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Immune Morphological Changes in the Body of Kazakh White Rock Calves after Vaccination with Bcg Vaccine and Izoniazid Drug Application

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Abstract: The study was conducted on monthly Kazakh white rock calves of LTD "zdens" farm of West Kazakhstan region, Taskalinskii district, to determine immune morphological, pathological changes in the organs and tissues of Kazakh white rock calves after application of BCG vaccine and izoniazid drug. When the BCG vaccine and isoniazid (tubazid) drug were used in combination, the distinct change was observed in the number of T and B-lymphocytes. Their number increased from the first day of using drugs and lasted one year. Combined use of BCG vaccine and isoniazid (tubazid) drug led to an immune morphological change of the internal organs and the emergence of a strong immunity to tuberculosis for 1 year. Immune morphological changes were detected at the site of BCG vaccine and in the right (submandibular, parotid) lymph nodes and local lymph nodes, kidneys, liver and lungs. Changes in parenchyma organs (liver, kidney and myocardium) were observed in the form of grain and fatty infiltration of hepatocytes. Hyperchromatic and diploid nucleus were found in the cells of liver. Hepatocytes increased in volume.

Key words: Vaccine BCG · Isoniazid · Immunomorphology · RBTL · Changes

INTRODUCTION

Tuberculosis is a global emergency. One third of the world's population is infected and although only about 5 - 10% develop active disease during the first few years following exposure [1].

Now tuberculosis is one of the major social problems of Kazakhstan. Since 1991, there was observed a sharp deterioration of the epidemiological situation of tuberculosis due to low living standards in the country. Only in one year (1995) 4, 5 thousand patients died of tuberculosis.

Bovine tuberculosis (caused by Mycobacterium bovis) is still an infectious disease that causes substantial damages in the agricultural sector in many developed countries; nowadays it is subjected to expensive eradication programs in most EU countries [2].

Bovine tuberculosis, caused by *Mycobacterium bovis*, is a major cause of economic loss in countries, where it is endemic and in some countries it is a significant zoonotic problem [3].

This disease is a significant zoonosis that can spread to humans, typically by the inhalation of aerosols or the ingestion of unpasteurized milk. In developed countries, eradication programs have reduced or eliminated tuberculosis in cattle and human disease is now rare; however, reservoirs in wildlife can make complete eradication difficult. Bovine tuberculosis is still common in less developed countries and severe economic losses can occur from livestock deaths, chronic disease and trade restrictions. In some situations, this disease may also be a serious threat to endangered species. Test and slaughter of infected cattle can eradicate the disease, although these control measures are less effective where wildlife reservoirs of bovine tuberculosis exist. Wildlife reservoirs of *M. bovis* infection have caused problems in the disease eradication from domestic animals in New Zealand, from infection of the brush tail possum, in the UK and Ireland from the badger and in the USA from white-tailed deer [4]. In many developing countries, test and slaughter programs are not economically viable and the disease is not controlled. Use of effective vaccination

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strategies against *M. bovis* for cattle and for wildlife species would be an attractive option for the disease control. Bacillus Calmette-Guerin (BCG) has been widely used for vaccination against human tuberculosis despite its variable efficacy. In cattle, BCG is used to induce a significant level of protection against *M. bovis* infection, when cattle are experimentally challenged, but results from field trials are less encouraging [5,6].

The activities on tuberculosis eradication, regulated by the guidelines, are the most effective in those households, where the infection level is low. Long disadvantaged TB farms, especially at the high incidence of TB animals, do not always achieve the goal. This encourages veterinary science to find new preventive and control methods for this infection and and to improve the existing funds.

Every year the problem of tuberculosis in Kazakhstan attracts more and more attention of scientists and practitioners. This is due to increased morbidity and the emergence of severe forms of the disease with a fatal outcome, which, unfortunately, is not always reflected in the report documentation.

This problem acquired particular urgency in the countries of Western Europe and the United States. The annual incidence of tuberculosis in the economically developed countries to some extent supported the idea of the process of eliminating tuberculosis as a disease of mass. In 1991 the General Assembly of the World Health Organization (WHO) was forced to admit that tuberculosis is still a priority of international and national public health problem not only in developing countries, but also in the economically advanced ones. There are more than 8 million new cases of tuberculosis in the world each year, 7 million 600 thousand (95%) of them occur in developing countries; 3 million people die each year from TB, so we may expect that in the next 10 years another 30 million patients will die from it. The current situation in WHO is described as a crisis of global policy on Tuberculosis [7].

