## Methodology

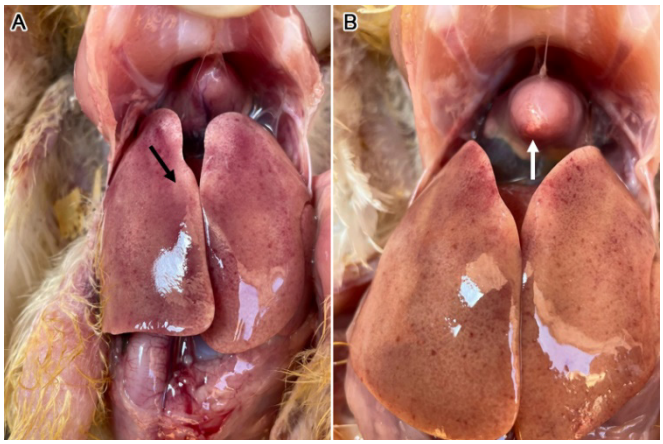
*Ethical statement*

Samples were collected from clinically diseased chickens from the corresponding flocks and processed at the Thabet Center for Veterinary Services (TCVS) diagnostic laboratory, Gaza Strip - Palestine for diagnosis of the disease as part of the usual veterinary service work in Palestine.

*Clinical samples*

Samples were collected during a recent outbreak in May 2022. This outbreak affected three major commercial broiler farms in Gaza Strip. Chicks from the infected flocks were of the same line origin (Ross 308) in Israel, imported and hatched at different times at the same hatchery in Gaza Strip, and might originate from different breeders. Chicken flocks I, II, and III were 11, 15, and 20 days old, respectively. The diseased flocks followed standard vaccination programs that include: spray vaccination against Newcastle disease at one day old (VIR 105, Biovac, Or Akiva, Israel), Infectious Bursal Disease vaccine in water at 12 days old (VIR 114, Biovac, Or Akiva, Israel), applied on flocks II and III, and further vaccination against Newcastle disease in water at 18 days old (VIR 105, Biovac, Or Akiva, Israel) was applied in flock III. Clinical symptoms of the disease and the postmortem lesions were recorded. Liver tissue samples were fixed in 10% neutral buffered formalin and processed by routine paraffin-embedding technique for histopathological examination. Five µm thick sections were deparaffinized and stained by hematoxylin and eosin staining method for detailed microscopic examination [16]. Pooled liver samples were collected from infected flocks together to achieve 30-40 liver samples per flock. DNA was extracted from the pooled liver samples and the existence of FAdVs was confirmed by polymerase chain reaction (PCR). Liver tissues were stored at –20 °C at the Thabet Center for Veterinary Services (TCVS) Diagnostic Laboratory, Gaza Strip-Palestine for further analysis.

**Figure 1.** Post-mortem of diseased birds showing typical IBH-HPS lesions.




**A:** Black arrow: multifocal areas of hepatic necrosis with necrotic hemorrhagic foci; **B:** White arrow: hydropericardium. Images were captured from different flocks.

*DNA extraction, PCR amplification, and sequencing*

Total genomic DNA was extracted from 2 mg of each liver sample from diseased birds using NucleoSpin tissue (reference number: EF 740952.50, Macherey-Nagel GmbH & Co. KG, Dueren, Germany) according to the manufacturer's instructions. To determine which FAdVs circulated in these flocks; PCR was performed using a primer set that targets the hexon gene including the L1 loop region: H1 (5'-TGGACATGGGGGCGACCTA-3') and H2 (5'-AAGGGATTGACGTTGTCCA3'). The molecular size of the anticipated amplicon was 1219 bp as previously described [17]. PCRs were performed using Biometra Tone® Thermal Cycler (Analytikjena, Jena, Germany), with the following cycling conditions: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles at 95 °C for 45 sec, 55 °C for 45 sec, and 72 °C for 1.5 min with a final extension at 72 °C for 10 min. The amplified PCR products were analyzed by electrophoresis using a 1.5% agarose gel stained with ethidium bromide. The PCR products of the hexon loop-1 gene with the expected size were further analyzed by Sanger sequencing (Syntezza Bioscience, IDT, Jerusalem).

*Sequence analysis*

Nucleotide sequence results were initially analyzed by FinchTV software version 1.4.0 and submitted to GenBank [18]. The Basic Local Alignment Search Tool (BLAST) website of the National Center for Biotechnology (NCBI) was used to identify the most closely related FAdVs (http://blast.ncbi.nlm.nih.gov/)

[19]. Genome sequences of the detected and reference FAdVs strains were aligned and analyzed using MEGA X ® software [20]. Phylogenetic trees were constructed using the neighbour-Joining (NJ) method with 1,000 bootstrap replications.

