

Histopathological and Immunohistochemical Study of Newcastle Disease in Chicken in Al- Najaf Province

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Abstract

The aim of the present study was histopathology examination and the distribution of Newcastle disease virus(NDV) in internal organs of broiler chickens from field case using immunohistochemistry staining. thirty female and male broiler chicken were collected from different poultry fields in Al-Najaf province. Spleen, lung, intestine, liver, kidney were removed for general histopathological examination and ribonucleoprotein expression measured by immunohistochemistry technique. The present study investigated the gross lesions in euthanized and dead chickens have been mainly hyperemia, liver enlargement , spleen enlargement, lung congestion and kidney congestions. The histopathological results showed pathological changes in various organs and over ribonucleoprotein was observed. In conclusion, Newcastle disease causes various histopathological changes in all examined organs, Also over expression of ribonucleoprotein in these organs.

Keyword: Chicken, Newcastle disease, broiler, Ribonucleoprotein, IHC.

Introduction

Newcastle sickness (ND) is an critical avian disease in Iraq that reason large loss in poultry industry.

The disease is spreading hastily; each morbidity or mortality of ND virus (NDV) reached one hundred percent ⁽¹⁾. NDV is a member of the genus *Avulavirus* in the family *Paramyxoviridae*. The

genome of NDV is RNA virus nonsegmented, single-stranded, negative-sense of 15,186 nucleotides (nt) ⁽²⁾. The genome carries at the least six genes, which encode the nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN), and massive RNA-based RNA polymerase protein (L). two additional proteins, V and W, can be produced by RNA enhancing at some stage in P gene transcription ⁽³⁾. Most of the NP is for that reason really important for virus replication because of multifold engagement of NP within the assembly and biologic interest of the RNP. As well as, NPs of poor strand RNA viruses are extremely immunogenic in nature and were used as antigens for diagnostic purposes, along with the NP of rabies ⁽⁴⁾ and NDV ⁽⁵⁾.

primarily based on its virulence, NDV is classed into 4 agencies, which might be velogenic

viscerotropic characterised by acute deadly infection with hemorrhage in the intestine, velogenic neurotropic characterised by means of breathing and nerve lesion without intestinal lesion with excessive mortality, mesogenic characterized by using breathing and nerve lesion with low mortality, and lentogenic characterised by asymptomatic signs occur in the gut ^(6,7). NDV is broadly spread through direct contact between healthy birds and the discharges of infected birds. The transmission of disease through infected birds droppings and secretions from the nose, mouth, and eyes. body discharges of the NDV infected birds containing high concentrations, consequently, the disease can be spread easily by mechanical means ⁽⁸⁾.

The powerful packages in disorder manipulate decrease the losses, but ND continues to be a big hazard to our chicken industry in numerous forms. The rapid detection and differentiation of

virulent and avirulent lines in the fowl may be very crucial with a view to provoke suitable manage measures.

Early analysis of ND In subclinical infection, might be useful in sickness prevention programs further to right rescheduling of vaccination. Monoclonal antibodies of mouse in opposition to NDV which have been advanced in some research laboratories have substantial diagnostic importance. The ones MAbs are capable of detecting antigenically splendid pathotypes of NDV ^(7,8).

Outbreaks of ND are often pronounced from many regions of the sector, with the most intense ailment being resulting from virulent lines. America is taken into consideration free of virulent NDV traces, but once in a while pathogenic strains enter via wild birds. The ultimate outbreak of ND happened in California in 2002–2003 and ended in depopulation of more than three million birds and containment fees exceeding a hundred and sixty million dollars ⁽⁹⁾.

In field case, ND lesions are frequently without problems confused with different fowl diseases, therefore to ascertain whether or not the tissue lesions are because of NDV contamination or now not, studies about antigen distribution is vital in watching the life of NDV inside tissue. Immunohistochemistry staining can be used to provide an explanation for pathogenesis of ND in subject instances. In immunohistochemistry staining, an antibody reacting to a selected antigen is used to localize the antigen inside affected tissue, which will increase the accuracy of a analysis as it allows identity of antigens within tissues ⁽¹⁰⁾. The present study conducted to investigated the histopathological changes that induced by Newcastle disease virus. Also, the ribonucleoprotein expression was examined in ND infected chicken.

