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**PREVENTIVE AND THERAPEUTIC EFFICIENCY OF A POLYVALENT
INACTIVATED VACCINE AGAINST DERMATOMYCOTOSIS
OF AGRICULTURAL AND CARNIVOROUS ANIMALS**

Abstract. In the Republic of Kazakhstan, the number of cattle is more than 7 000 000 heads. Every year, the number of livestock purchased from abroad is growing. According to the statistics Committee, there are 7,613. 9 thousand heads of cattle, 2,862. 6 thousand heads of horses and 337. 8 thousand heads of camels in Kazakhstan [1, p.1].

Currently, fungal infection is widespread among agricultural and carnivorous animals. This infection has recently been found in an associated form. Therefore, the preservation of the well-being of various animal species and the improvement of existing vaccine preparations for the prevention and treatment of dermatomycosis (trichophytia and microsporia) of agricultural and carnivorous animals is a necessary condition for the development of small and large livestock farms in the country.

Dermatomycosis infection of animals is included in the list of particularly dangerous diseases, prevention, diagnosis and elimination of which is carried out at the expense of budget funds (Annex 5 to the order of the Minister of agriculture of the Republic of Kazakhstan dated October 30, 2014 No. 7-1 / 559) [2, p. 1]. For the treatment and prevention of trichophytosis and microsporia of animals in the Republic of Kazakhstan, expensive vaccine preparations imported from abroad were purchased through public procurement.

Currently, we have developed a technology for manufacturing an improved polyvalent inactivated vaccine against dermatomycosis of agricultural and carnivorous animals, which will solve the issues of prevention (treatment) of the disease, which will affect the improvement of the epidemiological situation for dermatomycosis in the Republic of Kazakhstan, and ultimately will contribute to obtaining safe livestock products (milk, beef and leather cheese) of high sanitary quality.

The article presents the results of testing a polyvalent inactivated vaccine against dermatomycosis of agricultural and carnivorous animals made from local highly immunogenic vaccine strains *Trichophyton mentagrophytes* F-01, *Trichophyton verrucosum* F-02, *Trichophyton sarkisovii* F-03, *Trichophyton verrucosum variantis autotrophicum* F-04, *Trichophyton equinum* F-05, *Microsporum Canis* f-06 on rabbits, as well as the results of Commission testing on the basis of the farm «Zhaksylyk» of the Zhambyl district of Almaty region.

For «Polyvalent inactivated vaccine against dermatomycosis of agricultural and carnivorous animals», a registration certificate for the number of RK-VP was obtained-1-3458-17 dated October 30, 2017, as well as the patent of the Republic of Kazakhstan (No. 32633, Byul. No.1. from 08.01.2018) on the mass production of the vaccine.

Key words: vaccine, inactivation, strains, immunogenicity, efficacy, rabbits, cattle.

Introduction. Dermatomycosis (trichophytosis, microsporia, favus) is a particularly dangerous skin disease belonging to a large group of fungal infections found between the skin and the hair of animals. Scientific specialists have been struggling with this disease in Kazakhstan and abroad for decades to the present.

Trichophytosis of cattle is a widespread disease and is a chronic form of infection. Trichophytosis leads cattle farms (farmers, private farms) to large economic losses, which is due to the loss of animal

weight (often in young animals), reduces the value and quality of raw leather, as well as costs for therapeutic and quarantine measures.

In connection with the increase in the number of young cattle and other animal species, you can often find this disease among young animals in an associated form. The reason for the more frequent occurrence of trichophytosis and microsporia is excessive humidity of the room, not timely implementation of veterinary and sanitary measures, unsanitary conditions of animals, as well as untimely planned disinfection work, etc.

In the Republic of Kazakhstan, the livestock of cattle is more than 7 million heads, and the number of cattle purchased from further countries grows every year. The main customers for the company's products may be government agencies, including the Ministry of Agriculture of the Republic of Kazakhstan and the veterinary pharmacy network. According to the Statistics Committee, in Kazakhstan there are 7 613,9 thousand heads of cattle, 2 862,6 thousand heads of horses and 337,8 thousand heads of camels [1, p.1].

