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Molecular detection and genetic analysis of a virulent NDV strain causing high mortality in vaccinated broilers in Al-Najaf province, Iraq

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Abstract
Newcastle Disease (ND) outbreaks were investigated in vaccinated broiler flocks in Al-Najaf, Iraq, with mortality rates of 81-85%. Despite vaccination with Clone 30, LaSota, and inactivated ND and H9 vaccines, affected birds showed respiratory, digestive, and neurological signs, accompanied by characteristic lesions. ELISA revealed high antibody titers, PCR confirmed systemic infection, and sequencing of the fusion gene identified two virulent isolates (Hayder 1 and Hayder 2). These results confirm vaccine failure and highlight the importance of molecular surveillance and genotype-matched vaccines to improve ND control in Iraq.

Keywords: Newcastle disease virus, broiler chickens, vaccine failure, molecular detection, genetic characterization.

Introduction
Newcastle Disease (ND) is a highly contagious viral infection affecting poultry worldwide, caused by virulent strains of Newcastle Disease Virus (NDV), also known as Avian Paramyxovirus 1 (APMV-1). It poses one of the greatest threats to poultry production due to its rapid spread, high mortality rates, and systemic lesions affecting the digestive and nervous systems (Haddas, 2023) [7]. Historically, ND has caused at least four major global epidemics, each linked to distinct viral genotypes, leading to severe economic losses and disruptions in international poultry trade (Mao *et al*., 2022) [10]. Despite extensive vaccination programs, outbreaks continue to occur in vaccinated flocks, partly due to the emergence of novel strains and atypical clinical signs that complicate diagnosis, prevention, and control (Hu *et al*., 2022) [8]. Molecular studies have reclassified NDV under the genus Avian orthoavulavirus 1 in the Paramyxoviridae family The virus has a single-stranded, negative-sense RNA genome of approximately 15.2 kb, encoding eight proteins crucial for viral replication, transcription, and immune evasion (Suarez *et al*., 2020) [12]. Among these, the fusion (F) protein cleavage site (FCS) plays a vital role in determining viral pathogenicity, with velogenic and mesogenic strains carrying multibasic motifs that enable systemic infection, while lentogenic strains contain monobasic motifs associated with milder disease (Ji *et al*., 2024) [9]. Clinically, ND manifests in four main forms: viscerotropic velogenic, neurotropic velogenic, mesogenic, and lentogenic. The velogenic pathotypes cause severe hemorrhagic and neurological symptoms with high mortality, whereas lentogenic strains result in mild respiratory disease but still impact productivity (Ansari *et al*., 2024) [3]. The Vaccination of NDV failures, once attributed mainly to administration errors, are now increasingly linked to antigenic drift and concurrent infections that reduce vaccine efficacy (Shahid *et al*., 2020) [11]. In the Middle East and Iraq, ND remains a major challenge for poultry farming, with recurring outbreaks causing substantial economic losses despite vaccination efforts, continuous molecular surveillance and genetic characterization of circulating NDV strains are essential for developing effective, genotype-specific vaccines and improving disease control strategies in the region (AbdAllah, 2019) [1].

2. Materials and Methods
2.1. Outbreak Summary
The study was conducted on two broiler farms in Al-Najaf, Iraq (51,000 and 35,000 birds)

between April and July. Mortality reached 85% and 81%, with clinical signs appearing at 22 days of age. Both flocks were vaccinated with Clone 30 (day 1, eye drop), inactivated ND and H9 (subcutaneous), and LaSota (day 7, drinking water).

2.2. Clinical Signs and Sampling

Affected birds exhibited high mortality and marked depression, in addition to sudden deaths, anorexia, watery green diarrhea, fever, respiratory distress, and late-stage torticollis (Figure.1). Trachea, spleen, brain, and liver samples were aseptically collected and stored at 2-4 °C for PCR, while additional tissues were preserved on Whatman® Flinders Technology Associates (FTA) cards for F-gene sequencing.

