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Molecular detection and descriptive epidemiology of fowl adenovirus infection in broiler chickens

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Fowl adenoviruses represent substantial viral pathogens distributed across poultry sectors globally, being etiologically linked with severe disease complexes that induce devastating economic losses in broiler production. This study was conducted to establish definitive molecular evidence of fowl adenovirus infection in commercial broiler chickens within the Diyala Governorate using an optimized real-time polymerase chain reaction framework and to mathematically model its regional distribution boundaries. For this purpose, a total number of 96 clinical liver tissue specimens were aseptically collected from 32-day-old broiler chickens displaying clinical indications of hepatic dysfunction across various private operations. Following tissue processing and lysis, viral genomic DNA was isolated using standard silica-membrane extraction matrices. Molecular screening was executed via a qualitative real-time PCR (qRT-PCR) platform utilizing specific oligonucleotide primers (L1-F: 5′-GACGGCATTTGGGGAGGAATT-3′ and L1-R: 5′-GCTGAGGATTGAGCTTCACCC-3′) targeting the hypervariable loop L1 region of the viral hexon gene, followed by a continuous post-amplification dissociation gradient analysis from 58°C to 95°C. Out of the total number of examined specimens, 20 samples returned positive diagnostic outcomes, establishing a regional viral infection prevalence rate of 20.8%. Descriptive statistical variance modeling successfully projected the true expected prevalence limits within a stable 95% confidence interval ranging from 12.7% to 28.9%, maintaining an estimated standard error of 4.14. Furthermore, a non-parametric Chi-square goodness-of-fit test demonstrated an intense, highly significant deviation from theoretical uniform frequencies ($\chi^2 = 32.66$, df = 1, $P < 0.001$), determining a powerful localized infectious pressure across the regional poultry ecosystem despite the statistical predominance of negative samples (79.2%). The recorded kinetic amplification plots manifested rapid exponential fluorescence signal emergence between cycles 15 and 22, while melt curve evaluation demonstrated sharp, singular dissociation peaks centered at approximately 85°C, validating absolute amplicon specificity and confirming the total absence of primer-dimer artifacts or non-specific carryover products. These empirical data determine that fowl adenovirus circulation is firmly established at a substantial frequency within the regional poultry networks, confirming that real-time PCR remains an exceptionally rapid, high-fidelity, and dependable diagnostic screening tool for early epidemiological mapping.

Keywords: fowl adenovirus, real-time PCR, molecular prevalence, descriptive epidemiology, broiler chickens.

Молекулярна детекція та описова епідеміологія інфекції пташиного аденовірусу у курей-бройлерів

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Пташині аденовіруси належать до епідеміологічно важливих вірусних патогенів, широко поширених у промисловому птахівництві в усьому світі, та є етіологічними факторами низки тяжких захворювань, що завдають суттєвих економічних збитків господарствам бройлерного типу. Це дослідження було проведено з метою встановлення чітких молекулярних доказів наявності збудника пташиного аденовірусу у курчат-бройлерів у комерційних господарствах провінції Діяла за допомогою оптимізованої полімеразної ланцюгової реакції в реальному часі, а також математичного моделювання меж його регіонального поширення. Для цього септичн відібрано 96 клінічних зразків тканин печінки від 32-добових курчат-бройлерів із клінічними ознаками печінкової дисфункції на різних приватних підприємствах. Після обробки та лізису тканин вірусну геномну ДНК ізолювали за допомогою стандартних екстракційних матриць на основі силікагелевих мембран. Молекулярний скринінг виконували за допомогою платформи якісної ПЛР у реальному часі з використанням специфічних олігонуклеотидних праймерів (L1-F: 5′-GACGGCATTTGGGGAGGAATT-3′ та L1-R: 5′-GCTGAGGATTGAGCTTCACCC-3′), спрямованих на гіперваріабельну ділянку петлі L1 гена гексону вірусу, з подальшим безперервним аналізом градієнта дисоціації після ампліфікації від 58°C до 95°C. Дослідженнями встановлено, що із загальної кількості досліджуваних зразків 20 вивилися позитивно реагуючими, що дало змогу встановити регіональний рівень поширеності вірусної інфекції на рівні 20,8 %. Описове статистичне моделювання дисперсії успішно спрогнозувало межі істинного очікуваного поширення в межах стабільного 95 % довірчого інтервалу від 12,7 до 28,9 % при розрахунковій стандартній похибці 4,14. Крім того, непараметричний критерій згоди Хі-квадрат продемонстрував інтенсивне, високозначуще відхилення від теоретичних рівномірних частот ($\chi^2 = 32{,}66$, df = 1, $P < 0{,}001$), визначаючи високу активність циркуляції патогену в регіональній системі птахівництва, попри статистичне переважання негативних зразків (79,2 %). Зафіксовані кінетичні графіки ампліфікації продемонстрували швидку експоненціальну появу сигналу флуоресценції між 15 і 22 циклами, тоді як оцінка кривої плавлення виявила чіткі, поодинокі піки дисоціації, центровані приблизно на 85°C, що підтверджує абсолютну специфічність амплікону та повну відсутність артефактів праймер-димерів або неспецифічних продуктів переносу. Отримані дані визначають, що циркуляція пташиного аденовірусу чітко встановлена зі значною частотою в регіональних птахівничих мережах, підтверджуючи, що ПЛР у реальному часі залишається винятково швидким, високоточним і надійним інструментом діагностичного скринінгу для раннього епідеміологічного картування.

