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Detection and Identification of Avian Orthoavulovirus-1 in Broiler Chickens in Mid and South Areas of Iraq

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Abstract

This study investigates the presence of Avian Orthoavulovirus-1 (AOAV-1), which is previously called avian paramyxovirus-1, in Iraqi broiler chicken Newcastle disease, a highly contagious and economically important poultry disease, is caused by AOAV-1. A total of 250 samples were collected from 6 Iraqi provinces, Clinically, 150 samples indicated ND. Using PCR and clinical examination, the virus was examined in 150 broiler samples from farms in the north, center and south of Iraq that clinically indicated ND. According to the results (50), 33.3% of the samples tested were positive for PCR detection using the F and HN genes.The results of this investigation may have implications for regional disease control strategies and advance our understanding of the occurrence of AOAV-1 in Iraqi poultry.

Keywords: Detection, Identification, Iraq, Broiler Chickens.

Introduction

The essential nutrients found in red meat can also be found in white meat, however, white meat has the benefit of having lower levels of fats and cholesterol. because it is reasonably priced and contains high-quality protein and most poultry is raised in "backyard" subsistence settings and broiler meat is the least expensive source of animal protein in developing nations, non-destructive breathing New Castle Disease Virus (NDV) may significantly decrease the amount of protein consumed by an individual as well as harm the

microeconomy by preventing the sale of surplus chickens or eggs (1).

Avian orthoavulovirus-1, the primary cause of Newcastle disease (ND), is a serious viral disease that primarily affects broilers worldwide. The virus, which causes disruption of the nervous, digestive and, respiratory systems, results in significant mortality and financial losses (2). Morbidity and mortality Newcastle disease in poultry flocks may reach 90-100%, depending on the variant strains. Also, a wide range of captive and free-living birds are susceptible, and can sometimes act as the primary source of ND infection in chickens (3).

The NDV strains have been classified into 4 groups according to virulence and based on the clinical disease they caused in infected chickens, they were classified as velogenic (high virulence, up to 100% mortality), mesogenic (moderate virulence with respiratory signs and lower level of mortality), lentogenic (low virulence with mild or inapparent respiratory signs), and avirulent (asymptomatic) (4). Additionally, velogenic strains are classified as neurotropic, which causes encephalitis and respiratory and neurological clinical symptoms, and viscerotropic, that causes severe gastrointestinal and visceral hemorrhages (5, 6). The NDV virus is categorized as zoonotic because it infects people after sick birds, resulting in flu-like symptoms and moderate conjunctivitis. Furthermore, in serious instances, it can lead to chronic vision problems. A member of the Paramyxoviridae family, avian orthoavulovirus-1 infects chickens across many species and age groups. It possesses a variety of genotypes and pathotypes, each with varying degrees of virulence (7). Given that its primary routes of transmission are excrement and respiratory secretions are the routes of transmission. The NDV flexibility to a range of(2), the potential for repeating outbreaks is increased by environmental circumstances.

Molecular techniques such as PCR are currently the preferred method for NDV detection. PCR amplifies specific NDV DNA regions and permits quick and precise detection [8]. Reverse transcription PCR (RT-PCR) is a common technique for RNA viruses such as NDV. It makes it possible to identify infection even in its early stages (8). PCR has proven important in determining the virulent Newcastle disease virus isolates in broiler chicken farms in Iraq (9).

The viral genome is transported into the infected cell's cytoplasm, where replication occurs when the fusion protein promotes the fusion of the virion envelope and plasma membrane . the 1792nt long F gene encodes a precursor polypeptide fusion glycoprotein that is 553 amino acid long (10).

On the surface of NDV there is a functional glycoprotein called hemagglutinin-neuraminidase (HN). this versatile protein aids in determining the presence of receptors and functions of the virus's integrated neuraminidase (NA). It allows the virus to enter the cell by recognizing sialic acid receptors on the surface of the host cell and triggering the activity of fusion protein by acting as neuraminidase, it also helps to destroy sialic acid receptors, stopping surrounding virus particles from self –

agglutinating. Accordingly, the NH protein is crucial for the stage of viral infection (11). NDV has been detected in Iraq, according to much research (12, 13) with notable outbreaks occurring in commercial chicken farms. Through clinical symptoms, morphological lesions and a confirmed laboratory diagnosis of NDV, the study aims to isolate and identify avian paramyxovirus serotype 1. Broiler chicken in an RT-PCR (reverse transcription-polymerase chain reaction) assay .

