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GENOMIC VARIATION IN HEXON GENE OF INCLUSION BODY HEPATITIS VIRUS IN BROILER, IRAQ

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Abstract
Adenovirus is responsible for inclusion body hepatitis. Chicken adenovirus (FAdV), a member of the Adenoviridae family, is the virus that causes chicken inclusion body hepatitis (IBH). The first report of the disease in Iraq was in 1979, yet the virus is not characterized. During the period from September 2021 to May 2022, we tracked disease outbreaks in 110 broiler farms in the central of Southwestern, and Southern parts of Iraq. Precisely, these areas were Al-Daghara, Al-Amarah, Baghdad, and Al-Diwaniyah. Birds affected were with clinical signs including lethargy, huddling, ruffled feathers, a lack of appetite, and yellowish mucoid droppings. The carcasses showed pale skin or icteric, the liver was mottled and enlarged, and the kidney was enlarged with distended tubules in the postmortem examination.The histological examination showed large intranuclear inclusion bodies, congestion, and liver sinusoidal degeneration. There was a higher incidence of necrotizing pancreatitis. The virus was detected using reverse transcription-PCR (RT-PCR) of the hexon genes (RT-PCR). Using next-generation sequencing, the amplified fragments were confirmed to be authentic. According to the findings of the study, broiler chickens of all breeding ages were susceptible to infection and the IBH was widespread in the studied areas during that time period.

Keywords: Chicken adenovirus**,** IBH, Reverse Transcription-PCR, Necrotizing pancreatitis

Introduction

The causative agent of the inclusion body hepatitis (IBH) is a virus known as aviadenovirus (FAdV), which occurs only in chickens (12). Other illnesses in chicken that are caused by fowl adenoviruses (FAdVs) are hydropericardium hepatitis syndrome (HHS), adenoviral gizzard erosion AGE), and infectious broiler hemorrhagic fever (IBH). The closely related HHS is brought on by FAdV-4 (FAdV-C) strains, which are considered as highly contagious in chickens (4). In that case, the report described extreme necrotic hepatitis in chickens that were in their seventh week of age (6). HHS, which is a novel illness, was first reported in broilers from a place called Angara Goth, Karachi, Pakistan in 1988.The hydropericardium hepatitis syndrome shows clear-watery, straw-colored fluid in the pericardial sac, with massive multi-focal necrosis of the liver (1). In 1979, IBH was firstly reported in Iraq (3). Inclusion body hepatitis outbreaks have grown in number during the past 20 years, underscoring the diseases widespread frequency (8, 11).This disease primarily affects broilers up to 35 days old, while it has occasionally been seen in all age groups. Inclusion body hepatitis is characterized by 2–40% chicken mortalities during natural outbreaks typically. Younger birds (at the first three weeks of age) may experience the highest

mortality ratesFocal necrotic regions and intranuclear inclusion bodies in some hepatocytes are examples of histopathological abnormalities. They appear as sizable, round or asymmetrical objects with a distinct, pale aura (2). In the south area of Iraq, there haven`t been any reports of IBH or similar instances in poultrythat are characterized by hepatitis and hepatocytic intranuclear inclusion bodies. As a result, this is the first time cases of IBH that resemble an adenovirus have been documented in broiler farms in south, Iraq .

Materials and Methods

Sample Collection

A total of 110 broiler chickens were taken from four different farms in the south and center of Iraq involving 23 from Al-Daghara (Al-Diwaniyah governorate), 21 from Kumait (Al-Amarah governorate) 27 from Qal'at Sukar (Dhi-Qar) and 39 from Al-Lij (Baghdad governorate). The liver, spleen, bursa, and pancreas were removed from each chicken in a sterile dissection. During shipment and storage at -20ºC, samples of molecularassay were carefully protected from degradation.

Detection of FAdV Hexon Genes

Liver samples from infected chickens were prepared as 10% homogenates in minimum essential medium (Kylt®, Germany). DNA was extracted from the homogenates using Viral Gene kit (Kylt®, Germany) and used for PCR. Primers were designed according to (7). The primer design allows detection DNA with 1,319 bases from whole hexon gene of FAdV.

DNA extraction

The process for extracting DNA was exactly as described (reference). For example, 1 ml of 0.9% normal saline or 1 x TE buffer would be sufficient volume for storing swabs in a sterile environment. Swabs were given a sufficient amount of time in the TE buffer, after which they were given a thorough pulse-vortex. The DNA extraction procedure makes use of the supernatant. If necessary, lysis buffer can be used to submerge small swabs directly. Samples of tissue and organs were homogenized in sterile buffer (as described above) and an adequate amount of DNA was extracted.

