Қостанай, Қазақстан Республикасы, 110000, Қостанай қ., Маяковский көш, 99/1, тел.: 87058306206, e-mail: sveta.kz89@mail.ru.

Tegza Alexandra Alekseyevna – Doctor of Veterinary Sciences, Professor of the Department of Veterinary, Akhmet Baitursynuly Kostanay Regional University NLC, 110000, Kostanay, 99/1 Mayakovskiy Str., tel.: 87142558568, e-mail: tegza.4@mail.ru.*

Papusha Natalya Vladimirovna – Candidate of Agricultural Sciences, acting Associate Professor of the Department of food safety and biotechnology, Akhmet Baitursynuly Kostanay Regional University NLC, Republic of Kazakhstan, Kostanay, 99/1 Mayakovskiy Str. tel.: 87054115171, e-mail: natali.p82@inbox.ru.

Khassanova Madina Assylkhanovna -– PhD, Associate Professor of the Department of veterinary medicine, Akhmet Baitursynuly Kostanay Regional University NLC, 110000, Kostanay, 99/1 Mayakovskiy Str., tel.: 87082968802, e-mail: khassanova.madina@yandex.kz.

Nurzhanova Svetlana Anatolyevna – 1st year Doctoral student, "8D09101-Veterinary medicine" educational program, Akhmet Baitursynuly Kostanay Regional University NLC, Republic of Kazakhstan, Kostanay, 110000 Kostanay, 99/1 Mayakovskiy Str., tel.: tel.: 87058306206, e-mail: sveta.kz89@mail.ru.

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TUBERCULIN FOR ALLERGIC DIAGNOSTIC OF ANIMAL TUBERCULOSIS

Turgenbayev K.A. – Doctor of Veterinary Sciences, Professor, Chief Researcher, BioVet Research and Production Center LLP, Almaty, Republic of Kazakhstan.*

Ozatbekuly A. – Master of Veterinary Sciences, Junior Researcher, BioVet Research and Production Center LLP, Almaty, Republic of Kazakhstan.

Borsynbayeva A.M. – PhD, Senior Researcher, BioVet Research and Production Center LLP, Almaty, Republic of Kazakhstan.

Mikniene Z. – Associate Professor, PhD, Lithuanian University of Health Sciences, Kaunas, Lithuania.

The article addresses the challenges of allergic diagnosis of tuberculosis in cattle, focusing on nonspecific reactions in animals from tuberculosis-free farms and the insufficient detection of infected individual animals in tuberculosis-affected areas. The authors propose an improved and more affordable method for producing tuberculin based on alcohol precipitation of specific proteins from Mycobacterium bovis(BCG) culture fluid. The culture was grown on a Sauton's fluid medium, and the biomass was treated with Triton X-100, followed by ultrafiltration with a 15 kDa protein cutoff and subsequent ethanol precipitation at 4°C. The resulting precipitate was homogeneous, with a predominant protein of approximately 35 kDa. The final preparation contained protein at a concentration of 1 mg/cm³.

The biological activity and specificity of the obtained preparation were evaluated in a comparative test on guinea pigs sensitized with live cultures of M. bovis and M. Tuberculosis (BCG). Tuberculin was administered intradermally in the groin area at a dose of 25 IU, concurrently with commercial PPD tuberculin. In the first group of animals sensitized with M. bovis, the response to the experimental preparation was comparable to that elicited by PPD, followed by attenuation within 48 hours. In the second group, sensitized with M. tuberculosis, the reaction to the tested tuberculin was less pronounced, indicating a higher species-specificity of the preparation.

Thus, the proposed technology enables to obtain a more specific and standardized tuberculin, which makes it promising for improving the accuracy of allergic diagnosis of tuberculosis in farm animals.

Key words: *bovine tuberculosis, PPD tuberculin, specific proteins, allergic diagnosis, species specificity, homogeneous tuberculin.*

ЖАНУАРЛАРДЫҢ ТУБЕРКУЛЕЗІН АНЫҚТАУДА ҚОЛДАНЫЛАТЫН АЛЛЕРГИЯЛЫҚ ДИАГНОСТИКАЛАУҒА АРНАЛҒАН ТУБЕРКУЛИН ТУРАЛЫ

Тургенбаев К.А. – ветеринария ғылымдарының докторы, профессор, бас ғылыми қызметкер, "БиоВет" Ғылыми өндірістік орталығы ЖШС, Алматы қ., Қазақстан Республикасы.

Озатбекұлы А. – ветеринария ғылымдарының магистрі, кіші ғылыми қызметкер, "БиоВет" Ғылыми өндірістік орталығы ЖШС, Алматы қ., Қазақстан Республикасы.

Борсынбаева А.М. – PhD, аға ғылыми қызметкер, "БиоВет" Ғылыми өндірістік орталығы ЖШС, Алматы қ, Қазақстан Республикасы.

Микниене З. – PhD докторы, доцент, Литва денсаулық ғылымдары университеті, Литва.

Мақалада ірі қара малдағы туберкулезді аллергиялық диагностикалау мәселелері қарастырылады. Атап айтқанда, туберкулезден сау шаруашылықтарда жануарларда пайда болатын спецификалық емес реакциялар және туберкулез бойынша қолайсыз аймақтарда ауру малдарды жеткілікті деңгейде анықтай алмау мәселелері талқыланады. Авторлар Mycobacterium bovis (BCG) культуралық сұйықтығынан арнайы ақуыздарды алкогольдік тұндыру негізінде туберкулинді алудың жетілдірілген және қолжетімді әдісін ұсынады. Мәдени өсімдіктер Сотон ортасында өсірілді, биомасса Triton X-100 реагентінің көмегімен өңделді, содан кейін молекулалық массасы 15 кДа-ға дейінгі ақуыздарды бөлу үшін ультрасүзу жүргізілді және соңында 4 °C температурада этанолмен тұндыру әдісі қолданылды. Соңғы препараттағы ақуыз концентрациясы 1 мг / см3 болды.

