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وحماية المستهلك

DETECTION OF ADENOVIRUS ASSOCIATED WITH INCLUSION BODY HEPATITIS IN CHICKENS

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ABSTRACT

Inclusion body hepatitis is one of the disease that cause economic loses in poultry industry. Infection with the fowl adenovirus leads to the illness known as inclusion body hepatitis. Affected chickens showed dullness, depression, ruffled feathers and mild greenish diarrhea with low morbidity and high mortality. Seventy-five samples were collected from various areas around Iraq, including Diyala, Karbala and Tikrit, that were thought to be infected with the fowl adenovirus.

The infected chickens ranged in age from 25 to 45 days. After that viral nucleic acid (DNA) was isolated from collected livers. This was followed by testing using conventional PCR by amplification of the Loop 1 gene, which yielded a positive result for the presence of fowl adenovirus. Avian species are at risk of inclusion body hepatitis (IBH) infection, with subclinical infection being the most common type. The study reveals that the liver is affected by a disease that can be diagnosed through necropsy findings such as the presence of multiple genotypes such as FAdV-4, FAdV-8b and E. Molecular tools like PCR offer superior accuracy and sensitivity compared to seroprevalence techniques, which are not specific to the species or type of adenoviruses or their co-infecting illnesses. The current research aimed to clinical diagnosis of adenovirus in chickens and confirmed diagnosis by conventional PCR.

Keywords: Avian Viruses, Inclusion Body Hepatitis, Polymerase Chain Reaction, L1 gene.

التشخيص السريري والمختبري للفيروسات الغدية المرتبطة بالتهاب الكبد ذو الجسيمات الاشتمالية في الدجاج

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الخلاصة

يعتبر مرض التهاب الكبد ذو الجسيمات الاشتمالية احد الامراض التي تسبب خسائر اقتصادية كبيرة في صناعة الدواجن. تؤدي الإصابة بالفيروس الغدي للطيور إلى المرض المعروف باسم التهاب الكبد ذو الجسيمات الاشتمالية.. أظهرت الدجاجات المصابة بلادة واكتئاب وريش منتفخ وإسهال خفيف مخضر مع انخفاض معدلات الإصابة بالمرض وارتفاع معدل الوفيات. تم جمع خمسة وسبعين عينة من مناطق مختلفة في جميع أنحاء العراق، بما في ذلك ديالى وكربلاء وتكريت، والتي يعتقد أنها مصابة بالفيروس الغدي للطيور. وتراوحت أعمار الدجاج المصاب بين 25 إلى 45 يوما. بعد ذلك تم عزل الحمض النووي الفيروسي من عينات الاكباد التي تم جمعها. واعقب ذلك اختبار باستعمال تفاعل البلمرة المتسلسل التقليدي عن طريق تضخيم جين الحلقة 1, مما ادى الى نتيجة ايجابية لوجود الفايروس الغدي للطيور. أنواع الطيور معرضة لخطر الإصابة بمرض بالتهاب الكبد ذو الجسيمات الاشتمالية, مع كون العدوى تحت السريرية هي النوع الأكثر شيوعًا. وتكشف الدراسة أن الكبد يتأثر بمرض يمكن تشخيصه من خلال نتائج التشريح مع وجود أنماط وراثية متعددة مثل FAdV-4 وFAdV-8b وE. توفر الاختبارات الجزيئية مثل تفاعل البلمرة المتسلسل دقة وحساسية فائقة مقارنة بتقنيات الانتشار المصلي، والتي لا تقتصر على نوع أو نوع الفيروسات الغدية أو الأمراض المصاحبة لها. يهدف هذا البحث إلى التشخيص السريري للفيروس الغدي في الدجاج والتشخيص المؤكد بواسطة تفاعل البوليميراز المتسلسل التقليدي

الكلمات المفتاحية: فيروسات الطيور، التهاب الكبد ذو الجسيمات الاشتمالية، تفاعل البلمرة المتسلسل، جين L1.

