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Changes in blood cell count in chickens vaccinated as newly-hatched against Marek's disease using HVT FC 126 by means of nebulisation

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ABSTRACT

Marek's disease is one of the greatest problems in poultry production today and vaccination is one of the most important ways of prevention. An innvoative method of vaccine delivery by means of nebulisation was used for vaccination of newly hatched chicks using HVT FC 126 and its impact on blood cell count (WBC, RBC and differential cell count) and H/L ratio was measured. The trial was performed from day 1 to day 21 of life of newly hatched male chicks. Standard blood cell count was performed in a Neubauer hemocytometer, and differential cell count on blood smears stained with MayGrünwald-Giemsa. Blood for that purpose was taken from the jugular vein. The results show a significantly higher WBC count in the group vaccinated by means of nebulisation than in the non-vaccinated groups on day 5 of the trial, and from the group that received only physiological solution, on day 14. In the RBC count there were fluctuations but without any significant differences between the groups during the trial. In differential blood cell count, there were some significant differences on days 7 and 21 of the trial, but no differences in H/L ratio. These results show that vaccination by means of nebulisation significantly influenced the blood cell count but all changes were within the physiological range. Nebulisation as a method of vaccination could probably improve the immune response to the wild Marek's disease virus, mimicking natural infection via the respiratory system and as a mass form of vaccination could be a powerful method for delivery of recombinant HVT vaccine.

Key words: chicken, Marek's disease, blood cell count, nebulisation, vaccination

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Introduction

Marek's disease (MD) is one of the most important contagious viral diseases of chickens. Its lymphoprolypherative nature is characterized by pleomorphic lymphocyte infiltration of various tissues, as well as formation of lymphocyte tumors, often together with inflammatory and degenerative changes in neurons (neurolymphomatosis). MD is caused by alpha-herpesvirus of the genus *Mardivirus*, gallid herpesvirus 2, called MD herpesvirus 1 (MDV 1), but two herpes viruses, meleagrid herpesvirus 1 (HVT) and MD herpesvirus 2 (gallid herpesvirus 3) (MDV 2), are closely related non-pathogenic viruses used as specific vaccines against the MD (BAATEN et al., 2004). All these viruses infect the chicken organism through the respiratory route, and while strictly intracellular, they primarily infect lymphatic cells. MDV 1 infects B cells *via* macrophages, what leads to their lyses, and the same happens after activation and infection of T cells. Immune response, which is dominantly cellular, forces MDV into its latent phase, with later development of $CD4^+$ T-cell lymphomas (SCHAT and XING, 2000).

It is to be expected that infection with these viruses could cause changes in cellular subtypes. The results of previous research (OHASHI et al., 2001; MARKOWSKI-GRIMSRUD and SCHAT, 2002) have shown that infection with MDV's and HVT causes changes in cellular immune response, but none was able to demonstrate changes in basic blood cell count and heterophil/lymphocyte (H/L) ratio. Regarding knowledge of the molecular basics of MD pathogenesis, it is better known which mechanisms could influence erythro- and lymphopoiesis (YAMADA et al., 1998; BACHELDER et al., 2002), as well as immune response to infectious viruses (KARACA et al., 2004; SARSON et al., 2006; ABDUL-CAREEM et al., 2006 and 2008), and in that way also influence the number of those cells in the blood.

As lungs are the major route of MD virus entry, nebulisation as a method of vaccination *via* the respiratory system was developed for specific vaccine delivery (MAZIJA et al., 1994). HVT is the only virus in this group which can be produced as a freeze dried vaccine, and earlier research showed that Marikal® (Veterina d.o.o., Kalinovica, Croatia) was one of the vaccines that, because of its water solubility, can be easily used by means of nebulisation (MAZIJA et al., 1994). This method, besides the delivery of vaccine to the natural place of viral entry, is also less stressful. Earlier research showed that changes in cell count, especially in H/L ratio could be used as a measure of stress in chickens (GROSS and SIEGEL, 1983; GROSS, 1990; SHINI et al., 2008).

