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More on search for causes of sensitization to tuberculin PPD for mammals in cattle

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SUMMARY

Despite the large number of papers dealing with the description of proposed methods for bovine tuberculosis diagnosis and mechanisms of non-specific reaction development in diseased and healthy animals, various aspects require further study. Many specialists are still of the view, formulated when studying causes of pseudoallergic reactions, that the agents of actinomycosis, trematode infections etc. can cause sensitization of the animal body to tuberculin PPD for mammals. The possibility of sensitization of cattle body to *Actinomyces bovis* tuberculin was studied in 240 animals identified as actinomycosis diseased among 3,473 tested animals. Only 11 (4.6%) of the total number of diseased animals were reactors to tuberculin PPD for mammals. During bacteriological tests of material from animals euthanized for diagnostic purposes (tuberculin reactors and nonreactors with a confirmed postmortem diagnosis of actinomycosis), acid-tolerant nontuberculous mycobacterium (NTM) cultures were isolated. The results of the experiment conducted in 628 cows of a dairy holding confirmed that *Actinomyces bovis* lacks tuberculin-associated allergenicity: actinomycosis was detected only in one of 96 (15.2%) tuberculin reactors. The conducted clinical tests with high significance level ($P < 0.005$) showed that there is no association between allergic reaction to tuberculin PPD for mammals and actinomycosis infection. The obtained results are indicative of imperfections in differential diagnosis, and further studies in this field should significantly contribute to gaining a better insight into non-specific sensitization of cattle body to tuberculin.

Keywords: tuberculosis, actinomycosis, sensitization, diagnosis, differentiation, parallergy, pseudoallergic reactions, mycobacteria, atypical acid-tolerant mycobacteria

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К поиску причин сенсибилизации крупного рогатого скота к ППД-туберкулину для млекопитающих

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РЕЗЮМЕ

Несмотря на большое количество работ, посвященных характеристике предложенных методов диагностики туберкулеза крупного рогатого скота и механизмам проявления неспецифических реакций у больных и здоровых животных, многие аспекты этой проблемы требуют дополнительного изучения. До настоящего времени многие специалисты придерживаются точки зрения, сформулированной при изучении причин псевдоаллергических реакций, согласно которой возбудители актиномикозной инфекции, трематодозной инвазии и др. могут быть причиной сенсибилизации макроорганизма животных к ППД-туберкулину для млекопитающих. Возможность сенсибилизации организма крупного рогатого скота к туберкулину *Actinomyces bovis* изучали

на 240 больных актиномикозом животных из 3473 исследованных. Из числа больных только 11 голов (4,6%) реагировали на ППД-туберкулин для млекопитающих. При бактериологическом исследовании материала от убитых с диагностической целью животных (реагировавших и не реагировавших на туберкулин с подтвержденным на актиномикоз патолого-анатомическим диагнозом) изолированы культуры кислотоустойчивых нетуберкулезных микобактерий. Отсутствие аллергизирующих к туберкулину свойств у *Actinomyces bovis* было подтверждено результатами эксперимента, проведенного на 628 животных одного из молочных комплексов, где только у одной из 96 (15,2%) реагирующих на туберкулин коров выявили актиномикоз. Проведенные клинические исследования с высокой степенью достоверности ($P < 0,005$) позволили установить отсутствие взаимосвязи между аллергией на ППД-туберкулин для млекопитающих и актиномикозным инфекционным процессом. Полученные результаты свидетельствуют о несовершенстве дифференциальной диагностики, и дальнейшие исследования в этой области должны внести существенный вклад в развитие представлений о неспецифической сенсибилизации организма крупного рогатого скота к туберкулину.

Ключевые слова: туберкулез, актиномикоз, сенсибилизация, диагностика, дифференциация, парааллергия, псевдоаллергические реакции, микобактерии, атипичные кислотоустойчивые микобактерии

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INTRODUCTION

Non-specific reactions to tuberculin PPD for mammals constitute an issue that affects animal husbandry worldwide and has not been fully resolved despite the numerous differentiation methods proposed. According to official statistics, including those from the World Organisation for Animal Health, at present, tuberculin reactors make up about 54% of tested animals globally, in the CIS member states – from 12.6 to 62.4%, in the Subjects of the Russian Federation – from 7.8 to 49.3% [1–4].

Amongst the variety of microbial biota causing sensitization of the animal body to tuberculin, atypical acid-tolerant mycobacteria of groups II, III and IV (according to the classification of E. Runyon) are most significant as regards the number and levels of the caused skin reactions. Acid resistant saprophytes can also play a significant role in the allergization of the body [3, 5–10].

According to current conceptions, microoganisms that are closely related to mycobacteria, namely corynebacteria, *Nocardia* and rhodococci, also have tuberculin sensitizing properties. Their essential characteristics are genus and species specificity to mycobacteria, as well as common group specific antigens detected when cross reactivity occurs. Interest in these microorganisms has at present expanded due to their more frequent isolation from the biomaterial from tuberculin reactive animals. Experimental and clinical data show that corynebacterium cultures are isolated in 28.6% of cases [6, 11, 12], *Nocardia* cultures – in 16.9% of cases [12], and rhodococci – in 26.3% of cases [11, 13–15].