INTRODUCTION

One of the most important sectors in the world is the poultry industry. Due to this development, there are more opportunities for illnesses to spread, and in certain cases, there is no vaccine control. Several of these illnesses are caused by Newcastle disease virus, Infectious bronchitis, Avian influenza virus in addition to aviadenoviruses **(Mahmood & Allawe., 2021; Ali & Allawe.,2023)**. , which are members of the Adenoviridae family and have linear double-stranded DNA with a size range of 25 to 46 kilobase pairs **(Hess, 2013; Fenner *et al*., 2014)**. The main components of viruses are the structural proteins that enclose their genetic material **(Mijwil & Al-Zubaidi 2021)**. The majority of adenoviruses found in poultry are fowl adenoviruses (FAdVs), which are divided into five species (A-E) **(Niczyporuk, 2016)**. They can be isolated from diseased birds and birds without illness signs **(Abdulla *et al*., 2023)**. In addition to having the capacity to cause asymptomatic infections, it also has the capacity to act as an etiological agent for diseases like Inclusion Body Hepatitis (IBH) **(Dar *et al*., 2012; Oraibi & Abdalmaged, 2022)**. Common FAdV diseases that may affect hens include inclusion body hepatitis, Hepatitis-Hydropericardium Syndrome (HHS), and FAdV Gizzard Erosion (GE) **(Matos *et al*., 2016)**. IBH instances have mainly resulted in the isolation of strains of the species FAdV-D and FAdV-E in a number of different nations **(Li *et al*., 2016)**. Only a few studies have been conducted to examine the fowl adenovirus epidemic in grill chickens in Iraq, including in Nineveh and Kurdistan **(Jarjees *et al*., 2022; Abdulrahman *et al*., 2022)**. IBH can be diagnosed by the observation of macroscopic and histological changes, viral isolation, and polymerase chain reaction (PCR), which is thought to be the quickest and most reliable method **(Abdulsahib *et al*., 2015)**. Combinations of these procedures are another way to diagnose IBH. Using DNA sequencing and restriction enzyme analysis, FAdV typing may be done **(Mittal *et al*., 2014)**. There have only been a few studies conducted in Iraq about fowl adenoviruses (FAVs), the virus itself has not been well defined. As a result, the current research aimed to identify a clinical diagnosis of adenovirus in chickens and confirmed diagnosis by conventional PCR.

MATERIALS AND METHODS

Clinical examination:

Clinical signs was noticed for suspected infected chickens and recorded with case history.

Post-mortem examination:

Postmortem examination was conducted to detect main lesions on the suspected infected livers.

Collection of samples

From January to June of 2022, a total of seventy-five liver samples were taken from broiler chickens in Iraq that were thought to have avian adenovirus infection. The age of chickens from 25 to 45 days. These samples were taken from various locations in the country including the provinces of Karbala, Diyala (Baqubah), and Salahuddin.

Viral nucleic acid extraction:

Viral DNA was extracted from the obtained samples (livers) using the Favorgene (Tissue Genomic DNA Extraction Mini Kit) DNA isolation kit batch No. Fav20061. As directed by the manufacturer. Each sample yielded a 50µl DNA with a spectrophotometric purity of 1.8 that was frozen at -20°C for Polymerase Chain Reaction amplification.

Primers

The primer used during this study. which is responsible for the amplification of the L1 gene of the FAdV genome part, The sequences of nucleotide primers were as follows. Forward: 5AATGTCACNACCGARAAGGC3, reverse: 5CBGCBTRCATGTACTGGTA 3 **(Niczyporuk, 2018)**.

Conventional PCR to detect adenovirus:

Reagents from a Taq DNA Polymerase kit manufactured by Promega in the United States of America were used to produce the reaction mixture. Conventional PCR was used to detect the virus by amplification of the L1gene.

The following components were used in a final volume of 20 l for the PCR reaction: 10µl of a 2x Master mix, 5 µl of DNA template, 3µl of nuclease-free water, 1µl of F primer (10 pmol/µl), 1µl of R primer (10 pmol/µl). The following cycle profile was used for PCR amplification: Pre-denaturation at 95°C for five minutes was followed by extract denouement at 94°C for 45 sec., 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 2% agarose using an 8 volt/cm field strength for one hour. After being stained with ethidium bromide for 30 minutes, the bands were seen using a UV transilluminator MSE-280.

