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The population dynamics of T - and B-lymphocytes in blood of the cattle vaccinated against anthrax

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ABSTRACT

Anthrax remains a serious problem for many countries in the world. Containment of major outbreaks of this disease is possible only with the help of specific preventive measures as vaccinating all susceptible livestock. Immunization of animals should create solid immunity in the organism, which should resist the development of the infection process when introducing field strains of an agent. However, there are no data on the dynamics of the development of immunity in the literature. The purpose of this work is to study the cellular component of adaptive post-vaccinal immunity in animals at different stages after immunization against anthrax. The authors have studied the dynamics of the quantitative composition of T- and B-lymphocytes in cattle during a year. Isolation of T- and B-cells causes spontaneous rosetting of lymphocytes with erythrocytes of heterologous species of animals: sheep and mouse respectively. Recording of the reaction was carried out under a light microscope. 200 lymphocytes were counted, and the percentage of rosette-forming cells was calculated. It was found that immunization leads to an increase in the number of lymphoid cells and epiphylaixis. The number of T- and B-lymphocytes in the blood of vaccinated animals has been observed for 35 days after immunization and has been maintained at the same level for two months; 3 months later the number of cells decreased, which shows weakening of the immune system and immune memory by the 6th month after immunization of animals.

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INTRODUCTION

Anthrax is an extremely dangerous infectious disease of livestock, wild animals and men, which is caused by the spore-forming organism Bacillus anthracis. Despite the detailed and comprehensive study of the agent, its ecology and epizootology, the modern area of anthrax of livestock and wild animals comprises all continents. Every year the outbreaks of the infection are registered in the world (Doganay and Demiraslan, 2015).

The main factor that leads to the possible infection of animals is indefinite and long presence and reproduction of the agent in the environment (Carlson

et al., 2018).

The conducted specific vaccination, on the whole, restrains the development of epizootic but does not eliminate a threat of sporadic outbreaks of the disease caused by the overall state of the immune system of animals.

One of the ways to minimize the risks of sporadic outbreaks is to monitor the solidity of group immunity and to correct the conducted preventive measures.

It is known that the immunity against anthrax stimulated by vaccination is provided by the work of complementary components of the immune system: congenital and adaptive. The basis of the congenital immunity is phagocytosis, and the basis of the adaptive immunity is antitoxic antibodies and lymphocytes (Altmann, 2015). However, despite the seeming coverage of the problem, researchers continue to argue on the issues of improving preventing measures of the disease (Lv *et al.*, 2017).

Today the immune level against anthrax is defined by revealing specific antibodies in blood serum (Varshney *et al.*, 2019; Simbotwe *et al.*, 2018), but protection of animals against the disease is provided by the whole complex of the organism means, including lymphoid cells (Gars *et al.*, 2016).

It is known that the complex of T- and B-lymphocytes play an important role in the formation of immunity against anthrax (Ascough *et al.*, 2016; Colliou *et al.*, 2015). The quantitative changes of the population of these cells participating in the formation of the antitoxic immunity and immune memory are one of the indicators of the state of the immune system.

Consequently, the purpose of this work is to study the dynamics of the population of T- and B-lymphocytes in the blood of the cattle at different stages after vaccination against anthrax for further improvement of preventive measures.

MATERIALS AND METHODS

The work was conducted in the Federal Centre of Toxicological, Radiological and Biological Safety at the Department of Biological Safety, PLC «Biriuli» of the Vysokogorskiy District of the Republic of Tatarstan. 30 animals of one age-sex group vaccinated against anthrax were studied.

The live dry monovalent vaccine against anthrax (strain 55) produced by the enterprise «Orlovskaya Biofabrika» was used for immunization of the animals. The first vaccination was carried out in March, the second one – in October of 2018. The introduction of the vaccine was carried out according to the instructions for the use.

Blood of the studied animals was taken from the subcaudal vein and placed into plastic vacutainer tubes with the anticoagulant of ethylene diamine tetraacetate 7, 14, 21, 35, 60, 90, 150, 180 days after the first and second vaccination according to the timetable of the research.

The isolation of lymphocytes from peripheral blood was carried out by the fractionation method in a single-stage gradient of the density of the mixture of polyvinyl alcohol and urografin with the density of 1,077 g/ml. The lymphocytes isolated after centrifugation were washed out in the Hanks' solution with the addition of the embryonic serum.

The percentage of the living lymphocytes was counted by mixing a small amount of the cells with the equal amount of the 0,5 % solution of trypan blue; they were placed into the Gorjaev's chamber; the amount of all lymphocytes, including the achromous ones, was accounted on 20 lymphocytic squares.

The method of spontaneous rosetting of lymphocytes (E-POK) with erythrocytes of a sheep and a mouse respectively was used to determine the amount of T- and B-cells. It included preparation of the indicator system of erythrocytes (IS) and joint incubation of the IS with the isolated lymphocytes.

Preparation of the IS included: 1) blood sampling of the donor sheep and the mouse in the Alsever's solution; 2) three-time washing off of the received blood in the Hanks' solution by 1500 g during 10 minutes; 3) preparation of the 0,5 % suspension of erythrocytes of the sheep with the environment 199 (for T-lymphocytes), 10 % suspension of erythrocytes of the mouse with the environment 199 and the bovine serum albumin (for B- lymphocytes).

To isolate T-cells, 0,25 ml of the received suspension of lymphocytes fractionated from peripheral blood was mixed with the same amount of the IS; it was kept under the temperature of 20^0C during 25 minutes, then it was centrifuged by 1000 g during 5 minutes and incubated under the temperature of 4^0C during 18 hours. To isolate B-cells, 0,4 ml of the received suspension of lymphocytes was mixed with the same amount of the IS; it was incubated under the temperature of 37^0C during 30 minutes, then it was centrifuged by 1000 g during 5 minutes and incubated under the temperature of 4^0C during 1 hour. The immune connections were fixed by the 0,6 % gluteraldehyde during 20 minutes. The swabs of the received sediment were prepared and were stained according to the Romanovsky method.

