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Effect of low dose gamma-radiation upon Newcastle disease virus antibody level in chicken

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Background: The specific antibody response against Newcastle disease virus (NDV) in the blood serum of chickens hatched from eggs exposed to low dose gamma-radiation was studied. ***Materials and methods:*** Two groups of eggs of commercial meat chicken lines were irradiated with the dose of 0.30 Gy ^{60}Co gamma-rays before incubation and on the 19th day of incubation, respectively. The same number of eggs unexposed to gamma-radiation served as controls. After hatching the group of chicken hatched from eggs irradiated on the 19th day of incubation was not vaccinated while the group of chicken hatched from eggs irradiated before incubation was vaccinated on the 14 day. Specific serum anti-NDV antibodies were quantified by the hemagglutination inhibition assay with 4 HA units of NDV La Sota strain. ***Result:*** Specific antibody titres against NDV in the blood serum of chickens hatched from eggs irradiated before incubation and vaccinated on the 14th day significantly increased on the 28th day. Specific antibody titre against NDV in the blood serum of chickens hatched from eggs irradiated on the 19th day of incubation and non-vaccinated was significantly higher on the 1st and 14th day. ***Conclusion:*** Acute irradiation of heavy breeding chicken eggs with the dose of 0.30 Gy ^{60}Co gamma-rays before incubation and on the 19th day of incubation could have a stimulative effect on humoral immunity in chickens. **Iran. J. Radiat. Res., 2009; 7 (1): 27-31**

Keywords: *Gamma-radiation, low dose effects, broiler, Newcastle disease virus, antibody.*

INTRODUCTION

It has been shown that different biological effects can be obtained by using a low dose and low dose rate of ionizing radiation such as prolongation of life span [1, 2] in parallelly with activation of immune system [3], stimulation of growth rate and development [4], adaptive response and bystander effect [5, 6] and modulation of gene expression [7, 8]. Furthermore, low doses and low dose rates of ionizing radiation can stimulate humoral and cellular immunity in mice [9-13]. Previous studies regarding low dose radiation effects in chickens investigated mostly hatchability, body weight and egg fertility in commercial meat chicken lines [14-19]. Although these mentioned studies showed contradictory data, it is reported that dose of gamma radiation between 0.1 to 1 Gy, especially the dose between 0.1 and 0.5 Gy [17] or between 0.06 to 0.3 Gy, [15], could have stimulative effects on chicken egg hatchability and chicken body weight after egg irradiation before incubation. However, to date, no information of humoral immunity is known on the chicken after irradiation of eggs by acute low dose radiation.

Antigen that has been successfully used to promote humoral immune response in chickens is Newcastle disease virus [20-22]. Although the maternal antibody in the chickens is depended on the levels in their parents [22, 23] it is known that some substance could decrease [24, 25] or enhance [26, 27] humoral immunity in chickens.

Consequently, the aim of this study was to investigate the antibody response against vaccinal NDV in blood serum of

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chickens hatched from eggs exposed to the dose of 0.30 Gy gamma-rays before and on the 19th day of incubation.

MATERIALS AND METHODS

Investigations were carried out in two independent experiments.

Eggs

Eggs produced by commercial meat chicken lines were irradiated three hours prior to incubation (experiment 1) and on the nineteenth day of incubation (experiment 2). In each experiment, there were included the same number of eggs unexposed to gamma-radiation and served as controls. Non-irradiated eggs were retained in the same place for a period of time equal to that required for irradiated eggs. The study was reviewed and approved by an Ethics Comittee of the Faculty of Veterinary Medicine University of Zagreb.

Irradiation and dosimetry

In both experiments eggs were irradiated with the dose of 0.30 Gy gamma radiation from panoramic ^{60}Co source (activity about 3 PBq) of the Ruđer Bošković Institute, Zagreb, Croatia [28]. Dose rate was about 23.84 mGy/s, and a source axis-to-egg axis distance was 3.06 m. Dosimetric measurements were performed with an ionization chamber type 2581 and a Farmer Dosimeter type 2570 (NE Technology Limited). The dose is specified as absorbed dose to water (measued free in air).

