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Molecular Diagnosis of Adeno Virus Associated with Hydropericardium Hepatitis Syndrome of the Broiler Chickens in Nineveh Province, Iraq

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THIS study aimed to molecular diagnose hydropericardium hepatitis syndrome (HHS) in the broiler chickens of six areas in Nineveh Province, Iraq namely: Mosul, Talafer, Baaj, Bartela, Hamdanya and Qayara during the period October 2021-March 2022. Clinical and pathological manifestation of the disease in affected birds was investigated and observed. A total of 24 infected liver lesioned samples as 12 broiler farms (2 farms for each area) detected for hexon gene of FAdV by PCR and sequencing for accurate diagnosis. The results revealed that the PCR of liver samples for 12 broiler farms of the six regions in Nineveh Province were positive results of HHS adenovirus 897bp and the result of DNA sequencing revealed that the pathogenic HHS adenovirus of the six areas was serotype 4 of one strain E1B corresponding with sequencing XM_040669733.2 of National Center for Biotechnology Information NCBI. This study is the first of its kind for the molecular diagnosis and determination of the strain type of FAdV that causes HHS in broilers chicken in Nineveh Province, Iraq.

Keywords: Hydropericardium hepatitis syndrome, Fowl adenoviruses, Molecular Diagnosis, Broiler chickens, Nineveh Province.

Introduction

Fowl adenoviruses (FAdV) are members of the genus Aviadenovirus and the family Adenoviridae, which is divided into three divisions. According to serum cross-neutralization tests and restriction enzyme digest patterns, Group 1 is split into 12 serotypes (FAdV-1 8a and 8b - 11) and 5 species (FAdV A, B, C, D, and E). FAdV are linked to a variety of avian disorders, such as inclusion body hepatitis, hydropericardium hepatitis syndrome (HHS), and Egg Drop Syndrome EDS. FAdV subtypes 4 are the most common cause of these diseases in the chicken [1-3]. FAdV4 is the etiological agent for HHS which is a linear double stranded DNA virus. The genome is approximately 43 to 46 kb, non-enveloped and icosahedral in shape. Hexon, Penton, and the fiber protein are three structural proteins that are encoded by the FAdV genome [4, 5].

HHS was first observed in 1987 at Angara Goth, Pakistan and therefore it was named as Angara disease. Later, the disease was reported in other countries including India where the disease was first detected in Jammu followed by Punjab and Delhi in 1994 [6] and the disease was first report in Iraq in 1991[7].

Broiler chicken of 3-6 weeks of age are mostly affected by HHS [8, 9]. There are 12 serotypes belonging to the first group of poultry adenoviruses, which are widespread in various parts of the world, and the serotype 4 has been isolated from most of the cases of HHS, as it was isolated in Ecuador, Chile, Japan, Mexico and

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Pakistan10,11]]. The infected birds don't exhibit any obvious signs, though a sudden death was noticed between 2-5 days. Birds with sporadic cases may exhibit various postures, grow dull and dejected in the final stages, and gather in a corner. The eyelids were closed, and the beak and chest were resting on the ground. The gross lesions in 90% of the affected birds, are the pericardium accumulates fluids with a green tint or fluid that is colorless, watery to jellylike, heart malformation with floating in the pericardial sac's apex and petechial hemorrhage. The liver's alterations include ecchymotic or petechial hemorrhage, extensive areas of mottled focal necrosis, friable swelling, and yellow, paleness. Lung congestion is present [6,7, 11,12].

The risk of HHS outbreaks could be minimized by adopting proper management and biosecurity measures [13] in addition to this, vaccination might play a key role in preventing HHS. However, there is still dire need of a vaccine with higher efficacy and fewer sides to counter FAdVs in poultry [13]. For that, this study aimed to survey diagnosis for the HHS virus in broiler chicken farms in the six areas of Nineveh Province, namely Mosul, Tal Afar, Hamdaniya, Bartella, Al-Baaj and Qayyarah for the period October 2021to March 2022 by molecular diagnosis of the adeno virus that causes HHS in broilers by PCR technique, as well as gene sequencing to determine the strain of the virus.

