CLINICAL, PATHOLOGICAL AND MOLECULAR STUDY OF NEWCASTLE DISEASE IN LAYERS FARMS IN BASRAH, IRAQ

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ABSTRACT

Newcastle disease (ND) is an important viral disease of poultry which is a major economic concern. It has world-wide distribution and affects a variety of avian species. The disease is characterized by marked variation in morbidity and mortality. The present study conducted to monitoring the incidence of ND in layer hen poultry houses which located in Al Zubair -Basrah governorate, during 6 month starting from September 2019 to March 2020. The study was depending on clinical examination, monitoring antibodies titer by ELISA, postmortem lesions, histopathological changes, and polymerase chain reaction (PCR). Different type of samples as proventriculus, lung, trachea, intestine, spleen, cecal tonsils ,oviduct and uterus, were collected from 6 poultry houses according to the clinically suspected diagnosis of ND. The results of the present study recorded the disease in layer hen . The positive clinical case showed significant clinical signs of ND as nervous signs and digestive signs. Sharp drop in eggs production, loss eggs pigmentations loss eggs quality. while observing the herd disappears respiratory signs. Post mortem changes recorded in reproductive ,digestive and nerves system. The sever changes on female reproductive system especially appear on ovarian follicles. Histopathological changes was in proventiculus, cecal, tonsils and spleen. The result of ELISA test during study period indicated high level during and post 7 days of infection .

INTRODUCTION

Newcastle disease (ND) is an important viral disease of poultry which is a major economic concern. It has world-wide distribution and affects a variety of avian species. The disease is characterized by marked variation in morbidity and mortality.

The first documented outbreaks of ND occurred in 1926 in Java Indonesia and Newcastle-upon-Tyne, England(1). Etiology of the ND is avian paramyxovirus serotype-1 (APMV-1). Transmission is fecal–oral route, with the respiratory route playing a role where there are close bird-to-bird associations(2). Newcastle disease virus (NDV) infects chickens, guinea fowls, turkeys, and a large number of species of domestic and wild birds Sea birds are less susceptible but may act as reservoir(3). On the basis of their virulence in chickens, lentogenic , mesogenic and velogenic strains are distinguished. Lentogenic strains cause mild respiratory or enteric infections, whereas mesogenic strains induce mainly acute respiratory signs .lentogenic and mesogenic strains are used as vaccines . Velogenic strains are responsible for severe disease and high mortality in poultry. Depending on main clinical signs, these velogenic viruses are further sub-divided into viscerotropic and neurotropic strains (4). Layers infected with vNDV may only present with a decrease in egg production 1 week after infection, with the fewest eggs produced 2 to 3 weeks post infection, after which the number of eggs produced will start to increase (5).

In Iraq, the poultry industry has a significant economic contribution and involve different species of chicken. There limited (molecular) studies on this disease in Basrah-Iraq. The disease remains one of the problems in poultry farms and there are limited studies about the incidence and type of virus. The aim of this study is to investigate the clinical occurrence rate , Pathological and molecular detection of NDV in layers farms in Basrah.

MATERIALS AND METHODS

Case history, clinical signs, gross lesions, and histopathologic examination:.

The study was conducted to monitoring 6 poultry houses of layers hens which located in Al zubair -Basrah governorate, layers hens which named A, B, C, D, E and

F located in Al zubier - Basrah. The duration of the study was 6 month starting from September 2019 to march 2020. The samples collected randomly from different areas of Basrah according to the clinical sings of ND.Samples were included Proventriculus, lung, trachea, intestine, spleen, cecal tonsils ,oviduct and uterus.

Then all samples were presented to the department of veterinary Pathology and poultry diseases at the University of Basrah for necropsy examination. Chickens that were showing clinical signs were euthanized. All cases were examined for gross lesions . The preparation of postmortem lesions, was processed according to (6). Tissue samples from internal organs including (, lung, trachea, spleen, cecal tonsils and uterus were collected and preserved in 10% formalin solution for histopathological examination according to Luna (11).

Monitoring antibodies titer by ELISA:

Collected random samples of blood from laying hens are taken from wing vein and according to a commercial ELISA kit (Newcastle disease virus antibody test kit, SYNBIOTICS CORPORATION, Canada) was performed according to manufactures direction to determine Ab levels against ND vaccine.