## Results

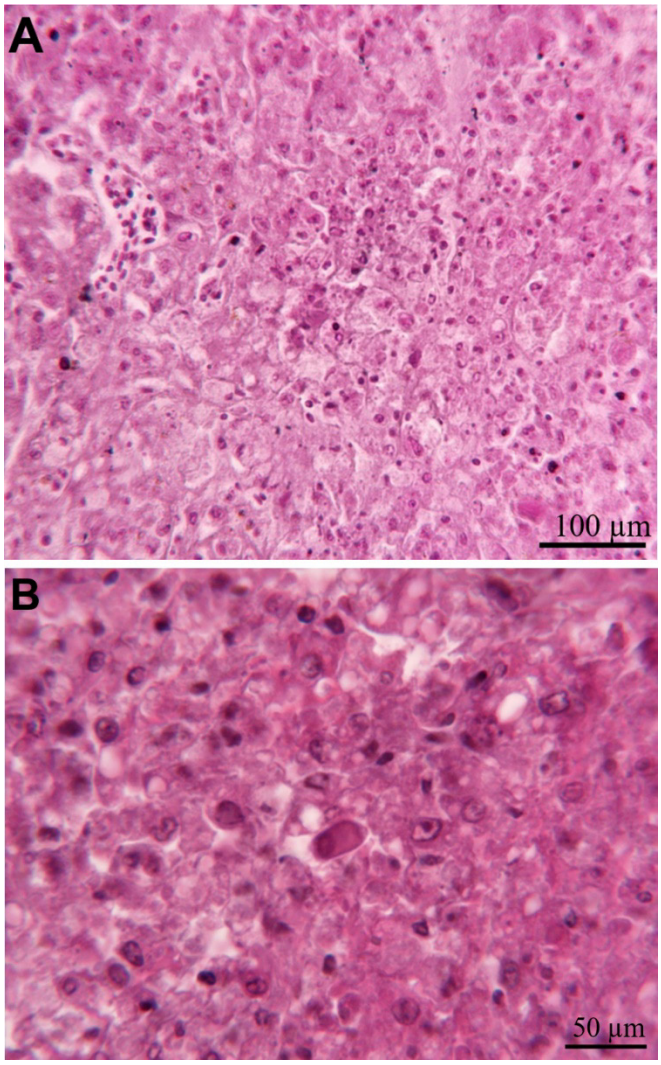
*Clinical symptoms and gross pathological lesions*

The diseased birds had variable clinical symptoms including mouth gasping, depression, stunting, growth retardation, yellowish diarrhea, pale comb and wattles. The mortality rates were 35%, 46%, and 20% for flocks I, II, and III respectively. Post-mortem lesions revealed severe hepatomegaly with multifocal pale areas of hepatic necrosis (Figure 1A). Furthermore, slight hydropericardium was demonstrated in all necropsied birds (Figure 1B). Other lesions observed included marked atrophy of the thymus and bursa of fabricius and splenomegaly. Histopathological examination revealed areas of scattered coagulative necrosis within the liver with mononuclear cell infiltration (Figure 2A). In addition, hepatocytes showed characteristic multiple basophilic intranuclear inclusions. (Figure 2B).

*Identification and typing of the FAdVs causing the diseases*

The PCR products amplified from the DNA extracted from infected chickens showed the expected amplification of 1219 bp (Figure 3). Sanger sequencing and BLAST search confirmed the presence of the FAdV-encoded L1 loop of the hexon gene in the investigated samples. Sequencing results were submitted to GenBank with accession numbers ON638995, ON872150 and ON872151. The nucleotide similarity of FAdV_isolate_Gaza-6_2022 (GenBank: ON638995) was 95.9% and 94.8% with FAdV_isolate_Gaza-7_2022 (GenBank: ON872150) and FAdV_isolate_Gaza-8_2022 (GenBank: ON872151) respectively (Table 1). The most closely related were FAdV E named FAdV_isolate_04-53357-

**Figure 2.** Histologic lesions of FAdV infection in the liver.




**A:** Areas of coagulative necrosis are scattered throughout the liver with mixed mononuclear cell infiltrates, (H & E, magnification 40X, Scale Bar = 100 µm). Necrotic hepatocytes showed pyknotic and karyorrhexic nuclei. **B:** Multiple basophilic intranuclear inclusion bodies on the background of severe degenerative necrobiotic parenchymal lesions, (H & E, magnification 400X, Scale Bar = 50 µm).