Material and Methods

Sample collection

A poultry population of total 24 samples from 12 broiler farms of six areas (2 farms for each area): Mosul, Talafer, Baaj, Bartela, Hamdanya and Qayara in Nineveh Province visited regularly to study outbreak of HHS during the period from October 2021 to march 2022 .Suspected flocks were clinically examined, clinical sign and symptoms were observed. A total of 24 samples of dead chickens were collected after necropsy examination observed, liver samples and stored in (-20) degree for molecular diagnosis by polymerase chain reaction PCR.

Diagnosis of hydropericarditis and hepatitis by polymerase chain reaction PCR

The first step was relied upon extraction of DNA from chicken liver tissue using the analysis kit supplied by Geneaid, Taiwan, then preparation of agarose gel and DNA electrophoresis: was done for DNA transfer and detection, 1% agarose gel was prepared. Then the gel solution was added in the basin of the Tray of the transfer device after the special comb was fixed to form the Wells at the edges of the gel. Then the Tray was placed in an electric relay tank containing an appropriate amount of X1 TBE solution, after which we lifted the comb softly. The gel was photographed under ultraviolet rays using a gel documentation device to be able to see the DNA bundles as well as the PCR reaction product.

In the PCR reactions the concentration of DNA in all samples was adjusted by dilution with TE solution to obtain the concentration required to perform the PCR reactions, and it was 50 ng/microliter for each sample. The presence of the amplified region was detected, as 4 µl (100 nanogram) of template DNA, and 1 µl (10 picompl) of each gene-specific primer were added to the contents of the master mix (Table 1).

Then we inserted the reaction tubes into the thermocycler to conduct the multiplication

TABLE 1. The sequence of hexon in DNA

Primer	Sequence
Hexon-A	5′- CAARTTCAGRCAGACGGT -3′
Hexon-B	5′- TAGTGATGMCGSGACATCAT -3′

TABLE 2. The special program for the PCR method

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	6 min.	1
2.	Denaturation	95	1.30 min.	
3.	Annealing	46	1.30 min.	35
4.	Extension	72	2 min.	
5.	Final extension	72	5 min.	1

reaction using the special program for the reaction as shown in the following (Table 2).

DNA sequencing analysis

The DNA sequencing technique is the basis for identifying and detecting genetic mutations, SNP variations and the strain of virus. Usually, the output of the PCR reaction is used to determine the amplified segment sequences in which genetic differences are required.

The sequence of nitrogenous bases of the amplified DNA pieces of the chicken liver tissue was determined. The PCR reaction products of the aforementioned samples were sent with the primers of the resulting package. The sequence of genes was read based on the 3130 Genetic Analyzer device supplied by the Hitachi Co., Japan. Gene-specific sequences were matched with those documented in the National Center for Biotechnology Information (NCBI) and the results were analyzed using BLAST.

Result

Diagnosis the adenovirus by PCR

All results showed that the PCR of liver samples for 12 broiler farms of the six regions in Nineveh Province, namely: Mosul, Talafar, Hamdaniya, Bartella, Al-Baaj and Qayyarah with a positive results of HHS adenovirus and the result was 897bp (Fig. 1), in the period (October 2021 - March 2022).

Molecular diagnosis of adenovirus by DNA sequencing

The result of molecular diagnosis of adenovirus by DNA sequencing revealed that the pathogenic HHS adenovirus causative agent of the six areas in Nineveh Province was adenovirus serotype 4 of one strain E1B for all of them according to the gene sequencing below and corresponding with sequencing ID: XM_040669733.2 of National Center for Biotechnology Information NCBI (Fig. 2).