Алынған Препараттың биологиялық белсенділігі мен ерекшелігі M. bovis және M. Tuberculosis (BCG) тірі дақылдарымен сенсибилизацияланған теңіз шошқаларындағы салыстырмалы сынақта бағаланды. Туберкулин шап аймағында тері ішіне 25 ХБ дозада, коммерциялық ППД-туберкулинмен қатар енгізілді. M. bovis(BCG) сезімтал жануарлардың бірінші тобында авторлық препаратқа реакция PPD реакциясымен салыстырылды, содан кейін 48 сағаттан кейін әлсіреді. Екінші топта Сенсибилизацияланған M. tuberculosis(BCG), зерттелетін туберкулинге реакция аз байқалды, бұл препараттың түрге жоғары ерекшелігін көрсетеді.

Осылайша, ұсынылған технология нақтырақ және стандартталған туберкулинді алуға мүмкіндік береді, бұл оны ауылшаруашылық жануарларындағы туберкулездің аллергиялық диагностикасының дәлдігін жақсартуға мүмкіндік береді.

Түйінді сөздер: *ірі қара мал туберкулезі, ППД-туберкулин, арнайы ақуыздар, аллергиялық диагностика, түрге тән, біртекті туберкулин.*

ТУБЕРКУЛИН ДЛЯ АЛЛЕРГОДИАГНОСТИКИ ТУБЕРКУЛЕЗА ЖИВОТНЫХ

Тургенбаев К.А. – доктор ветеринарных наук, профессор, главный научный сотрудник, ТОО «Научно-производственный центр «БиоВет», г. Алматы, Республика Казахстан.

Озатбекулы А. – магистр ветеринарных наук, младший научный сотрудник, ТОО «Научно-производственный центр «БиоВет», г. Алматы, Республика Казахстан.

Борсынбаева А.М. – PhD, старший научный сотрудник, ТОО «Научно-производственный центр «БиоВет», г. Алматы, Республика Казахстан.

Микниене З. – ассоциированный профессор, доктор PhD, Литовский университет наук здоровья, г. Каунас, Литва.

В статье рассматриваются проблемы аллергической диагностики туберкулеза у крупного рогатого скота, связанные с неспецифическими реакциями у животных в благополучных хозяйствах и недостаточной выявляемостью больных особей в неблагополучных по туберкулезу зонах. Авторы предлагают усовершенствованный и более доступный метод получения туберкулина на основе спиртового осаждения специфических белков из культуральной жидкости Mycobacterium bovis (BCG). Культура выращивалась на среде Сотона, биомасса обрабатывалась с использованием тритона X-100, после чего проводилась ультрафильтрация с отсечкой белков до 15 кДа и последующее осаждение этанолом при 4 °C. Полученный осадок был однородным, с преобладающим белком массой около 35 кДа. Концентрация белка в конечном препарате составляла 1 мг/см³.

Биологическая активность и специфичность полученного препарата оценивалась в сравнительном тесте на морских свинках, сенсибилизированных живыми культурами M. bovis и M. Tuberculosis. Туберкулин вводился внутрикожно в области паха в дозе 25 МЕ, параллельно с коммерческим ППД-туберкулином. В первой группе животных, сенсибилизированных M. bovis, реакция на авторский препарат была сопоставима с реакцией на ППД, с последующим ослаблением через 48 часов. Во второй группе, сенсибилизированной M. tuberculosis, реакция на исследуемый туберкулин была менее выраженной, что свидетельствует о более высокой видоспецифичности препарата.

Таким образом, предложенная технология позволяет получить более специфичный и стандартизованный туберкулин, что делает её перспективной для повышения точности аллергической диагностики туберкулеза у сельскохозяйственных животных.

Ключевые слова: *туберкулез крупного рогатого скота, ППД-туберкулин, специфические белки, аллергическая диагностика, видоспецифичность, гомогенный туберкулин.*

Introduction

Two main challenges arise in the allergic diagnosis of bovine tuberculosis: (i) nonspecific reactions in animals from tuberculosis-free herds and (ii) the incomplete detection of infected animals in herds with confirmed cases of the disease. The diagnostic efficiency of PPD tuberculin for mammals produced by the Kursk Biofactory reaches 80% or higher in herds with active tuberculosis. However, in herds undergoing recovery, and in some tuberculosis-free herds, animals exhibited para-allergic reactions to PPD tuberculin. These findings have prompted numerous researchers to investigate factors such as the site of injection [1, pp.

87–101], the number of tuberculin injections [2, p. 304], routes of allergen administration [3, pp. 70–73], injection dose [4, pp. 36–37], and, finally, improvements in both the composition of tuberculin [5, p. 20] and methods of allergen production.

It is established that culture filtrates are the primary material for the preparation of PPD tuberculin, containing up to 23 antigens. Many of these antigens are common across all *Mycobacterium* species; some are shared by several species, while others are species-specific [7, pp. 74–76]. In the production of PPD tuberculin from culture fluid, proteins are precipitated using trichloroacetic acid followed by reprecipitation with ammonium sulfate. However, this procedure does not separate common antigens from species-specific ones.

According to T.G. Baitubaev et al. [8, pp. 4–9], electrophoresis of PPD tuberculin reveals eight protein fractions, including three proteins above 94 kDa, one at 94 kDa, one at 67 kDa, two at 30 kDa, and one below 14 kDa. Various purification techniques have been applied to isolate type-specific antigens. For example, A.P. Lysenko [7, pp. 74–76] employed gel filtration on Sephadex G-150 to extract three allergenic fractions from PPD tuberculin with higher specificity than commercial preparations. A more specific component of tuberculin (SCT) for mammals was obtained through double gel filtration of PPD tuberculin using Sephadex G-50 and G-150 [5, p. 20].

Although these methods aim to improve the purification of commercial PPD tuberculin, they rely on multi-step procedures in which heterogeneous tuberculin is first produced and homogeneous fractions are subsequently extracted. Such approaches remain inefficient and technically demanding.