The aim of this research was to examine changes in blood cell count in chickens vaccinated as newly-hatched against MD by means of nebulisation, using the HVT FC 126 strain. Knowing the changes in blood cell count induced by this virus as potential carriers of vector vaccines against different virus diseases (e.g. Newcastle disease virus,

infectious bronchitis, infectious bursal disease, avian influenza etc.) will give new and necessary information on the nature of the immunity expected to be developed.

Materials and methods

Vaccine. For vaccination of newly-hatched chicks a commercial vaccine was used. Vaccine Lyomarex® (Merial, France), containing HVT FC 126, was diluted in distilled water, two vials with 1000 doses in 40 mL ddH_2O for nebulisation, and a vial with 1000 doses in 200 mL ddH_2O for s/c application, according to the manufacturer and one dose was given per chicken.

Vaccine delivery. Vaccine was applied by means of ultrasonic nebulisation using SONOVAC 095 (MAZIJA and ŠTIMAC, 1999; MAZIJA et al., 2009) or by standard s/c route to the nape of the neck. Vaccine for nebulisation was diluted whereby around 70 doses were present in a 1.5 mL of vaccine solution, which was aerosolized during 60 seconds of nebulisation. In the Sonoavc 095 the vaccine solution was treated with ultrasound which produces an aerosol with 95 % of particles ranging between 2-5 μm in diameter. During the nebulisation chicks were placed in a closed chamber in which the nebulized aerosol is delivered in that one dose of vaccine is provided per chicken.

Chicks and experimental design. Newly-hatched commercial male Lohmann light hybrid chicks were used in the experiment. During the trial, 300 chicks were held in cages, and water and feed were offered *ad libitum*. The chicks were separated into four groups (A, B, C, D), with 70 chicks per group, which were divided into 8 cages with a maximum of 9 chicks per 0.25 m^2. Newly-hatched chicks in group A were vaccinated by means of nebulisation and exposed for 60 seconds to the HVT vaccine, while group B received the same vaccine by parenteral route (s/c) according to the manufacturer's instructions. Group C received a physiological solution by means of nebulisation (60 seconds exposure), while group D was not treated and served as a negative control. The trial was performed over 21 days. Blood samples with heparin anticoagulant were taken from the jugular vein and blood smears were also made before vaccination (day 0) and on days 3, 5, 7, 10, 14 and 21 of the experiment.

Blood cell count and differential cell count. The number of white blood cells (WBC), as well as red blood cells (RBC) from the collected samples was counted in a Neubauer hemocytometer, according to Natt and Herrick (FUDGE, 2000; CRAY and ZAIAS, 2004), while differential WBC count was made on the blood smears stained with May-Grünwald-Giemsa. One hundred white blood cells were counted to determine differential WBC count, as well as to calculate the H/L ratio.

Statistical analysis. Data were tested for normality of distribution and analysed using the nonparametric Kruskall-Wallis test using Statistica 7.1 (Statsoft, 2005), and significant differences ($P<0.05$) were marked.

Results

White blood cell count. The WBC count was elevated on day 3 compared to day 0, without any significant differences between the groups (Table 1). On day 5 it was still rising in all groups, but in group A it rose to the of level 36.28 ± 7.72×10^9/L, which is significantly different only to the non-vaccinated groups C and D. From day 5 to day 7 WBC count declined to almost the original level before vaccination, without significant difference between groups. From day 7 to day 21 WBC count slightly rose in groups B, C and D, without significant differences between groups. The same count in group A rose significantly on day 14 compared to group C only, and declined slightly on day 21 without any significant difference compared to the other groups of chickens in the trial.

Table 1. White blood cell count in chicken blood during the trial (×10^9/L)

	Days after vaccination						
Groups	0	3	5	7	10	14	21
A	10.60 ± 1.92	22.50 ± 2.80	36.28 ± 7.72^a	12.24 ± 3.50	11.68 ± 3.08	18.08 ± 3.35^a	14.73 ± 2.76
B		22.83 ± 3.66	31.90 ± 9.38^{ab}	13.08 ± 2.98	12.38 ± 2.37	14.66 ± 3.61^{ab}	17.20 ± 2.96
C		19.08 ± 4.15	23.63 ± 6.98^b	10.73 ± 3.47	11.32 ± 2.78	12.96 ± 1.71^b	14.08 ± 2.94
D		21.70 ± 3.90	25.44 ± 2.35^b	11.03 ± 3.00	11.96 ± 3.12	14.48 ± 2.34^{ab}	14.24 ± 3.24