Sequencing of fowl adenovirus PCR products

Forty-eight amplicons (PCR product) were sent to Macrogen com. Korea for DNA sequencing by the sanger method, then the Basic Local Alignment Search Tool BLAST used for reading the results, available on the Uniport database.

RESULTS AND DISSCUSION

Results of clinical examination

All seventy-five samples were collected from different farms in different provinces in Iraq (Karbala, Diyala and Salahaddin). These samples were collected based on case history and clinical signs. The clinical signs included dullness, depression, ruffled feathers and mild greenish diarrhoea with low morbidity and high mortality. These findings are consistent with those that were reported by **(Dinesh *et al*., 2011)**. They are also comparable to those discovered by **(Laanani *et al*., 2015)**. they found that the affected chickens showed ruffled feathers, depression, watery droppings and some of them limping.

Results of post mortem examination:

The main post-mortem lesions used as indicative for inclusion body hepatitis is enlargement of the liver, pale to pale yellow discoloration with scattered petechiae as shown in **(Figure1)**. These findings are consistent with those that were reported by **(Dinesh *et al*., 2011)**. They are also comparable to those discovered by **(Laanani *et al*., 2015)**. They found that the most common pathological lesions seen enlargement, swollen and pale liver with hemorrhage.

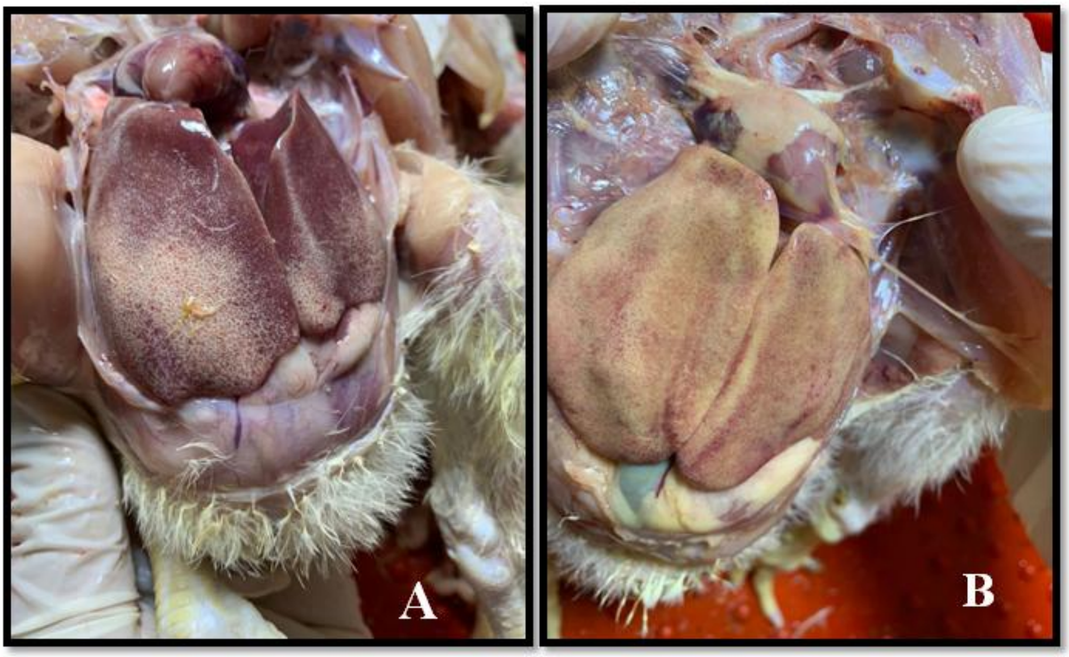

Figure (1): (A) enlargement and congestion of liver with necrotic foci in broiler infected with IBH. (B) enlargement and yellowish pale liver with hemorrhagic and necrotic foci in broiler infected**.**

