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Research article

First detection of FAdV serotype 8b associated with Inclusion Body Hepatitis in broiler flocks in the southern part of Iraq during, 2022-2023

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Abstract

Field observations reported many outbreaks of IBH infection in poultry flocks in different regions of Basra province leading to high economic losses, and the diagnosis of the disease is based on clinical signs and gross pathological lesions. Little information on FAdV recently circulating in the southern part of Iraq; thus, partial sequencing of hexon gene of four FAdV has been detected in the present study during the period between mid-2022 to the beginning of 2023 from various FAdV outbreaks in commercial broiler farms in southern parts of Iraq. The results showed the four Iraqi field strains of FAdV were closely related and shared a high identity of 100% to each other and to previous Iraqi strains from the northern part of Iraq. Phylogenetic analysis indicated that the four viruses obtained from this study and two additional viruses from Ninawa province are classified as serotype 8b of FAdV group E, as a predominant serotype related to inclusion body hepatitis, and clustered along with the same 8b serotype of FAdV circulating in Indonesia, Turkey, and Israel. The results provided useful genetic information on FAdV that recently reemerged in Iraq in 2022 and 2023 and might assist in the production of a local vaccine for FAdV serotype 8b.

Keywords: : Fadv, Hexon, Inclusion Body Hepatitis, Iraq.

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INTRODUCTION

Fowl adenoviruses (FAdVs) are non-enveloped viruses containing double-stranded linear DNA genomes and belonging to the genus aviadenovirus. This viruses are have 5 species (FAdV-A B,C,D and E) with 12 serotypes (FAdV-1 to −8a and −8b to −11) based on restriction enzyme digest patterns and serum cross-neutralization tests (Hess, 2000). Inclusion body hepatitis (IBH), /hydropericardium syndrome (HPS), and adenoviral gizzard erosion are the most poultry disease associated with FAdVs infection (Wells and Harrigan, 1974; Nakamura et al., 1999; Domanska-Blicharz et al., 2011). During the period between 1977 and 1978, eighteen outbreaks of inclusion body hepatitis (IBH) were first documented in broiler flocks in Baghdad (Al-Sheikhly and Mutalib, 1979). While in 1989 the first outbreaks of HPS were recorded in Iraq by Abdul-Aziz and Al-Attar (Abdul-Aziz and Al-Attar, 1991), who mentioned that the disease appeared widely in 1990 in broiler flocks causing a mortality rate ranging between 10-30% they suggested that the disease is similar to a condition reported from Iraq which is thought to be due to adenovirus. The first emerged of HPS as sporicidal cases early in 1985, however, the first disease outbreak was recorded in the Angara region near Karachi in Pakistan in October 1987, and then it gradually spread to other areas of Karachi in March 1988, followed by spreading of the disease in all regions of poultry farming in Pakistan (Cheema et al., 1989). IBH is mainly associated with FAdV-2-11 (species D), 8a, and 8b (species E), whereas HPS is associated with serotype 4 species C FAdV. The grossly pathological lesions of IBH infection include pale, enlarged, hemorrhagic, and friable liver; swollen and hemorrhagic kidneys; and mottled spleen, similar gross lesions have been reported in HPS infection in addition to the accumulation of a clear, watery, gelatinous, yellow or greenish-yellow liquid in the pericardial sac, with a volume ranging between 2-20 ml in 90% of affected birds (Mahmood et al., 2014; Cizmecigil et al., 2020). Genetic analysis of FAdV in different areas of the world, particularly in boundary countries to Iraq, has been well documented, however, little information on FAdV currently reemerged in Iraq poultry flocks; thus, hexon gene sequences of four FAdV have been identified during the period between mid-2022 to the beginning of 2023 from various FAdV outbreaks in commercial broiler farms in southern parts of Iraq. The aim of the current research was to carry out the molecular detection and typing of FAdV strains from IBH outbreaks that recently remerged in Basrah province (southern part of Iraq).

