

EFFECTS OF DIFFERENT VACCINATION METHODS AGAINST NEWCASTLE DISEASE ON IMMUNE RESPONSE AND SOME BLOOD PARAMETERS IN LOCAL CHICKEN (*GALLUS GALLUS DOMESTICUS*) IN SHIRQAT CITY

ABDULJABBAR M.H. ALJOBURI¹, QUSAI S. JUMMA¹ AND SABAH M.H. AL-SHAMMARI²

¹ Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine, Tikrit University, Iraq

² Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine, Diyala University, Iraq

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ABSTRACT

The current study aimed to investigate the effects of different vaccination programs against Newcastle disease (ND) on both immune response and some blood parameters in local chickens in Shirqat city. Ninety local chicks were divided into three groups, 30 chicks each. The first group (G1) left unvaccinated, while the second and third groups (G2, G3) were vaccinated with ND strains using different methods of administration. A significant decrease was recorded in both the number of red blood cells (RBCs), white blood cells (WBCs) and packed cell volume (PCV) in G3 at 14 days of age. Additionally, a significant decrease in antibody levels in the G2 at 24 days of age. A significant decrease was also noted in both the number of RBCs in the G2 and the hemoglobin in both G2 and G3. A significant decrease in both PCV and mean corpuscular hemoglobin concentration (MCHC) was recorded in the G3. Furthermore, a significant decrease in antibody levels was observed in both the ELISA and hemagglutination inhibition (HI) tests in G3, along with a significant difference in the number of RBCs in G2 and G3. There was also a significant difference in hemoglobin and PCV levels in G3 at 34 days, and a significant decrease in PCV value was noted in G3 at 42 days. In conclusion, the different vaccination programs against Newcastle disease led to a significant decrease in antibody levels in the second and third groups at 24, 34, and 42 days of age. The study also revealed that some blood parameters in the vaccinated groups showed a significant decrease at the level of $P \leq 0.05$.

Key words: Different vaccination, Newcastle disease, local chicken

INTRODUCTION

The local chicken sectors provide a significant contribution to human livelihoods and food security in rural areas (Walugembe *et al.*, 2019). One of the most

deadly epidemic diseases affecting poultry farms and many types of wild birds is Newcastle disease (Walugembe *et al.*, 2020). Although this disease does not typically infect ducks and they are considered vectors, chickens are more vulnerable to infection and the development of clinical symptoms (Mpenda *et al.*, 2019).

Corresponding author: Abduljabbar M.H. Aljoburi

E-mail address: abduljabar1981@tu.edu.iq

Present address: Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine, Tikrit University, Iraq

The disease is characterized by medical and pathological signs that vary depending on the virulence of the virus strain, dosage,

mode of entry, age, and the immune status of the birds following infection (Ferreira *et al.*, 2019; Aljubori and Jumma, 2024). Newcastle disease is one of the prevalent diseases in Iraq, with the virus first isolated in the Abu Ghraib region in 1968, where the strain was named (Ahmed and Odisho, 2018). In recent years, the disease has spread, causing significant economic losses. It recurs globally, triggering frequent outbreaks (Bessel *et al.*, 2020).

Newcastle disease is caused by a member of the Paramyxoviridae family. The Newcastle disease virus (NDV) has biological and physical properties that distinguish it from other Paramyxoviridae viruses, including the ability to agglutinate red blood cells (Berghof *et al.*, 2019). The incubation period following exposure to NDV ranges from 2 to 15 days, with an average of 5 to 6 days or more. The virus is also characterized by antigenic variation (Zhan *et al.*, 2020). However, it is not possible to distinguish between strains based solely on these variations.

The virus is classified into virulent strains (Velogenic Strains), moderate strains (Mesogenic Strains), and mild strains (Lentogenic Strains), according to the

severity of the disease (Ferreira *et al.*, 2019). This study aimed to determine the effects of different vaccination programs against Newcastle disease on the immune response and some blood parameters in local chickens in Shirqat city.