**Table 1.** Estimates of evolutionary divergence between hexon loop-1 gene sequences.

| Hexon loop-1 gene sequences | ON872151 | ON872150 | ON638995 |
|---|---|---|---|
| ON872151_FAdV_isolate_Gaza-8_2022 | | | |
| ON872150_FAdV_isolate_Gaza-7_2022 | 94.8 | | |
| ON638995_FAdV_isolate_Gaza-6_2022 | 95.9 | 95.2 | |
| AF339924.1_FAdV_10_Belgium_2010 | 94.6 | 94.5 | 94.6 |
| KU981139.1_FAdV_isolate_FAV-LNDL-121011_China_2017 | 94.1 | 93.0 | 93.9 |
| EF685489.1_FAdV_isolate_04-53357-122_Canada_2007 | 97.0 | 96.7 | 97.9 |
| MT274428_FAdV_D_Palestine_2018 | 23.8 | 21.5 | 23.7 |
| MT380196__FAdV__isolate_IS/3114/2020_Israel_2020 | 25.2 | 23.0 | 25.2 |
| AF508947.1_FAdV_2_Belgium_2004 | 25.2 | 23.0 | 25.1 |

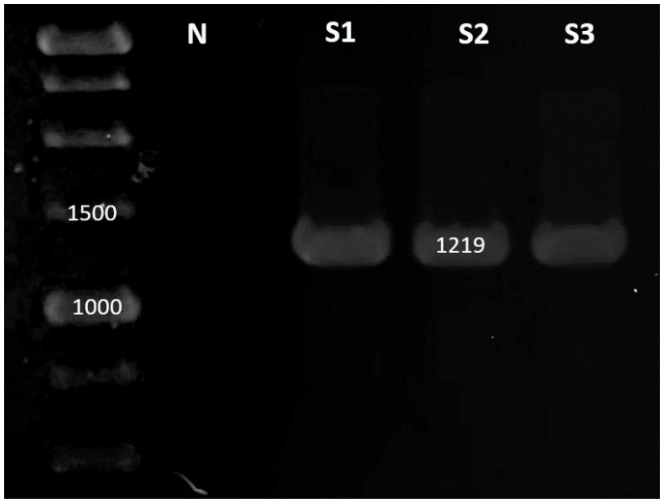
The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model of MEGA X ® software [20].

122, detected in highly pathogenic IBH from chickens in Canada in 2007 (GenBank: EF685489) and FAdV E strain_10_Belgium_2010 isolate 11-15941 (GenBank: AF339924.1) from chickens in Belgium. Low similarities were observed with locally circulating highly pathogenic FAdV D detected in West Bank-Palestine (GenBank: MT274428) in 2018 and Israel in 2020 (GenBank: MT380196) [15,21]. The nucleoid homology of the hexon loop-1 gene of the different flocks with highly similar global sequences and the previously detected IBH from Palestine and Israel are shown in Table 1. The phylogenetic tree of the analyzed and reference sequences is shown in Figure 4. Phylogenetic analysis of the hexon loop-1 gene sequences showed that the currently reported FAdVs are in the same clade and closely related to the sequences of the European FAdV-E, closely related to sequences from Canada (8b), China (8b) and Belgium (strain 10) (Figure 4).