Incubation

All eggs of each experiment were placed in the same commercial incubator Victoria (Pavia, Italy), capacity of 22100 eggs for 19 days. Incubators had automatic control of temperature (37.8 ^{0}C), humidity (60-62 % relative humidity), and incubation rack turning (each hour). On the 19th day of incubation the eggs were transferred to hatching trays located in the same incubator.

Chickens

After the hatch chickens were housed on the floor at a temperature appropriate to each age interval. Thus, the initial temperature was 33 °C and decreased at a rate of 2 °C per week. During the experimental period the chickens were given feed and water *ad libitum.* Both groups were kept under the same conditions.

Vaccination schedule

Chicks were vaccinated against Newcastle disease only in the experiment 1 with a commercial Newcastle disease vaccine (PESTIKAL® La Sota SPF, VETERINA d.o.o., Zagreb, Croatia) by oculonasal route on the 14th day.

Serological examination

From all chickens in both experiments blood samples were collected for serological analyses from day 1 up to day 28 in weekly interval. Approximately 1 ml of blood was taken from the right jugular vein. Serum was separated by centrifugation and stored at -18 ^{0}C until use. Specific serum anti-NDV antibodies were quantitated by the hemagglutination inhibition (HI) assay as described by OIE [29]. The HI titre was defined as the reciprocal of the highest serum dilution completely inhibiting agglutination. Serum antibody titres were expressed as a $\log_2$ values.

Statistical analysis

Obtained results of antibody titre against NDV were expressed as mean value (mean ± SE) and the significance of differences between the control and irradiated groups was analysed in STATISTICA [30] using Student's t-test whereas p value <0.05 was selected to indicate significance.

RESULTS

Experiment 1

The results of specific HI antibody titre against NDV in the blood serum of chickens hatched from eggs irradiated before incubation and vaccinated on the 14th day are presented in figure 1. Antibody titre against NDV was significantly increased in the blood serum of chickens hatched from irradiated eggs on the 28th day ($p < 0.05$).

Experiment 2

The results of specific HI antibody titres against NDV in the blood serrum of chickens hatched from eggs irradiated on the 19th day of incubation are presented in figure 2. Antibody titre against NDV in the blood serum of chickens hatched from irradiated eggs was higher than it was in the control group during first three weeks. The difference was statistically significant on the 1st day ($p < 0.05$) and the 14th day ($p<0.05$).

DISCUSSION

Our results demonstrate that acute low-dose radiation (LDR) exposure could enhance maternal HI antibody titre against NDV in chicks as well as antibody titre synthesized in chickens after vaccination (figure 1 and 2). These results suggest that enhanced antibody titre in chicken blood serum might be due to increased antibody transport from yolk sac into embryo circulation or to the direct effect on chicken B-cell development. Namely, it is known that maternal IgG (antibodies) are transferred to offspring across the yolk sac IgG receptors without degradation [31-34]. Furthermore, HAMAL *et al.* [22] reported that level of IgG in chicken plasma directly corresponds to

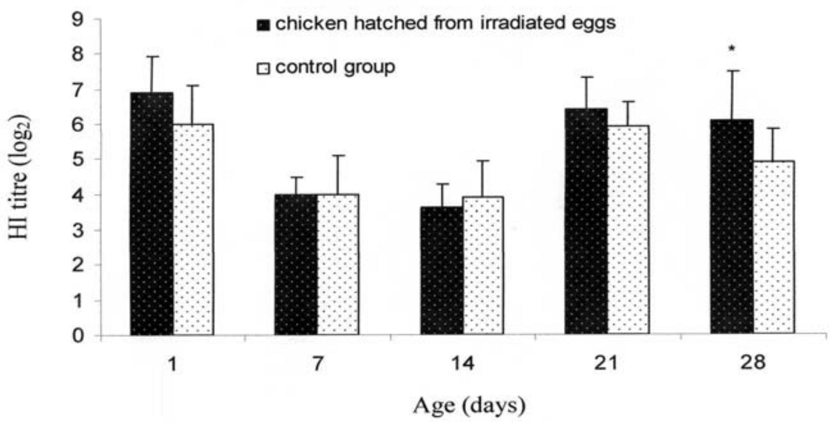

Figure 1. Serum antibody titres to Newcastle disease virus of chickens hatched from eggs irradiated with the dose of 0.30 Gy gamma-radiation before incubation and vaccinated on the 14th day of age by oculonasal route. Results are expressed as the mean ± standard error (SE) of 10 chickens. *Star marks represent significant differences (P<0.05) between means of irradiated group and control group.