Values are expressed as mean value ± SD. Values with a different letter are significantly different (P<0.05) between groups on the same day of experiment

Table 2. Red blood cell count in chicken blood during the trial (×10^{12}/L)

	Days after vaccination						
Groups	0	3	5	7	10	14	21
A	2.22 ± 0.24	2.20 ± 0.24	1.79 ± 0.13	1.92 ± 0.20	2.01 ± 0.32	2.19 ± 0.23	1.95 ± 0.23
B		2.29 ± 0.34	1.92 ± 0.32	1.89 ± 0.23	1.83 ± 0.22	1.88 ± 0.17	2.11 ± 0.26
C		2.18 ± 0.20	2.08 ± 0.25	1.94 ± 0.21	2.12 ± 0.39	2.01 ± 0.18	2.21 ± 0.40
D		2.14 ± 0.17	1.94 ± 0.32	1.92 ± 0.20	1.92 ± 0.22	2.16 ± 0.25	2.29 ± 0.31

Values are expressed as mean value ± SD

Red blood cell count. The RBC cout dropped from day 3 to day 5 in all groups of chickens during the experiment (Table 2), but without any significance between the groups. On day 7 it rose slightly in group A, and declined in group C, and was at a similar level to the other two groups. On day 10 the RBC count was elevated to the level of 2.12 ± 0.39×10^{12}/L in group C and to 2.01 ± 0.32×10^{12}/L in group A, without any significant difference. It rose again in groups A and D on day 14 to 2.19 ± 0.23×10^{12}/L and 2.16 ± 0.25×10^{12}/L, respectively, while in group C it slightly declined without any significance. The RBC count rose on day 21 in all groups except A, without any significance between groups.

Table 3. Relative (%) count of lymphocytes in chicken blood during the trial

Groups	Days after vaccination						
	0	3	5	7	10	14	21
A	37.10 ± 9.07	63.00 ± 10.35	76.00 ± 4.24	74.42 ± 3.95^{b}	88.00 ± 2.82	79.50 ± 6.07	84.50 ± 6.71^{a}
B		64.50 ± 9.35	71.75 ± 4.52	77.62 ± 3.33^{ab}	90.42 ± 4.50	76.50 ± 7.23	76.50 ± 3.02^{b}
C		67.12 ± 9.14	73.75 ± 6.96	79.50 ± 3.89^{ab}	91.12 ± 4.15	80.87 ± 3.31	81.25 ± 3.19^{ab}
D		69.12 ± 12.17	74.50 ± 5.73	81.25 ± 4.52^{a}	87.75 ± 5.11	79.87 ± 4.38	80.75 ± 4.16^{ab}

Values are expressed as mean value ± SD. Values with a different letter are significantly different ($P<0.05$) between groups on the same day of experiment

Table 4. Relative (%) count of neutrophils in chicken blood during the trial

Groups	Days after vaccination						
	0	3	5	7	10	14	21
A	61.90 ± 9.09	36.37 ± 9.82	22.62 ± 4.53	24.42 ± 3.40^{a}	11.62 ± 3.15	19.75 ± 6.04	14.00 ± 6.78^{b}
B		36.00 ± 8.15	27.50 ± 4.53	21.75 ± 3.10^{ab}	10.42 ± 3.73	23.00 ± 7.05	22.37 ± 2.77^{a}
C		32.37 ± 9.13	25.12 ± 6.35	19.75 ± 3.57^{ab}	8.87 ± 5.71	16.87 ± 4.67	18.25 ± 3.45^{ab}
D		30.12 ± 11.92	21.87 ± 3.72	18.12 ± 4.29^{b}	12.12 ± 4.70	18.75 ± 4.74	18.12 ± 4.05^{ab}

Values are expressed as mean value ± SD. Values with a different letter are significantly different ($P<0.05$) between groups on the same day of experiment

