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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, 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 glutaraldehyde test 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. Anthropozoonotic 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.

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.

Palpebral test

The palpebral tuberculin test was conducted on cattle reacting to SAT in accordance with the appropriate method (Ovdiyenko, Nuritinov, and Naymanov 1987).

“ZhAT” test

In order to achieve the estimated effect and eliminate negative aspects, an allergy depressant was administered for four days, which involved intravenous (slow) injection of 100 cm³ of 20% Calcium Borogluconate solution heated to 37 °C. A single intramuscular injection of 5 cm³ of 20% Nitamin solution was made to enhance the allergy depressant effect. In addition, 2 cm³ of caffeine 20% solution was administered. Subcutaneous and palpebral tuberculin tests were done simultaneously after four days with reaction measurement after 72 h. The comparison of the repeat readings of the subcutaneous reaction with the initial and additional assessment of the palpebral test result showed the specific or nonspecific nature of tuberculin reactions in cattle that originally reacted to tuberculin. The statistical analysis was carried out using the EXCEL program (Microsoft, USA).

Results

The developed ZhAT express technique was implemented in the Talgar and Ile Districts (Almaty Region, Kazakhstan) since 2012 to determine the actual epizootic situation in terms of cattle tuberculosis in three farms that were healthy in terms of said infection. The allergy study at the first farm (Baiserke Agro) was conducted on 2076 black-and-white and white-headed cattle units; it detected 15 units (0.7%) that reacted to tuberculin with allergic reactions of up to 13 mm.

The ZhAT express technique was used on these isolated cows with subsequent subcutaneous and palpebral tuberculin tests.

The measurement of the reaction to these tests after 72 h found no subcutaneous reaction in four cows (26.7%), reduced reaction by 3 mm or more in 11 cows (73.3%) in the presence of a questionable reaction of one and two pluses to the palpebral test (Fig. 1).

Of the latter, two cows (IN454 and IN1058) were slaughtered. Typical tuberculosis alterations were not found in their internal organs (lungs, liver, spleen, and kidneys) and regional (prescapular, external inguinal, supramammary, mesenteric,

mandibular, retropharyngeal, bronchial, and mediastinal) lymph nodes. Besides echinococcosis of the lungs and liver, mycobacterial alterations were found in the retropharyngeal, bronchial, mediastinal, and external inguinal lymph nodes in the form of severe hyperplasia with striate hemorrhage (with pus particles). In addition, purulent hemorrhagic endometritis caused by abortion was diagnosed in IN458; limb mastitis and necrobacteriosis with inguinal lymph node hypertrophy was diagnosed in IN1058.

The bacteriological examination of the biomaterial from these cows in terms of tuberculosis yielded negative results. At the same time, group II and IV (according to Runyon) atypical mycobacteria were isolated.

At the second farm (Plemzavod Almaty, Kazakhstan), the allergy test of 2432 Alatau cattle units detected 17 cows (0.7%) that reacted to tuberculin (reaction size ranged from 3 mm to 13 mm). After the cows were isolated, the ZhAT express technique was used on them (Fig. 2).

The simultaneous reading of reactions after 72 h found negative reactions to these tests in nine cows (52.9%), 3–5 mm weaker reactions in eight cows (47.1%), and unclear reactions to the palpebral test (with a four-plus assessment system) in the form of mild conjunctiva swelling and hyperthermia with low mucous flux from the angulus oculi (++ and +).

Intravenous tests with 3 cm³ of undiluted tuberculin of the same lot and reading after 6–9 and 12 h found a 0.8 °C and higher temperature rise, but not higher than the maximum normal level (39.5 °C), in the latter seven cows, two of which (IN8626 and IN7195) were slaughtered for examination. The autopsy did not find alterations typical for tuberculosis in internal organs (lungs, liver, spleen, and kidneys) and lymph nodes. At the same time, besides echinococcosis of the lungs and liver, nodes with blood and pus without “caseation” in lungs and severe hyperplasia with striate hemorrhage in regional retropharyngeal, bronchial, mediastinal, supramammary, and inguinal lymph nodes were found in five cases. In addition, purulent hemorrhagic endometritis and keratoconjunctivitis sinister was diagnosed in IN8626; suppurative mastitis was diagnosed in IN7195. The inoculation of the 24 samples of biomaterial from these cows isolated group II, III, and IV (according to Runyon) atypical mycobacteria.

