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Impact of multiple *Lasota* vaccinations on the haematological parameters of pullets infected with velogenic Newcastle disease virus

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Abstract

Newcastle disease (ND) is an important disease of poultry worldwide. This study aimed to evaluate the impact of multiple ND *Lasota* vaccinations on haematological parameters of pullets before and after experimental infection with velogenic ND virus (vNDV). A total of 100-day-old chicks were used for the study. They were randomly assigned into five groups, as follows: L1, L2, L3, unvaccinated unchallenged (UU) and unvaccinated challenged (UC). The L1, L2 and L3 were the groups vaccinated once, twice and thrice, respectively, while the UU and UC groups served as negative and positive control groups, respectively. At three weeks of age, birds in groups L1, L2 and L3 were given 0.5 ml of *Lasota* vaccine orally. At five weeks of age, L2 and L3 were given the second dose. Only the L3 birds were given the third dose at seven weeks of age. Groups L1, L2, L3 and UC were experimentally challenged with 0.2 ml of vNDV intramuscularly at 10 weeks of age. Blood samples were collected from the pullets for haematological evaluations before vaccination commenced (Day 0), and two, four, six and seven weeks post commencement of vaccination (PCoV). Results showed that in all the groups, there was no significant ($P>0.05$) difference in the packed cell volume (PCV), red blood cell, (RBC), haemoglobin concentration (HBC), total white blood cell (TWBC) and absolute lymphocytic counts on day 0. At weeks two and four, PCoV, TWBC, lymphocyte and heterophils counts were significantly ($P<0.05$) higher in the vaccinated groups compared to the control. On week six PCoV, L1 had significantly ($P<0.05$) higher TWBC and heterophil counts compared to L2, L3, UU and UC. It was concluded that vaccinating pullets once with *Lasota* vaccine was more effective in eliciting a sustainable leukocytic response than vaccinating twice or three times, especially before viral challenge.

Keywords: Haematology, *Lasota* vaccination, Leukocytic profile, Newcastle disease, Pullets

Introduction

Newcastle disease (ND) remains a big threat to the poultry industry worldwide, and specifically in Nigeria and Africa, despite the use of ND vaccination. The ND virus (NDV) has a wide host range and has been reported to infect many species of birds, and besides poultry, species specific virulent NDV (vNDV) strains are commonly found in pigeons and double crested cormorants, geese, pheasants, quails, and even certain raptor species, but generally, study and preventive studies and preventive efforts are usually mainly targeted at chickens – pullets, laying birds and broilers (Pchelkina *et al.,* 2013; Getabalew *et al.,* 2019; McMullin, 2020; Ramsubeik *et al.,* 2023). Broilers have been reported to be more susceptible to ND than pullets (Omeke *et al.,* 2018). The causative agent of ND is a non-segmented, single-stranded RNA virus of the genus *Orthoavulavirus* 1 subfamily *Avulavirinae* within the family *Paramyxoviridae* and order *Mononegavirales* (Amarasinghe *et al.,* 2019; Walker *et al.,* 2019). Protection of birds against ND is through the use of vaccines manufactured with low virulence NDV strains. Vaccination against ND is mainly by use of the ND Lasota vaccine, which is highly antigenic and easy to administer through drinking water (Omeke *et al.,* 2021). An important setback for ND control in developing countries such as Nigeria is the lack of a ''cold chain'' to preserve the vaccines at 4℃. It has been posited that even the best live vaccine will not induce an immune response if it is not viable due to improper storage during transportation to the point of need (Kapczynski *et al.,* 2013).

Clinical signs and pathological changes presented by the affected birds depend on many factors such as the virus strain, host species, host age, secondary infections, stress, environmental conditions, host immune status, viral dose, and route of infection (Getabalew *et al.,* 2019; WOAH, 2022). Newcastle disease must be promptly diagnosed and treated with the proper management techniques, including vaccination, isolating affected birds and strict biosecurity measures whenever it occurs (Al-Rasheed, 2024).

Despite the use of vaccination against the disease, ND continues to adversely affect the poultry industry worldwide (Xu *et al.,* 2019; Mousa *et al.,* 2020; Joshi *et al*., 2021; Hu *et al*., 2022). Even though current vaccines can checkmate the outbreak of the disease and death from the disease when given properly, the virus can infect vaccinated animals and replicate, leading to its spread (Miller and Koch, 2013; Bello *et al.,* 2018; Mahamud *et al.,* 2022). It has further been reported that vaccination with ND *Lasota* vaccine may not stop drop in egg production in laying chickens affected with NDV and this alone causes heavy economic losses to farmers annually (Igwe *et al.,* 2020).