Ключові слова: пташиний аденовірус, ПЛР у реальному часі, молекулярна поширеність, описова е підеміологія, курчата-бройлери.

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Introduction

Fowl adenoviruses (FAdVs) are significant non-enveloped, double-stranded DNA viral pathogens that belong to the family *Adenoviridae* and are known to infect a wide range of avian species globally. In commercial poultry production, these viruses are etiologically linked to several economically devastating diseases, most notably inclusion body hepatitis (IBH), hydropericardium syndrome (HPS), and severe avian gizzard erosion [1]. Outbreaks of fowl adenovirus infection can lead to profound economic distress in commercial broiler chicken operations due to sudden spikes in flock mortality, poorly developed growth performance parameters, reduced body weight gain, compromised feed conversion efficiency, and generalized immunosuppression that dramatically elevates the susceptibility of the birds to secondary bacterial or viral pathogens [2].

Based on their distinct molecular, structural, and antigenic properties, fowl adenoviruses are officially classified into five distinct species, designated from *Fowl aviadenovirus A* to *Fowl aviadenovirus E*, all belonging to the genus *Aviadenovirus*. The pathogenicity, clinical manifestations, and specific tissue tropisms vary extensively among the different serotypes within these species. For instance, strains belonging to FAdV-4 are recognized as the primary etiological agents responsible for hydropericardium syndrome, which causes high mortality and severe fluid accumulation in the pericardial sac, whereas specific serotypes such as FAdV-8a, FAdV-8b, and FAdV-11 have been heavily implicated as the main causative agents of inclusion body hepatitis in poultry flocks worldwide [3].

Adenovirus entry into susceptible host cells is initiated through a coordinated two-step process, beginning with targeted attachment to surface receptors, which is rapidly followed by structural internalization via receptor-mediated endocytosis. Once inside the intracellular compartment, the virion is systematically transported toward the host cell nucleus, where genomic DNA replication and active transcription take place [4]. Following structural genome translocation, the replication cascade is chronologically programmed by the synchronized synthesis of early regulatory and late structural proteins, which are essential for viral DNA duplication and correct capsomeric assembly. In avian hosts, the advanced progression of this intracellular replication cycle ultimately triggers profound cellular injury and localized tissue necrosis, manifesting predominantly as severe gross and microscopic lesions within the target liver and spleen tissues of infected broiler chickens [5].

It is well established that the liver serves as the primary target organ for fowl adenovirus replication in chickens, reflecting the highly hepatotropic nature of these pathogens. Infected poultry flocks display a variety of characteristic micro- and histopathological lesions during post-mortem examination, including diffuse hepatocellular necrosis, severe degeneration of hepatocytes, hepatic inflammation, and the formation of prominent basophilic intranuclear inclusion bodies within the affected liver cells [6]. Due to rapid systemic viral dissemination via the bloodstream and the intense immune suppression caused by structural damage to lymphoid tissues, fowl adenovirus infections also frequently spread to cause severe degenerative alterations and apoptotic changes within the splenic tissues [7].

Fowl adenovirus infections have been increasingly reported on commercial poultry operations globally, threatening sustainable animal production. The high prevalence and rapid spread of these disease outbreaks are significantly exacerbated by inadequate biosecurity measures on farms, poor sanitary regimes, intense environmental stressors, and synergistic co-infections with other avian immunosuppressive pathogens, such as the infectious bursal disease virus. Consequently, ensuring early, highly sensitive molecular diagnosis remains a paramount prerequisite for the success of preventative management strategies and control programs in commercial poultry production [8].

Given the lack of comprehensive molecular data from Iraq, there is a critical need to understand the circulation of fowl adenovirus within intensive poultry operations in the Diyala Governorate. Investigating these distribution patterns via targeted molecular tools is essential for establishing reliable regional diagnostic data and managing infection risks in commercial broiler facilities [1].

