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THE INFLUENCE OF BCG VACCINE TO THE CALVES'S SKIN AFTER ADMINISTRATION

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Annotation

The article presents the results of a study on calves after application of BCG vaccine. in the experiment, the calves of the Kazakh white breeds, 1-2 months of age. Using electron microscopy allowed establishing pronounced ultrastructural changes in the organism of calves immunized with BCG vaccine. They are characterized by: the destruction of the muscle fibers of the dermis and the appearance of necrotic detritus; macrophage reaction followed fibrotization tissue at the injection site; destruction of mitochondria in hepatocytes and epithelial tubules uriniparous; swelling of podocytes in renal vascular glomeruli. and also develop less serious violations: serous edema, partial fragmentation of striated fibers in the tissue at the site of these biologics, swelling of mitochondria with cristae fuzzy pattern in epithelial cells of the liver and kidneys.

Key words: tuberculosis, electronic microscope, vaccine, BCG, pharenchyma.

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 [2,3].

BCG (BacilleCalmette-Guerin), an attenuated IV. bovis strain, has been the live vaccine widely used for immunoprophylaxis against human tuberculosis. Although there has been considerable debate about the degree of protection conferred by BCG, this vaccine has played a major role in controlling the spread of human tuberculosis . Trials to evaluate the effectiveness of BCG for control of tuberculosis in cattle have been under- taken since 1920sW9, with doses ranging from 1 to 100 mg of live BCG being used. The results of these trials were inconclusive and often difficult to interpret, as many cattle were challenged by unnatural routes including subcutaneous inoculation. However, in some situations, vaccination appeared to decrease the severity of the disease'**. A major problem associated with BCG vacci- nation of cattle was that vaccination interfered with diagnostic intradermal skin testing [4,5].

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 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 [6,7].

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 [8].

As per College of Veterinary Medicine of Iowa 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 [9].

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 oneis 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. [10].

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 izoniazidon 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 [11].

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].

Work novelty:there was first investigated the ultrastructure of calve's bodies after the application of BCG vaccine.

The aim of the work: Study the ultrastructure of organs after application of BCG vaccine.

The studies of ultrastructuralchanges in the tissues of Kazakh white breed calves after use of BCG vaccine 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 ultrastructural changes in the tissue of calves after the application of BCG vaccine.

The materials and methods or research.

The research work was conducted at Zhangir khan West Kazakhstan Agrarian-Technical University on faculty of veterinary medicine and biotechnology, non infectious disease department in 2009-2015 years according to the scientific plan of faculty. There were used 1-2 month old, well-fed averaged, Kazakh white head breed calves. The diagnostic works, anti infectious actions and veterinary sanitary actions were conducted with approved recommendations as a ``Recommendation to liquidation and prevention of cattle tuberculosis`` and ``Recommendation to diagnostics of tuberculosis`` [13,14,15].

There were used 40 calves. The research works were conducted on 4 groups 10 calves in each group. First group were immunized with vaccine BCG .Before the research all calves were checked by allergic method. All results were negatively. In order to do researches by electron microscope we killed 3 calves in each group on 3, 7, 15, 30, 60, 120, 240, 360 days after vaccination. In the present study we bring just results from the first group studies which calves of first group were vaccinated with BCG.

The methodology of how to do ultra-cuts:

Firstly we must know how to take materials right. The basic requirements for taking the material:

1. Have the necessary tools and equipment for animal dissection, fixing solutions (at t + 4 ° C) is poured on the Eppendorf instruments (PTFE or wax pla¬stinka blade, pipettes to transfer samples in Eppendorf) for selection tissue samples.

2. Quick slaughtered animal and material selection.

3. Strict adherence to the size of the pieces of material.

Fixing material

The purpose of fixing - to stop the post-mortem changes, fabric in the state closest to the lifetime.

To do this while taking a sample (sample studies) should to know two basic rules:

1. Immediate fixation - cut an object to lock the drop, the pieces immediately transferred to the eppendorf with a lock.

2. Strict compliance to the size of the pieces. It is important that the volume of pieces less than 1 mm3. Lock volume should exceed 1000 times the volume of tissue. One Eppendorf can fold 5-15 pieces.

In electron microscopy used with great success hell-additivity clips that got this name due to the fact that they can be integrated into the structure of recorded materials before proteid. Ideal clamps no. Any of the applied has positive and negative sides. To study the ultrastructure of the cells are successfully special aqueous solutions of aldehydes (glutaraldehyde, formaldehyde), potassium permanganate (commonly used for tissue fixation now largely superseded aldehyde retainers and osmium). Glutaraldehyde is a universal lock in their ability to maintain the ultrastructure of cells.

After that goes dual fixing and de watering, fabric impregnation sealing medium and polymerization units. In brief about sharpening blocks.