Haematology evaluations remain indispensable diagnostic tools for evaluating health status in healthy and diseased individuals, monitoring the progress of diseases, evaluating the response to vaccines and therapy and making a prognosis (Samour, 2009). Due to lack of proper preservation and frequent vaccine failure, farmers constantly re-vaccinate their birds to hopefully induce an adequate immune response. There is a paucity of information on the effects of multiple ND *Lasota* vaccinations on chickens. The present study evaluated the effects of multiple ND *Lasota* vaccinations on the haematology parameters of pullets before and after experimental infection with velogenic Newcastle disease virus.

Materials and Methods

Study location

The study was carried out at the Poultry Unit of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

Study animals

One hundred day-old pullets were procured from a reputable hatchery for the study. The birds were raised in a deep litter system. They were vaccinated against infectious bursal disease (IBD) on days 10 and 21 of age. The birds were also treated against Eimeria infection (coccidiosis) and helminth infestation at two weeks of age.

Study design

The birds were randomly assigned to five groups of 20 birds each. The groups were designated as *Lasota* 1× (L1), *Lasota* 2× (L2), *Lasota* 3× (L3), unvaccinated unchallenged (UU) and unvaccinated challenged (UC). L1, L2 and L3 served as vaccinated groups, while UU and UC served as negative and positive control groups, respectively. The groups were housed in separate pens at a bio-secured experimental facility of the Veterinary Pathology Department, they were fed with commercial feed and water provided *ad libitum*.

Vaccination and Infection: The ND *Lasota* vaccine used for the study was procured from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The 200-dose *Lasota* vaccine was

diluted with 20 ml of phosphate-buffered saline (PBS). It had a median embryo infective dose (EID50) of 106.2 per ml. At three weeks of age, pullets in vaccination groups (L1, L2 and L3) were given 0.5 ml of the vaccine while pullets in UU and UC group received 0.5 ml of PBS used in diluting the vaccine orally as placebo. At five weeks of age, groups L2 and L3 received second dose of the vaccine. Then at seven weeks of age, only group L3 pullets received the third dose of the vaccine. The vaccine was administered through drenching using 1ml syringes.

Infection of the pullets

One millilitre of the inoculum (KUDU- 113) was reconstituted with 20 ml of phosphate-buffered saline (PBS) to give a median embryo lethal dose (ELD50) of 106.46 per ml (Echeonwu *et al.,* 1993). At 10 weeks of age, groups L1, L2, L3 and UC were experimentally challenged with 0.1 ml velogenic Newcastle disease virus (vNDV) through the intramuscular route.

Clinical signs and pathological changes of Newcastle disease

Birds in all the groups were observed/monitored for clinical signs before and after challenge with the virus. The clinical signs, body weight of the birds, gross pictures, histopathology changes and serology results were recorded.

Haematological evaluations

At three weeks of age, before first vaccination, five birds from each group were randomly selected for blood collection, and 1 ml of blood sample was collected from each bird, into sample bottles containing ethylenediaminetetra acetic acid (EDTA). The blood sample collection was repeated on the 5th, 7th, 10th and 11th weeks of age (that is, before each vaccination and one week after infection with NDV), corresponding to weeks two, four, six and seven post commencement of vaccination (PCoV). Parameters that were evaluated on the blood samples were total white blood cell (TWBC) counts, differential white blood cell counts, packed cell volume (PCV), red blood cell count (RBC) and haemoglobin concentration (Hb). Total white blood cell (TWBC) counts were done by the hemocytometer method (Schalm *et al.,* 1975), using Natt and Herrick's avian blood cell diluting fluid (Campbell, 1994) and an improved Neubauer counting chamber (Hawksley and Sons Ltd. West Sussex, UK) on a light microscope (Leica Gallen, New York, USA). The differential leukocyte counts (DLC) was done on air-dried thin blood smears stained by the Leishman technique (Campbell, 1994). Packed cell volume (PCV) was determined by the microhematocrit method (Thrall & Weiser, 2002), using a Hematospin 1400® microhaematocrit centrifuge and a Hawksley Microhaematocrit Reader® (Hawksley and Sons Ltd. West Sussex, UK). The hemoglobin concentration was determined by the cyanomethemoglobin method (Higgins *et al.,* 2008), using an automated blood biochemistry analyzer (Wuxi HiwellDiatek Instruments Co. Ltd., Wuxi, China). Red blood cell (RBC) counts was done by the hemocytometer method (Schalm *et al.,* 1975), using Natt and Herrick's avian blood cell diluting fluid (Campbell, 1994) and an improved Neubauer counting chamber (Hawksley and Sons Ltd. West Sussex, UK) on a light microscope (Leica Gallen, New York, USA).