Materials and Methods

Sample Collection and Preparation: The current investigation focused on a prospective NDV outbreak that occurred in vaccinating chickens on broiler farms in Iraq between November 2022 to February 2024. A total of 250 specimens were collected in a row throughout this period from several different Iraqi provinces: Basrah 175, Maysan 15, Karbala 4, Wasit 40, Baghdad 3, and Kirkuk 13. The examined herds suffered from respiratory distress, digestive disorders, and sometimes nervous symptoms. These clinical signs and symptoms of birds suspected of having Newcastle disease have been observed and recorded during outbreaks. Deceased and moribund chickens were subjected to necropsy, and tissue samples including the brain, trachea, lungs, proventriculus, liver, spleen, and cecal tonsils were collected for gross examination. The samples stored at -20 °C for molecular detection. The tissue were placed in the cold mortar and doused with a small quantity of liquid nitrogen, then the tissue was (carefully) grounded to a fine powder (most frozen cloths are very brittle). More liquid nitrogen was added as needed until the material was completely pulverized. The liquid N_2 was allowed to evaporate and the powder was carefully scooped into the pre-labeled, pre-cooled tubes.

Molecular Methods

extraction of viral RNA and synthesis of CDNA

Extraction of viral RNA was performed from harvested tissue (trachea, liver, proventriculus, spleen using a total RNA isolation kit (Promega, USA) and stored at (-18°C) until use. cDNA synthesis kit (Promega, USA) according to the manufacturer's instructions. Then cDNA was synthesized and stored at a temperature of (-18 °C).

PCR Reaction For The Detection Of Avian Paramyxovirus 1

RT-PCR amplification was performed with (Promega, USA) to detect the virulent NDV strain according to the manufacturer's instructions using the following primers, Table (1).

After being separated by electrophoresis on a 1.5% agarose gel using TAE buffer and stained with ethidium bromide, the PCR products were compared to a molecular marker (a base pair of DNA). The UV light revealed the gel.

RESULTS

Clinical Signs and Postmortem

The birds under examination displayed neurological and/or respiratory disorders,

depression, and greenish diarrhea. Typical postmortem lesions are presented in Figure (1) and Table (2) Necrotic lesions were found to be present in the mucosa of the intestine, proventriculus, and gizzard during the necropsy of dead birds. The proventriculus mucosa had significant amounts of hemorrhagic lesions. Congestion of the trachea and lungs and enlargement of the spleen and liver have also been observed.

Table (1): PCR primer sequences of F and HN genes

Gene	Forward primer	Reverse primer	RT-PCR Product	Reference
F gene	F:5'-TGGAGCCAAACCGCGCACCTGCGG-3'	R:5'-GGAGGATGTTGGCAGCAT-3'	767 bp	[14]
HN gene	F:5'-GGTTCCCAGTTTACGGAGGG-3'	R:5'-CTGGCAAGGGACACTACCTG -3'	431 bp	Design primer

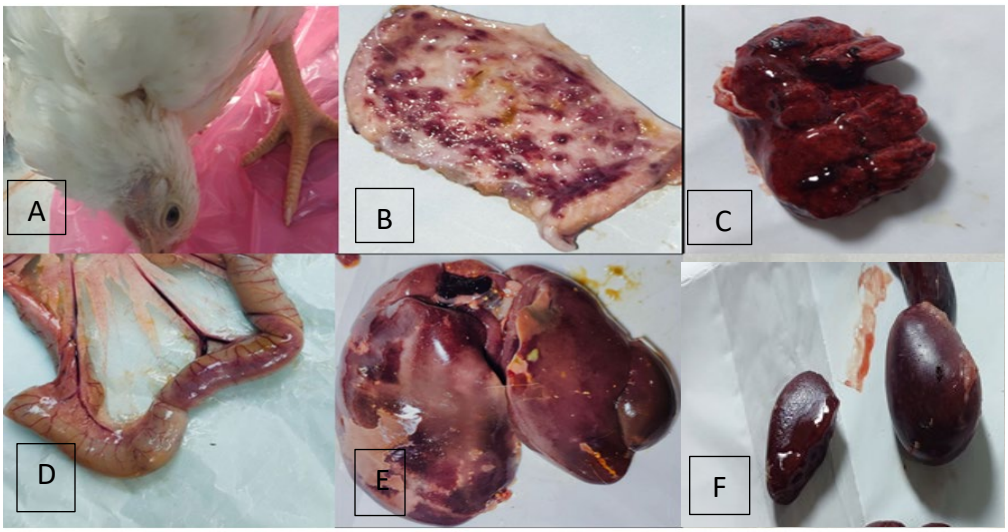

Figure (1): Postmortem and clinical signs of affected birds. Neurological symptoms such as A. Torticollis, B. Autopsy shows bleeding in the mucous membrane of the proventriculus, C. Congestion of lung D. Bleeding in the intestine E. Necrosis of the liver F. Enlarge size of spleen than normal.