MATERIALS AND METHODS

Sampling

The Samples were collected from three broiler chicken farms located in the north and middle areas of Basrah province from September 2022 to January 2023. Each broiler farm contains 9000-111000 chicks, with ages ranging from 20-25 days old and a mortality range (of 10%-12%). The samples of liver, kidney, and heart were taken from chickens that showed clinical signs, and lesions related to inclusion body hepatitis showed lethargy, huddling, ruffled feathers, and loss of appetence. Abnormal consistency and yellowish

discoloration of the droppings. While the lesions; are enlarged pale and friable liver sometimes with necrotic foci. Ecchymotic hemorrhages may be also seen in the liver. Pale and enlargement of kidneys. The samples were collected with an aseptic technique and frozen at -70 C. The authors declare that this work has not previously been published. The current study was conducted on September 01, 2022 and performed under the permission of the ethical committee in the Faculty of Veterinary Medicine, University of Basra (Ref. No. 87/2022)

DNA extraction

DNA of liver tissue specimens was extracted by the gSYNC™ DNA Extraction Kit and according to the manufacturer's instructions. Transfer up to 25 mg of bird tissue (0.5 cm mouse tail x 2 or 0.5 cm rat tail x 1) to a 1.5 ml microcentrifuge tube. If the tissue has a higher number of cells (e.g. spleen or liver), reduce the starting material to 10 mg. Add 200 µl of GST Buffer and 20 µl of Proteinase K then vortex thoroughly. Incubate at 60ºC overnight or until the sample lysate becomes clear.Polymerase chain reaction (PCR). Finally, the extracted DNA was stored at -70°C until it used.

PCR reaction

A part of 590 bps of the Hexon gene was subsequently amplified by PCR, using one pair of specific primers (Table 1) as described previously by (Nateghi et al., 2014). The PCR was performed in a 20 µL reaction volume containing AccuPower® PCR PreMix. The PCR was performed in a 20 µL reaction volume containing AccuPower® PCR PreMix. Template DNA 5 µl). Forward Primer (10 pmole/ µl) 1.5 µl, Reverse Primer (10 pmole/ µl) 1, 5 µl and 12 µl PCR water, (Top DNA polymerase 1.0 U). The thermocycler (Thermos Fisher Scientific, Applied Biosystems) was configured as 94°C for 5 min followed by 35 cycles of 94°C for 60 s, 54.2°C for 45 s, 72°C for 60 s, and a final step at 72°C for 5 min. The PCR products were visualized via gel electrophoresis on 1.5% agarose gel (Figure 1). The products were purified by the PCR AccuPrep® PCR purification kit (Bioneer Co., Korea). Sequencing was implemented for both directions by the same primers that were used for the amplification of Hexon gene.

Table 1 primer sequences used for PCR identification

Primer	Sequence	Genome location	Size (bp)
Hex L1-F	5′ATGGGAGCSACCTAYTTCGACAT-3′	301-323	590
Hex L1-R	5′AAATTGTCCCKRAANCCGATCTA-3′	868-890	

Table 2 FAdv viruses used for phylogenetic analysis in present study

	Viruses	Accession No.	Location
1	FAdV_8b_strain_FAV8b/Hubei/duck/H2138/2019	ON502589.1	China
2	FAdV_E_isolate_MSL	MT104456.1	Indonesia
3	FAdV_isolate_M41	MK642682.1	Indonesia
4	FAdV_E_isolate_ID.MSL.424.21	OK236344.1	Indonesia
	FAdV_E_isolate_vsn045bdl18	MK692964.1	Indonesia
5	FAdV_E_isolate -ISR/4346/2021	MZ368700.1	Israel
6	FAdV_DDO-2007	EF685515.1	Canada
7	FAdV_E_isolate_	MK937074.1	Turkey
8	FAdV-E strain	MN052902.1	Turkey
9	FAdV_8b_strain_20489-M/2015	MG953210.1	Hungary
10	Fowl_adenovirus_Z-M_gene/ Nineveh/Iraq/2022	LC721874.1_	Iraq
11	Aviadenovirus sp. A-M-D/ Nineveh/Iraq/2022	LC727624.1	Iraq
12	FAdV_isolate_FAdV/Basrah1/Iraq/2022	study	Iraq
13	FAdV_isolate_FAdV/Basrah2/Iraq/2022	study	Iraq
14	FAdV_isolate_FAdV/Basrah3/Iraq/2022	study	Iraq
15	FAdV_isolate_FAdV/Basrah4/Iraq/2022	study	Iraq

RESULTS

Virus detection

During the period between mid-2022 to the beginning of 2023, three outbreaks of IBH were recorded in broiler flocks in Basrah province (southern part of Iraq). All tested samples in the present study showed molecular positive results as it's shown in (Figure 1). On the other hand, the DNA sequencing results revealed four positive samples from different flocks. Subsequently, these strains in addition to two previously Iraqi strains which have been reported in Ninawa province (northern part of Iraq) were included for genetic analysis (Figure 2).