Due to the increase in livestock numbers in the Republic of Kazakhstan, the number of animals suffering from ringworm infection is also increasing. Currently, ringworm (trichophytosis and microsporia) infection of animals is on the list of especially dangerous diseases, prevention, diagnosis and elimination of which is carried out at the expense of budget funds (Appendix 5 to the Decree of the Minister of Agriculture of the Republic of Kazakhstan dated October 30, 2014 No. 7-1 / 559) [2, p.1]. For the treatment and prevention of trichophytosis and microsporia of animals in the Republic of Kazakhstan, government purchased expensive vaccine preparations brought from abroad.

Today, in the Republic of Kazakhstan, we have developed mono-, bivalent, and trivalent vaccines against fungal infection. The requirement of today is the creation of an improved polyvalent vaccine.

The messages of the first President of the Republic of Kazakhstan N. Nazarbayev said, that the state, together with business, should find strategic niches in international markets and promote domestic products, and also instructed to increase labor productivity in the agro-industrial sector and export of processed agricultural products at least 2,5 within 5 years 5 times. According to the state program of development of the agroindustrial complex until 2021, in Kazakhstan is planned to increase the number of cattle to 15 million heads [3, p.1].

Therefore, we offer domestic universal complex polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals. Farmers, breeding and private individual livestock farms are in acutely need of this vaccine.

The implementation and use of this vaccine will contribute to obtaining competitive and safe products (milk, koumiss, shubat, beef, horse meat and leather raw materials of animals) for export.

The advanced polyvalent inactivated vaccine against dermatomycosis of agricultural and carnivorous animals recommended by us is made from highly immunogenic vaccine strains isolated in the territory of the Republic of Kazakhstan and cheaper compared to similar foreign vaccines.

Produced domestic polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals has two important properties, it is prophylactic and therapeutic, as well as a triple prophylactic dose of vaccine is inoculated depending on the extent of infection of animals with trichophytosis and microspory. The domestic biological product in the Republic of Kazakhstan is primarily aimed at the treatment and prevention of trichophytosis of cattle and other animal species.

The proposed "Polyvalent inactivated vaccine against dermatomycosis of agricultural and carnivorous animals" is made from local highly immunogenic vaccine strains circulating in the Republic of Kazakhstan. All vaccine strains, in particular: *Trichophyton mentagrophytes* F-01, *Trichophyton verrucosum* F-02, *Trichophyton sarkisovii* F-03, *Trichophyton verrucosum* variantis autotrophycum F-04, *Trichophyton equinum* F-05, *Microsporum canis* F-06 deposited in RGC «Republican collection of microorganisms», Central Museum of Microorganisms.

Analogues of the proposed vaccine are available in Russia (PolyvakTM), which is manufactured using chemical preparations for inactivating pathogens of trichophytosis and microsporia.

Until today, live fungal vaccines LTF-130, the multivalent Vermet vaccine have been successfully used in Russia [4, p.1-5; 5, p. 11-13], as well as inactivated PolivakTM vaccines and other drugs for ringworm of agricultural and carnivorous animals, where spore cells of aleria (macro- and microconidia) are responsible for immunogenicity. Small doses of these vaccines prepared from microides, have preventive properties, double and triple doses treat diseases caused by dermatophytes.

Our improved vaccine is made using ultrasound, that is, after vaccination on the animal's body and the environment has no negative effect.

This vaccine passed state approbation at the RGC "Committee for Veterinary Control and Supervision" of the Ministry of Agriculture of the Republic of Kazakhstan; registration certificate No. RK-VP-1-3458-17 dated October 30, 2017 and Patent RK No. 32633 dated January 8, 2018 have been received. 100% ready for mass production and sale [6, p.1; 7, p.1].

