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Actual bovine tuberculosis situation in the Republic of Dagestan

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SUMMARY

Lack of statistical data and inconsistences in science and practice make it impossible to give at least approximate tuberculosis prevalence rates in the Republic of Dagestan. Every year the number of tuberculin reacting animals is increasing. For example out of 2,944 tested heifers of breeding age in 2014–2019, up to 30% of animals had positive reactions in tests. During this period out of 1,166 emergency slaughtered animals, tuberculosis was confirmed in 326 animals (28%). Bacteriological tests revealed 291 mycobacterium cultures, 107 out of them were *Mycobacterium bovis*, the other 184 cultures were identified as atypical ones. Based on the species differentiation of 58 cultures, 22 Group II cultures (according to Runyon classification) were isolated; 18 out of them belonged to *Mycobacterium gordonae*, 2 to *Mycobacterium flavescens*, and species of two cultures could not be identified. Four cultures of Group III were species of *Mycobacterium intracellulare*. Out of 32 cultures of Group IV, two belonged to *Mycobacterium smegmatis*, seven to *Mycobacterium fortuitum* and one to *Mycobacterium phlei*, 22 cultures were not identified. To elucidate the role of milk in tuberculosis epidemiology 82 samples of milk from reactors from two farms were tested. In the farm, where reactors were awaiting their removal for a long time, mycobacteria were detected in 20% of milk samples, whereas in the recently infected farm the detection rate was 4%, which suggests that long awaiting periods present high risks. Microscopic, conventional phenotypic and targeted biochemical tests indicate that pseudo-allergic reactions, revealed by tests, result from the atypical mycobacteria of the mentioned groups and species, which present in the animal organism, and seem to be responsible for the tuberculin sensibilization. Timely and comprehensive diagnostic and animal health measures will improve the situation.

Keywords: tuberculosis, cattle, mycobacteria, atypical mycobacteria, infected farms, tuberculin, allergy tests, differentiation, identification, pseudo-allergic reactions

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Актуализированная эпизоотическая ситуация по туберкулезу крупного рогатого скота в Республике Дагестан

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РЕЗЮМЕ

Из-за несовершенства статистических данных и несоответствия расчетных и практических показателей не представляется возможным привести хотя бы приблизительные цифры о заболеваемости животных туберкулезом в Республике Дагестан. С каждым годом число реагирующих на туберкулин животных растет. Так, из 2944 исследованных телок случного возраста в 2014–2019 гг. выявлено до 30% особей, реагирующих на введение туберкулина. За этот период из 1166 подвергнутых вынужденному убою животных диагноз на туберкулез подтвержден у 326 (28%). При проведении бактериологических исследований удалось изолировать 291 культуру микобактерий, из них к *Mycobacterium bovis* отнесено 107 культур, остальные 184 идентифицированы как атипичные. Во многих хозяйствах одновременно с *Mycobacterium bovis* выделялись и нетуберкулезные кислотоустойчивые микобактерии. При видовой

дифференциации 58 культур изолировано 22 культуры второй группы (по Раньону), 18 из которых отнесены к *Mycobacterium gordonae*, 2 – к *Mycobacterium flavescens*, у двух видовую принадлежность установить не удалось. Четыре культуры третьей группы являются представителями вида *Mycobacterium intracellulare*. Из 32 культур четвертой группы 2 отнесены к *Mycobacterium smegmatis*, 7 – к *Mycobacterium fortuitum* и 1 – к *Mycobacterium phlei*, у 22 культур вид не установлен. Для выяснения роли молока в эпизоотологии туберкулеза исследовано 82 пробы от реагирующих на туберкулин животных двух хозяйств. В одном, где реагирующие животные передерживались длительный период, микобактерии в молоке выявлялись в 20% случаев, в другом, где туберкулез выявлен недавно, доля обнаружения составляла 4%, что говорит о большой опасности длительной передержки животных с положительной аллергической реакцией. Проведенные микроскопические, традиционно фенотипические и узкие биохимические исследования свидетельствуют, что выявляемые в процессе диагностики парааллергические реакции обусловлены наличием в организме животных атипичных микобактерий отмеченных групп и видов, которые, по-видимому, обуславливают сенсибилизацию организма к туберкулину. Своевременное и полное выполнение диагностических и ветеринарно-санитарных мероприятий позволит улучшить ситуацию в республике.

