Part 1. Veterinary medicine

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THE MACROPHAGES ACCUMULATION IN CHICKENS VACCINATED AGAINST AVIAN MYCOPLASMOSIS

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Summary. The goal of our investigations was determination of the dynamics and intensity of macrophages accumulation in the immunocompetent organs of chickens vaccinated against Avian mycoplasmosis.

For creation of experimental series vaccines we have applied two technologies. In the first series as an antigenic bases used formaldehyde inactivated bacterin of production strain Mycoplasma gallisepticum VK (VB); in the second series – ultrasound disintegrated bacterial mass of cells of the same strain (VS). Experiments were carried out on chickens. Birds of first experimental group (n = 30) were immunized intramuscularly twice at an interval of 30 days by vaccine VB (VB group). Birds of second experimental group (n = 30) were immunized at the same scheme by vaccine VS (VS group). Birds of control group (n = 30) was not vaccinated.

On the 7th, 10th, 14th and 21st days after the second injection of vaccines 5 individuals from each group were euthanized; from birds were taken lungs, trachea, spleen and caecum tonsil. Preparations were stained by immuno-histochemistry method using labeled streptavidin-biotin. Presence and percentage of cell populations macrophages into organ samples account in the process of smear microscopy.

Macrophages are actually the first link of cellular immunity. After immunization the activation occurs in a relatively short time after injection of immunizing substance. It is this process we observed in the study of the internal organs of immunized chickens.

It was found that injection of inactivated vaccines in chickens promoted stimulation for primary link of cellular immunity. The population of macrophages increased rapidly during the first 10 days after the second injection of both vaccines. The highest value of this indicator was recorded in the spleen and lungs of birds (24.125 % and 22.280 % in the VB group; 21.010 % and 20.333 % in the VS group). Over the next 11 days, their number gradually decreased and on 21st day almost reached the level of the Control group. However, in VB group, this process was more intense, as evidenced by high values recorded during the study.

Keywords: inactivated vaccine, avian mycoplasmosis, macrophages

The main condition for the successful conduct of poultry industry is the prevention of infectious diseases, which cause significant economic losses, these include avian mycoplasmosis (Georgiades, 2002; Olanrewaju, Collier and Branton, 2011). Effective method to prevention of this infection is vaccination by inactivated vaccines (Kleven, 2008; Hussein et al., 2007; Ferguson-Noel et al., 2012; Branton et al., 2000). A clear indicator of immune reactivity of bird is intensity of macrophages accumulation in the immunocompetent organs. However, the dynamics and intensity of this process varies and depends on many factors, particularly important for determining the level of activation of the immune system is considered the first three weeks after vaccination (Bolotnikov and Konopatov, 1993; Parker et al., 2002; Halvorson, 2011).

The goal of our investigations was determination of the dynamics and intensity of macrophages accumulation in the immunocompetent organs of chickens vaccinated against avian mycoplasmosis.

Materials and methods. For creation of experimental series vaccines against avian mycoplasmosis we have applied two technologies. In the first series as an antigenic bases used formaldehyde inactivated bacterin of production strain Mycoplasma gallisepticum VK (VB); in the second series – ultrasound disintegrated bacterial mass of cells of the same strain (VS). Vaccines contained 30 % of antigenic substrate (3×10^7 CFU) and 70 % adjuvant (Mantanide ISA 70 VG).

Experiments were carried out on 3 groups of chickens. Birds of first experimental group (n = 30) were immunized intramuscularly twice at an interval of 30 days (at the age of 30 and 60 days, respectively) by vaccine VB (VB group). Birds of second experimental group (n = 30) were immunized at the same scheme by vaccine VS (VS group). Birds of control group (n = 30) was not vaccinated.

On the 7th, 10th, 14th and 21st days after the second injection of vaccines 5 individuals from each group were

euthanized; from birds were taken lungs, trachea, spleen and caecum tonsil.

Organ samples were fixed in 10 % neutral formalin solution, and pouring paraffin carried, histological sections prepared by standard methods. Preparations were stained by immuno-histochemistry method using labeled streptavidin-biotin. Presence and percentage of cell populations macrophages into account by using the «Video Test Morphology - 5» in the process of smear microscopy using a microscope Axiskop 40 / 40FL (Carl Zeiss).

Statistical processing of the data was performed using the program SPASS Statistics 17.0.