A G C G G C G G G A A G C G C G G G G C -
C A T G G C G G G C G G C G G C G A C G A C G -
G C T C T G G G T G G A G C T G C G C T G C G A C C C C -
G C G C C C T T C T C G T G C C A C G C C G A C G T G -
G A G C G G A T G C T G C T G G A G G C G C A G C T G -
G A G C C G G A C G G C G C C G A C G G G G G C -
G C T G C C G G C G C G G C C C C C C G G C C C -
G G G A C G A G G A A G A C G C C G C A A A G G T -
G A G C G A G A G G C T C C G C C C C G C C C C -
G G G C C G C C C G C A G A C C C C G C C G G G C C -
G G G A A G G A G C G C C G A A G T C C C G C G T G -
G A G C C C C C C G C G C C G C G A G A C G C C C C G -
C A G G A C G C C G A G C G G A C C G A G C G C C -
G T C C G C C C G C A G C G C T C A G C C G G A G C T -
G C G C G G A G C C G C G C C G C G C G A A G G A C -
C A C C T C C C C T T C G C G T G T C C C C C C C C T C -
C G G T G C G G T G G G C G C T G C G G G A C G -
G C G C G G T G C T G A G G A A G A A G G G G C -
C G T T C A G C T C C G A G C T G C T G C T G C T C -
G T C A T C C C C T C G C T G C T G C T C A G C C A C -
G T G C T G A C C C T G G G C C T G G G G A T C T A -
C A T C G G G A A G C G G C T G G C G G C C T C C T C G -
G C A A A C C C G C T G T G A G C G C C G G G C C C G -
G C G G G G A G C G G C C T C C G T T C C G C T C T G C -
G G G G G G G T G C A G C G C G G T G C T C A G C G C -
G G C C G T C T G T C C G C G G A C C C C G C G G C T -
G T G G C A C G C A G C G C C G T C T G C C C C T C C