Table 5. Relative (%) count of monocytes in chicken blood during the trial

Groups	Days after vaccination						
	0	3	5	7	10	14	21
A	0.90 ± 0.99	0.37 ± 0.74	1.12 ± 1.12	0.71 ± 0.75	0.37 ± 1.06	0.50 ± 0.75	0.62 ± 0.74
B		0.75 ± 1.16	0.62 ± 0.51	0.62 ± 0.74	0.57 ± 0.78	0.50 ± 0.53	0.75 ± 1.38
C		0.37 ± 0.51	1.00 ± 1.30	0.62 ± 0.74	0.25 ± 0.46	2.25 ± 1.90	0.37 ± 0.51
D		0.75 ± 0.88	0.87 ± 0.83	0.62 ± 0.74	0.12 ± 0.35	1.37 ± 1.30	1.00 ± 1.06

Values are expressed as mean value ± SD

Table 6. H/L ratio in chicken blood during the trial

Groups	Days after vaccination						
	0	3	5	7	10	14	21
A	1.97 ± 0.62	0.71 ± 0.40	0.34 ± 0.08	0.34 ± 0.13	0.13 ± 0.04	0.32 ± 0.16	0.15 ± 0.15
B		0.57 ± 0.23	0.36 ± 0.12	0.28 ± 0.03	0.13 ± 0.08	0.41 ± 0.19	0.31 ± 0.04
C		0.71 ± 0.20	0.48 ± 0.07	0.29 ± 0.05	0.03 ± 0.02	0.16 ± 0.01	0.22 ± 0.03
D		0.66 ± 0.58	0.33 ± 0.05	0.23 ± 0.10	0.14 ± 0.07	0.23 ± 0.09	0.22 ± 0.07

Values are expressed as mean value ± SD

Differential blood cell count. The relative lymphocyte count rose gradually until day 10 with a slight drop in the count on days 14 and 21 in all experimental groups (Table 3). A significant difference was found on day 7 between groups A and D, with percentages of 74.42 ± 3.95 and 81.25 ± 4.52, respectively, while on day 21 a significant difference was found between groups A and B with percentages of 84.50 ± 6.71 and 76.50 ± 3.02, respectively.

In contrast, the relative number of heterophils (Table 4) declined in all experimental groups up to day 10 of the trial, with a slight rise on days 14 and 21. A significant difference was found between groups A and D on day 7, with percentages of 24.42 ± 3.40 and 18.12 ± 4.29, respectively, and on day 21 between groups A and B, with percentages of 14.00 ± 6.78 and 22.37 ± 2.77, respectively.

During the whole experiment eosinophils and basophils were not detected or were in a very low percentage (below 0.5 %) (data not shown), without any significant differences.

Relative monocyte count (Table 5) was below 1 % throughout the whole experiment, with a slight elevation on day 14 in groups C and D compared to groups A and B, but without any significant differences between any of these groups.

H/L ratio. During the trial, the H/L ratio declined from 1.97 ± 0.62 to physiological values around 0.20 (Table 6), from day 0 to day 21, respectively. There were no significant differences between groups during the trial, but slightly higher levels of H/L ratio in group B during the last two days of the trial were recorded.

Discussion

Marek's disease is one of the most important diseases of modern poultry production. The main problem of its control are the very complex viral kinetics and pathogenesis of MDV in relation to genetics, stress, age, gender, environment, as well as simultaneous infection with other microorganisms, especially immunosuppressant viral infections (BAIGENT and DAVISON, 2004). HVT has been used for decades to protect chickens against virulent MDV. Today its solitary immunogenic impact against vv+MDV strains is significantly reduced, but as non-cell-associated MDV vaccine it could be resurrected in the form of a recombinant vaccine. To develop a recombinant vaccine of good quality, it is important to determine all the features of the vector virus. The importance is much more significant if the vector virus is delivered by respiratory route by means of nebulisation (MAZIJA et al., 2009) that mimics a natural infection. One of the first steps is to detect blood cell count changes that could give standard levels of response to the vector virus after delivery by nebulisation. Changes in blood cell count could be caused by immune response as well, because of the complexity of the viral cycle, by viral avoidance and weakening of the same. Vaccination is the most significant method of protection and could effectively protect chickens against vv+MDV, and thereby improve production as well as the welfare of animals. HVT given to broiler chickens improved weight gain, combined with lower mortality (BAATEN et al., 2004), which may be explained by its influence on growth hormones (LIU et al., 2001), enzymatic systems of cell energy delivery (LI et al., 1998a, 1998b), cytokines (KAISER et al., 2003), expression of genes involved in signal transduction and transcription regulation (KARACA et al., 2004; ABDUL-CAREEM et al., 2008).

