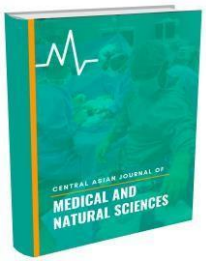

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Article

Newcastle Disease Hemato-immune Reaction Following Immunization

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Abstract: One hundred unsexed Ross broiler chicks, one day old, were used in this experiment divided four groups as a control group, the first group (G1) was not immunized. The Newcastle Lasota strain was sprayed into the second group (G2) was revaccinated with drinking water with a second and third vaccine, third group (G3) was inoculated with the inactivated oil adjuvant vaccine via subcutaneous injection on the tenth day of the birds' age precede eye instillation attenuated live vaccine it is reinforced with live vaccine attenuated via drinking water, fourth group (G4) was administered the vaccine via eye instillation as a first vaccine then it was vaccinated with a second and a third with a live attenuated vaccine using the drinking water method. Newcastle antibody levels were determined using a hem agglutination inhibition (HI) test and an ELISA test for chick blood on 7, 14, and 28 days from all groups at the probability level (P 0.05), Hemoglobin inhibitory antibodies to the Newcastle antigen were significantly higher in the vaccinated groups (G2, G3, and G4) than control group (G1). When the vaccinated groups (G2, G3, and G4) were compared to the control group, the Newcastle antibody standard as determined by the ELISA test increased significantly (P 0.05). In terms of antibody levels as assessed by the agglutination inhibitor and ELISA tests, G3 did better than the other groups, followed by G4. White blood cell counts (WBC) (cells/mm3) and the ratio of Heterophils/Lymphocytes (H/L) were measured at 7, 14, and 28 days. When the vaccinated groups were compared to the negative control group, the rate of WBC increased significantly (P 0.05) and decrease the ratio of heterophils to lymphocytes implies stress as substantial (P 0.05) which reflected positively on the birds' health. G3 was significantly superior of the amount of white blood cells at value 28.1 2 ± 0.1 2 , 32.11 ± 0.16 on 14, 28 days of age , as well as a considerable drop in the percentage of heterophil cells H/L. especially on 14, 28 days from age at value 0.21 ± 0.006 , 0.18 ± 0.007 respectively.

Keywords: ND ,Vaccine Route, Antibodies, WBC Count , H/L Ratio

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1. Introduction

Newcastle disease is an enzootic and occasionally epizootic disease in hens [1]. Pneumoencephalitis in young chickens, turkeys, and other domestic and wild birds is caused by the Newcastle disease virus, an avian Paramyxovirus that causes hemorrhagic lesions, neurological symptoms, enteritis, and respiratory symptoms in addition to high mortality [2].

It has been postulated that both cellular and humoral immune responses play important roles in the host's resistance to NDV infection [3]. Cell-mediated immunity (CMI) is a type of adaptive immunity that involves T cells. It has been proposed that CMI plays an important role in the development of protection in hens immunized against NDV and contributes to viral clearance. Cell-mediated stimulation can be found as soon as 2-3 days following NDV infection or immunization [4]. T cells have a role in immune responses that defend against viruses. The cytokines interleukin-2 and interferon-gamma

are expressed by bursal T cells [5]. IFN-gamma activates the lymphokine interleukin-2 (IL-2), which is mostly generated by CD4+ T cells and natural killer (NK) cells. In order to eradicate the virus infection, it encourages the growth of CD8+ T cells and cytotoxic T lymphocytes (CTL) [6], [7].

The most prevalent ND virus strains used in commercial live virus injections are lentogenic vaccines, which include Hitchner B1, Lasota, V4, Clone 30, and F strains. Mesogenic vaccine strains include Roakin, Mukteswar, and Komarov [8]. B1, F, and Lasota lentogenic strains are used in very young chicks without harming the host and can be administered in a variety of ways, including eye drops, beak dipping, spray, intranasal drops, and egg injections [9]. Because of their virulence, mesogenic strains are only employed in places where severe Newcastle disease is prevalent. They are also suited for secondary immunization in growing and adult birds following a first vaccination with a lentogenic vaccine. These immunizations are administered via drinking water, injection, and twitching wings [10]. Alexander and Senne, Inactivated vaccines are frequently prepared from infectious allantoic fluid that has been treated with propiolactone or formalin to kill the virus and then mixed with a carrier adjuvant to boost immunogenicity [11]. After a single vaccination, the oily emulsion vaccine (Water in Oil Emulsion) has the highest immunological response of the vaccinal strain [12]. Inactivated vaccines are commonly made from infective allantoic fluid that has been destroyed with propiolactone or formalin and then mixed with a carrier adjuvant to increase immunogenicity Grimes, 2002. The oily emulsion vaccine (water in oil emulsion) is the most successful in terms of the vaccine strain's immune response following a single vaccination Jansen et al., 2005.