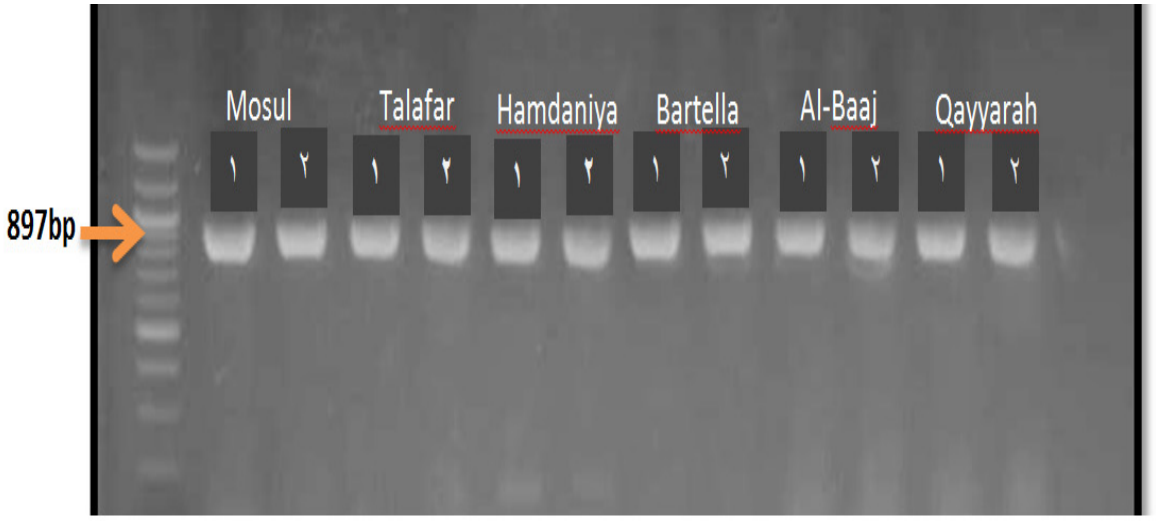

Fig. 1. PCR Result of Liver Tissue Samples Containing HHs Adenovirus 897 bp

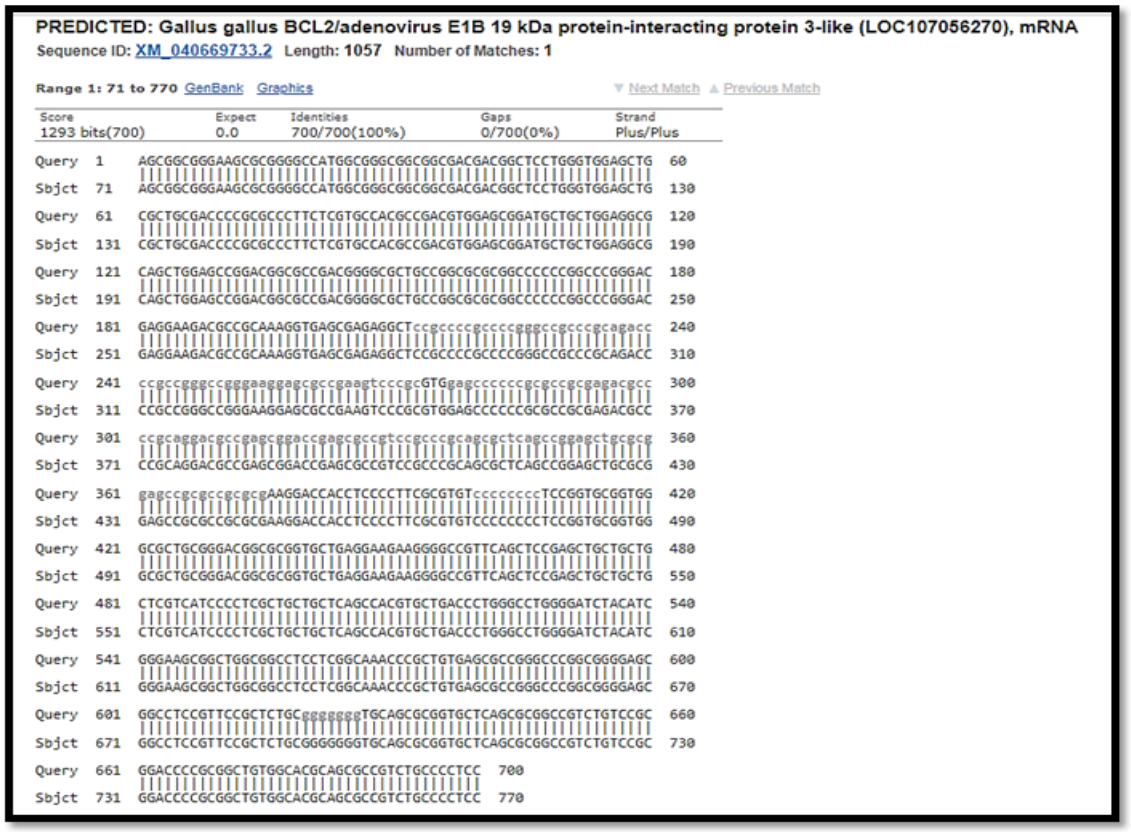

Fig. 2. Molecular diagnosis of HHS adenovirus by gene sequencing in the broiler farms of Nineveh Province, Iraq

Disscusion

Although the disease has been reported in almost all the areas of Iraq, the objective of the current study was to accurate diagnose of HHS fowl adenovirus in the broilers farms of six regions in Nineveh Province, namely: Mosul, Talafar, Hamdaniya, Bartella, Al-Baaj and Qayyarah by PCR technique and DNA or gene sequencing to detect the accurate strain of adenovirus. Adenoviruses are among the viruses that are widely spread in all types of birds, as shown by numerous studies, where the presence of antibodies to adenoviruses was observed in healthy chickens and these viruses were isolated from uninfected natural birds as well [14].

Two significant illnesses known as inclusion body hepatitis IBH and hydropercardium hepatitis are caused by chicken adenoviruses. Although in certain situations, each disease is seen separately, the two cases have been consistently seen together, hence this pathological condition is known as hydropercardium hepatitis syndrome. This sickness affects young chickens and is a severe illness that is accompanied by anaemia, and fluid accumulation surround the heart (Hydropercardium) [15, 16].

There are 12 different avian adenovirus serotypes, but the majority of the viruses found in cases of hydropercardium hepatitis belong to the serotypes 4 and 8 because which they have the ability to cause disease without the immunosuppression caused by some viruses, for example, infectious bursal disease IBD virus or any other factor that suppresses the immunity of chickens, but coincidence with infection with viruses that cause immunosuppression such as IBD and Chicken infectious anemia CIA results in severe concurrent diseases in broiler chickens [17]. Although, some strains of fowl adenovirus may produce a mild infection [17, 18].