As per College of Veterinary Medicine of Lowa State University bovine tuberculosis is still common in less developed countries. In some cases, the disease can also be a serious threat to endangered species [8].

Bovine tuberculosis is a chronic bacterial disease of cattle that is sometimes observed among the other species of mammals. It is a significant zoonotic disease that can spread to humans, through inhalation of aerosols or the use of raw milk. In developed countries, the program of eliminating tuberculosis in cattle was reduced or closed, but these diseases are still found in wildlife.

An important management strategy for the TB diseases prevention is the use of effective vaccines. Bacilli Calmette-Guerin (BCG), an attenuated strain of M. Bovis, is widely used for human tuberculosis control, despite the raging about the protective efficacy.

Among tuberculostatic drugs, widely used in medical practice, the most active one is isoniazid (tubazid - drug, anti-Ttuberculoses drug, isonicotinic acid hydrazide (Ginko). Isoniazid is highly toxic to dogs and cats. It is the most effective anti-TB drug for treatment of active tuberculosis [9].

Isoniazid is effective in all forms of pulmonary and out pulmonary tuberculosis. The advantage of this drug is the speed and uniformity of penetration into the organs and tissues of the patient body. It is strictly specific to the mode of action of the agent, selectively concentrating lesions in organs. Currently, the drug is more widely used in veterinary medicine as an additional tool in the fight against the tuberculosis of cattle, pigs, poultry and fur animals.

The action mechanism of isoniazid is associated with inhibition of synthesis of mikolice acid in the cell wall of Mycobacterium tuberculosis (MT). The effect of izoniazid on Mycobacterium tuberculosis in the stage of reproduction is bactericidal and it is bacteriostatic for the rest Mycobacterium tuberculosis. Isoniazid is highly effective, but monotherapies quickly develop resistance.

The drug is well absorbed in the gastrointestinal tract, the therapeutic blood concentrations are reached in 1-2 hours after taking. The drug has hepatotoxicity that may cause isoniazid-associated hepatitis (in some cases). Combined with ethambutol, isoniazid can be used in the treatment of cutaneous tuberculosis of cats and dogs [10].

Tuberculosis is still widespread in the world. It caused great economic damage, millions of livestock died from it. The causative agent of bovine tuberculosis is the most pathogenic for all types of farm and wild animals; it also causes the disease in dogs and cats easily [12].

The studies of immune morphology changes in the tissues of Kazakh white breed calves after use of BCG vaccine and izoniazid (tubazid) drug are not enough. Big perspectives of using the izoniazid drug and BCG vaccine in veterinary medicine for tuberculosis (TB) of productive animals were considered; we aimed to study immunomorphological, patho-morphological changes in the tissue of calves after the application of BCG vaccine and tubazid drug.

MATERIALS AND METHODS

The work was performed at the Department of Non-Communicable Diseases at the Faculty of Veterinary Medicine and Agricultural Biotechnology in 2009 to 2013, at the West Kazakhstan Agrarian Technical University, at limited partnership «Izdenis» farm, Scientific Research Institute at Western Kazakhstan Agrarian Technical University named Zhangir Khan.

Experiments were held in four groups. There were 10 animals in each group. We used monthly aged calves of Kazakh white breed, belonging to farms limited partnership «Izdenis». The body weight of calves was 30-40 kg. All calves were exposed to an allergic test before the experimental work, all the results were negative. Calves were well provided with water and food. The ration consisted of silage, hay and salt.

We used BCG vaccine and tubazid drug in order to prevent tuberculosis, which showed immunomor phological changes in the body of calves.

The first group of calves inoculated with BCG vaccine intradermally (1 mg which was diluted with 0.2 isotonic sodium chloride solution).

The calves of the second group received isoniazid orally with milk within 30 days. Ñalves received 10 mg of isoniazid per 1 kg of body weight.

Then, after 1 month, calves were subcutaneously injected with BCG vaccine in a dose of 1 mg, which was diluted with 0.2 isotonic sodium chloride solutions. The fourth group served to control us. Three calves from each group were killed for pathological study.