Statistical analysis

Data generated in the study were subjected to one-way analysis of variance (ANOVA) using SPSS statistical package version 15.0 computer software (IBM, USA). Variant means were separated post hoc using the least significant difference methods. The level of significance was accepted at $P<0.05$. A summary of the data from all groups was presented as mean ± SEM in tables.

Results

Birds in all groups were observed for clinical signs of ND after challenge (PC). The morbidity and mortality rates were monitored. Pullets in the unvaccinated challenge showed clinical signs of ND, and mortality was also recorded. In haematology examination, there were no significant ($P>0.05$) variations between all the groups in all the parameters at day zero (before vaccination commenced). On weeks two and four post commencement of vaccination (PCoV), the TWBC counts of the L1, L2 and L3 groups were significantly ($P<0.05$) higher than those of the UU and UC (Table 1). But on week six PCoV, only group L1 had TWBC counts significantly ($p < 0.05$) higher than those of all other groups (Table 1). At week seven, PCoV, however, the TWBC counts of L1, L2, and L3 groups became significantly ($P<0.05$) higher than those of Group UU (Table 1).

The lymphocyte counts of the L1, L2 and L3 groups were significantly ($P<0.05$) higher than those of the UU and UC groups on weeks two and four PCoV, but on week six PCoV, the lymphocyte counts of the groups L1 and L2 were significantly ($P<0.05$) higher

Table 1: The total white blood cell counts of pullets given *Lasota* vaccine and challenged with velogenic Newcastle disease virus (vNDV)

Experimental groups	Means of total white blood cell counts (10^3/µl) with standard deviation in brackets				
	Day 0	Week 2	Week 4	Week 6	Week 7
L1 (*Lasota* 1×)	72.33 (10.97)	106.17[a] (18.06)	105.50[a] (5.27)	103.83[a] (3.40)	110.00[a] (5.72)
L2 (*Lasota* 2×)	67.67 (8.18)	104.50[a] (15.27)	101.17[a] (16.66)	80.50[b] (7.40)	104.67[a] (6.09)
L3 (*Lasota* 3×)	70.67 (4.73)	110.33[a] (13.01)	99.17[a] (7.75)	65.83[b] (6.37)	114.67[a] (10.53)
UU (Unvaccinated Unchallenged)	68.67 (6.66)	67.00[b] (7.55)	73.83[b] (7.42)	72.00[b] (17.35)	74.00[b] (7.55)
UC (Unvaccinated Challenged)	69.64 (7.11)	66.32[b] (7.86)	72.67[b] (7.78)	73.33[b] (6.43)	–

[a b] Different alphabetical superscripts in a column indicate significant differences between the means of the groups, P<0.05

Table 2: The absolute lymphocyte counts of pullets given *Lasota* vaccine and challenged with velogenic Newcastle disease virus (vNDV)

Experimental groups	Means of lymphocyte counts (10^3/µl) with standard deviation in brackets				
	Day 0	Week 2	Week 4	Week 6	Week 7
L1 (*Lasota* 1×)	42.67 (7.68)	60.56[a] (13.03)	54.48[a] (5.14)	48.80[a] (7.15)	58.91[ab] (6.06)
L2 (*Lasota* 2×)	39.94 (5.16)	61.38[a] (10.92)	63.53[a] (11.69)	48.60[a] (8.30)	58.79[ab] (6.07)
L3 (*Lasota* 3×)	42.40 (4.06)	62.81[a] (10.39)	62.56[a] (8.81)	35.67[b] (3.39)	65.62[a] (13.37)
UU (Unvaccinated Unchallenged)	40.25 (3.39)	38.89[b] (5.93)	39.95[b] (3.38)	36.35[b] (4.73)	39.08[b] (0.46)
UC (Unvaccinated Challenged)	40.57 (3.89)	39.16[b] (4.18)	39.84[b] (2.11)	36.83[b] (5.33)	–

