



## Characteristics of the *Trichophyton mentagrophytes* F-01 Strain Used for the Manufacture of Biologics against Bovine *Trichophyton*

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### Abstract

According to the Committee on Statistics, there are 7,613.9 thousand heads of cattle in Kazakhstan. Currently, the 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 (*Trichophyton* and *Microsporum*) of agricultural and carnivorous animals is a necessary condition for the development of small and large farm livestock farms in our country.

Dermatomycosis infection of animals is on the list of particularly dangerous diseases, the prevention, diagnosis and elimination of which is carried out at the expense of budget funds. For the treatment and prevention of trichophytosis 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 domestic polyvalent inactivated vaccine against dermatomycosis of agricultural and carnivorous animals, which will solve the issues of prevention (treatment) of the disease, which will improve the epidemiological situation for dermatomycosis in the Republic of Kazakhstan, and ultimately will contribute to the production of safe animal products (milk, beef and leather raw materials) of high sanitary quality.

Using the *Trichophyton mentagrophytes* F-01 vaccine strain, a domestic live and "Polyvalent inactivated vaccine against *Trichophyton* in agricultural and carnivorous animals" was developed.

The vaccine strain *Trichophyton mentagrophytes* F-01 was obtained by step-by-step selection based on sporulation of fast-growing colonies with active accumulation of microconidia. The resulting vaccine strain differs from epizootic strains in weak virulence, high sporogenicity, and intramuscular activity. Currently, the highly immunogenic strain *Trichophyton mentagrophytes* F-01 is used for the manufacture of biological preparations against *Trichophyton* in farm animals.

**Keywords:** Vaccine; Inactivation; Strain; Immunogenicity; Rabbits; Cattle

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Received Date: 21 Jun 2022

Accepted Date: 02 Aug 2022

Published Date: 10 Aug 2022

#### Citation:

Abdiramanova BA, Umizhanov M, Imanbaev AA, Omarbekova GK, Bakirov NZ. Characteristics of the *Trichophyton mentagrophytes* F-01 Strain Used for the Manufacture of Biologics against Bovine *Trichophyton*. *World J Surg Surgical Res.* 2022; 5: 1397.

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### Introduction

In the Republic of Kazakhstan, the number of cattle is more than 7,000,000 heads. Every year, the number of livestock purchased from foreign countries is growing. According to the Committee on Statistics, 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].

Currently, the 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 *Trichophyton* and *Microsporum* of agricultural and carnivorous animals is a necessary condition for the development of all types of farm livestock farms in our country.

*Trichophyton* is a particularly dangerous skin disease that belongs to a large group of fungal infections that occur between the skin and hair of animals. Scientists in Kazakhstan and abroad have been fighting this disease for decades and to this day (Figures 1-4).

Bovine *Trichophyton* is a widespread disease and refers to a chronic form of infection. Trichophytosis leads cattle farms (farm, peasant and private) to large economic losses, which is composed of weight loss of animals (more often in young animals), reduces the value and quality of raw leather, as well as the cost of therapeutic and quarantine measures.

Due to the increase in the number of young cattle and other animal species, this disease can often be found among young animals in an associated form. The reason for the more frequent occurrence of *Trichophyton* and *Microsporium* is excessive humidity in the room, not timely implementation of veterinary and sanitary measures, unsanitary conditions for keeping animals, as well as untimely scheduled disinfection work, etc.

For the treatment and prevention of *Trichophyton* and *Microsporium* of animals in the Republic of Kazakhstan, expensive vaccine preparations imported from abroad were purchased through public procurement [2].

To obtain high-quality meat and milk by cattle farms of the Republic of Kazakhstan, it is necessary to prevent skin diseases that spoil the quality of raw leather and the resulting products. In the future, in order to obtain competitive and safe products (milk, beef and leather raw materials) for export and import, it is necessary to prevent such an infectious disease as *Trichophyton*.

For the prevention and treatment of bovine *Trichophyton*, our country has developed an inactivated vaccine against bovine *Trichophyton*, as well as a live, inactivated polyvalent vaccine against dermatomycosis of agricultural and carnivorous animals.