Materials and Methods

Study design

According to the clinical signs thirty female and male infected chicken of Newcastle disease observation were collected from fields in Najaf government. The 30 infected Newcastle disease chicken were euthanized and the spleen, lung, intestine, liver and kidney were removed and fixed by neutral buffered formalin ten percent for Histopathology examination and immunohistochemical technique. Ribonucleoprotein expression were measured using polyclonal antibody via immunohistochemical technique. Tissue samples were dehydrated, cleared, embedded in paraffin and cut into 1 cm × 1 cm × 0.5 cm histopathological section.

Microscopic observation

The visceral organs tissue like spleen, lung, intestine, liver and kidney were taken in order to measure expression of ribonucleoprotein via immunohistochemical staining and histological examination. This samples were collected in 10% neutral buffered formalin and processed by paraffin embedding technique then sectioning with automatic section cutting machine about 5-6 μ in thickness (Leica, Germany) and stained with haematoxylin and eosin (H and E)⁽¹¹⁾. After the H and E staining the observation of sections under the light microscope, and lesions were recorded. Intestine and trachea, lung tissues were stained with Alcian blue using Alcian blue solution.

Immunohistochemistry Examination

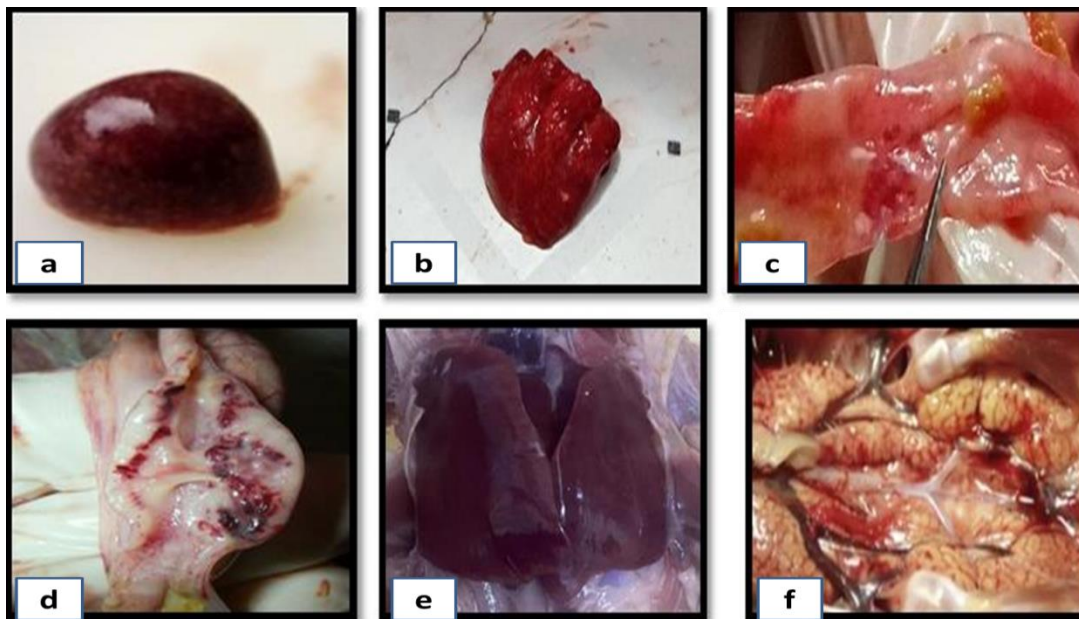
All suspected tissues have been examined by using IHC to discover viral ribonucleoprotein. In brief, after deparaffinization, tissue sections had been subjected to antigen retrieval by microwaving for 20 minutes at minimum energy in Vector antigen unmasking solution (Vector Laboratories, Burlingame, CA) accompanied via application of normal blocking reagent (Biogenex, San Ramon, CA) as advocated by using the manufacturer. The primary antibody made in rabbit was raised against a artificial nucleoprotein peptide (TAYETADESETRRIC) and used at 1:8000 dilution.