Virus detection by PCR

Conventional PCR was used to detect FAdV in collected samples by amplification of the Loop1 (L1) gene. which is considered an indicator gene responsible for encoding viral capsid protein. Forty-eight samples were positive out of 75 samples. L1 gene with 600pb when they were seen in agarose gel electrophoresis **(Figure, 1).**

In the current study, molecular detection tests were used to look for the chicken adenovi rus (FAdV). The L1 region of the hexon gene, which is the most hypervariable area on the hexon gene and may be utilized to differentiate between the species of FAdVs, was the focus of the primers developed. These results are consistent with those found by **(Adel *et al*., 2021; Niczyporuk *et al*., 2021)**. who discovered that the majority of the samples tested positive for FAdV by conventional PCR, with the L1 region of the hexon gene as the target. PCR was useful method to detect viruses **(Atta & Allawe., 2018).**

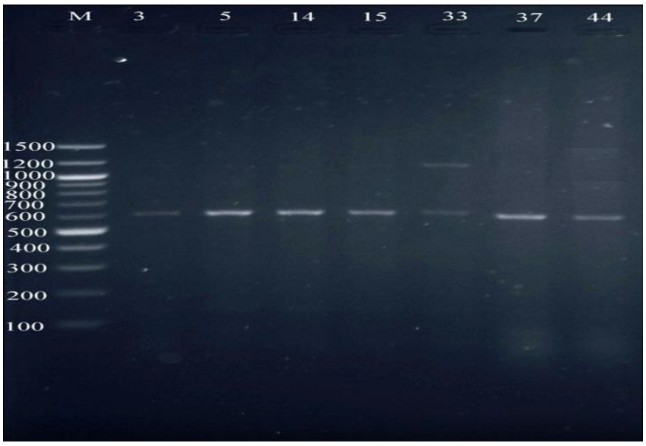

Figure (2): Electrophoresis was performed in 2% agarose gel by amplification of L1 gene.

Results of sequencing

PCR amplicons obtained from the L1 segment were sent to be sequenced. Seven of all 48 isolates were selected based on similarity to other strains through analysis. The result showed the presence of multiple genotypes such as FAdV-4, FAdV-8b and E, this result agreement with **(Fenner *et al*., 2014)**. Results obtained from BLAST against globally identified strains are shown in figures (3, 4, 5, 6, 7, 8 and 9).