In our experiment there were no clinical reactions to the vaccine given by any means of delivery (nebulisation or parenteral). Blood cell count (WBC, RBC, differential WBC, H/L ratio) showed differences, but they were all within the physiological range.

Results showed that WBC count was significantly influenced by vaccination, especially by nebulisation (Table 1). Values were within physiological range, from 9.00 to 32.00×10^9/L (BOUNOUS and STEDMAN, 2000; POLJIČAK-MILAS et al., 2004), with a significant increase in group A, vaccinated by means of nebulisation, on days 5 and 14. Such results indicate significant activation of immune cells in the group vaccinated by means of nebulisation, compared to parenteral vaccination (s/c), and especially to the non-vaccinated groups. It is already known that HVT, like MDV, induces activation of immune cells, of innate and active immunity (BAATEN et al., 2004), of different intensity, depending mainly on genetic resistance. The same influence has been confirmed at the molecular level, with changes in expression of cytokines and their receptors, as well as other molecules that participate in immune reaction (KARACA et al., 2004; SARSON et al., 2006; ABDUL-CAREEM et al., 2006, 2008; HAQ et al., 2010b).

RBC count (Table 2) was in the range of 1.3 to 4.5×10^{12}/L, which is within the physiological range (BOUNOUS and STEDMAN, 2000; POLJIČAK-MILAS et al., 2007). In group A, vaccinated by means of nebulisation, there was a non-significant reduction in RBC count on day 5 compared to the other groups, which could be explained by the higher activation of immune mechanisms that might slightly slow the erythropoiesis and delivery of mature cells to the blood stream. This could also induce elevation of RBC in group A up to day 14 and the subsequent reduction up to day 21. Earlier research showed that HVT had an impact on factors important for signal transduction into the cell (KARACA et al., 2004), such as CXCR4 connected to tyrosine kinase (CHERNOCK et al., 2001; PTASZNIK et al., 2002), or transcribing factors, such as Lmo2 (WADMAN et al., 1997). As already mentioned, these factors have a very important role in haematopoiesis and angiogenesis (YAMADA et al., 1998) and cell migration (BACHELDER et al., 2002.), so it is possible that HVT, by influencing the level of expression of these molecules in the cells, indirectly causes the erythropoiesis and delivery of mature cells to the blood stream, in synergy with other systems of signalling in and around the cells, such as cytokines (HEIDARI et al., 2008; KAISER et al., 2009; HAQ et al., 2011).

Differential cell count showed significant differences in lymphocyte and heterophil cell count between some groups during the trial, while no significant differences were detected in monocyte, eosinophil and basophil cell count. On day 7 the drop in the lymphocyte cell count in the vaccinated groups is in a way consistent with the transient cell drop caused by cytolysis after MDV and HVT infection (ISLAM et al., 2002) which could change the relative cell count. The results on day 21 showed a significant difference between the vaccinated groups, where the relative lymphocyte number was higher in nebulized group A compared to the s/c vaccinated group B, which could be a sporadic finding, but also the result of different vaccine delivery and stimulation of immune cells (HAQ et al., 2010a), which should be elucidated by further research.

The H/L ratio shows elevation in group B, vaccinated s/c, on the last two days of the experiment, but without any significant statistical difference, compared to the other groups (Table 6). The levels measured are around the physiological values for chickens in all groups, but because of their juvenile organism, with the cellular and immune systems in development, those values vary in the same groups (SHINI et al., 2008), and are not standardized. It is known that stress, especially chronic, can be immunosuppressant (POLJIČAK-MILAS et al., 2004), adapting the organism to new conditions, and by metabolic changes can even lower weight gain (PUVADOLPIROD and THAXTON, 2000; POST et al., 2003). In the case of infection (including response to vaccine) it could be characterized as a prolonged insult, with probably different pathways between microbiological and environmental insult (SHINI et al., 2008). On the other hand, the act of vaccination (application) is characterized as acute stress, which according to DHABHAR (2002), could have an immune-enhancing effect. In our case, because of the probably mixed stressors and juvenile system, H/L ratio results could not be compared to other research results.