Research methods. Experimental studies were conducted in the laboratory of Mycology LLP "IP Vetinvest".

The selection of pathological material was taken according to the standard technique. For microscopic examination, the material was placed in a sterile Petri dish according to GOST 1770-74, which was placed on a dark background. Using a dissection needle or an ocular scalpel, the thickened root parts of the coat of wool covered with white bloom and skin flakes were cut off. The length of the segments prepared for microscopy is 1-2 mm. Then several pieces of wool and flakes (8-10) were transferred onto a glass slide according to State standard 9284-75 in a drop of 10-15% caustic potassium or sodium according to State standard 4328-77, slightly heated over the burner flame until a white halo appeared around the drop, after which a drop of a 50% aqueous solution of glycerin according to State standard 6259-75 was added and the preparation was covered with a cover glass. We looked through a microscope at first with an $\times 10$ lens, and then $\times 40$. Microscopy according to State standard 8074-82 of infected hair from animals with dermatomycosis, found a case of arthrospores around and at the root of the hair, arranged in chains, inside the hair or the development of mycelium around and inside the hair, in the skin scales noted mycelium of the fungus and arthrospor chain. The size of arthrospores in the material from animals with trichophytia was 2.5-7 microns.

Detection of fungal elements in the material (arthrospores, mycelial filaments) makes it possible to make a preliminary diagnosis for trichophytosis. To accurately determine the type of pathogen, it is necessary to isolate the fungus in a pure culture. To obtain a pure culture of the fungus and determine its type, root parts of wool and skin scales were sown.

Sowing of bacteria was made on the following nutrient medium:

- wort agar and meat-peptone-glycerin agar with 2% glucose (MPGA) according to State standard 17206-96; State standard 20730-75 - for isolation of pathogen cultures from cattle, zebu, buffaloes, sheep, and reindeer;

- wort agar and agar Saburo - for isolating test tubes from horses, fur-bearing animals, rabbits, guinea pigs, mouse-like rodents, cats and dogs.

In case of primary isolation of dermatophytes, in order to suppress the growth of concomitant microflora, antibiotics were added to the indicated media: penicillin with streptomycin ($100-200 \text{ U} / \text{cm}^3$). For each examination, 7-10 tubes were taken. Tubes were kept in a thermostat at a temperature of 28°C for up to 30 days.

In case of severe contamination of the material by extraneous microflora, they resorted to treatment with 70° - ethyl alcohol. The formation of colonies of dermatophytes of different types occurred at different times - on the 10-14th day after the start of growth for *Tr. mentagrophytes*, *Tr. equinum*, *M. canis*, *M. equinum* and on the 20-21st day for *Tr. verrucosum*. Description of cultures was carried out in this period.

By the nature of the growth of pathogens of dermatomycosis of animals on nutrient media of MPGA, wort, agar Saburo and according to microscopy with a description of cultural and morphological properties and with the use of determinants [8, p.192-195; 9, pp.54-60; 10, pp. 283-285; 11. p. 126-130] conducted the identification of selected cultures of dermatophytes.

The concentration of the fungus (microconidia) in 1 cm^3 was determined in a Goryaev chamber using a microscope.

For the luminescent analysis of the pathological material used a PRK-2, PRK-4, L-60 mercury-quartz lamps and other luminescent devices equipped with a Vud filter were used. The material was viewed 10-15 minutes after the lamp was turned on; material (wool, skin scales) infected with microsporia pathogens emitted a characteristic emerald green glow. During trichophytosis the affected coat does not have such an emerald glow.

Sterility was established according to the requirements of State Standard 28085-89, appearance - by visual examination.

The study of the basic biological properties of cattle trichophytosis and identification were carried out according to P.N. Kashkin [8, p.192-195] and others, L.G.Ivanova [9, p.54-60; 10, p. 283-285], D. Sutton, A. Fotergilla et al. [11. p. 126-130], Z.R. Khismatullina [12. p. 32-36], T.N. Titova [13. p.320], T.N. Titova [14. p. 38], F.I. Yazdanov [15. p.49].