Table (2): Distribution of samples by site and postmortem results

Province	No. of sample	Positive Postmortem	Negative Postmortem	Percent of clinically positive sample
Basrah	175	75	100	42.8
Maysan	15	15	-	100
Karbala	4	4	-	100
Wasit	40	40	-	100
Baghdad	3	3	-	100
Kirkuk	13	13	-	100
Total	250	150	100	

Confirmation Of NDV By RT-PCR: The tissue samples were successfully amplified using two-step RT-PCR. The genes were amplified using a set of primers. All results showed single and clear bands at 767 bp for the F gene and the NH gene at 431 bp (Figures 3, 4). The number and percentage of positive PCR tests to positive postmortem samples are shown in (Table 3, Figure 2).

Table (3): Number and percentage of positive PCR test to positive postmortem samples

Province	Presence of postmortem changes	Positive PCR test	Percentage rate
Basrah	75	23	30.6
Maysan	15	8	53.3
Karbala	4	0	0
Wasit	40	12	30
Baghdad	3	3	100
Kirkuk	13	4	30.7
Total	150	50	33.3

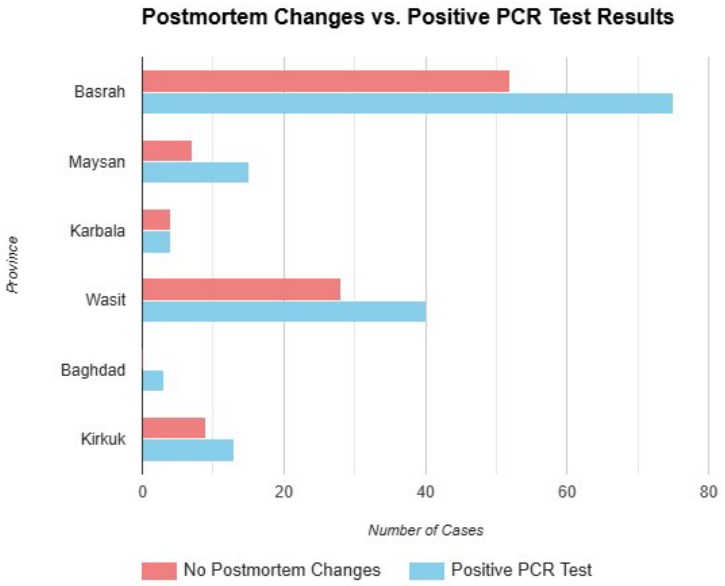

Figure (2): Number of clinical cases confirmed by PCR test.

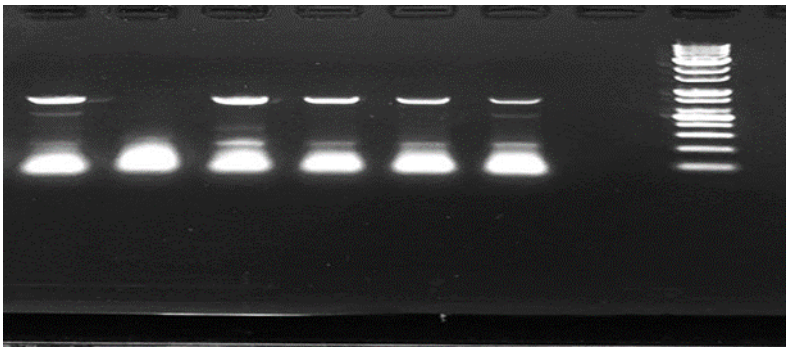

Figure (3): Agarose gel electrophoresis shows RT-PCR results of the NDV fusion protein gene on a 767-bp PCR product. Lane 2: Negative results, no amplification.