In conclusion, vaccination by means of nebulisation induced significant changes in the WBC count in chicken blood, compared to the control groups, in that way inducing a significant immune response. On the other hand, it also slightly changed the RBC count, but probably as a regular response to such an immunogen, and like the WBC and differential WBC count, it remained within the physiological range. The H/L ratio, because of the numerous influences on it, could not be used as a credible result, but may show that nebulisation, as well as infection with vaccinal HVT, is not a stressful insult.

References

ABDUL-CAREEM, M. F., B. D. HUNTER, A. J. SARSON, A. MAYAMEEI, H. ZHOU, S. SHARIF (2006): Marek's disease virus-induced transient paralysis is associated with cytokine gene expression in the nervous system. Viral. Immunol. 19, 167-176.

ABDUL-CAREEM, M. F., B. D. HUNTER, A. J. SARSON, P. PARVIZI, H. R. HAGHIGHI, L. READ, M. HEIDARI, S. SHARIF (2008): Host responses are induced in feathers of chickens infected with Marek's disease virus. Virology 370, 323-332.

BAATEN, B. J. G., C. BUTTER, T. F. DAVISON (2004): Study of host-pathogen interactions to identify sustainable vaccine strategies to Marek's disease. Vet. Immunol. Immunopathol. 100, 165-177.

BACHELDER, R. E., M. A. WENDT, A. M. MERCURIO (2002): Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. Cancer Res. 62, 7203-7206.

BAIGENT, S. J., T. F. DAVISON (2004): Marek's disease virus: biology and life cycle. In: Marek's Disease: An Evolving Problem. (Davison T. F., V. Nair, Eds.), London, Academic Press, 62-77.

BOUNOUS, D. I., N. L. STEDMAN (2000): Normal avian hematology: chicken and turkey. In: Schalm´s Veterinary Hematology (Feldman, B. F., J. G. Zinkl, N. C. Jain, Eds.), Lippincott, Williams and Wilkins, Philadelphia, 1147-1154.

CHERNOCK, R. D., R. P. CHERLA, R. K. GANJU (2001): SHP2 and cbl participate in alpha-chemokine receptor CXCR4-mediated signaling pathways. Blood 97, 608-615.

CRAY, C., J. ZAIAS (2004): Laboratory procedures. Vet. Clin. Exotic. Anim. 7, 487-518.

DHABHAR, F. S. (2002): Stress-induced augmentation of immune function—the role of stress hormones, leukocyte trafficking, and cytokines. Brain Behav. Immun. 16, 785-798.

FUDGE, A. M. (2000): Avian complete blood count. In: Laboratory Medicine-Avian and exotic pets. (Fudge A. M., Ed.), WB Saunders company, Philadelphia, 9-18.

GROSS, W. B., H. S. SIEGEL (1983): Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27, 972-979.

GROSS, W. B. (1990): Effect of exposure to a short-duration sound on the stress response of chickens. Avian Dis. 34, 759-761.

HAQ, K., M. F. ABDUL-CAREEM, S. SHANMUGANTHAN, N. THANTHRIGE-DON, L. R. READ, S. SHARIF (2010a): Vaccine-induced host responses against very virulent Marek's disease virus infection in the lungs of chickens. Vaccine 28, 5565-5572.

HAQ, K., J. T. BRISBIN, N. THANTHRIGE-DON, M. HEIDARI, S. SHARIF (2010b): Transcriptome and proteome profiling of host responses to Marek's disease virus in chickens. Vet. Immunol. Immunopathol. 138, 292-302.

HAQ, K., I. ELAWADLI, P. PARVIZI, A. I. MALLICK, S. BEHBOUDI, S. SHARIF (2011): Interferon-γ influences immunity elicited by vaccines against very virulent Marek's disease virus. Antivir. Res. 90, 218-226.

HEIDARI, M., M. HUEBNER, D. KIREEV, R. F. SILVA (2008): Transcriptional profiling of Marek's disease virus genes during cytolytic and latent infection. Virus Genes 36, 383-392.

