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DERMATOMYCOSIS VACCINE

This invention relates to the preparation of vaccines and their use in preparing compositions for specifically preventing and treating dermatomycosis.

Dermatomycoses in animals are anthropozoonosic diseases of the skin and related tissue. Clinical symptoms are characterised by loss of hair in the affected area, hyperemia, scaling and asbestos-like scabs. Inflammation is often accompanied by suppuration. Dermatomycoses are often also characterised by localised infection of the skin.

Dermatomycoses in animals carry a substantial socio-economic impact. Diseased animals required prolonged treatment and can spread infection to both animals and humans.

Up till now, dermatomycoses have been treated using various types of medication applied locally to affected areas of the skin. These included the ointments YaM, Yuglon(I) and a number of other ointments, liniments, solutions and other substances containing fungicides and fungistatic agents.

The disadvantages of such treatments were:

- they were not very effective;
- they required the adoption of quarantine measures and disinfection of areas where animals were kept (rearing pens, vivaria, farms, zoos, circuses, etc.);

- they required substantial funds to be spent on drug preparations and veterinary treatment;
- they posed difficulties in immobilising the animals (for wild animals held in captivity).

Later vaccines were developed to treat trichophytosis in cattle (see USSR Patent No. 268593, 1970), fur-bearing animals and rabbits (see USSR Patent No. 835446, 1980), camels (see USSR Patent No. 1190574, 1985) and others.

A vaccine had also been developed earlier for the prevention and treatment of trichophytosis in horses: S-P-I (see USSR Patent No. 548947, 1976)(2).

The S-P-I vaccine contains the vaccinal strain Trichophyton equinum No. 2251/71, deposited with the USSR All-Union State Scientific Control Institute of Veterinary Preparations, which is cultivated in agar/wort for 20-25 days at a temperature of 26-28°C. The fungal mass is then lifted from the surface of the nutrient medium, mixed with sterile distilled water and homogenised, and the concentration of cells is brought to 600-900 million per ml. The homogenate is transferred to a separate flask and stabilised with a mixture containing 2-8% gelatine (gelatose) and 10-40% sucrose in the ratio 1:1 (±25%), then lyophilised.

For prophylactic and treatment purposes the vaccine is injected into the muscle tissue of the neck area of juvenile and mature horses in two doses of 1-2 cc, depending on the age of the horse, with an interval of 10-14 days. For therapeutic use the dosages were doubled.

Vaccines obtained using this method have the disadvantage that they do not provide immunity against microsporiae and trichophytiae caused by other agents.

It has also been noted that the areas where a live vaccine is injected may become a specific focus in which cultures of vaccinal strains may at certain times be produced. Vaccinal strains have residual virulence. Given that some species of domestic animals come into frequent contact with humans, the occurrence of such specific foci in these animals is unacceptable.

This invention now provides universal vaccines for the specific treatment and prevention of dermatomycosis in animals and corresponding immunogenic fungal strains.

This aim has been achieved by using the following fungal strains as vaccinal strains: Trichophyton verrucosum, (especially VKPGF-931/410), T. mentagrophytes (especially No. VKPGF-930/1032), T. equinum (especially No. VKPGF-929/381), T. sarkisovii (especially No. VKPGF-551/68), Microsporum canis (especially No. VKPGF-928/1393), M. canis var.obesum (especially No. VKPGF-727/1311), M. canis var. distortum (especially No. VKPGF-728/120), M. gypseum (especially No. VKPGF-729/59). Vaccines can be produced by using various combinations of antigenic material from the above strains together with a suitable carrier.

A preferred combination consists of Trichophyton verrucosum No. VKPGF-931/410, Trichophyton mentagrophytes No. VKPGF-930/1032, Trichophyton equinum No. VKPGF-551/68, Microsporum canis No. VKPGF-928/1393, Microsporum canis var. obesum No. VKPGF-727/1311, Microsporum canis var. distortum No. VKPGF-728/120 and Microsporum gypseum No. VKPGF-729/59, particularly for use in dogs, cats and horses.