Results. Macrophages (mononuclear phagocytes) are the population of "long lifetime phagocytes". The role of macrophages in shaping the immune response is important, they provide phagocytosis of heterogenous protein components, processing and presenting antigens for T-lymphocytes. This is unique group of so-called "antigen-presenting cells". However, they have two important features: the ability to form complex antigenic peptide with molecules I and II MHC class, serving as the first signal to the proliferation and differentiation of T-lymphocytes; ability to initiate expression of co-stimulants to ensure the passage of the second signal to activate of T-lymphocytes (Lam, 2002).

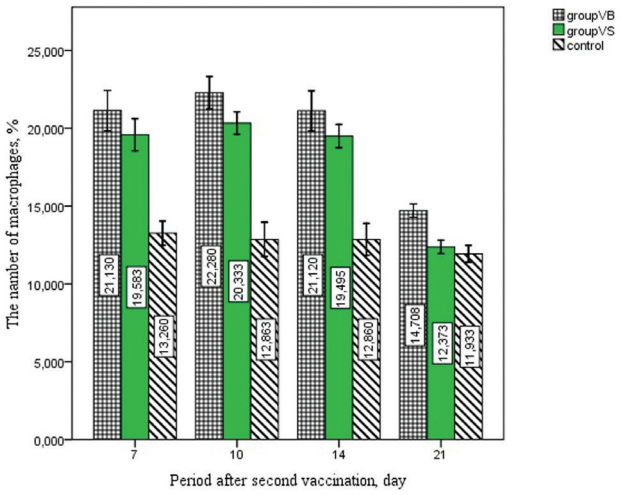

Figure 1 – Changes in the number of macrophages in the chicken lungs

Thus, macrophages are actually the first link of cellular immunity. After immunization the activation occurs in a relatively short time after injection of immunizing substance. It is this process we observed in the study of the internal organs of immunized chickens.

The number of macrophages in the lungs of chickens VB group grew rapidly in the first 10 days after the second vaccination. On the 7th day it reached 21.130 %, and the 10th day acquired the highest value – 22.280 %, which is almost twice as analog in Control group. Then the number of these cells began to gradually decrease and 14th day nearly equal to the level that was recorded on the 7th day.

A week later indicator value decreased to almost 14.708 % in this period exceeded the reference value only 2.775 %.

In the VS group observed similar changes, but the number of macrophages in the lungs of chickens was smaller. On the 10th day it was equal to 20.333 %, which is higher than the value in Control group of almost 1.6 times, but was lower than in VB group in 2 %. Further population of these cells decreased and on 21st day almost reached the level of the Control group (12.373 % against 11.933 %, respectively). This is lower than in the VB group on 2,335 %.

In trachea of both groups of birds macrophages accumulated by a similar scheme, but this process was not as intense as in the lungs, is shown in Fig. 2.

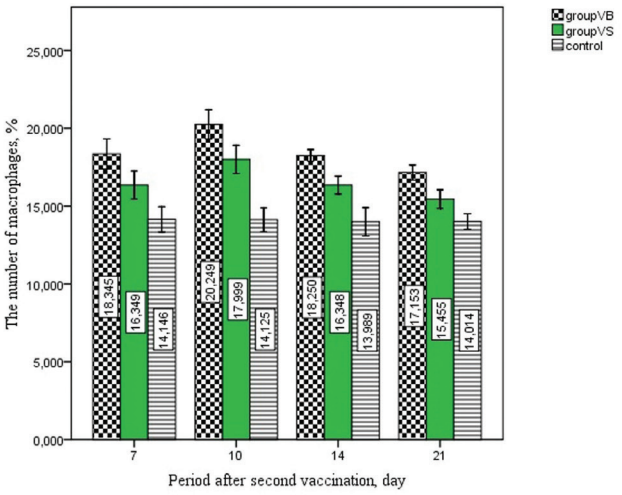

Figure 2 – Changes in the number of macrophages in the chicken trachea

On the 7th day the number of macrophages in the trachea of chickens VB group exceeded analogue for the Control group to 1.3 times in VS group this difference was 1.15 times.

The maximum value of this index in both groups reached 10th day and reached for the VB group – 20.249 % and for VS group – 17.999 % (against 14.125 % in Control group). Then we observed a gradual decline and at the 21st day in VB group the population of these cells was equal to 17.153 %, which was on 3.139 % higher than in the Control group and in the VS group the difference was smaller – 1.441 % (15.435 % vs. 14.014 %, respectively).