## Discussion

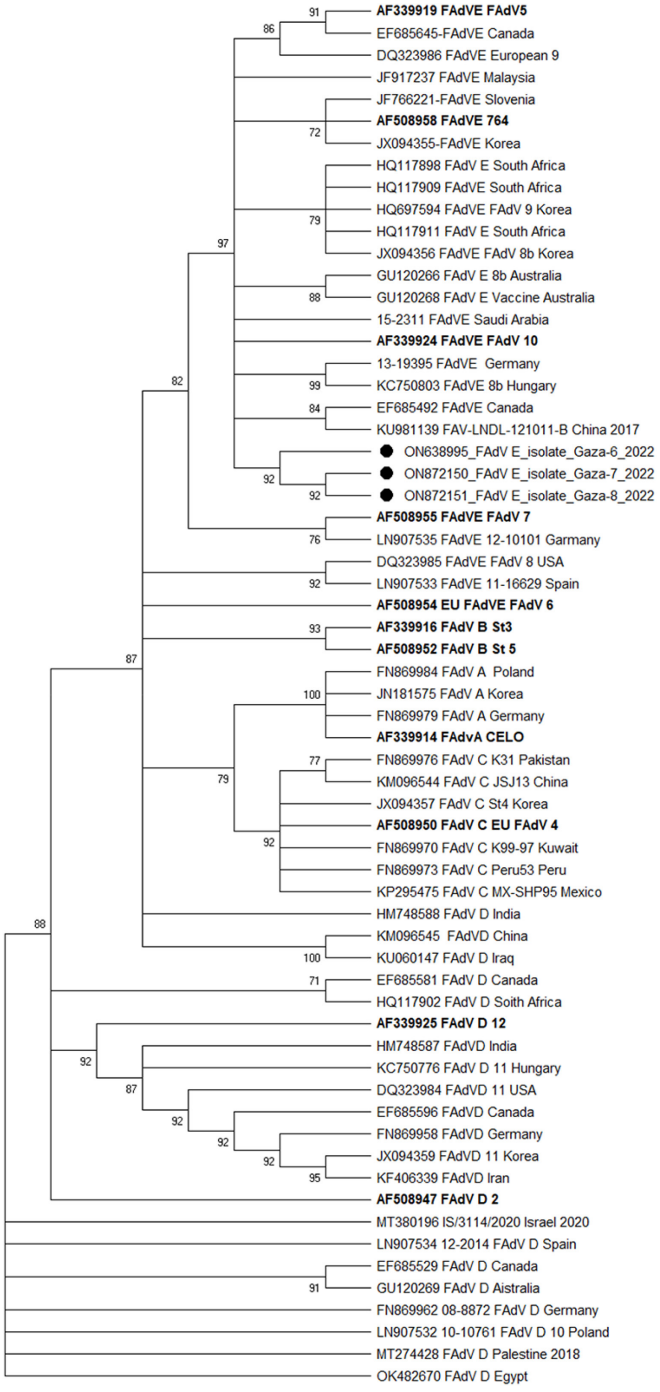
The poultry sector is one of the largest agricultural industries in Gaza Strip - Palestine [22]. Intensive commercial farming and improper control measures have led to the emergence and re-emergence of new viral infections [23]. Therefore, epidemiological research is needed to monitor disease outbreaks and develop vaccines. Although FAdVs are distributed globally, they were first reported in West Bank - Palestine in 2018 [15]. The West Bank and Gaza Strip

**Figure 3.** Detection of FAdV genome by polymerase chain reaction (PCR) in the tissue of the diseased chickens.




Agarose gel electrophoresis showing the three PCR products of IBH-hexon loop-1gene from three broiler flocks. Lane 1: 50 bp DNA Molecular Weight Marker (GeneDireX, Inc., Taoyuan, Taiwan), lane 2 (N): negative control. Lanes 3-5 (S1-S3): 1219 bp of IBH- hexon loop-1gene (including primers) sample from infected animals.

**Figure 4.** Phylogenetic analysis of the common fowl adenoviruses (FAdVs) using nucleotide sequences encoding for the hexon loop-1 gene.




The data included Gaza Strip isolates (indicated with a black circle) and field and reference strains as mentioned by Schachner *et al.* (2016) [11]. Reference FAdV strains are classified on the species level e.g. (A-E) in bold. The evolutionary history was inferred using the neighbor-joining method of MEGA X ® software [19]. Numbers indicate the bootstrap values (1000 replicates). Only bootstrap values of more than 70% are shown.

are Palestinian territories geographically separated by Israeli territories. Until recently, no FAdV disease was reported in Gaza Strip. Therefore, this is the first study in which FAdV has been detected and identified in commercial broiler farms in Gaza Strip - Palestine. The current study investigated three broiler farms in Gaza Strip - Palestine with typical clinical symptoms and necropsy findings of FAdV infection as described by others [4-6]. The clinical findings and microscopic lesions observed in the liver and heart were similar to pathogenic FAdV-E (serotypes 8b, 11 and 12) that cause IBH and HPS in broilers, which have been described previously [24-26].

Detection of FAdV in birds less than 3 weeks old might indicate vertical transmission since FAdV can be reactivated when the maternal antibody levels fall below the protective levels [27]. Although the infected chickens were of the same breed and the farms followed the same biosecurity programs; the infected flocks had varied mortality rates. The mortality rate increases with the accumulation of a straw-colored fluid in the pericardial sac [6]. FAdV-induced diseases can be primary or secondary to immunosuppression with predisposing infection of other viruses such as Marek's disease virus, Infectious Bursal disease virus, and chicken anemia virus [8]. We couldn't exclude these factors, however; the chickens did not display any specific postmortem lesions indicating infection by any of these viruses.