Results of the research A technology has been developed for the production of experimental polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals. Positive results were obtained in rabbits on the effectiveness of a polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals. The preventive and therapeutic efficacy of a polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals are shown in the 1st and 2nd tables.

Table 1 – Preventive efficacy of a polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals in rabbits

№ I/o	Name of vaccine	Number of animals (heads)	Vaccine dose (cm ³)	Order of vaccination	Dose of infection (LD ₅₀), 2 million/cm ³	The results of research		Efficiency of vaccine (%)
						Sick	Not sick	
1	Polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals	10	1,0	2 times with an interval of 14 days	5LD ₅₀	–	10	100
2	Control group (saline)	10	1,0	2 times with an interval of 14 days	5LD ₅₀	10	–	–

Table 2 – Therapeutic efficacy of a polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals in rabbits

№ I/o	Name of vaccine	Number of animals (heads)	Vaccine dose (cm ³)	Order of vaccination	The results of research		Efficiency of vaccine (%)
					sick	cured	
1	Polyvalent inactivated vaccine against ring-worms of agricultural and carnivorous animals	10	2,0	2 times with an interval of 14 days	–	10	100

From dates on tables 1 and 2 it can be seen that the prophylactic and therapeutic efficacy of this vaccine after 21 days after the last immunization of rabbits of the control group, simultaneously infecting 18 daily homologous (6 types of dermatophytes) epizootic culture (5LD₅₀) in the dorsal part (5 x 5 cm²) by rubbing in. Experimental and control groups of animals were observed for 20 days. In the experimental group of rabbits, no clinical signs of trichophytia and microsporia were observed, and in the control group, apparent clinical signs of trichophytia and microsporia were observed.

To determine the therapeutic dose of polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals were tested on patients in the control group of rabbits with a double prophylactic dose of the polyvalent inactivated vaccine intramuscularly 2 times with an interval of 14 days. The therapeutic efficacy of the vaccine was observed 28-30 days after the last immunization. Crusts falling away, healing and growth of new hair were observed in specially infected areas.

According to the results of scientific studies, it was found that a polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals is recommended for determining preventive and therapeutic efficacy under production conditions.

In the period from May 10 to June 30, 2017, the immunogenic activity of a polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals was determined (series No. 1, control No. 1, manufactured by Vetinvest Innovative Enterprise LLP on February 25, 2017).

Testing of the “Polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals” was tested by the commission under production conditions at the «Zhaksylyk» farm in the Zhambyl district, Almaty region.

Immunization of animals was carried out according to the schedule on calves aged from 6 to 12 months in the amount of 30 animals. At the time of the experiment, all calves suffered from moderate trichophytosis; the vaccine was injected intramuscularly twice with an interval of 14 days at a dose of 4,0 cm³. After the last vaccination on the 25-30th day, we conducted a survey of the results of the work done, made up a commission Report of approbation. By the end of the observation period, all calves had recovered from a fungal infection, in particular from cattle trichophytosis.

As a result of the research, it was established that the immunogenic activity of the veterinary drug «Polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals» was 100%, that is, healing and spontaneous falling off of crusts, flakes and the growth of new hair occurred on the places of dermatophytic foci.

There are commission reports on the results of a positive decision of vaccine tested in sick conditions and contingently healthy with trichophytosis of calves of different ages.

Regulatory and technical documentation has been prepared for the developed polyvalent inactivated vaccine. A patent of the Republic of Kazakhstan was received for a polyvalent vaccine against dermatomycosis of agricultural and carnivorous animals, as well as a registration certificate.

Conclusion. «Polyvalent inactivated vaccine against ringworms of agricultural and carnivorous animals» in a prophylactic dose protects, and the therapeutic dose of the vaccine cures patients with ringworm, it has immunogenic activity and is commission recommended for veterinary use.