The aim of the study

The aim of this study was to molecularly detect fowl adenovirus infection in broiler chickens using a targeted Real-Time PCR platform and to analyze the apparent prevalence and cycle threshold (Ct) distribution patterns across hepatic tissue samples collected from 32-day-old broiler chickens.

Materials and methods

Sample collection and clinical screening

A total of 96 liver tissue samples were collected from young commercial broiler chickens at 32 days of age, obtained from various private poultry farms located across the Diyala province. Slices of hepatic tissue were systematically extracted from birds that were clinically suspected of harboring avian adenovirus disease based on flock-level parameters, including acute depression, uneven growth, and varying degrees of hepatomegaly or pale liver discoloration observed during preliminary post-mortem screenings [9]. To establish a rigorous differential profile and confirm the viral etiology behind these suspicious hepatic manifestations, all clinical specimens were gathered under strict aseptic conditions using pre-sterilized surgical tools to prevent external cross-contamination. Immediately following dissection, each tissue specimen was placed into an individual sterile container, properly labeled, and instantly transferred into a specialized thermal icebox maintaining a temperature of approximately 4°C for rapid and secure transportation to the molecular biology laboratory for further diagnostic verification.

Tissue processing and homogenization

In the laboratory setting, the processing of the collected liver specimens was conducted within a certified laminar flow cabinet to maintain a completely sterile environment. Approximately 25 to 50 mg of each individual liver tissue sample was carefully dissected and sliced into small fragments using sterile instruments. The finely minced tissue fragments were subsequently transferred into sterile containers containing cold, sterile phosphate-buffered saline (PBS) or a specialized extraction lysis buffer, and thoroughly homogenized to establish a uniform, consistent tissue suspension optimized for downstream nucleic acid isolation. To separate the target viral particles from large host cellular debris, the resulting tissue homogenates were subjected to centrifugation for 5 minutes. The clear supernatant was carefully collected and immediately subjected to viral genomic DNA extraction [10].

Viral DNA extraction

The isolation of viral genomic DNA from the previously clarified liver tissue supernatants was executed using the commercial EasyPure® Viral DNA/RNA Kit, strictly adhering to the specialized extraction protocols and guidelines provided by the manufacturer. Briefly, the extraction pipeline involved efficient chemical lysis of viral envelopes and capsids, followed by the selective adsorption of nucleic acids onto silica-membrane mini-columns. After serial washing steps to remove potential enzymatic inhibitors, cellular proteins, and residual salts, the highly purified viral DNA templates were eluted in nuclease-free elution buffer. The concentration and structural purity of the isolated DNA were immediately quantified using spectrophotometric analysis, and the templates were subsequently stored in labeled microcentrifuge tubes at a constant temperature of −20°C to preserve genomic stability until downstream molecular amplification.

Oligonucleotide primers and target region

For the molecular screening and specific detection of fowl adenovirus DNA, a set of published oligonucleotide primers targeting the hypervariable loop L1 region of the viral hexon gene was utilized. The forward and reverse primer sequences synthesized for this study were designated as follows:

L1-F (Forward): 5′–GACGGCATTTGGGGAGGAATT–3′
L1-R (Reverse): 5′–GCTGAGGATTGAGCTTCACCC–3′

These specific primer sequences were selected to ensure the universal amplification of a conserved diagnostic fragment within the hexon gene locus across circulating fowl aviadenovirus species, yielding an expected distinct amplicon size suitable for qualitative evaluation [2].

Qualitative real-time PCR (qRT-PCR) amplification protocol

The molecular detection and qualitative profiling of fowl adenovirus genomic DNA were carried out on a high-performance Real-Time thermal cycler system utilizing fluorescent SYBR Green chemistry. The amplification assays were executed in optical-grade PCR reaction wells containing genomic region-specific oligonucleotide primers. The amplification reaction was performed in a standardized final volume, consisting of the extracted viral DNA template, the optimized forward and reverse primers, a commercial SYBR Green master mix containing Taq DNA polymerase, dNTPs, and structural reaction buffers, and filled to the final volume with nuclease-free water.

The thermal cycling profile programmed for the optimal amplification of the target hexon gene fragment consisted of an initial denaturation step at 95°C for 5 minutes to fully activate the hot-start polymerase enzyme and ensure the complete denaturation of the double-stranded DNA templates. Following this initial step, the reaction mixture was subjected to 40 continuous amplification cycles under the following regulated parameters: denaturation at 95°C for 30 seconds to separate the newly synthesized strands, primer annealing at 60°C for 30 seconds to allow specific primer attachment to the target L1 locus, and extension at 72°C for 30 seconds for optimal nucleotide polymerization. A final terminal extension step was executed at 72°C for 1 minute to complete the synthesis of any partial amplicons. Fluorescence intensity signals resulting from the accumulation of the targeted PCR products were recorded automatically by the optical sensors of the thermal cycler system at the end of each extension phase. Positive samples were definitively identified based on the emergence of characteristic sigmoidal amplification curves crossing the mathematically calculated baseline threshold line within defined threshold cycle (Ct) values.

