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Histopathological lesions and partial F gene sequencing of Newcastle disease virus in pigeons

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Abstract
This investigation aimed to study histopathological changes, molecular, and phylogenetic approaches to unveil both the virulence and genetic identity of circulating viral strains of Newcastle Disease Virus (NDV) in pigeons in Babil province. Histopathological evaluations revealed hallmark lesions of velogenic NDV strains, including submeningeal edema and cerebral vascular congestion, hepatic amyloidosis with thrombus formation, hyperplasia of respiratory epithelium, depletion of splenic lymphoid follicles with amyloid-like material, and severe enteric damage marked by villous atrophy and glandular loss. These lesions underscore the virus's systemic pathogenic potential and tissue tropism. At the molecular level, RT-PCR amplification of a 767 bp segment of the F gene confirmed NDV infection in all sampled pigeons. Subsequent sequencing and genetic analysis revealed remarkable homology (99.5-99.8%) between the Iraqi field isolates (NDV.IQ.2-6) and reference strains from Iraq and Turkey. This study strongly advocates for the integration of histopathology with molecular diagnostics as a robust platform for NDV characterization and outbreak investigation. It offers pivotal insights into viral evolution, epidemiology, and pathogenicity, and lays the groundwork for developing targeted control strategies. Future directions should explore the pathobiology of chronic NDV infection, particularly amyloidogenesis, and assess the ecological role of wild birds in sustaining and disseminating NDV within and beyond national borders.

Keywords: Newcastle disease virus (NDV), pigeons, histopathology, fusion (F) gene, RT-PCR, phylogenetic analysis.

Introduction
Newcastle disease virus (NDV), also known as avian paramyxovirus type 1 (APMV-1), is a highly contagious pathogen affecting a wide range of avian species, including pigeons (*Columba livia*), and poses significant economic and ecological threats to poultry industries worldwide (Dimitrov *et al*., 2019) [8]. NDV belongs to the genus *Orthoavulavirus* within the family *Paramyxoviridae* and is classified into three pathotypes based on virulence: lentogenic (low virulence), mesogenic (moderate virulence), and velogenic (high virulence) (Nooruzzaman *et al*., 2021) [28]. The velogenic strains, particularly those causing neurotropic and viscerotropic forms, result in high mortality rates in susceptible birds, including pigeons, which are considered natural reservoirs and potential disseminators of the virus (Aldous *et al*., 2004; Brown & Bevins, 2017a) [1, 5]. The fusion (F) protein, a major determinant of NDV pathogenicity, plays a crucial role in viral entry by mediating membrane fusion, and its cleavage site sequence is a key genetic marker for virulence prediction (Dortmans, 2011; Huang *et al*., 2021) [10, 11, 20]. Pigeons infected with NDV often exhibit severe histopathological lesions, including multifocal necrosis in the spleen, pancreas, and liver, along with perivascular cuffing and gliosis in the brain, encephalitis, hemorrhagic tracheitis, and lymphoid depletion in the bursa of Fabricius, indicative of systemic infection and neurotropism (Wang *et al*., 2018; Thomazelli *et al*., 2021; Hamouda *et al*., 2024) [38, 37, 15]. Furthermore, immunohistochemical studies have demonstrated widespread viral antigen distribution in neuronal tissues, gastrointestinal mucosa, and lymphoid organs, confirming the pantropic nature of virulent NDV strainsb (He *et al*., 2018; Rehman *et al*., 2020) [18, 31]. The emergence of pigeon-specific NDV variants (pigeon paramyxovirus type 1, PPMV-1) has raised concerns due to their ability to cross species barriers and infect domestic poultry, leading to outbreaks with substantial economic losses (Hayes, 2023) [17]. Molecular characterization of the partial F gene has been instrumental in tracking viral evolution,

identifying genotype shifts, and understanding the epidemiology of NDV in pigeons (Wang *et al*., 2024) [39]. Recent studies have highlighted the predominance of genotype VI NDV strains in pigeons, characterized by unique mutations in the F protein cleavage site (e.g., 112RRQKRF117), which enhance viral fitness and host adaptation (Liu *et al*., 2015; Sabra *et al*., 2017) [25, 32]. The role of pigeons in NDV epidemiology is further complicated by their migratory behavior, facilitating long-distance viral transmission and complicating disease control measures (Bansal *et al*., 2022; Snoeck *et al*., 2013) [3, 35]. Despite extensive vaccination programs in poultry, PPMV-1 outbreaks continue to occur in pigeon populations, underscoring the need for continuous surveillance and genetic monitoring to detect emerging variants (Hamouda *et al*., 2024) [15]. The partial F gene sequencing approach has proven valuable in differentiating field isolates from vaccine strains, assessing viral evolution, and informing vaccine development strategies (Samy *et al*., 2018) [33]. Additionally, phylogenetic analyses based on F gene sequences have revealed the global distribution of PPMV-1 strains and their evolutionary divergence from classical NDV genotypes (Esmaeelzadeh Dizaji *et al*., 2020) [13]. Understanding the histopathological manifestations and molecular characteristics of NDV in pigeons is critical for disease prevention and control (Hossain *et al*., 2023) [19]. This study aims to investigate the histopathological lesions associated with NDV infection in pigeons and perform partial F gene sequencing to elucidate viral genetic diversity, virulence markers, and epidemiological trends, thereby contributing to improved NDV surveillance and management strategies in avian populations.