For sharpening the pyramid on the ultra-thin cutting, follow these steps:

1. View semithin sections under a light microscope and sketch them with a designation of traces on the knife;

2. Remove from the ultratome unit with the drug and put it under a stereo microscope cut side up;

3. Turn the illuminator and cut as long as the cut surface of the block will not be the most clearly visible details of the object required for ultra-studies;

4. Cut a razor blade those portions of the drug, which will not be explored;

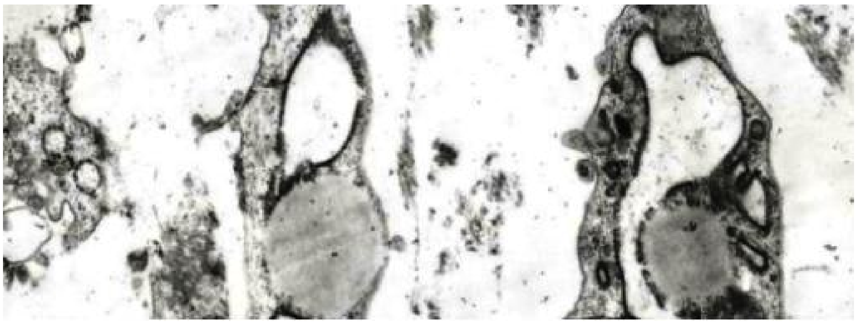
5. making the final sections of the new sharp blade, to all faces were smooth. Biological structures are mostly electron-optically transparent. Image contrast in transmission electron microscope can be increased by decreasing the accelerating voltage and the present-aperture diaphragm, increasing the focal length of the races-standing objective lens. The most effective impregnation of cuts atoms of heavy elements that are intensively scatter electrons, and thus increase the contrast of the image. Greater contrast can be achieved by increasing the density ultrastructures.For non-specific staining is most often used uranyl acetate (UA) and lead nitrate (CA).

Contrast tissue using UA immediately as possible after fixation osmium or during dewatering in alcohols. In the first case, a solution of 0.25-2% (5% less) the V solution in water or in maleate or Na-acetate buffer (pH 5). The final pH may vary from 4.2 to 5.2 depending on the con centration UA. Application UA solution pH 5-5,2 leads to better co-Security of DNA, cell contacts, mitochondria, myofibrils, nucleoproteins and phospholipids [16].

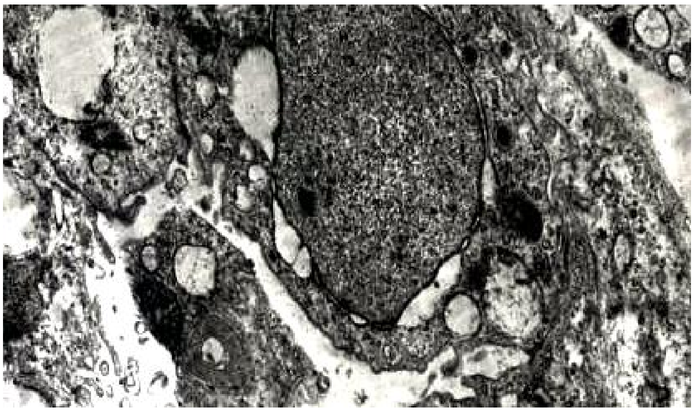
The main results of research work.

After vaccination, in muscle tissue (cross-striated muscle tissue) at the site of administration of BCG vaccine at low magnification there were visualized the structure of the dermis of the skin and cross-striped muscle tissue. in the sarcoplasm of muscle fibers quite clearly contoured transverse striations.

On 3-15 days after immunization with BCG were found everywhere fragments of nuclei, organelles (picture 1), fibers, components of necrotic dendrite actively disposed of by macrophages (picture 2).

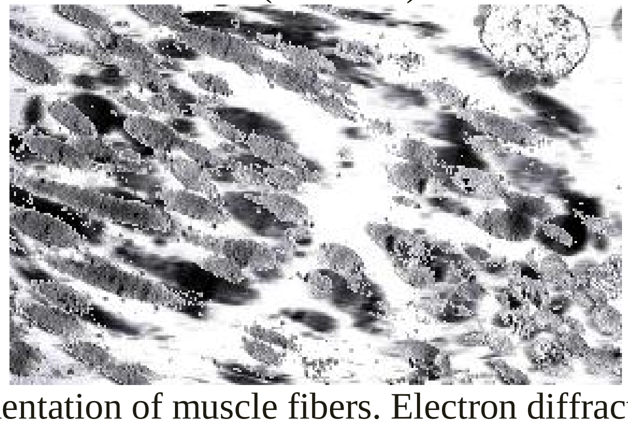


Picture 1- The destruction of the cell and tissue elements (nuclei fragments, organelles fibers) at the site of injections after 7 days of immunization, electron diffraction X 16,000.



