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Problems and prospects of bovine tuberculosis serological diagnosis

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SUMMARY
For the purposes of tuberculosis eradication on any tuberculosis-infected farm, it is necessary to identify tuberculin anergic animals, being a potential source of the infection. The purpose of this study was to analyze the role of complement fixing and haemagglutinating antibodies for the detection cattle infected with bovine tuberculosis (TB). 977 cattle of different sex and age groups on two tuberculosis-infected farms were tested thrice over time. After 35 days all tuberculin reactive cattle (132 animals; 13.5%) were subjected to complex testing using allergy and serology methods. After 40 days (Stage 3), animals demonstrating apparent specific antibody activity and low cell immunity were tested. Allergy tests were proved to be non-informative to diagnose tuberculosis on infected farms. Complement fixing and haemagglutinating antibodies were found to be active in tuberculin anergic animals. A higher antigenicity of Ukrainian RIEVM TB antigen complex as compared to Siberian RVI one was revealed by complement fixation test as well as by indirect haemagglutination test using VIEV polysaccharide antigen; the detection rate was 68 (7.0%), 28 (2.9%) and 299 (30.6%) respectively. The correlation between seropositivity and immunoreactivity was not established. Animals, positive in complement fixation and indirect haemagglutination tests, did not react to tuberculin. Nineteen out of twenty tuberculin reactive animals showed post-mortem lesions, consistent with their seropositivity during post-mortem inspection; moreover, the post-mortem lesions of animals, positive in complement fixation test using Siberian RVI antigen, were consistent in all cases. The results obtained suggest a high performance of allergy test and serological test combination and a promising potential of their complex use for tuberculosis diagnosis in cattle.

Keywords: Tuberculosis, serological tests, allergen, anergy, antibodies, cattle, allergy test, post-mortem tests.

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Проблемы и перспективы серологической диагностики туберкулеза крупного рогатого скота

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РЕЗЮМЕ
При оздоровлении каждого неблагополучного по туберкулезу хозяйствующего субъекта необходимо выявлять анергичных к туберкулину животных, являющихся потенциальным источником инфекции. Целью настоящего исследования было изучение роли комплементсвязывающих и гемагглютинирующих антител при выявлении больного туберкулезом крупного рогатого скота. В двух неблагополучных по туберкулезу хозяйствах исследовано трехкратно в динамике 977 голов скота разных половозрастных групп. Всех реагирующих на туберкулин животных (132 головы; 13,5%) через 35 дней подвергли комплексному исследованию с использованием аллергических и серологических методов. На третьем этапе с интервалом 40 дней исследовали животных с выраженной специфической активностью антител и низким функциональным состоянием клеточного иммунитета. Показана низкая информативность аллергических методов диагностики туберкулеза в неблагополучных по заболеванию хозяйствах. Установлена активность комплементсвязывающих и гемагглютинирующих антител в организме анергичных к туберкулину животных. Более высокая антигенная активность выявлена в реакции связывания комплемента у комплексного туберкулезного антигена Украинского НИИЭВ в сравнении с антигеном Сибирского НИВИ, а также в реакции непрямой гемагглютинации с полисахаридным антигеном ВИЭВ, показатель выявляемости при этом составил 68 (7,0%), 28 (2,9%) и 299 (30,6%) случаев соответственно. Коррелятивной связи между серопозитивностью и иммунореактивностью обнаружить не удалось. Животные с положительными показаниями в реакции связывания комплемента и реакции непрямой гемагглютинации не реагировали на туберкулин. При послеубойном осмотре

у 19 из 20 не реагировавших на туберкулин коров патолого-анатомические изменения совпадали с серопозитивностью, причем у животных с положительными показаниями в реакции связывания комплемента с антигеном Сибирского НИВИ – во всех случаях. Полученные результаты свидетельствуют о высокой результативности комбинации аллергического теста с серологическими методами и перспективности их сочетанного использования для диагностики туберкулеза крупного рогатого скота.