Before using the drug, all animals were subjected to an allergic reaction to PPD tuberculin, intended for mammals.We used anti TÂ drug izoniazid (tubazid) in tablet form. Manufactured «macleods pharmaceuticals ltd», produced in Mumbai (India) and Japanese freeze-dried glutamate vaccine in the skin injection (japan BCG laboratory, 4-2-6, kohinata, Bunkyo-ku, 112-0006, Tokyo, Japan).

Experimental animals were slaughtered after 14 days spent in the hospital of West Kazakhstan Scientific Research Veterinary Station. Carcasses and tissues were detailed post-mortem examination for TB change. Then the organs were sent for laboratory tests (for histological and ultra structural studies). Laboratory studies were carried out on the interior of parenchymal organs (liver, kidneys, myocardium, lungs) and lymph nodes (submandibular, mediastinal, subscapularis, bronchial, parotid).

For histological examination, pathological material was preserved in 10% - rated neutral aqueous solution of formalin. The resulting pieces of bodies after appropriate treatment embedded in paraffin and celloidin. Paraffin sections were obtained from paraffin blocks of 4 mm thick; they were stained with haematoxylin-eosin and Van Gieson and examined under microscope at 200-400 times. Histological microscopic materials were studied by means of binocular microscope Leica DM 4000 B. Thin sections were obtained on a semiautomatic microtome HEOSTION ER M 3100.

The functional activity of peripheral blood lymphocytes of animals after using drugs was determined by A.Averbah (1974) RBTL (reaction of blast transformation of lymphocytes of animals). We used the purified (PPD) tuberculin, intended for mammals, as antigen. For the experiments, we took the blood of calves from the jugular vein in the volume of 10 mg to a sterile tube size 20x200 mm. The tube contained heparin at a dose of 16 units per 1 ml of blood

Tubes were mixed well, left for 3-5 hours at room temperature under gorizantal angle of 10 degrees. This time a thin film between erythrocytes and plasma lymphocytes concentrated in significant volumes.

The lymphocytes of the blood plasma were carefully collected with a pipette to the vials and sent to the laboratory for analysis RBTL.

RESULTS

Immune Morphological Changes in the Body of Calves Kazakh White Rock after Using BCG Vaccine: The experiment shows the state of cellular immune system in 7,15,30,60,120,240,360 days after vaccination with BCG with the method Blast Ttransformation reaction (RBTL). BTLR response rate is shown in Table 1

As shown in Table 1, the rate of RBTL by 7-15 days of studies in the experimental group was equal to 18,29 ± 0,69 and in the control group it decreased to 1,45 ± 0,04. 2,24 ± 0,02. The rate increased after 2 months by 22, 55 ± 0, 52 and it decreased in the control group. The rate of RBTL was 17, 13 ± 0, 43 by year end. In this connection, the study shows specific antigenic irritability of cellular elements, stored for 1 year.

Rosette formation of T- lymphocytes was equal to 61,6 ± 0,7 percent of the calves of the control group. After the 15 days of vaccination with BCG vaccine, the category of functional activity of the lymphocytes in calves of the experimental and control groups increased. The number of Rosette formation of T- lymphocytes count 63, 4 ± 0, 3% (Table 1). The number of

Table 1: Indicators of T and B- cells and datas of reaction of blast transformation of lympocytes (RBTL) after using the vaccine BCG.

	T - lymphocytes		B - lymphocytes		Indicators of RBTL	
Time	Experimental group (BCG)	Control group	Experimental group (BCG)	Control group	Experimental group (BCG)	Control group
7	62,0±1,4	62,3±0,9	47,3±1,4	46,7±0,4	3,23 ±0,47	2,33±0,42
15	62,8±0,6	62,6±0,8	47,0±1,2	46,4±0,9	3,03 ±0,60	3,46±0,02
30	62,4±0,9	62,4±0,3	47,9±0,6	46,9±0,6	2,32 ±1,32	2,61±1,63
60	62,8±1,9	63,3 ± 1,6%	46,8±0,6	47,4±1,3	3,22 ±0,97	2,53±1,49
120	62,1±0,5	62,5±0,6	48,2±1,6	47,1±1,4	2,13 ±0,63	2,41±0,65
240	62,9±0,2	62,3±0,5	47,4±1,8	47,0±1,1	3,41 ±1,73	2,49±0,93
360	62,6±0,7	61,8±0,7	47,2±0,9	46,9±0,3	3,22 ±1,65	1,63±0,65