2. Materials and Methods

Experiment birds

100 Ross broiler chicks one day of age were used in this experiment. The chicks were randomly divided into four equal groups and placed in specially constructed wooden cages, with 25 chicks in each cage. the first group (G1) was not immunized. The Newcastle Lasota strain was sprayed into the second group (G2) was revaccinated with drinking water with a second and third vaccine, third group (G3) was inoculated with the inactivated oil adjuvant vaccine via subcutaneous injection on the tenth day of the birds' age precede eye instillation attenuated live vaccine it is reinforced with live vaccine attenuated via drinking water, fourth group (G4) was administered the vaccine via eye instillation as a first vaccine then it was vaccinated with a second and a third with a live attenuated vaccine using the drinking water method.

Vaccines

a. Live Newcastle Disease vaccine (La Sota strain). Volvac® Boehringer Ingelheim 10 5.5 EID 50.
b. ND in activated vaccine emulsion for injection Vaxxon ND_Flu®

Blood collection

Blood samples were taken from all groups at 7, 14, and 28 days. Blood samples were drawn from the wing and brachial veins of five birds from each group at random, and placed in tubes. To conduct serological and immunological tests, serum was extracted and kept at -18 degrees Celsius. To obtain serum, samples were centrifuged for 10 minutes at 3000 rpm, then separated into aliquots and stored in Eppendorf tubes at -20°C until analysis.

Serological tests

A commercial NDV-ELISA from BioChek Immunoassays was used in accordance with the manufacturer's instructions to assess the titer of anti-NDV antibodies in the tested chicken serum. The sera were tested using an Enzyme Linked Immunosorbent Assay (ELISA) and a Hemagglutination Inhibition Test (HI-test). Given its low cost, the HI test is often used to assess immunization efficacy, while the ELISA test is commonly used to detect infections [13], [14] .With a cut-off value of 0.35, results below this threshold were viewed as unfavorable and non-protective, while scores above this threshold were viewed

as protective. Hemagglutination inhibitory antibodies to Newcastle Disease Virus were found in serum samples using an HI test. The test samples were combined with the same amount of NDV antigen after being serially diluted twice. Complete hemagglutination inhibition was assessed in the dilutions after chicken red blood cells (CRBC) were added. OIE 2012 [15] states that four hemagglutination units (HAU) of NDV antigen (LaSota strain) and 1% chicken red blood cells were used to assess the antibody titer of maternal and NDV-vaccinated chickens. Measurements were made of blood parameters.

The dates are the 7th, 14th, and 28th. The antigen's haemagglutination units were used. The antigen was serially diluted into serum samples using a twofold dilution method. Each well was given 0.5% RBC and incubated for 40 minutes. The reciprocal of the final dilutions that demonstrated total suppression of RBC agglutination was used to estimate the HI.

WBC Count (Cell / mm3) and Lymphocytes/ heterophils H/I ratio

For each group , blood was drawn from the brachial vein medial to the wing and placed in tubes with the anticoagulant EDTA. These cells were then diluted in the blood of birds using a specific Natt and Herrick's solution [16] Campbell,1988, calculated the ratio of lymphocytes to heterophils. In summary according to Zhang et al. [17].

Aclean glass slide was stained with five liters of blood. Wright-Giemsa staining was then applied to the smears. A competent person used an oil-immersed microscope set to 100 magnification to count the cells. A total of 100 cells, including lymphocytes and heterophils, were present in each visual area. Next, the ratios of heterophils to lymphocytes were computed.

To determine whether there were any significant differences between groups , Leech [18] states that one and two-way ANOVA and a least significant test (P 0.05) were used to statistically analyze the data.

Table 1. Statistically analyze.