Similar studies were conducted in 2012–2014, with a view to implementing the developed express technique for lifetime differentiation of tuberculosis diagnostics and mycobacteriosis in cattle. The results of the differentiated and diagnostic studies of cattle over the course of three years are presented in Fig. 3.

The figure shows that 155 cattle units (1.2%) reacting to tuberculin were detected out of the 13,252 cattle units tested for tuberculosis during the implementation of the developed technique (2012–2014). After these units were isolated, the ZhAT express technique was used for four days, followed by simultaneous subcutaneous and palpebral tuberculin tests. No positive reactions were found during the reading of the tests after 72 h after the administration of tuberculin. At the same time, so-called unclear reactions (+ and ++) to the palpebral test were found in five cows and retained the original intensity of the reaction and in 95 cows that had a weaker reaction.

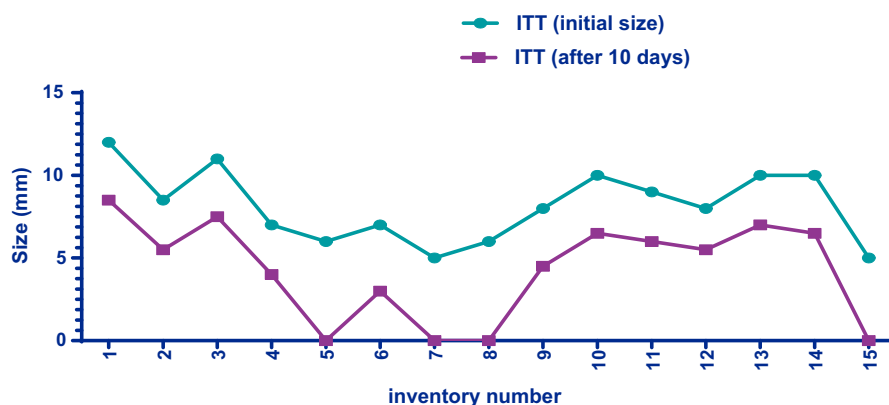


Fig. 1 – Results of the complex allergy depressant used on 15 cows (Baiserke Agro). Statistical analysis: Mean = 8.166; standard deviation = 2.168 – for initial size: Mean = 5.86; standard deviation = 1.598 – after 10 days.

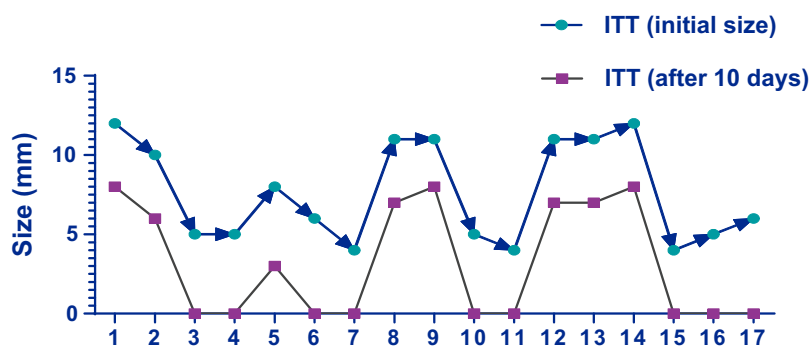


Fig. 2 – Results of simultaneous subcutaneous and palpebral tuberculin tests after using the allergy depressant in 17 cows. Statistical analysis: Mean = 7.64; standard deviation = 3.18 – for initial size; Mean = 6.75; standard deviation = 1.66 – after 10 days.

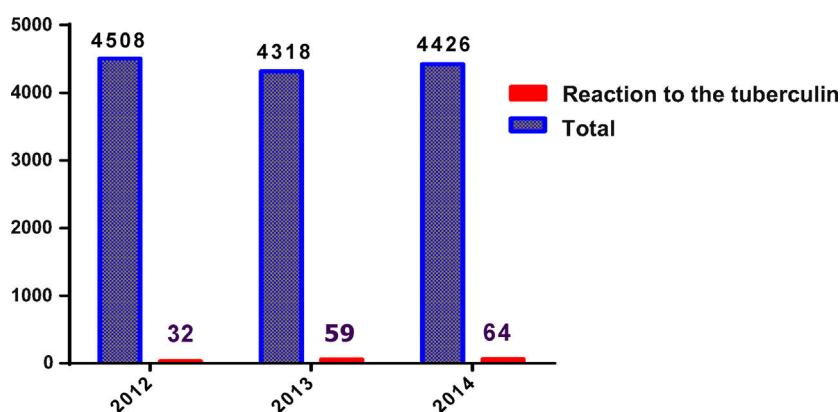


Fig. 3 – Results of re-examination of cattle reacting to subcutaneous tuberculin tests after seven days of administering the allergy depressant in 2012–2014. Statistical analysis:

Year	Mean	Standart deviation
2011	16	1414
2012	32	4242
2013	32	14,142
2014	77.5	23,334

The single test of these animals (100 units) with undiluted tuberculin was negative after 6–9 and 12 h. At the same time, an unclear reaction in the form of a 0.8 °C and higher temperature rise, but not higher than the maximum normal level (39.5 °C), was found in 21 cows with reduced reaction size with SAT and + or ++ reaction to the palpebral test; 15 of these units were slaughtered for examination. The autopsy did not find alterations typical for tuberculosis. Besides echinococcosis of the lungs and liver and pancreatic eurytrematosis, mycobacterial alterations were found in the upper lobes of lungs with blood and pus particles and in regional lymph nodes (prescapular, inguinal, mandibular, retropharyngeal, bronchial, mediastinal, supramammary, and mesenteric) in the form of hyperplasia with petechial or striate hemorrhage. In addition, hemorrhagic or purulent hemorrhagic endometritis, mastitis, hepatitis, nephritis, enteritis (most often in the ileocolic junction), and necrotic foci in certain organs were diagnosed in most cases.

The simultaneous bacteriological examination of the biomaterial from these cows in terms of tuberculosis yielded negative results. At the same time, group II, III, and IV (according to Runyon) atypical mycobacteria were isolated.

The allergy test of 1007 Alatau and Holstein Friesian cattle units in 2013 detected 29 cows (2.4%) that reacted to the subcutaneous tuberculin test. The re-examination in seven days with simultaneous subcutaneous and palpebral tuberculin tests for four days, the ZhAT test found no reaction to both tests in 16 cows (53.2%) and weaker subcutaneous tuberculin reaction with an unclear diagnosis of the palpebral test in 13 cows (46.8%); two of the latter cows (IN5098 and IN7089) were slaughtered for examination.

The autopsy did not find alterations typical for tuberculosis. Besides echinococcosis of the lungs and liver and pancreatic eurytrematosis, mycobacterial alterations (with blood and pus) were found in regional lymph nodes (retropharyngeal, bronchial, mediastinal, mesenteric, inguinal in one unit and supramammary in the second unit) in the form of hyperplasia with petechial or striate hemorrhage. In addition, purulent hemorrhagic endometritis, mastitis, and enteritis was diagnosed. The simultaneous bacteriological examination of the biomaterial from these cows in terms of tuberculosis yielded negative results. At the same time, group III (according to Runyon) “avian” atypical mycobacteria were isolated.

Out of the 46 and 29 cows that reacted to tuberculin, which were detected in 2013 and 2014, respectively: loss of reaction was found in 17 and 11 cows, respectively; reduction of reaction intensiveness with an unclear result of the palpebral test was found in 29 and 11 cows, respectively. Four latter cows (IN2097, 7089; 0347 and 9014) were slaughtered for examination; alterations that were found in said cows were similar to those in cows that were slaughtered in 2014.

The bacteriological examination of the biomaterial from these cows in terms of tuberculosis yielded negative results. At the same time, group III (according to Runyon) non-photochromogenic atypical mycobacteria were isolated, which was a prerequisite for the simultaneous test with tuberculin mammalian and avian on all cows at respective farms in 2014.

In 2013, parallel tests with tuberculin mammalian and avian were conducted on 543 cows to determine the specific weight of nonspecific tuberculin reactions in cattle caused by non-photochromogenic atypical mycobacteria. The tests used mammalian and avian tuberculin PPD (Biovet, Kazakhstan). The tests detected 30 cows (5.5%) that reacted to tuberculin mammalian and avian. The size of reactions ranged from 3 to 11 mm to tuberculin mammalian and reached 17 mm to tuberculin avian. The size of reactions to tuberculin avian in 24 cows were 2 mm larger than those to tuberculin mammalian; in six cows, the reactions to both allergens were identical.

Two cows (IN0347 and IN9014) with the most intensive reactions to tuberculin mammalian (10 and 8 mm) and tuberculin avian (15 and 11 mm), the alterations wherein were similar to those in the seven cows slaughtered in previous years, were slaughtered for examination. The bacteriological examination of the biomaterial from these cows in terms of tuberculosis yielded negative results. At the same time, group III (according to Runyon) non-photochromogenic atypical mycobacteria were isolated.

