Tikrit Journal of Veterinary Sciences (2021) 1(0): 1-8.

IRAQI Academic Scientific Journals

TJVS Tikrit Journal of Veterinary Sciences

Tikrit Journal of Veterinary Sciences

Genetic Diagnosis and Histological Effects of Newcastle Disease Virus in liver and spleen of broiler chickens in Tikrit city

Saad T. Rasheed[1], Ismael I. Hasan[2], N.A. Jassim[2], A. Al-Jabbar M. H Aljoburi[2] , Mohammed K. Shakor[3]

[1]Department of Public Health, College of Veterinary Medicine, Tikrit University, Tikrit, Iraq.

[2]Department of Pathology and Poultry diseases, College of Veterinary Medicine, Tikrit University, Tikrit, Iraq.

[3]Slah-adin Educational Veterinary Hospital, Tikrit, Iraq.

ARTICLE INFO.

Article history:
-Received: 3 / 4 / 2021
-Accepted: 22 / 6 / 2021
-Available online:

Corresponding Author:
Name: Ismael I. Hasan
E-mail: Ismailhasa@tu.edu.iq
Tel: : +9647717908910

Keywords: Virus, Newcastle disease, poultry, liver, spleen, pathological lesions

ABSTRACT

Newcastle Disease (ND) is a viral disease that infects poultry and causes serious losses in commercial flocks worldwide. The disease had different forms of outbreak in different regions, depending on the vilogenicity of the virus. Different ranges of mortality in commercial poultry flocks correlated with respiratory signs were reported in the area of study. The aim of this study to identify the presence of NDV depending on HN gene at Tikrit city with the histopathological effect of the virus in spleen and hepatic tissues. During the period of June –July 2018, Twenty-five broiler chicken tissue (Liver and Spleen) samples of each farm were collected from two farms at Tikrit city. Clinical signs and postmortem lesions were recorded, then the samples were sent for molecular and histological study. Chickens showed signs of ND included gasping, lacrimation with postmortem lesions of inflammation. The presence of ND virus was confirmed in hepatic and spleen tissue using the conventional Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test. Histopathological study results showed signs of inflammation, degeneration, and necrosis in the liver and spleen. The study concludes that the ND virus was positively found in poultry and causes clear pathological lesions in hepatic and splenic tissues.

1.Introduction

Newcastle disease (ND) one of the most contagious high mortality rate diseases in the poultry industry [1]. ND virus also called avian paramyxovirus type 1 (APMV-1), is a member of the Paramyxoviridae family, Avulavirus genus [2]. It causes severe pathological and economic losses, even in vaccinated farms [3]. The non-segmented single-stranded, negative-sense RNA genome of the virus [4] consists of six genes encoded six proteins. The nucleoprotein (NP), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin-

neuraminidase (HN), and the large protein (L) [5].

According to location and severity of clinical signs, the disease classified into five categories included (i) viscerotropic with intestinal lesions (ii) neurotropic velogenic with a neurologic lesion (iii) mesogenic respiratory with low mortality (iv) lento- genic with mild respiratory signs (v) asymptomatic enteric within apparent signs [6]. NDV is also classified into two strains named class I and class II, according to genome sequence analysis [7]. Class I include the avirulent serotype of wild waterfowl, while class II represents the virulent strains of the virus that include about 18 genotypes [8]. According to the virulence used HN protein for NDV classification in which virulent strain count 571 amino acids and 616 amino acids in lentogenic strains [9, 10]. ND virus, as an enveloped virus, enters the cell by envelope fusion of the virus with the plasma membrane in which the nucleocapsid translocates into the host cell [11]. The virus fusion process occurred by F protein and its cleavability important in virus infectivity. However, it does not determine the virulence of a strain, which appears to be depending on differences in amino acids of HN protein [12]. The HN protein located on the outer surface of virions and infected cells allows the F protein to be in close contact and penetrate the host cell membrane as well as removing the sialic acid from the race of virus so as not to agglutinate with each other[13, 14]. Several systemic pathological changes are caused by the ND virus in variable organs [15]. The main changes include congestion with multi-focal necrosis, fibrin exudates, and accumulation of lymphocytes in the perivascular area of the spleen [16], while oxidative stress associated with virus replication leads to hepatic damage [17]. The aim of this study to identify the presence of NDV depending on HN gene at Tikrit city with the histopathological effect of the virus in spleen and hepatic tissues.