Genomic RNA extraction and cDNA synthesis (RT product:

According to AccuPower RocketScript Cycle RT PreMix tubes . A velogenic specific primer probe designed by (7) was used to amplify a wide range of velogenic NDVs.

Table 1: A velogenic specific primer as follows

Sr. No.	Gene	Primer	Primer sequence	PCR Product
I	F	NDV-F	5'-GGTGAGTCTATCCGGARGATACAAG-3'	202bp
II	F	NDV-R	5'-TCATTGGTTGCRGCAATGCTCT-3'	202bp

RESULTS

Clinical history, gross lesions, and histopathological examination:

This study was conducted to monitoring 6 poultry houses of layers hens. The clinical signs which were recorded, include firstly nervous signs of torticollis showed

in (figure 1) . greenish diarrhea (figure 2). Sharp drop in eggs production, loss eggs pigmentations loss eggs quality (figure 3).

A decrease in egg production a week after infection, with the least number of eggs being produced two to three weeks after infection, after which the number of eggs produced begins to increase with gradually returned with a gradual improvement in the shell eggs and quality .

Figure 1: Chicken suffering from torticollis

Figure 2: Chicken suffering from greenish diarrhea

Figure 3: Loss of eggs pigmentations

Post mortem inspection shown different lesions. The disease affected firstly on reproductive , digestive and nervous system. The sever changes on female reproductive system especially appear on ovarian follicles as hemorrhagic stigmata (figures 4). In digestive system were hemorrhage of proventricular mucosa (figure 5)

and necrotic hemorrhage area on the mucosal surface of the intestine especially cecal tonsils(figure 6).