MATERIALS AND METHODS

1. Experimental Chicks-

Ninety local chicks were obtained from a local hatchery in Al-Shirqat city, Salah Al-Din Governorate, Iraq. The study was conducted from the beginning of November 2022 until the end of January 2023. The chicks were divided into three groups, with each group consisting of 30 chicks, which were housed in sterile rooms at the animal house of the College of Veterinary Medicine, University of Tikrit. The chicks were fed commercial poultry feed and given drinking water. The first group did not receive any vaccinations and was considered the control group, while the remaining two groups were vaccinated with ND vaccines using different methods, coarse spray, drinking water, subcutaneous injection and eye drops (Table 1).

Table 1: Experimental design and approved vaccination schedule for immunization:-

Groups	Day of vaccination	Type of vaccine	Time of blood collection	Vaccination methods
Group1 (control group)			2 days old	
			24 days old	
			31 days old	
Group2	1	Hitchner B1	2 days old	Coarse spray
	14	Clon 30	24 days old	Drinking water
	28	LaSota	14-28-32 days old	Drinking water
Group3	1	Clon 30	2 day old	S/C injection
	18	Rinnovac-Eli7	14 days old	Eye drops
	32	LaSota	24-34-42 days old	Drinking water

2. Clinical pictures:

Clinical symptoms included lacrimation, swelling around the eyes and head, loss of appetite, conjunctivitis, sneezing, coughing, nasal discharge, greenish diarrhea, and paralysis of the wings and legs. Some dead chickens were found suffering from torticollis.

Observed gross lesions: conjunctival tears, hemorrhages in the trachea, hemorrhages on the proventricular glands, enteritis, congestion in the lungs, hemorrhages in Peyer's patches, and swollen cecal tonsils.

3. Serological Methods: -

Blood samples were obtained at the ages of 2, 14, 24, 28, 34, and 42 days post-immunization via heart puncture. To eliminate non-specific inhibitors, all serum samples were heat-inactivated for 30 minutes at 56 °C. Estimation of antibodies against NDV in serum samples was carried out using the hemagglutination inhibition (HI) test with 4 hemagglutination units of inactivated La Sota virus (freeze-dried product suspended in distilled water), as described by Sultan *et al.* (2020). The results were expressed in terms of the geometric mean titer (GMT). Anti-ND antibody titers were calculated according to the manufacturer's instructions using the ProFLOK-ND ELISA Kit (Synbiotics Company, USA). The ELISA titers were determined using the formula below:

ND titer = antilog of \log_{10} titer;

\log_{10} titer = $(1:172 \times \log_{10} SP) + 3.164$

$Sp = \frac{\text{(sample absorbance - average normal control absorbance)}}{\text{(corrected positive control absorbance)}}$

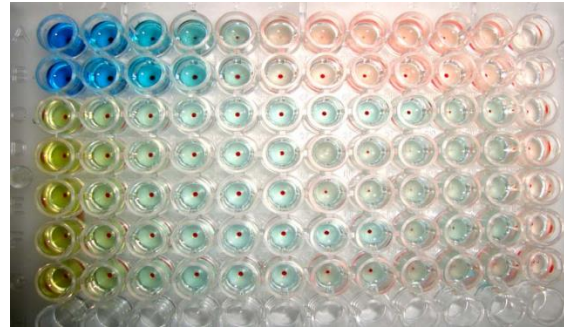


Figure 1 : The HI test result on a micro titre plate.

4. Evaluation Of Hematological Parameters:-

Blood samples (1.5 ml) with anticoagulant EDTA were collected from different groups of experimental chicks to examine hematological parameters at 2, 14, 24, 28, 34, and 42 days after vaccination with NDV. Total Red Blood Cells (RBCs), total White Blood Cells (WBCs), and packed cell volume (PCV) were determined. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were also calculated (Wajid *et al.*, 2018).

5. Vaccinated Strains Which Used Against Newcastle Disease:-

- **Hitchner B1** :- Virus of Newcastle Disease, strain Hitchner B1 $\geq 10^{6.0}$ EID₅₀.. produced by the company Ceva Nobilis-France.

- **LaSota**:- Virus of Newcastle Disease, strain LaSota $10^{6.0}$ EID₅₀.. produced by the company Luhmen Nobilis-Germany.

- **Clone30**:- Virus of Newcastle Disease, strain Clone30 $10^{9.5}$ EID₅₀. produced by the company Intervet Nobilis-Holland.