Results

Gross Lesions

The organs of NDV suspected chickens had been examined and pathological adjustments have been recorded. Lesions in Spleen in some cases showed moderately enlarged (figure 1,a), while the lungs (Figure 1,b) showed pulmonary hemorrhages, congestion, and consolidation. In the gut, lesions characterised by way of thickened, edematous and hemorrhagic at various sites of intestine (Figure 1,c). Cecal tonsils showed ecchymotic hemorrhages (Figure 1,d). Other visceral organ the liver showed congestion, and hemorrhage (Figure 1,f). and the kidneys showed swollen (Figure 1,e).

Figure 1: Gross pathological changes in organs of infected chickens with NDV: a. spleen of infected chickens shows enlarged and diffusely mottled spleen b. congestion, hemorrhages and consolidation of lung.c. multiple thickened, edematous button ulcer in intestinal mucosa. d. ecchymotichemorrhage and inflammation of Cecal tonsil.e. congestion and hemorrhages of liver.f.swollen kidney



Histopathology result

Necropsy made on a complete of 30 bird carcasses, gross and histopathology lesions of numerous ailment conditions have been found and their frequency of prevalence turned into recorded. Lesions of ND turned into constantly discovered in bird tested, this cases which have been tentatively diagnosed and final confirmation the use of immunohistochemistry.

Histopathology section of spleen revealed multifocal lymphocytic aggregation, hemorrhage and macrophages infiltration (Figure 2). While changes in lung showing necrosis, fibrosis, infiltration of mononuclear cells and ruptured alveoli (Figure.3). Lymphoid hyperplasia, mononuclear cell infiltration and congestion with sloughing of villi showed in intestine (Figure.4). Histopathologically, the multifocal inflammatory cell infiltration,

congestion and necrotic areas observed in the liver parenchyma (Figure.5). While kidney histology change a lack of tubular epithelial cells and an accumulation of cell particles within the lumen. and infiltration of inflammatory cells (Figure.6).

Distribution and predominance of goblet cells. It has been seen mainly among the columnar cells and supplied with the standard goblet form (figure 7) shows that increase the numbers of its cells and increase mucin production in the intestine segments were increased in the NDV-infected chicken.

Figure 2: Histopathological section of spleen of (NDV) infected chickens shows multifocal lymphocytic aggregation and macrophages infiltration (H&E, 10X).

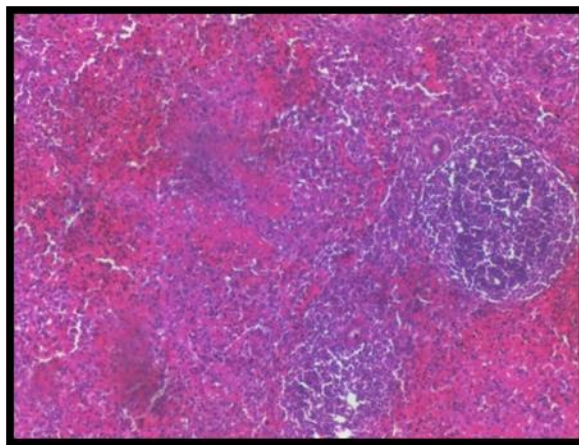


Figure 3: Histopathological section of lung of (NDV) infected chickens shows necrosis, fibrosis, infiltration of mononuclear cells and ruptured alveoli (H&E, 10X)

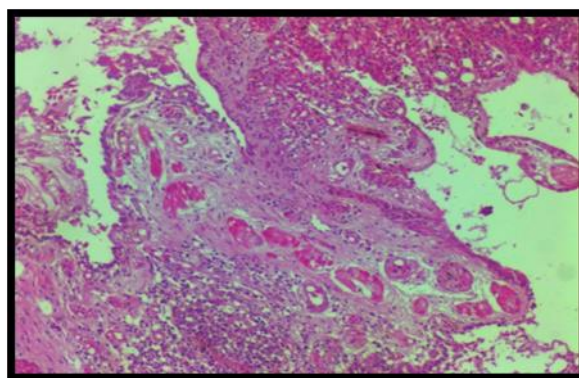


Figure 4: Histopathological section of Intestine of (NDV) infected chickens shows lymphoid hyperplasia Mononuclear cell infiltration and congestion with sloughing of villi. (H&E, 10X).

