Research Article

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Isolation and Molecular Detection of Fowl Adenovirus Associated with Inclusion Body Hepatitis in Broiler Chickens in Iraq

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Abstract

Inclusion body hepatitis is caused by an infection with fowl adenovirus and is documented all over the globe. The current research aimed to first isolate fowl adenovirus in embryonated chicken eggs by allantoic sac inoculation and then use PCR to identify the virus. Seventy-five samples suspected to be infected with fowl adenovirus were collected from various locations around Iraq, including Diyala, Tikrit, and Karbala. The age of the hens in which infection was detected varied from 25 to 45 days. For two passages, prepared samples (0.1ml) from infected livers were injected into embryonated chicken eggs (nine days old). The pathological alterations were not observable at the time of the first injection; nevertheless, the second passage revealed congestion, subcutaneous tissue haemorrhage, and embryo's death. After collecting the allantoic fluid from the two passages in the biohazard safety equipment, viral nucleic acid (DNA) was isolated. It was followed by viral detection using conventional PCR by amplification of the Loop1 gene, which yielded a positive result for the presence of fowl adenovirus. The results show that chicken embryos are sensitive methods for detecting fowl adenovirus and molecular instruments like PCR offer higher accuracy and sensitivity.

Keywards: Embryonated chicken, Fowl adenovirus, hepatitis, L1 gene, polymerase chain reaction

العزل والكشف الجزيئي للفيروس الغدي في الطيور المصاحب لالتهاب الكبد ذو الجسيمات الاشتمالية في الدجاج اللاحم في العراق.

يحدث التهاب الكبد الوبائي الشامل بسبب الإصابة بالفيروس الغدي للطيور ويتم توثيقه في جميع أنحاء العالم.

هدف البحث الحالي أولاً إلى عزل الفيروس الغدي للطيور في أجنة بيض الدجاج عن طريق التلقيح بالكيس السقاء ثم استخدام تفاعل البوليميراز المتسلسل للتعرف على الفيروس. تم جمع خمسة وسبعين عينة يشتبه في إصابتها بفيروس غدي الطيور من مواقع مختلفة في جميع أنحاء العراق، بما في ذلك ديالى وتكريت وكربلاء. وتراوح عمر الدجاج الذي تم اكتشاف الإصابة فيه من 25 إلى 45 يومًا. تم حقن العينات المحضرة (0.1 مل) من الكبد المصابة في بيض دجاج أجنة (عمره تسعة أيام) . تم التكرار مرتين. لم تكن التغيرات المرضية ملحوظة في وقت الحقنة الأولى؛ ومع ذلك، أظهر المقطع الثاني احتقانًا ونزيفًا تحت الجلد وموت الجنين. بعد جمع السائل السقاء من الممرين في معدات السلامة البيولوجية، تم عزل الحمض النووي الفيروسي.(DNA) . وأعقب ذلك الكشف عن الفيروس باستخدام تفاعل البوليميراز المتسلسل التقليدي عن طريق تضخيم جين Loop1، مما أدى إلى نتيجة إيجابية لوجود الفيروس الغدي للطيور. أظهرت النتائج أن أجنة الدجاج هي طرق حساسة للكشف عن الفيروس الغدي للطيور وأن الأدوات الجزيئية مثل تفاعل البوليميراز المتسلسل توفر دقة وحساسية أعلى.

Introduction

The poultry industry is one of the most important industries in the world's nations. Because of this expansion, there are more possibilities for diseases to spread, and there is no vaccination control in certain situations. Aviadenoviruses, which are members of the family Adenoviridae, are linear double-stranded DNA ranging in size from 25 to 46 kilobase pairs, are the causative agents of several of these disorders (1,2). Viruses comprise genetic material and structural proteins that encapsulate them (3).

Adenoviruses that have been identified from poultry are almost exclusively fowl adenoviruses (FAdVs), which are classified into five species (A–E) and they are further classified into 12 serotypes (FAdV-1 to 8a and 8b to 11) by cross-neutralization tests (4). The function that adenoviruses play in pathogenesis is not well understood. They can be separated from infected birds and birds that do not exhibit disease symptoms (5,6). There is evidence that FAdV had a role in acting as an opportunistic factor (7). It is also an etiological agent of illnesses such as inclusion body hepatitis (IBH) and the ability to induce asymptomatic infections (8,9). Inclusion body hepatitis, hepatitis–hydropericardium syndrome (HHS), and FAdV gizzard erosion (GE) are all examples of typical FAdV disorders that may affect chickens (10,11). These diseases are often brought on by certain viral strains (12). IBH cases have resulted mainly in isolating strains of FAdV – D and FAdV – E in several countries (13); few researches dealt with studying the outbreak of fowl adenovirus in broiler chickens in Iraq, such as in Nineveh and Kurdistan (14,15). HHS epidemics are caused by pathogenic strains of FAdV – 4, which belong to the species FAdV – C, while GE is connected with FAdV – 1 (16,17).