- **Rinnovac-Eli7** :-Virus of Newcastle Disease, strain Rinnovac-Eli7 10^5 EID₅₀. produced by the company MEVAC for Vaccines – Egypt.

6. Ethical approval:- The study was approved by the ethical committee of veterinary medicine college, Tikrit University.

7. Statistical Analyses:-

The results were statistically analyzed using SPSS Edition 15 (2008). Descriptive analysis of the data was conducted, which included the highest value, lowest value,

RESULTS

1. Clinical signs and post mortem examinations:-

Clinical signs: Depression, anorexia, edema around the eyes and head, conjunctivitis, lacrimation, nasal discharge, coughing, sneezing, green diarrhea, paralysis of the legs and wings, and torticollis. The most common post-mortem lesions observed in infected chickens were hemorrhagic ulcers in the intestinal wall (group 2) and hemorrhagic ulcers with mild enteritis in the intestinal wall of group 3 (Figures 1 and 2). Additionally, hemorrhagic ulcers were present in the cecal tonsils (Figure 3), with pinpoint hemorrhages at the tips of the proventriculus glands (group 2) and hemorrhages in the mucosal layer of the proventriculus (group 3) (Figures 4 and 5), as well as hemorrhages in the lungs (group 2) (Figure 6).

2. Serological and blood parameters tests

The results of the ELISA test at two days old showed no significant difference in the level of antibodies to Newcastle disease between groups, and there were also no significant variations in the results of the hemagglutination inhibition test among the groups. Additionally, no major differences were observed in the blood parameters.

At 14 days old, the ELISA test results indicated no significant differences between the groups. However, there was a non-significant difference favoring the second and third groups. This may be attributed to the acquired passive immunity, which can affect the

mean, and standard error. A one-way analysis of variance (ANOVA) was performed, followed by the LSD test, to detect significant differences between group means.

effectiveness of the vaccine when administered during the first four weeks of life. In contrast, the blood parameter results showed a significant decrease in red blood cell count, packed cell volume (PCV), and white blood cell count in the third group.

At 24 days old, the ELISA test recorded a significant decrease in the second group, while the hemagglutination inhibition test showed a delay in the immune response in the third group compared to the other groups, which did not show significant differences among them. However, there was an arithmetic superiority in favor of the first group over the second and third groups. The low level of antibodies in the second group is likely due to the last administered vaccine in drinking water at 14 days old, resulting in a short protection period.

The results of the blood parameter measurements indicated a significant decrease in red blood cell counts in the second group, as well as a significant decrease in hemoglobin (Hb) levels in both the second and third groups. Additionally, a significant decrease in PCV and mean corpuscular hemoglobin concentration (MCHC) was observed in the third group at the level of $p < 0.05$. The erythrocyte response in the chickens displayed varying patterns in this study, with a significant depression of RBC, PCV, and Hb levels in vaccinated chickens, indicating possible anemia.

At 34 days old, the ELISA and hemagglutination inhibition tests showed a significant increase in antibodies level in the second group compared to the third group. This superiority can be attributed to the short interval between vaccination and

measuring antibody levels. The improved responses in the second and third groups were likely due to repeated vaccinations in close intervals. Meanwhile, the blood parameter measurements showed a significant decrease in red blood cell counts in both the second and third groups, as well as a significant decrease in both Hb and PCV parameters in the third group at a probability level of $p < 0.05$.

The results of the ELISA and hemagglutination inhibition tests at 42 days old indicated that the second group significantly outperformed the third group ($p < 0.05$), which recorded a lower level of antibodies. The results of the ELISA and

HI tests consistently demonstrated the significant superiority of the second and third groups.

However, the blood parameter tests at 42 days showed a significant decrease in packed cell volume (PCV) in the third group at a probability level of $p < 0.05$. Additionally, a non-significant increase in white blood cell count was observed in both the second and third groups, while the mean corpuscular volume (MCV) showed a non-significant change in the third group. The MCV values recorded in this study are shown in Tables (2, 3, 4) and Figures (8, 9).



Figure (2): Bleeding ulcers in the intestinal wall (group 2)



Figure (3): Hemorrhagic ulcers and mild enteritis in the intestinal wall of (group 3).