Research purpose

To develop an improved method for obtaining tuberculin based on ethanol precipitation of specific proteins from Mycobacterium bovis and to evaluate its diagnostic efficacy and species specificity in comparison with commercial PPD tuberculin [22–25].

Research objectives

1. To obtain tuberculin from the culture fluid of *M. bovis* using ultrafiltration and ethanol precipitation of proteins [6].
2. To determine the protein composition and homogeneity of the obtained preparation [7, pp. 74–76; 8, pp. 4–9].
3. To evaluate the biological activity and species specificity of the new tuberculin in laboratory animals sensitized with different *Mycobacterium* species [12, pp. 537–544; 13, 17].
4. To compare the diagnostic efficiency of the experimental tuberculin with commercial PPD tuberculin in tuberculosis-affected and tuberculosis-free herds [22–25, 28].

Material and methods

We developed a simplified and accessible method for producing tuberculin by ethanol precipitation of specific proteins from the culture fluid [6]. For this purpose, *M. bovis* strain No. 8 was cultivated for 8 weeks at 38.5° on Sauton's medium in 3-liter biocarboys sterilized by autoclaving at 120 °C for 1.5–2 h. After cultivation, the bacterial biomass was separated from the culture fluid. The biomass was homogenized at 1,000 rpm for 5 min using a laboratory homogenizer in the presence of the surfactant Triton X-100. The centrifuged culture fluid was subsequently concentrated by ultrafiltration using an AP-2.0 unit equipped with a hollow-fiber membrane (VPU-15PA) housed in an acrylic chamber. Filtration was carried out under a pressure of up to 0.2 MPa, retaining proteins with a molecular weight above 15 kDa.

The culture fluid was concentrated 3–5 fold, chilled to 4 °C, and mixed with cold rectified ethanol at a ratio of 1:3. The mixture was kept under constant stirring at 4 °C for 18 h. The resulting precipitate was collected by continuous centrifugation (C-44 centrifuge) at 24,000 rpm. Protein concentration (mg/cm³) was determined by the Lowry method [12] (1951), and protein properties were analyzed by disk electrophoresis [8] (G. Maurer, 1971). The resulting protein preparation was homogeneous in structure, with a molecular mass of approximately 35 kDa. The precipitate was resuspended in 10% glycerol-saline solution to achieve a final protein concentration of 1 mg/cm³.

Results and discussions

The biological activity and specificity of the preparation were evaluated in a comparative trial with commercial PPD tuberculin for mammals [12, 13], conducted at the Kazakh Research Veterinary Institute. Twenty guinea pigs, aged two months and weighing no less than 350–400 g, were divided into four groups of five animals each. Animals were sensitized with live cultures at a dose of 1 mg per animal. Thirty days after sensitization, guinea pigs were tested with tuberculin derived from the culture fluid of *M. bovis*. The experimental tuberculin was administered intradermally into the groin area at a dose of 0.0001 mg of protein (equivalent to 25 IU). Commercial PPD tuberculin, administered at 25 IU into the opposite groin, served as the control. Reactions were assessed at 24 and 48 h by measuring the diameter of the resulting papule.

As shown in Figure 1, guinea pigs sensitized with *M. bovis* exhibited reactions of comparable intensity to both the experimental tuberculin and PPD tuberculin at 24 h. However, by 48 h the responses had declined. In the second group, sensitized with *M. tuberculosis*, the mean reaction at 24 h was 7.2 mm for the experimental tuberculin and 8.6 mm for PPD tuberculin; by 48 h, the corresponding reactions decreased to 5.4 mm and 4.4 mm, respectively [18, 19].

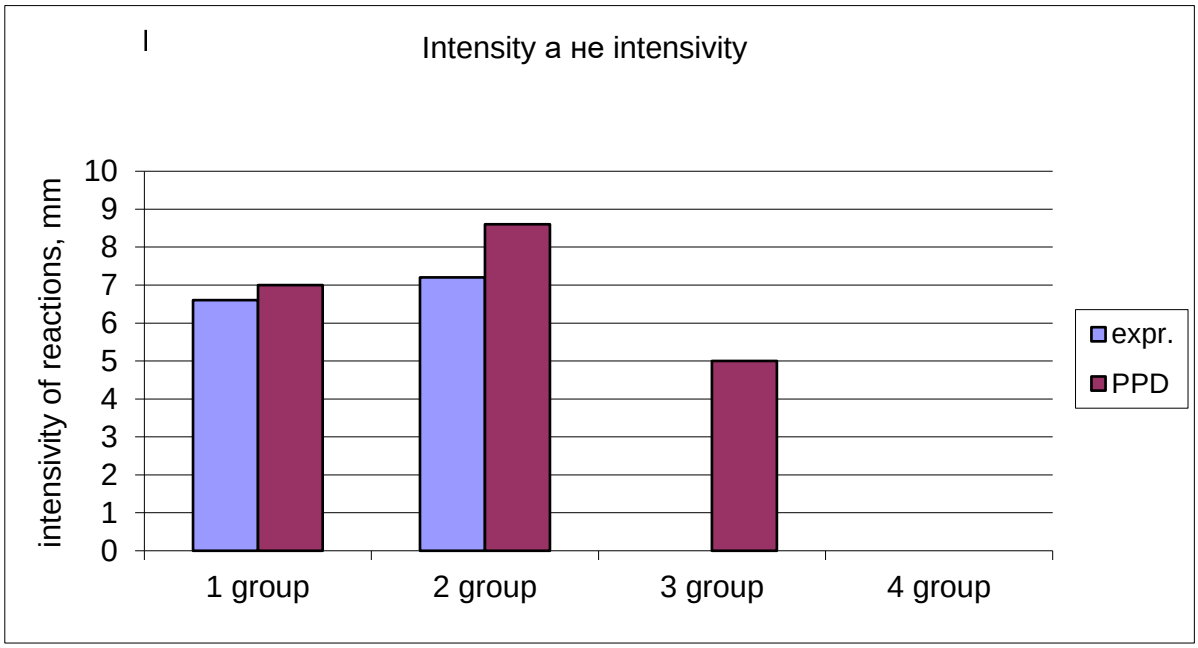

Figure 1. In the third group of Guinea pigs sensitized with *M. avium*, within 24 hours reactions were observed only to PPD tuberculin. In the fourth group of Guinea pigs sensitized with *M. kansasii*, no hyperaemia reactions to the tested allergens were recorded in any of the animals