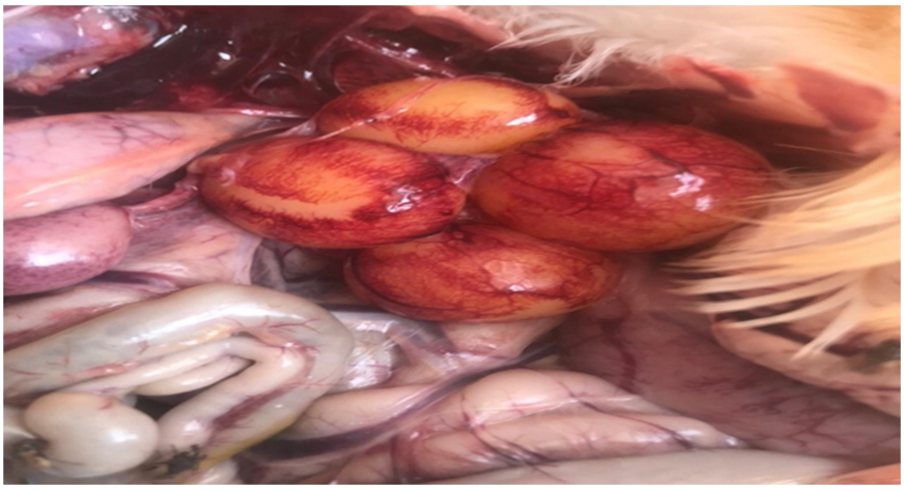

Figure 4: Ovarian follicles with hemorrhagic stigmata

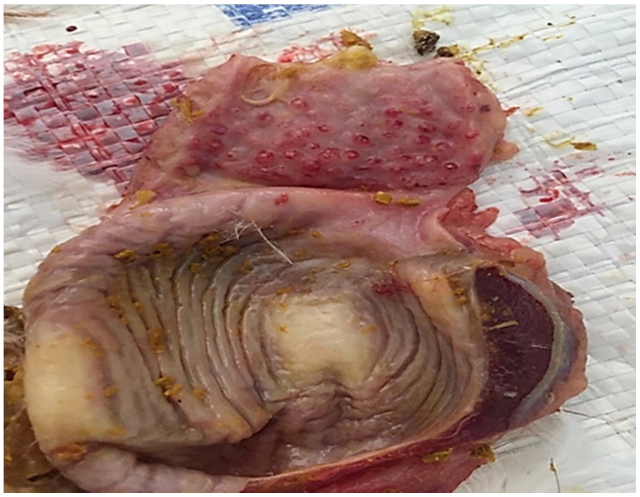

Figure 5: Hemorrhage of proventriculus mucosa

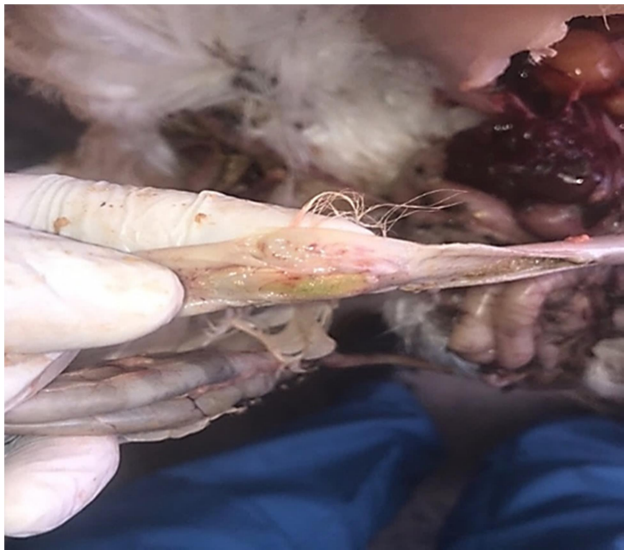

Figure 6: Necrotic hemorrhage area on the mucosal surface of the intestine especially cecal tonsils

Histopathological lesions, after staining with hematoxylin eosin of the tissues showed changes in these figures :

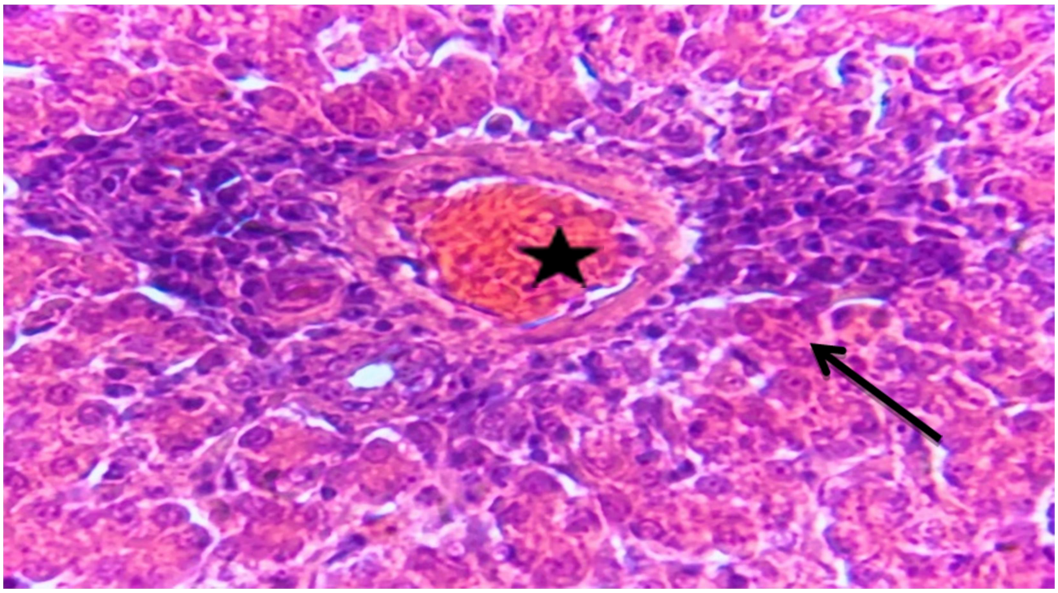

Figure 7: Histological section of liver congestion of portal vein(star) and inflammation in portal area (arrows) H& E, 400x