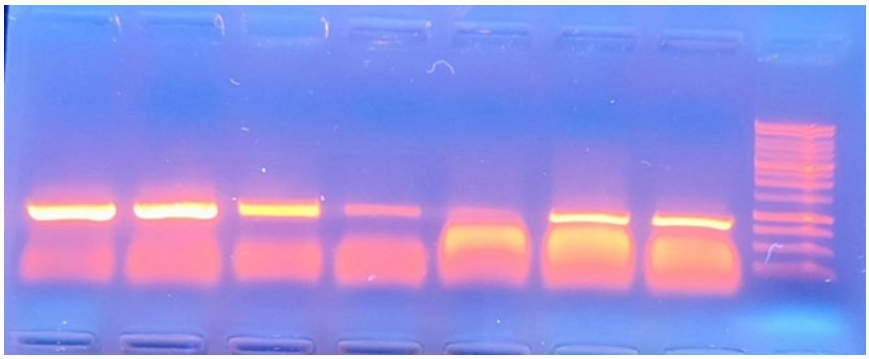

Figure (4): Agarose gel electrophoresis of PCR products. Amplification of the HN gene fragment (431 bp) of NDV by RT-PCR.

Discussion

Newcastle disease is a worldwide infection that affects domestic poultry economically. Many bird species are susceptible to infection by the NDV, which can cause varying degrees of clinical symptoms in various age groups ((15). Newcastle disease remains a major obstacle to poultry production in backyard or commercial systems. More than 250 species of domestic and wild birds suffer from the infectious disease known as ND (16).

The severity of different NDV strains varies greatly, ranging from infection without symptoms to fatal disease. Although vaccination programs have significantly reduced the risk of NDV outbreaks, ND virus infections have become more common worldwide in recent years. Rapid and precise identification of NDV will undoubtedly contribute to the effective treatment of the disease. Unfortunately, the current diagnosis of NDV infection by conventional virus isolation and serological testing has always been hampered by the lack of a sensitive rapid detection strategy (17, 18).

The clinical signs reported in the current study in chickens infected with Newcastle disease virus represented the general digestive, respiratory and neurological disorder, The clinical signs reported in the this study who described the depression, hunching over a heat source, breathing difficulties, gasping, coughing, tracheal rales, nasal discharge, fatigue, watery eyes, and, in more severe cases, slightly swollen eye, compared with the others studies that isolated NDV strains are highly pathogenic to all susceptible species and cause severe mortality with typical clinical signs because this virus can entry to these organs , replicate and then damage occur . The results are consistent with those of previous researchers (9, 19).

At postmortem, Infected Chickens with NDV were necropsied after death, congestion of trachea, ulcerative hemorrhage may be observed throughout the digestive system especially at the proventriculus and cecal tonsils, swollen and necrotic foci may also present in spleen and liver. These finding are similar to that reported by (20, 21).

A number of samples were collected from Basra and Wasit governorates (170 and 45) due to the large number of chicken farms there compared to the rest of the governorates mentioned in Table 2. It was found that the number of positive samples due to post-mortem lesions in Basra and Wasit were 75 and 40, respectively, compared to the other cities. The clinical symptoms and pathological lesions of ND vary depending on the age and species of birds, the immune status of the host, and environmental conditions (22). The total number of migratory birds in the area, sampling periods that include migratory and non-migratory seasons, and the occurrence and history of ND outbreaks in nearby locations were also taken into consideration (23).

The regions in concern have a significant percentage of positive sample based on RT-PCR testing, and chickens collected from fields of Basrah and Wasit exhibited a high number of postmortem lesions diagnostic of the disease. This could be due to the large number of broiler farms and sampling period, which is the first days following infection and is characterized by high viral levels and visible lesions.

The present study's significant morbidity and mortality rates suggest that immunization may not always increase immunity that protects against NDV. Although vaccinations normally provide good protection from symptomatic disease and mortality. There is concern that they may not provide sufficient defense against viral transmission to prevent ND outbreaks (9, 24) .

Different rates of Newcastle disease virus infection – related mortality was reported among the broiler flocks in the current study; tracheitis and proventiculus hemorrhage were prevalent symptom (25). This could be brought on by disorders that depress the immune system, like viral infections or anemia, severe infection such as mycotoxicosis, or the inability to provide the herd with a vaccination (26).

Through the use of two specific gene primer (the F gene and the HN gene) covering the cleavage site of the F protein gene, the molecular component of the current study was able to demonstrate the presence of NDV, including the detection of such virus in tissue samples by RT-PCR (14). The start of reverse transcription –PCR molecular techniques as standard, accurate laboratory procedure offers significant advantages over conventional techniques of Newcastle disease virus genome detection (27). One of the two glycoprotein on the surface of NDV that has both hemagglutinating and neuraminidase activity is the HN protein (28).