Another preferred combination of vaccine strains consists of Trichophyton verrucosum No. VKPGF-931/410,

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Trichophyton mentagrophytes No. VKPGF-920/1032 and Trichophyton sarkisovii No. VKPGF-551/68, particularly for use in cattle.

The antigenic material may comprise a single antigen of at least one, and more particularly of all of the above-mentioned dermatophytes or from a plurality of antigens, provided that a sufficient immune response is stimulated to give resistance to a dermatophyte infection. Antigenic material for such a purpose can be prepared using methods known from the prior art, e.g. homogenising the above-mentioned dermatophytes or parts thereof, fractionation of dermatophyte preparations, production of antigenic dermatophyte material by recombinant DNA technology, etc. It is preferable to use homogenised culture material having 40 to 120 million, preferably 90 million microconidiae.

Suitable physiologically acceptable carriers for administering the vaccines are known from the prior art and may include buffers, gels, microparticles, implantable solids, solutions and other adjuvants.

To kill off the dermatophytes it is possible to use thiomersal ($C_9H_9O_2SNaHg$), formaldehyde or 2-propyolactone.

In order to prepare a vaccine the following procedure may be used, for example:

Cultures of the strains are homogenised in an aqueous solution containing 0.2 to 2.0% fermented, hydrolysed muscle protein (FGM-s), 5 to 12% glucose and 0.1 to 1.2% yeast extract. The concentration of the microconidiae is adjusted to 40 to 120 million per millilitre and after 1 to 2 days the mixture is inactivated, e.g. with thiomersal ($C_9H_9O_2SNaHg$) in the ratio 1:10,000 to

1:25000, or with another substance known from the prior art. The resulting suspension is packaged and is ready for use in animals.

The preparation of the vaccines, the dosage to be given and the method of administration for prevention and therapeutic treatment are explained in Examples 1 to 3.

The invention now makes it possible to prepare an inactivated vaccine which reduces the probability of reinfection and also implants a high degree of immunity. Unlike the known vaccines, the vaccine according to the invention in practice gives immunity to all important causes of dermatomycosis in animals.

Briefly, the vaccine according to the invention offers the following advantages:

- in many species of disease-prone animals it establishes immunity after intramuscular injection,
- it grants immunity against almost all causes of dermatomycosis in animals,
- it has stable immunogenic properties,
- it is easy to prepare,
- it has a complete set of exo- and endo-antigens of dermatophyte cultures and shows no side reactions in animals.

The vaccine has been successfully tested on over 500 animals of different species, predominantly in affected regions.

The strains used to produce the vaccine have been deposited at the "All-Union Collection of Pathogenic Fungi within the USSR, Ministry of Health Centre for Deep Mycoses" in Leningrad and at the "DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen",

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Mascheroder Weg 1B, W-3300 Braunschweig, Germany.

Their characteristics are set out below:

TRICHOPHYTON VERRUCOSUM, No. VKPGF-931/410

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on .... under the Serial No. ....

The strain was obtained by directed selection based on spore production and attenuation of the epizootic strain No. 410, which was identified on a deer in 1978. The strain was identified using the Rebell-Taplin key (Rebell, G., Taplin, D.: Dermatophytes, their recognition and identification, 1978) and according to Kashkin, P.N. et al. (Opredelitel patogennykh, toksigenykh vrednykh dlya cheloveka gribov, 1979).

The biological properties of the strain are described in Table 1.

Strain No. VKPGF-931/410 differs from the epizootic strain in its faster growth in nutrient medium, the enormous production of microconidiae, lower virulence and the absence of any reaction with its antigens.

TRICHOPHYTON MENTAGROPHYTES, No. VKPGF-930/1032

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on .... under the Serial No. ....

The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain

No. 1032 which was found on a horse in 1985. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 2.

The Strain No. VKPGF-930/1032 differs from the epizootic strain by its faster growth in nutrient medium, the enormous production of microconidiae, its lower virulence and the absence of any reaction with its antigens.