2. Materials and Methods:

Sample collection and histological preparation: tissues of the liver and spleen collected from suspected farms chickens stored at -85°C for molecular diagnoses while the whole organs kept in formaldehyde for one day then rinsed with tap water for two hours and placed in 70% ethanol solution before the samples completely processed [18].

RNA extraction of the virus: Viral RNA extracted by using TRIZOL® reagent (Qaigene-germany), 150 mg of tissues were smashed with liquid nitrogen and mortal the resulting powder then placed in 1.5 ml tube included QIAzol Lysis Reagent, the remaining protocol performed as described by the manufacture.

cDNA Preparation: cDNA prepared with two steps Reverse Transcriptase kit (Tonkbio-USA) 10 µg of RNA mixed with 1µl (20 pmol) of OligodT primer and 1µl (20 pmol) of Random primer mixed in 0.25 ml PCR tube the final volume made up to 12.5 µl, the mixture incubated in a thermocycler at 65°C. After 5 minutes, the tubes spanned and placed in ice and the flowing ingredient added primarily 4 µL of 5 × Reaction buffer added before 0.5 µLRNase Inhibitor, 2 µLdNTP mix (10 mM each), and 1 µL of TonkBioTM M-MLV (200 U/ µL) added. The final mixture was placed in the thermocycler. The program was applied as flow 25°C for 5 minutes, 42°C for 1 hour, and 70°C for 5 minutes according to the manufacturer's protocols.

Amplification of HN gene with PCR: One pair of primers (FW NDV-ACA CAT GCC CCG ATA AGC AA; RV NDV-CAT TGT CCC GAA GAC CCC TC) used in this study (Al-shammari *et al*., 2014), targeting HN gene to amplify PCR product about 499 bp. The reaction in thermocycler was subjected to one cycle of initial denaturation at 92°C for 60s, flowed by 40 cycles of denaturation at 92°C for 20s, annealing at 48.5°C for 20s, extension 72°C for 30s, and final extension of 72°C for 5 minutes. Electrophoresis of PCR products occurred in 2% agarose stained with ultraviolet stain (Redsafe®-USA), and were documented by using the gel documentation system.

3. Results and discussion:

Poultry farms in Tikrit city continuously show signs of ND infection-causing economic losses, although ND vaccination routinely achieved during first-week olds in most local farms

vaccination programs. For confirming that NDV was in affected chickens, a RT-PCR test was performed using a primer designed by Iraqi researchers tested on two local virus strains (Najaf APMV1/ Chicken/ Iraq-najaf/ ICCMGR/2012 and Baghdad ICCMGR) amplify a piece of NDV HN gene [19]. Tissue samples of trachea and spleen collected from suspected broiler farms were subjected to one-step RT-PCR. The test showed positive results depending on the HN gene. The primer successfully amplifies the specific band of 499 base pairs of HN gene that were observed after horizontal gel electrophoresis (Fig-1). Another band also observed clearly in the current study not appeared at the same density in the primer designer study. The band consisted of 180 base pairs, which suggests the presence of a new strain with deletion mutation between the sites that the primer-linked.

Infection with NDV stills the common cause of the outbreak in both backyards and commercial flocks [20]. Recent studies perform a diagnosis of ND depending on the causative agent genome using RT-PCT technique [21]. The molecular results of RT-PCR in this paper agreed with the research of primer designers, especially in the presence of 499 bp band, but not the 180 bp [19]. Unfortunately, not all bands clearly visible in the research, the appearance of DNA smear and non-specific bands neglected and not mentioned in the designer research and may consider as primer dimer [22]. While the selection of primer specific to the intending target important for further nucleic acid applications[23].