The diagnosis of IBH may be made by observing macroscopic and histological alterations, viral isolation, and polymerase chain reaction (PCR), which consider the fastest and most accurate approach (18). Other methods of diagnosing IBH include a combination of these methods. FAdV typing may be accomplished by using DNA sequencing and restriction enzyme analysis (19).

Only a few studies have been conducted in Iraq about fowl adenoviruses (FAVs), and the virus itself needs to be better defined. As a result, the current research was designed to identify FAVs and determine their link to pathogenic isolates found in Iraqi farms using both traditional and molecular approaches.

MATERIALS AND METHODS Collection of Samples

In the year 2022 (from January to June), 75 liver samples were collected from broiler chickens in Iraq suspected to be infected with avian adenovirus (FAdV). These samples were taken from various locations in the country, including the provinces of Karbala, Diyala (Baqubah), and Salahuddin. Commercial embryonated chicken eggs (ECE): Chicken embryonated eggs were used for cultivation of fowl adenovirus (FAdV) and were obtained from Private Hatcheries in (Salah Alden) and (Baghdad) at (9-11) days.

Kit of DNA extraction:

The DNA extraction kit used Favorgene batch no. Fav20061 to extract DNA from liver samples.

Primers:

The primer used during this work is responsible for amplifying the L1 gene of the FAdV genome part, as shown in Table (1).

Table (1). Primer used for the amplification of

the L1 gene.

Gene		Sequence PCR primers 5'-3'	Ref
L1	F	aatgtcacnaccgaraaggc	[20]
	R	cbgcbtrcatgtactggta	

Conventional PCR: The program of the L1 gene by PCR program is in Table (2).

Steps	**Temp. (℃)**	**Time**	**No. of cycles**
Initial denaturation	95℃	5min	1
Denaturation	94℃	45s	35
Annealing	55℃	1min	35
Extension	72℃	2min	35
Final extension	72℃	10min	1

Gel electrophoresis: Agarose 2% was used at 10 volts/cm for 1hour to detect the PCR product.

Preparation of samples:

One (gm) of the (10 selected liver samples) was collected and placed in a mortar. It was then chopped into small pieces, and nine (ml) of PBS was added before the tissue was crushed into a fine paste. After that, the suspension was placed into a sterile tube and then centrifuged at 3500 (RPM) for 10 minutes using a cold centrifuge; then, the supernatant fluid was transferred into a sterile tube, which was repeated three times to precipitate coarse particles. Additionally, ten mg of streptomycin and 10000 IU of crystalline penicillin per ml were added to the solution, and it was kept at room temperature for half an hour. The suspension fluid was kept at -20 ℃ until it was used.

Isolation of FAdV in the embryonated chicken eggs According to (21), the supernatant from each sample was used to inoculate a 9-day-old chicken embryo via the allantoic cavity. About 40 chicken embryos was used, the amount of inoculum administered to each embryo was 0.1 ml. 5 embryos were used for each inoculum. Moreover, using the artificial air-sac approach, the PBS solution was used to inoculate the control chicken embryos (20 eggs). The inoculated eggs were examined daily by candling inspections. After that, the dead embryo before 24 hr. post inoculation were discarded. Dead embryos after 24 hr. post inoculation were kept in the refrigerator at 4℃ for 1 hr. After that, the infected allantoic fluid was taken, and lesions were noticed on the infected embryos in contrast to the control chicken embryo. The injection of chicken embryos with harvested allantoic fluid was repeated for two passages.

Viral DNA extraction and PCR The harvested fluid from the second passage of inoculated embryos was processed for extraction of viral DNA using Favorgene DNA isolation kit batch no. Fav20061. According to the manufacturer's instructions. A 50 µl DNA sample was obtained from each sample with a purity of 1.8 as measured spectrophotometrically and freezed under – 200C for PCR amplification.