TRICHOPHYTON EQUINUM, No. VKPGF-929/381

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on .... under the Serial No. ....

The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 381 which was found on a horse in 1986. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 3.

Strain No. VKPGF-929/381 differs from the epizootic strain by its faster growth in nutrient medium, lower virulence and the absence of any reaction with its antigens.

MICROSPORUM CANIS, No. VKPGF-928/1393

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on .... under the Serial No. ....

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The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 1393 which was found on a cat in 1988. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 4.

Strain No. VKPGF-928/1393 differs from the epizootic strain by its faster growth in nutrient medium, its enormous capacity to carry spores, lower virulence and the absence of any reaction with its antigens.

MICROSPORUM CANIS VAR. OBESUM, No. VKPGF-727/1311

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on .... under the Serial No. ....

The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 1311 which was found on a tiger in 1986. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 5.

Strain No. VKPGF-727/1311 differs from the epizootic strain by its faster growth in nutrient medium, its enormous capacity to carry spores, its lower virulence and the absence of any reaction with its antigens.

MICROSPORUM CANIS VAR. DISTORTUM, No. VKPGF-728/120

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on .... under the Serial No. ....

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The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 120 which was found on a black panther in 1987. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 6.

Strain No. VKPGF-728/120 differs from the epizootic strain by its faster growth in nutrient medium, the enormous production of microconidiae, its lower virulence and the absence of any reaction with its antigens.

MICROSPORUM GYPSEUM, No. VKPGF-729/59

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on .... under the Serial No. ....

The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 59 which was found on a horse in 1985. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 7.

Strain No. VKPGF-729/59 differs from the epizootic strain by its faster growth in nutrient medium, the enormous production of microconidiae, the lower virulence and the absence of any reaction with its antigens.

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TABLE 1

Properties and characteristics of strains	Strain No. VKPGF-931/410	Epizootic Strain No. 410
Description of culture	Mature 10-15 day single-spore colony in agar/wort; white, velvety, convex, narrow growing margin undersurface colourless, diameter of colony 10-15 mm	Mature 25-30 day colony in agar/wort; cream; leathery/velvety, folded, undersurface colourless, diameter of colony 9-13 mm
Morphological characteristics	Mature 10-15 day culture with septate branching hyphae 1 to 3 μm wide, numerous oval, pyriform microconidiae measuring 1.5 to 3 x 3 to 5 μm, no macroconidiae	Mature 25-30 day culture with septate branching mycelium 1 to 3 μm wide, few oval, pyriform, cylindrical microconidiae measuring 1 to 3 x 3 to 7 μm, single elongate irregular shape macroconidiae with 2-5 septates measuring 3 to 5 x 25 to 30 μm, numerous arthrospores in chains 6 to 8 μm in diameter, chlamydospores 10 to 12 μm in diameter
Pathogenic characteristics	The result 12 to 15 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	Thin necrotic scabs	Dense asbestos-like scabs, possible suppuration
	spontaneous recovery after:	
	19 - 20 days	25 - 30 days

TABLE 1 (Continued)

Properties and characteristics of strains	Strain No. VKPGF-931/410	Epizootic Strain No. 410
Reaction response	Results of subcutaneous and intramuscular injection of inactivated corpuscular antigens from cultures:	
	no observed changes in clinical state	inflammation at point of injection, oedema
Antigen response	20 to 25 days after injecting rabbits with corpuscular antigens, antibody titres observed in blood serum: by Passive Haemagglutination Reaction (PHR):	
	1 : 320 to 1 : 640	1 : 320 to 1:640
	by Enzyme-linked Immunosorbent Assay (ELISA):	
	1 : 400 to 1 : 1600	1 : 400 to 1 : 1600
Immunogenic response	Results of immunisation of a group of rabbits with inactivated antigen from cultures (repeated not less than 5 times):	
	establishes immunity	establishes immunity