Figure (4): Hemorrhagic ulcers in the cecal wall of (group 3).



Figure (5): Pin-point hemorrhages at the tips of proventriculus glands (group 2)



Figure (6): Hemorrhage in the mucosa for proventriculus (group 3)



Figure (7): Hemorrhage in the lungs (group 2)

Table 2: Results of the antibodies levels for Newcastle virus using ELISA test.

Groups	Age (day)				
	2 days	14 days	24 days	34 days	42 days
Group1	7754±472.52a	389.7±56.37a	0.0	0.0	0.0
Group2	7652±365.21a	3532±45.32a	3231±165.51b	4532±457.15a	8010±657.45a
Group3	7434±435.67a	3578.6±138.79a	5387±382.78a	3644±261.74b	3531±298.34b

a,b: The presence of the different letters vertically represents the presence of significant differences at the level (p <0.05) between the groups.

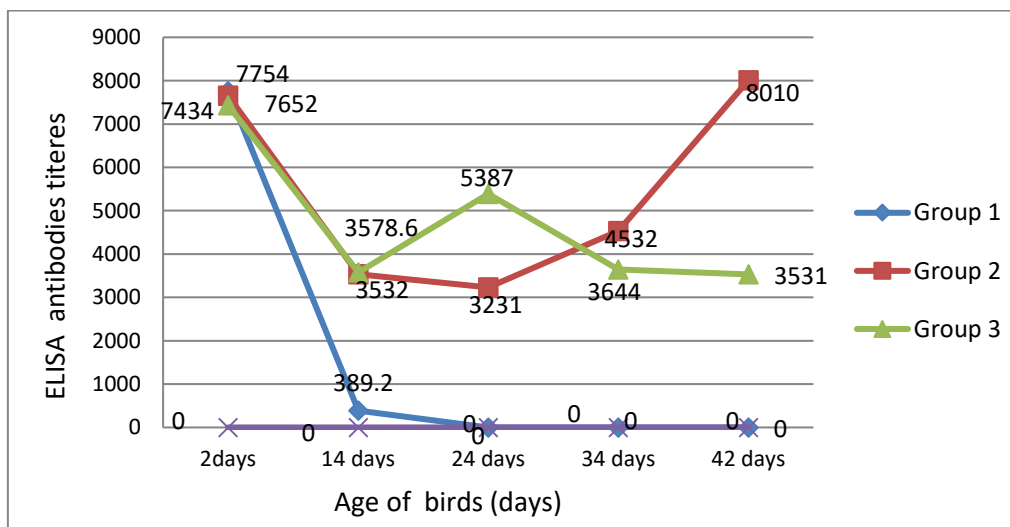


Figure (8): Graphical representation of antibody levels for Newcastle virus using ELISA test.

Table 3: Results of antibody levels for Newcastle virus using hemagglutination inhibition test.

Groups	Age (day)				
	2 days	14 days	24 days	34 days	42 days
Group1	20.2±5.520a	7.7±2.300a	0.0	0.0	0.0
Group2	22.3±3.651a	10.4±2.400a	35.3±8.760a	36.1±18.860a	74.1±16.45a
Group3	21.2±4.878a	8.8±1.967a	27±3.828a	28±8.788b	64±17.786b

a,b: The presence of the different letters vertically represents the presence of significant differences at the level (p <0.05) between the groups.