Group Days	G1	G2	G3	G4
1 days	Un vaccinated	ND(live vaccine) spray	ND vaccine Lasota ocular	ND vaccine Lasota ocular
10 days	Un vaccinated	ND (live vaccine) Drinking water	Sub cutaneous Injection	ND (live vaccine) Drinking water
20 days	Un vaccinated	ND (live vaccine) Drinking water	ND (live vaccine) Drinking water	ND (live vaccine) Drinking water

3. Results

The volumetric standard rate of agglutination-inhibiting antibodies to Newcastle antigen in chick serum is presented in Table No. 1 after vaccinations. The statistical analysis found substantial differences, with the three vaccinated groups showing the greatest rise after the first, second, and third group vaccines compared to the first (control group). The G4 demonstrated a substantial superiority in the volumetric amount of inhibitory antibodies after the first vaccine at a value of 6.78 ± 0.8, but the G3 demonstrated a significant superiority over the G2 and G4 after the second and third vaccines at values of 8.43 ± 0.25 and 8.73 ± 0.17, respectively.

Table 2. Newcastle agglutinin antibodies that inhibit volumetric agglutination.

Group	Standard Antibodies Log 10 (7 Days)	Standard Antibodies Log 10 (14 Days)	Standard Antibodies Log 10 (28 Days)
G1	3.01 ± 0.18	2.2 ± 0.15	1.42 ± 0.12
G2	4.82 ± 0.2 2	5.25 ± 0.15	6. 9 ± 0.15
G3	4.81 ± 0.9	8. 43 ± 0.25	8.73 ± 0.17
G4	6.78 ± 0.8	7.00 ± 0.18	7.51 ± 0.18

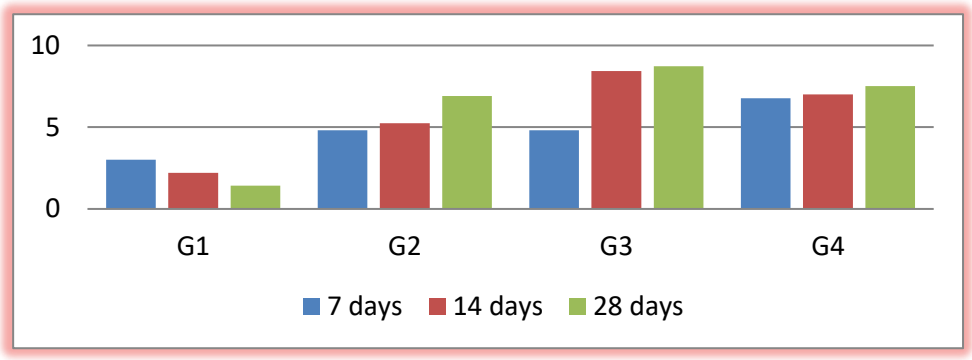

Figure 1. The volumetric standard rate of agglutination-inhibiting Newcastle agglutinin antibodies.

Table 2 The volumetric standard rate of Newcastle antibodies in the experimental ELISA test for the first, second, and third vaccination groups is depicted in the figure. Statistical analysis revealed that the amount of antibodies detected by the ELISA test increased significantly in the second, third, and fourth groups when compared to the control group.

A significant increase in the amount of antibodies measured by this test was observed in the fourth group following the first vaccine at value (8.78 ± 0.7), and a significant increase in the amount of antibodies was also observed in the G3 following the second and third vaccines at values (9.33 ± 0.13) and (9.53 ± 0.16), respectively.

Table 3. ELISA volumetric Newcastle antibody titer rate test.

Group	Standard Antibodies Log ^{10} (7 Days)	Standard Antibodies Log ^{10} (14 Days)	Standard Antibodies Log ^{10} (28 Days)
G1	4.01 ± 0.17	3.1 ± 0.15	1.7 2 ± 0.12
G2	5.9 2 ± 0.2 2	6.1 5 ± 0.15	6.8 ± 0.91
G3	6.6 1 ± 0.65	9. 33 ± 0.13	9.53 ± 0.16
G4	8.78 ± 0.7	8.01 ± 0.19	8.72 ± 0.13

