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Hematological and biochemical parameters of ostriches after vaccination against Newcastle disease

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Abbreviations:
ND – Newcastle disease

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Abstract

Background and Purpose: *Although chickens are routinely vaccinated against Newcastle disease, vaccination of ostrich is less well understood. To assess the effect of vaccination on the health of ostriches, key biochemical parameters and differential blood count were monitored after vaccination by La Sota strain of the Newcastle disease virus, which is widely used in chickens.*

Materials and Methods: *The investigation was performed in 24 adult ostriches divided into three study groups and a control group, each comprising six ostriches. In the study groups birds were vaccinated via different routes: drinking water, oculo-nasally or by spraying. Blood samples were collected immediately before the vaccination and on days 7, 14, 21 and 28. Total erythrocyte counts, hemoglobin concentrations, hematocrit values, as well as total and differential leukocyte counts were assessed. Total albumins and globulins in serum were quantitated spectrophotometrically.*

Results: *Erythrocyte count, hemoglobin concentration, hematocrit values and total leukocyte count were not significantly changed in any group. Only leukocyte differentiation yielded a significant decrease in eosinophiles in all groups and a significant monocyte increase in groups vaccinated via drinking water and oculo-nasally. While the lower eosinophil count could be attributed to the experimental stress, increased monocyte percentage indicates successful immunological reaction against the vaccine virus. In all groups, total serum proteins were elevated within physiological boundaries, with albumin to globulin ratio suggesting stimulation of antibody production.*

Conclusion: *The results did not indicate any adverse health effects. Therefore, the vaccine which is already routinely used for chickens can be safely applied in ostrich.*

INTRODUCTION

Newcastle disease (ND) is caused by the avian paramyxovirus and affects various species of poultry. It is also an important disease of ostriches *(1)*, often incurring significant losses in ostrich farming since the mortality rate is around 30%. Despite the measures undertaken worldwide in order to eradicate the disease, outbreaks are a common occurrence even today *(2)*. Since the year 2000, all poultry in Croatia must be vaccinated against ND by the La Sota lentogenic strain of the virus.

Vaccination has been successfully used for protection of chickens, and similar procedures have been developed for ostriches. Although the widely used La Sota vaccine has been proven harmless to chickens, physiological reaction of adult ostriches is less well understood. Due to the marked difference in body size and to specificities of the immune system, results obtained in chickens cannot be directly extrapolated to ostriches *(3)*. This holds true especially because the farming conditions and climate in Europe differ considerably from the natural habitat of ostriches, which is central and southern Africa *(4)*.

In order to test if the La Sota vaccine has any harmful effect on the health of ostriches, we administered the vaccine in the ways adapted to the ostrich anatomy: via drinking water, oculo-nasally and by spraying. To assess the effect on health of the birds, we monitored key hematological and biochemical parameters. Differential blood count was used to gain additional insight into the way the ostrich immune system reacted to the vaccine. Standard ostrich blood parameter values reported by different researchers vary significantly *(5),* so the results were interpreted not only based on comparison to a set of reference values selected from the available literature, but the overall trend of parameter variation was taken into account as well.

MATERIALS AND METHODS

We conducted the investigation on 24 clinically healthy adult ostriches, hybrids between blue-neck and African black ostriches, weighing approximately 120 kg each. They were divided into four groups, each group having two families of one male and two female birds. A single family was kept in an enclosure with 800 square meters of open space and 12 square meters of covered space. The animals were fed once daily and had free access to drinking water, except the night before the vaccination.

In this study we used the live vaccine against ND (PESTIKAL® LA SOTA SPF, Veterina Ltd., Zagreb, Croatia). We administered the vaccine to the first group via drinking water as suspension in five liters per family. The second group received the vaccine oculo-nasally as suspension in 1 ml water per bird. We applied five drops into each nostril and two drops into each eye. We administered the vaccine to the third group by spraying using an atomizer, while the fourth group served as the control and received no treatment. Each vaccinated ostrich received approximately $20x10^6$ EID_{50} of the La Sota ND virus.

Blood samples were collected from the wing vein (*v. cutanea ulnaris*) into test tubes with potassium citrate immediately before the vaccination and on days 7, 14, 21 and 28 after the vaccination. We assessed total erythrocyte counts, hemoglobin concentrations, hematocrit values and total and differential leukocyte counts in the collected blood, as well as total serum albumins and globulins. The chemicals used in this investigation were produced by Herbos dijagnostika (Sisak, Croatia).

For measuring hemoglobin concentration in whole blood samples we used the cyanomethemoglobin method with spectrophotometer Helios Delta (Thermo Spectronic, Mercers Row, Cambridge, United Kingdom).

To determine hematocrit values, we centrifuged the samples in an HC 240 Tehtnica instrument at 2000 rpm, after which results could be read on capillary microhematocrit tubes.