ISLAM, A. F., C. W. WONG, S. W. WALKDEN-BROWN, I. G. COLDITZ, K. E. ARZEY, P. J. GROVES (2002): Immunosuppressive effects of Marek's disease virus (MDV) and herpesvirus of turkeys (HVT) in broiler chickens and the protective effect of HVT vaccination against MDV challenge. Avian Pathol. 31, 449-461.

KAISER, P., G. UNDERWOOD, F. DAVISON (2003): Differential cytokine responses following Marek's disease virus infection of chickens differing in resistance to Marek's disease. J. Virol. 77, 762-768.

KAISER P., Z. WU, L. ROTHWELL, M. FIFE, M. GIBSON, T. -Y. POH, A. SHINI, W. BRYDEN, S. SHINI (2009): Prospects for understanding immune-endocrine interactions in the chicken. General Comp. Endocrin. 163, 83-91.

KARACA, G., J. ANOBILE, D. DOWNS, J. BURNSIDE, C. J. SCHMID (2004): Herpesvirus of turkeys: microarray analysis of host gene responses to infection. Virology 318, 102-111.

LI, S., S. E. AGGREY, D. ZADWORNY, W. FAIRFULL, U. KUHNLEIN (1998a): Evidence for a genetic variation in the mitochondrial genome affecting traits in White Leghorn chickens. J. Hered. 89, 222-226.

LI, S., D. ZADWORNY, S. E. AGGREY, U. KUHNLEIN (1998b): Mitochondrial PEPCK: a highly polymorphic gene with alleles co-selected with Marek's disease resistance in chickens. Anim. Genet. 29, 395-397.

LIU, H. C., H. J. KUNG, J. E. FULTON, R. W. MORGAN, H. H. CHENG (2001): Growth hormone interacts with the Marek's disease virus SORF2 protein and is associated with disease resistance in chicken. Proc. Natl. Acad. Sci. U. S. A. 98, 9203-9208.

MARKOWSKI-GRIMSRUD, C. J., K. A. SCHAT (2002): Cytotoxic T lymphocyte responses to Marek's disease herpesvirus-encoded glycoproteins. Vet. Immunol. Immunopathol. 90, 133-144.

MAZIJA, H., S. ČAJAVEC, Đ. NEMARNIK, E. PRUKNER-RADOVČIĆ (1994): Immunogenicity of Marek's disease vaccine HVT FC-126 applied by aerosol of fine particles. Proc. 9th European Poultry Conf. WPSA, Glasgow, UK.

MAZIJA, H., T. ŠTIMAC (1999): P950425A Ultrasonic atomizer for vaccination against Marek's and other poultry diseases. Croatian Intellectual Property Gazette 6, 877.

MAZIJA, H., S. ČAJAVEC, E. PRUKNER-RADOVČIĆ, N. ERGOTIĆ, I. CIGLAR GROZDANIĆ, Ž. GOTTSTEIN, A. KOKIĆ, W. L. RAGLAND (2009): Immunogenicity and safety of La Sota strain of Newcastle disease virus administered to newly hatched chicks by nebulization. Acta Vet. (Brno). 78, 137-144.

OHASHI, K., Y. MAEDA, S. I. LEE, M. MIZUTANIA, C. SUGIMOTO, M. ONUMA (2001): The kinetic changes of lymphocyte populations and cytokine profiles in chickens genetically susceptible and resistant to Marek's disease. Proceedings of 6th International Symposium on Marek's Disease, Montreal, Canada, 295-302.

POLJIČAK-MILAS, N., S. MILINKOVIĆ-TUR, Z. STOJEVIĆ, Z. BIĐIN (2004): Vergleich des weißen Blutbildes bei Junghähnen und Junghennen während des Fastens und nach der erneuten Fütterung. Tierärzt. Umschau 59, 464-469.

POLJIČAK-MILAS, N., S. MILINKOVIĆ-TUR, T. S. MARENJAK, M. ZDELAR-TUK, Z. STOJEVIĆ, Z. BIĐIN (2007): Auswirkungen des Fastens und der erneuten Futteraufnahme auf die Zahl und Eigenschaften der Erytrozyten und den gesamten antioxidativen Status bei Junghähnen und Junghennen. Arch. Geflügelk. 71, 68-73.