[a b] Different alphabetical superscripts in a column indicate significant differences between the means of the groups, P<0.05

Table 3: The absolute heterophil counts of pullets given *Lasota* vaccine and challenged with velogenic Newcastle disease virus (vNDV)

Experimental groups	Means of heterophil counts (10^3/µl) with standard deviation in brackets				
	Day 0	Week 2	Week 4	Week 6	Week 7
L1 (*Lasota* 1×)	23.49 (4.69)	36.63[a] (3.41)	40.53[a] (4.73)	49.03[a] (4.14)	49.36[a] (12.40)
L2 (*Lasota* 2×)	23.38 (1.43)	35.68[a] (12.73)	31.85[ab] (4.42)	25.25[b] (6.29)	40.98[ab] (9.80)
L3 (*Lasota* 3×)	22.64 (2.39)	37.46[a] (3.26)	31.99[ab] (3.51)	23.93[b] (7.14)	43.04[ab] (8.85)
UU (Unvaccinated Unchallenged)	23.00 (1.53)	23.51[b] (3.96)	27.47[b] (5.99)	29.03[b] (5.15)	28.25[b] (8.57)
UC (Unvaccinated Challenged)	22.37 (1.36)	22.88[b] (3.92)	27.05[b] (8.06)	30.03[b] (4.81)	–

[a b] Different alphabetical superscripts in a column indicate significant differences between the means of the groups, P<0.05

than those of Groups L3, UU and UC (Table 2). At week seven, PCoV, however, the lymphocyte counts of Group L3 pullets were significantly (P<0.05) higher than those of the UU group only (Table 2).

At week two PCoV, the heterophil counts of groups L1, L2 and L3 pullets were significantly (P<0.05) higher than that of groups UU and UC, but at week 4 PCoV, only the heterophil counts of the group L1 pullets was significantly (P<0.05) higher than those of the UU and UC groups (Table 3). Further at week six PCoV, the heterophil counts of the group L1 pullets were significantly (P<0.05) higher than those of all other groups, but at week seven PCoV, the heterophil counts of the group L1 was significantly (P<0.05) higher than that of group UU only (Table 3). There were no significant differences (P>0.05) between the groups in their eosinophil counts throughout the study, except in week 6 PCoV, when the eosinophil count of the group L1 pullets was significantly (P<0.05) higher than that of the group L2 pullets (Table 4). There were also no significant differences (P>0.05) between all the groups in their monocyte counts throughout the study (Table 5).

The PCV and Hb of the groups did not significantly vary (P>0.05) throughout the study, except at week seven PCoV, when the PCV and Hb of the group L2 pullets were significantly (P<0.05) lower than those of the group UU pullets (Tables 6 and 7). The RBC counts

Table 4: The absolute eosinophil counts of pullets given *Lasota* vaccine and challenged with velogenic Newcastle disease virus (vNDV)

Experimental groups	Means of eosinophil counts (10^3/µl) with standard deviation in brackets				
	Day 0	Week 2	Week 4	Week 6	Week 7
L1 (*Lasota* 1×)	5.14 (1.57)	7.63 (3.79)	8.16 (6.22)	6.56[a] (0.38)	2.91 (0.72)
L2 (*Lasota* 2×)	4.71 (0.79)	5.44 (5.30)	3.43 (1.16)	2.93[b] (1.57)	3.49 (0.68)
L3 (*Lasota* 3×)	4.25 (0.93)	5.72 (3.66)	3.44 (1.10)	4.56[ab] (0.32)	4.76 (3.03)
UU (Unvaccinated Unchallenged)	3.96 (1.65)	4.15 (1.64)	3.70 (1.96)	4.27[ab] (2.58)	3.00 (2.03)
UC (Unvaccinated Challenged)	4.50 (1.26)	4.29 (1.16)	4.02 (1.43)	3.92[ab] (1.96)	–

[a b] Different alphabetical superscripts in a column indicate significant differences between the means of the groups, P<0.05

Table 5: The absolute monocyte counts of pullets given *Lasota* vaccine and challenged with velogenic Newcastle disease virus (vNDV)