Materials and Methods: The current study was conducted in the department of Pathology and poultry diseases, Collage of Veterinary Medicine, Al-Qasim Green University.

A total of 100 local-breed pigeons, varying in sex and age, were collected from various sources, including live bird markets and private veterinary clinics in Babil province, central Iraq, during the period from November 2024 to January 2025. The birds were selected based on the presence of clinical signs suggestive of Newcastle disease infection, such as neurological manifestations, respiratory distress, and greenish diarrhea. These pigeons were subsequently subjected to the necessary diagnostic examinations. The samples were collected aseptically from affected tissues including trachea, lungs, spleen, brain, and liver of the pigeons. These specimens were placed in plastic containers containing 10% neutral-buffered formalin for histopathological analysis according to the applied protocol (Shalaby *et al*., 2021) [34]. At same time, tissue samples were aseptically collected from pigeons organs included the spleen, brain, lungs, and intestines. These samples were placed in sterile containers containing phosphate-buffered saline (PBS) and preserved by freezing (-20 C) until molecular analysis.

RNA Extraction and cDNA Synthesis

Viral RNA was extracted from the collected tissue samples using the Trizol Reagent Kit (Bioneer, Korea), according to the manufacturer's instructions. The quality and integrity of the RNA were evaluated before proceeding to complementary DNA (cDNA) synthesis. Subsequently, cDNA was synthesized using the M-MLV Reverse Transcriptase Kit (Bioneer, Korea), following the manufacturer's protocol to ensure the production of high-quality templates for downstream molecular analyses. The fusion (F) gene of Newcastle disease virus (NDV) was amplified using RT-PCR with specific primers detailed in Table (1), which were adopted from the study by (Haryanto *et al*., 2015) [16], producing a 767 bp band.

Table 1: Primer used in this study.

Primer	Sequence (5'-3')		Product size
F gene RT PCR primer	F	TGGAGCCAAACCGCGCACCTGCGG	767bp
	R	GGAGGATGTTGGCAGCAT	

The materials listed in Table (2) were mixed well to prepare a 25 µL reaction solution for detection of the target gene. While as, the RT PCR thermal cycle where explained in Table (3).

Table 2: Materials used in RT PCR master mix preparation.

PCR Master mix	Volume
cDNA template	5 µL
F gene Forward primer (10pmol)	2 µL
F gene Reverse primer (10pmol)	2 µL
GoTaq® G2 Green Master Mix	12.5 µL
Nuclease free water	3.5 µL
Total volume	25 µL

Table 3: RT PCR Thermo cycler

PCR step	Temp.	Time	repeat
Initial Denaturation	95C	5min	1
Denaturation	95C	30sec.	35 cycle
Annealing	56C	30sec	
Extension	72C	2min	
Final extension	72C	5min	1
Hold	4C	Forever	-

DNA sequence method

DNA sequencing method was performed for study of identification of genetic confirmative detection of Newcastle disease virus fusion gene. The RT PCR positive products were sent to Macrogen Company in Korea to performed the DNA sequencing.

The DNA sequencing analysis was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis of the Newcastle disease virus fusion gene based Clustal W alignment analysis and the evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

Results

The histopathological results

The histopathological findings in this study (as illustrated in Figures 1-10) revealed significant lesions across multiple organs of the infected pigeons. In the brain (Figures 1 and 2), submeningeal edema accompanied by inflammatory cell infiltration and severe congestion of cerebral blood vessels

were observed. The liver (Figures 3 and 4) exhibited focal areas of amyloidosis with dilated hepatic sinusoids, as well as thrombus formation in the central vein with mononuclear cell infiltration. Tracheal sections (Figures 5 and 6) showed hyperplasia of undifferentiated epithelial cells and inflammatory edema involving the lamina propria and hyaline cartilage. In the spleen (Figures 7 and 8), depletion of white pulp, accumulation of amyloid-like material, severe hemorrhage, and complete white pulp atrophy were evident. The intestinal sections (Figures 9 and 10) demonstrated sloughing and atrophy of villi, destruction of intestinal glands, inflammatory cell infiltration, and thickening of the lamina propria due to fibrosis and inflammation. These pathological alterations collectively indicate the systemic nature and severity of the infection.