To prevent further Newcastle disease virus in Iraq, it is necessary to identify and detect avian orthoavulovirus -1 in broiler chicken, prospective research and monitoring are required to decrease the adverse consequences of the virus on the health of chicken and farming sector.

Conclusion

In the present study, RT-PCR confirmed the existence of observable pathogenic changes which caused by Newcastle disease virus, which replicates in target organs when

determining the virus's identification and their pathogenicity, RT-PCR turned out to be most effective method.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

The Research Ethical Committee approves this work.

References

1. Melkamu, S. and M. Asrat, 2018 Review on economic importance and current diagnostic techniques of Newcastle disease in poultry. Int. J. Adv. Res. Biol. Sci,. 5(3): 117-125, DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.03.012.

2. Brown, V.R. and S.N. Bevins, 2017 A review of virulent Newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. Veterinary research,. 48: 1-15, https://doi.org/10.1186/s13567-017-0485-7.

3. RM, S.P., et al., 2020 An effective feature engineering for DNN using hybrid PCA-GWO for intrusion detection in IoMT architecture. Computer Communications,. 160: 139-149, 10.1016/j.comcom.2020.05.048.

4. Kabiraj, C.K., et al., 2020Sequential pathology of a genotype XIII Newcastle disease virus from Bangladesh in chickens on experimental infection. Pathogens,. 9(7): p. 539,https://doi.org/10.3390/pathogens9070539.

5. Mariappan, A.K., et al., 2018 Pathological and molecular investigation of velogenic viscerotropic Newcastle disease outbreak in a vaccinated chicken flocks. Virusdisease,. 29: 180-191, https://doi.org/10.1007/s13337-018-0445-5.

6. Ali, A., et al., 2022 A mini-review on Newcastle disease virus in Egypt, with particular references to common vaccines and their development. *Zagazig Veterinary Journal,. 50*(1): 19-36,https://doi.org/10.21608/zvjz.2022.104225.1161.

7. Miller, P.J., E.L. Decanini, and C.L. Afonso, 2010 Newcastle disease: evolution of genotypes and the related diagnostic challenges. I*nfection, genetics and evolution,. 10*(1): 26-35, https://doi.org/10.1016/j.meegid.2009.09.012.

8. Mao, Q., et al., 2022 Review detection of Newcastle disease virus. *Frontiers in Veterinary Science,. 9:* 936251, https://doi.org/10.3389/fvets.2022.936251.

9. Alazawy, A.K. and K.S. Al Ajeeli, 2020. Isolation and molecular

identification of wild Newcastle disease virus isolated from broiler farms of Diyala Province, Iraq. *Veterinary World, 13*(1): 33,doi: www.doi.org/10.14202/vetworld.2020.33-39.

10. Chambers, P., N.S. Millar, and P.T. Emmerson, 1986. Nucleotide sequence of the gene encoding the fusion glycoprotein of Newcastle disease virus. *Journal of General Virology, 67*(12):. 2685-2694, https://doi.org/10.1099/0022-1317-67-12-2685.

11. Lu, X., et al., 2024 The haemagglutinin–neuraminidase protein of velogenic Newcastle disease virus enhances viral infection through NF-κB-mediated programmed cell death. *Veterinary Research,. 55*(1): 58,https://doi.org/10.1186/s13567-024-01312-y.

12. Ahmed ,A. and S.M. Odisho, 2018 Isolation identification and pathotyping of Newcastle disease viruses form naturally infected chickens in Iraqi Kurdistan Region. I*raqi Journal of Agricultural Sciences,. 49*(1), https://doi.org/10.36103/ijas.v49i1.216.

13. Al-Shammari, A.M., et al., 2020 Clinical, molecular and cytopathological characterization of a Newcastle disease virus from an outbreak in Baghdad, Iraq. *Veterinary Medicine and Science,. 6*(3): 477-484, https://doi.org/10.1002/vms3.262.

14. Mase, M. and K. Kanehira, 2012 Simple differentiation of avirulent and virulent strains of avian paramyxovirus serotype-1 (Newcastle disease virus) by PCR and restriction endonuclease analysis in Japan. *Journal of Veterinary Medical Science,. 74*(12): 1661-1664,https://doi.org/10.1292/jvms.12-0178.