For erythrocyte and leukocyte counting, we used Neubauer chamber, as described by Natt and Henrick *(5)*. To determine differential leukocyte count, we stained the blood smears using the May-Grünwald-Giemsa method. In this way we assessed interrelations of, and ratios between heterophils, eosinophils, basophils, lymphocytes and monocytes *(6)*.

We determined the concentration of serum proteins spectrophotometrically using the Helios Delta instrument. Serum was prepared by removing the clot from the whole blood sample and subsequent centrifugation for five minutes at 2000 rpm. We used the biuret method for determination of total serum protein concentration, while brom cresol green binding was used to assess the albumin fraction. We supposed that the fraction of total serum proteins not assigned to albumins represents the globulin fraction. From this data we calculated the albumin to globulin ratio.

Differences between individual parameters and their significance in repeated measurements were assessed using general linear model (GLM) procedure of repeated measurements method. Linear correlation was used to assess interrelations between individual groups. The assessment of the value of an individual characteristic (the dependent variable) was done on the basis of values of several independent variables and analyzed using multiple regression. We used the SAS statistical programme, version 8 (SAS Institute Inc., Cary, N. C., USA) and Statistics 5.0 (SPSS Inc., Chicago, USA) to process the experimental data.

RESULTS

Values for erythrocyte count, total leukocyte count, hemoglobin concentration and hematocrit showed no statistically significant change throughout the experiment, and they remained within their physiological limits. This indicates that no adverse reaction to vaccination which would affect the said parameters had taken place.

Differential leukocyte count revealed a picture consistent with a healthy immune system stimulated by an antigen. Monocyte count started to elevate slightly in all groups after the third or the fourth week (Figure 1A). Although the trend can be seen for all groups (Figure 1A), this change was found to be statistically significant only in groups which received the vaccine in drinking water ($F=3.03$, $p<0.0363$) and oculo-nasally ($F=4.86$, $p<0.0062$). The decrease in eosinophyl count (Figure 1B) was statistically significant in all groups (drinking water: $F=7.47$, $p<0.0004$; spraying: $F=3.56$, $p<0.0197$;

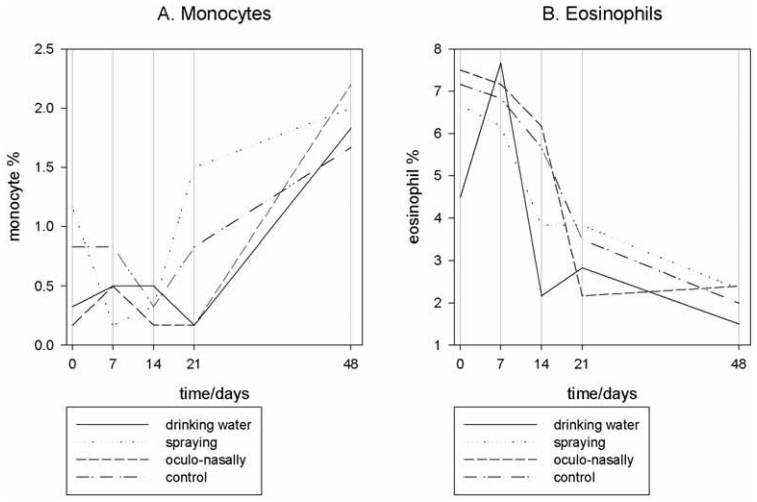

Figure 1. *Monocyte count has increased (A) and the eosinophil count decreased (B) after vaccination.*

oculo-nasally: $F=8.67$, $p<0.0002$; control: $F=8.37$, $p<0.0002$). This change might represent a stress reaction caused by experimental handling.

Total serum protein concentration increased in all groups (Table 1), and this increase was statistically significant (drinking water: $F=4.46$, $p<0.0074$; spraying: $F=5.81$, $p<0.0019$; oculo-nasally: $F=8.87$, $p<0.0002$; control: $F=6.10$, $p<0.0014$). The albumin fraction (Table 1) increased as well, although this change was significant only in groups vaccinated via drinking water ($F=3.51$, $p<0.0209$) and oculo-nasally ($F=3.74$, $p<0.0167$). The rest of serum proteins were treated as globulins, and the albumin to globulin ratio was calculated for the groups with significant albumin increase (Figure 2). This ratio decreased after vaccination, showing that globulins had an even higher impact on the total protein increase than albumins. This was expected, supposing that the increase is due to the gamma-globulin fraction representing the antibodies formed in response to the vaccine virus. Other serum proteins remained within the physiological range, thus indicating a healthy status of the vaccinated birds.