Ключевые слова: Туберкулез, серологические реакции, аллерген, анергия, антитела, крупный рогатый скот, аллергический метод, патолого-анатомические исследования.

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INTRODUCTION

In tuberculosis-infected farms, diseased animals may develop anergy to tuberculin. Such animals do not respond to tuberculin intradermal administration and are a source of infection [1–4].

In the literature, there is enough information about the detection of tuberculin anergic animals using serological tests. Thus, studies of bovine sera by complement fixation test, conducted by I. A. Karkadinovskaya, show the presence of 19.2% of such animals in the herd [5]; according to the studies by E. D. Lakman – from 2 to 3% [6]; V. I. Rotov et al. detected 12% of such animals [7], Yu. Ya. Kassich et al. – 1.5–7.8% [8]. To clarify the results of the allergic test and to detect anergic animals, the complement fixation test (CFT) and the indirect hemagglutination test (IHA test) are mainly used [9].

A. S. Donchenko et al. when studying the specificity of various antigens by CFT on TB free farms, found that in comparison with the phenolic antigen of the Siberian Research Veterinary Institute (Siberian RVI), the tuberculosis antigen complex of the Ukrainian Research Institute of Experimental Veterinary Medicine (Ukrainian RIEVM) had a higher specificity [10].

Indirect hemagglutination test for the diagnosis of TB was studied by such researchers as E. I. Buryak [11], A. I. Kuzin and N. P. Ovdienko [9], V. L. Solodovnikov [12] and others. N. P. Ovdienko et al. established the possibility of application of IHA test using polysaccharide antigen of the All-Union Institute of Experimental Veterinary Medicine (VIEV) for the detection of TB-infected animals as well as tuberculin anergic animals [13].

When conducting CFT and IHA tests, the researchers used a variety of antigens for *in vivo* diagnosis of tuberculosis on farms with different TB epidemic situations, but the results were contradictory.

The aim of the work was to evaluate the effectiveness of serological tests in detecting of cattle anergic to tuberculin on TB-infected farms.

MATERIALS AND METHODS

The practical significance of serological tests was determined on two farms, where the animal health situation was improved by systematic diagnostic studies and removal of reacting animals. In total, 977 cattle of different sex and age groups were tested: on the first farm – 563 animals, of which 269 cows aged 3–4 years and young animals of 1.5–2 years of age (294 animals); on the second farm – 414 animals (219 and 195 animals, respectively).

After 35 days, animals reacting to tuberculin were subjected to a complex study using intradermal, palpebral and ocular tests as well as serological tests.

The studies were conducted in accordance with the "Manual on the diagnosis of animal tuberculosis" (approved by the Veterinary Department of the RF Ministry of Health on 18.11.2002), mammalian tuberculin was administered in the amount of 10,000 international units.

The complement fixation test was performed using Ukrainian RIEVM complex tuberculosis antigen and Siberian RVI phenolic antigen according to the approved methods, and the indirect hemagglutination test using VIEV polysaccharide antigen was carried out by the method of M. Lorid.

Animals that did not respond to tuberculin administration and showing positive results in complement fixation and indirect hemagglutination tests were re-tested using skin test in 40 days. 20 cows, anergic to tuberculin and positive in both tests, were autopsied. The internal organs and lymph nodes were examined and, regardless of the presence or absence of tuberculous changes, the samples were taken for laboratory testing according to the generally accepted method.

RESULTS AND DISCUSSION

As a result of allergic studies, it was found that 132 (13.5%) of 977 animals reacted to tuberculin administration.

When conducting a second complex study after 35 days, various allergic and serological methods revealed: 27 (2.8%) animals responding to a skin test; 24 (2.5%) – to a palpebral test; 4 (0.4%) – to an eye test (Table 1). All animals positive in the eye test, responded in parallel to skin and palpebral tests. Only 5 animals were positive in a palpebral test and 8 animals were positive in a skin test.