Fig 1: Broiler chicken showing respiratory signs (gasping and depression) associated with Newcastle disease

2.3. Gross Lesion

Gross lesions characterized by petechial hemorrhages in the proventriculus, congestion and button-like ulcers in the cecal tonsils, tracheal congestion, splenic congestion and necrosis (Figure 2, 3, 4, 5)

Fig 2; Gross Lesion (trachea) showing hemorrhages along the trachea.

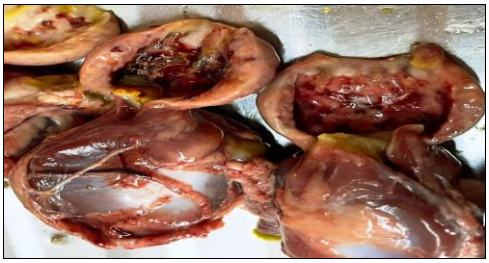

Fig 3: Gross Lesion (proventriculus) showing distinct petechial hemorrhages at the glandular tips and along the mucosal surface

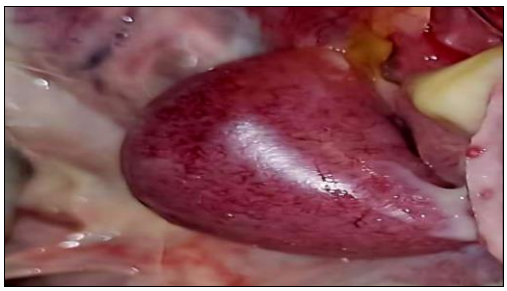

Fig 4: Gross Lesion (spleen) showing enlarged and markedly congested of spleen, with multifocal petechial to ecchymosis hemorrhages on the surface.

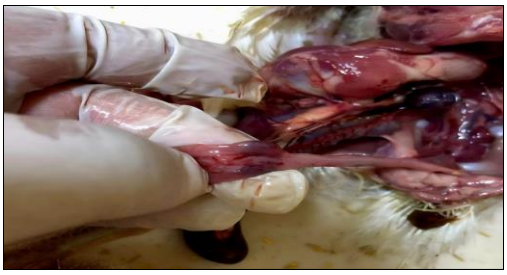

Fig 5: Gross Lesion (Cecal tonsils): The cecal tonsils exhibited severe congestion and petechial to ecchymosis hemorrhages.

2.4. ELISA

An indirect ELISA test was performed using the ID Screen® Newcastle Disease Indirect kit (Innovative Diagnostics, France). Serum, plasma, and yolk samples were diluted 1:500 and the optical density was measured at 450 nm. Samples with S/P ratio > 0.3 (titer > 993) were considered positive.

2.5. Real-time PCR

RNA was extracted using the Kylt® RNA/DNA Purification Kit (AniCon Labor GmbH, Germany) and tested by real-time RT-PCR for NDV detection. The assay was performed as a one-step real-time RT-PCR targeting the (M gene) of NDV.

Table 1: Primers and probe used for real-time RT-PCR detection of NDV (M gene).

Name	Sequence (5’ → 3’)	Target gene	Label/Quencher
Forward primer (APMV-1 F)	AGTGATGTGCTCGGACCTTC	M gene	-
Reverse primer (APMV-1 R)	CCTGAGGAGAGGCATTTGCTA	M gene	-
Probe (HEX APMV-1 LNA)	gggaCrGChTgCtatCc	M gene	5’-HEX / 3’-BHQ3

2.6. Sequencing

FTA card samples prepared at Ugene Laboratory (Iraq) were submitted to a specialized sequencing facility in Korea for partial sequencing of the fusion (F) gene.

3. Results

3.1 ELISA Test

All samples tested positive for NDV antibodies. Mean titers were high, reaching 19,500 and 21,000 (Figure.6) in the two flocks, suggesting natural infection despite vaccination.