As a result of the conducted research, it was established that the experimental tuberculin demonstrated activity and specificity comparable to commercial PPD tuberculin in Guinea pigs sensitized with *M. bovis* and *M. tuberculosis*. At the same time, the experimental tuberculin was inert in Guinea pigs sensitized with *M. avium* and *M. kansasii*, whereas PPD tuberculin also elicited responses in animals sensitized with *M. avium* [13, 17].

Thus, the experimental tuberculin is suitable for intravital diagnosis of tuberculosis in animals, similarly to PPD tuberculin, and allows differentiation of para-allergic reactions caused by sensitization with *M. avium* and *M. kansasii*.

We further conducted a qualification test to evaluate the diagnostic value, activity, and specificity of the experimental tuberculin in comparison with PPD tuberculin, using rabbits sensitized with different species of mycobacteria. For this purpose, 40 rabbits weighing 2.5–3.0 kg were divided into 9 groups and immunized with different mycobacterial species. Rabbits in groups 1–7 were immunized by subcutaneous injection of 1 cm³ of a suspension of killed mycobacterial cultures in paraffin oil at a dose equivalent to 0.2 mg of dry bacterial mass per animal. Rabbits of group 8 received 0.5 cm³ of paraffin oil only. Rabbits in group 9 served as intact, non-immunized controls [22–25].

Thirty days after immunization, the rabbits were injected intradermally on a shaved area of the back. They were treated with rectified 70% ethyl alcohol and tested with prototype allergens at a dose of 40 IU in 0.1 cm³ of sterile saline solution, administered in the following order on each animal: experimental tuberculin, commercial PPD tuberculin (PPD), as well as other allergens developed in the tuberculosis department—SCT allergen [25, 26, 28], KJ allergen, and others.

At 24 and 48 hours, the reactions were evaluated by measuring the areas of hyperaemia on the skin in millimeters. The results are presented in Figure 2 and Table 1.

Table 1. Intensity of rabbits' skin reactions to allergen injection (mm) at 24 and 48 hours

Group No.	Number of animals	expr.		PPD		BA		expr.		KJ	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
1	2	3	4	5	6	7	8	9	10	11	12
1 *M. bovis*	71	13	11,5	14	14,5	16	16	9	11	14	16
	52	13	13,5	15	13,5	18	14,5	9	6	15,5	16
	82	10,5	17	10	13,5	10	8,5	11	-	11	9
	122	12	11	12	12	15	11	10,5	7	-	11,5
	M=	12,1	13,3	12,8	13,4	14,8	12,5	9,9	6	10,1	13,1
	мМ±	0,59	0,68	1,1	0,52	0,56	1,69	0,51	2,27	3,5	1,73
	P≥,<	>0,99	>0,99	>0,99	>0,99	>0,99	>0,99	>0,99	<0,95	>0,95	>0,99

Continuation of Table 1

M. tuberculosis	2	99	9	6	10	-	8	-	11	-	12,5	-
		64	12,5	5	11,5	7	-	-	-	-	8,5	-
		51	8,5	-	6,5	-	5,5	-	10	-	5	-
		61	11	-	10,5	-	10	-	10	5	11,5.	5,5
		M=	10,3	2,75	9,63	1,8	5,88	-	7,75	1,25	9,4	1,4
		мМ±	0,92	1,6	1,1	-	2,16	-	2,59	-	0,92	-
		P≥,<	>0,99	<0,95	>0,99	-	>0,95	-	>0,99	-	>0,99	-
1		2	3	4	5	6	7	8	9	10	11	12
M. avium	3	110	6,5	-	6,5	-	7	-	-	-	6	-
		119	-	-	-	-	-	-	-	-	-	-
		114	7	-	-	-	6	-	-	-	6	7
		115	-	5	7	-	12	-	8	-	14	-
		127	12	5	10	-	9	-	-	-	9,5	-
		M=	5,1	2	4,7	-	6,8	-	1,6	-	7,1	1,4
		мМ±	2,29	-	2	-	1,98	-	-	-	2,3	-
		P≥,<	<0,95	-	<0,95	-	>0,95	-	-	-	-	<0,95
M. kansassii	4	112	-	-	-	-	13	-	5	3	-	-
		113	13	12	13,5	5	15	8	13,5	5	16,5	8
		124	-	-	7	-	11,5	-	11	-	9,5	-
		126	7	-	7	-	9	-	-	-	-	-
		M=	5	3	6,88	1,25	12,1	2	7,4	2	6,5	2
		мМ±	-	-	2,76	-	1,9	-	3	-	-	-
		P≥,<	-	-	<0,95	-	>0,99	-	>0,99	-	-	-
M. phley	5	100	-	-	-	-	-	-	-	-	-	-
		107	7,5	-	-	-	-	-	-	-	8,5	-
		120	-	-	-	-	5,5	-	-	-	-	-
		123	-	-	-	-	-	-	-	-	6,5	-
		125	-	-	-	-	-	-	-	-	-	-
		M=	1,5	-	-	-	1,1	-	-	-	3	-
		мМ±	-	-	-	-	-	-	-	-	-	-
M.scrofula-ceum	*6*	108	-	-	-	-	-	-	-	-	9	-
		109	8	-	-	-	8,5	-	-	-	9	6
		111	-	-	-	-	-	-	-	-	-	6
		116	-	-	-	-	-	-	-	-	-	-
		117	-	-	-	-	-	-	-	-	-	-
		M=	1,6	-	-	-	1,7	-	-	-	3,6	2,4
M.xeno-pii	*7*	102	-	-	-	-	-	-	-	-	--	-
		103	-	-	-	-	-	-	-	-	-	-
		104	-	-	-	-	-	-	-	-	-	-
		105	-	-	-	-	-	-	-	-	-	-
		106	-	-	-	-	-	-	-	-	-	-
8		22	-	-	-	-	-	-	-	-	-	-
Paraffin oil		26	-	-	-	-	-	-	-	-	-	-
		59	-	-	-	-	-	-	-	-	-	-
		65	-	-	-	-	-	-	-	-	-	-
		128	-	-	-	-	-	-	-	-	-	-
Conntrol	9	9	-	-	-	-	-	-	-	-	-	-
		42	-	-	-	-	-	-	-	-	-	-
		69	-	-	-	-	-	-	-	-	-	-
		79	-	-	-	-	-	-	-	-	-	-
		81	-	-	-	-	-	-	-	-	-	-