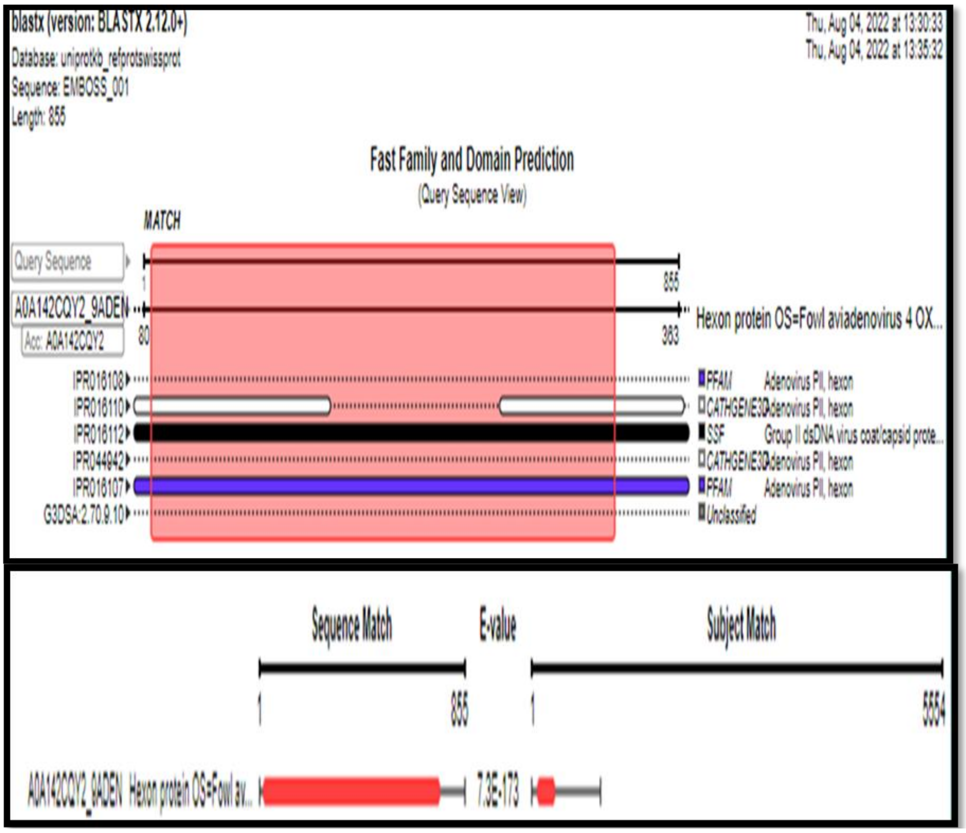

Figure (3): Graphical representation of blast results of L1 DNA. Isolate No.3.

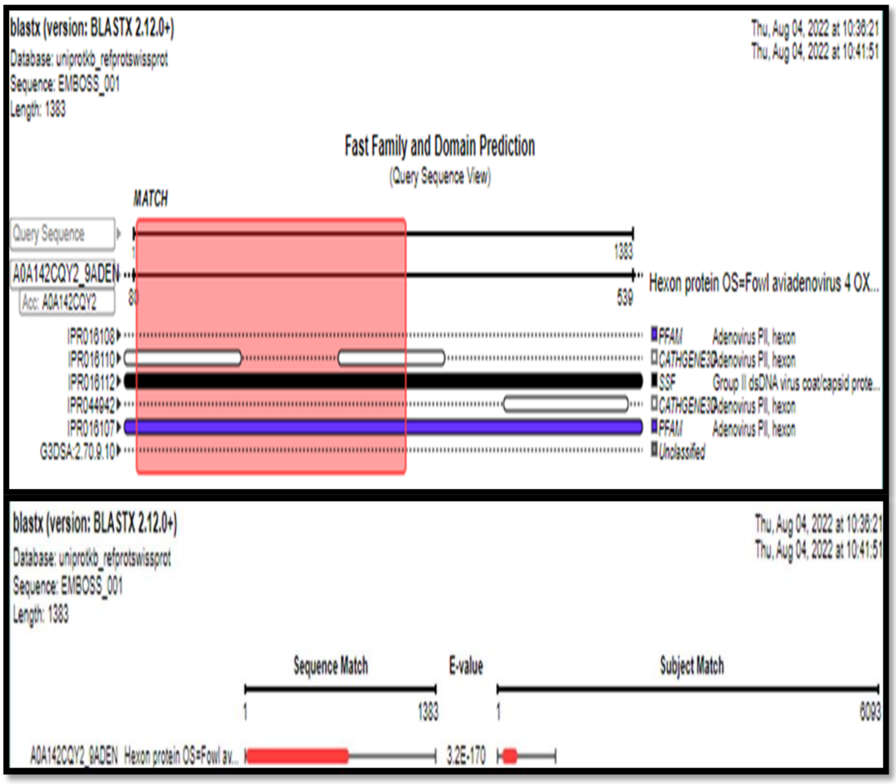

Figure (4): Graphical representation of blast results of L1 DNA. Isolate No.5.