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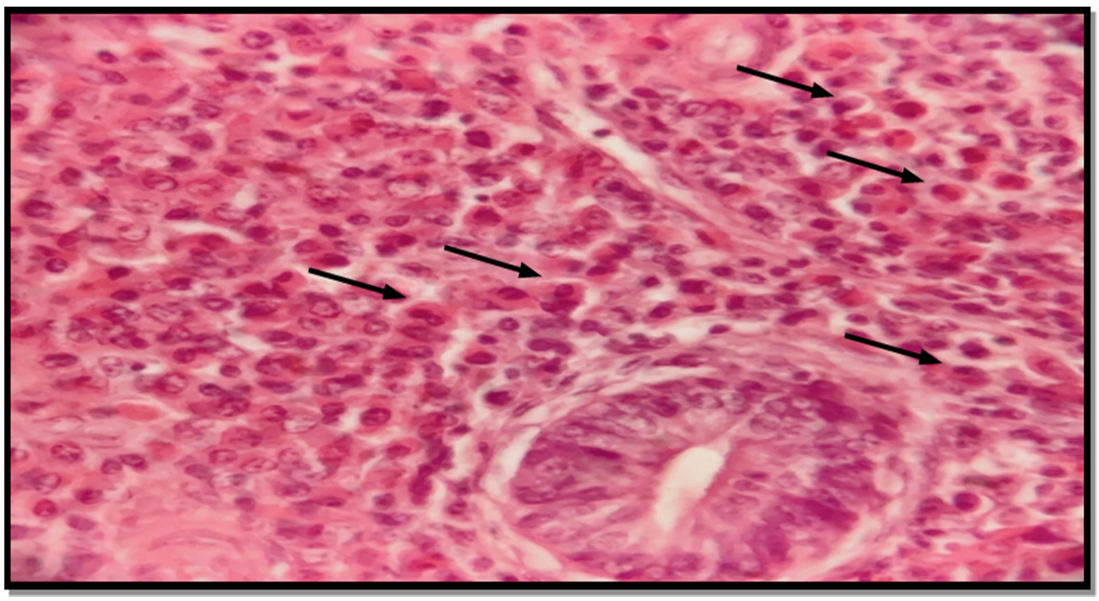

Figure 8 :Histological section of cecal tonsils increase of lymph tissue (stars) and congestion of blood vessel between muscle cells (arrows), H& E, 40X.

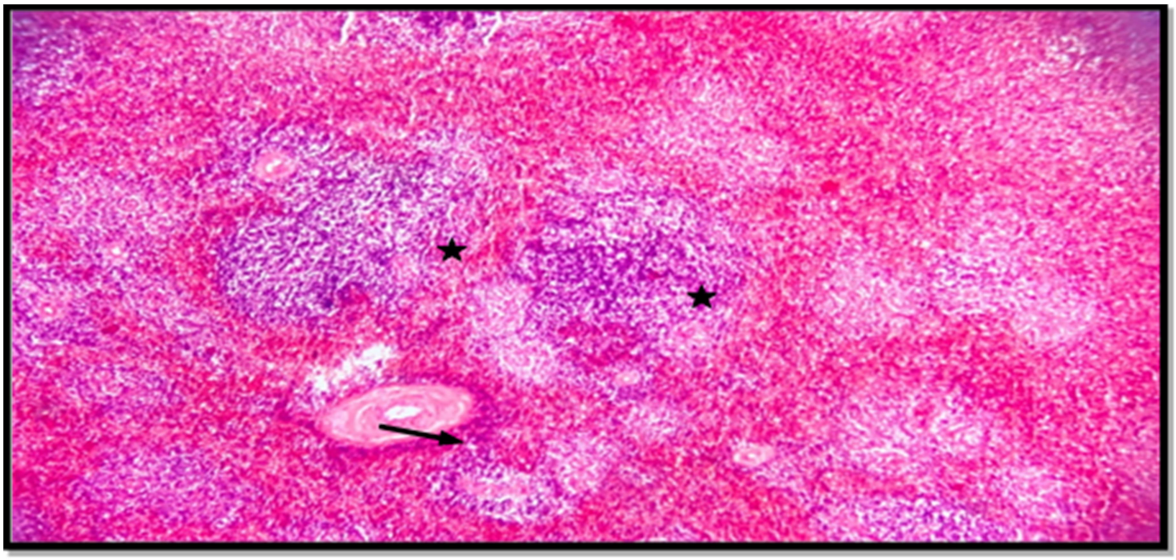

Figure 9: Histological section of spleen deposit of hyaline materials in wall of blood vessel (arrow) and dysplasia of white pulp not lymphnode (star) (cortex and germinal center) H& E, 40x.