There is no doubt that parallergies hold a key position among the causes of non-specific sensitization of the animal body. At the same time, characteristic reactions can be attributable to other causes not associated with mycobacteria, various purulent processes caused by transient microorganisms, parasitic diseases (trematode infections), stress factors, protein load [3, 4, 9, 16].

Allergy is also attributed to different pregnancy pathologies, animal diets with an unbalanced proportion of micro- and macronutrients, feeding with mold fungus infected feedstuffs, etc. [16–18].

However, the further studies of allergic reactions to tuberculin in animals affected with trichophytosis, dicroceliosis, paramphistomatosis, echinococcosis did not reveal any correlation. No association is found between sensitization to tuberculin and stress factors, in particular in cows in the antepartum and postpartum periods [19–21].

Convincing experimental evidence was provided with respect to the absence of allergy in animals receiving urea [16, 22].

The comparison between the results of coprological tests of samples collected from fascioliasis affected animals and intradermal tuberculin test results yielded mutually exclusive data. Tuberculin reactors made up 5.8% of the tested fascioliasis affected animals, the coprological test results were negative in 22% of tuberculin reactive animals [5, 7].

The interest in examining the possibility of animal body sensitization to tuberculin by microorganisms having common genus specific characteristics with mycobacteria, in particular A*ctinomyces bovis* of the genus *Actinomyces* of the order *Actinomycetales* with characteristic high population genetic homogeneity with the genus *Mycobacterium*, has at present expanded [2, 3, 11, 16, 18, 23].

Many specialists are currently of the view, formulated when studying the genetic characteristics of these microorganisms, that evolutionary commonality and possible presence of common antigens can be responsible for sensitization to tuberculin [24, 25].

Table 1
Distribution of tuberculin reactor animals by actynomycosis occurrence level

No.	Farm	Number of tested animals	Intradermal tuberculin test reactors		Actinomycosis diseased cattle		Out of these			
							tuberculin reactors		tuberculin nonreactors	
			number of animals	%	number of animals	%	number of animals	%	number of animals	%
1	SPK "Ordzhonikidze"	740	–	–	43	5.8	–	–	43	100.0
2	SPK "Lenina"	859	78	9.1	45	5.2	2	4.4	43	95.6
3	KFKh "Paraulsky"	300	8	2.7	19	6.3	1	5.3	18	94.7
4	SPK "Buynaksky"	500	66	13.2	69	13.8	5	7.2	64	92.8
5	SPK "Gelinsky"	300	48	16.0	59	19.7	3	5.1	56	94.9
6	KFKh "Rassvet"	543	–	–	4	0.7	–	–	4	100.0
7	KFKh "Tavrida"	231	–	–	1	0.4	–	–	1	100.0
	Total	3,473	232	6.7	240	6.9	11	4.6	229	95.4

According to the data from some clinical studies, actinomycosis diseased animals are reactive to tuberculin for mammals. However, no experimentally proven and laboratory-confirmed study results have been found.

The purpose of the study was to substantiate experimentally a possible association between reactions to tuberculin tests and actinomycosis infection.

MATERIALS AND METHODS

Clinical tests were carried out in 3,473 cattle of different age and sex groups on seven farms of the Karabudakhkentsky and Novolaksky Raions of the Republic of Dagestan.

Tests for assessment of tuberculin sensitizing properties of *Actinomyces bovis* were carried out in 628 cattle at a dairy complex located in the Babayurtovsky Raion of the Republic of Dagestan.

Allergy tests of cattle were carried out in accordance with the "Veterinary rules for implementation of preventive, diagnostic, restrictive and other measures, imposition and lifting of quarantine and other restrictions aimed to prevent the spread and eradicate the outbreaks of tuberculosis" approved by Order of the Ministry of Agriculture of the Russian Federation No. 534 of 08 September 2020[1].

The identification of the isolated mycobacterium cultures was performed according to GOST 26072-89 "Agricultural animals and poultry. Methods of laboratory diagnostics of tuberculosis"[2] and GOST 27318-87 "Agricultural animals. Methods of identification of atypical mycobacteria"[3].

The animals were subjected to clinical examination for actinomycosis, including palpation of the affected area; laboratory tests of tissues collected from the infected areas were carried out. The diagnosis was considered to be confirmed only when *Actinomyces bovis* was isolated from the material. In case of detection of neoplasms of unknown etiology, histological analysis was performed in accordance with the generally accepted procedures.

The obtained data were statistically processed using parametric techniques; statistical significance of the results was determined with Student's test.

TEST RESULTS

Allergy tests carried out on the farms revealed non-specific sensitization of animals to tuberculin PPD for mammals. The percentage of reactor animals varied from 2.7 to 16.0%. Actinomycosis diseased animals made up from 0.4 to 19.7% (Table 1).

The tests showed that 232 (6.7%) out of 3,473 animals reacted to intradermal tuberculin tests, the number of actinomycosis diseased animals amounted to 240 (6.9%).