To rigorously evaluate the physical specificity of the resulting amplicons and verify the absence of any technical artifacts, a post-amplification melt curve analysis (dissociation curve analysis) was systematically performed immediately following the completion of the 40 cycling phases. The dissociation protocol was executed across a continuous temperature gradient rising from 58°C to 95°C, with simultaneous monitoring of fluorescence decrease. The resulting dissociation profiles were evaluated to confirm that clear, singular melting temperature (Tm) peaks appeared exclusively at the expected thermal point of the target gene fragment, demonstrating that no non-specific genomic amplifications were produced and confirming the total absence of primer-dimer artifacts or external contamination within the reaction matrices.

Statistical analysis and descriptive epidemiological modeling

The empirical raw datasets generated from the qualitative real-time PCR diagnostic screenings were subjected to rigorous non-parametric and descriptive statistical processing using IBM SPSS Statistics software. The baseline regional molecular distribution of fowl adenovirus was mathematically expressed as an apparent prevalence percentage, calculated by dividing the total frequency of confirmed positive instances by the overall number of examined specimens (n = 96). To project the mathematical stability of the sampling distribution and evaluate variance boundaries, the estimated standard error was defined, and the limits of the true expected regional prevalence were modeled within a strict 95 % confidence interval (CI bounds).

To evaluate whether the observed frequencies of positive and negative molecular outcomes deviated significantly from a theoretical uniform distribution model predicated upon equal probability (50/50 null hypothesis), a non-parametric Chi-square (χ^2) goodness-of-fit test was applied to the operational matrices. The mathematical weight contributions of individual cells were accumulated to compute the final test metric, with the statistical degrees of freedom (df = 1) and the corresponding asymptotic significance values (P) determined accordingly. Statistical significance thresholds throughout all modeled epidemiological parameters were strictly fixed at a confidence level of $P<0.05$

Results and discussion

Fowl adenovirus detection and baseline distribution

The molecular screening of clinical specimens using a targeted qualitative Real-Time PCR (qRT-PCR) platform provided baseline epidemiological data regarding the circulation of the viral pathogen across intensive poultry farming networks within the region. Out of the total cohort of liver tissues gathered from suspected broiler flocks displaying clinical indications of hepatic dysfunction, a notable portion of the population tested positive for the presence of the fowl adenovirus genomic DNA. The definitive breakdown of the diagnostic screening outcomes, including the total sample frequencies and the corresponding detection rates, is systematically structured in ***Table 1***.

Table 1

Epidemiological distribution and detection rates of fowl adenovirus in clinical liver specimens, n=96

Diagnostic screening parameter	Frequencies of tested samples	Detection rate, %
Positive samples (FAdV-DNA detected)	20	20.8
Negative samples (FAdV-DNA not detected)	76	79.2
Total evaluated cohort	96	100.0

The diagnostic outcomes demonstrate a significant regional circulation of fowl adenovirus within the intensive broiler production sectors of the governorate. Although the clear majority of the screened suspected birds were ultimately confirmed to be uninfected, the discovery that more than one-fifth of the evaluated flock cohort actively harbored the viral hexon gene highlights a persistent biosecurity threat. This baseline detection level confirms that aviadenovirus infection is not an isolated or sporadic event, but rather a established epidemiological entity within local commercial operations. Furthermore, the substantial proportion of negative outcomes among clinically suspected birds indicates a complex etiology for general hepatitis signs on local farms, reinforcing the conclusion that subjective post-mortem gross observations are insufficient for definitive diagnosis and highlighting the absolute necessity of utilizing highly sensitive molecular assays to prevent misidentification.

To evaluate the replication intensity and determine the geometric scale of viral colonization within the target hepatic cells, a comprehensive analysis of the generated cycle threshold (Ct) values was executed across the positive cohort. The monitored amplification cycles provided an indirect quantitative assessment of the initial viral nucleic acid concentration, established upon the physical principle that the onset of exponential fluorescence signals shares a strict inverse proportional correlation with the baseline target DNA template copy number. To characterize the severity of the infection profiles, the confirmed positive instances were systematically stratified into distinct classification ranges reflecting high, moderate, or low viral load parameters. The definitive breakdown of this kinetic distribution and its corresponding biological interpretations are detailed in ***Table 2***.