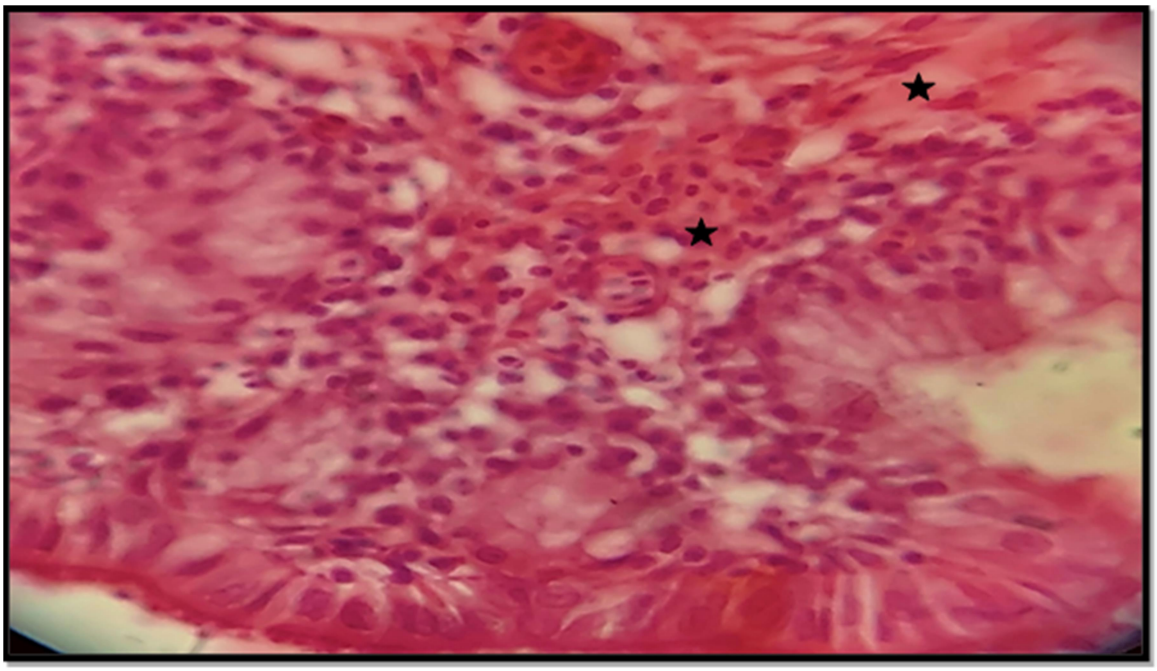

Figure 10: Histological section of trachea necrosis in connective tissue of lamina propria under epithelial lining cells (arrows) H& E, the magnification not true.

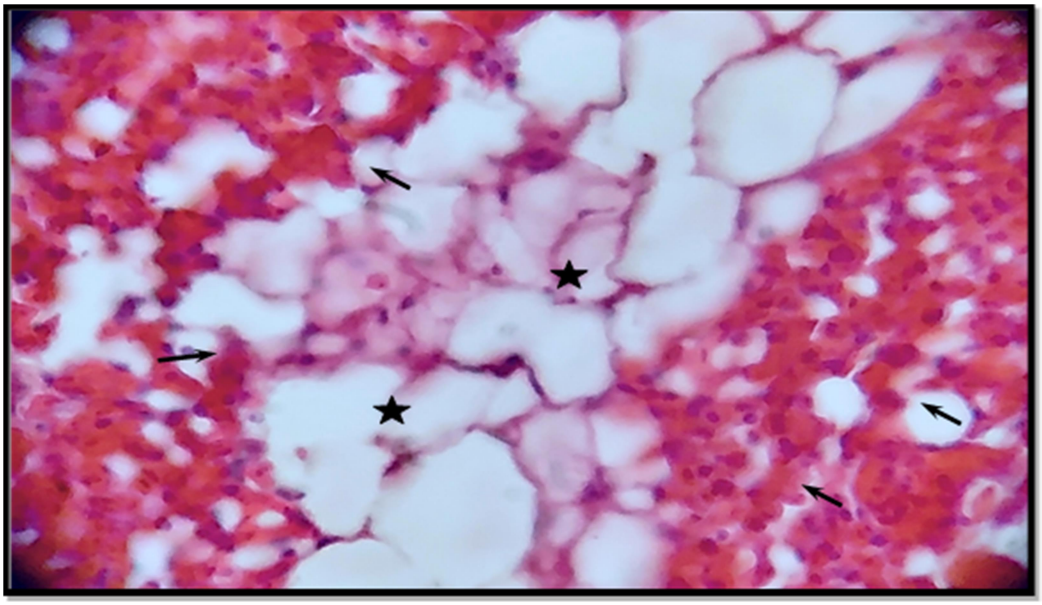

Figure 11:histologica section of lung edema in alveolar lumen (stars),and congestion of blood vessels of alveolar walks(arrows) , H& E.