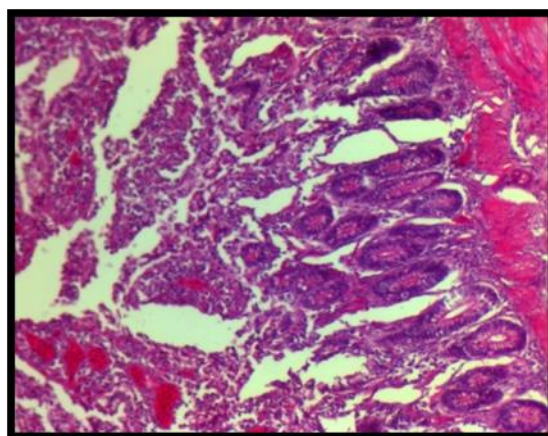


Figure 5:Photomicrograph of liver of (NDV) infected chickens showing multifocal infiltration of inflammatory cell, congestion and necrotic areas (H&E,10X).

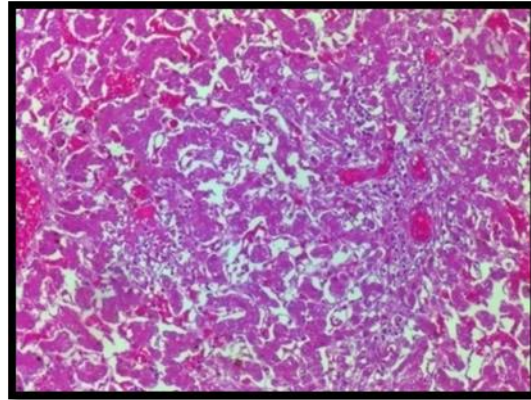


Figure 6:Histopathological section of kidney of (NDV) infected chickens showing a loss of tubular epithelium and waste accumulation within the lumen and infiltration of inflammatory cells (H&E ,10x)

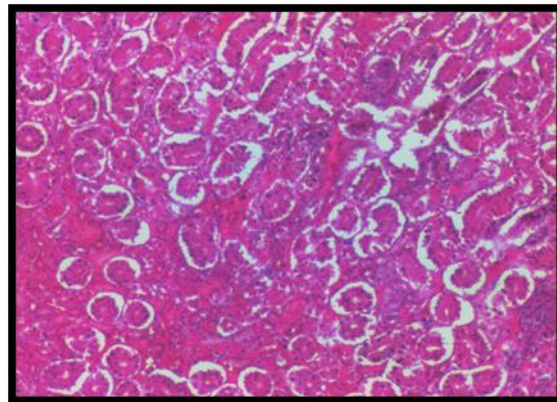
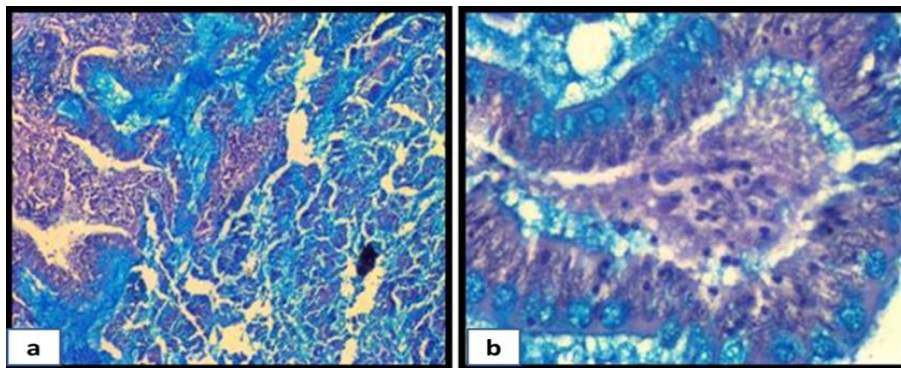


Figure 7:Histopathological section of the intestine (a,b)of chicken stained with Alcian blue-periodic acid-Shiff (ABPAS) showing Goblet cell production could be observed at every segment.