The above results of the autopsy and bacteriological examination of biomaterial were indicative of a sensitization of the cattle's organism by AM, which caused nonspecific tuberculin reactions in 5.5% of cases.

Discussion

Postmortem diagnosis based on examination of gross lesions, followed by histopathology and culture, is widely used for surveillance of bTB in wild animals, but this method is time-consuming.²²

The intradermal tuberculin test has been in use for almost a century and, despite the technological advances of the last two decades, is still the only prescribed test for the diagnosis of tuberculosis in cattle.²³ The significant error of this test is related to several factors, such as allergic reaction or persistence of related mycobacteria in the organism, which do not cause complications in animals. Reassessment of the state of animals with suspected mycobacterial contamination should provide as accurate a diagnosis as possible, confirm or refute a false-positive result, with regard to the factors that could distort the result. TST can also cause false-negative reactions due to immunosuppression, desensitization toward tuberculin, subpotent use of tuberculin, and lengthy exposure to a field strain.²⁴

Thus, the effectiveness of tuberculin skin testing ranges from 75.0% to 95.5%.²⁵

SICTT with specific antigens, such as a cocktail of ESAT-6/85B²⁶ and ESAT-6/CFP-10,⁸ are used to improve the specificity of reactions. However, these studies still do not offer a solution for commercial use at large farms. At the current stage, these methods, alongside flow cytometry analysis of the IFN- γ system, remain only at the level of laboratory studies.

The implementation of the ZhAT express technique, which involves the administration of a complex allergy depressant with subsequent re-examination on day 7–10 and simultaneous subcutaneous and palpebral tuberculin

tests, at three farms (Baiserke, Plemzavod-Almaty, and Mezhdurechensk-Agro) in the Almaty Region, identified non-specific tuberculin reactions in 279 cattle units. Follow-up tests on 100 cattle units with persistent tuberculin reactions and unclear results of palpebral tests using a single intravenous test with undiluted tuberculin and simultaneous test with tuberculin mammalian and avian, results of the veterinary and sanitary examination of the internal organs and regional lymph nodes of animals that were slaughtered for examination, and the bacteriological examination of the biomaterial from said cattle units conclusively ruled out the tuberculosis diagnosis and diagnosed mycobacteriosis. This prevented premature slaughtering of 258 units of pedigree (Alatau and Holstein Friesian), mostly highly productive, cattle.

An express technique for accelerated lifetime differentiated diagnostics of animal tuberculosis and mycobacteriosis was developed; this technique accelerates the lifetime differentiated diagnosis of tuberculosis and mycobacteriosis in animals that react to tuberculin, rules out the short-term nonspecific sensitization of their organism toward tuberculin mammalian, and improves the specificity of the subcutaneous tuberculin test using a complex allergy depressant and a simultaneous subcutaneous and palpebral tuberculin test.

The scientific novelty is confirmed by patent “Means of Improving the Specificity of the Subcutaneous Tuberculin Reaction Using a Complex Allergy Depressant for Accelerated Lifetime Differentiated Diagnostics of Animal Tuberculosis and Mycobacteriosis” No. 2010/0926.1 dated 14.07.2014.

Conclusion

A dependence of the isolation rate and species of acid-fast cultures of pathogenic and potentially pathogenic mycobacteria on the nature of internal organ and lymph node lesion in cattle reacting to tuberculin was found.

Specific tuberculosis changes in cattle were found in regional lymph nodes more often than in internal organs.

Seasonal emergence of nonspecific tuberculin sensitivity in cows aged 4–9 years was found in Almaty Region farms that were healthy in terms of tuberculosis.

The implementation of the developed ZhAT express technique at Almaty Region farms that were healthy in terms of tuberculosis accelerated the lifetime differentiated diagnostics of tuberculosis and mycobacteriosis in animals that reacted to tuberculin and ruled out the short-term nonspecific sensitization of their organism toward tuberculin.

The implementation of this technique at Almaty Region farms excluded the “Tuberculosis” diagnosis and diagnosed “Mycobacteriosis”; this prevented premature slaughtering of 258 units of pedigree (Alatau and Holstein Friesian), mostly highly productive, cattle.

The developed ZhAT technique is implemented at farms in the Republic of Kazakhstan, which have stationary isolation facilities and detect animals that react to tuberculin, as well as at healthy farms that experience cases of recurrent outbreaks of the disease or retain the threat thereof.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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