Diagnostic titers of complement fixing antibodies in the complement fixation test using Ukrainian RIEVM complex TB antigen were established in 68 (7.0%) cases,

and using Siberian RVI phenolic antigen in 28 (2.9%) cases. When testing sera by indirect hemagglutination test using VIEV polysaccharide antigen, the hemagglutinating antibodies were detected in 299 (30.6%) animals.

Positive results in the complement fixation test were established more often in sera of cows than of heifers. Thus using Ukrainian RIEVM complex TB antigen, diagnostic antibody titers were established on average in 10.5% of cows and in 3.5% of heifers, and using Siberian RVI antigen in 4.5 and 1.2%, respectively. Of the 27 animals with positive skin reactions, the results of complement fixation test using Ukrainian RIEVM complex TB antigen coincided in 3 cases, and using Siberian RVI antigen in 2 cases, while the results of indirect hemagglutination test showed the coincidence in 10 cases. The serological testing of sera by complement fixation test using both antigens demonstrated coincidence of positive results in 26 animals. The results obtained by CFT and IHA test using Siberian RVI antigen coincided in 22 cases, and using Ukrainian RIEVM complex TB antigen in 41 cases.

Skin test-positive animals were isolated. The rest 950 animals with positive results in palpebral and ocular tests, CFT and IHA test were left in herds. After 40 days, they were re-tested by skin test, which revealed 18 (1.9%) reacting cows: 9 out of 471 (1.9%) cows and 9 out of 479 (1.9%) heifers. Of the 96 animals with complement-fixing antibodies in serum, none reacted to the skin test, and of the 299 animals with hemagglutinating antibodies in blood, 10 animals reacted.

A comparative analysis of allergic and serological test results showed no correlation between seropositivity and immunoreactivity. From the animals with positive results in CFT using Ukranian RIEVM complex TB antigen (65 animals), Siberian RVI antigen (26 animals) and in IHA test (299 animals), no tuberculin reacting animals were detected.

In order to compare the positive results of CFT and IHA test with post-mortem lesions in organs, 20 tuberculin anergic cows were killed (Table 2).

In 19 of 20 animals, diagnostic antibody titers were detected by CFT using Ukrainian RIEVM antigen, and in all animals by CFT using Siberian RVI antigen and in 16 animals by IHA test using VIEV polysaccharide antigen. Post-mortem examination revealed TB-characteristic post-mortem lesions in 19 of 20 animals; moreover "pearl disease" was found in 3 animals, a generalized form was revealed in 4 animals, and the remaining 12 animals showed local changes in organs and lymph nodes.

Autopsy revealed no TB-characteristic lesions in one animal with high diagnostic antibody titers in tests using all studied antigens; the diagnosis was established by the bacteriological method. The sera of 14 animals showed positive results in tests using all antigens, each of them had TB consistent changes.

Of the 20 animals killed, showing positive results in CFT using Siberian RVI antigen, tuberculosis was diagnosed in 100% of cases. No antibodies were detected in one animal, showing TB typical changes, by CFT using Ukrainian RIEVM antigen. Also, 4 animals with established tuberculosis, showed negative results in IHA test using VIEV polysaccharide antigen. It was not possible to kill the remaining animals with positive serological tests; tuberculin reacting animals were not killed as well.

The direct correlation between antibody titers and TB severity was not established. The post-mortem examination revealed apparent tuberculosis in animals with both high and low antibody titers. Thus, in 3 animals, showing titers not exceeding 1:20 and 1:10 in CFT using Ukrainian RIEVM and Siberian RVI antigens, respectively, generalized tuberculosis was detected. A similar pattern was observed in one animal, showing negative results in IHA test.

Thus, the use of tuberculin skin test alone for TB diagnosis in infected herds does not allow to identify all diseased animals, which suggests the need for complex studies.