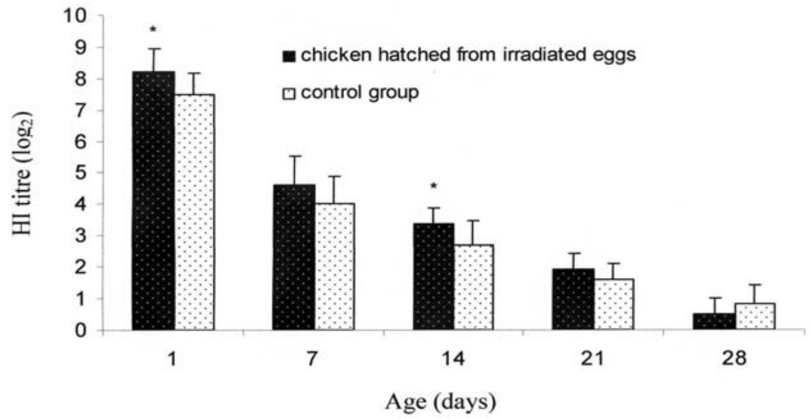

Figure 2. Serum antibody titres to NDV of chickens hatched from eggs irradiated with the dose of 0.30 Gy gamma-radiation on the 19th day of incubation and non-vaccinated. Results are expressed as the mean ± standard error (SE) of 10 chickens. *Star marks represent significant differences (P<0.05) between means of irradiated group and control group.

IgG level in hen plasma and is about 30% of it. Our experiment showed that acute LDR exposure on the 19th day of incubation, when the absorption of IgG is the most intensive [35], caused significant change of antibody titre against NDV in newly hatched chicks (figure 2). However, it is still unclear what mechanisms are involved in significant increase of maternal as well as specific antibodies level to NDV in irradiated groups. Yet, we suppose the acute LDR exposure could be responsible for activation of antibody transfer over the yolk sac receptors or expression of genes for mentioned receptors. Namely, WEST *et al.* [34] firstly purified and characterized chicken yolk sac membrane IgG binding protein in 18-19 day old chicken embryos which they named FcRY receptor and sequenced its gene. Furthermore, they reported an avian protein receptor present on the chicken yolk sac membrane to be responsible for transferring of antibodies from yolk to the embryonic circulation. Since low doses of radiation can modulate protein expressions as well as new, before radiation unknown proteins in mammals [36, 37], it is not to be excluded that dose of 0.3 Gy could not have effect upon the gen expressions of receptors.

On the other side, in the experiment 1 chicken hatched from eggs irradiated before incubation and vaccinated on the 14th day after hatch had a significantly higher specific antibody titre against NDV on the 28th day compared to non-vaccinated chickens. We presume that such result could be caused by stimulating effect of acute LDR exposure on: (i) many mediators, like cytokines, which have ability to stimulate the ontogeny and activation of neonatal host defenses [38] or (ii) expression of other molecules which participate in simultaneous reactions between cells during immune response [12].

In conclusion we want to emphasize that acute exposure of 0.30 Gy gamma-rays applied before and on the 19th day of incubation of hatching eggs seems to stimulate the humoral immunity by enhancing transport of antibodies from eggs to embryonic circulation as well as by stimulating antibody production after vaccination.

After this, certainly, lots of questions stay open and answers to them will be results of many researches of LDR in the future on humoral immunity in birds and further extending the knowledge in the field of molecular basis of antibody and IgG transport in hens.

Our further studies are in progress to test specific anti-NDV antibodies and IgG transfer from parents to eggs and from eggs to embryonic circulation and newly hatched chicks after egg exposures by different low dose radiation in different time of incubation.

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