Experimental groups	Means of Monocyte counts (10^3/µl) with standard deviation in brackets				
	Day 0	Week 2	Week 4	Week 6	Week 7
L1 (*Lasota* 1×)	2.02 (0.46)	2.03 (0.21)	2.75 (1.05)	2.44 (1.62)	1.82 (0.67)
L2 (*Lasota* 2×)	1.73 (0.56)	2.01 (0.65)	2.35 (0.62)	3.22 (0.30)	1.40 (0.63)
L3 (*Lasota* 3×)	1.84 (0.41)	2.15 (0.26)	2.30 (0.57)	1.51 (0.20)	1.79 (1.28)
UU (Unvaccinated Unchallenged)	1.95 (0.54)	1.95 (0.23)	2.94 (0.68)	2.02 (1.44)	2.01 (1.58)
UC (Unvaccinated Challenged)	1.97 (0.25)	2.20 (0.53)	2.77 (0.92)	1.98 (1.31)	–

Table 6: The packed cell volume (PCV) of pullets given *Lasota* vaccine and challenged with velogenic Newcastle disease virus (vNDV)

Experimental groups	Means of PCV (%) with standard deviation in brackets				
	Day 0	Week 2	Week 4	Week 6	Week 7
L1 (*Lasota* 1×)	26.73 (2.11)	28.03 (1.45)	29.70 (4.05)	29.97 (1.27)	28.70[ab] (1.25)
L2 (*Lasota* 2×)	27.70 (2.15)	29.07 (2.41)	29.77 (1.94)	25.40 (1.60)	24.03[a] (2.97)
L3 (*Lasota* 3×)	26.53 (2.14)	29.97 (0.74)	29.57 (1.61)	28.33 (2.63)	26.67[ab] (3.89)
UU (Unvaccinated Unchallenged)	27.07 (1.19)	27.90 (0.56)	28.03 (1.65)	29.10 (4.00)	29.07[b] (1.35)
UC (Unvaccinated Challenged)	26.93 (1.81)	27.90 (0.75)	28.20 (1.15)	29.03 (2.30)	–

[a b] Different alphabetical superscripts in a column indicate significant differences between the means of the groups, P<0.05

Table 7: The haemoglobin concentration (Hb) of pullets given *Lasota* vaccine and challenged with velogenic Newcastle disease virus (vNDV)

Experimental groups	Means of Hb (g/dl) with standard deviation in brackets				
	Day 0	Week 2	Week 4	Week 6	Week 7
L1 (*Lasota* 1×)	9.73 (0.96)	10.26 (0.95)	9.88 (1.13)	9.61 (0.14)	10.24[ab] (0.70)
L2 (*Lasota* 2×)	9.99 (0.84)	10.14 (0.97)	9.60 (0.59)	9.03 (0.16)	8.82[a] (0.78)
L3 (*Lasota* 3×)	9.39 (1.18)	10.26 (0.85)	9.63 (0.56)	9.45 (0.66)	9.41[ab] (1.62)
UU (Unvaccinated Unchallenged)	9.70 (0.78)	9.44 (0.13)	9.04 (0.46)	9.76 (1.26)	11.43[b] (1.34)
UC (Unvaccinated Challenged)	9.71 (0.61)	9.16 (0.11)	9.11 (0.18)	10.12 (1.13)	–

[a b] Different alphabetical superscripts in a column indicate significant differences between the means of the groups, P<0.05

of the groups also did not significantly vary (P>0.05) all through the experiment except at week seven PCoV, when the RBC count of the group L2 pullets was significantly (P<0.05) lower than those of the groups L1 and UU pullets (Table 8).

Discussion

The results of TWBC and differential WBC counts showed that single Lasota vaccination of birds led to the highest enhancement of white blood cells mobilization from the bone marrow reserves, and this is a positive attribute because enhanced white blood cells mobilization will put the animal in a better position to effectively withstand infection challenge (Stockham & Scott, 2008; Wakenell, 2010). This significant higher TWBC and differential WBC observed in this study have been reported by early

Table 8: The red blood cell (RBC) counts (10^6/µl) of given Lasota vaccine and challenged with velogenic Newcastle disease virus (vNDV)