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TABLE 2

Properties and characteristics of strains	Strain No. VKPGF-930/1032	Epizootic Strain No. 1032
Description of culture	Mature 10-15 day colony in agar/wort; cream, velvety/powdered, flat with slight flat elevation in centre, narrow growing margin, fringed, undersurface light brown, diameter of colony 25 to 30 mm	Mature 25-30 day colony in agar/wort; white, flat, narrow growing margin, undersurface reddish-brown, diameter of colony 15 to 20 mm
Morphological characteristics	Septate, branching hyphae 1 to 3 μm wide, numerous pyriform, oval microconidiae measuring 1 to 3 x 2 to 6 μm, no macroconidiae	Septate, branching straight and spiral hyphae 1 to 3 μm wide, round, flattened pyriform micronconidiae measuring 1 to 3 x 2 to 6 μm, few elongate-oval macroconidiae with 2 to 5 septates, measuring 2 to 6 x 15 to 25 μm
Pathogenic characteristics	The result 9 to 10 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	necrotic scabs	dense, asbestos-like scabs
	spontaneous recovery after:	
	22 - 25 days	30 - 35 days

TABLE 2 (Continued)

Properties and characteristics of strains	Strain No. VKPGF-930/1032	Epizootic Strain No. 1032
Reaction response	Results of subcutaneous and intramuscular injection of inactivated corpuscular antigens from cultures:	
	no observed changes in clinical state	inflammation at point of injection, oedema
Antigen response	20 to 25 days after injecting rabbits with corpuscular antigens, antibody titres observed in blood serum by PHR:	
	1 : 320 to 1 : 640	1 : 320 to 1 : 640
	by ELISA:	
	1 : 400 to 1 : 1600	1 : 400 to 1 : 1600
Immunogenic response	Results of immunisation of a group of rabbits with inactivated antigens from cultures (repeated not less than 5 times):	
	establishes immunity	establishes immunity

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TABLE 3

Properties and characteristics of strains	Strain No. VKPGF-929/381	Epizootic Strain No. 381
Description and culture	Mature 10-15 day colony in agar/wort; white, velvety/powdery, flat with slight elevation in centre, narrow growing margin, fringed, undersurface light-brown, diameter of colony 15 to 20 mm	Mature 15 day colony in agar/wort; white, velvety, slightly creased centre, narrow growing margin, undersurface reddish-brown, diameter of colony 13 to 15 mm
Morphological characteristics	Septate, branching hyphae 1 to 3 µm wide, numerous oval pyriform microconidiae measuring 2 to 3 x 3 to 6 µm, no macroconidiae	Septate, branching hyphae with coil end, 1 to 4 µm wide, few oval, pyriform microconidiae measuring 2 to 3 x 3 to 7 µm, lobar macroconidiae measuring 4 to 7 x 15 to 25 µm
Pathogenic characteristics	The result 10 to 12 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	necrotic scabs	asbestos-like scabs
	spontaneous recovery after:	
	20 - 22 days	25 - 30 days

TABLE 3 (Continued)

Properties and characteristics of strains	Strain No. VKPGF-929/381	Epizootic Strain No. 381
Reaction response	Results of subcutaneous and intramuscular injection of inactivated corpuscular antigen from cultures:	
	no observed changes in clinical state	inflammation at point of injection, oedema
Antigen response	20 to 25 days after injecting rabbits with corpuscular inactivated antigens, antibody titres observed in blood serum:	
	by PHR:	
	1 : 320 to 1 : 640	1 : 320 to 1 : 640
	by ELISA:	
	1 : 800 to 1 : 1600	1 : 800 to 1 : 1600
Immunogenic response	Results of immunisation of a group of rabbits with inactivated antigens from cultures (repeated not less than 5 times):	
	establishes immunity	establishes immunity