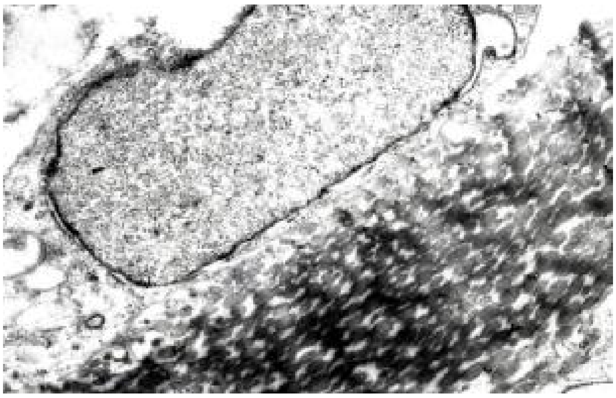
Picture 2 - Recycle necrotic detritus in the vaccine 14 days after immunization. Electron diffraction. X 46000.

On the 30th day of the experiment at the injection site there were continued identifying macrophages and fibroblasts that replace to the damaged areas. In the study of tissues at 3 and 7 days of the experiment everywhere revealed the characteristic degradation of cross fibers (Picture 3).



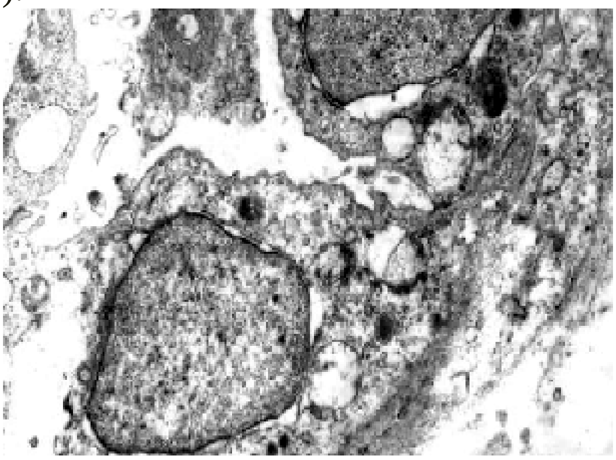
Picture 3 - Fragmentation of muscle fibers. Electron diffraction. X 46000.

Among the fragments of the past there were recorded damaged mitochondria. In subsequent periods of the study there was dominated mainly fibroblastic reaction (picture 4).

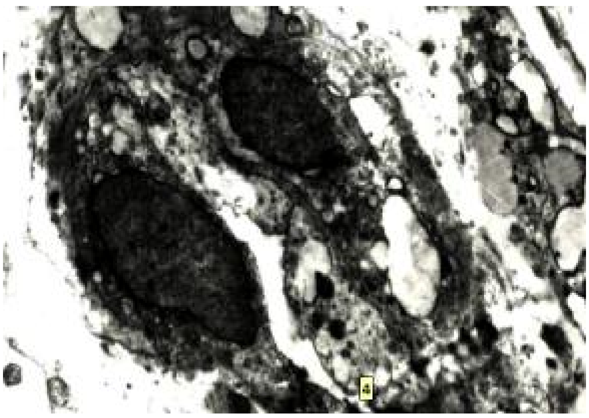


Picture 4 - The accumulation of fibroblasts at the site of vaccine administration. Electron diffraction.X 24 000.

It noted the emergence of a large number of macrophage phagosomes (picture 5). Among the last recorded fragments and damaged mitochondria. In subsequent periods the study was dominated mainly fibroblastic reaction. It noted the emergence of a large number of macrophage phagosomes. 30 day study noted isolated fibroblasts from secretory vesicles developed plate complex. On the 30th day there were observed single fibroblast secretory vesicles which has developed complex plate (Picture 6).



Picture 5 - The accumulation of fibroblasts at the site of vaccine administration. Electron diffraction.X 24 000.

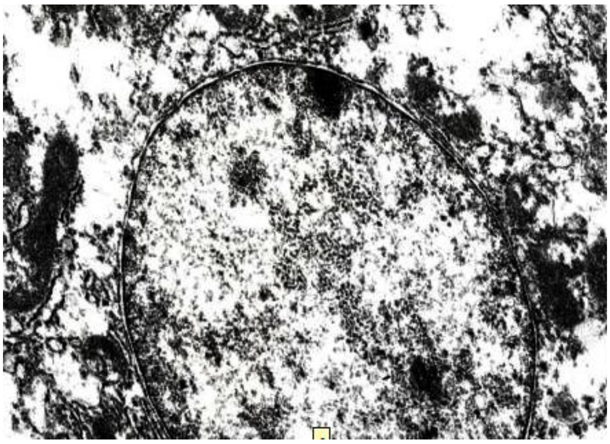


Picture 6 - The accumulation of fibroblasts at the site of vaccine administration. Electron diffraction.X 24 000.