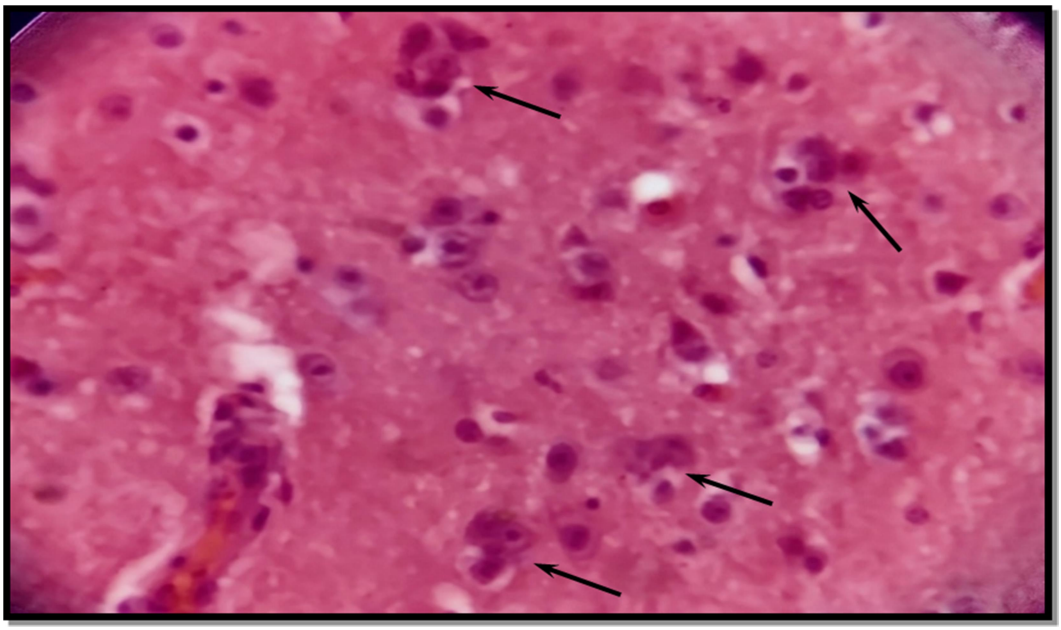

Figure 12: histological section of brain aggregation of glial cells (arrows), and formed numerous masses in white mater H& E.

Monitoring antibodies titer by ELISA:

The antibody titers were monitor by ELISA test during study period in normal conditions and after infection with ND . Checking antibodies were depended on six poultry houses .

Table (1) Mean antibody titre during 6 month for all poultry house

Poultry house name	Incidence of ND	Mean titre antibody during 6 month
A	–	13390
B	–	16992
C	+	20391
D	–	15111
E	–	13264
F	–	13795

polymerase Chain Reaction (PCR) Results for NDV:

PCR expansion to NDV was performed by extracting RNA from samples after cDNA and post-DNA conversion by your anterior pre-primer and reverse primer from the presence of a DNA progenitor (approximately 203 bps long). A total of 25 samples were examined by PCR technology .The total expected samples by PCR reached 13 samples: five positive brain samples , three positive tracheal samples and five positive of cecal tonsil samples . The total of negative samples by PCR reached 12 samples which the result observed in figure 13&14.