Imunohistochemistry of Newcastle infected tissue reveals high expression of Nucleoprotein but little/no Nucleoprotein

In this study, Examined the expression of Ribonucleoprotein in Newcastle infected tissue obtained from chicken spleen, lung, intestine, liver and kidney. IHC performed by using polyclonal antibodies. In the cells infected NDV, the immune reaction within the cytoplasm and finely to coarsely granular. Infected birds had the widest distribution of virus and the most intense signal. The organs with the powerful sign were the organs of lymphatic system, and the lymphoid aggregation in the mucosa of multiple organs. In those tissues, the positive cells were consist specifically from lymphocytes and macrophages. in the spleen, the

immunoreactivity became confined to the fixed-macrophage structured regions across the penicillary arteries, while the lymphocyte-established regions were without signal (Fig. 8). In the lung, the positive signal was restricted to scattered lymphoid aggregation strongly related with the bronchial tree(BALT) (Fig. 9). whereas in the intestines, immune reaction was also present within submucosa (Fig. 10). Kupffer cells Scattered in the liver (Fig. 11), and in the kidney, the positive signal was present of numerous cells of epithelium in the distal tubules (Fig. 12).

Figure8:Representative IHC images of a/ normal tissue, b/ staining of spleen tissue of ND infected chicken shows high expression of cytoplasmic ribonucleoprotein . Note NDV antigen indicated by the presence of brown precipitates in the cytoplasm (20X).

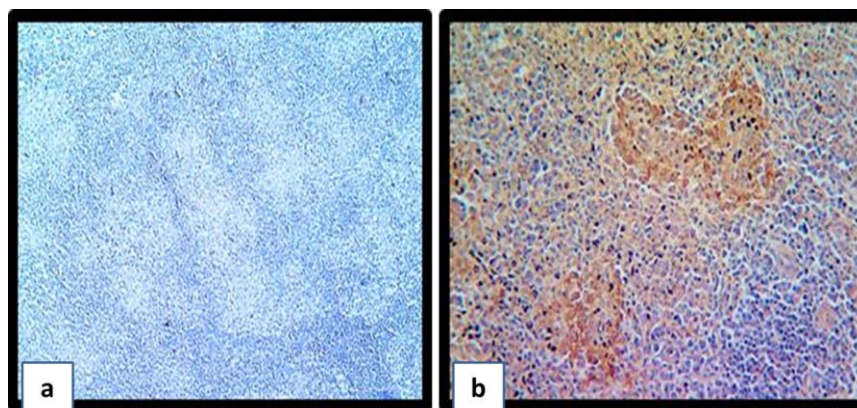


Figure9: Representative IHC images of a. normal tissue, b. staining of lung tissue of ND infected chicken shows high expression of cytoplasmic ribonucleoprotein. Note NDV antigen indicated by the presence of brown precipitates in the cytoplasm (20×).

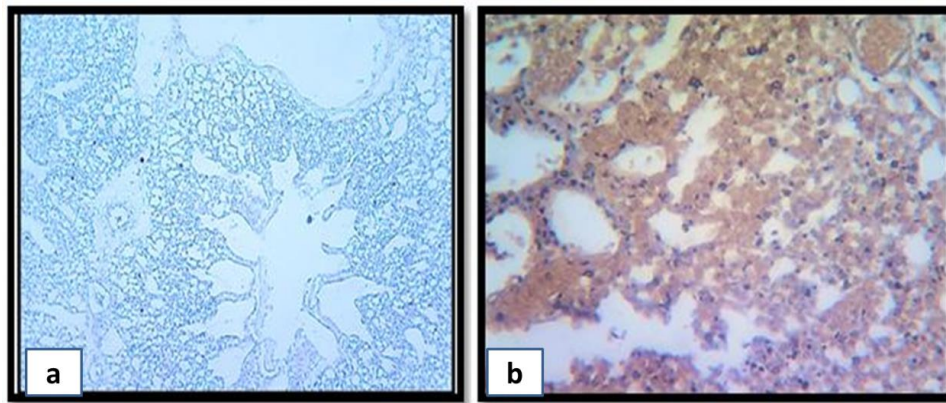


Figure 10: Representative IHC images of a. normal tissue, b. staining of intestine tissue of ND infected chicken shows high expression of cytoplasmic ribonucleoprotein. Note NDV antigen indicated by the presence of brown precipitates in the cytoplasm (20×).