In a comparative evaluation of the diagnostic efficiency of allergens, it was established that all rabbits of the first group, immunized with *M. bovis* cultures, reacted to almost all types of allergens 24 hours after injection. This observation is explained by the fact that *M. bovis* served as the basis for the production of all allergens. The mean hyperemia response of the skin to tuberculin in this group was 12.1 mm, while the response to PPD tuberculin was 12.8 mm. At 48 hours after injection, the mean skin hyperemia measured

13.3 mm for tuberculin and 13.4 mm for PPD tuberculin. The results were statistically processed and found to be significant.

In the second group of rabbits, immunized with *M. tuberculosis*, the mean skin hyperemia at 24 hours was 10.3 mm for tuberculin and 9.63 mm for PPD tuberculin, demonstrating the clear superiority of tuberculin (10.3 mm) over other allergens. Statistical analysis confirmed the significance of these findings. At 48 hours, skin hyperemia persisted in 2 of 4 rabbits (50%) in response to tuberculin, as well as to PPD tuberculin.

In the third group of rabbits, immunized with *M. avium*, three out of five animals (60%) reacted to tuberculin after 24 hours, with a mean hyperemia of 5.1 mm. Similarly, three rabbits (60%) reacted to PPD tuberculin, with a mean hyperemia of 4.7 mm. In both cases, statistical analysis did not confirm significance. At 48 hours, most rabbits showed no response to either allergen, and the data were not suitable for statistical analysis. These results demonstrate that neither tuberculin nor PPD tuberculin elicited reliable allergic responses in rabbits immunized with *M. avium*, which was expected [22, 27, 29–31].

In the fourth group of rabbits, immunized with *M. kansasii*, two out of four animals (50%) responded to tuberculin at 24 hours, with a mean hyperemia of 5.0 mm, while three rabbits (75%) reacted to PPD tuberculin with a mean hyperemia of 6.9 mm. By 48 hours, most rabbits no longer exhibited reactions to either allergen.

In the fifth group of rabbits, immunized with *M. phlei*, only one out of five animals (20%) responded to tuberculin at 24 hours, with a hyperemia of 7.5 mm.

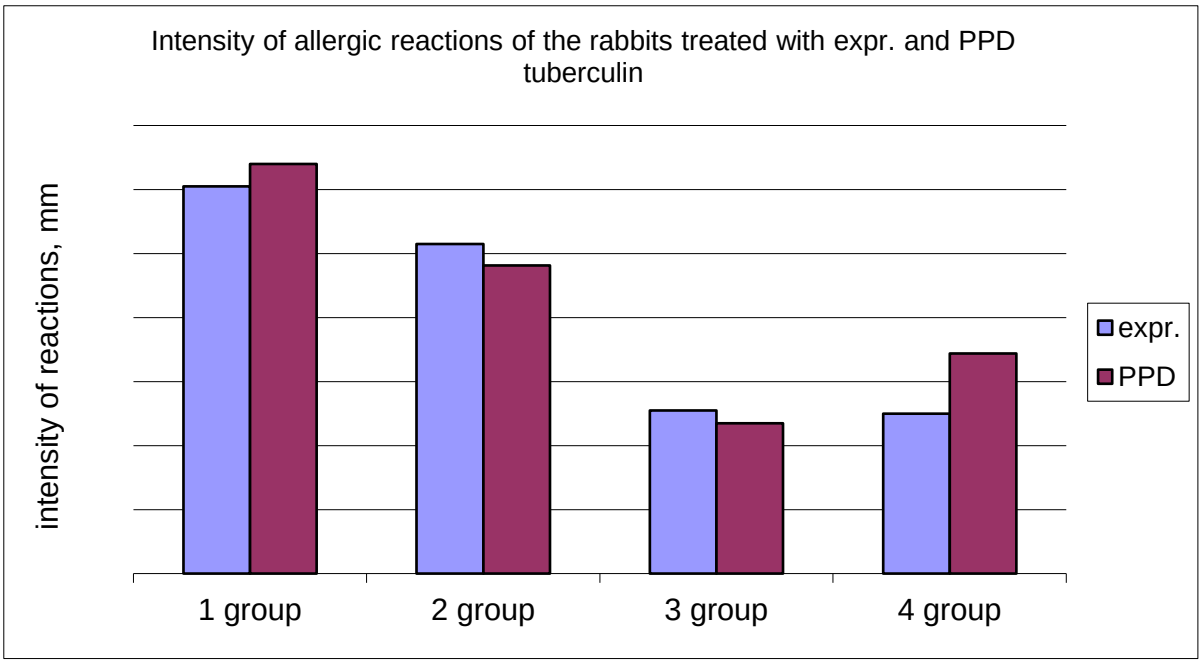

Figure 2

In the sixth group of rabbits immunized with *M. scrofulaceum*, one out of five animals responded to the experimental tuberculin, showing a weak hyperaemia reaction.