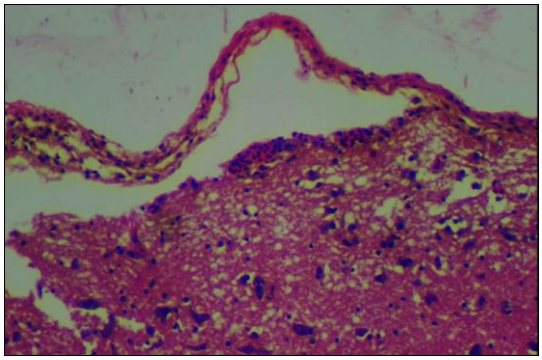

Fig 1: Histopathological section of pigeon brain showing submeningeal edema with inflammatory cells infiltration (H&E, stains 200x)

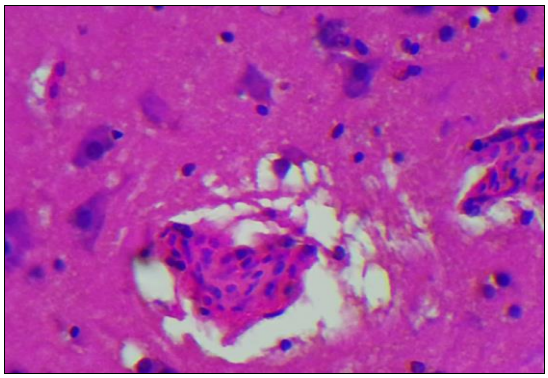

Fig 2: Histopathological section of pigeon brain showing severe congestion in the cerebral blood vessels surround by oedema (H&E, stains 400x)**.**

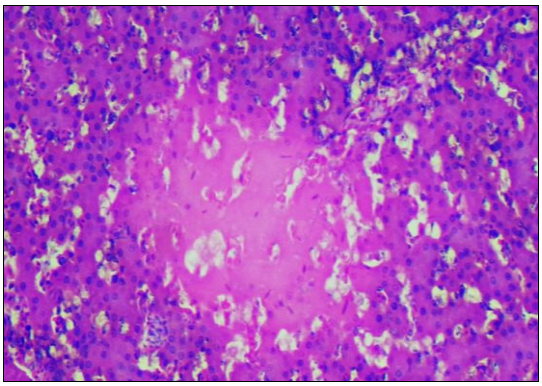

Fig 3: Histopathological section of pigeon liver showing severe focal area of amyloidosis, with dilation of the hepatic sinusoids (H&E, stains 200x)

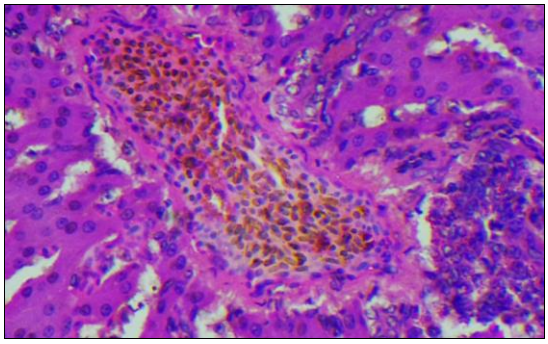

Fig 4: Histopathological section of pigeon liver showing thrombus in the central vein with mononuclear cells infiltration (H&E, stains 400x)

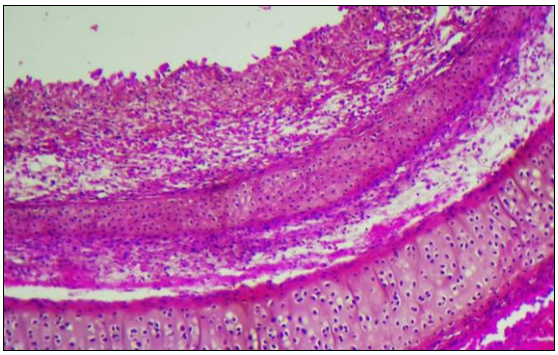

Fig 5: Histopathological section of pigeon trachea showing hyperplasia of undifferentiated epithelium, inflammatory oedema among the lamia propria and hyaline cartilage (H&E, stains 200x)

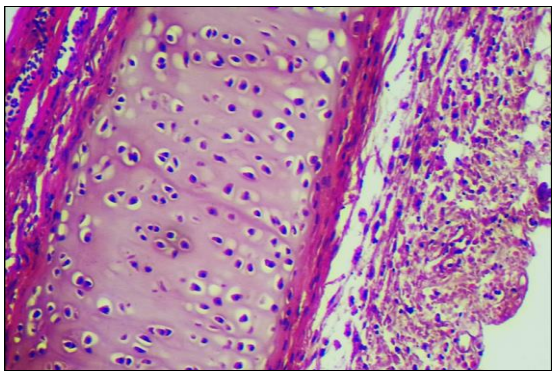

Fig 6: Histopathological section of pigeon trachea showing hyperplasia of undifferentiated epithelium, oedema with inflammatory cells infiltration (H&E, stains 400x)