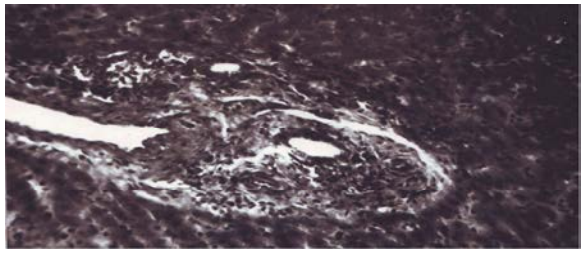

Fig. 1: The liver. Patchy lymphocytic histiocytic infiltration of the connective tissue after using vaccine BCG. Colored Gemotoksilenom and eosin. X 240

B-lymphocytes was equal to 51,3 ± 0,9% in the first month after vaccination with BCG. The number of B-lymphocytes also increased 2 months later. The category of functional activity of B-lymphocytes was 52,1 ± 0,7 percent and it was 64,4 ± 0,6 for T-lymphocytes (Table1). The presented results indicate that the number of immune components of blood cells varied from the first days of vaccination and recovered after 1 year. After 3 days of vaccination, the injection site was swollen, somewhat painful to the touch. After the slaughter of animals for pathomorphological examination, the changes were similar in all animals. In connection with this, we have provided a general characterization. The most pronounced pathological changes in organs and tissues were local to the site of the vaccine in the right inguinal and local counter in the left inguinal lymph nodes and spleen. Mild hyperemia of vessels in the lungs and thickening of inter alveolar cells were detected. We carried out the immune morphological assessment of organs and tissues to determine the strength of immunity after the vaccination. The plasmacytic and hyper plastic lymphoid macrophage reaction was observed in the spleen and lymph nodes (lymph nodes (submandibular, mediastinal, subscapularis and bronchial nadvymennye, parotid). The increased degradation of immature lymphocytes was also revealed. Lymph nodes (submandibular, mediastinal, subscapularis and bronchial nadvymennye, parotid) were enlarged and there were a large number of reticular and blast cells, lymphocytes and macrophages within them. The reticular stroma of many follicles was displaced to the periphery due to the expansion of the reactive centers. Mature lymphocytes accumulated in the peripheral zone of the reactive centers. They were found between cells in the form bare nuclei. The liver's structure stored in, a moderate blood filling of vessels was revealed, hepatocytes were grainy dystrophy. Number of glycogen and RNA decreased. Moderate proliferation of stellate reticulo endotheliocyte was observed in the parenchymatous organs. Nucleus of hepatocytes was hyperchromic and diploid (Figure 1).

Granular dystrophy and decreased glycogen were observed in the myocardiumm and there was also a focal accumulation of lymphoid histiocytic cells in the interstitial tissue. The number of affected nephrons increased in the kidney of experimental animals, but obvious pathological damage was detected in glomeration vessel. An increase in the amount and moderate blood filling were mostly observed in the vessel glomeruli. A considerable amount of granular, eosinophilic, protein substance contained in Bowman-shumlyansky capsules. The irregular neuronal damage was also observed. Finally, the hyperplastic - proliferative processes in tissues, activation of stellate reticulo endotheliocyte system, increase of capillary permeability, lymphostasis manifestations and the presence of homogeneous basophilic masses in the sinuses were observed after applying BCG. The changes, such as fibrinous tissue proliferation in the lymph nodes and the presence of homogeneous basophilic masses in the sinuses, occurred only in the body of calves Kazakh white rock vaccinated with BCG.

Table 2: Indicators of T and B- cells and data on reaction of blast transformation of lympocytes (RBTL) after taking the drug izoniazid (tubazid).

	T - lymphocytes		B - lymphocytes		Indicators of RBTL	
Time	Experimental group (tubazid)	Control group	Experimental group (tubazid)	Control group	Experimental group (tubazid)	Control group
7	62,3 ± 0,5	61,8±0,9	49,9±0,6	47,3±1,2	17,69± 0,47	2,31± 0,35
15	63,4±0,3	61,7±0,8	50,2±1,3	47,8±0,1	18,29± 0,69	2,24± 0,02
30	64,4±0,6	61,6±0,1	51,3±0,9	47,0±0,4	18,93± 1,61	1,61 ±0,03
60	64,2±0,2	61,8±0,8	52,1±0,7	47,9±0,7	22,55± 0,97	1,45± 0,04
120	62,7±0,3	61,3±0,5	49,9±1,3	47,7±0,5	20,32± 0,52	1,75 ±0,04
240	62,8±0,6	61,7±0,7	48,1±1,8	47,5±0,9	17,62 ±1,63	1,47±0,03
360	62,9±0,9	61,2±0,5	48,3±1,2	47,3±0,6	17,13± 0,43	1,63 ±0,02

Immunomorphological Changes in the Body of Calves Kazakh White Rock after Taking the Tubazid Drug: As indicated in Table 2, the activity of lymphocyte function was not revealed after the application of tubazid. Only in the experimental group, at 60 and 120 days after the application of tubazid, there was a quantitative increase in T and B lymphocytes. There were also no major changes in terms of RBTL (Table 2).