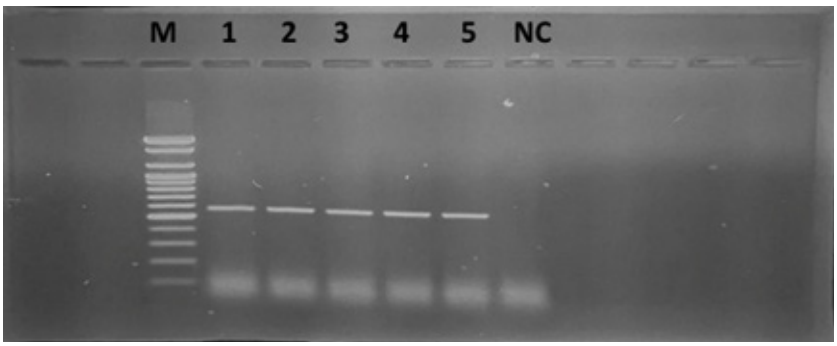

Figure 1 FAdV Hexon gene Amplification, M: 100 bp molecular Ladder. Lane: 1-5 studied samples, Lane 6: Negative Control (NC).

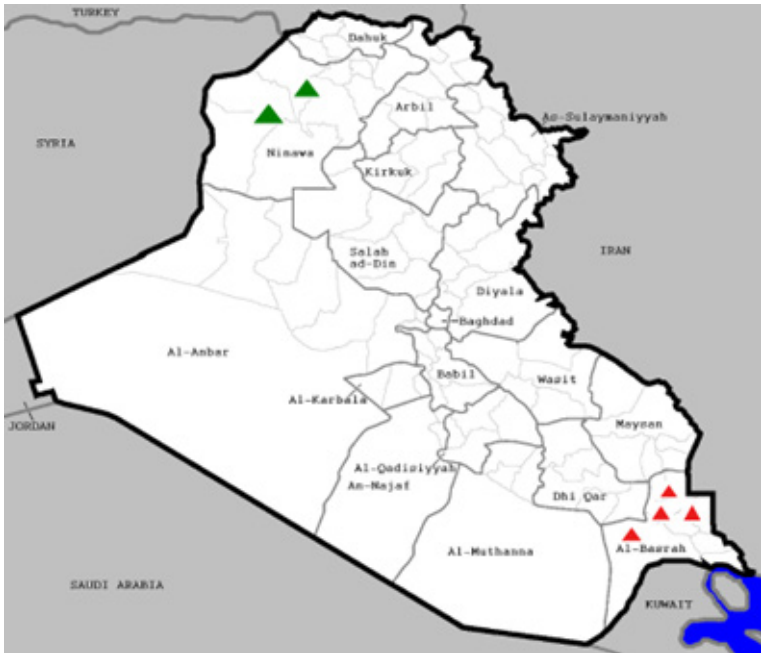

Figure 2 Distribution of Iraqi FAdV strains which are indicated in a red and green triangle in AL-Basrah and Ninawa provinces, respectively.

Phylogenetic analysis of FAdV

partial sequencing analysis of the Hexon gene for the four Iraqi FAdV field strains stated that all strains were closely related and shared a high identity of 100% to each other and to previous Iraqi strains from the northern part of Iraq; moreover, the blast analysis of Hexon gene sequencing obtained in the current study exhibited high percentages of identity with FAdV serotype 8b species E reference strains available in NCBI. Interestingly, all viruses from Indonesian, Turkish's and Israeli strains shared a high nucleotide sequence identity of 100% with all Iraqi viruses (Table 3) and failed within the same cluster belonged to FAdV E serotype 8b (Figure 3), in addition, Hungarian, Canadian and Chinese strains revealed lower identities 98.78%,98.54% and 97.54%, respectively.

Table 3 similarity (%) among the nucleotide sequences of the hexon gene of Iraqi FAdv with reference strains from different countries.