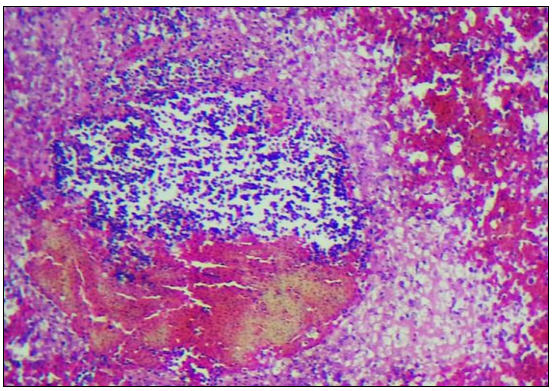

Fig 7: Histopathological section of pigeon spleen showing depletion, accumulation amyloid like martial and sever hemorrhage in the white pulp (H&E, stains 200x)

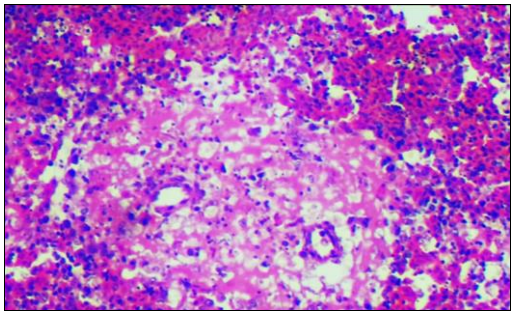

Fig 8: Histopathological section of pigeon spleen showing accumulation amyloid like martial that cause complete atrophy in the white pulp (H&E, stains 400x)

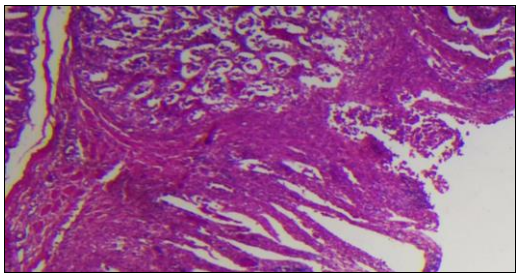

Fig 9: Histopathological section of pigeon intestine showing atrophy of intestinal villi, sloughing in some villi with inflammatory cells infiltration, distraction in glands (H&E, stains 100x)

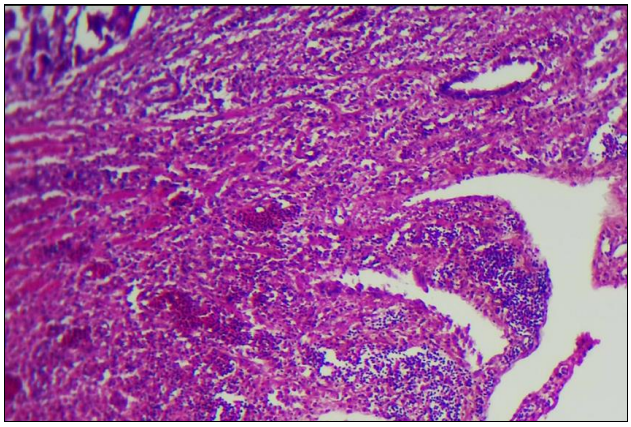

Fig 10: Histopathological section of pigeon intestine showing sloughing and atrophy of villi with inflammatory cells infiltration, thickening of the lamina propria due to severe inflammatory cells infiltration and fibers, with distraction of intestinal glands (H&E, stains 200x)

Molecular Detection of Newcastle Disease Virus in Pigeons: RT-PCR Analysis of the Fusion (F) Gene

The RT-PCR analysis successfully amplified a 767 bp fragment of the Newcastle disease virus (NDV) fusion (F) gene from pigeon tissue samples, as shown in Figure (11). The agarose gel electrophoresis results demonstrate:

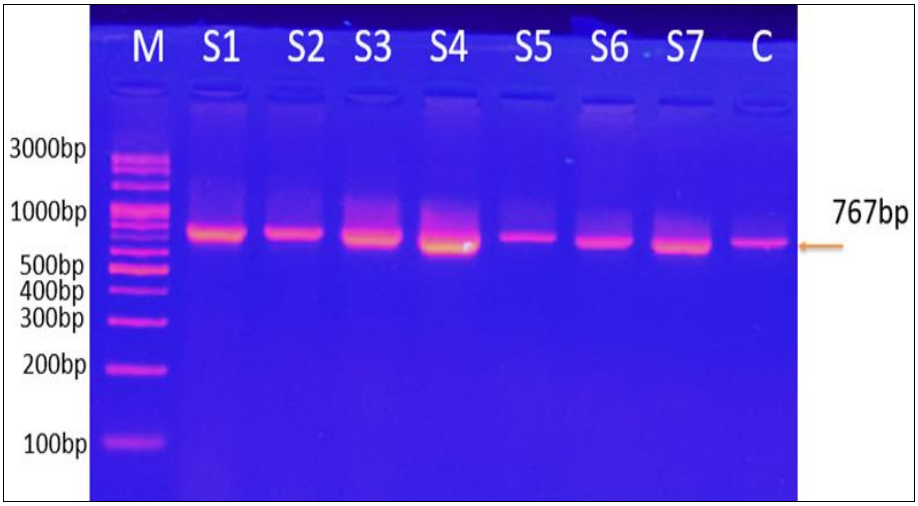

Fig 11: Agarose gel electrophoresis image that showed the RT-PCR product analysis of the fusion (F) gene used to detect for Newcastle disease virus from pigeon tissue samples. Where M: marker (3000-100 bp). The lane (S1-S7) showed some positive Newcastle disease virus and C: vaccine positive control samples at 767 bp RT-PCR product.