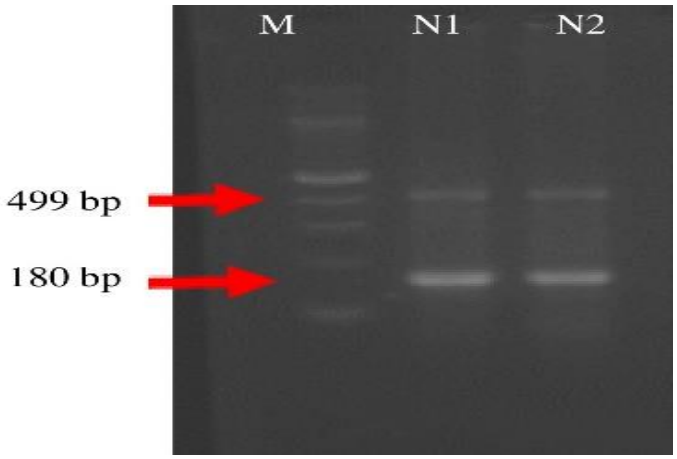

Figure 1: Agarose gel (2%) electrophoresis of PCR product (250v/35 minute) showing the amplified region of HN gene using Al-Shammari *et. al.* primer. Lane M: ladder marker (100-1000). Lane N1: Spleen sample, Lane N2: liver sample

Hepatic tissue (Figure-2) showed pathological signs of inflammation, degeneration, and necrosis, in which the parenchyma of the liver was demonstrating liver cells in groups. In the form of clusters (a), these cells were containing spherical nuclei (b), and the whole groups were surrounded by blood sinusoids (c). In comparison with infected organ, the study shows that liver (Figure-3) cells were demonstrated as atrophy, scattered from each other (a); also, degeneration was shown for certain liver cells (b), the blood sinusoids were containing a residue of the degenerated cell (c). The portal vein (Figure-4) engorged with a huge number of WBC (a), and the endothelium of the vein was thick (b). The central vein (Figure-5) included partial congestion of RBC with thickening of the central vein wall (a), partial congestion with R.B.C within the central vein (b). The periphery of liver tissue revealed necrosis of a certain number of cells which appeared as caralte because of the presence of NDV in the liver (a) (Figure-6).

Histological results showed that the pathological effect of NDV in hepatic tissue was included several stages of cell injury and inflammation; researchers attribute these lesions to the alteration in the pro-antioxidant status caused by the virus (Venkata Subbaiah *et al.*, 2015;Ravendra Babu *et al.*, 2012). The

oxidation role is well known in the pathological effect of viruses [25]. NDV, also another virus, causes hepatic damage by the same major mechanism of oxidative stress by the production of superoxide radical and Xanthine oxidase [17]. The viral oxidation causes serious damage by distraction to the proteins of aromatic and cysteine-rich amino acids with the content of busted phagocytic cells [26]. Cell death during viral infection is also reported as a result of mitochondrial damage, which could prevent by the supplementation of antioxidants [27].

Figure 2: Healthy liver section; hepatocytes as groups "clusters" (a), hepatocytes nuclei (b), blood sinusoids (c). (H&E x40).

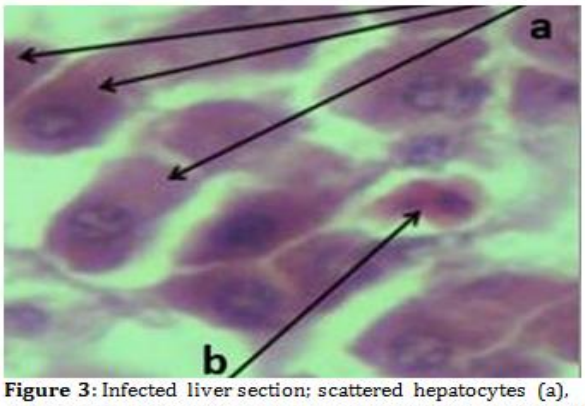

Figure 3: Infected liver section; scattered hepatocytes (a), degenerated hepatocytes (b), sinusoid containing degenerating hepatocytes (c). (H&E x40).