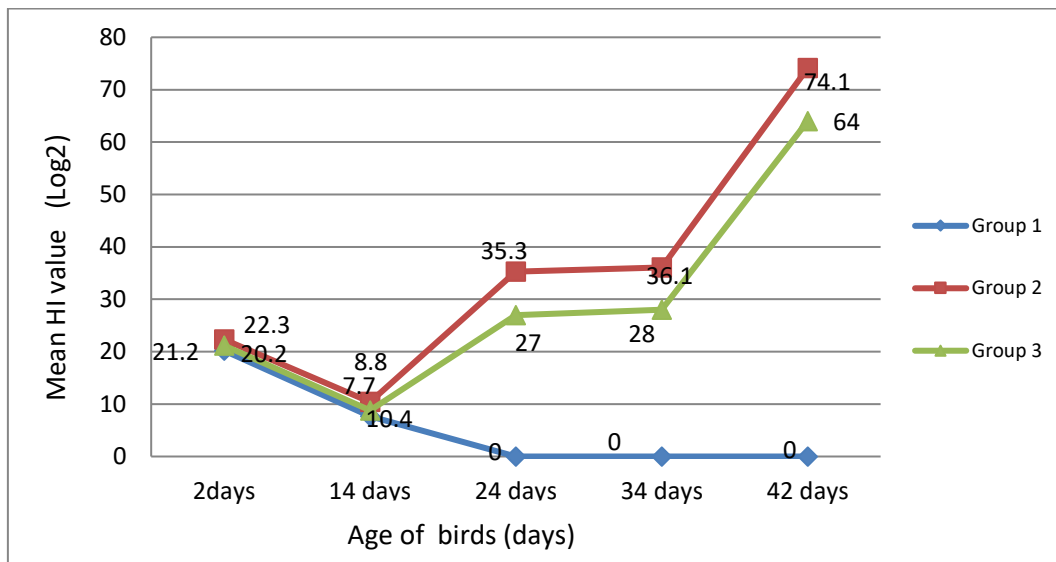


Figure (9): Graphical representation of antibody levels for Newcastle virus using ELISA test.

Table 4: Values of some blood parameters of local chickens during the experimental period.

Age /day	Groups	RBCs (x10 ⁶ / mm ³)	Hb (g/dl)	PCV (%)	MCV (fl)	MCHC (%)	WBCs (g/dl)
2 days	Group 1	11.25±2.87a	11.25±2.13a	34.54±0.52a	103.15±3.76a	27.05±0.01 a	11.04 ± 0.34a
	Group 2	10.21±0.89 a	9.63 ± 0.85a	34.59±0.06a	98.31±0.97a	26.50±1.54a	10.78 ± 1.8a
	Group 3	11.27 ± 0.30a	8.25 ± 0.76a	34.61±0.02a	100.1±1.5 a	24.±0.15 a	9.28 ± 0.06a
14 days	Group 1	10.18 ± 1.53a	11.7±0.7 9a	32.4±4.58 a	90±1.38a	27.65±0.13 a	10.49±0.17a
	Group 2	10.74±0.19 a	10±2.42 a	32.4±4.8 a	91.0±3.68a	26.70±0.05a	10.03±0.15a
	Group 3	7.68±0.42b	9.6±0.4 4a	34.8±4.76b	92.1±7.12 a	20.78±0.08 a	8.15±0.22b
24 days	Group 1	10.71±0.54a	10.2±1.60 a	34.7±6.92 a	89.2±1.0 a	28.83±0.04a	11.46±0.26a
	Group 2	8.09±0.22 b	7.7±0.86 b	32.3±5.1 a	103.8±4.28a	25.78±0.3 a	10.39±0.17a
	Group 3	9.21±0.32 a	8.7±1.24 b	30±1.06 b	94.1±9.91 a	24.03±0.04b	12.91±0.14a
34 days	Group 1	9.24±0.38 a	11.1±5.07a	30.4±5.05 a	100.7±8.4 a	24.20±0.11 a	10.90±0.21a
	Group 2	7.82±0.32 b	10.32.78 a	34.4±5.57a	98.5±3.6 a	26.94±0.18 a	8.8 ± 0.29a
	Group 3	7.02±1.52 b	8.17±0.6 2b	26.2±4.28 b	102.89±0.11a	26.39±0.16a	10.78±1.29a
42 days	Group 1	11.45±2.71 a	8.30±5.48a	32.56±1.87a	90.9±5.12a	25.25±2.87a	11.15±3.27a
	Group 2	10.81±3.85 a	8±0.54a	29.1±1.5a	86.9±4.52a	24±0.38a	9.25±2.82a
	Group 3	9.85±2.54 a	7±6.78a	27.52±0.22b	89.25±2.87a	24.39±0.05a	10.65±0.05a

a, b, means with different superscript with in row are significant at P-value <0.05; RBCs: red blood cells count; Hb: hemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume ;MCHC: mean corpuscular hemoglobin concentration; WBCs: white blood cells.