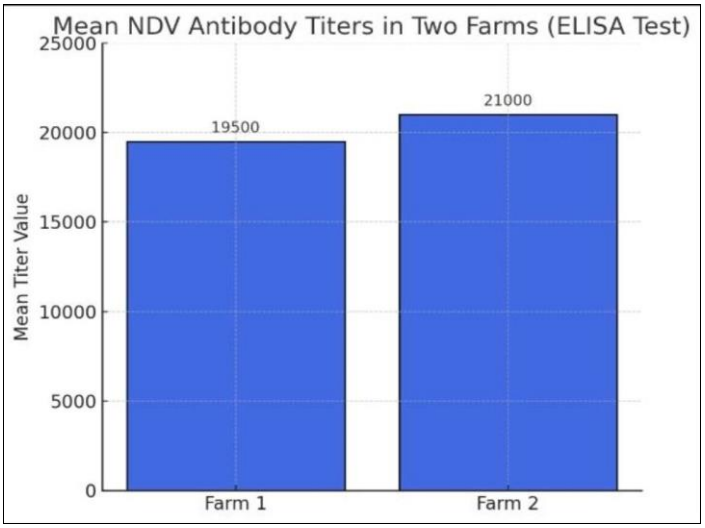

Fig 6: Mean antibody titers against Newcastle disease virus (NDV) in two broiler farms as measured by ELISA

3.2. Real-time PCR

All organ samples were positive by real-time RT-PCR. Ct values ranged from 18.5-23 across spleen, trachea, and brain samples, confirming high viral loads and systemic distribution. (Figure.7).

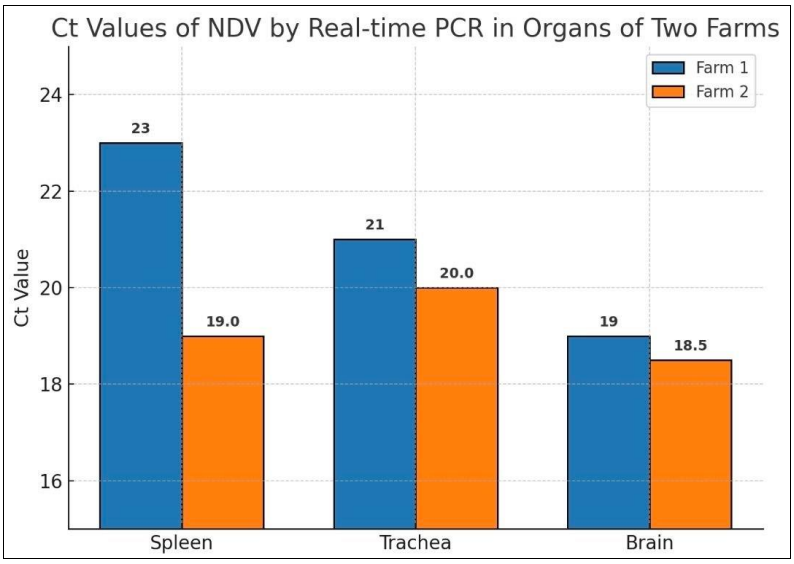

Fig 8: Comparison of Ct values of NDV detected by real-time PCR in spleen, trachea, and brain samples from Farm 1 and Farm 2

3.3. Sequencing

Partial sequencing of the fusion (F) gene identified two NDV isolates, designated Hayder 1 (GenBank accession no. PQ800135.1) and Hayder 2 (GenBank accession no. PV153708.1). These isolates were deposited in GenBank to support future molecular and epidemiological studies.

4. Discussion

This study confirmed severe ND outbreaks in vaccinated broiler flocks. High antibody titers indicated exposure to field strains rather than vaccine-induced immunity, consistent with earlier reports of vaccine failure in endemic regions (Dimitrov *et al*., 2020; Miller & Koch, 2021) [6, 12]. The low Ct values obtained by real-time PCR demonstrated high viral loads and systemic dissemination, consistent with the viscerotropic and neurotropic nature of velogenic NDV (Absalón *et al*., 2019; Bello *et al*., 2021) [2, 5]. Sequencing further confirmed NDV involvement. The identification of Hayder 1 (PQ800135.1) and Hayder 2 (PV153708.1) provides molecular evidence of strain circulation in Iraq and enriches the global GenBank database. Continuous molecular surveillance and vaccine updates are necessary to improve NDV control.