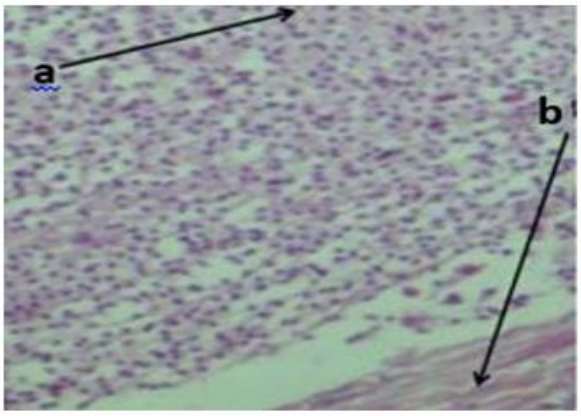

Figure 4: Infected liver section; engorgement portal vein with large number of W.B.C. (a), thickening of portal vein wall (b). (H&E x10).

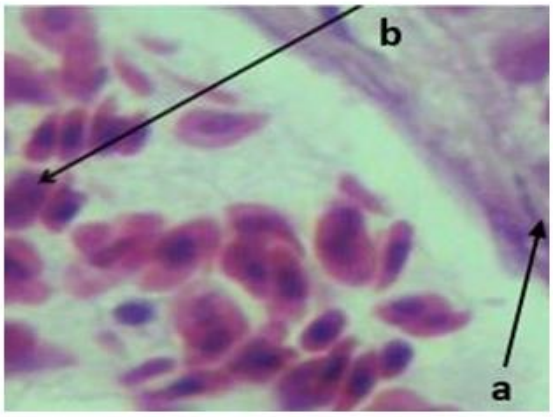

Figure 5: Infected liver section; thickening of central vein (a), partial congestion with R.B.C. within central vein (b). (H&E x40).

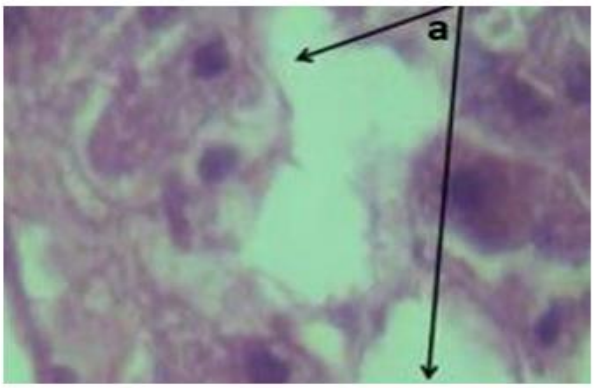

Figure 6: Infected liver section; Hepatocytes necrosis (a). (H&E x40).

The parenchyma of the spleen included megakaryocyte (a), associated with other WBC in the white pulp (b) (Figure-7). The red pulp had blood with abundant lymphocyte (Figure-8). The capsule of the spleen appeared normal (a), but the tissue of the nearby spleen capsule demonstrated extensive necrosis of cell (b) (Figure-9). The demarcation between the red and white pulp was apparent (a), as well as the spherical forms of white pulp (Figure-10). In comparing with the infected organ, the study shows that the parenchyma of the spleen in both white and red pulps included a mass of cells with degeneration (b). In contrast, the splenic artery revealed a prominent thickening in its wall (a) (Figure-11). The results also showed that the parenchyma of the spleen intensively engorged with lymphocytic aggregation in both red and white pulps, which appeared as a plug (a) or packed groups with the present thickened wall of the splenic artery (b) (Figure-12).