Ключевые слова: туберкулез, крупный рогатый скот, микобактерии, атипичные микобактерии, неблагополучные пункты, туберкулин, аллергические исследования, дифференциация, идентификация, парааллергические реакции

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INTRODUCTION

In recent years, certain success has been achieved in many districts of the Republic of Dagestan due to comprehensive organizational, economic, veterinary and sanitary measures aimed at eradication and prevention of bovine tuberculosis (BT). There has been a noticeable improvement in hygiene practices employed across animal production and increase in the number and quality of diagnostic tests. Diseased cattle are timely isolated and slaughtered. At the same time, bovine tuberculosis in some regions of the Republic still poses a serious threat to animal production and human health [1, 2].

Comprehensive research conducted earlier on the BT epidemic situation in all natural and climatic zones of the Republic showed the highest prevalence of the disease in the lowland farms and a steady increase in the incidence due to fundamentally different factors that compromise the immunobiological status of animals. Out of 26 infected farms, identified in the Republic in recent years, only three were located in the mountainous area. This is explained not by the resistance to tuberculosis of local cattle, but by the fact that in mountainous areas, due to the small size of farms, the delivery of feed and contacts between animals, including in the private sector, are restricted, and abundant vegetation of alpine and subalpine meadows, significant solar aeration, and large pastures, etc. are available [1–5].

The results of the study suggest that farms become infected due to various reasons: the introduction of infected young replacement animals, feeding with raw milk, pooling and regrouping of young replacement animals and cows from farms with different animal health statuses, long awaiting periods for diseased animals, inappropriate veterinary, preventive and organizational and management measures, etc. [5–12].

Along with the study of various pathogen introduction ways, indicators of the incidence rate and the influence of various factors on the spread of tuberculosis are of considerable interest. These data are directly related to the organization of preventive measures, investigation of the likely timing of the pathogen introduction into the farm, as well as evaluating of the performance and reliability of diagnostic tests [13–17].

The incidence rate of cattle re-infection in the infected areas depends mainly on the quality of feeding and management conditions. Severe violations of hygienic conditions, micro- and macroclimate, unbalanced diet lead to a decrease in individual resistance of the macroorganism, contribute to the transmission of the pathogen from one animal to another and reduce the incubation period [18–23].

In this regard, a large group of transient nontuberculous acid-fast mycobacteria and mycobacteria-like microorganisms related to mycobacteria, which are widespread in nature and are not fastidious to environmental conditions and resistant, is of particular interest. Under these conditions, it is acceptable that infection of cattle with non-tuberculosis microorganisms occurs with a frequency no less than infection with tuberculosis-causing pathogens, which ultimately results in sensitization of the body to mammalian PPD-tuberculin and false positive results due to the low specificity of the diagnostic tests used [1, 2, 24–27].

In general, the problem of diagnosing tuberculosis, including in Dagestan, involves nonspecific reactions to tuberculin. Reacting animals are often identified among livestock purchased outside the Republic [1, 2, 7].

In this regard, the aim of the work was to obtain additional data on bovine tuberculosis situation in the Republic of Dagestan and the causes of nonspecific sensitization to mammalian PPD-tuberculin.

MATERIALS AND METHODS

In total, from 2014 to 2019, 2,944 heifers of breeding age were subjected to skin test. For the post-mortem examination, 1,166 animals were slaughtered using a humane method. 291 cultures of mycobacteria were isolated from pathological material obtained from 67 farms. Identification and differentiation of 104 cultures was carried out based on the Runyon classification.

The intradermal tuberculin skin test, post-mortem examinations of animals slaughtered for diagnostic purposes and laboratory tests of pathological material were performed in accordance with the "Manual on the Diagnosis of Animal Tuberculosis" (2002)[1]. The mammalian tuberculin produced by the FKP "Kursk Biofactory" (Russia) was injected intradermally using a needle-free BI-7 injector (JSC MIZ-Vorsma, Russia). The reactions were recorded and evaluated 72 hours after injection by measuring the thickness of the skin using TB caliper. Animals with increased skin thickness by three or more millimeters in comparison with a healthy area were considered reactors [3, 5, 11].