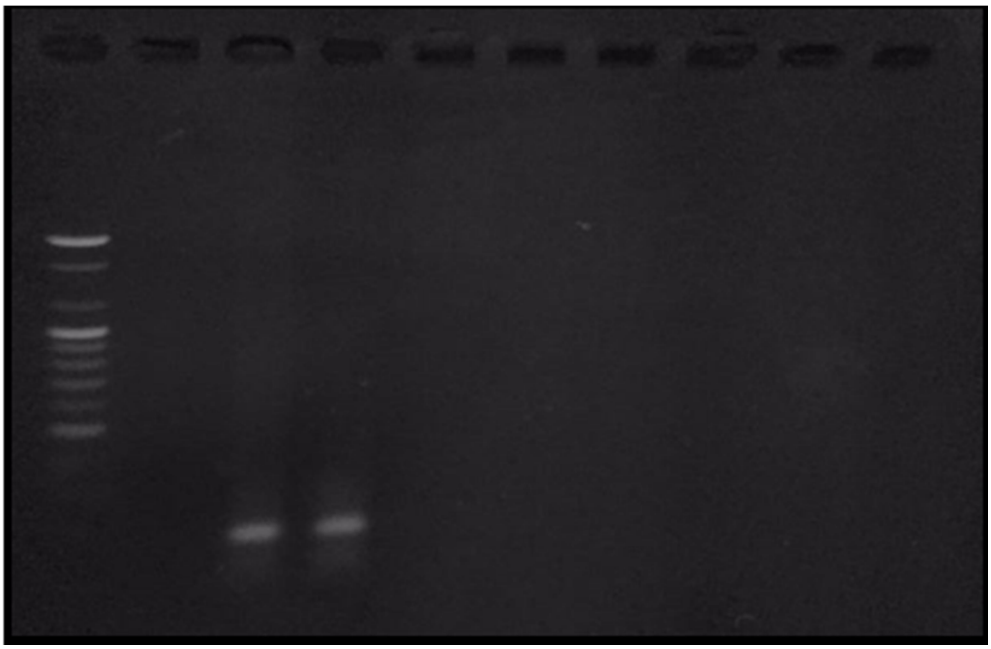

Figure 13: Agarose gel electrophoresis design of NDV partial F gene PCR produces (approximately 203bp). 2& 3: Field samples were positive

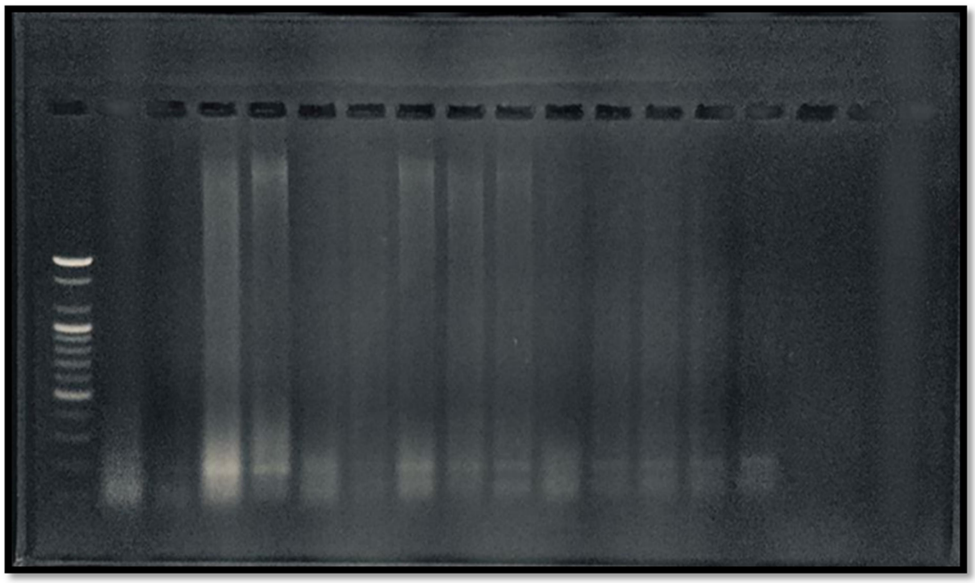

Figure 14: Agarose gel electrophoresis design of NDV partial F gene PCR produces (approximately 202bp).2,3,4 and 6-13 Field samples were positive

DISCUSSION

Newcastle disease is a contagious, highly fatal viral infection affecting many species of domestic and wild birds worldwide, and caused huge economic impact on poultry industry precipitated following outbreaks of the disease. In our study we recorded occurrence of ND. Checking antibodies were depended on six poultry houses layers hen.

This result agreement with (13). While disagreement with(14) duo to the infection resulting from irregular vaccination programs in the poultry house C lead to increase of anti-body titer post infection. The results of clinical monitoring, clinical signs of NDV were showed nervous signs (tremors ,paralyzed wing and leg) and digestive signs like greenish-dark diarrhea, with reproductive systems effected were the eggs production sharp drop and misshapen and bleached eggs after two weeks from infection this results in line with mentioned (15) .