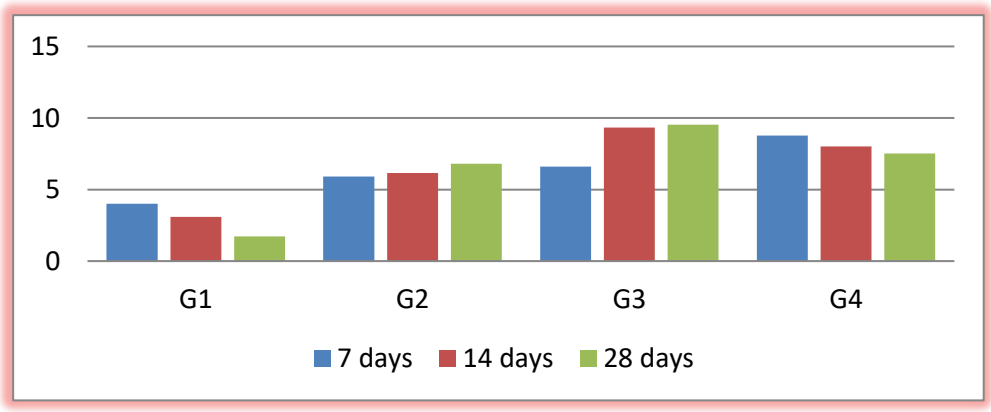

Figure 2. Statistical analysis WBCs (103 /mm3).

The ratio of heterophil/lymphocyt (H/L) is shown in Table 4. The statistical analysis found that there was a substantial drop in this proportion in the vaccinated groups compared to the control group, and there were significant differences between the vaccinated groups. There is also a significant decrease in this percentage in the G4 after the first vaccination compared to the percentages in the other groups at value (0.34 ± 0.03), while the G3 appears to have a significant decrease in this percentage at value (0.21 ± 0.006) (0.18 ± 0.007) after the second and third vaccinations, while the control group appears to have a significant increase in this ratio at value (0.42 ± 0.09).

Table 4. A ratio of heterophil/lymphocyt (H/ L).

group	7days WBCs (103 /mm3)	14 days WBCs (103 /mm3)	28 days WBCs (103 /mm3)
G1	17.9 ± 0.17	14.21 ± 0.15	6.27 ± 0.12
G2	19.1 ± 0.91	22.17 ± 0.35	25.13 ± 0.36
G3	20.91 ± 0.07	28.1 2 ± 0.1 2	32.11 ± 0.16
G4	24.8 ± 0.14	26.89 ± 0.65	28.97 ± 0.15

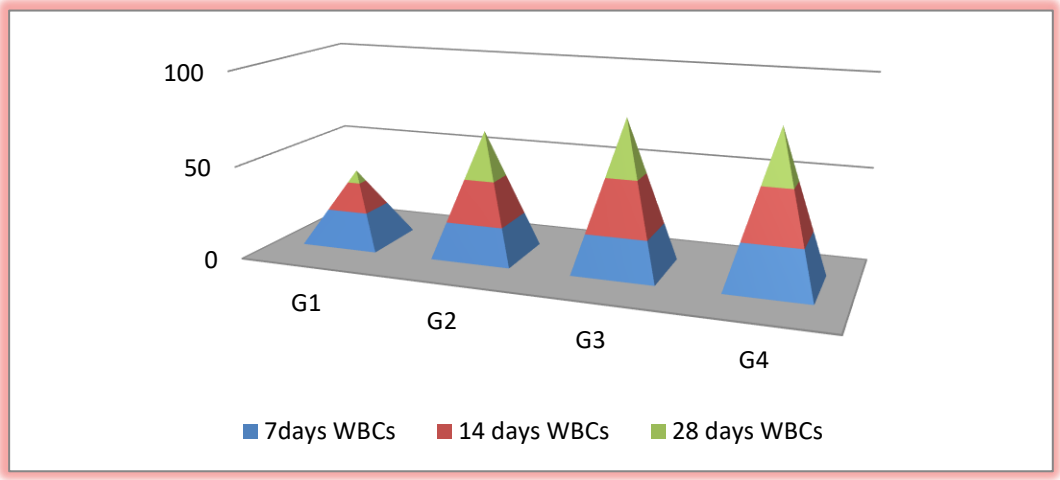

Figure 3. Statistical analysis ratio of heterophil/lymphocyt (H/ L).

4. Discussion

Cellular immunity is represented by the antibody titer while humoral immunity is represented by lymphocyte proliferation. The kind (immunoprotective, immunoregulatory/inhibitory, or immunopathological), duration and immune system components evaluated all affect the immunological result [19], [20].

The results obtained from our study showed a significant increase in the volumetric amount of antibodies that inhibit the virus that causes Newcastle disease in poultry in the vaccinated groups compared to the negative control group. showing that the humoral immune system had been activated. A second or third dosage of the vaccine increases the immune response. Immune memory is responsible for this [21]. Beginnings the control group had an increase in immunological antibodies after being measured on day 7 of the bird's life, which was linked to the persistence of maternally derived antibody (MDA) against NDV transmission. It is well known that certain IgG is transferred from the serum of the hen to the yolk and subsequently to the circulation of the embryo, and that the breeder's vaccination regimen has a significant impact on the amount of this antibody.