The histological section of the spleen showed engorgement, thickening of the artery with depletion of cells, and necrosis with abundant lymphocytes. Researches agreed with previous results showed that the cytokines and chemokines inflammatory mediators guided by viral load play a vital role in the regulation of inflammatory cells activity leading to tissue damage; furthermore, they found that gene regulation of mediators depends on the type of viral strain and type of tissue [28]. Other researchers suggest that there are different regulation responses between two chicken lines and one line of breed more resistant than the other one [29]. Previous studies revealed that different virus genotype showed different pathological lesions, and genotype VIId of NDV have more potency in splenic tissue [30, 31]. Reports also associate the M, F, and HN genes of genotype VIId of NDV with severe pathological changes as results of strong innate immunity in the spleen of chicken [32].

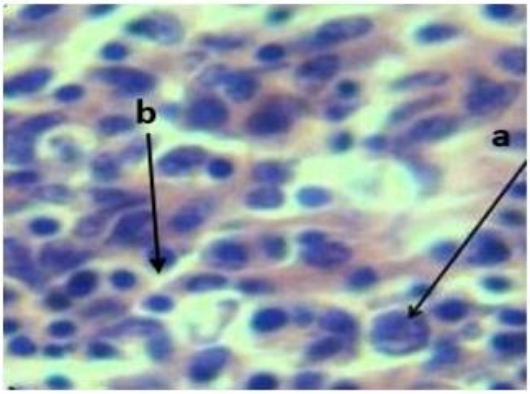

Figure 7: Healthy spleen section; megakaryocyte (a), W.B.C (b). H&E x40.

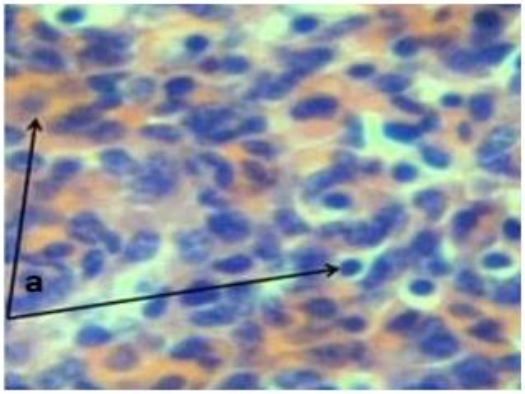

Figure 8: Healthy spleen section; Red blood cells and W.B.C (a). H&E x40.

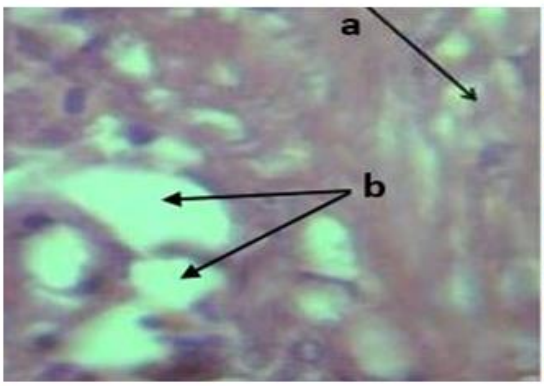

Figure 9: Infected spleen section; normal capsule (a), necrotic cells (b). H&E x40.

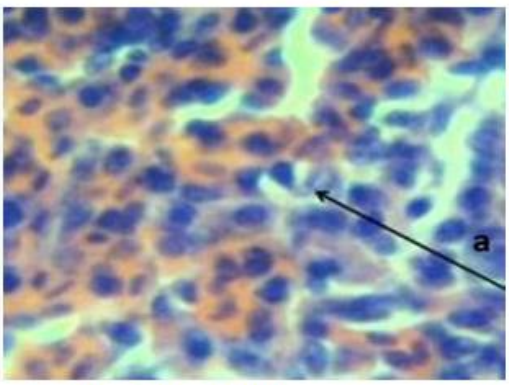

Figure 10: Healthy spleen section; demarcation between red and white pulp (a). H&E x40.