This nucleic acid has been purified using the Kylt ® RNA/DNA purification kit (AniCon Labor GmbH, Muehlenstr, Germany). The avian adenoviruses are closely related to the chicken adenovirus, but this kit is only intended to detect the viral DNA of the chicken adenovirus. During RT-PCR, fluorescently labeled probes were used to look for amplification of the target gene fragment, the hexon, which was already amplified by PCR.

Pure isolates, swabs, tissues, and organs were all viable sample matrices that can be processed with the right DNA preparation kits or in-house procedures. Statistical analysis was carried out using the GraphPad prism Software at a significant variation of $P<0.05$ (19).

Results and dissection

PCR Analysis, Sequencing, and Phylogeny

Amplification of the hexon gene fragment resulted in a 590 bp PCR product (Fig. 1). Results from the nucleotide BLAST for the sequences revealed 96%-99.48% similarity with the genomic

sequence of the FAdV isolates present in the BLAST database (BLAST, NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi.). Nucleotide sequences from the liver matched FAdV species E isolates of serotype FAdV 8b The sequencing analysis of the partial hexon gene revealed the high similarity of the FAdV-E (FAdV-8b) strain characterized in this study to those from Indonesia, Canada, Peru, and China (MK692992, EF685489, KX755572, and KU981139), and its difference from Egyptian, Iranian, Lebanese, Pakistani, Austrian, Italian, Brazilian, and Australian strains reported to GenBank see the link (https://www.ncbi.nlm.nih.gov/nuccore/LC729408). In this study, samples were also screened for the presence of possible mixed viral infections as part or routine diagnostic work using RT-PCR. However, none of the samples were found to be positive for the tested field viruses (20).

Molecular Results

Liver samples were collected from the naturally-infected flocks (110 samples). The samples were pooled and submitted to perform PCR assay. The original experimental design was involving primer design for hexons. However, lack of facilities made me move for using FTA kit instead. All the taken samples were PCR positive for FAdV (Figure 1). These samples were sequenced and analyzed using NCBI free software to create the phylogenic tree of our isolates. Our results showed that there was a single serotype (FAdV8) depending on the hexon gene PCR and the sequencing results. The sample codes were A2122617.001 - A2122617.004 Assay information. The results showed that there was positive amplification of all the taken samples from the infected flocks. This reveals that the PCR is a potent tool for IBH diagnosis (1)

In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The CT (cycle threshold) is defined as the number of cycles required for the fluorescent signal to exceed background levels. CT levels are inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the CT level the greater the amount of target nucleic acid in the sample).

The sequenced DNA showed that there was a single viral serotype striking in the studied areas. This serotype is designed as FADV 8b (Table 1). This serotype was also described in the neighboring areas and countries (14, 13) .The sequencing analysis of the partial hexon gene revealed the high similarity of the FAdV-E (FAdV-8b) strain characterized in this study to those from Egyptian, Iranian, Lebanese, Pakistani strains , and difference from Canada, Peru, and China strains (13). Mixed viral infections were also considered by using RT-PCR in this study's routine diagnostic work. None of the samples tested positive for any of the field viruses (13, 20).

Table (6) : Sequencing and typing (species A-E, serotype 1-7, 8a, 8b, 9-11; acc. to ICTV) (a)

Sample code *	Sample Description	Result	Typing
A2122617.001	Anicard (spots 1-3 pooled: Sample 1)	Positive	FADV E,FADV 8b
A2122617.002	Anicard (spots 4-6 pooled: Sample 2)	Positive	FADV E,FADV 8b
A2122617.003	Anicard (spots 7+8 pooled: Sample 3)	Positive	FADV E,FADV 8b

A2122617.004	Anicard (spots 9+10 pooled: Sample 4)	Positive	FADV E,FADV 8b

*This code as it has been supplied by (AniCon labor GmbH)