In the messages of the President of the Republic of Kazakhstan, Nazarbayev NA, it is 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 complex and export of processed agricultural products at least 2.5 times within 5 years. According to the state program for the development of the agro-industrial complex until 2021, it is planned to increase the number of cattle in Kazakhstan to 15 million heads [3].

There are no analogues of the proposed vaccine, but there are in Russia (Polyvac), which is made with the use of chemical preparations for inactivating the pathogens of *Trichophyton* and *Microsporium*.

To date, Russia has successfully used live fungal vaccines LTF-130, polyvalent vaccine «Vermet» [4-6], as well as inactivated vaccines «Polyvac™» and other drugs against dermatomycosis of agricultural and carnivorous animals, where the immunogenicity is responsible for the spore cells of aleurium (macro - and micro-conidia). Small doses of these vaccines, prepared from Aleyria, have preventive properties, and double and triple doses treat diseases caused by dermatophytes.

Therefore, we offer a domestic inactivated polyvalent vaccine against dermatomycosis of agricultural and carnivorous animals (Figure 5).

Our advanced vaccine is made using ultrasound, that is, after vaccination; it does not have a negative harmful effect on the animal's body and the environment. This vaccine has passed the state approbation in the RSU "Committee of Veterinary Control and Supervision" of the Ministry of Agriculture of the Republic of

Kazakhstan, received the registration Certificate No. RK-VP-1-3458-17 dated 30.10.2017 and Patent of the Republic of Kazakhstan No. 32633 dated 08.01.2018. The developed vaccine is 100% ready for mass production and sale. Currently, the vaccine strains *Trichophyton mentagrophytes* F-01 and *Trichophyton verrucosum* F-02 and others are used for the production of a vaccine against bovine *Trichophyton* [7-9]. The above-mentioned vaccine strains of the fungus, deposited in 2017 in the RSE «Republican Collection of Microorganisms», the Central Museum of Microorganisms in Astana.

## Materials and Methods

This strain was isolated from a rabbit with *Trichophyton*, belonging to a private individual resident of the Ili district (Baisek village) of the Almaty region and obtained by directional step selection.

The identification of the fungus strain was carried out by the main morphological, cultural and biological properties, according to the determinant of pathogenic, toxigenic and harmful to human's fungi, as well as a number of other researchers [10-12].

After purification of the obtained pat-material (mushroom culture), it was sown in a culture medium (suslo-agar) and placed in a thermostat for 18 days at a temperature of 28°C. Further, from one colony of the grown fungus, repeated replanting was done for 7 times. By the method of breeding, clones of the named fungus were isolated, which grew in Petri dishes on suslo-agar, which gave the greatest growth during further sowing. The accumulation of biomass was determined in the Goryaev chamber according to the generally accepted method. The vaccine strain *Trichophyton mentagrophytes* F-01 was obtained by directed stepwise selection based on the spore formation of fast-growing colonies with active accumulation of microconidia. The resulting strain differs from epizootic strains in weak virulence, high sporogenicity and activity when administered intramuscularly.

## Results and Discussion

This strain is characterized by the following characteristics. Young cells of the fungus strain are gram-positive; the mycelium is smooth, branching, 0.6 to 3 microns wide, there are spiral and ring-shaped endings of hypha.

The strain of the fungus *Trichophyton mentagrophytes* F-01 has an unpainted smooth, branched, septic mycelium that breaks up into fragments; terminal hypha sometimes form spirals (up to 5 whorls).

Microconidia are numerous, round, less often pear-shaped and elongated, with a diameter of 2 to 4 microns. On suslo-agar, the strain forms well-developed white, flat, mealy colonies with a smooth edge.

Macroconidia are few, with rounded ends, elongated-oval up to 45 microns long, 5-10 × 30-45 microns in size, divided from 3 to 5 cells. Chlamydospores are rare, rounded with a diameter of up to 10 microns. Arthrospores are absent.

On the suslo-agar by the 14<sup>th</sup> day, colonies are formed: White, yellowish, pinkish, flat, even, powdery, granular or velvety, the growing edge is pink or starry, the reverse side of the colony is yellowish or red-brown, and the colony covers the entire surface of the nutrient medium. Young colonies are white, turning slightly yellow with age. There are raised colonies, represented mainly by white air mycelium. The culture needs vitamins, amino acids and minerals to increase growth and spore formation.



Figure 1: Sick and conditionally ill calves with trichophytosis.