It should be noted that most researchers have studied various serological methods, including CFT and IHA test for the diagnosis of bovine tuberculosis in sera from tuberculin-responsive animals. Herewith, the lack of their effectiveness was noted. It is known that the tuberculin test and serological methods reveal different stages of the infectious process.

Table 1
Results of animal complex testing for tuberculosis

Таблица 1
Результаты комплексного исследования животных на туберкулез

Farm number	Sex-age group of animals	Number of animals	The number of tuberculin reacting animals			Antibody diagnostic titers		
			skin test	palpebral test	eye test	CFT (Ukranian RIEVM antigen)	CFT (Siberian RVI)	IHA test (VIEV antigen)
1	Cows	269	9 (3.3)*	6 (2.2)	2 (0.7)	46 (17.1)	21 (7.8)	96 (35.7)
	Heifers	294	3 (1.0)	4 (1.4)	–	9 (3.0)	1 (0.3)	57 (19.4)
2	Cows	219	6 (2.7)	6 (2.7)	–	5 (2.3)	1 (0.5)	48 (21.9)
	Heifers	195	9 (4.6)	8 (4.1)	2 (1.0)	8 (4.1)	5 (2.6)	98 (50.3)
Total		977	27 (2.8)	24 (2.5)	4 (0.4)	68 (7.0)	28 (2.9)	299 (30.6)

CFT – Complement fixation test (РСК – реакция связывания комплемента);

IHA test – Indirect haemagglutination test (РНГА – реакция непрямой гемагглютинации);

* Percentage is indicated in parenthesis (в скобках указаны проценты).

Table 2
Results of serological and post-mortem studies

Таблица 2
Результаты серологических и патолого-анатомических исследований

Animal number	Antibody titer			TB consistent lesions
	CFT (Ukranian RIEVM antigen)	CFT (Siberian RVI antigen)	IHA test (VIEV antigen)	
1	1:40	1:40	1:64	in the mediastinal lymph nodes
2	1:20	1:10	1:32	in the pharyngeal, portal lymph nodes, liver
3	1:20	1:40	1:64	in the pharyngeal, mediastinal, bronchial lymph nodes
4	1:20	1:10	1:64	generalized tuberculosis
5	1:160	1:40	1:128	"pearl disease"
6	1:80	1:20	1:32	in the parotid lymph nodes, pulmonary tuberculosis
7	1:160	1:40	1:16	"pearl disease"
8	1:20	1:20	1:16	"pearl disease"
9	–	1:10	1:16	bronchial tuberculosis, pulmonary tuberculosis
10	1:320	1:40	1:16	not found (tuberculosis / diagnosis established by bacteriological method)
11	1:20	1:40	1:16	in the pharyngeal lymph nodes
12	1:40	1:10	1:16	in the bronchial lymph nodes
13	1:40	1:10	1:16	in the bronchial lymph nodes
14	1:20	1:10	–	in the bronchial lymph nodes, lungs, udder
15	1:160	1:10	–	in the lungs
16	1:80	1:40	1:128	generalized tuberculosis
17	1:20	1:10	1:64	generalized tuberculosis
18	1:20	1:10	–	generalized tuberculosis
19	1:80	1:10	–	in the liver
20	1:80	1:10	1:128	in the pharyngeal, submandibular lymph nodes, liver, lungs

The data obtained in the course of this study suggest a possible blockade of the immunity T-system and, as a result, the malfunctioning of the antigen-dependent lymphocyte differentiation in response to mycobacteria activitiy. The results coincide with the data of other authors, suggesting that T-system blockade occurs in some diseased animals, followed by temporary or permanent lack of allergic reactions while humoral antibodies are maintained.

The decrease in the cellular immunity functioning of individual TB-infected animals requires a more extensive research.

CONCLUSION

In herds infected with TB for longer periods, CFT using TB antigens and IHA test using VIEV polysaccharide antigen allows additional detection of diseased animals that are anergic to tuberculin. On these farms, it is advisable to perform complex diagnostic tests using serological methods.

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