Table 2

Quantitative stratification of fowl adenovirus viral loads based on kinetic cycle threshold (Ct) profiles, n=20

Cycle threshold (Ct) range	Biomolecular interpretation	Frequency of positive samples / %
15.0 – 20.0	High viral load (elevated nucleic acid concentration)	18 / 90
20.1 – 25.0	Moderate viral load (intermediate nucleic acid concentration)	2 / 10
25.1 – 30.0	Low viral load (minimal nucleic acid concentration)	0

The kinetic stratification reveals an overwhelming predominance of advanced, high-density replication profiles among the infected commercial poultry individuals. The finding that the vast majority of the positive clinical specimens triggered exponential signal emergence well before the twentieth thermal cycle indicates an intense state of active viral duplication and massive genomic accumulation within the hepatic parenchyma. Conversely, intermediate replication profiles were rarely encountered, and cases of low-level or trace viral presence were entirely absent from the analyzed dataset. This uniform trend toward elevated target DNA concentrations suggests that the circulating fowl adenovirus strain exhibits high replication efficiency, or that the evaluated broiler chickens were sampled during the acute, peak phase of the clinical disease process, during which hepatocellular destruction and viral shedding reach their maximum biological velocity.

Mathematical Modeling and Descriptive Epidemiology

To map the definitive epidemiological boundaries of the infection and determine the mathematical reliability of the survey within the regional poultry networks, a rigorous descriptive statistical evaluation was performed on the raw screening frequencies. This mathematical characterization focused on establishing the variance boundaries and calculating the standard error to project the potential viral circulation dynamics beyond the immediate sample pool. By defining the limits of variance and modeling the ratio parameters, the dataset provides a standardized framework for regional disease forecasting. The consolidated descriptive statistical parameters generated from the total evaluated broiler cohort are structured in ***Table 3***.

Table 3
Descriptive epidemiological parameters and variance modeling of fowl adenovirus detection

Descriptive statistical parameter	Modeled mathematical value
Total evaluated sample size (n)	96
Confirmed positive frequency	20
Confirmed negative frequency	76
Calculated apparent prevalence	20.8 %
Estimated standard error	4.14
95% confidence interval (CI bounds)	12.7–28.9 %
Modeled positive-to-negative ratio	1 : 3.8

The descriptive configuration patterns illustrate that the infection is firmly anchored at a substantial mathematical frequency within the local poultry ecosystem. The calculated standard error reflects a highly stable sampling distribution, which directly validates the accuracy of the overall screening methodology. Most importantly, the calculated lower and upper boundaries of the 95% confidence interval mathematically demonstrate that if similar commercial broiler flocks under identical production pressures were surveyed within the region, the true viral prevalence would consistently fall within this defined range of 12.7 % to 28.9 %. This mathematical modeling firmly refutes any assumption that fowl adenovirus is a transient or minor threat, confirming instead that the pathogen exerts a constant, uniform infectious pressure across more than one-fourth of the local commercial poultry population at any given time.

Non-parametric modeling and goodness-of-fit evaluation

To evaluate whether the observed distribution of positive and negative diagnostic frequencies deviates significantly from a theoretical uniform distribution, a non-parametric Chi-square goodness-of-fit test was applied to the dataset. This mathematical analysis modeled the actual laboratory frequencies against an expected null-hypothesis framework predicated upon an equal probability distribution profile. The comprehensive non-parametric parameter configuration, reflecting the individual cell weight contributions and the final accumulated test metric, is systematically structured in ***Table 4***.

The non-parametric statistical evaluation demonstrates an intense, mathematically validated deviation from the theoretical uniform distribution model. The high accumulated empirical test value ($\chi^2 = 32.66$, df = 1) confirms that the predominant frequency of uninfected individuals over active viral cases is statistically highly significant ($P<0.001$), thereby firmly rejecting the random variation hypothesis.

Table 4
Chi-square analysis and empirical weight modeling of qualitative real-time PCR outcomes

qRT-PCR result	Observed number	Expected number	Chi-square contribution
Positive	20	48	16.33
Negative	76	48	16.33
Total	96	96	32.66

From a veterinary epidemiology perspective, the extreme weight contribution generated by both operational groups emphasizes that while negative outcomes statistically dominate the flock population, the persistent identification of active viral replication remains highly alarming. This clear statistical imbalance indicates that while the absolute majority of birds successfully avoided clinical colonization, the virus exerts a substantial, uniform infectious pressure within the commercial poultry ecosystem, necessitating strict molecular monitoring protocols regardless of high baseline negative sample frequencies.

Real-time amplification kinetics and curve analysis

The qualitative validation of the generated fluorogenic datasets was established by analyzing the continuous geometric monitoring profiles recorded during the thermal processing phases. The real-time tracking of the accumulation of the targeted hexon gene fragments provided definitive biomolecular evidence regarding the polymerization velocity and execution efficiency of the assay across the clinical samples. The characteristic kinetic configurations, representing the exponential progression of active viral DNA amplification alongside the completely non-reactive baseline references, are systematically displayed in ***Fig. 1***.