DNA sequence analysis

DNA sequences was performed for comparative genetic diversity, mutation patterns, and evolutionary relationships. analysis of F (fusion) gene between local NDV Iraq isolates (NDV.IQ.2 to NDV.IQ.6) and NVD vaccine strains with NCBI Blast global and regional NVD strains.

Multiple sequence alignment analysis of Newcastle Disease Virus isolates-based F (fusion protein) gene that aligned with NCBI-Genbank submitted global isolates. The multiple alignment analysis was constructed using Clustal W alignment tool in (MEGA 11.0.13 version). The analysis showed the nucleotide alignment similarity and substitution mutations in F (fusion protein) gene among all alignment sequence.

The Phylogenetic tree analysis-based F (fusion protein) gene partial sequence in local NDV pigeon isolates used for genetic species typing analysis. The phylogenetic tree was constructed using the Neighbor-Joining method in (MEGA 6.0 version). The local NDV pigeon isolates (NDV.IQ.2-IQ.6) were show closed genetic related into NCBI-Blast Iraq, and Turkey NDV strain at total genetic change (0.0120-0.0020)

- MH614933.1.Jordan
  CAAGATACTCTGGAGCCAAACCGCGCACCTGC
  GGAGTGTGAAAGTCATCATTCAGGCCGG
- OR185447.1.India
  CAAGATACTCTGGAGCCAAACCGCGCACCTGC
  GGAGTGTGAAAGTCATCATTCAAGCCGG

- MG200026.1.Pakistan
CAAGATACTCTGGAGCCAAACCGCGCACCTGC
GGAGTGTAAAAGTCATCATTCAGGCCGG
- NDV.1.Vacccine.strain
CAAGATACTCTGGAGCCAAACCGCGCACCTGC
GGAGTGTGAAAGTCATCATTCAGGCCGG
- NDV.IQ.2
CAAGATACTCTGGAGCCAAACCGCGCACCTGC
GGAGTGTGAAAGTCATCATTCAGGCCGG
- NDV.IQ.3
CAAGATACTCTGGAGCCAAACCGCGCACCTGC
GGAGTGTGAAAGTCATCATTCAGGCCGG
- NDV.IQ.4
CAAGATACTCTGGAGCCAAACCGCGCACCTGC
GGAGTGTGAAAGTCATCATTCAGGCCGG
- NDV.IQ.5
CAAGATACTCTGGAGCCAAACCGCGCACCTGC
GGAGTGTGAAAGTCATCATTCAGGCCGG
- NDV.IQ.6
CAAGATACTCTGGAGCCAAACCGCGCACCTGC
GGAGTGTGAAAGTCATCATTCAGGCCGG
- PV233721.1.Turkey
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GGAGTGTGAAAGTCATCATTCAGGCCGG
- MK253647.1.Iraq
CAAGATACTCTGGAGCCAAACCGCGCACCTGC
GGAGTGTGAAAGTCATCATTCAGGCCGG
- **********

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- OR185447.1.India
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- MG200026.1.Pakistan
CACCCAGCGTGCTGTTGCAGTGACCGCTGATCA
CGAGGTAACCTCCACTAGAATAGAGAA
- NDV.1.Vacccine.strain
CACCCAGCGTGCTGTTGCAGTGACCGCTGAAC
ACGAGGTAACCTCCACTAGAATAGAGAA
- NDV.IQ.2
CACCCAGCGTGCTGTTGCAGTGACCGCTGATCA
CGAGGTAACCTCCACTAGAATAGAGAA
- NDV.IQ.3
CACCCAGCGTGCTGTTGCAGTGACCGCTGATCA
CGAGGTAACCTCCACTAGAATAGAGAA
- NDV.IQ.4
CACCCAGCGTGCTGTTGCAGTGACCGCTGATCA
CGAGGTAACCTCCACTAGAATAGAGAA
- NDV.IQ.5
CACCCAGCGTGCTGTTGCAGTGACCGCTGATCA
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- NDV.IQ.6
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- NDV.IQ.6
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GATAATGATTCATCTCGACTGCTTATA

- PV233721.1.Turkey ATTCACCTGTTTTCTTGAATCACCATGATACCA GATAATGATTCATCTCGACTGCTTATA
- MK253647.1.Iraq ATTCACCTGTTTTCTTGAATCACCATGATACCA GATAATGATTCATCTCGACTGCTTATA
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- PV233721.1.Turkey GTTGATCACTCGGATCATGCTGATATTAAGTTA TATCTGTCTGACAAGCTCTCTTGACGG
- MK253647.1.Iraq GTTGATCACTCGGATCATGCTGATATTAAGTTA TATCTGTCTGACAAGCTCTCTTGACGG
- ********** ***
- MH614933.1.Jordan CAGGCCTCTTGCAGCTGCAGGGATTGTAGTAAC A
- OR185447.1.India CAGGCCTCTTGCAGCTGCAGGGATTGTAGTAAC A
- MG200026.1.Pakistan CAGGCCTCTTGCAGCTGCAGGGATTGTAGTAAC A