A regulatory and technical documentation was prepared for the developed polyvalent inactivated vaccine (ST LLP 1603400169-001-2016), which was approved by the Director of Vetinvest Innovative Enterprise LLP and approved by the acting Chairman of the State Institution «Committee for Veterinary Control and Supervision» Ministry of Agriculture of the Republic of Kazakhstan on December 23, 2016. Patent of the Republic of Kazakhstan (No. 32633, Bul. No. 1. dated January 8, 2018), as well as registration certificate No. 30 RC-VP-1-3458-17, was received for the «Polyvalent inactivated vaccine against dermatomycosis of agricultural and carnivorous animals». 30 October, 2017.

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АУЫЛ ШАРУАШЫЛЫҚ ЖӘНЕ ЕТ ҚОРЕКТІ ЖАНУАРЛАРДЫҢ ДЕРМАТОМИКОТОЗЫНА ҚАРСЫ КӨПВАЛЕНТТІ ИНАКТИВИРЛЕНГЕН ВАКЦИНАНЫҢ АЛДЫН АЛУ ЖӘНЕ ЕМДІК ТИІМДІЛІГІ

Аннотация. Қазақстан Республикасында ірі қара мал басы 7 000 000 бас құрайды. Жыл сайын алыс шетелден сатып алынатын мал басының саны өсуде. Статистика комитетінің мәліметтері бойынша Қазақстанда 7 613,9 мың бас ірі қара, 2 862,6 мың бас жылқы және 337,8 мың бас түйе бар. [1, б.1].

Қазіргі уақытта саңырауқұлақ жұқпасы ауыл шаруашылығы және ет қоректілер арасында кең таралған. Бұл індет соңғы уақытта ассоциацияланған түрде кездеседі. Сондықтан ауыл шаруашылығы және ет қоректі жануарлардың дерматомикоздарын (трихофития мен микроспория) алдын алу және емдеу үшін жануарлардың әр бір түрлерін аталмыш аурулардан сақтау және қолданыстағы вакциналық препараттарды жетілдіру, елдің ұсақ және ірі фермерлік мал шаруашылықтарын дамыту үшін қажетті шарт болып табылады.

Жануарлардың дерматомикоздық індетін алдын алу, балау және оның жойылуы бюджет қаражаты есебінен жүзеге асырылатын аса қауіпті аурулар тізімінде тұр (Қазақстан Республикасы Ауыл шаруашылығы министрінің 2014 жылғы 30 қазандағы №7-1/559 бұйрығына сәйкес 5-қосымша) [2, б.1]. Қазақстан Республикасында жануарлардың трихофитиясы мен микроспориясын емдеу және алдын алу үшін мемлекеттік сатып алу бойынша шетелден әкелінген қымбат вакциналық препараттар сатып алынады.

Қазіргі уақытта біз ауыл шаруашылығы және ет қоректілер жануарларының дерматомикоздарына қарсы жетілдірілген поливалентті инактивирленген вакцина дайындау технологиясын әзірледік, ол аурудың алдын алу (емдеу) мәселелерін шешуге мүмкіндік береді, бұл Қазақстан Республикасындағы дерматомикоз бойынша эпидемиологиялық жағдайдың жақсаруына ықпал етеді және сайып келгенде жоғары санитарлық сапалы қауіпсіз мал шаруашылығы өнімдерін (сүт, сиыр еті және тері) алуға ықпал етеді.

Макалада *Trichophyton mentagrophytes* F-01, *Trichophyton verrucosum* F-02, *Trichophyton sarkisovii* F-03, *Trichophyton verrucosum variantis autotrophycum* F-04, *Trichophyton equinum* F-05, *Microsporum canis* F-06 жергілікті вакциналық штамдарынан дайындалған ауыл шаруашылық және ет қоректі жануарлардың дерматомикоздарына қарсы көп валентті инактивтендірілген вакцина қояндарға, сондай-ақ Алматы облысы Жамбыл ауданының «Жақсылық» шаруа қожалығының базасында комиссиялық апробациядан өткізу нәтижелері жарияланған.