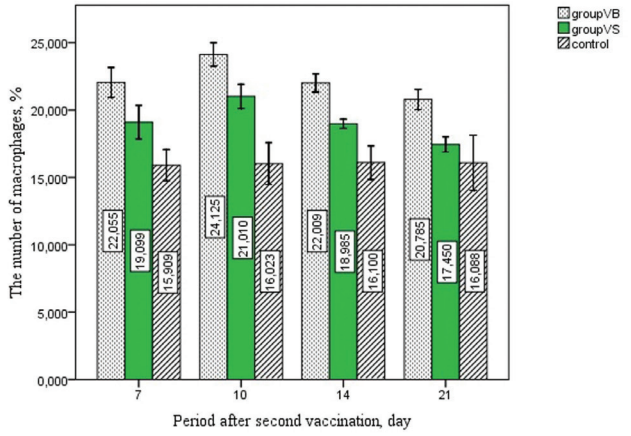

Figure 3 – Changes in the number of macrophages in the chicken spleen

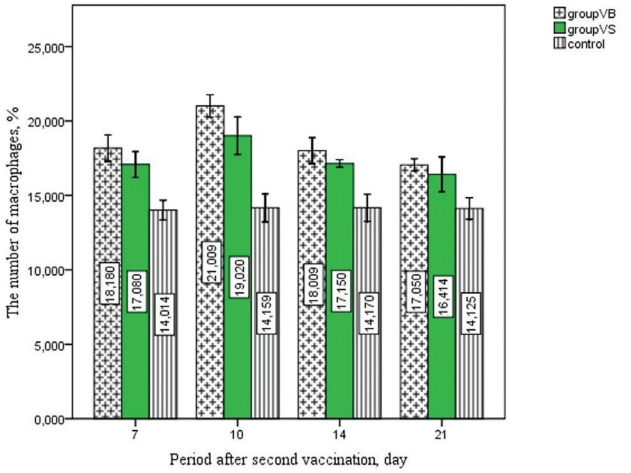

Figure 4 – Changes in the number of macrophages in the chicken caecum

The most striking and intense was the dynamics of accumulation of macrophages in the spleen of immunized birds. Thus, on the 10th day in VB group population of these cells were 24.125 % of the other cells, in the lungs compared to this period there were 22.280 %, and in the trachea - 20.249 %. Reducing the number of macrophages was recorded on the 14th and 21st day (up to 22.009 % and 20.785 %, respectively). In the VS group maximum value was also on 10th day, but their number was lower (21.010 %). Later their value decreased to 17.450 % on 21st day (against 16.088 % in the Control group). In caecum tonsils macrophage accumulation occurred at a high level, but they showed a slightly lower number than in the spleen. Thus, the highest value of this indicator was found in both experimental groups revealed on the 10th day after the second vaccination in the number of 21.009 % and 19.020 %, respectively (Fig. 4).

At the 14th day noted the decrease in the number of these cells almost to the level of the 7th day. The declining trend in the population of these cells was observed by us and over the next week. On 21st day the number of it in the BV group amounted to 17.050 %; in VS group – to 16.414 %, which is higher than the analog in the Control group only on 2.925 % and 2.289 %, respectively.

Thus, it was found that injection of inactivated vaccines in chickens promoted stimulation for primary link of cellular immunity. The population of macrophages increased rapidly during the first 10 days after the second injection of both vaccines. The highest value of this indicator was recorded in the spleen and lungs of birds (24.125 % and 22.280 % in the VB group; 21.010 % and 20.333 % in the VS group). Over the next 11 days, their number gradually decreased and on 21st day almost reached the level of the Control group. However, in VB group, this process was more intense, as evidenced by high values recorded during the study.

Conclusions. It was found that level of macrophages in chickens increased rapidly during the first 10 days after the second injection of inactivated vaccines against avian mycoplasmosis. The highest value of this indicator was recorded in the spleen and lungs of birds treated by vaccine with Mycoplasma gallisepticum bacterin (24.125 % and 22.280 %, respectively). In group treated by vaccine with subunit Mycoplasma gallisepticum antigen this process was less intense. Generally it was shown that injection of inactivated vaccines against avian mycoplasmosis in chickens promoted stimulation for primary link of cellular immunity.

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