When conducting a simultaneous test, a complex allergen from atypical mycobacteria (CAM) was used together with mammalian PPD-tuberculin. The results were evaluated by the intensity of responses to tuberculin and CAM. A more intense reaction to tuberculin indicated homologous infection.

During the post-mortem examination, attention was paid to the site and size of granulomas (tubercles), the nature of inflammation in the lymph nodes, vessels and capsules surrounding the tubercles. The necrosis color, the density of the junction with the surrounding capsule, the capsule inner surface as well as, the consistency of the nodule contents when incised were analyzed. Post-mortem focal catarrhal and catarrhal-purulent inflammations were detected in the lungs of cattle from farms infected with tuberculosis for a long period. Extensive lesions in the lungs, caused by lobular and lobar pneumonia with multiple necrotic foci, along with lymphadenitis of the bronchial and portal lymph nodes, became a visible confirmation of advanced tuberculosis.

Isolated mycobacteria were identified in accordance with GOST 26072-89 (ST SEV 3457-81) "Agricultural animals and poultry. Methods of laboratory diagnostics of tuberculosis"[2] and GOST 27318-87 (ST CMEA 5627-86) "Agricultural animals. Methods of identification of non-typical microbacteria"[3].

The lymph nodes (parotid, submandibular, pre-scapular, bronchial, portal, supramental) served as the material for laboratory testing. The seed was pre-treated using the Sumiyoshi-Löwenstein and Hohn method by acid exposure for 30 minutes.

Primary identification was performed taking into account cultural characteristics: colony growth rate on dense nutrient media, colony color, pigmentation and colony morphology.

The material was seeded on blood agar by spreading 2–4 drops of the suspensions from the Löwenstein – Jensen medium. Next, the plates with blood agar were placed in an incubator at a temperature of 37 °C with oxygen. The results were analyzed visually after 24–48 hours of incubation.

The relation of the isolated culture to *Mycobacterium tuberculosis complex* or to non-tuberculous acid-fast mycobacteria was confirmed by specific laboratory and conventional phenotypic, microscopic and targeted biochemical methods. Among the biochemical methods, niacin, nitrate reduction and heat stable catalase tests were used [28].

RESULTS

TB skin tests of 2,944 heifers of breeding age, supplied in 2014–2019, showed up to 30% reactors in some groups. Reactions in individual animals persisted for up to a year, tuberculin allergic reactions disappeared and occurred from time to time.

In one of the farms, 29 reactors were identified out of the purchased 136 animals by the end of the quarantine. These animals were tested simultaneously after 40 days, with 20 reacting and 13 of them repeatedly reacting. The control slaughter of three animals did not reveal any post-mortem lesions consistent with tuberculosis. The results of bacteriological tests of animals slaughtered for diagnostic purposes were negative. After 45 days, the animals were re-tested and only five animals re-reacted. The next testing was performed after 6 months, all previously reacting animals showed no reactions, on the contrary, positive reactions to tuberculin were registered in 43 animals, who had not reacted before.

A similar situation was observed in other farms that purchased genetically improved or breeding heifers.

In 2014, 28 of the imported animals were slaughtered for diagnostic purposes, tuberculosis was not detected in any case, but the introduced animals continued to react to tuberculin.

To clarify the results of skin tests in 2014–2019, 1,166 animals were subjected to control slaughter. At the same time, tuberculosis lesions were found in the lymph nodes (pharyngeal, bronchial, mediastinal, submandibular), as well as generalization involving parenchymal organs in 326 animals, representing 28.0% (Table).

The test results showed a decreasing coincidence of the skin test results with post-mortem results. Thus, during control tests of reactors in 2014, tuberculosis lesions were detected in 77.0% of animals. In 2019, thanks to targeted actions, including keeping of livestock in isolators, on-farm processing, introduction of healthy animals and the implementation of veterinary and sanitary measures pursuant to regulations, the proportion of diseased animals with tuberculosis lesions decreased to 9.2%.

291 cultures of mycobacteria were isolated during laboratory tests of pathological material of slaughtered animals from 67 farms. Their differentiation enabled to reveal that 107 cultures in 31 farms were *Mycobacterium bovis*, 184 isolated cultures in 36 farms belonged to atypical mycobacteria. Atypical mycobacteria were isolated simultaneously with *Mycobacterium bovis* in 15 farms.