The result of PCR as an accurate diagnosis for HHS fowl adenovirus FAdV Hexon gene in the broilers farms of six regions in Nineveh Province, was 897bp which is positive for all liver

tissues of broilers farms and is consistent and compatible with what was previously diagnosed by PCR technique targeting the FAdV Hexon gene. Interestingly, hexone protein and fiber proteins were described to play a major role in the pathogenicity of serotype 4 of chicken adenovirus, specifically because of the amino acid residues. At position 188 of the hexone protein is responsible for the pathogenicity of serotype 4 of chicken adenovirus [19, 20].

In recent years, the results presented by the DNA Sequencing technology be highly accurate in identifying genetic mutations [21], so the result of the molecular diagnosis by genetic sequencing of the DNA amplified pieces of adenovirus from chicken livers, showed that the causative agent of the disease in the six regions was serotype 4 of one strain E1B for all of them according to the XM_040669733.2 gene sequence from NCBI. The serotype 4 considered the dominant serotype associated with the HHS and IBH in Iraq in particular and the Middle East in general, as it was observed that strains of chicken adenovirus FAdV in the Emirates clustered together with the same serotype of the virus and spread in Saudi Arabia (KY606586.1), Pakistan (MH151202.1 and EU931693.1), Nepal (MN604721.1), and China (MK629523.1, KY426988.1 and MH006602.1) (22, 23), this is consistent with what was stated with the study of Abdulrahman et al.[22], where isolates of chicken adenovirus were detected between the years 2013-2021 in Iraqi Kurdistan that there are two different types of the virus, and both genotypes have a different genetic evolution, namely FAdV-D and FadV-E. This is agree with other studies in Korea during the outbreak of chicken adenovirus [24].

It is necessary to continue investigating the spread of diseases caused by the chicken adenovirus FAdV and to understand the genetic epidemiology of the viruses associated with it, as co-infections from multiple serotypes have been observed in other regions of the world, such as China [14].

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Conflict of interest

There is no conflict of interest with personal financial statement.

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التشخيص الجزيئي لفيروس أدينو الدواجن المرتبط بمتلازمة موه التامور والتهاب الكبد في فروج اللحم في محافظة نينوى، العراق

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هدفت هذه الدراسة إلى التشخيص الجزيئي لمتلازمة موه التامور والتهاب الكبدHHS في فروج اللحم في ست مناطق في محافظة نينوى بالعراق وهي: الموصل ، تلعفر ، البعاج ، برطلة ، الحمدانية والقيارة خلال الفترة من تشرين الأول (أكتوبر) ٢٠٢١ إلى آذار (مارس) ٢٠٢٢. تم التحري وملاحظة المرض في الحقول المذكورة . تم جمع عينات اكباد فروج اللحم بما مجموعه ٢٤ عينة بواقع ١٢ حقل فروج لحم للمناطق الستة (حقلين لكل منطقة) لتحديد جين Hexon لـفايروس أدينو الدواجن FAdV بواسطة تقنية تفاعل البلمرة المتسلسل PCR والتسلسل الجيني DNA sequencing من أجل التشخيص الدقيق. كانت نتائج تفاعل البلمرة المتسلسل PCR لعينات الكبد في ١٢ حقل فروج لحم في المناطق الست في محافظة نينوى موجبة لـفايروس أدينو الدواجن 897bp ، وأظهرت نتيجة تسلسل الحمض النووي أن لـفايروس أدينو الدواجن المسبب لمتلازمة موه التامور والتهاب الكبد في المناطق الست كان من النمط المصلي ٤ لعترة واحدة E1B ذو التسلسل XM_040669733.2 للمركز الوطني لمعلومات التكنولوجيا الحيوية NCBI . تعد هذه الدراسة هي الأولى من نوعها للتشخيص الجزيئي وتحديد نوع عترة فايروس أدينو الدواجن المسبب لمتلازمة موه التامور والتهاب الكبد في فروج اللحم في محافظة نينوى في العراق.

الكلمات المفتاحية: متلازمة موه التامور والتهاب الكبد، ـفايروس أدينو الدواجن، التشخيص الجزيئي، فروج اللحم، محافظة نينوى.