5. Conclusion

This study confirmed severe outbreaks of Newcastle Disease in broiler farms in Al-Najaf Province despite the use of routine vaccination programs. Serological tests, PCR, and sequencing revealed the presence of virulent NDV strains, which explain the vaccine failure and high mortality rates. These findings highlight the need for continuous molecular monitoring and updating of vaccines to match locally circulating strains, along with improving preventive measures and vaccination programs to reduce the economic losses caused by the disease.

6. Acknowledgments

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7. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

8. Author Contributions

H.S.K. designed and conducted the experiments, analyzed the data, and wrote the manuscript. F.H.K.A. assisted with sample collection, laboratory work, and manuscript revision. Both authors approved the final version of the manuscript.

References

1. AbdAllah FSS. Survival of Newcastle disease virus in poultry farm environment [Master's thesis]. Cairo: Cairo University; 2019.
2. Absalón AE, Cortés-Espinosa DV, Lucio E, Miller PJ, Afonso CL. Epidemiology, control, and prevention of Newcastle disease in endemic regions: Latin America. Trop Anim Health Prod. 2019;51(5):1033-48. https://doi.org/10.1007/s11250-019-01843-z
3. Ansari S, Patel A, Dabas R, Pradhan TR. Newcastle disease: Strategies for effective management. In: Innovative poultry science: Trends, disease management, and future perspectives. Singapore: Springer; 2024. p. 96-112. https://doi.org/10.1007/978-981-99-3117-5_6
4. Bello I, Abdullah A, Adelakun O, Agbato O, Okunrinkoya B. Coconut water enhances immunity of cockerels before and after vaccination against Newcastle disease virus. J Anim Sci Vet Med. 2021;6(2):58-63. https://doi.org/10.31248/JASVM2021.271
5. Bello MB, Yusoff K, Ideris A, Hair-Bejo M, Peeters BP, Omar AR, Aini I. Diagnostic and vaccination approaches for Newcastle disease virus in poultry. Front Vet Sci. 2021;7:575237. https://doi.org/10.3389/fvets.2020.575237
6. Dimitrov KM, Abolnik C, Afonso CL, Albina E, Bahl J, Berg M, Briand FX, Brown IH, Choi KS, Chvala I, Diel DG, Durr PA, Ferreira HL, Fusaro A, Gil P, Goujgoulova GV, Grund C, Hicks JT, Joannis TM, *et al*. Updated unified phylogenetic classification system for Newcastle disease virus. Infect Genet Evol. 2020;79:104169. https://doi.org/10.1016/j.meegid.2020.104169
7. Haddas R. Newcastle disease virus. In: Swayne DE, Pantin-Jackwood M, Afonso CL, editors. Infectious diseases of poultry. Cham: Springer; 2023. p. 427-40. https://doi.org/10.1007/978-3-031-14224-8_24
8. Hu Z, He X, Deng J, Hu J, Liu X. Current situation and future direction of Newcastle disease vaccines. Vet Res. 2022;53(1):99. https://doi.org/10.1186/s13567-022-01090-3
9. Ji Y, Chen A, Zhu Q. Biological characteristics of Newcastle disease virus and control strategies. In: Veterinary virology of domestic and pet animals. Singapore: Springer; 2024. p. 1-30. https://doi.org/10.1007/978-981-97-1139-6_1
10. Mao Q, Ma S, Schrickel PL, Zhao P, Wang J, Zhang Y, Li S, Wang C. Review of detection methods for Newcastle disease virus. Front Vet Sci. 2022;9:936251. https://doi.org/10.3389/fvets.2022.936251
11. Shahid N, Samiullah TR, Shakoor S, Latif A, Yasmeen A, Azam S, Shahid AA, Husnain T, Rao AQ. Early-stage development of a Newcastle disease vaccine candidate in corn. Front Vet Sci. 2020;7:499. https://doi.org/10.3389/fvets.2020.00499
12. Suarez DL, Miller PJ, Koch G, Mundt E, Rautenschlein S. Newcastle disease, other avian paramyxoviruses, and avian metapneumovirus infections. In: Swayne DE, editor. Diseases of poultry. 14th ed. Hoboken: Wiley-Blackwell; 2020. p. 109-66. https://doi.org/10.1002/9781119371199.ch4