DISCUSSION

1. Clinical signs and post mortum examinations:-

Depression, anorexia, edema around the eyes and head, conjunctivitis, lacrimation, runny nose, coughing, sneezing, green diarrhea, paralysis of the legs and wings, and torticollis were the main clinical signs observed, consistent with the findings of Deist *et al.* (2017). The most common post-

mortem lesions observed in infected chickens included bleeding ulcers in the intestinal wall (group 2) and hemorrhagic ulcers with mild enteritis in the intestinal wall of group 3. Additionally, hemorrhagic ulcers were present in the cecal tonsils, pinpoint hemorrhages were observed at the tips of the proventriculus glands, and there were hemorrhages in the mucosal layer of the proventriculus (group 3), as well as hemorrhages in the lungs (group 2). These findings agree with the studies conducted

by Han *et al.* (2017) and El-Shall *et al.* (2020).

2-Serological and blood parameters tests

Different vaccination programs were used in this study to compare Newcastle virus antibody titers in local chickens from two days to 42 days of age, assess immunity using hemagglutination inhibition and ELISA measures, and monitor certain blood parameters. The results of the ELISA test at two days of age showed no significant difference in the level of antibodies to Newcastle disease between groups. Additionally, there were no significant variations in the hemagglutination inhibition test results between groups, which agrees with the findings of Simaraks *et al.* (2018). Furthermore, no major differences were observed in the blood parameters test results, aligning with the research by Odunitan *et al.* (2018).

At 14 days old, the results of the ELISA test showed no significant differences between the groups, but there was a non-significant difference favoring the second and third groups. This may be attributed to the acquired maternal immunity, which can affect the effectiveness of the vaccine when administered during the first four weeks of life. This finding agrees with the conclusions of Kabiraj *et al.* (2020), who indicated that maternal immunity can complement vaccination and that responses may vary according to the method of vaccine administration and the strain used in the vaccination program. The hemagglutination inhibition test yielded similar results for this reason. However, the blood parameters test results indicated a significant decrease in red blood cell count, PCV, and WBC count in the third group. This finding aligns with Kim *et al.* (2017), who suggested these reductions may be due to hemolysis or blood loss, often seen as periventricular bleeding and gut ulcers (Odunitan *et al.*, 2018).

At 24 days old, the results of the ELISA test recorded a significant decrease in the second group, while the hemagglutination inhibition test showed a delay in the immune response in the third group compared to the other groups, which did not show significant differences between them, although there was a numerical superiority favoring the second group over the first and third groups. The low level of antibodies in the second group may be attributed to the last vaccine administered in drinking water at 14 days of age, indicating that the protection provided by this program is short-lived (Anjum *et al.*, 2020). The results of the blood parameters showed a significant decrease in RBCs number in the second group, along with a significant decrease in hemoglobin values recorded in both the second and third groups. Additionally, significant decreases in PCV and MCHC were observed in the third group at a probability level of ($p < 0.05$). The erythrocyte response in the chickens exhibited varying patterns in this study, despite the significant decreases in RBC, PCV, and Hb in vaccinated chickens, indicating possible anemia, similar results were reported by Okoroafor *et al.* (2018).

The results of the ELISA and hemagglutination inhibition tests at 34 days of age showed that the second and third groups had significant increases in antibody levels compared to the first group. This superiority is likely due to the short interval between vaccination and measuring antibody levels. The increase in antibody levels in the second and third groups can be attributed to the close administration of vaccinations, consistent with the findings of Saelao *et al.* (2019), which indicated that repeated vaccinations lead to increased antibody levels in addition to the immunity generated by oil-based vaccines, which last longer than other types. Meanwhile, the results of the blood parameters showed significant decreases in red blood cell counts in the second and third groups, as well as

significant decreases in both hemoglobin and PCV in the third group, at a probability level of ($p < 0.05$). The results obtained in this study support the findings of Musa *et al.* (2018).

The results of the ELISA and hemagglutination inhibition tests at 42 days of age indicated that the second group significantly outperformed the third group ($p < 0.05$), which recorded a lower level of antibodies. The consistent significant superiority of the second group results in higher antibody standards compared to the third group (Mpenda *et al.*, 2020). In contrast, the results of the blood parameters test at 42 days showed a significant decrease in PCV in the third group at a probability level of ($p < 0.05$). These results are similar to those reported by Igwe and Eze (2016). The lower levels of total red blood cell count (Hb) and PCV observed in our study may be caused by increased permeability of capillaries and venules, leading to significant fluid loss.