«Ауыл шаруашылығы және ет қоректі жануарлардың дерматомикоздарына қарсы көп валентті инактивтендірілген вакцинасынаға» тіркеу куәлігі (№ҚР-ВП-1-3458-17) 30 қазан 2017 жылы және жаппай өндіріске шығару үшін Қазақстан Республикасының патенті (№32633, Бюл.№1. 08.01.2018 ж.) алынды.

Түйін сөздер: вакцина, инактивация, штамдар, иммуногендігі, тиімділігі, қояндар, ірі қара мал.

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ПРОФИЛАКТИЧЕСКАЯ И ТЕРАПЕВТИЧЕСКАЯ ЭФФЕКТИВНОСТЬ ПОЛИВАЛЕНТНОЙ ИНАКТИВИРОВАННОЙ ВАКЦИНЫ ПРОТИВ ДЕРМАТОМИКОТОЗА СЕЛЬСКОХОЗЯЙСТВЕННЫХ И ПЛОТОЯДНЫХ ЖИВОТНЫХ

Аннотация. В Республике Казахстан поголовье крупного рогатого скота составляет более 7 000 000 голов. С каждым годом растет численность поголовья, закупаемые из дальнего зарубежья. По данным Комитета по статистике в Казахстане насчитываются 7 613,9 тыс. голов КРС, 2 862,6 тыс. голов лошадей и 337,8 тыс. голов верблюдов [1, с.1].

В настоящее время грибковая инфекция широко распространена среди сельскохозяйственных и плотоядных животных. Данная инфекция в последнее время встречается в ассоциированной форме. Поэтому сохранение благополучия различных видов животных и усовершенствование существующих вакцинных препаратов для профилактики и терапии дерматомикозов (трихофитии и микроспории) сельскохозяйственных и плотоядных животных, является необходимым условием для развития мелких и крупных фермерских животноводческих хозяйств страны.

Дерматомикозная инфекция животных состоит в списке особо опасных болезней, профилактика, диагностика и ликвидация которых осуществляется за счет бюджетных средств (Приложение 5 к приказу Министра сельского хозяйства Республики Казахстан от 30 октября 2014 года №7-1/559) [2, с.1]. Для лечения и профилактики трихофитии и микроспории животных в Республике Казахстан по государственным закупкам приобретались дорогие вакцинные препараты, привезенные из-за рубежа.

В настоящее время нами разработана технология изготовления усовершенствованной поливалентной инактивированной вакцины против дерматомикозов сельскохозяйственных и плотоядных животных, которая позволит решить вопросы профилактики (лечения) болезни, что повлияет на улучшение эпидемиологической обстановки по дерматомикозу в Республике Казахстан, и в конечном итоге будет способствовать получению безопасной животноводческой продукции (молоко, говядина и козье сырье) высокого санитарного качества.

В статье приведены результаты испытания поливалентной инактивированной вакцины против дерматомикозов сельскохозяйственных и плотоядных животных, изготовленной из местных высокоиммуногенных вакцинных штаммов *Trichophyton mentagrophytes* F-01, *Trichophyton sarkisovii* F-03, *Trichophyton verrucosum* F-02, *Trichophyton verrucosum variantis autotrophycum* F-04, *Trichophyton equinum* F-05, *Microsporum canis* F-06 на кроликах, а также результаты комиссионной апробации на базе крестьянского хозяйства «Жақсылық» Жамбылского района Алматинской области.

На «Поливалентную инактивированную вакцину против дерматомикозов сельскохозяйственных и плотоядных животных» получено регистрационное удостоверение за № РК-ВП-1-3458-17 от 30 октября 2017 года, а также патент Республики Казахстан (№32633, Бюл.№1. от 08.01.2018 г.) на массовое производство вакцины.

Ключевые слова: вакцина, инактивация, штаммы, иммуногенность, эффективность, кролики, крупный рогатый скот.

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