In connection with this, tubazid does not cause the quantitative changes in the activity of the functions of lymphocytes blastransformation.

The results of our study show the following tubazid pathological changes in the organs and tissues after treatment: granular dystrophy in parenchymal organs (lung, liver, kidney, spleen), focal accumulations of lymphoid histiocytic cells and fatty degeneration of hepatocytes.

Protective reaction of tissue and organs was found in animals, treated with tubazid mainly in mesenteric lymph nodes and liver. The structure of liver was saved, hemorrhages of organ's vessels were found. Granular dystrophy took place in hepatocytes. The satellites reticule - endothelial cells were uniformly profiled. The focal congestion of lymphocytic histiocytic cells was observed in some places. Fatty infiltration was observed in hepatocytes and in the cytoplasm. The histiocytic and lymphocytic infiltration was found in the lungs. Therefore, there was a congestion of the alveolar cells. The structure of lungs was saved. The changes in the mesenteric lymph nodes and liver were similar. The lymphoid tissue of the cortex was swollen and hyperplastic; there was also a focal accumulation of epithelial cells. The central sinus had a high capillary permeability. A phenomena of lymph stasis was observed. The blood vessels of liver, kidneys and in the myocardium were filled with blood. Granular degeneration was seen in cells of the parenchyma. There were focal accumulations of lymphoid histiocytic cells. Using the isoniazid drug, the receptacle was filled with blood. A granular dystrophy in parenchyma cells was also observed. Figure 2 for focal proliferation of lymphoid cells and fatty infiltration in hepatocytes.

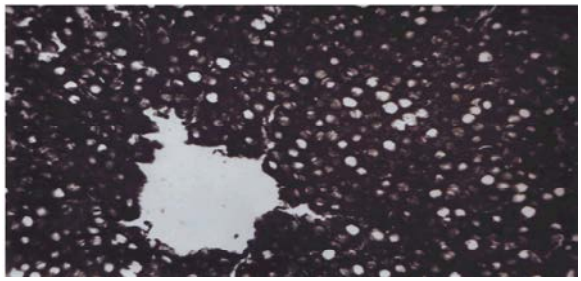

Fig. 2: Liver. Fatty infiltration of hepatocytes after taking the isoniazid.The sections were stained with gemotoksilen and eosin. X 240.

Studies of Immunomorphological Changes in the Body of Calves, Treated with Bcg Vaccine and Isoniazid Drug: The results of the study of the immune system during co-administration tubazid combined with BCG vaccine are given in Table 3.

The content of T rosette forming cells from a control group of calves was 61,3±0,6 % and the one of B - lymphocytes was 47,3 ± 0,7%.

It was noted that after 7 days T - lymphocytes increased up to 62,2 ± 0,8% and B - lymphocytes increased up to 48,9 ± 1,3%. The indicators of BTLR peripheral blood of the animals of the experimental group averaged 18,41 ± 0,58%, while the ones of the control group were 2,43 ± 0,63%. After 30 days there was an increase compared to the control group. The above data indicate the specificity of the reaction, where the dynamics of digital indicators of T and B lymphocytes and RBTL match each other (Table 3).

During the use of BCG vaccine and tubazid farms in the organs and tissues of calves, immunomorphological obvious changes were observed in the injection site, however, they occur in the right submandibular,

Table 3: Indicators of T and B- cells and data on reaction of blast transformation of lymphocytes (RBTL) after using the vaccine BCG and taking the drug izoniazid (tubazid)..