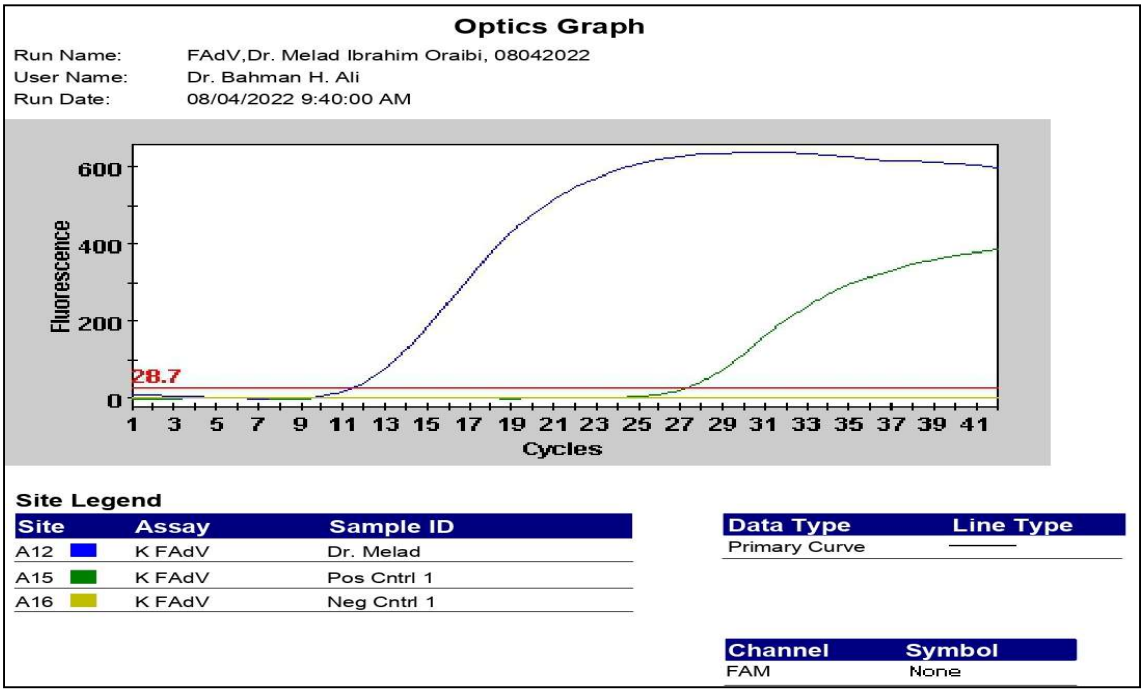

Figure (1) : Optics graph and site legend

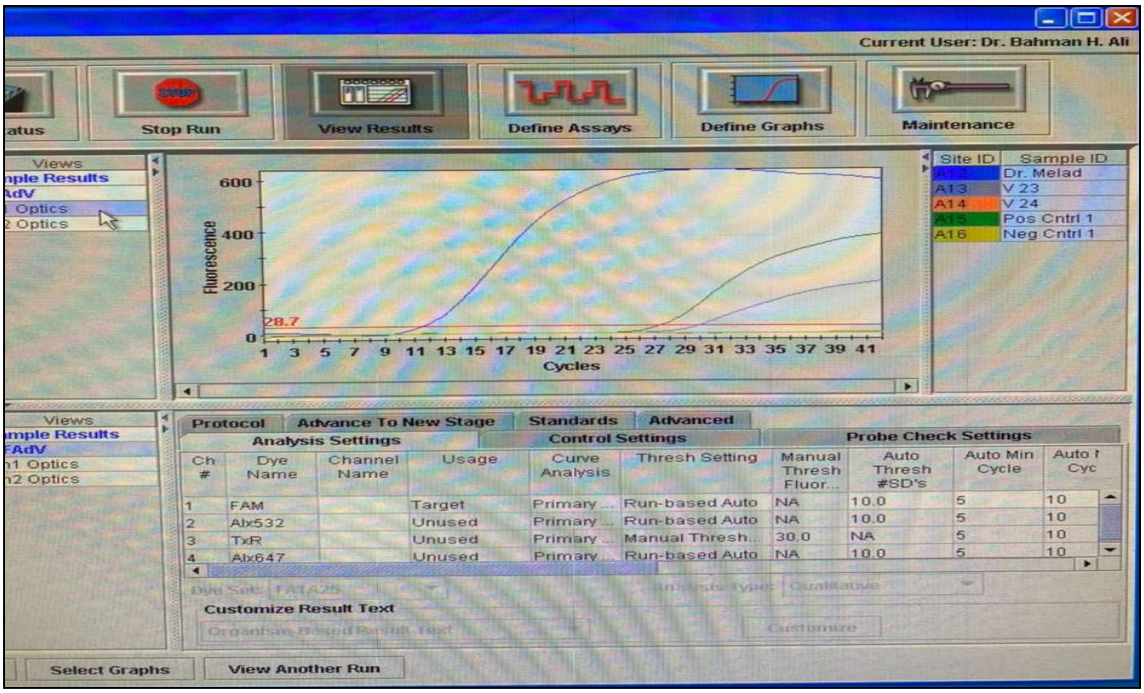

Figure (2): Optics graph in PC

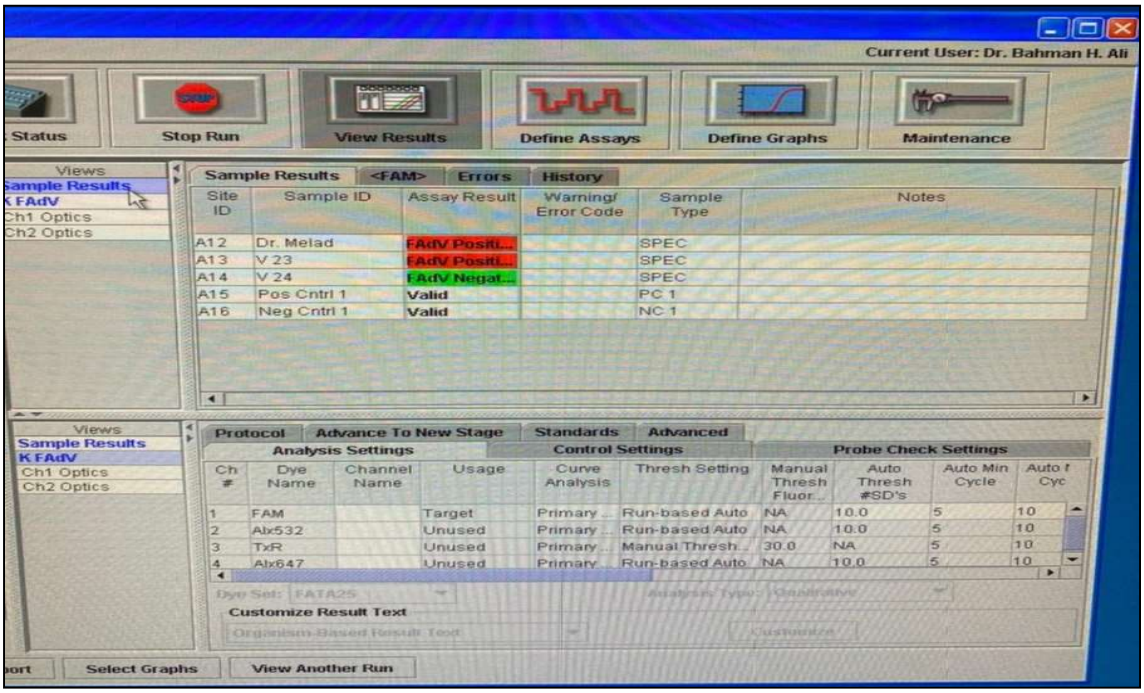

Figure (3): Sample result in PC

Adenovirus tree

Using the NCBI free software, the phylogenic tree of the adenovirus has been created (https://www.ncbi.nlm.nih.gov/blast/treeview/treeView.cgi?request=pageand blastRID=N5YP9616013 and queryID=dbj|LC729408.1 and entrezLim=and ex=and exl=and exh=and ns=100and screenWidth=1366and screenHeight=768). The tree shows that our serotype, which is closely-related to other fowl adenoviruses. These closely-related serotypes are strain X11-A and E strain X11-A (15). Data were available at (https://www.ncbi.nlm.nih.gov/nuccore /Lc729409). Fowl aviadenovirus E MELAD gene for hexon assembly protein .The complete protein sequence and assembly is with 100K, complete CDC, and can be reached at (https://www.ncbi.nlm.nih.gov/nuccore/LC729408). The evolutionary history was inferred using the UPGMA method (16). The optimal tree with the sum of branch length = 0.13529415 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (17, 18, 21) and are in the units of the number of base substitutions per site. The analysis involved 18 nucleotide sequences. Codon positions included were $1^{st} + 2^{nd} + 3^{rd}$ + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 3187 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (22-24).

Conclusion

In conclusion, the IBH is striking in most parts of the Middle, Midwestern, and Southern part of Iraq. The Iraqi isolate (Referenced as Melad in NCBI), is closely related to Chinese isolate, the Japanese isolate and Brazilian isolate. The source of these isolated could be contaminated vaccines,

feed additives or chicken products. No Middle Eastern isolated has been detected in Iraq within this study.

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