Post mortem inspection shown different lesions in layers hens which are severely affected characterized by trachea with congestion, pin point hemorrhages at the tip of proventriculus glands, hemorrhagic ulcers in the intestinal wall and cecal tonsils, necrosis and focal ulceration of intestinal lymphoid aggregates. These results

are in agreement with (16). Histopathological changes with ND in present study were showed in the of infected chickens as infiltration of inflammatory cells and necrosis of lining epithelial cells metaplasia of epithelial cells and dilation of lumen which have numerous sloughing cells and necrosis in other tubule sloughing of epithelial cells into lumen hyperplasia of lining epithelia this agreement with (17). The changes with ND in cecal tonsils increase of lymph tissue and congestion of blood vessel between muscle cells and increased of plasma cells this result agree with(18). Spleen Enlarge of e chicken don't have lymph node and dysplasia of their region (cortex and germinal center). deposit of hyaline materials in wall of blood vessel and dysplasia of lymph node (cortex and germinal center) this result agreement with (19). Trachea necrosis in connective tissue of lamina propria under epithelial lining cells as well to bleeding in lamina propria .

Histological section of lung is hyperplasia of lining epithelial of alveolar , bleeding infiltration of inflammatory cells and congestion of blood vessels of alveolar walks. Edema in alveolar lumen and congestion of blood vessels of alveolar walks this result similar to mentioned by(20).In my study the histological section of brain aggregation of glial cells and formed numerous masses in white mater, ring cells appear in white matter with flatting nucleus and degeneration of white matter this finding agree with (21). The rapid diagnosis is possibly increased by using methods based on molecular biology e.g. PCR reaction (22). who applied the PCR method for the first time to detect the presence of nucleic acids of NDV in samples of chicken by using specific primers that enabled rapid differentiation of the pathotype . In this study PCR method was applied to identify NDV in homogenized organ sample of chickens naturally infected with NDV .To confirm the presence of pathogenic NDV via PCR were used to detection of NDV from the homogenized tissue obtained from organ samples were taken directly using the pair of primers and probes' targeting the F gene was specific to the virulent strains of NDV, which showed out of 30 pooling samples only13 were positive in PCR, this results were in lined with mentioned by (23, 24).

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Ethical consideration:

This study was carried out in accordance to the ethical rules for samples handling and animal's managements and researches, College of Veterinary Medicine, University of Basrah, Ministry of Higher Education and Scientific Researches, Republic of Iraq.

دراسة سريرية ومرضية وجزيئية لمرض نيوكاسل في حقول الدجاج البياض في البصرة ، العراق

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الخلاصة

مرض نيوكاسل هو مرض فيروسي مهم للدواجن وهو مصدر قلق اقتصادي كبير ومنتشر في جميع أنحاء العالم ويصيب مجموعة متنوعة من أنواع الطيور. يتميز المرض باختلاف ملحوظ في معدلات الاصابة والوفيات. أجريت الدراسة الحالية لرصد حدوث مرض نيوكاسل في بيوت الدواجن البياضة الواقعة في الزبير - محافظة البصرة ، خلال ٦ أشهر ابتداء من سبتمبر ٢٠١٩ إلى مارس ٢٠٢٠. اعتمدت الدراسة على الفحص السريري ، ومراقبة معيار الأجسام المضادة بواسطة ELISA ، وآفات ما بعد الوفاة. والتغيرات النسيجية المرضية وتفاعل البلمرة المتسلسل (PCR). تم جمع أنواع مختلفة من العينات مثل المعده الحقيقية والرئة والقصبة الهوائية والأمعاء والطحال واللوز الاعورية وقناة البيض والرحم من ٦قاعات دواجن وفقًا للتشخيص المشتبه به سريريًا .سجلت نتائج الدراسة الحالية المرض في الدجاج البياض. أظهرت الحالة السريرية الإيجابية علامات سريرية مهمة لـ مرض نيوكاسل مثل العلامات العصبية والتغيرات على الجهاز الهضمي. انخفاض حاد في إنتاج البيض ، وفقدان تصبغات البيض ، وانخفاض جودة البيض أثناء مراقبة القطيع بين عدم ظهور

علامات تنفسية. تم تسجيل التغيرات بعد الوفاة في الجهاز التناسلي والجهاز الهضمي والأعصاب. التغيرات الحادة في الجهاز التناسلي للأنثى تظهر بشكل خاص على بصيلات المبيض. حدثت تغيرات في الأنسجة المرضية في واللوز الاعورية والطحال والدماغ. أشارت نتيجة اختبار ELISA خلال فترة الدراسة إلى مستوى عالٍ خلال وبعد ٧ أيام من الإصابة.

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