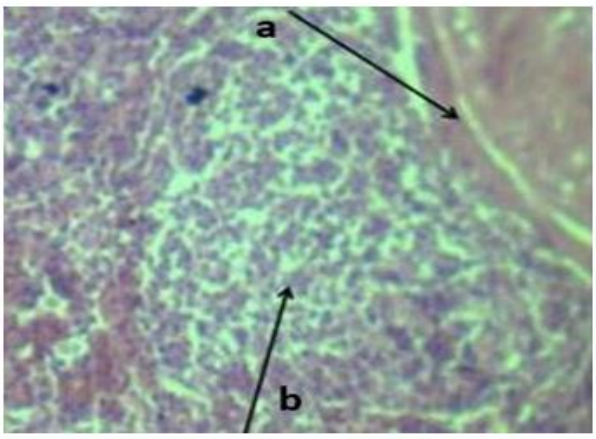

Figure 11: Infected spleen section; thickening of splenic artery (a), degeneration of the cells in white and red pulp. H&E x10.

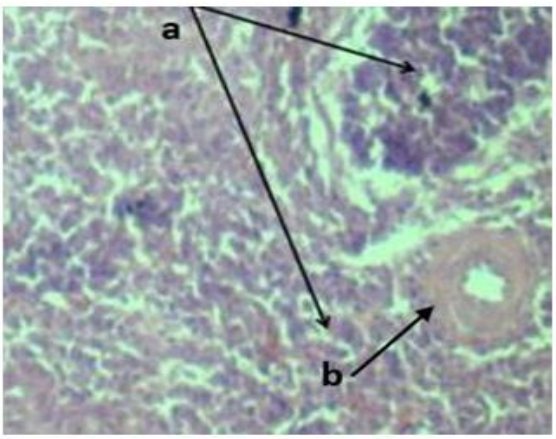

Figure 12: Infected spleen section; engorgement of red and white pulp with lymphocytes as a plugs (a), thickening of splenic artery (b). H&E x40.

Conclusion

The NDV was the causative agent that causes respiratory problems in the affected chickens. The virus also causes a serious problem because of its effect on both the spleen and hepatic tissues. Precaution techniques and more researches on molecular and immunological sections must be developed to protecting the local and global poultry industry from this virus.

Acknowledgments:

We have to thank the College of Veterinary Medicine, and Central Laboratory of Tikrit University for them technical and supporting, and also we thank Salahadin Veterinary Teaching hospital for their cooperation.

References:

(1) Snoeck, C. J. et al. (2009). Newcastle disease virus in West Africa: New virulent strains identified in non-commercial farms, *Archives of Virology*, 154 (1): 47–54.

(2) Mayo, M. A. (2002). Virus taxonomy - Houston 2002., *Archives of Virology*, 147 (5): 1071–1076.

(3) Awan, M. A., Otte, M. J., and James, A. D. (1994). The epidemiology of Newcastle disease in rural poultry: A review, *Avian Pathology*, 23 (3): 405–423.

(4) Domingo, E. (1997). Rna Virus Mutations, *Annual Review of Microbiology*, 51 151–178.

(5) Liu, M. et al. (2015). Characterization and sequencing of a genotype VIId Newcastle disease virus isolated from laying ducks in Jiangsu, China, *Genome Announcements*, 3 (6): 3–4.

(6) ox, J. G. et al. (2015). *Laboratory Animal Medicine American College of Laboratory Animal Medicine 3rd Series*

(7) Dimitrov, K. M. et al. (2019). Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus, *Infection, Genetics and Evolution*, 74: 103917.

(8) Snoeck, C. J. et al. (2013). High genetic diversity of newcastle disease virus in poultry in west and central Africa: Cocirculation of genotype XIV and newly defined genotypes XVII and XVIII, *Journal of Clinical Microbiology*, 51 (7): 2250–2260.