The kinetic configuration patterns illustrate a highly reproducible and specific synchronization during the enzymatic polymerization phase. The emergence of classical, well-defined sigmoidal profiles crossing the mathematically calculated baseline threshold line within a uniform window between cycles 15 and 22 confirms successful targeted duplication of the viral DNA in 20 confirmed positive specimens. The steep, vertical shift observed in these fluorescence intensity curves reflects optimal primer annealing and high enzymatic efficiency of the polymerase chain reaction. Conversely, uninfected negative controls and the remaining non-reactive clinical specimens from the total cohort of 96 samples remained completely flat, preserving a steady horizontal line well below the critical threshold limit throughout the entire 40-cycle sequence. This absolute lack of signal elevation mathematically rules out the presence of non-specific carryover artifacts or primer-dimer interference during data logging, validating the reliability of the baseline setup and confirming an overall viral detection rate of 20.8 % across the investigated broiler chicken population.

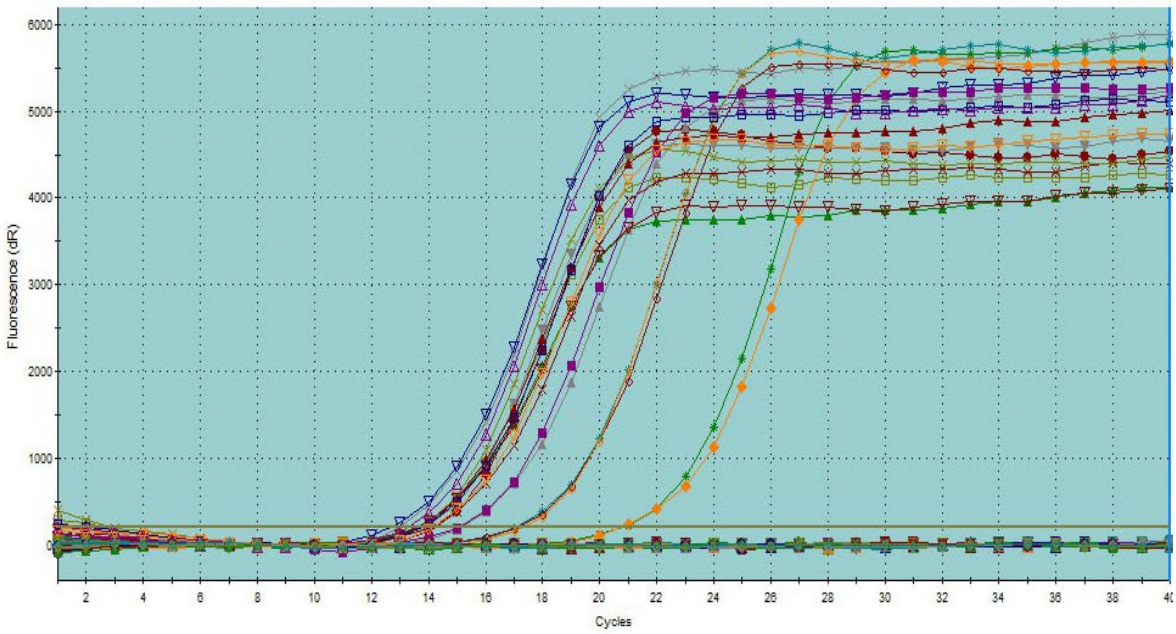

Fig. 1. Real-time polymerase chain reaction amplification plots of fowl adenovirus positive samples

Dissociation profiling and amplicon specificity analysis

To rigorously verify the thermodynamic purity and physical homogeneity of the generated nucleic acid products, a post-amplification melt curve analysis was performed across all reactive wells immediately following thermal cycling [11]. Monitoring the precise temperature at which the double-stranded DNA amplicons denature into single strands provides a reliable non-gel method to evaluate target validation and confirm enzymatic accuracy. The resulting dissociation profiles, representing the singular thermal melting curves of the targeted L1 hexon gene fragment alongside the absence of any non-specific reaction artifacts, are systematically illustrated in ***Fig. 2***.