DISCUSSION

Erythrocyte count, hemoglobin concentration and hematocrit values did not change significantly over the whole study period and they were within the physiological range characteristic for the ostrich age *(7, 8, 9)*. Only when leukocytes were differentiated into heterophils, eosinophils, basophils, lymphocytes and monocytes, statistically significant changes were observed.

The basic role of eosinophils is the defense of the body against allergens and some parasites. Their count increases in the state of anaphylactic shock, while their percentage decreases under stress *(10)*. Since handling of experimental animals included vaccination and repeated blood sampling, we hold that the stress which it induced was responsible for the observed drop in the eosinophil count.

Increased monocyte percentage might indicate a successful defense of the body against a pathogen *(5)*. In a separate study, we found an increase in hemagglutination inhibition antibody titers for ND virus in the two groups mentioned (Šimpraga, personal communication).

TABLE 1

Total serum protein and albumin concentration. Mean values are given.

	Group	0 days	7 days	14 days	21 days	42 days
Total protein (g/L)	water	43.00	54.00	54.00	60.00	58.67
	o/n	48.33	55.33	57.83	58.17	61.67
	spraying	42.83	54.00	52.83	55.50	56.60
	control	43.00	58.00	56.67	56.33	55.00
Albumin (g/L)	water	23.33	24.00	23.50	25.33	25.67
	o/n	24.00	24.67	24.00	23.50	26.00
	spraying	22.17	24.17	22.50	24.17	27.40
	control	24.83	25.00	24.33	24.50	27.50

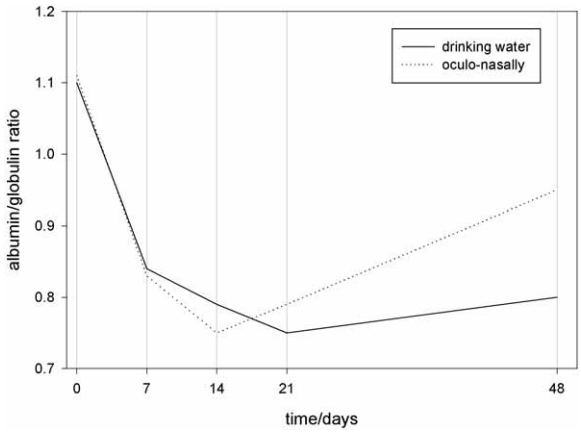

Figure 2. *Albumin to globulin ratio in groups where the change in albumin concentration was statistically significant.*

Heterophils, called »the window of the health status« by Maxwell and Robertson *(10)*, did not change during the whole investigation period. Generally, heterophils, being phagocytic cells, react during early stages of infection preventing bacterial growth and eliminating them in the course of attaining immunity. Heterophils can control bacterial proliferation, but not eliminate them as long as the birds do not develop immunity *(10)*. As no bacterial infection developed during the experiment, no heterophil increase was expected, and the obtained heterophil values were in accordance with those reported in literature *(8, 9)*.

Basophil count in peripheral blood was below the detection limit in some groups and trial periods, so the obtained data were insufficient for statistical analysis in those cases. Otherwise, the values were within the physiological range reported by Levy *(11, 12),* Fudge *(8)* and Green *(9)*.

Over the entire course of the experiment, lymphocyte percentage did not significantly change in any study group. Although the role of B lymphocytes in immune reactions is production of antibodies, their percentage in peripheral blood does not significantly change *(5)* after administration of lentogenic vaccines. Only certain pathogenic viruses and microorganisms which cause severe inflammations can induce lymphopenia, especially in young ostriches, during the first three days of infection *(13)*. In our experiment, lymphocyte values conformed to those reported by Green *(5),* as expected for the lentogenic vaccine that we used.

The increase of total serum proteins was observed in all groups, including the control. The increase was statistically significant, but remained within physiological limits throughout the experiment *(14)*. This variation could be explained by additional feeding which the birds received because of the season in which the experiment was conducted (winter). However, more interesting was the relative proportion of albumin to globulin fraction. The change in this ratio was found to be statistically significant in the groups vaccinated via drinking water and oculo-nasally. In these groups, albumin fraction increased at a rate roughly matching total protein increase, while globulin fraction increased even more. This led to decreased albumin to globulin ratio after vaccination, which can be an indicator of antibody production. Although globulin fractionation was not done, attributing their relative increase to immune response was corroborated by the already mentioned parallel study where the titer of specific antibodies against ND virus was assessed (Šimpraga, personal communication).

Finally, we conclude that vaccination of ostriches against Newcastle disease by a lentogenic ND virus does not cause adverse effects on the birds' health, since the key hematological and biochemical blood parameters remained well within the physiological boundaries *(11, 13, 15)*. Increase in monocyte count accompanied by an increase in the globulin fraction proportion represents the expected reaction to stimulation of the immune system, while decrease in the eosinophil count can be attributed to experiment-induced stress.

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