(9) Yuan, P. et al. (2012). Structure of the Ulster Strain Newcastle Disease Virus Hemagglutinin-Neuraminidase Reveals Auto-Inhibitory Interactions Associated with Low Virulence, *PLoS Pathogens*, 8 (8): 1-14.

(10) Dhaygude, V. S. et al. (2017). Molecular characterization of velogenic viscerotropic Ranikhet (Newcastle) disease virus from different outbreaks in desi chickens, *Veterinary World*, 10 (3): 319–323.

(11) Rey, F. A., and Lok, S. M. (2018). Common Features of Enveloped Viruses and Implications for Immunogen Design for Next-Generation Vaccines, *Cell*, 172 (6): 1319–1334.

(12) Huang, Z. et al. (2004). The Hemagglutinin-Neuraminidase Protein of Newcastle Disease Virus Determines Tropism and Virulence, *Journal of Virology*, 78 (8): 4176–4184.

(13) Jin, J. H. et al. (2017). Different origins of newcastle disease virus hemagglutinin-neuraminidase protein modulate the replication efficiency and pathogenicity of the virus, *Frontiers in Microbiology*, 8 (1): 1–17.

(14) Sun, J. et al. (2017). Chicken galectin-1B inhibits Newcastle disease virus adsorption and replication through binding to hemagglutinin–neuraminidase (HN) glycoprotein, *Journal of Biological Chemistry*, 292 (49): 20141–20161.

(15) Etriwati et al. (2017). Pathology and immunohistochemistry study of Newcastle disease field case in chicken in Indonesia, *Veterinary World*, 10 (9): 1066–1071.

(16) Susta, L. et al. (2014). Separate evolution of virulent Newcastle disease viruses from Mexico and Central America, *Journal of Clinical Microbiology*, 52 (5): 1382–1390.

(17) Venkata Subbaiah, K. C. et al. (2015). Newcastle disease virus (NDV) induces protein oxidation and nitration in brain and liver of chicken: Ameliorative effect of Vitamin E, *International Journal of Biochemistry and Cell Biology*, 64 97–106.

(18) Suvarna, K. S., Layton, C., and Bancroft, J. D. (2013). *Bancroft's Theory and Practice of Histological Techniques*, (K. S. Suvarna, C. Layton, & J. D. Bancroft, Eds.) (seventh.), Churchill Livingstone, China

(19) Al-shammari, A. M., Al-nassrawei, H. A., and A.M.H. Murtadha. (2014). Molecular diagnosis of Newcastle disease Iraqi Virulent strain virus HN gene by specific primers design., *Kufa J. Vet. Med. Sci.*, 5 (2): 196–203.

(20) Dimitrov, K. M. et al. (2019). Pathogenicity and transmission of virulent Newcastle disease virus from the 2018–2019 California outbreak and related viruses in young and adult chickens, *Virology*, 531 : 203–218.

(21) Triosanti, L. S., Wibowo, M. H., and Widayanti, R. (2018). Molecular characterization of hemagglutinin-neuraminidase fragment gene of Newcastle disease virus isolated from periodically-vaccinated farms, *Veterinary World*, 11 (5): 657–666.

(22) Brownie, J. et al. (1997). The elimination of primerdimer, *Nucleic Acids Research*, 25 (16): 3235–3241.

(23) Vallone, P. M., and Butler, J. M. (2004). AutoDimer: A screening tool for primer-dimer and hairpin structures, *BioTechniques*, 37 (2): 226–231.

(24) Ravendra Babu, K. et al. (2012). Identification of substituted [3, 2-a] pyrimidines as selective antiviral agents: Molecular modeling study, *Antiviral Research*, 95 (2): 118–127.

(25) Kavouras, J. et al. (2007). Herpes simplex virus type 1 infection induces oxidative stress and the release of bioactive lipid peroxidation by-products in mouse P19N neural cell cultures, *Journal of NeuroVirology*, 13 (5): 416–425.