[1] https://files.stroyinf.ru/Data2/1/4293744/4293744181.pdf.
[2] https://docs.cntd.ru/document/1200025492.
[3] https://base.garant.ru/5917269.

Table
Results of post-mortem and bacteriological tests for tuberculosis

Year	Slaughtered	Detected	%	Samples tested	Cultures isolated			Tested among atypical ones	Group by Runyon			
					in total	including			I	II	III	IV
						Mycobacterium bovis	atypical					
2014	122	94	77.0	15	10	10	–	–	–	–	–	–
2015	115	47	40.9	11	7	3	4	–	–	–	–	–
2016	165	59	35.8	167	105	24	81	55	–	22	1	32
2017	348	91	26.1	195	105	50	55	26	–	17	1	8
2018	243	19	7.8	116	48	11	37	16	–	12	1	3
2019	173	16	9.2	150	16	9	7	7	–	1	1	5
Total	1,166	326	28.0	654	291	107	184	104	–	52	4	48

In a number of farms, despite a significant number of reactors, tuberculosis was not established by post-mortem examinations and bacteriological tests. In most cases, atypical mycobacteria were isolated from the pathological material of these animals.

Of 104 cultures of atypical mycobacteria being differentiated according to the Runyon classification, 52 were classified as Group II (scotochromogens), 4 as Group III (nonchromogens) and 48 as Group IV (rapid growers), Fig. 1.

Detections of reactors not showing any visible pathological lesions in internal organs are frequent in Dagestan. Isolation of such animals does not stop the detection of new reactors. Therefore, the observed phenomenon is of interest and is the reason for its comprehensive study.

According to various publications, laboratory tests of biological materials often reveal atypical mycobacteria belonging to Ranyon IV organisms – *Mycobacterium fortuitum* and *Mycobacterium chelonae,* which can be potentially pathogenic for both animals and humans.

At the same time, the sensitizing and pathogenetic roles of rapid growers for cattle remain understudied and controversial. In this regard, when assessing the tuberculosis situation, special attention should be paid to identification of isolated mycobacteria, including atypical ones, since knowing the species has a great practical and theoretical importance for successful prevention and eradication of tuberculosis on farms.

Nontuberculous acid-fast mycobacteria have a number of common features with true tuberculosis agents (morphology, tinctorial properties, acid-, alcohol- and alkali-resistance), but at the same time they have a number of properties similar to saprophytic mycobacteria (colony shape, growth rate, enzymatic activity, drug resistance).

In order to differentiate species within groups, 58 cultures were subjected to a more detailed testing.

As a result, out of 22 cultures of Group II, 18 were identified as *Mycobacterium gordonae*, 2 as *Mycobacterium flavescens*, two species could not be identified.

It was found that all 4 cultures of Group III are representatives of *Mycobacterium intracellulare* species.

Of 32 cultures of Group IV, 2 were classified as *Mycobacterium smegmatis*, 7 as *Mycobacterium fortuitum* and 1 as *Mycobacterium phlei*, 22 cultures were not identified (Fig. 2).

The data obtained reflect the high species heterogeneity of atypical mycobacteria in cattle reactors.

Of all the tested cultures, 34 different species were established in 34, which is 58.6%.

The results of quantitative distribution show that the largest number of identified species was classified as Group II. They can probably play a significant role in the sensitization of cattle to mammalian PPD-tuberculin, but further studies using a larger number of strains are needed to confirm the correlation between the species and sensitization.

The distribution analysis of the identified species showed that the highest number of different species of atypical mycobacteria isolated from reactors' pathological material was observed in Group IV, which is an experimental confirmation of numerous published data. At the same time, considering that only 31.3% of cultures

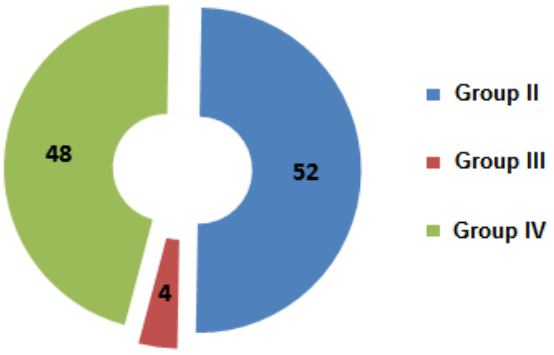

Fig. 1. Grouping of isolated atypical mycobacteria based on Runyon classification