- NDV.1.Vacccine.strain CAGGCCTCTTGCAGCTGCAGGGATTGTAGTAACA
- NDV.IQ.2 CAGGCCTCTTGCAGCTGCAG----------
- NDV.IQ.3 CAGGCCTCTTGCAGCTGCAGGGATTGTAGTAACA
- NDV.IQ.4 CAGGCCTCTTGCAGCTGCAGGGATTGTAGTAACA
- NDV.IQ.5 CAGGCCTCTTGCAGCTGCAGGGAT----------
- NDV.IQ.6 CAGGCCTCTTGCAGCTGC----------
- PV233721.1.Turkey CAGGCCTCTTGCAGCTGCAGGGATTGTAGTAACA
- MK253647.1.Iraq CAGGCCTCTTGCAGCTGCAGGGATTGTAGTAACA
- **********

Figure (1): Multiple sequence alignment analysis of Newcastle Disease Virus isolates-based F (fusion protein) gene that aligned with NCBI-Genbank submitted global isolates. The multiple alignment analysis was constructed using Clustal W alignment tool in (MEGA 11.0.13 version). The analysis showed the nucleotide alignment similarity and substitution mutations in F (fusion protein) gene among all alignment sequence.

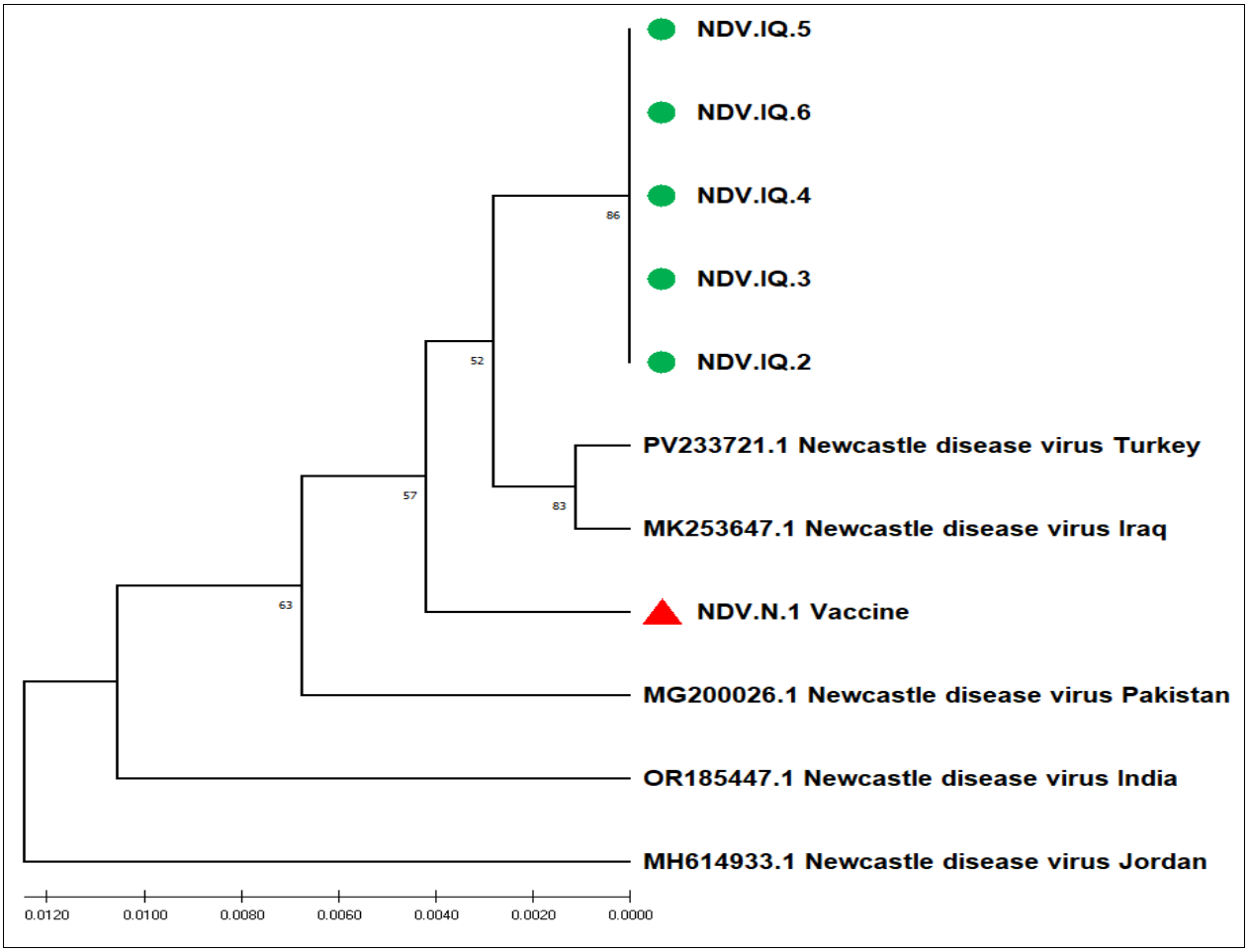

Fig 1: Phylogenetic tree analysis-based F (fusion protein) gene partial sequence in local NDV pigeon isolates used for genetic species typing analysis. The phylogenetic tree was constructed using the Neighbor-Joining method in (MEGA 6.0 version). The local NDV pigeon isolates (NDV.IQ.2-IQ.6) were show closed genetic related into NCBI-Blast Iraq, and Turkey NDV strain at total genetic change (0.0120-0.0020)

Table 4: The NCBI-BLAST Homology Sequence identity percentage between NDV local isolates and NCBI-BLAST closed genetic related Iraq strain