In the seventh group of rabbits immunized with *M. xenopi*, almost none of the animals reacted to the tested allergens, which indicates the specificity of the preparation with respect to this species of mycobacteria.

In the control eighth group of rabbits, no animals showed any reactions to the injected allergens, confirming the absence of allergenic properties of paraffin oil used in the immunization procedure.

In the ninth, intact group of rabbits, no allergic responses to any of the tested allergens were observed, which indicates the satisfactory sanitary conditions and proper isolation of all animals throughout the experiment.

The experimental tuberculin demonstrated the highest allergic activity in Groups 1 and 2, immunized with *M. bovis* and *M. tuberculosis*. In other groups of rabbits immunized with *M. avium* and atypical mycobacteria, it showed little to no activity, thereby confirming its species specificity.

Based on the presented experimental results, it can be concluded that the newly obtained tuberculin is not inferior in terms of specificity and activity to commercial PPD tuberculin for mammals, while showing reduced para-allergic responses.

The production-line testing of the experimental tuberculin was carried out through a qualification trial in comparison with PPD tuberculin on 233 cows from tuberculosis-free farms and 100 cows from chronically tuberculosis-affected farms in the Kostanay oblast (region).

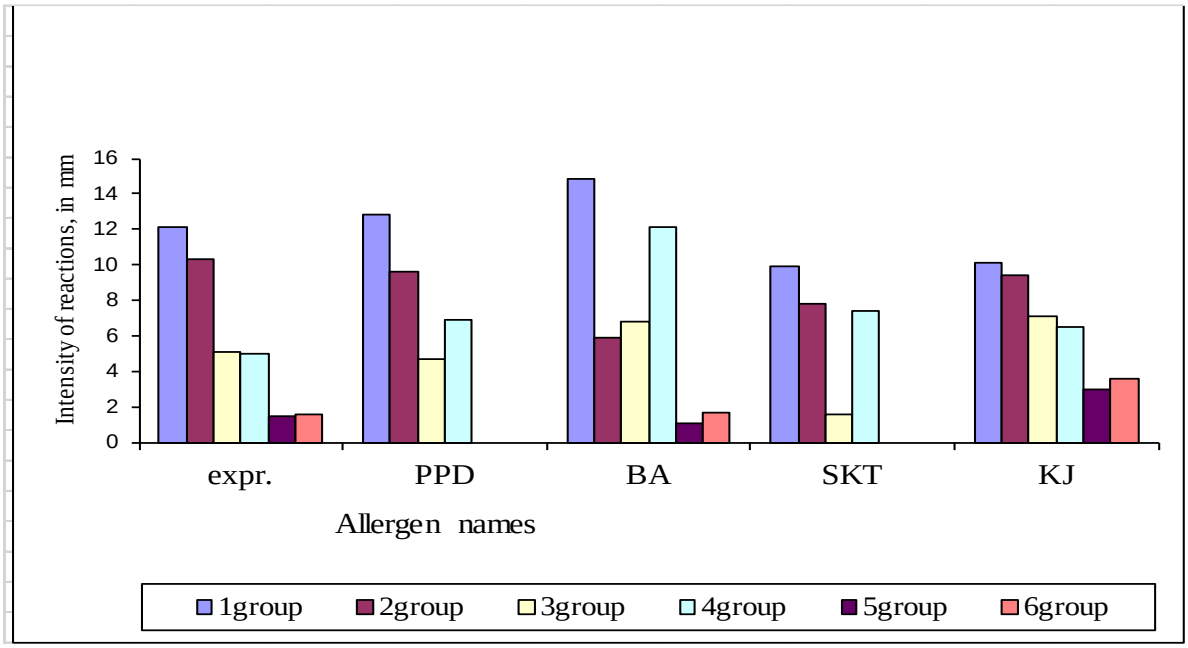

Figure 3

Allergic tests were performed simultaneously with both tuberculins. Each animal received an injection of the standard solution of PPD tuberculin for mammals, produced by the Kursk biofactory, on the right side, and the experimental tuberculin developed in our laboratory on the left side. Both preparations were administered into the upper third of the neck using a needleless injector at a dose of 0.2 cm³ (10,000 IU). The injection sites were shaved and disinfected with 70% ethyl alcohol prior to administration.

The evaluation of allergic reactions was conducted 72 hours after injection. Measurements included the thickness of the skin fold at the injection site using a cutometer (in mm) and the size of the inflammatory oedema.

Table 2 – Intensity of allergic reactions in cattle from Division №5 of CF “Peshkovskoe” following allergen injection

Number of animals	Intensity of allergic reactions in mm, on tuberculin injection		Reaction «+», «-», or «=»	Number of animals	Intensity of allergic reactions in mm, on tuberculin injection		Reaction «+», «-», or «=»
	PPD	expr.			PPD	expr.	
1	21	6	-	55	10	14	+
5	6	5	-	56	10	9	-
348	14	6	-	58	43	8	-
12	4	-		60	35	4	-
13	10	10	=	1984	5	-	
1716	14	8	-	65	17	11	-
15	20	9	-	66	10	9	-
21	6	-		67	18	8	-
22	42	16	+	68	18	11	
23	11	10	-	71	12	7	-
1121	14	-		74	10	7	-
25	22	12	-	75	10	6	-
28	24	15	-	76	9	7	-
31	11	8	-	78	17	8	-
33	17	8	-	80	9	9	=
34	6	4	-	83	10	12	+
36	9	9	=	85	9	9	=
38	8	8	=	86	18	10	-
40	9	13	+	87	10	8	-
41	14	9	-	88	11	9	-
42	7	5	-	89	42	15	-
1713	3	7	+	92	43	19	-

Continuation of Table 2

44	17	8	-	93	8	7	-
45	27	13	-	96	9	7	-
46	10	3	-	98	14	9	-
47	18	13	-	99	8	-	
50	17	10	-	100	6	-	
51	9	5	-				
Total					55	49	«-»-38, «+»-5

Table 3 – Results of controlling and diagnostic slaughter of animals, reacting on tuberculin