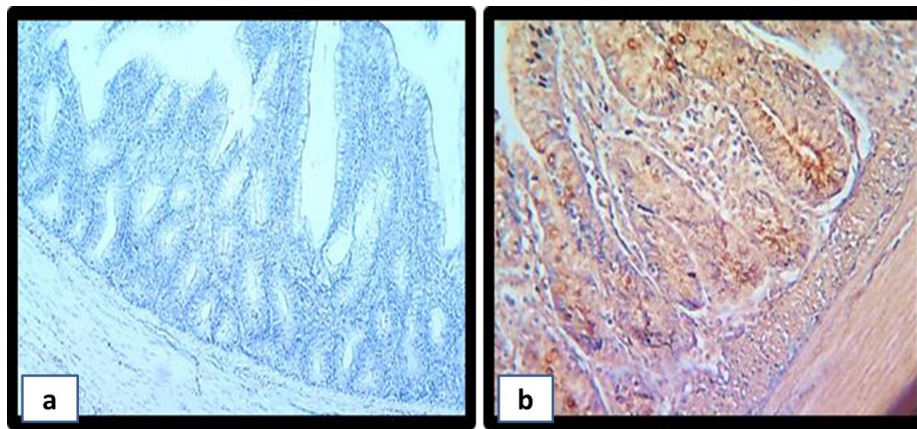


Figure 11: Representative IHC images of a. normal tissue, b. staining of liver tissue of ND infected chicken shows high expression of cytoplasmic ribonucleoprotein. Note NDV antigen indicated by the presence of brown precipitates in the cytoplasm (20×).

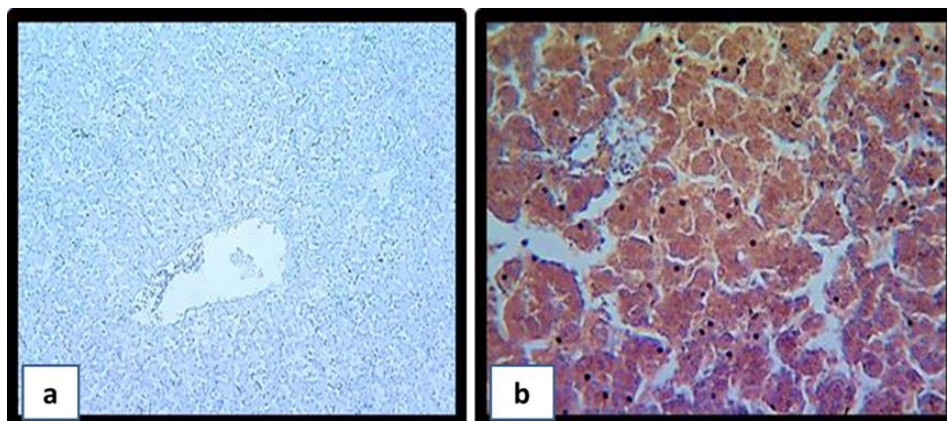
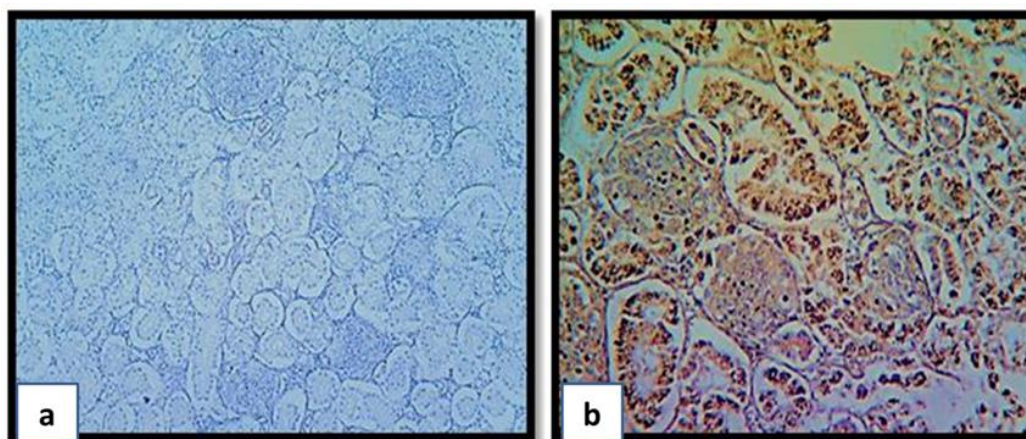


Figure 12: Representative IHC images of a. normal tissue, b. staining of tissue of ND kidney infected chicken shows high expression of cytoplasmic ribonucleoprotein. Note NDV antigen indicated by the presence of brown precipitates in the cytoplasm (20×).