15. Balachandran, P., et al., 2014. Isolation and characterization of Newcastle disease virus from vaccinated commercial layer chicken. *Veterinary World, 7*.(7)

16. Dimitrov, K.M., et al., 2019 Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. Infection, *Genetics and Evolution,. 74:* p. 103917, https://doi.org/10.1016/j.meegid.2019.103917.

17. Kant, A., et al., 1997 Differentiation of virulent and non-virulent strains of Newcastle disease virus within 24 hours by polymerase chain reaction. *Avian pathology,. 26*(4): 837-849, https://doi.org/10.1080/03079459708419257.

18. DJ., A., 1977:Newcastle disease and other paramyxovirus infections. Diseases of Poultry, Iowa State University Press, Ames, Iowa;, 541-569,https://doi.org/10.20506/rst.19.2.1231.

19. Spackman, E., et al., 2013Optimal specimen collection and transport methods for the detection of avian influenza virus and Newcastle disease virus. *BMC veterinary research, :9* .1-12, https://doi.org/10.1186/1746-6148-9-35.

20. Cattoli, G., et al., 2011 Newcastle disease: a review of field recognition and current methods of laboratory detection. *Journal of veterinary diagnostic investigation,. 23*(4): p. 637-656, https://doi.org/10.1177/104063871140 7887.

21. Bello, M.B., et al., 2018 Diagnostic and vaccination approaches for Newcastle disease virus in poultry: The current and emerging perspectives. *BioMed research international, (1)*:. 7278459, https://doi.org/10.1155/2018/7278459.

22. Jaganathan, S., et al., 2015. Observation of risk factors, clinical manifestations and genetic characterization of recent Newcastle Disease Virus outbreak in West Malaysia. *BMC veterinary research, 1*1: 1-9, https://doi.org/10.1186/s12917-015-0537-z.

23. Eid, A.A., et al., 2022Newcastle disease genotype VII prevalence in poultry and wild birds in Egypt. *Viruses,. 14*(10): 2244, https://doi.org/10.3390/v14102244.

24. Esaki ,M., et al., 2013.Protection and antibody response caused by turkey herpesvirus vector Newcastle disease vaccine. *Avian diseases, 57*(4): 750-755, https://doi.org/10.1637/10540-032613-reg.1.

25. Alexander, D. and D. Senne, 2003, Newcastle disease, other avian paramyxoviruses, and pneumovirus infections .Diseases of Poultry. Ames, IA: Iowa State Press, https://doi.org/10.1002/9781119421481.ch3.

26. Bano, S., K. Naeem, and S. Malik, 2003 Evaluation of pathogenic potential of avian influenza virus serotype H9N2 in chickens. *Avian diseases,. 47*(s3): 817-822., https://doi.org/10.1637/0005-2086-47.s3.817.

27. Wise, M.G., et al., 2004. Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. *Journal of clinical microbiology, 42*(1): 329-338, https://doi.org/10.1128/jcm.42.1.329-338.2004.

28. Gravel, K.A. and T.G. Morrison, 2003.Interacting domains of the HN and F proteins of Newcastle disease virus. Journal of *Virology, 77*(20):11040-11049, https://doi.org/10.1128/jvi.77.20.11040-11049.2003.

كشف وتشخيص فيروس Orthoavulovirus-1 في الدجاج اللاحم في وسط وجنوب العراق

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الخلاصة

تبحث هذه الدراسة في وجود وتحري فيروس Orthoavulovirus-1 (AOAV-1) ، المعروف سابقاً باسم avian paramyxovirus-1، في الدجاج اللاحم العراقي. مرض نيوكاسل، وهو مرض دواجن شديد العدوى ومهم اقتصاديًا، يسببه AOAV-1 . تم جمع 250 عينة من 6 محافظات عراقية. سريريا، أشارت 150 عينة إلى مرض نيوكاسل. باستخدام تفاعل البوليميراز المتسلسل والفحص السريري، تم فحص الفيروس في 150 عينة من دجاج اللاحم من مزارع شمال ووسط وجنوب العراق والتي تشير سريرياً إلى مرض النيوكاسل. وفقًا للنتائج كانت (50) 33.3% من العينات التي تم اختبارها إيجابية للكشف عن تفاعل البوليميراز المتسلسل باستخدام جينات F و HN. إن نتائج هذه الدراسة قد يكون لها تأثير في استراتيجيات مكافحة الأمراض المحلية وتعزز فهمنا لحدوث AOAV-1 في الدواجن العراقية.

الكلمات المفتاحية: كشف, تشخيص, العراق, الدجاج اللاحم.