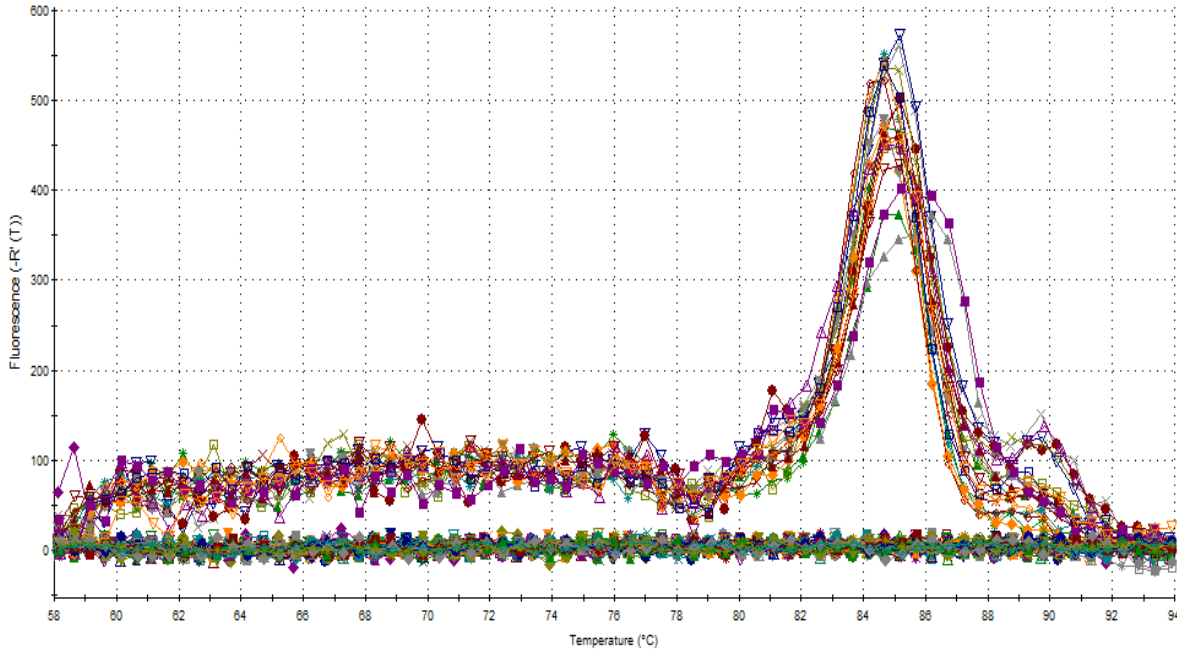

Fig. 2. Post-amplification dissociation profiles and melt curve analysis of fowl adenovirus positive specimens

The thermodynamic dissociation patterns demonstrate a highly specific and uniform amplification profile across the evaluated clinical matrices. The emergence of a singular, sharp melting peak occurring exclusively at a highly specific melting temperature region between 84°C and 86°C, centered at approximately 85°C, reflects a completely homogeneous PCR product population. This uniform thermal stability indicates that the oligonucleotide primers successfully locked onto the specific target sequence without creating mismatched hybrid structures. Furthermore, the complete flatness of the baseline across lower temperature ranges mathematically demonstrates the absolute absence of non-specific amplification products, primer-dimer artifacts, or external genomic contamination during the thermal profiling phase, establishing the high diagnostic fidelity of the molecular screening protocol.

The successful molecular detection of fowl adenovirus genomic DNA in clinical liver specimens via a targeted qualitative real-time PCR platform underscores the persistent circulation and epidemiological importance of this pathogen within local commercial poultry production networks. The documented apparent prevalence rate of 20.8% established in the current investigation provides clear, empirical evidence that the virus actively circulates within the evaluated broiler chicken population in the region. These data strongly indicate that fowl adenovirus continues to represent a highly significant, threatening viral pathogen capable of compromising sustainable animal production and inducing severe economic stress within modern commercial poultry management systems.

The epidemiological patterns and pathological trends observed in our dataset share close biological alignment with the findings reported by Schachner et al. [2]. Our results further support the comprehensive investigations of Tamam et al. [12], who demonstrated that infectious fowl adenovirus strains are heavily distributed across commercial broiler operations and manifest a tight etiological association with destructive macro- and microscopic liver lesions linked directly to severe inclusion body hepatitis (IBH) outbreaks, localized lymphoid depletion, severe immunosuppression, and elevated mortality rates within intensive production systems. Furthermore, the frequent and highly stable isolation of viral DNA from hepatic samples confirms that the liver parenchyma must be regarded as the primary biological target organ for fowl adenovirus colonization and replication, directly matching the classic hepatotropic nature of the genus Aviadenovirus established during comprehensive transmission profiles by Hess [7].

The specific infection rate documented in the current study (20.8 %) is also in highly consistent alignment with the baseline molecular observations published by Marek et al. [3]. In their multi-center molecular diagnostic surveys, prevalence rates ranging between 18 % and 27 % were frequently encountered within infected broiler chicken flocks. They demonstrated that subclinical or acute viral circulation is highly influenced by deficient biosecurity standards and poor microclimate management practices on localized private farms, which significantly accelerate the regional transmission dynamics and active circulation of the virus among susceptible bird cohorts.