Several studies have shown that the classification strategy of FAdVs infection mainly depends on PCR detection and sequencing of the hexon loop 1 gene [3, 6, 10]. Hexon loop- 1 nucleotide composition analysis revealed that the strains detected in broiler flocks in Gaza Strip were highly similar in between and unrelated to the locally circulating FAdV serotypes in West Bank-Palestine and Israel; suggesting that new strains were recently introduced into Palestine [15]. Phylogenetic tree analyses of the FAdV identified in the current study showed that they clustered with FAdV-E, which is different from the FAdV-D clade of West Bank-Palestine and Israel. These results indicate that they might perhaps be of different origins and that more than one serotype is circulating in chicken flocks in the country. Currently, FAdV-E species dominate in Europe, Australia, and the Middle East [28].

Highly similar FAdV-E species were described previously from Europe, Canada, and China, suggesting that viruses circulating in these countries might be related to trades and primary breeders or breeder replacements [8,29,30]. Mixed infections with FAdV-E and FAdV-D serotypes have already been described in other countries [8,29,30]. Further studies are needed to isolate the viruses and evaluate their pathogenicity. To date, there are no commercial vaccines available in Palestine that can protect chickens from this deadly pathogen. Therefore, it is strongly recommended to use vaccines as soon as possible.

## Conclusions

FAdVs are a concern for the poultry production sector in Gaza Strip-Palestine. Detection and identification of circulating FAdV strains in Palestine based on the sequence of the hexon loop-1 gene confirm the emergence of different strains in our region. The precise analysis and establishment of a phylogenetic tree reveal the possible origins of the virus spread and shed light on how the virus can be introduced to our commercial production systems. Further, we recommend the introduction of FAdV vaccines in Palestine to minimize losses and to control the circulation of the viruses to other regions.

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### Authors' contributions

Ahmed Thabet reported the clinical symptoms and performed molecular detection. Ibrahim Alzuheir carried out the design and drafting of the manuscript. Adnan Fayyad performed histopathology. Ahmed Kheimar analyzed the sequences and edited the manuscript. Nasr Jalboush performed the necropsy and drafted the manuscript. All authors edited the manuscript and accept the final version of the manuscript.

### Data availability statement

All data from this study are available from the corresponding author upon request. The partial hexon loop-1 gene sequences in this study are available in the NCBI database under accession numbers mentioned in the manuscript.

### References

1. Areayi Haiyilati XL, Shijun J Zheng (2021) Fowl adenovirus: pathogenesis and control. Int J Plant Animal Env Sci 11: 566-589.
2. Benkő M, Aoki K, Arnberg N, Davison AJ, Echavarría M, Hess M, Jones MS, Kaján GL, Kajon AE, Mittal SK (2022) ICTV virus taxonomy profile: Adenoviridae 2022. J Gen Virol 103: 001721.
3. Hess M (2000) Detection and differentiation of avian adenoviruses: a review. Avian Pathol 29: 195-206.