	T - lymphocytes		B - lymphocytes		Indicators of RBTL	
Time	Experimental group (BCG)	Control group	Experimental group (BCG)	Control group	Experimental group (BCG)	Control group
7	62,2±0,8	61,3±0,6	48,9±1,3	47,3±0,7	18,41±0,58	2,43±0,63
15	64,2±0,7	61,3±0,8	50,1±1,8	47,7±0,1	19,23±0,16	2,31±1,13
30	65,3±0,3	61,9±0,9	53,9±1,6	47,2±0,4	23,24±1,29	2,76±0,63
60	64,0±1,8	61,3±0,1	52,3±1,2	47,3±0,4	23,18±1,32	2,39±0,21
120	64,6±0,8	61,7±0,6	50,1±1,6	47,7±0,2	22,24±1,29	2,46±0,60
240	64,0±0,5	61,0±0,8	49,9±1,6	47,9±0,7	18,45±0,21	2,53±0,31
360	63,9±0,7	61,5±0,3	49,6±2,4	47,8±1,3	18,33±0,74	2,73±0,31

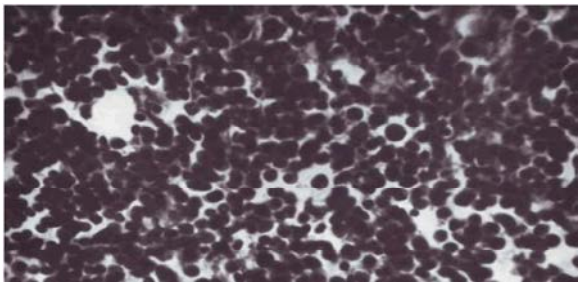

Fig. 3: The submandibular lymph node. The reaction of plasma cell. Stained according to the method Brush. X 240

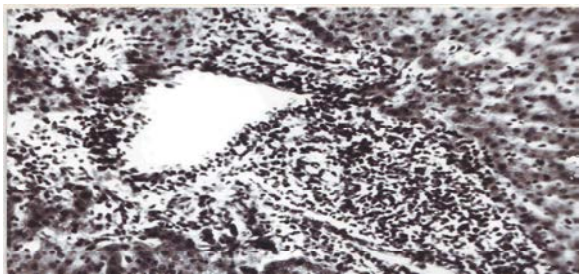

Fig. 4: The focal accumulations of lymphoid histiocytic cells in the perivascular tissue of the live. The sections were stained with gemotoksilen and eosin. X 240.

parotid lymph nodes and the local lymph nodes, as well as in the kidneys and lungs. The structure of the follicular pattern stored in the lymph nodes and in the follicles observed mild germinal centers. Lymphoid cortical substance was slightly hyper plastic. Tissue fibers were thickened, plasma cells filtered in a large volume. The walls of the arterioles, veins, capillaries were in mucoid swelling. The central sinuses were expanded; macrophages and lymphocytes were identified in some areas. The perivascular tissues of trabeculae were increased and fibred. There were lymphostasis of sinuses in lymph nodes and lymphoid - hyperplastic response Figure 3.

Obvious decay of the old cells took place in certain areas of the lymph nodes. However, lymphostasis of sines was observed. One of the features of the control group was the growth of tissue on the way of granulation tissue trabeculae; sometimes they took up the entire area of sine. The vessels of the liver were not fully filled with blood. Hepatocytes exposed to granular dystrophy. Stellate retikuloendoteliotsity weakly spread. There were focal accumulations of lymphoid - histiocytic cells and connective tissue in parenchymal organs. Figure 4.

In some places their volume increased significantly. The number of hepatocytes with hyperchromatic and diploid nuclei rised.

In animals, the pathological changes in the organisms of calves after using the vaccine BCG izoniazid drug were similar. In addition, permeability of the walls of the small capillaries in the central of sines lymphostasis increased.

Expansion of the sinuses, preservation of cellular elements follicles and focal eosinophilia were found in all examined lymph nodes. In addition, there was observed an increased collapse of the old lymphocytes in the form of sound-clusters hromotino. The follicles of the lymphoid tissue of the cortex and germinal center uniformly benign enlarged sinuses. In addition there were focal hemorrhage of tissues, accumulation of eosinophils, mucoid swelling of arterioles, veins and capillaries. The granular distrophy of parenchymal elements and the lobular proliferation of lymphoid lymphocytic-histiocytic cells of liver, kidney and adrenal gland were found out. The structure of lung was saved, in some places alveoli were thickened by filling the capillaries and infiltration lymphocytic - histiocytic cells. The granular dystrophy, uniformity proliferation of stellate reticuloendothelial cells and equal

vascular perfusion in the liver were observed. Fat in some sections of cytoplasmic hepatocytes was found. The germinal centers of lymph nodes (submandibular, bronchial parotid) contained the limited number of cellular elements; lymphocytes were found besides the blast elements. Clot accumulated lymphocytes were detected in per follicular areas of follicle.