№	Number of animal	Intensity of allergic reaction in mm, after injection of		Results of pathological study of bulk
		PPD	expr.	
1	1	21	6	Tuberculosis of pneumomediastinum lymph nodes
2	3513	6	4	Tuberculosis of pneumomediastinum lymph nodes
3	5	6	4	Tuberculosis of pneumomediastinum lymph nodes
4	12	4	-	Tuberculosis of pneumomediastinum lymph nodes
5	21	6	-	Tuberculosis of retropharyngeal, portal lymph nodes
6	22	42	16	Tuberculosis of prescapular lymph nodes
7	112	14	-	Tuberculosis of submaxillary pneumomediastinum lymph nodes
8	1713	3	7	Tuberculosis of bronchial and pneumomediastinum lymph nodes
9	55	10	14	Tuberculosis of pneumomediastinum lymph nodes
10	2	-	-	-

Based on conducted tests, it was established that on tuberculosis-free farms no cows responded to the injection of either the experimental tuberculin or PPD tuberculin. In contrast, on a tuberculosis-affected farm, 55 out of 100 cows (55%) reacted to PPD tuberculin, while 49 animals (49%) reacted to the experimental tuberculin. Among them, 49 cows showed coinciding allergic reactions to both allergens, and only 6 animals developed diagnostic-level responses exclusively to PPD tuberculin.

During control diagnostic slaughter of 10 cows, pathological and anatomical examinations revealed characteristic tubercular lesions of varying severity in 9 animals that had reacted to either tuberculin or PPD tuberculin. In one cow, which had not reacted to either allergen, no visible tubercular changes were identified (Table 2).

Analysis of the obtained allergic test results using the experimental tuberculin, in comparison with the commercial PPD tuberculin, and the outcomes of pathological examinations confirmed the diagnostic reliability of the new preparation. Based on the findings, the expert commission recommended the tuberculin for broad production line testing.

Currently, large-scale production line testing of the experimental tuberculin is being conducted in the Kostanay region of the Republic of Kazakhstan. 2,000,000 doses of the preparation have been produced, and 776,200 animals have already been examined. Upon completion of these trials and based on their results, an official resolution regarding the approval and application of the new tuberculin in the Republic of Kazakhstan will be issued.

Conclusion

This study demonstrated that tuberculin obtained by alcohol precipitation of specific proteins from the culture fluid of *Mycobacterium bovis* represents a homogeneous preparation, with a predominant protein fraction of approximately 35 kDa, standardized to a protein concentration of 1 mg/cm³. Electrophoretic analysis confirmed the reproducibility and purity of the protein composition, which is an essential prerequisite for the stability of diagnostic reagents [7, p.74–76; 8, p.4–9].

Experimental evaluation in guinea pigs sensitized with different mycobacterial species showed that the new tuberculin has biological activity comparable to that of commercial PPD tuberculin when tested against *M. bovis*. Moreover, the experimental preparation demonstrated a clear advantage in species specificity, producing weaker or absent reactions in animals sensitized with atypical mycobacteria, such as *M. avium* and *M. kansasii*. These findings confirm that the alcohol precipitation method preserves species-specific antigens while minimizing non-specific components that commonly cause cross-reactivity [12, 13, 17–19].

Further testing in rabbits and large-scale field trials in cattle herds demonstrated that the diagnostic efficiency of the experimental tuberculin was equivalent to that of commercial PPD tuberculin under natural infection conditions. In tuberculosis-affected herds, the new tuberculin identified diseased animals with the

same sensitivity as PPD, while in tuberculosis-free herds it produced no false-positive responses. Notably, the number of para-allergic reactions was lower compared with the commercial preparation, thereby improving the reliability of the diagnostic process [22–25, 28].

Overall, these results indicate that the developed method effectively addresses the long-standing challenge of improving the specificity of allergic diagnostics for bovine tuberculosis. The combination of ultrafiltration and alcohol precipitation makes it possible to obtain a standardized, safe, and reproducible diagnostic reagent that retains high biological activity while reducing cross-reactivity. Such properties fully meet the objective of this study and demonstrate the feasibility of using this approach to produce advanced tuberculin preparations.

The findings provide a solid basis for recommending the developed tuberculin for further validation in large-scale veterinary practice. Its application has the potential to improve the accuracy of tuberculosis detection in cattle, reduce diagnostic errors in tuberculosis-free herds, and strengthen epidemiological surveillance programs. Future research should focus on long-term stability studies, comparative evaluation across different livestock populations, and integration of this preparation into national tuberculosis control strategies [26–31].

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Information about the authors:

Turgenbayev Kairat Altynbekovich – Doctor of Veterinary Science, Professor, Chief Researcher, BioVet Research and Production Center LLP, Republic of Kazakhstan, 050008, Almaty, 191-A Karasay batyr Str., e-mail: biovet.kaz@mail.ru. https://orcid.org/0000-0002-0982-1863.*

Ozatbekuly Amanbek – Master of Veterinary Sciences, Junior Researcher, BioVet Research and Production Center LLP, Republic of Kazakhstan, 050008, Almaty, 191-A Karasay batyr Str., e-mail: ozatbekamanbek@gmail.com. https://orcid.org/0009-0006-0627-5680.

Borsynbayeva Assiya Madenovna – PhD, Senior Researcher, BioVet Research and Production Center LLP, Republic of Kazakhstan, 050008, Almaty, 191-A Karasay batyr Str., e-mail: asiajan@mail.ru. https://orcid.org/0000-0002-2722-2020.

Mikniene Zoja – Associate Professor, PhD, Lithuanian University of Health Sciences, Lithuania, 03154, Kaunas, 18 Tilžės Str., tel.: +37061029223, e-mail: zoja.mikniene@lsmuni.lt. https://orcid.org/0000-0001-5165-837X.