4. Hess M, Raue R, Prusas C (1999) Epidemiological studies on fowl adenoviruses isolated from cases of infectious hydropericardium. Avian Pathol 28: 433-439.
5. Guy JS, Barnes HJ (1997) Characterization of an avian adenovirus associated with inclusion body hepatitis in day-old turkeys. Avian Dis 41: 726-731.
6. Shah M, Ashraf A, Khan M, Rahman M, Habib M, Chughtai M, Qureshi J (2017) Fowl adenovirus: history, emergence, biology and development of a vaccine against hydropericardium syndrome. Arch Virol 162: 1833-1843.
7. 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 Pathol 47: 111-126.
8. Gomis S, Goodhope R, Ojkic D, Willson P (2006) Inclusion body hepatitis as a primary disease in broilers in Saskatchewan, Canada. Avian Dis 50: 550-555.
9. Grgic H, Philippe C, Ojkic D, Nagy E (2006) Study of vertical transmission of fowl adenoviruses. Can J Vet Res 70: 230-233.
10. Sohaimi NM, Hair-Bejo M (2021) A recent perspective on fiber and hexon genes proteins analyses of fowl adenovirus toward virus infectivity — a review. Open Vet J 11: 569–580.
11. Schachner A, Marek A, Grafl B, Hess M (2016) Detailed molecular analyses of the hexon loop-1 and fibers of fowl aviadenoviruses reveal new insights into the antigenic relationship and confirm that specific genotypes are involved in field outbreaks of inclusion body hepatitis. Vet Microbiol 186: 13-20.
12. Radwan MM, El-Deeb AH, Mousa MR, El-Sanousi AA, Shalaby MA (2019) First report of fowl adenovirus 8a from commercial broiler chickens in Egypt: molecular characterization and pathogenicity. Poult Sci 98: 97-104.
13. Morshed R, Hosseini H, Langeroudi AG, Fard MHB, Charkhkar S (2017) Fowl adenoviruses D and E cause inclusion body hepatitis outbreaks in broiler and broiler breeder pullet flocks. Avian Dis 61: 205-210.
14. Mohamed MHA, El-Sabagh IM, Abdelaziz AM, Al-Ali AM, Alramadan M, Lebdah MA, Ibrahim AM, Al-Ankari AS (2018) Molecular characterization of fowl aviadenoviruses species D and E associated with inclusion body hepatitis in chickens and falcons indicates possible cross-species transmission. Avian Pathol 47: 384-390.
15. Alzuheir IM, Jalboush N, Fayyad A, Daibes R (2021) Phylogenetic analysis and pathological characterization of fowl adenovirus isolated during an outbreak of inclusion body hepatitis in Tubas-Palestine. Vet Res Forum 14: 511.
16. Bancroft JD, Gamble M (2008) Theory and practice of histological techniques, 6th edition. London: Churchill Livingstone 83p.
17. 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: 211-217.
18. Geospiza, Inc (2006) FinchTV Version 1.4.0. Available: https://digitalworldbiology.com/sites/default/files/Basic%20page/upfiles/Windows_FinchTV_1_4_0.zip. Accessed: 10 October 2016.
19. Ye J, McGinnis S, Madden TL (2006) BLAST: improvements for better sequence analysis. Nucleic Acids Res 34: W6-9.
20. Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9: 299-306.
21. Ojkic D, Martin E, Swinton J, Vaillancourt JP, Boulianne M, Gomis S (2008) Genotyping of Canadian isolates of fowl adenoviruses. Avian Pathol 37: 95-100.
22. Palestinian Central Bureau of Statistics (2014) Livestock Survey, 2013 / Main Results. Available: https://www.pcbs.gov.ps/Downloads/book2042.pdf. Accessed: 24 August 2022.
23. Kuiken T, Fouchier R, Rimmelzwaan G, Osterhaus A (2003) Emerging viral infections in a rapidly changing world. Curr Opin Biotechnol 14: 641-646.
24. De la Torre D, Nunez LFN, Santander Parra SH, Astolfi-Ferreira CS, Piantino Ferreira AJ (2018) Molecular characterization of fowl adenovirus group I in commercial broiler chickens in Brazil. Virus Dis 29: 83-88.
25. Rahul S, Kataria JM, Senthilkumar N, Dhama K, Sylvester SA, Uma R (2005) Association of fowl adenovirus serotype 12 with hydropericardium syndrome of poultry in India. Acta Virol 49: 139-143.
26. Mittal D, Jindal N, Tiwari AK, Khokhar RS (2014) Characterization of fowl adenoviruses associated with hydropericardium syndrome and inclusion body hepatitis in broiler chickens. Virus Dis 25: 114-119.
27. Mazaheri A, Prusas C, Voß M, Hess M (2003) Vertical transmission of fowl Adenovirus serotype 4 investigated in specified pathogen-free birds after experimental infection. Archiv fur Geflugelkunde 67: 6-10.
28. Niczyporuk JS, Kozdrun W, Czekaj H, Piekarska K, Stys-Fijol N (2021) Characterisation of adenovirus strains represented species B and E isolated from broiler chicken flocks in eastern Poland. Heliyon 7: e06225.
29. Meulemans G, Boschmans M, Berg TP, Decaesstecker M (2001) Polymerase chain reaction combined with restriction enzyme analysis for detection and differentiation of fowl adenoviruses. Avian Pathol 30: 655-660.
30. Changjing L, Haiying L, Dongdong W, Jingjing W, Youming W, Shouchun W, Jida L, Ping L, Jianlin W, Shouzhen X (2016) Characterization of fowl adenoviruses isolated between 2007 and 2014 in China. Vet Microbiol 197: 62-67.

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**Conflict of interests:** No conflict of interests is declared.