A pronounced lymphoid - hyperplastic reaction was identified, but it was observed from immunopathological reaction to capillary permeability. In the central sinus the cells and homogeneous basophils of similar blasts were detected in small quantities. Immunomorphological changes in parenchymal organs (liver, kidneys, myocardium) were in the form of granular degeneration and fatty infiltration of parenchymal cells. Stretching of the renal cortex was due to the hyperplasia of papulose zones.

Thus, the effective use of BCG vaccine and izoniazid in the farms caused the morphological changes in the internal organs of calves and influenced the emergence of a moderately active immunity.

DISCUSSION

The results, obtained by no susceptibility of animals to infectious diseases, including tuberculosis, present a method of great future. Our research proves it.

The method of chemo-prevention using tubazid drug is effective, but only thanks to this drug. There is information on the effect of isoniazid for real fat metabolism in rabbits at the injection into the abdominal cavity, a reduction of cholesterol took place in 30 days [12].

According to the veterinary [13, 14] and medical researcher [15], tubazid has a demonstrable activity; the inactivation was similar to the process of inactivation in the liver, so we decided to investigate the impact of tubazid on functions of various organs. Liver pathology changes the biological process, as a result blood cell functions and biochemical properties are also changed. When applying izoniazid drug, the activity of lymphocytes in peripheral blood are changed; only the testimony of T lymphocytes in the 30 day study reached 62,4 ± 0,9 and B lymphocytes in 120 days reached 48,2 ± 1,6. Similar results were in terms of the reaction of blast transformation of lymphocytes (RBTL). The obtained results indicate no change in the level of T and B lymphocytes; the activity of RBTL after tubazid application was not revealed, that is why it follows that tubazid will not make changes in the cells, immunocompetent cells and RBTL. At present, tubazid is the main tuberculostatic drug, used in the treatment of tuberculosis of calves. After immune morphological research of myocardium, kidney, liver and adrenal glands of experimental animals, we found blood filling of vessels, granular degeneration of parenchyma cells and focal histiocytic proliferation of lymphoid cells. The animals, treated with tubazid, had the following changes: varying degrees of blood filling, proliferation of hepatic stellate reticular endothelial cells, fatty dystrophy of hepatocytes, granular dystrophy of parenchyma elements, focal lymphocytic proliferation of histiocytic cells.

After the application of the BCG vaccine, we observed the hyperplastic - proliferative processes in tissues, activation of stellate reticulo endotheliocyte system, increase of capillary permeability, lymphostasis manifestations and the presence of homogeneous basophilic masses in the sinuses. The change, such as fibrinous tissue proliferation in the lymph nodes and the presence of homogeneous basophilic masses in the sinuses, occurred only in the body of calves Kazakh white rock, vaccinated with BCG. By 7-15 days studies the rate of RBTL in the experimental group was equal to 18,29 ± 0,69 and in the control group it decreased to 1,45 ± 0,04. 2,24 ± 0,02. The rate increased after 2 months by 22, 55 ± 0, 52 and in the control group decreased. The rate of RBTL was 17, 13 ± 0, 43 by year end. In this connection, the study shows specific antigenic irritability of cellular elements, stored for 1 year. Rosette formation of T- lymphocytes was 61,6 ± 0,7 percent of the calves of the control group. After the 15 days of vaccination with BCG vaccine, the category of lymphocyte functional activity in calves of the experimental and control groups increased. The number of Rosette formation of T- lymphocytes was equal to 63, 4 ± 0, 3% (Table 1). The number of B-lymphocytes was 51,3 ± 0,9% in the first month after vaccination with BCG. The number of B-lymphocytes also increased 2 month later. The category of functional activity of B-lymphocytes was 52,1 ± 0,7 percent and the one of T-lymphocytes was 64,4 ± 0,6 (Table 1). The presented results indicate that the number of immune component of blood cells varied from the first days of vaccination and recovered after 1 year.