Name of virus strain	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1- /Basrah1	100	100	100	100	100	100	100	100	100	100	100	100	98.78	98.54	97.54
2- Basrah2		100	100	100	100	100	100	100	100	100	100	100	98.78	98.54	97.54
3- Basrah3			100	100	100	100	100	100	100	100	100	100	98.78	98.54	97.54
4- Basrah4				100	100	100	100	100	100	100	100	100	98.78	98.54	97.54
5- Z-M Nineveh					100	100	100	100	100	100	100	100	98.78	98.54	97.54
6- A-M-D/ Nineveh						100	100	100	100	100	100	100	98.78	98.54	97.54
7- MSL							100	100	100	100	100	100	98.78	98.54	97.54
8- M41								100	100	100	100	100	98.78	98.54	97.54
9- ID.MSL									100	100	100	100	98.78	98.54	97.54
10- vsn045bdl18										100	100	100	98.78	98.54	97.54
11-ISR/4346/											100	100	98.78	98.78	97.54
12- TR/BVKE												100	98.78	98.54	97.54
13- FAdV-E strain													98.78	98.54	97.54
14- 8b20489														98.78	98.78
15- DDO-2007															97.54
16-8b/Hubei/duck															

Reference strains that appeared in red and green colors represent strains of present and previous studies, respectively, while others represent the reference strains as mentioned in (Table 1)

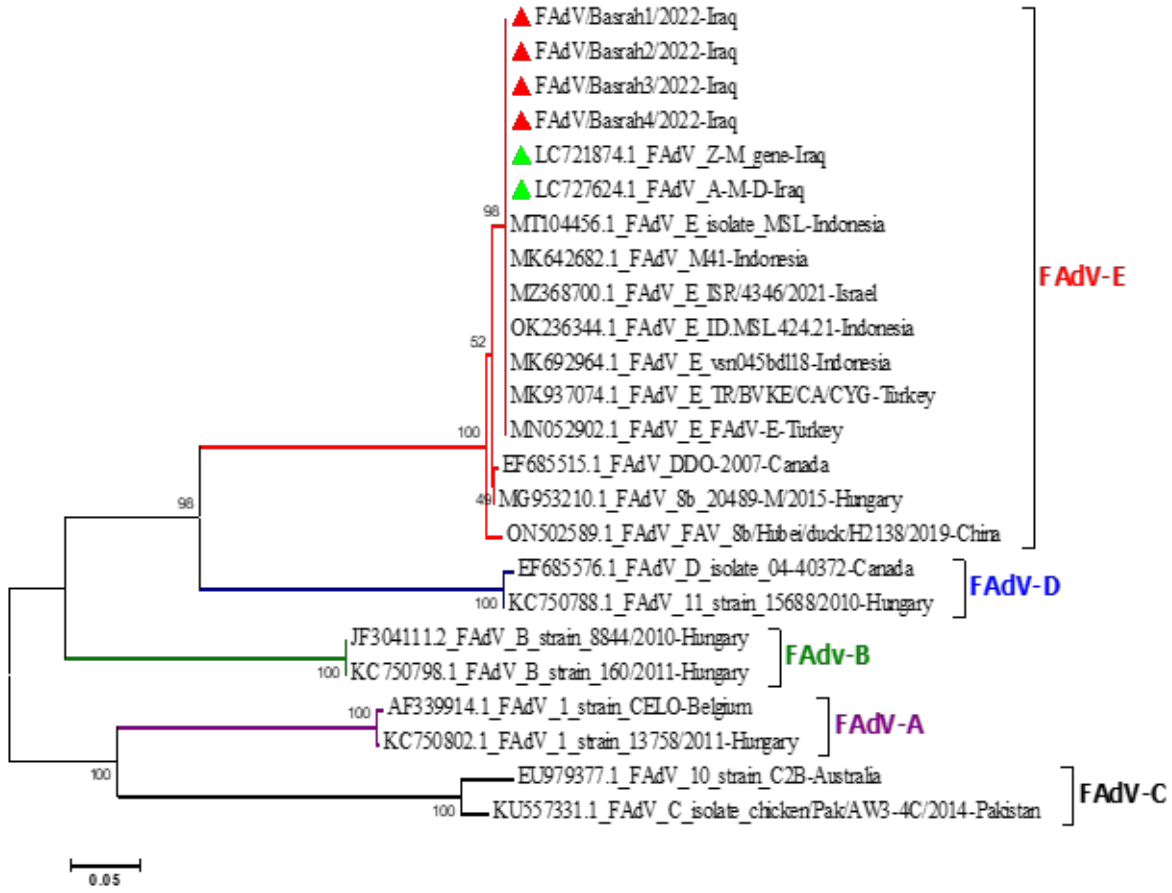

Figure 3 Phylogenetic relationships of Iraqi FAdv with reference strains from different countries based on hexon gene during 2022-2023, the strains indicated in a red and green triangle represent southern and northern Iraqi strains, respectively.