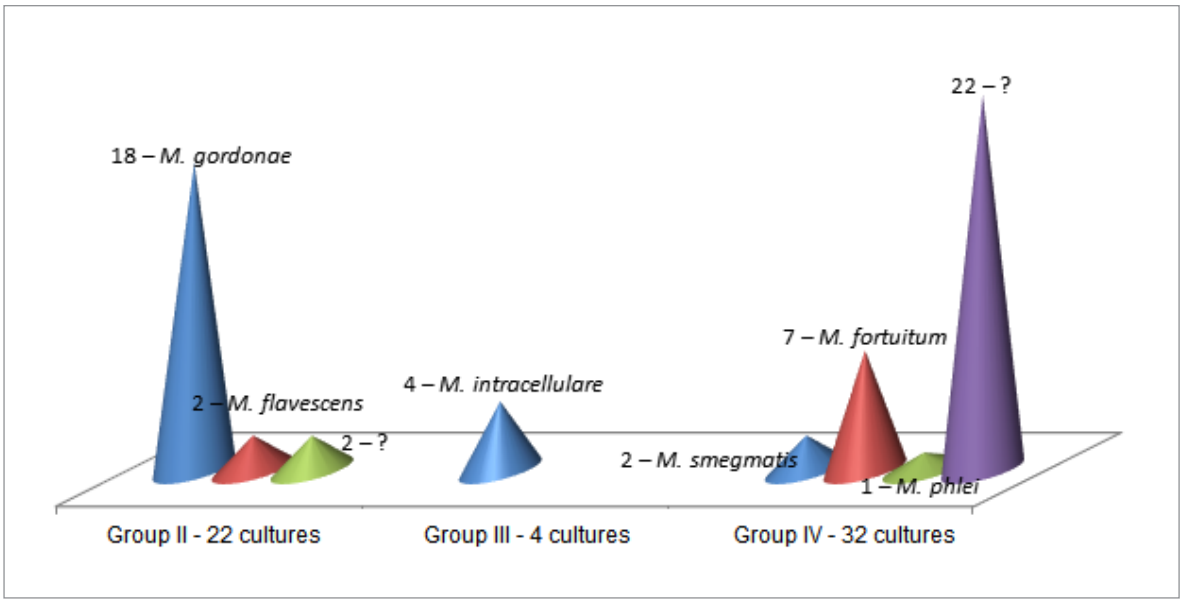

Fig. 2. Species differentiation of atypical mycobacteria

(10 out of 32) were identified in this group, it becomes obvious that representatives of this group play a particularly important role in the sensitization of the macroorganism to tuberculin.

In general, the data obtained became the basis for further dynamic monitoring of the circulation of nontuberculous acid-fast microorganisms in biological material in order to optimize differential diagnosis of bovine tuberculosis.

To clarify the role of milk in the epizootology of tuberculosis, 82 samples from reactors from two farms were tested. One of the farm had been infected for a long period; the other had been infected recently.

In the first farm, where reactors awaited their removal for long periods, mycobacteria in milk were detected in 20% of cases; in the second farm, the detection rate was 4%, which suggests the great danger of prolonged awaiting periods.

CONCLUSION

The data obtained give grounds to believe that the pseudoallergic reactions in skin tests are caused by the presence of atypical mycobacteria of the above mentioned groups and species in animals, which apparently cause sensitization of the organism to tuberculin.

The results of species differentiation did not allow identification of a certain group of atypical mycobacteria species causing increased sensitization to tuberculin.

Isolation of pure mycobacterium cultures from pathological material and their identification should be carried out in close connection with the detection of allergic reactions to tuberculin.

Due to the complex contradictory BT situation in the Republic of Dagestan, a comprehensive plan of anti-tuberculosis measures was drawn up. At the same time, the main attention was paid to the protection of BT free farms from the introduction of tuberculosis, timely and complete identification and removal of diseased animals and reactors from farms, measures to destroy the pathogen in the environment and raising healthy young animals to replace diseased livestock. Diagnostic activities were strengthened in free farms and farms where the disease is eradicated under veterinary control.

Timely and comprehensive anti-tuberculosis measures will allow achieving positive results in the disease control.

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