Figure 4: Black-and-white calves with trichophyton from the farm "Beibit", Fabrichny village, Almaty region.



Figure 2: Calves with trichophytosis.



Figure 5: Domestic inactivated polyvalent vaccine against dermatomycosis of agricultural and carnivorous animals.



Figure 3: 85%-90% of calves with trichophytosis in the khozyaystvo K/H "Beibit" are calves with trichophytosis.

The maximum level of spore formation on the suslo-agar is 350.0 million/cm<sup>3</sup>. The optimal growth rate at a temperature of 26°C to 28°C, pH 7.2 to 7.4. The mycelium is smooth branching with a diameter of 0.6 to 3.0 microns, there are spiral and ring-shaped endings of hypha, has whorls up to 4 to 5 times.

The enzymatic activity of the fungus in relation to proteins, carbohydrates and fats is more vivid than in other types of dermatophytes.

On the introduction of the *Trichophyton mentagrophytes* F-01 strain culture, specific agglutinins are formed in the body of rabbits in the titers 1: 40-1: 1280.

The strain of the fungus *Trichophyton mentagrophytes* F-01 is weakly reactogenic for rabbits when administered intramuscularly. In vaccinated animals, after 14 days at the injection site, a specifically localized, superficial lesion with a diameter of 1.0 cm to 2.0 cm develops, which is spontaneously cured during the next 12 to 15 days.

With a double intramuscular injection of the *Trichophyton*

*mentagrophytes* F-01 strain at a dose of  $20 \times 10^6$  microconidia, a tense immunity is created in rabbits to subsequent infection with trichophytia, lasting at least 24 months.

The strain of the fungus *Trichophyton mentagrophytes* F-01 differs from the epizootic strain in weak virulence, high sporogenicity and activity when administered intramuscularly to animals.

The main biological properties of the strain of the fungus *Trichophyton mentagrophytes* F-01 are stably preserved for 5 years in a lyophilically dried state (protective medium skimmed milk) and for more than 6 months when stored in the native state (seeded on test tubes with wort) at a temperature of 2°C to 8°C. After dissolution from the lyophilized state with a physiological 0.85% solution of sodium chloride at a pH of 7.4, the *Trichophyton mentagrophytes* F-01 mushroom retains its biological activity at a temperature of 26°C to 28°C for 6 h, and at 4°C 3 days.

Marker features of the *Trichophyton mentagrophytes* F-01 strain.

Physiological - chemoheterotroph.

Biochemical - from carbohydrates constantly and well assimilates sucrose.

Serological-in the reactions of RSC and RNG, the antibody titers range from 1: 40 to 1: 1280.

The *Trichophyton mentagrophytes* F-01 strain is pathogenic for wild, domestic and laboratory animals. It is easily inoculated when cultures and pathological material are rubbed into the skin, causing an inflammatory process with exudation, various thicknesses of crusts, torn off by animals with severe itching of lesions. Rubbing of the pathogenic strain is accompanied by lighter, transient lesions, with slight peeling and redness at the site of rubbing.

For preventive immunization, the lyophilized and inactivated

vaccine is used twice at intervals of 14 days, dissolved in a saline solution of sodium chloride (pH 7.2 to 7.4), and the sick animals are administered twice at intervals of 14 days in a doubled or tripled preventive dose.

The homogenate prepared in this way from the vaccine strain makes it possible to reliably prevent the incidence of cattle from *Trichophyton* infection.

## Conclusion

Currently, the 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 *Trichophyton* and *Microsporium* of agricultural and carnivorous animals is a necessary condition for the development of all types of farm livestock farms in our country.

Using the *Trichophyton mentagrophytes* F-01 vaccine strain, a domestic live and «Polyvalent inactivated vaccine against *Trichophyton* in agricultural and carnivorous animals» was developed.

The vaccine strain *Trichophyton mentagrophytes* F-01 was obtained by step-by-step selection based on sporulation of fast-growing colonies with active accumulation of microconidia. The resulting vaccine strain differs from epizootic strains in weak virulence, high sporogenicity, and intramuscular activity.

Currently, the highly immunogenic strain *Trichophyton mentagrophytes* F-01 is used for the manufacture of biological drugs against bovine *Trichophyton*.

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