DISCUSSION

Development of the poultry industry in Iraq, particularly in the province of Basrah, where many poultry farms were established. The intensive poultry farming and lack of awareness of a biosecurity program facilitated emerging of different viral diseases. Field observations reported many outbreaks of IBH infection in poultry flocks in different regions of Basra province leading to high economic losses, and the diagnosis of the disease is based on clinical signs and gross pathological lesions. Thus, the current investigation was conducted for molecular detection, to monitor disease outbreaks and vaccine development. Partial sequencing analysis of the Hexon gene for the four Iraqi FAdV field strains stated that all strains were closely related and shared a high identity of 100% to each other and to previous Iraqi strains from the northern part of Iraq. Phylogenetic analysis indicated of 4 viruses obtained from this study and 2 additional viruses from Ninawa province are classified as FAdV group E, serotype 8b as a predominant serotype related to IBH. It was noted the southern and northern Iraqi FAdV strains clustered along with the same serotype of FAdVs circulating in Indonesia, Turkey, and Israel. The results of the current study are in accordance with those of (Silaen et al., 2020) who mentioned that phylogenetic analysis of the Indonesian isolate Broiler/MSL/Ciputat-29/19 has 100% similarty with TR/BVKE/R/D-1 isolate of FAdV-E from Turkey. Also (Şahindokuyucu et al., 2020) described that Turkish field isolates (TR/BVKE/CA/CYG and TR/BVKE/R/D-1) were most similar to isolates from Canada, China, Slovenia, Peru, and South Africa. FAdV-8b has been identified in many countries in association with IBH in Korea, Canada, and Slovenia (Ojkic et al., 2008; Lim et al., 2011; Zadravec et al., 2013). The reemergence of FAdV group E, serotype 8b in Iraq is a predictable episode as a result of the global circulating of the virus, particularly in boundary countries to Iraq, and also due to halted Vaccination campaign programs in Iraq. It is, however, complex to determine the route FAdA serotype 8b entry to Iraq. The identical homology between the Iraqi strains and turkey viruses may refer to potential epidemiological links and the fact that these viruses may stem from the same origin. In recent years, Iraq imports poultry products and everything related to the chicken industry, including live chicken from the boundary countries such as Turkey, which may have assisted in virus transmission. It's well known that the FAdV is readily transmitted horizontally since they are present in all excretions, and at high titers in the feces, fomites, personnel, and transport may also be important contributors to the transmission of the virus. other researchers have mentioned that IBH viruses could be transmitted vertically through embryonated eggs and that the virus could be reactivated in young chicks, especially if the chicks are immunosuppressed (Fadly et al., 1980; Arazi et al., 2020). Most of the examined poultry feed ration samples from some broiler flocks in Basrah province were complexly contaminated with both types of afla and T-2, higher than the allowed limit (Kraidi et al., 2019). The second scenario related to the cause of entry of FAdV includes the migration of wild birds as a reservoir in the spread of FAdV (Kumar et al., 2010).

CONCLUSIONS

Overall, our study revealed the circulation of FAdV group E, serotype 8b, and genetically identical to the northern part of Iraq in addition to isolated FAdV from Indonesia, Turkey, and Israel. The results provided useful information on the genetic epidemiology of FAdV circulating in Iraq in 2022 and 2023 and would help to develop a local vaccine against FAdV serotype 8b.

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AUTHOR CONTRIBUTIONS

Qayssar Ali kraidi: genetic analysis of the results and to the writing of the manuscript.
Waleed Majeed Almayahi: contributed to the design of the research and field diagnosis of the disease.
Harith Abdulla Najem: molecular detection.

CONFLICT OF INTEREST

We declare that they have no conflict of interest.