Тургенбаев Қайрат Алтынбекұлы – ветеринария ғылымдарының докторы, профессор, бас ғылыми қызметкер, Ғылыми өндірістік орталығы "БиоВет" ЖШС, Қазақстан Республикасы, 050008, Алматы қ, Қарасай батыр көш 191 литр А, e-mail: biovet.kaz@mail.ru, https://orcid.org / 0000-0002-0982-1863.*

Озатбекұлы Аманбек –- ветеринария ғылымдарының магистрі,кіші ғылыми қызметкер, "БиоВет" ғылыми өндірістік орталығы ЖШС, Қазақстан Республикасы, 050008, Алматы қ, Қарасай батыр көш 191 литр А, e-mail: ozatbekamanbek@gmail.com, https://orcid.org/0009-0006-0627-5680.

Борсынбаева Асия Маденовна – PhD, аға ғылыми қызметкер, Ғылыми өндірістік орталығы "БиоВет" ЖШС, Қазақстан Республикасы, 050008, Алматы қ, Қарасай батыр көш 191 литр А, e-mail: asiajan@mail.ru, https://orcid.org/0000 0002-2722-2020.

Микниене Зоя – PhD докторы, доцент, Литва денсаулық ғылымдары университеті, LT44001, Каунас, Тильзес көш, 18, тел.: +37061029223, e-mail: zoja.mikniene@lsmuni.lt, https://orcid.org/0000-0001-5165-837X.

Тургенбаев Кайрат Алтынбекович – доктор ветеринарных наук, профессор, главный научный сотрудник, ТОО «Научно-производственный центр «БиоВет», Республика Казахстан, 050008, Алматы, ул. Карасай Батыра 191 литер А, e-mail: biovet.kaz@mail.ru, https://orcid.org/ 0000-0002-0982-1863.*

Озатбекулы Аманбек – магистр ветеринарных наук, младший научный сотрудник, ТОО «Научно-производственный центр «БиоВет», Республика Казахстан, 050008, Алматы, ул. Карасай Батыра 191 литер А, e-mail: ozatbekamanbek@gmail.com, https://orcid.org/0009-0006-0627-5680.

Борсынбаева Асия Маденовна – PhD, старший научный сотрудник, ТОО «Научно-производственный центр «БиоВет», Республика Казахстан, 050008, Алматы, ул. Карасай Батыра 191 литер А, e-mail: asiajan@mail.ru, https://orcid.org/0000- 0002-2722-2020.

Микниене Зоя – ассоциированный профессор, доктор PhD, Литовский университет наук здоровья, Литва, LT44001, г. Каунас, ул. Тилзеса 18, тел.: +37061029223, e-mail: zoja.mikniene@lsmuni.lt, https://orcid.org/0000-0001-5165-837X.

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ҚОСТАНАЙ ОБЛЫСЫ БОЙЫНША ЖАНУАР ӨНІМДЕРІНЕН БӨЛІНІП АЛЫНҒАН S.AUREUS ИЗОЛЯТТАРЫНЫҢ ТОКСИГЕНДІЛІГІН ЖӘНЕ АНТИБИОТИККЕ ТӨЗІМІДІЛІГІН ЗЕРТТЕУ

Чужебаева Г.Д. – ветеринария ғылымдарының кандидаты, қауымдастырылған профессор, ҚБ ҒЗИ ғылыми қызметкері, «Ахмет Байтұрсынұлы атындағы Қостанай өңірлік университеті» ҚЕАҚ, Қостанай қ., Қазақстан Республикасы.

Мәлікзада Қ.М. – ветеринария ғылымдарының магистрі, ветеринарлық медицина бағыты бойынша 1 курс докторанты, «Ахмет Байтұрсынұлы атындағы Қостанай өңірлік университеті» КЕАҚ, Қостанай қ., Қазақстан Республикасы.*

Байменов Б.М. – ветеринария ғылымдарының магистрі, ҚБ ҒЗИ молекулалық-генетикалық зерттеулер зертханасының меңгерушісі, «Ахмет Байтұрсынұлы атындағы Қостанай өңірлік университеті» ҚЕАҚ, Қостанай қ., Қазақстан Республикасы.

Досова А.Д. – техника және технологиялар магистрі, ҚБ ҒЗИ кіші ғылыми қызметкері, «Ахмет Байтұрсынұлы атындағы Қостанай өңірлік университеті» КЕАҚ, Қостанай қ., Қазақстан Республикасы.

Мақалада Қостанай облысы бойынша жануарлар өнімдерінен бөлініп алынған S. aureus-тың токсигенділігін және антибиотикке төзіміділігін зерттеу бойынша нәтижелер көрсетілген.

Тағамдық токсикоинфекциялар патогенді тірі микроорганизмдердің және олардың токсиндерінің әсерінен дамиды. Бұл токсиндер микроағзалардың өздері арқылы өндіріледі немесе олардың жойылуы кезінде бөлінеді. Тағам арқылы таралатын ең қауіпті патогенді бактериялардың бірі – S.aureus, өйткені олар барлық елдер мен өңірлерде жоғары өлім-жітім деңгейіне әкелуге бейім екені көптеген еңбектер мен ғылыми зерттеулерде анықталған.

Зерттеу жұмысының негізгі мақсаты Қостанай облысы аумағында жануарлардан алынатын өнімдерден бөлініп алынған S.aureus-тың токсигенді штаммдарына мониторинг жүргізу, энтеротоксиндерді иммунды-ферменттік талдау (ИФТ) әдісімен анықтау, биоқабықша түзілу қабілетін және антибиотиктерге төзімділік деңгейін зерттеу болып табылады.

Жүргізілген зерттеу нәтижелерінде жануарлар өнімдерінен таңдалған 178 сынамадан, S.aureus-тың 40 штаммы бөлініп алынды. Оқшауланған изоляттарды энтеротоксиндерге зерттеу барысында анықталған токсиндердің ең көп саны А және Д типтері болып табылады. А типті токсин 16 үлгіде (72,7%), Д типі 11 сынамада (50%) анықталды. БҚП тексеру барысында S.aureus-