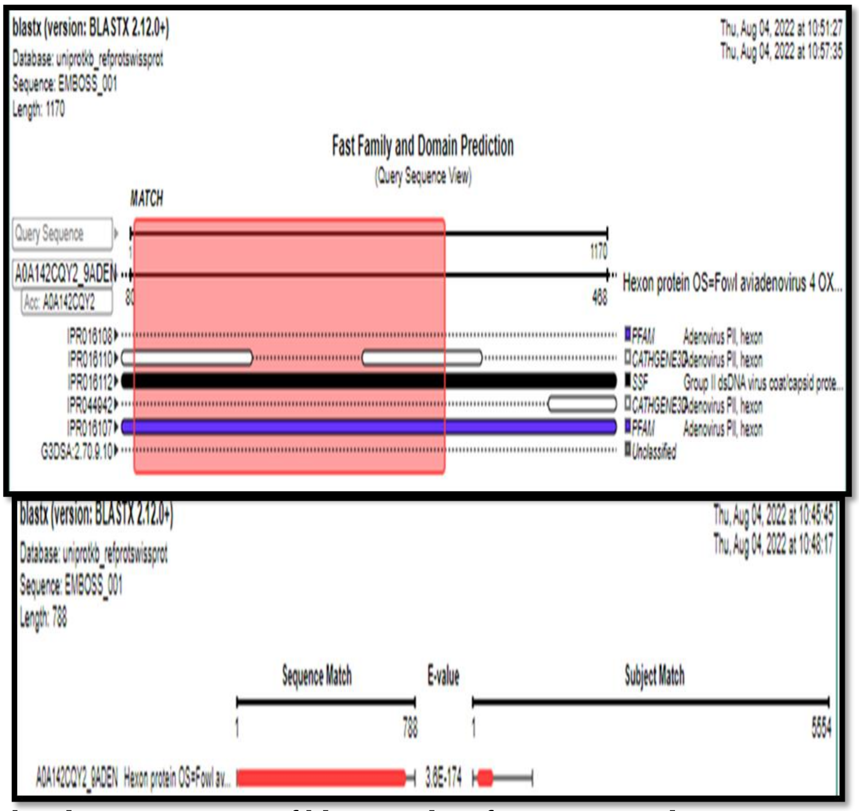

Figure (5): Graphical representation of blast results of L1 DNA. Isolate No.14.

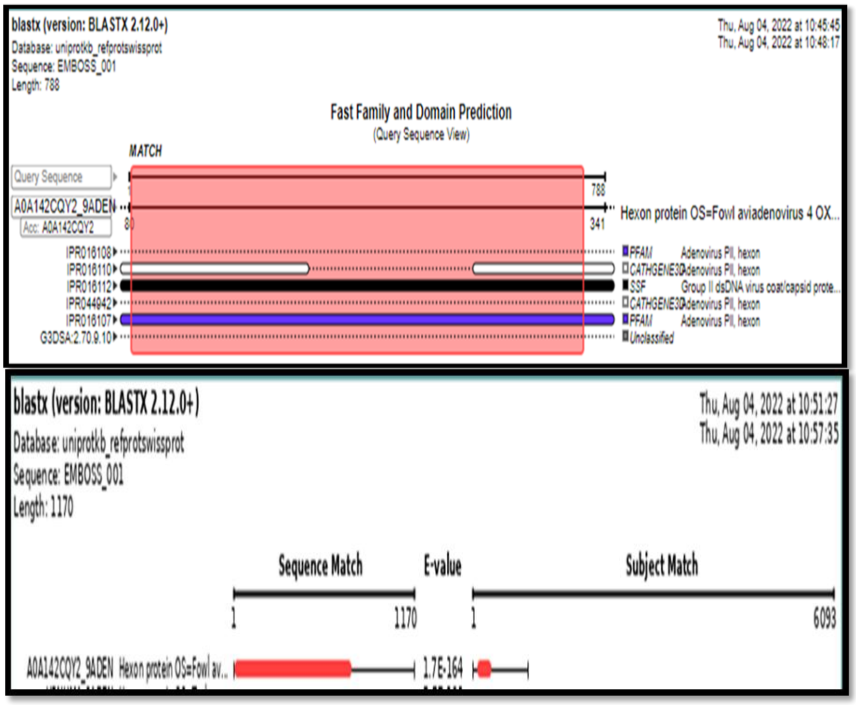

Figure (6): Graphical representation of blast results of L1 DNA. Isolate No.15.