Among actinomycosis diseased cattle, only 11 animals (4.6%) tested tuberculin PPD positive, 229 animals (95.4%) were nonreactors.

On the farm SPK "Ordzhonikidze", only 43 animals (5.8%) out of 740 tested cattle were actinomycosis diseased, no tuberculin reactors were detected. Similar results were obtained for the small scale farms KFKh "Rassvet" and KFKh "Tavrida" located in the Karabudakhkentsky Raion.

The following actinomycosis diseased animals were euthanized and subjected to postmortem examination: 11 tuberculin reactors and 10 animals with negative allergy test results (Table 2).

As a result of the tests performed, *Mycobacterium scrofulaceum* culture was isolated from the biomaterial from tuberculin positive animals, 7 strains were identified (63.6%). This indicates that the sensitization of the body was caused

[1] https://docs.cntd.ru/document/565721619.
[2] https://docs.cntd.ru/document/1200025492.
[3] https://base.garant.ru/5917269.

Table 2
The results of postmortem examination and bacteriological tests of biomaterial from actinomycosis diseased animals

Actinomycosis diseased animals	Number of animals	Post-mortem diagnosis	Bacteriological test results
Tuberculin reactors	11	actinomycosis	7 *Mycobacterium scrofulaceum* strains were isolated
Tuberculin nonreactors	10	actinomycosis	2 *Mycobacterium scrofulaceum* strains and 1 *Mycobacterium phlei* strain were isolated

by atypical mycobacteria. The true cause of sensitization was not identified for 4 animals, and this is indirectly indicative of imperfections of the proposed methods for tuberculosis diagnosis in animals, in this particular case – of laboratory methods. This suggests that sensitization can be due to the presence of hydrocarbon oxidizing microorganisms (corynebacteria, *Nocardia*, rhodococci, etc.) in the cattle body, the isolation of which requires specific test procedures.

On the other hand, the isolation of two *Mycobacterium scrofulaceum* strains and one strain of saprophytic mycobacteria (*Mycobacterium phlei*) from the biomaterial samples from animals with negative allergy test results should be considered as confirmatory evidence for the existing view that atypical mycobacteria are not always able to cause the sensitization of the animal body to tuberculin.

Extensive literature data, as well as the results of the study show that acid-tolerant nontuberculous mycobacteria are able to persist in the body latently, without manifesting themselves in any way, for long periods of time; this explains negative laboratory test results in 8 of 10 animals.

Tests for assessment of tuberculin sensitizing properties of *Actinomyces bovis* were carried out in 628 cattle at a dairy complex located in the Babayurtovsky Raion of the Republic of Dagestan. Among the tested cattle, 96 animals (15.2%) were tuberculin reactors, no actinomycosis diseased animals were detected.

Based on the comparison of clinical signs and data from the laboratory tests of animals for actinomycosis with allergy test results, it can be concluded that the obtained results show with high significance level ($P < 0.005$) that there is no correlation between actinomycosis infection and allergic reactions to tuberculin.

DISCUSSION AND CONCLUSION

The test results are consistent with extensive literature data on the studies of specificity of tuberculin PPD for mammals that demonstrate apparent specificity of the allergen towards homologous sensitization.

Less intense in manifestation, short term reactions to tuberculin are reported in animals infected with acid-tolerant nontuberculous mycobacteria (NTM), as well as with mycobacterium-like (hydrocarbon oxidizing) microorganisms (corynebacteria, *Nocardia*, rhodococci, etc.).

According to the statistics available, cross-reactions are often detected, and this is indicative of the high degree of the structural homogeneity of antigens, hence the need for using more effective differential diagnosis methods (postmortem examination, bacteriological testing).

It is important to note that, in some cases, negative laboratory test results call into question the effectiveness of the diagnostic method applied, since mycobacteria are characterized by continuous variation (in the opinion of the majority of researchers, owing to antigen drift), which results in new serological pathogenic variants.

In view of this, as well as taking into account culture method imperfections, a complex of diagnostic methods, in particular molecular genetic techniques (ELISA, PCR, etc.), should be used for isolation of commonly occurring transformed forms of mycobacteria (L-forms, spheroplasts, protoplasts, filterable forms, etc.), as well as for detection of latently persistent forms.

In the light of the obtained unequivocal evidence presented in this paper, when identifying the causes of pseudoallergic reactions, one should give due consideration to the fact that actinomycosis and parasitic infections are characterized by mixed clinical signs. In particular, atypical mycobacteria (*Mycobacterium* subsp.) and mycobacterium-like microorganisms (*Corynebacterium*, *Nocardia* and *Rhodococcus* subsp.), being typical representatives of gastrointestinal microbiota, can cause sensitization of the body to tuberculin in case of immunity status decline in animals.

The results of the study are consistent with the previously obtained data on identification of association between reactions to tuberculin and invasive diseases (trichophytosis, echinococcosis, dicroceliosis, fascioliasis, etc.) that show the absence of statistically significant correlation between them.

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