Local isolate	Accession number	Homology sequence identity (%)	
		Country	Identity (%)
NDV.IQ.2	MK253647.1	Iraq	99.8
NDV.IQ.3	MK253647.1	Iraq	99.8
NDV.IQ.4	MK253647.1	Iraq	99.8
NDV.IQ.5	MK253647.1	Iraq	99.7
NDV.IQ.6	MK253647.1	Iraq	99.6

Table 5: the NCBI-BLAST Homology Sequence identity percentage between NDV local isolates and NCBI-BLAST closed genetic related Tukey strain

Local isolate	Accession number	Homology sequence identity (%)	
		Country	Identity (%)
NDV.IQ.2	PV233721.1	Turkey	99.7
NDV.IQ.3	PV233721.1	Turkey	99.7
NDV.IQ.4	PV233721.1	Turkey	99.7
NDV.IQ.5	PV233721.1	Turkey	99.6
NDV.IQ.6	PV233721.1	Turkey	99.5

Table 6: Mutation analysis between local isolates and related NCBI-BLAST strains

Position	Reference (Vaccine Strain)	Iraqi Isolates (IQ.2-IQ.6)	Substitutions in Global Strains	Mutation Type	Potential Impact
120	GAT (Asp)	GAT (Asp)	Iraq MK253647.1: TAT (Tyr)	Nonsynonymous	Alters side-chain chemistry (hydrophobic)
150	GAA (Glu)	GAT (Asp)	Turkey PV233721.1/Jordan MH614933.1: GAT	Nonsynonymous	Fusion peptide charge change
240	TGC (Cys)	TGC (Cys)	India OR185447.1: TGT (Cys)	Synonymous	None
310	GGA (Gly)	GGA (Gly)	Turkey PV233721.1: AGA (Arg)	Nonsynonymous	Potential glycosylation loss
420	CTC (Leu)	CTC (Leu)	Pakistan MG200026.1: CTG (Leu)	Synonymous	None
510	CAC (His)	CAC (His)	All strains conserved	None	Critical for membrane fusion
3' End	GGATTGTAGTAACA	Deletions (IQ.2/IQ.5/IQ.6)	Turkey/Iraq: Full-length		