Discussion

The diagnosis of ND suspected chicken by immunohistochemistry staining proves That this method can be used to diagnosing Newcastle Disease appropriately, speedy, and economically than other diagnostic methods. in current study the present gross lesion in the organs of chicken infected by NDV showed severe lesions that manifested as multifocal organ lesions. Mild hemorrhage, congestion was the most notable and frequent gross lesion seen in these organs agree with Dewidareta⁽¹³⁾. Also, based on the virulence, NDV has been divided into three types: mild lentogenic, moderate mesogenic, and severe velogenic forms, which can manifest as viscerotropic or neurotropic forms ⁽¹⁴⁾. The feathers of the malnourished birds were ruffled, and the vents were stained with greenish material⁽¹⁵⁾. NDV replication in the intestine lymphoid follicle results in internal organ bleeding and edema due to blood vessel damage. Ratih, et al⁽¹⁶⁾ and Bwala et al. have both reported on NDV distribution in internal organs ⁽¹⁷⁾.

The current results of the NDV Ag test, which confirmed the NDV, were consistent with earlier reports by Rakibul Hasan *et al*⁽¹⁸⁾ and the NDV in the current investigation. The main

present histopathological analysis revealed marked presence of necrosis, fibrosis, infiltration of mononuclear cells in the lung while Lymphoid hyperplasia, mononuclear cell infiltration and congestion with sloughing of villi showed in intestine. Similar observation found by ¹⁶.

Also there is multifocal inflammatory cell infiltration, congestion and necrotic areas observed in the liver parenchyma While kidney histology change a Loss of tubular epithelium and an waste accumulation within the lumen and infiltration of inflammatory cells. these result are concurrent with previous studies which reported gross lesions in poultry birds affected with viscerotropic velogenic ND⁽¹⁶⁻¹⁹⁾ However, the hemorrhage in the cecal tonsils due to the virus replication and viral entrance into the systemic circulation through these tonsils following nasopharyngeal inoculation^(20,21).

Respiratory tract is the main important entry of NDV, so the respiratory impairment was noticeable in every chicken. Virus adhere to the respiratory epithelium by sialic acid utilization on epithelial cell as receptor⁽²²⁾. The virus present on breathing epithelium will encourage regional immunity to

provide unique immunoglobulin A(IgA) antibody so that you can phagocyte virus. Immune reaction visible at the breathing tract by means of the presence of congestion and increase of mucous exudate secretion into the breathing airways, then the body try to virus antigen removal from respiration tract, protection the epithelial floor from virus adherence and invasion⁽¹¹⁾. Viremia and secondary bacterial infection that lead to circulatory disturbance which cause lung lesions⁽²³⁾.

Thinking about the preliminary position that goblet cells appear to play in host defense in opposition to enteric pathogens, we detect the response of these distribution and predominance of goblet cells that lead to increase mucin production in the intestine segments were increased in the NDV-infected chicken similar observation found by⁽²⁴⁾.

In the current study, NDV ribonucleoprotein are mainly localized within the cytoplasm of necrotic cells of the different organs, mainly in the lining epithelium of the pulmonary alveoli, bronchi, tubules of kidney, intestines, and liver hepatocytes, inflammatory cells. those findings are concurrent with previous research of their immunohistochemical take a look at of the pathogenesis of virulent viscerotropic ND in fowl, the team stated that ND virus triggered excessive damage to the lymphoid tissues. They greater stated that the finest quantity of viral antigen found become in small intestines, spleen. moreover, immunohistochemical labeling was restrained to large mononuclear cells. Newcastle disease diagnosis based on gross lesions, histopathology, immunohistochemistry and clinical history indicated^(27,28,29).

Conclusion

In conclusion the distribution pattern of thirty field samples used in this studies remains similar with preceding reported

Newcastle Disease virus distribution pattern which spread systemically in the internal organs of chicken. The degree of NDV severity was velogenic viscerotropic,. Highest NDV was present in the spleen, intestine lungs, liver and kidney.

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