The rosetting was observed in the immersion field under the microscope. 200 lymphocytes were counted. The percentage of the rosette-forming cells was calculated. A lymphocyte united not less than 3 erythrocytes was counted as a rosette.

The statistical analysis of the research results was carried out with the help of the programme Microsoft Excel.

RESULTS AND DISCUSSION

The research on the dynamics of the lymphocytes population in the blood of the cattle vaccinated against anthrax with the help of the rosetting method (Figures 1 and 2) at different stages of the post-vaccinal period showed a periodical increase and decrease of the amount of immunocompetent cells presented on Figure 3.

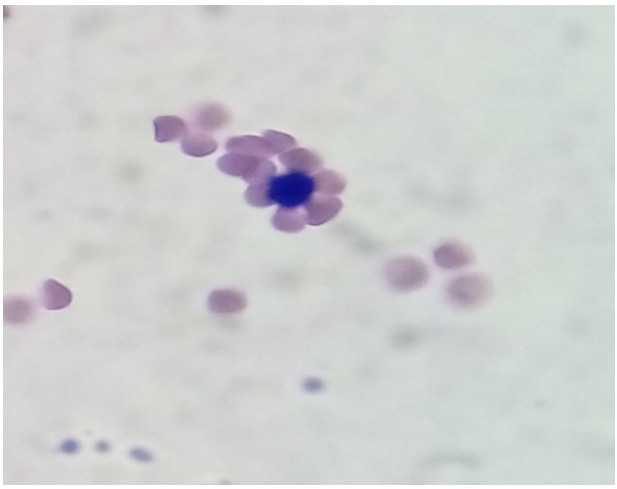

Figure 1: Rosette formation by T-lymphocytes (E-POK method)

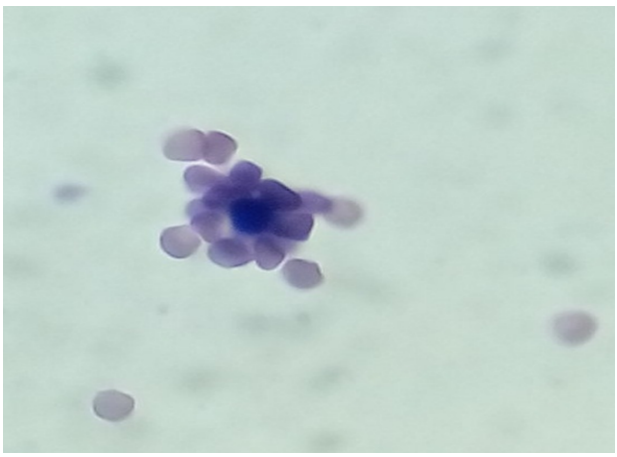

Figure 2: Rosette formation by B-lymphocytes (E-POK method)

The analysis of the population dynamics of lymphocytes showed that during a year, the cattle vaccinated against anthrax had a wave-like range of the

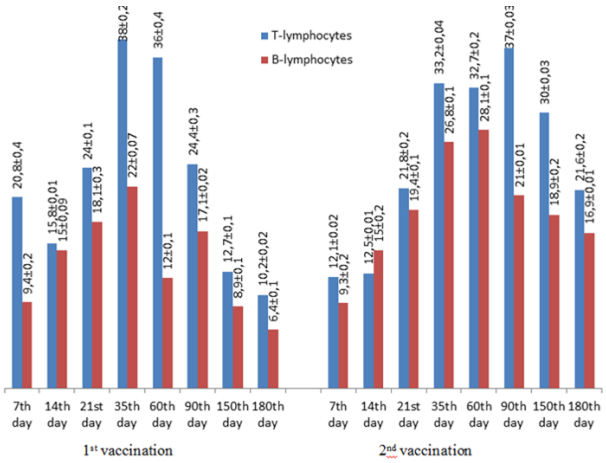

Figure 3: Dynamics of the population of lymphocytes in blood of the cattle after vaccination against anthrax

amount of T- and B-lymphocytes. After vaccination, it was observed that the amount of the cells under study increased, and, consequently, the solidity of the cellular component of the adaptive immunity enhanced. Most of the lymphocytes were recorded from the 35^{th} to the 60^{th} day, then the amount was gradually decreasing, and by the 180^{th} day, it reached its minimum. It shows that the level of specific resistance of the organism and immune memory of the cells decreased. After the second immunization followed by the pasture season, the amount of the cells in the animals increased. Most of the cells were recorded from the 35^{th} to the 90^{th} day after the vaccination that outreaches the duration of the increased amount of lymphocytes in the post housing season. It can be connected with the improvement of the general state of the animals' organism after the pasture season. However, by the 180^{th} day, the amount of the cells had significantly decreased after the second vaccination.

CONCLUSION

Thus, the received data show that immunization leads to an increase in the amount of lymphoid cells and promotes the improvement of the organism's resistance. The increase of lymphocytes participating in decontamination of the toxin and the cells changed by the agent in the organism as well as the immune memory gives ground to talk about the improvement of the immune level in the animals vaccinated against anthrax. The most T- and B-lymphocytes in the blood of the animals were observed on the 30^{th} day after immunization and had been kept on the same level during two months; 3 months later the amount of the cells gradually decreased, and by the 180^{th} day it reached its mini-

mum. It shows the decrease in the immune level and the immune memory.

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