REFERENCES

Abdul-Aziz, T.A., al-Attar, M.A., 1991. New syndrome in Iraqi chicks. Vet. Rec. 129(12), 272.
Al-Sheikhly, F., Mutalib, A.A., 1979. Inclusion body hepatitis in broiler chickens in Iraq. Avian Dis. 23(3), 763-767.
Arazi, S., Mayahi, M., Talazadeh, F., Boroomand, Z., Jafari, R., 2020. Molecular identification of aviadenoviruses in broiler chick,ens suspected to inclusion body hepatitis in Golestan province, Iran. Iran J. Vet. Med. 21(3), 198-202.
Cheema, A.E., Ahmad, J., Afzal, M., 1989. An adenovirus infection of poultry in Pakistan. Rev Sci Tech. 8(3), 789-795.
Cizmecigil, U.Y., Umar, S., Yilmaz, A., Bayraktar, E., Turan, N., Tali, B., Aydin, O., Tali, H.E., Yaramanoglu, M., Yilmaz, S.G., 2020. Characterisation of fowl adenovirus (FAdV-8b) strain concerning the geographic analysis and pathological lesions associated with inclusion body hepatitis in broiler flocks in Turkey. J. Vet. Res. 64(2), 231-237.
Domanska-Blicharz, K., Tomczyk, G., Smietanka, K., Kozaczynski, W., Minta, Z., 2011. Molecular characterization of fowl adenoviruses isolated from chickens with gizzard erosions. Poult. Sci. 90(5), 983-989.
Fadly, A., Riegle, B., Nazerian, K., Stephens, E., 1980. Some observations on an adenovirus isolated from specific pathogen free chickens. Poult. Sci. 59(1), 21-27.
Hess, M., 2000. Detection and differentiation of avian adenoviruses: a review. Avian Pathol. 29(3), 195-206.
Kraidi, Q.A., Abbas, S.S., Chayan, M.A., 2019.Study of pathological changes caused by mycotoxin in broilers in AL-Qurna city, Basra, Iraq. Plant Arch. 19(2), 2579-2584.

Kumar, R., Kumar, V., Asthana, M., Shukla, S., Chandra, R., 2010. Isolation and identification of a fowl adenovirus from wild black kites (Milvus migrans). J. Wildl. Dis. 46(1), 272-276.

Lim, T.H., Lee, H.J., Lee, D.H., Lee, Y.N., Park, J.K., Youn, H.N., Kim, M.S., Youn, H.S., Lee, J.B., Park, S.Y., 2011.Identification and virulence characterization of fowl adenoviruses in Korea. Avian Dis. 55(4), 554-560.

Mahmood, M.S., Ali, S., Hussain, I., Aslam, A., Rafique, A., 2014. The development of hydropericardium syndrome vaccines. Worlds. Poult. Sci. J. 70(2), 355-364.

Nakamura, K., Mase, M., Yamaguchi, S., Shibahara, T., Yuasa, N., 1999. Pathologic study of specific-pathogen-free chicks and hens inoculated with adenovirus isolated from hydropericardium syndrome. Avian. Dis. 43(3), 414-423.

Nateghi, E., Razmyar, J., Bassami, M.R., 2014. Molecular characterization of avian adenoviruses in Iranian broiler flocks. Iran. J. Vet. Res. 15(2), 164-167.

Ojkic, D., Martin, E., Swinton, J., Vaillancourt, J.P., Boulianne, M., Gomis, S., 2008. Genotyping of Canadian isolates of fowl adenoviruses. Avian. Pathol. 37(1), 95-100.

Şahindokuyucu, İ., Çöven, F., Kılıç, H., Yılmaz, Ö., Kars, M., Yazıcıoğlu, Ö., Ertunç, E., Yazıcı, Z., 2020. First report of fowl aviadenovirus serotypes FAdV-8b and FAdV-11 associated with inclusion body hepatitis in commercial broiler and broiler-breeder flocks in Turkey. Arch. Virol. 165(1), 43-51.

Silaen, O.S.M., Murtini, S., Pamungkas, J., Nugroho, C.M.H., 2020. Isolation and molecular characterization of fowl aviadenovirus associated with inclusion body hepatitis from poultry in Banten and West Java, Indonesia. Vet. World. 13(9), 1940-1946.

Wells, R., Harrigan, K., 1974. A fatal adenovirus infection of broiler chickens: inclusion body hepatitis. Vet. Rec. 94(21), 481-482.

Zadravec, M., Slavec, B., Krapež, U., Kajan, G., Račnik, J., Juntes, P., Juršič-Cizerl, R., Benkõ, M., Zorman-Rojs, O., 2013. Inclusion body hepatitis (IBH) outbreak associated with fowl adenovirus type 8b in broilers. Acta Vet. 63(1), 101-110.

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