(26) Maeda, H., and Akaike, T. (1991). Oxygen Free Radicals as Pathogenic Molecules in Viral Diseases, *Proceedings of the Society for Experimental Biology and Medicine*, 198 (2): 721–727.

(27) Chang, C. W. et al. (2011). Betanodavirus induces oxidative Stress-Mediated cell death that prevented by Anti-Oxidants and zfcatalase in fish cells, *PLoS ONE*, 6 (10):.

(28) Rasoli, M. et al. (2014). Alteration in lymphocyte responses, cytokine and chemokine profiles in chickens infected with genotype VII and VIII velogenic Newcastle disease virus, *Comparative Immunology, Microbiology and Infectious Diseases*, 37 (1): 11–21.

(29) Zhang, J. et al. (2018). Transcriptome analysis in spleen reveals differential regulation of response to newcastle disease virus in two chicken lines, *Scientific Reports*, 8 (1): 1–13.

(30) Wang, Y. et al. (2012). Lack of detection of host associated differences in Newcastle disease viruses of genotype VIId isolated from chickens and geese, *Virology Journal*, 9 (1): 1.

(31) Hu, Z. et al. (2015). High levels of virus replication and an intense inflammatory response contribute to the severe pathology in lymphoid tissues caused by Newcastle disease virus genotype VIId, *Archives of Virology*, 160 (3): 639–648.

(32) Kai, Y. et al. (2015). The M, F and HN genes of genotype VIId Newcastle disease virus are associated with the severe pathological changes in the spleen of chickens, *Virology Journal*, 12 (1): 1–10.

Tikrit Journal of Veterinary Sciences (2021) 1(0): 1-8.

تشخيص جزيئي و دراسة نسجية لتأثير مرض فيروس نيوكاسل في كبد و طحال الدجاج اللاحم في مدينة تكريت

سعد توفيق رشيد [1]، اسماعيل ابراهيم حسن[2]، نوار علي جاسم[2]، عبد الجبار محمد الجبوري[2] ، محمد خورشيد شكور[3]

[1] فرع الصحة العامة – كلية الطب البيطري- جامعة تكريت – تكريت - العراق
[2] فرع الامراض وامراض الدواجن – كلية الطب البيطري- جامعة تكريت– تكريت - العراق
[3] مستشفى صلاح الدين البيطري التعليمي – تكريت - العراق

الخلاصة

مرض نيوكاسل هو مرض فيروسي يصيب الدجاج ويؤدي الى خسائر اقتصادية كبيرة في حقول الدجاج التجارية حول العالم. يحتوي المرض على عدة اشكال من الاوبئة في مختلف الاماكن والتي تعتمد على ضراوة الفيروس نفسه. ان وجود طيف مختلف من الهلاكات في قطعان الدجاج اللاحم التجاري ترتبط مع العلامات السريرية للمرض في منطقة الدراسة، لذا فان الدراسة هدفت الى تأكيد وجود الفيروس في قطعان الدجاج اللاحم و دراسة تأثيره المرضي على انسجة الكبد و الطحال. خلال الفترة حزيران-تموز 2018، جمع خمسة وعشرون عينة نسجيه من كبد وطحال الدجاج في مدينة تكريت. وتم عمل تشخيص جزيئي للنماذج مع دراسة نسجيه. اظهرت الدواجن علامات سريرية لمرض نيوكاسل شملت لهاث وسوائل عينية. بينما اظهرت العلامات التشريحية التهابات حادة ومتوسطة. تم تأكيد وجود فيروس مرض نيوكاسل باستعمال تقنية تفاعل البلمرة المتسلسل الناسخ التقليدي. بينت الدراسة النسجية وجود علامات التهابية وتنكس خلوي في انسجة الكبد والطحال. تستنتج الدراسة ان فيروس مرض نيوكاسل يوجد فعلا في منطقة الدراسة و له تأثير مرضي واضح.