From a diagnostic methodology perspective, the execution kinetics recorded in our real-time PCR assay are technically supported by the universal validation trials conducted by Günes et al. [13]. They verified that real-time PCR platforms targeting specific hexon loci exhibit exceptional sensitivity, analytical accuracy, and high specificity for the rapid screening of poultry tissues. The dynamic behavior of the amplification curves logged in our investigation, which consistently manifested distinct exponential fluorescence signals between thermal cycles 15 and 22, closely mirrors the rapid amplification kinetics, low threshold cycle characteristics, and enzymatic efficiency documented in their standardized optimization models.

Furthermore, the significant infection parameters established in the current screening are heavily corroborated by the epidemiological reports of Toro et al. [14]. Their research established that clinical and subclinical aviadenovirus infections on commercial meat-type chicken operations are frequently driven by inadequate husbandry systems, intensive immune-suppressive stressors, high stocking densities, and mixed co-infections with other avian pathogens. These overlapping operational factors and management deficiencies help explain the high frequency of positive molecular outcomes detected across the evaluated private facilities in this study. To address these management-driven challenges, Mittal et al. [15] verified that advanced molecular diagnostic profiling and phylogenetic characterization targeting specific fowl adenovirus strains provide the necessary analytical framework for tracking field outbreaks of inclusion body hepatitis (IBH) and hydropericardium syndrome under compromised rearing conditions. Their molecular evaluations demonstrated that isolating and identifying specific viral genomic fragments directly from infected target tissues remains an essential tool to monitor regional pathogen distribution and evaluate structural variations within intensive poultry operations.

The diagnostic performance and physical specificity of our qualitative profiling are in complete accordance with the methodology validated by Kardoudi et al. [16], who confirmed that real-time PCR coupled with high-resolution melting curve analysis represents an optimal, high-fidelity molecular framework to rapidly detect and differentiate fowl adenovirus DNA directly from infected avian liver and hepatic tissues.

In contrast to the parameters documented in the current survey, our baseline prevalence data differ from the outcomes published by Elbestawy et al. [17], who recorded a substantially higher infection rate exceeding 40% in commercial broiler networks in Egypt. Such elevated regional detection rates are typically associated with catastrophic clinical outbreaks, the presence of localized vaccination failures, or the rapid geographic spread of highly virulent endemic viral strains. Conversely, the apparent prevalence established in our study was notably lower than the epidemic parameters documented by Zia et al. [18] in Pakistan, who reported extensive pathogen distribution and heightened detection rates during intense field outbreaks of hydropericardium hepatitis syndrome in industrial broilers. These continuous variations in epidemiological parameters are common in veterinary survey literature and are primarily driven by localized differences in sample size configurations, distinct environmental conditions, seasonal shifts, and variations in localized biosecurity management systems. Ultimately, the implementation of continuous, high-throughput genomic typing frameworks combined with robust monitoring of viral shedding dynamics during pathogen interactions remains the most critical requirement to control serotype diversification and mitigate the long-term economic impact of evolving fowl aviadenovirus strains within commercial poultry networks [19, 20].

Conclusions

Fowl adenovirus circulation was firmly established through definitive molecular confirmation within commercial poultry networks in the region. Out of the total number of examined specimens consisting of 96 clinical liver samples collected from young broiler chickens, a targeted real-time PCR platform identified a regional infection positivity rate of 20.8 % (with 20 samples returning positive diagnostic outcomes), while descriptive statistical modeling projected the true expected prevalence limits within a 95 % confidence interval of 12.7 % to 28.9 %. Non-parametric test analysis demonstrated a highly significant deviation from uniform frequencies ($\chi^2 = 32.66$, df = 1, $P<0.001$), indicating a strong infectious pressure despite the statistical predominance of negative samples.

DECLARATIONS

Ethical Statement

The author confirms that the studies were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), national bioethical norms, and international standards for the humane treatment of animals. The research protocol and sampling design were formally reviewed and approved by the Ethics Committee of the College of Veterinary Medicine at the University of Diyala, Iraq (Approval No. Vet Medicine (251) from January, 2026, Z). The clinical liver tissue specimens investigated in this research were recovered exclusively from deceased or suspected broiler chicken cases during routine diagnostic, post-mortem, and sanitary screening for fowl adenovirus detection.

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Conflict of Interest

The author states that there is no conflict of interest.

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Declaration of AI and AI-assisted technologies

The author declares that no generative AI or AI-assisted technologies were used during the preparation, writing, translation, or language polishing of this manuscript. The final content was fully authored and reviewed by the researcher, who takes full responsibility for the integrity of the work.

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