After taking the tubazid, there were focal accumulations of lymphoid histiocytic cells. Using the drug isoniazid, the filled receptacle with blood was seen. There was also observed a granular dystrophy in parenchyma cells. The focal proliferation of lymphoid cells was observed, as well as the fatty infiltration in hepatocytes. The results of our study show the following

tubazid pathological changes in the organs and tissues after treatment: granular dystrophy in parenchymal organs (lung, liver, kidney, spleen), focal accumulations of lymphoid histiocytic cells and fatty degeneration of hepatocytes.

Protective reaction of tissue and organs was found in animals, treated with tubazid, mainly in mesenteric lymph nodes and liver. The structure of liver was saved, there were found hemorrhages of organ's vessels and granular dystrophy in hepatocytes. The satellites reticule - endothelial cells were uniformly profiled. The focal congestion of lymphocytic histiocytic cells was observed in some places. Fatty infiltration was observed in hepatocytes and in the cytoplasm

When we use of BCG vaccine combined with drug isoniazid (tubazid), the number of T lymphocytes changed distinctly: in the first few days the number of T lymphocytes was 62, 2 ± 0,8 and B lymphocytes was 48,9 ± 1,3%. The data of reaction of lymphocytes blast transformation in peripheral blood of animals composed 18, 41 ± 0, 58%, while in the control group it was 2, 43 ± 0, 63%. After 30 days, the number of T lymphocytes increased to 65, 3 ± 0, 3, in the control group it was 61, 9 ± 0, 9; the number of B lymphocytes was 53, 9 ± 1, 6, in the control group it was 47,7 ± 0,2 %. The highest rate of blast cells was 23, 24 ± 1, 29% after 30 days, while in the control group it was 2, 76 ± 0, 63. The activity and expression of post-vaccination immunity is a direct data of the reaction blast transformation of lymphocytes. The figure rises when the body's resistance to the causative agent of tuberculosis increases and it decreases when the resistance is suppressed. Conformity of quantitative dynamics of these T and B lymphocytes with RBTL figures points to the specificity of the reaction.

The main immune morphological changes were observed at the injection site and counter regional lymph nodes, liver and lungs after combined using of BCG vaccine and drug isoniazid (tubazid). Follicular pattern of lymph nodes was saved, germinative center of follicles was poorly expressed. Lymphoid tissue of the cortex were weakly hyperplastic, cellular elements localized scattered in Para cortical area, muscle fibers were thickened. Immune morphological changes in liver, kidney and myocardium were in the form of granular and fat dystrophy. In the kidney, due to hyperplasia of the beam and the grid zone, the cortex expansion was observed. So, after using the vaccine BCG and izoniazid, we observed the morphological changes in the internal organs of calves and the emergence of a moderately active immunity. It is a very effective to use the BCG vaccine and drug tubazid in tuberculosis disadvantaged households. Calves get the immunity against the tuberculosis, which is preserved for 1 year.

CONCLUSIONS

- The hyperplasic lymphoid, macrophage and plasma cell reaction of lymph nodes and liver were observed after application of BCG vaccine and drug izoniazid. Also, there were immune morphological changes in the body, including the activation of the reticulo histiocytic cells of internal organs.
- Combined use of BCG vaccine and drug tubazid caused activation of T and B lymphocytes in the experimental group of calves. The highest content of these cells was observed 30 days after the drug application in the peripheral blood of calves.
- Combined use of BCG vaccine and tubazid drug to Kazakh white breed calvesof disadvantaged TB farms, protects calves from tuberculosis during the year.
- The combined use of BCG vaccine and isoniazid drug in production conditions in case of moderate spread of TB animals creates a high protection level against the epizootic.
- The reticulo - proliferative protection elements of the reticuloendothelial system of the internal organs (RES) showed its high biological activity after combined use of BCG vaccine and tubazid drug. We came to this conclusion as per the following changes: the accumulation of glycogen and RNA in the cytoplasm and the increase of acid phosphatase activity in the cells.
- The lymphoid hyperplasia reaction of the lymphoid organs and fatty infiltration of the hepatocytes were observed after tubazid application in tuberculosis advantaged households.
- We have developed a preventive project to use BCG vaccine and tubazid drug. It provides an opportunity to get healthy young stock animals from tuberculosis disadvantaged households and to shorten the recovery from tuberculosis farms.

At the event aimed to combat tuberculosis, we propose to use a combination of BCG vaccine and tubazid drug. Newborn calves are provided with 10 mg of tubazid per 1 kg for 30 days, then after 10 days the calves are vaccinated with BCG vaccine.

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