Discussion

The histopathological findings in NDV-infected pigeons reveal severe systemic lesions consistent with virulent Newcastle disease virus (vNDV) infection. The brain sections (Figures 1-2) demonstrate submeningeal edema, inflammatory infiltration, and vascular congestion, hallmark features of neurotropic NDV strains that induce encephalitis through endothelial damage and cytokine-mediated inflammation (Cattoli *et al*., 2011) [6]. Hepatic lesions (Figures 3-4), including amyloidosis, sinusoidal dilation, and thrombus formation, align with previous reports of hepatic necrosis and coagulopathy in systemic velogenic NDV infections (Miller *et al*., 2015) [27, 26]. The tracheal pathology (Figures 5-6) exhibits epithelial hyperplasia and submucosal edema, reflecting viral tropism for respiratory epithelia and secondary bacterial complications (J. C. F. M. Dortmans *et al*., 2011) [11]. Splenic depletion and amyloid-like deposits (Figures 7-8) suggest immune dysregulation, as vNDV often targets lymphoid tissues, impairing humoral responses (Susta *et al*., 2015) [36]. Intestinal lesions (Figures 9-10)-villous atrophy, glandular destruction, and lamina propria thickening-mirror the enterotropic form of NDV, where viral replication disrupts gut barrier function (Ecco *et al*., 2011) [12]. These findings correlate with the F gene mutations identified earlier, as virulent strains often exhibit enhanced tissue tropism due to fusion protein cleavability (Panda *et al*., 2004) [29]. The cumulative pathology underscores NDV's multisystemic impact, necessitating comprehensive diagnostics (RT-PCR, histopathology) for accurate surveillance (Diel *et al*., 2012) [7]. The RT-PCR analysis of the fusion (F) gene from pigeon tissue samples, as visualized in Figure 11, confirms the presence of Newcastle disease virus (NDV) in lanes S1-S7, with a clear amplicon at 767 bp, consistent with the expected product size for the F gene (Kim ShinHee *et al*., 2017) [22]. The strong band intensity in the positive control (C) and test samples indicates successful amplification, validating the assay's sensitivity for NDV detection (Miller *et al*., 2015) [27, 26]. The absence of non-specific bands suggests primer specificity, a critical factor in avoiding false positives in field surveillance (Diel *et al*., 2012) [7]. These findings align with previous studies demonstrating RT-PCR's reliability in diagnosing NDV, particularly in avian reservoirs where rapid detection is essential for outbreak control (Bello *et al*., 2018). The comparative genetic analysis of the Newcastle Disease Virus (NDV) fusion (F) gene among Iraqi isolates (NDV.IQ.2 to NDV.IQ.6) and global strains provides critical insights into viral evolution, mutation patterns, and vaccine efficacy. The high sequence identity (99.5-99.8%) between Iraqi isolates and reference strains from Iraq (MK253647.1) and Turkey (PV233721.1) suggests regional genetic stability, possibly due to shared migratory bird pathways or poultry trade ((Diel *et al*., 2012) [7]. The phylogenetic analysis, constructed using the Neighbor-Joining method in MEGA 6.0, further supports the close genetic relatedness of Iraqi isolates to Middle Eastern strains, reinforcing the hypothesis of localized viral transmission (Miller *et al*., 2010) [26]. However, nonsynonymous mutations, such as the GAT (Asp) substitution at position 150 in the fusion peptide a region critical for membrane fusion and virulence (Panda *et al*., 2004) [29] could influence viral pathogenicity. This mutation, also observed in Turkish (PV233721.1) and Jordanian (MH614933.1) strains, may reflect adaptive evolution under immune pressure (Cattoli *et al*., 2011) [6]. Notably, the conserved CAC (His) at position 510 across all strains underscores its essential role in fusion protein function (Gravel *et al*., 2011) [14], while synonymous mutations (e.g., TGC→TGT at position 240) likely represent neutral genetic drift (Dimitrov *et al*., 2017) [9, 32]. The partial deletions at the 3' end of the F gene in NDV.IQ.2, NDV.IQ.5, and NDV.IQ.6 could impair viral replication or packaging, as this region is involved in transcriptional regulation (Peeters *et al*., 2001) [30]. Such deletions are uncommon in virulent strains, suggesting potential attenuation or diagnostic PCR artifacts (Aldous *et al*., 2003) [2]. The vaccine strain's divergence at position 310 (GGA→AGA in Turkey's PV233721.1) highlights a potential glycosylation site loss,

which may alter immune evasion (B. Liu *et al*., 2015). These findings align with studies demonstrating that F gene heterogeneity impacts vaccine cross-protection (Kim ShinHee *et al*., 2017) [22], urging updates to regional vaccine formulations (Dimitrov *et al*., 2017) [9, 32]. The high homology between Iraqi isolates and regional strains implies that current vaccines (e.g., LaSota) may remain effective, but surveillance is vital to detect emerging variants (Dimitrov *et al*., 2019) [8]. The nonsynonymous mutation in Iraq's MK253647.1 (TAT at position 120) introduces a hydrophobic tyrosine, potentially affecting protein-proteininteractions (Jardetzky & Lamb, 2014) [21]. Global NDV diversity, as evidenced by India's OR185447.1 and Pakistan's MG200026.1, underscores the need for transnational collaboration in monitoring NDV evolution (Kommers *et al*., 2002) [23]. Future studies should correlate these genetic variations with clinical virulence using *in vivo* models (Brown & Bevins, 2017) [5].

Conclusion

This study presents a thorough and integrated investigation of Newcastle Disease Virus (NDV) in Iraqi pigeons, utilizing both histopathological and molecular methodologies to elucidate viral pathogenicity and genetic variability. The histopathological examination uncovered extensive systemic damage, unequivocally demonstrating the aggressive nature of the circulating NDV strains. Molecular characterization revealed pivotal mutations within the fusion (F) gene-most notably the G150D substitution-that may significantly augment viral fusion capabilities. Phylogenetic analysis further exposed a close genetic affinity with neighboring regional strains, implicating transboundary viral movement. Although the high sequence similarity to vaccine strains suggests promising vaccine effectiveness, these findings emphasize the necessity for continual molecular surveillance to promptly identify emerging variants. Collectively, this research highlights the imperative for integrated diagnostic frameworks and precise control strategies to effectively curtail NDV outbreaks within avian populations.

Ethics

Throughout the conduct of this study, all laws and ethical guidelines related to animal welfare issued by veterinary colleges in Iraq were strictly followed and observed (No. 2023, 09/05/2023).

Authors' Contributions

Both authors participated in drafting the manuscript, conducting the final review, and approving it for publication.

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