In the spleen of animals immunized with BCG at day 3 of experiment showed an increase in macrophages (Picture 7).

Hepatocytes have clear form. immunized with BCG vaccine on the 3rd and 7th day of the experiment there was an almost complete destruction of the mitochondria.

In the. Electron diffraction of kidney tubule there were stood out most clearly epithelial cells of urea-forming.



Picture7 - Severe destruction of the hepatocytes.Electron diffraction.X 15 000.

Discussion of findings and conclusion Bibliography.

In recent years in commercial dairy farms and farms as well as at the reception LLP working milkmaids and cattlemen to work has decreased passing medical examinations and checks of medical books. Also there are no admission from doctor to work. If animal infests with tuberculosis from person, the 55 percent of checking animals responds to tuberculin [17].

The human and bird type of TB during the development process in the body it caused no change in body. But causative agent is excreted in milk and therefore is dangerous to human. The epidemiological situatuation of tuberculosis in the country is very heavy [18].

If you count the 100 people that in 1991 the incidence of people was 66 people in 2001 amounted to 155.7. And in 2002 the incidence of tuberculosis was 24765 people. 2008 year the incidence is 12500 [19]. The above data does not correspond with the statistical data. In some farms conceal animals reacting positively to a tuberculosis of animals. This should be strictly follow TB control and especially strictly necessary to diagnose animals. Prevention of animal tuberculosis drugs has not lost its relevance as well as the exercise is cost-effective.

Using transmission electron microscopy allowed establishing pronounced ultrastructural changes in the organism of calves immunized with BCG vaccine. They are characterized by: the destruction of the muscle fibers of the dermis and the appearance of necrotic detritus; macrophage reaction followed fibrotization tissue at the injection site; destruction of mitochondria in hepatocytes and epithelial tubules uriniparous; swelling of podocytes in renal vascular glomeruli. and also develop less serious violations: serous edema, partial fragmentation of striated fibers in the tissue at the site of these biologics, swelling of mitochondria with cristae fuzzy pattern in epithelial cells of the liver and kidneys.

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Түйін

Мақалада БЦЖ вакцинасы егілген бұзау ағзасындағы өзгерістердің нәтижесі берілген. Тәжірибеге бір және екі айлық қазақтың ақ бас сиыр бұзаулары қолданылды. Сәулелік электронды микроскоптың көмегімен келесідей ультрақұрылымдық өзгерістерді анықтауға мүмкіндік алдық:

Дерманың бұлшықетті талшықтарының бұзылуы, некротикалық өзгерістердің пайда болуы, егілген жердегі ұлпаның фибриотизацияланған, реакцияланған макрофагтардың реакциясы анықталды. Сонымен қатар, гепатоциттердегі митохондрияның деструкциясы, несеп түзгіш каналдың эпетиальды каналының деструкциясы анықталды. Подоциттердің ісінуі, буйрек клубочектерінің ісінуі, серозды ісінуілер, вакцина егілген жердегі ұлпаның көлденең жолақты талшықтардың бөлшектенуі, кристаларымен бәрге митохондрияның ісінуі және бүйрек пен бауырың эпителиальды жасушалырының анық емес суреті анықталды.

Резюме

В статье представлены результаты исследования на телят после применения вакцины БЦЖ.В эксперименте были использованы телята казахской белоголовой породы. С помощью просвечивающей электронной микроскопии позволило установить выраженные ультраструктурные изменения в организме телят, иммунизированных вакциной БЦЖ. Они характеризуются: разрушением мышечных волокон дермы и появление некротической щебнем; реакция макрофагов с последующим фибротизация ткани в месте инъекции; Разрушение митохондрий в гепатоцитах и эпителиальных канальцев мочеобразующий; отек подоцитах в почечных сосудов клубочков.а также было отмечено не менее серьезные нарушения: серозный отек, частичное дробление полосатых волокон в ткани на месте

иньецированиявакцины, набухание митохондрий с крист, нечетким рисунком в эпителиальных клетках печени и почек.

Summary

The article presents the results of a study on calves after application of BCG vaccine. in the experiment, the calves of the Kazakh white breeds, 1-2 months of age. Using electron microscopy allowed establishing pronounced ultrastructural changes in the organism of calves immunized with BCG vaccine. They are characterized by: the destruction of the muscle fibers of the dermis and the appearance of necrotic detritus; macrophage reaction followed fibrotization tissue at the injection site; destruction of mitochondria in hepatocytes and epithelial tubules uriniparous; swelling of podocytes in renal vascular glomeruli. and also develop less serious violations: serous edema, partial fragmentation of striated fibers in the tissue at the site of these biologics, swelling of mitochondria with cristae fuzzy pattern in epithelial cells of the liver and kidneys.