POST, J., J. M. REBEL, A. A. HUURNE (2003): Physiological effect of elevated plasma corticosterone concentrations in broiler chickens, an alternative means by which to assess the physiological effects of stress. Poult. Sci. 82, 1313-1318.

PTASZNIK, A., E. URBANOWSKA, S. CHINTA, M. A. COSTA, B. A. KATZ, M. A. STANISLAUS, G. DEMIR, D. LINNEKIN, Z. K. PAN, A. M. GEWIRTZ, (2002): Crosstalk between BCR/ABL oncoprotein and CXCR4 signaling through a Src family kinase in human leukemia cells. J. Exp. Med. 196, 667-678.

PUVADOLPIROD, S., J. P. THAXTON (2000): Model of physiological stress in chickens. 1. Response parameters. Poult. Sci. 79, 363-369.

SARSON, A. J., M. F. ABDUL-CAREEM, H. ZHOU, S. SHARIF (2006): Transcriptional analysis of host responses to Marek's disease. Viral Infect. Viral Immunol. 19, 747-758.

SCHAT, K. A., Z. XING (2000): Specific and nonspecific immune responses to Marek's disease virus. Dev. Comp. Immunol. 24, 201-221.

SHINI, S., P. KAISER, A. SHINI, W. L. BRYDEN (2008): Biological response of chickens (*Gallus gallus domesticus*) induced to corticosterone and a bacterial endotoxin. Comp. Biochem. Physiol. Part B: Biochem. Molecular Biol. 149, 324-333.

STATSOFT, INC. (2005): STATISTICA (data analysis software system), version 7. 1.

WADMAN, I. A., H. OSADA, G. G. GRUTZ, A. D. AGULNICK, H. WESTPHAL, A. FORSTER, T. H. RABBITTS (1997): The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1 E47, GATA-1 and Ldb1/NLI proteins. EMBO J. 16, 3145-3157.

YAMADA, Y., A. J. WARREN, C. DOBSON, A. FORSTER, R. PANNELL, T. H. RABBITTS (1998): The T cell leukemia LIM protein Lmo2 is necessary for adult mouse hematopoiesis. Proc. Natl. Acad. Sci. U. S. A. 95, 3890-3895.

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SAŽETAK

Marekova bolest predstavlja jedan od najvećih problema u uzgoju peradi pri čemu je cijepljenje jedan od najvažnijih postupaka suzbijanja. Korišten je novi postupak primjene cjepiva od soja FC 126 herpesvirusa purana jednodnevnim pilićima postupkom nebulizacije pri čemu je analiziran njegov utjecaj na broj stanica u krvi (broj leukocita, broj eritrocita i diferencijalnu krvnu sliku) kao i omjer neutrofila i limfocita (N/L). Pokus je proveden na jednodnevnim muškim pilićima od prvog do 21. dana života. Korišten je standardni postupak brojenja krvih stanica u Neubauerovoj komorici, dok je DKS načinjen na razmazima pune krvi obojene May-Grünwald-Giemsom. Krv za navedene postupke uzeta je iz jugularne vene. Rezultati pokazuju značajan porast broja leukocita u krvi kod skupine cijepljene postupkom nebulizacije u odnosu na necijepljene skupine petog dana pokusa, dok od skupine koja je primila samo fiziološku otopinu ima značajno više leukocita 14. dana pokusa. Broj eritrocita pokazuje varijacije u pokusnih skupina, ali bez značajnih razlika tijekom čitavog pokusa. Značajne razlike u diferencijalnoj krvnoj slici bile su ustanovljene 7. i 21. dana pokusa, dok kod N/L omjera nije bilo značajnih razlika. Navedeni rezultati pokazuju da je cijepljenje postupkom nebulizacije značajno utjecalo na broj krvnih stanica te moguće potaknulo specifičan imunosni odgovor, ali su sve promjene bile u fiziološkom rasponu. Nebulizacija kao postupak primjene cjepiva, iz navedenog, pretpostavlja se može poboljšati imunosni odgovor na virus Marekove bolesti oponašajući prirodan način zaražavanja putem dišnog sustava, a može biti izvrstan u masovnoj primjeni rekombinantnih cjepiva pripravljenih od herpesvirusa purana.

Ključne riječi: pilići, Marekova bolest, broj krvnih stanica, nebulizacija, cijepljenje