Polymerase chain reaction (PCR). The reaction mix was prepared using reagents from a Taq DNA Polymerase kit made by Promega in the United States of America. The PCR reaction was carried out in a final volume of 20µl with the following components: 10µl of a 2x Master mix, 5 µl of DNA template, 3µl of nuclease-free water, 1µl of F primer (10 pmol/µl), and 1µl of R primer (10 pmol/µl). PCR amplification was carried out using the cycle profile shown below: Predenaturation at 95 °C for five minutes was followed by extract denaturement at 94 °C for 45 seconds, primer annealing at 55 °C for one minute, product elongation at 72 °C for two minutes, and final elongation at 72 °C for ten minutes. A simple gradient thermocycler was used to complete 35 amplification cycles.

Gel electrophoresis

PCR amplicons were separated by electrophoresis in agarose with a concentration of 2% using a field strength of 10 volts/cm for one hour. The bands were visualized using a UV transilluminator MSE-280 after being stained with EtBr for thirty minutes.

Results and disscution

Results of inoculation of chicken embryos with prepared samples. The genus Aviadenovirus is home to the fowl adenoviruses, sometimes known as FAdVs. Most of the time, FAdVs are to blame for spontaneous epidemics of respiratory system disorders, including inclusion body hepatitis (IBH), hepatitishydropericardium syndrome (HHS), and others. Additionally, some strains of FAdV-1 have been linked to gizzard erosions in hens (22). After three days post-infection, the allantoic fluid was harvested for analysis. The results of the first passage did not reveal any pathological changes in the embryo, nor did the control embryonated eggs exhibit any changes. However, the results of the second passage indicated death, hemorrhagic and edematous body of the infected embryo (figure 1B). While the control embryo showed no changes, as seen in Figure (1-A). These findings are consistent with those that were reported by (23). They are also comparable to those discovered by (24), who found that the embryos were congested and hemorrhagic and that the infected embryos were smaller than those of the control group, even though there were no evident exterior lesions.

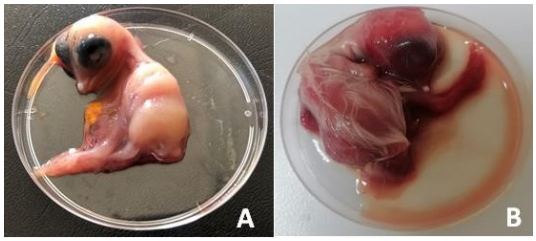

Figure 1: Infected chicken embryo with FAdV showed edematous embryo with severe congestion and haemorrhage (2nd passage) (B) normal chicken embryo (A)

Virus detection by PCR

Amplified DNA products approximately 400bp for the L1 gene were detected in all of the DNA samples from the supernatant of second passages from liver homogenates of infected embryos by the PCR findings when they were seen in agarose gel electrophoresis (Fig. 2). In the present investigation, molecular detection assays sought the chicken adenovirus (FAdV). These results are consistent with those found by (25) and (26), who discovered that most samples tested positive for FAdV by conventional PCR, with the L1 region of the hexon gene as the target.

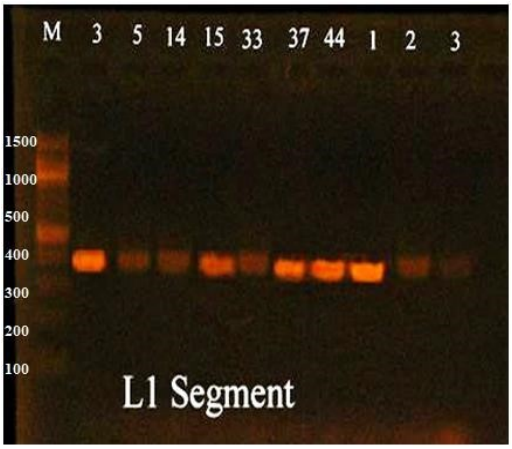

Figure 2: PCR-specific amplification of L1 gene. Electrophoresis was performed in 2% agarose gel at ten v/cm field strength for 1h

Conclusion

Avian species are susceptible to infection with inclusion body hepatitis (IBH). Subclinical

infections are the most common kind of infection. Chicken embryos were a suitable and sensitive method for isolating fowl adenovirus. The accuracy and sensitivity of molecular instruments like PCR are much higher than other methods.

Conflict of interest

The authors declared no conflict of interest .

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