Additionally, there was a non-significant increase in white blood cell counts in both the second and third groups, as well as a non-significant MCV value recorded in the present study, consistent with the findings of Igwe and Agbakwuru (2019). Leukocytosis is associated with the severity of injury, as it reflects the body's protective response through the inflammatory process (Okwor *et al.*, 2018), which can be linked to the presence of infectious agents, infections, chemical mediators released from inflammatory cells, immune-mediated injury, infarction, and damaged tissues that induce heterogeneity by stimulating the production of blood components from heterogeneous precursors when there is an increased demand for leukocytes in various tissues.

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تأثير طرق التلقيح المختلفة ضد مرض النيوكاسل على الاستجابة المناعية وبعض مؤشرات الدم في الدجاج المحلي (*Gallus gallus domesticus*) في مدينة الشرقاط

عبد الجبار محمد حسين الجبوري¹، قصي صالح جمعة¹، صباح محمود حمد الشمري²

¹ فرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة تكريت، العراق

² فرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة ديالى، العراق

Email: abduljabar1981@tu.edu.iq Assiut University web-site: www.aun.edu.eg

هدفت الدراسة الحالية إلى معرفة تأثير برامج التطعيم المختلفة ضد مرض النيوكاسل على الاستجابة المناعية وبعض مؤشرات الدم في الدجاج المحلي في مدينة الشرقاط. تم شراء ٩٠ كتكوت محلي من مفرخ محلي في مدينة الشرقاط. حيث تم تقسيم الكتاكيت إلى ثلاث مجموعات، كل مجموعة تتكون من ٣٠ كتكوتاً. تم الاحتفاظ بالكتاكيت في غرفة معقمة ومعزولة، وتم تغذيتها بغذاء طبيعي وتوفير المياه الطبيعية لها طوال فترة الدراسة من بداية سبتمبر وحتى نهاية نوفمبر ٢٠٢٢. وتركت المجموعة الأولى بدون أي لقاح، بينما أعطيت المجموعتان الثانية والثالثة لقاح سلالات ND بطرق تعاطي مختلفة. تم تسجيل انخفاض معنوي في كل من عدد خلايا الدم الحمراء و PCV وكذلك عدد خلايا الدم البيضاء في المجموعة الثالثة عند عمر ١٤ يوماً. كما سجلت النتائج انخفاضاً معنوياً في مستوى الأجسام المضادة لدى المجموعة الثانية عند عمر ٢٤ يوماً، بينما سجل انخفاض ملحوظ في كل من عدد كريات الدم الحمراء والهيموجلوبين في المجموعة الثانية وكذلك الهيموجلوبين في المجموعة الثالثة. كما تم تسجيل انخفاض كبير في كل من PCV و MCHC في المجموعة الثالثة. كما سجلت النتائج انخفاضاً معنوياً في مستوى الأجسام المضادة في كل من اختباري الاليزا والاختبار المانع للتلازن في المجموعة الثالثة ولوحظ فرق معنوي في عدد الخلايا الحمراء في المجموعتين الثانية والثالثة بينما كان هناك فرق معنوي في كل من الهيموجلوبين و PCV في المجموعة الثالثة عند عمر ٣٤ يوماً. بينما سجلت النتائج انخفاضاً معنوياً في مستوى الأجسام المضادة في كل من ELISA و HI في المجموعة الثالثة، كما لوحظ انخفاض معنوي في قيمة PCV في المجموعة الثالثة عند عمر ٤٢ يوماً. استنتجنا من هذه الدراسة أن برامج التطعيم المختلفة ضد مرض النيوكاسل أدت إلى انخفاض ملحوظ في مستوى الأجسام المضادة لدى المجموعتين الثانية والثالثة عند أعمار ٢٤ و ٣٤ و ٤٢ يوم، شهدت بعض مؤشرات الدم في المجموعات الملقحة انخفاضاً معنوياً عند مستوى $P \leq 0.05$.

الكلمات المفتاحية: التلقيحات المختلفة، مرض النيوكاسل، الدجاج المحلي