According to our studies and those of others (Elankumaran et al., 1996 [22] , the concentrations of these maternally derived IgG gradually decline in serum, tears, and bile. They are also partially transferred to the bile and the lachrymal fluid Toro et al., 1993 [23] [Kitaguchi et al., 2008] [24]. Live NDV vaccination has long been demonstrated to be hampered by MDA (Ganapathy et al., 2006) [25]. This is in line with earlier research

findings. Bell and associates [26] In fact the antibodies resulting from vaccination through drinking water may interfere with the maternal antibodies, which leads to the vaccine failing. Therefore, we did not give the vaccine to the birds in the first days of the bird's age, as the groups were vaccinated on the tenth and twenty days of age. Researchers in this field explained [27] that where.

the presence of IgA and IgM secreting cells in the duodenum's lamina propria, caecal tonsils, and chicken caecum is now widely recognized this explains the increase in immunological antibodies observed after avian immunization with drinking water. When measured after the initial immunization,immunological antibodies increased in the group that was vaccinated via the ocular route compared to the other two groups in our study. The findings back up recent studies that demonstrated stronger antibody responses in hens vaccinated intraocular [28], high levels in the Para nasal and Harderian glands, and maternal antibodies don't affect the immune system [29]. The development of immunological responses depends critically on the chicken Harderian gland [30]. In parts of the Harderian glands, eye drop vaccinations cause a considerable increase in plasma cells, which leads to the synthesis of the required humoral and local antibodies [31], [32]. When compared to ocular vaccination, the direct migration of vaccinal viruses into the Harderian and paranasal glands permits significant numbers of viruses to escape the chickens' humoral and phagocytic antiviral systems, resulting in improved immune responses. Our results are comparable with those of [33] Okwor et al. (2013), who discovered that chickens injected intraocularly had higher antibody levels than those vaccinated orally.The amount of immunological antibodies to the Newcastle vaccine does not rise for at least 14 to 21 days in addition, vaccination by injection with a killed vaccine preceded by a live, attenuated vaccine administered by eye and boosted with another live vaccine using the drinking water method for the third group provided excellent protection for the Newcastle virus, as the live vaccine is considered a starter . Vaccination by spraying also provided protection for group tow , but to a lesser extent, as this is due to g-secreting cells were found in avian lung tissue by Jeurissen et al. 1994 [34] , who believed that they were the main source of Ig in the lungs. However, the lungs get the Ig generated in the trachea and upper respiratory tract [35]. Lung-associated antibody-mediated immunity is boosted by tracheal vaccination. When the vaccinated groups were contrasted with the control groups, the quantity of WBCs (103/mm3) increased dramatically. The formation of various white blood cell types and immune system activation may be the reason Seyedeh Ameneh 2012 [36]; these results are in line with those of Eisa and S. Abdel-Hamied 2003 and other researchers [37]. The results were particularly positive for lymphocytes, which were linked to higher amounts of immunoglobulins, especially beta and gamma types. McFarland and Curtis claim that because an increase in the H/L cell ratio signifies the presence of stress, it provides a comprehensive indicator of one's actual health status [38].On the other hand, because stress works, a lower ratio means that the bird is in the best possible breeding health and is not under any stress. The decomposition of lymphocytes produced in lymphoid tissues and the spleen is directly impacted by it, as it stimulates the adrenal gland and increases the secretion of special hormones like corticosteroid hormone [39]. This leads to an increase in heterogeneous cells and, consequently, increase L/H. Our results consist with many researchers in this flied such as Ellakany et.al.2018, Wu et.al,2023 [40], [41].

5. Conclusion

Newcastle disease is one of the most dangerous viral diseases due to the economic losses it causes due to high mortality in poultry flocks or vaccine programs, as it is difficult to find a consistent vaccination program against the disease on a large scale in all countries around the world, and even within one country. This is owing to differences in the virulence of the strains producing the disease or the presence of genetic alterations between different strains .It is possible to use a preventive program represented by using

vaccination using the killed vaccine method supplemented with another vaccine using the drinking water method as an applied method in poultry farming fields, with good results in our study.

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