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Diagnostics of tuberculosis and differentiation of nonspecific tuberculin reactions in animals

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ABSTRACT

Tuberculosis is a serious disease of humans and animals, caused by bacteria of the *Mycobacterium* genus. This leads to complications in the life of the sick person, and subsequently to death. The cattle, who have been diagnosed with this bacterium, are usually sent to the slaughter, with the result that their livestock is reduced. Mycobacteriosis is also a disease, after determining which cattle are most often sent to slaughter. Such a reduction in livestock numbers has a negative effect on the economy. Of the 300 samples from the animals, 25 cultures of atypical bacteria responding to tuberculin were isolated. A series of tests – intravenous tuberculin test (IVTT), ophthalmic test, palpebral test, “ZhAT” test, showed that most of the tuberculosis changes in cattle were found in regional lymph nodes more often than in internal organs. In healthy for tuberculosis cows, at the age of 4–9 years, seasonal nonspecific sensitivity to tuberculin is observed. Implementation of the developed express method of GAT on farms in healthy tuberculosis will speed up the diagnosis of tuberculosis and mycobacteriosis in animals that reacted to tuberculin and will exclude short-term nonspecific sensitization of their organism to tuberculin. The introduction of this methodology can be used to diagnose and clearly differentiate the diagnoses of “tuberculosis” and “mycobacteriosis” in cattle. This will cure part of the livestock and reduce the amount of slaughter.

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Introduction

Timely diagnosis of epidemic diseases is an important aspect of the beef and dairy industry. The speed of the

diagnosis generally affects the possibility of therapy and damage minimization. Anthrozoonic tuberculosis is a serious international problem, since the rate of this disease started growing in the early twenty-first century due to the progressive course that is difficult to treat with complex antibacterial therapy.¹ Multiple-drug resistant strains were often isolated from such patients.

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The isolation rate of such strains was 10% in Japan,² 17% in Canada,³ 24.5% in Russia,⁴ and 23% in Kazakhstan.⁵ Animals that reacted to tuberculin with skin swelling of 3 mm and more are considered having tuberculosis and slaughtered.⁶ When such animals are detected, lifetime and post-slaughter examinations are conducted with a view to ruling out or confirming the "Tuberculosis" diagnosis. Even multiple examinations of cattle with subcutaneous allergy tests (SAT) are insufficiently informative (the effectiveness is 54.2%).⁷ If no infectious agents of tuberculosis were isolated from the post-slaughter material of animals that reacted to tuberculin, this indicated a nonspecific nature of tuberculin reactions.⁸ This causes economic damage to the development of animal husbandry due to the unjustified slaughter of apparently healthy, often pedigree and highly productive, cattle and unjustified anti-tuberculosis sanitary measures.⁶

Such reactions are often associated with the sensitization of animals to infectious agents of tuberculosis avian and atypical mycobacteria, which can cause a certain immunobiological reorganization of the allergic reactions of the macroorganism.⁹ Sanitary and hygienic conditions of animal management facilitate the penetration into the organism and reproduction of potentially pathogenic mycobacteria when said mycobacteria become capable of developing and growing excessively in a weakened animal organism.¹⁰

The assumption is that nonspecific reactions in cattle reacting to tuberculin are caused by the similarities between the antigenic structure of tuberculosis infectious agents and non-tuberculosis acid-fast cultures.¹¹ Some of their species have at least one common antigen, while others – two and more. It was found that out of 20 antigens of *Mycobacterium bovis* that are immunodominant for cattle, at least 16–17 are common antigens of atypical mycobacteria.¹²

Nonspecific tuberculin reactions in animals can also be caused by stress agents, purulent and necrotic processes, and even antigens of necrosis germs and actinomycetes.¹³ Lifetime differentiation of nonspecific tuberculin reactions requires palpebral tests with tuberculin mammalian.¹⁴ The effectiveness of simultaneous subcutaneous and palpebral injection of tuberculin was determined on animals that were artificially infected with virulent cultures of mycobacteria, animals with tuberculosis, and animals immunized with the BCG vaccine.¹⁵ At that, positive reactions to this test were found only in the group of animals that were infected with agents of bovine or human tuberculosis.

An additional intravenous test with undiluted or diluted (50% of concentration) tuberculin is used to select animals that react to tuberculin for diagnostic slaughter. All the above measures require a lot of time and effort.¹⁶

Thus, the emergence of nonspecific reactions to tuberculin mammalian in healthy or recovering from tuberculosis cattle requires extended research.

In order to expand the matter at hand, this research developed an accelerated (within a week) technique for diagnosing or ruling out tuberculosis and differentiating nonspecific reactions in animals by administering a complex allergy depressant for four days to animals that react to tuberculin, followed by re-examination with simultaneous subcutaneous and palpebral tuberculin tests. This express technique, called

"ZhAT", aims to identify specific and nonspecific tuberculin reactions. The prerequisite for the development of this express method was the offered "Booster Effect",¹⁷ which implies only the re-examination of animals reacting to tuberculin after seven days without using the allergy depressant.

The purpose of this research is to improve the differentiated diagnostics of tuberculosis and mycobacteriosis in animals, to analyze the epizootic situation in terms of tuberculosis in the Republic of Kazakhstan, to determine the role of atypical mycobacteria in animal pathology and manifestation of parasitocenosis in cattle reacting to tuberculin, to develop and improve the methods of lifetime differentiated diagnostics of tuberculosis and mycobacteriosis in animals, and to implement the "ZhAT" express technique for lifetime differentiated diagnostics of tuberculosis and mycobacteriosis in cattle.

Methods

Ethics statement

This research followed the international recommendation for experiments involving animals.¹⁸ The research was approved by Minutes No. 45 of the meeting of the Bioethics Committee of the Kazakh National Agrarian University dated 24.04.2012.

Examined animals

The examination of animals was carried out in 2012–2014 at the Baiserke Agro and Plemzavod Almaty farms (Almaty Region, Kazakhstan). The experimental study used 18,303 cattle units to confirm the tuberculosis diagnosis.

Bacteriological observation

A total of 300 bacteriological observations were conducted. The inoculation on the Lowenstein-Jensen medium in 2012–2014 isolated 25 cultures of atypical mycobacteria of groups II, III, and IV¹⁹ from 300 test samples of pathologic material from cattle reacting to tuberculin.

Intravenous tuberculin test (IVTT)

IVTT was conducted in accordance with the standard method.²⁰ It implies an intravenous administration into the jugular vein of 50% diluted solution of tuberculin mammalian at 1 mL per 100 kg of live weight, but not more than 4 cm³ per one unit, and the measurement of body temperature at the moment of administration and in three, six, nine, and 12 h, and then according to the improved method – undiluted tuberculin.

Ophthalmic test

The double ophthalmic tuberculin test was carried out in accordance with²¹ and according to improved methods that involve a shorter interval of three days and a simplified and accurate reaction six and nine hours after the diagnostic agent was administered.

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