Experimental groups	Means of RBC counts (10^6/µl) with standard deviation in brackets				
	Day 0	Week 2	Week 4	Week 6	Week 7
L1 (*Lasota* 1×)	3.27 (0.41)	3.88 (0.28)	3.68 (0.31)	3.82 (0.08)	3.79[a] (0.08)
L2 (*Lasota* 2×)	3.61 (0.28)	4.06 (0.25)	3.80 (0.09)	3.42 (0.19)	3.37[b] (0.16)
L3 (*Lasota* 3×)	3.40 (0.44)	3.95 (0.27)	3.74 (0.06)	3.66 (0.30)	3.63[ab] (0.33)
UU (Unvaccinated Unchallenged)	3.53 (0.22)	3.65 (0.16)	3.67 (0.21)	3.69 (0.34)	3.85[a] (0.08)
UC (Unvaccinated Challenged)	3.46 (0.26)	3.72 (0.15)	3.70 (0.16)	3.72 (0.29)	–

[a b] Different alphabetical superscripts in a column indicate significant differences between the means of the groups, P<0.05

researchers (Malik *et al.,* 2018). The total WBC count was significantly high only in the vaccinated groups; in the unvaccinated unchallenged and unvaccinated challenged the total white blood cell count was significantly low when compared to vaccinated groups.

The vaccination of the birds brought about the increase in TWBC, which eventually led to an increase in antibody production in these groups. It is worthy to note that the birds in group that received vaccine once elicited higher TWBC, lymphocyte and heterophil counts, and considering the functions of these specified cell types in blood of birds (Campbell, 1994; Wakenell, 2010), birds given vaccine once should be considered the ones best prepared for any form of pathogen challenge than others that received twice or thrice. The increase in lymphocytes will help in identifying the viral antigen, which will signal B lymphocytes to produce more of antibodies against the antigen. The principal function of lymphocytes is immunological activity. This might have contributed to the higher antibody titre production observed in the vaccinated groups, especially in groups L1 and L2 post-infection. Heterophils count, which is equivalent of mammalian neutrophil, is critical to the immune defence of birds. Single *Lasota* vaccination led to sustainably high heterophil counts throughout the study in comparison to vaccination two or three times; this is believed to be a plus for single vaccination when the role of heterophils in the defence of the body against pathogens is put into consideration. Vaccinations have had various side effects between men and women, with the human papillomavirus (HPV) vaccine being a good example (Meites *et al*., 2019). Evaluation of the association between the COVID-19 vaccine (CoronaVac-BNT162B2) and haematological abnormalities showed thrombocytopenia, leukopenia and neutropenia (Sing *et al.,* 2022). Granulocytopenia or leukocytopenia have been reported in patients who received Pfizer vaccine (Veerman *et al.,* 2022). The incidence of thrombocytopenia observed in patients with SARS-CoV-2 has been attributed to the infection of the bone marrow cells that leads to abnormal hematopoiesis. WBC counts are involved in the antibodies response, making an investigation of the effects of multiple *Lasota* vaccinations on WBC level very important. Newcastle disease is a disease of primarily the respiratory system therefore it is worth investigating the connection between the NDV vaccine and possible alterations in RBC and HB. Any level of alteration of these parameters may affect the ability of the RBC and HB to deliver oxygen to different areas of the body. Severe changes in RBC membrane homeostasis may result in a decreased ability of RBC to respond to environmental variations in oxidative stress when travelling to the body. In this study, a significantly high level of RBC and HB concentration was observed in birds in the group that received *Lasota* vaccine once, while those that received twice and thrice had low RBC and Hb concentration. This is also believed to be a plus for single *Lasota* vaccination when the roles of these parameters are considered. Vaccination is considered the best method of controlling the pandemic. Many researchers have observed haematological abnormalities in humans and animals following vaccination. Vaccination has been shown to cause an inflammatory response, which results in homeostatic changes, including changes in renal and hepatic functions. Andersen *et al.* (2012) have reported that too-frequent vaccination may have adverse effects, among other things due to an inflammatory response elicited with subsequent alterations in homeostasis. The decrease of RBC and Hbc observed in L2 and L3 after infection of the birds have been reported earlier by Marlik *et al.* (2018).

In conclusion, the results of the study showed that the

pullets vaccinated once with Lasota vaccine mounted a more sustainable leukocytic (Total WBC, heterophil and lymphocyte) response than those vaccinated twice or thrice, before viral challenge. Multiple vaccinations with *Lasota* vaccine did not seem relevant. Put together with the clinical signs following viral challenge, single *Lasota* vaccination of pullets is advised.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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