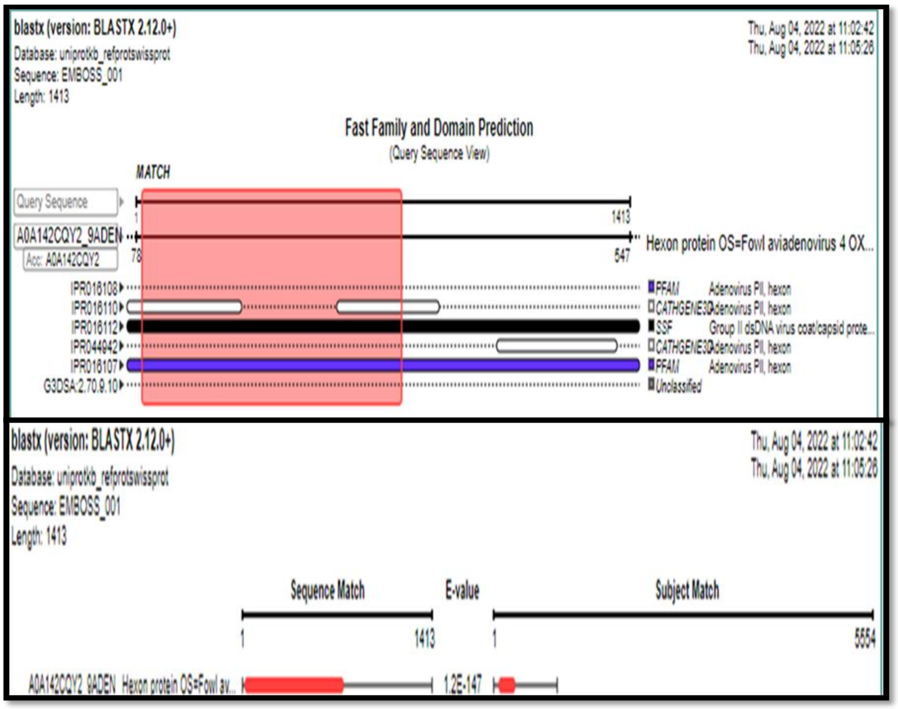

Figure (7): Graphical representation of blast results of L1 DNA. Isolate No.33.

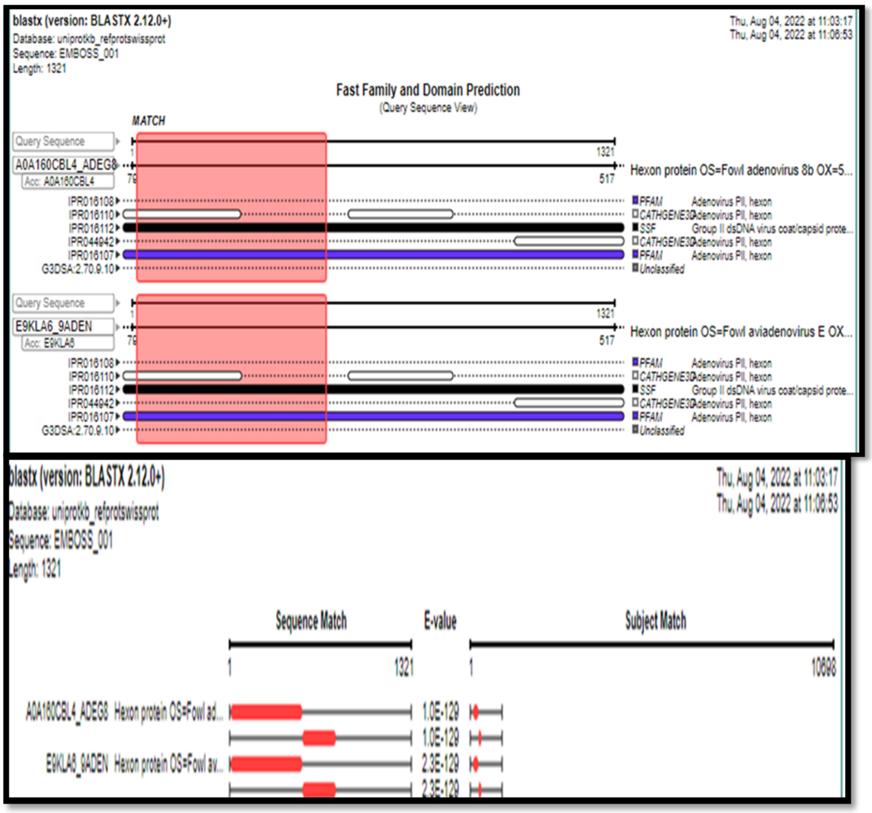

Figure (8): Graphical representation of blast results of L1 DNA. Isolate No.37.

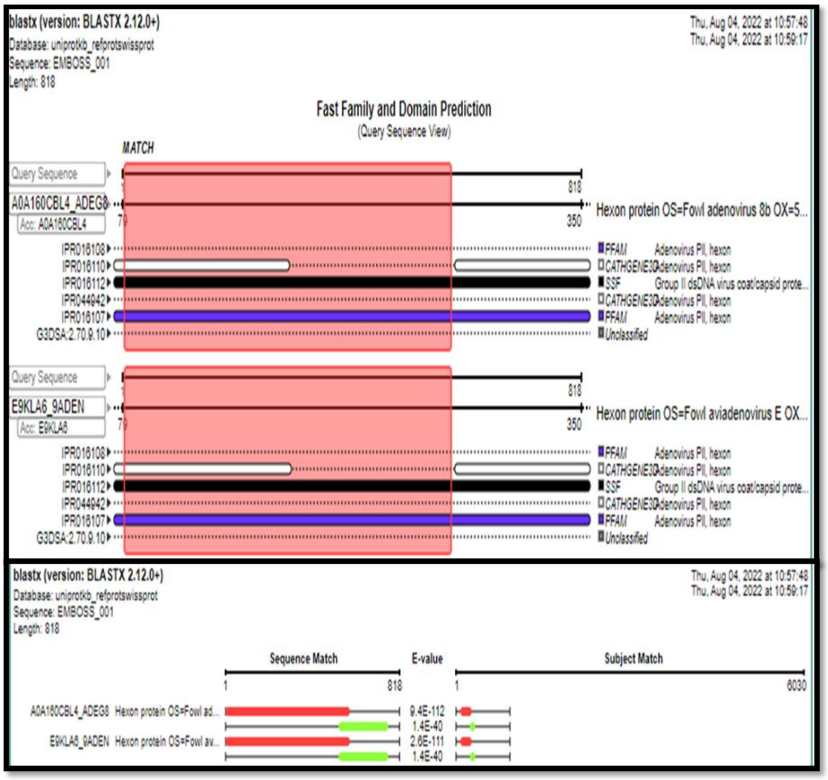

Figure (9): Graphical representation of blast results of L1 DNA. Isolate No. 44.

Data interpretation of Figure (3, 4, 5, 6, 7, 8 and 9) are illustrated in table (1).

Table (1): BLAST data illustration of FAdV local isolates.

Accession No. match	Isolate No.	Gene	Protein	Organism	Query coverage	e-value
A0A142CQY2	3	L – gene	Hexon	Fowl aviadenovirus 4	17 – 753	7.3E-173
A0A142CQY2	5	L – gene	Hexon	Fowl aviadenovirus 4	10 – 765	3.2E-170
A0A142CQY2	14	L – gene	Hexon	Fowl aviadenovirus 4	2 – 751	3.6E-174
A0A142CQY2	15	L – gene	Hexon	Fowl aviadenovirus 4	9 – 770	1.7E-164
A0A142CQY2	33	L – gene	Hexon	Fowl aviadenovirus 4	17 – 760	1.2E-147
A0A160CBL4 E9KLA6	37	L – gene	Hexon	Fowl adenovirus 8b Fowl aviadenovirus E	13 – 534	1.0E-129 1.0E-129
A0A160CBL4 E9KLA6	44	L – gene	Hexon	Fowl adenovirus 8b Fowl aviadenovirus E	3 – 587	9.4E-112 1.4E-40

CONCLUSION

There is a risk of inclusion body hepatitis (IBH) infection in avian species. The most typical type of infection is subclinical infection. This study revealed that the disease caused pathological changes in the liver and the disease can be diagnosed by necropsy findings and the presence of multiple genotypes such as FAdV-4, FAdV-8b and E. Molecular tools like the PCR have substantially greater accuracy and sensitivity than seroprevalence techniques. Techniques based on seroprevalence are also not specific to the species or type of adenoviruses or the illnesses they co-infect.

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