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TABLE 4

Properties and characteristics of strains	Strain No. VKPGF-928/1393	Epizootic Strain No. 1393
Description of culture	Mature 10-15 day colony in agar/wort; white, fluffy, convex, narrow growing margin, arachnoid, undersurface brown, diameter of colony 30 to 35 mm	Mature 15 day colony in agar/wort; greyish-beige, arachnoid, powdery in centre, growing margin fringed, undersurface yellowish, diameter of colony 20 to 25 mm
Morphological characteristics	Septate, branching hyphae 1 to 4 µm wide, numerous pyriform, cylindrical microconidiae, few fusiform macroconidiae with 3 to 11 septates, measuring 10 to 20 x 40 to 75 µm	Septate, branching hyphae 2 to 6 µm wide, few pyriform, cylindrical microconidiae measuring 1 to 3 x 3 to 7 µm, numerous fusiform macroconidiae with 3 to 11 septates, measuring 10 to 20 x 45 to 85 µm
Pathogenic characteristics	The result 9 to 11 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	necrotic scabs	dense asbestos-like scabs
	spontaneous recovery following:	
	20 - 24 days	25 - 45 days

TABLE 4 (Continued)

Properties and characteristics of strains	Strain No. VKPGF-928/1393	Epizootic Strain No. 1393
Reaction response	Results of subcutaneous and intramuscular injection of inactivated corpuscular antigen from cultures:	
	no observed changes in clinical state	oedema and inflammation at point of injection
Antigen response	20 to 25 days after injecting rabbits with corpuscular antigens, antibody titres observed in blood serum:	
	by PHR:	
	1 : 320 to 1 :640	1 : 320 to 1 : 640
	by ELISA:	
	1 : 400 to 1 : 1600	1 : 400 to 1 : 1600
Immunogenic response	Results of immunisation of a group of rabbits with inactivated antigens from cultures (repeated not less than 5 times):	
	establishes immunity	establishes immunity

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TABLE 5

Properties and characteristics of strains	Strain No. VKPGF-727/1311	Epizootic Strain No. 1311
Description of culture	Mature 10-15 day colony in agar/wort; white, fluffy, flat with a denser central dome-like elevation, narrow growing margin, fringed, undersurface colourless with brown centre, diameter of colony 30 to 35 mm	Mature 15 day colony in agar/wort; greyish, fasciculate/arachnoid with pieces of cottony white mycelium, growing margin fringed, undersurface brownish, diameter of colony 23 to 28 mm
Morphological characteristics	Sepatate, branching hyphae 1 to 3 μm wide, numerous pyriform, oval and cylindrical microconidiae measuring 1 to 3 x 3 to 7 μm, few short, elliptical, fusiform, elongate-oval macroconidiae, some irregular shapes, less frequently "beaked", with 2 to 5 septates, measuring 11 to 20 x 25 to 50 μm	Septate, branching hyphae 1 to 5 μm wide, few oval, cylindrical microconidiae measuring 1 to 3 x 3 to 8 μm, numerous elliptical, fusiform, elongate-oval or irregularly-shaped macroconidiae with 2 to 5 septates, measuring 11 to 20 x 25 to 55 μm
Pathogenic characteristics	The result 12 to 15 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	thin necrotic scabs	dense asbestos-like scabs
	spontaneous recovery following:	
	10 - 25 days	25 - 30 days

TABLE 5 (Continued)

Properties and characteristics of strains	Strain No. VKPGF-727/1311	Epizootic Strain No. 1311
Reaction response	Results of subcutaneous and intramuscular injection of inactivated corpuscular antigens from cultures:	
	no observed changes in clinical state	inflammation and oedema at point of injection
Antigen response	20-25 days after injecting rabbits with corpuscular antigens, antibody titres observed in blood serum:	
	by PHR: 1 : 320 - 1 : 640	1 : 320 - 1 : 640
	by ELISA: 1 : 800 - 1 : 1600	1 : 800 - 1 : 1600
Immunogenic response	Results of immunisation of a group of rabbits with inactivated antigens from cultures (repeated not less than 5 times):	
	establishes immunity	establishes immunity

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TABLE 6

Properties and characteristics of strains	Strain No. VKPGF-728/120	Epizootic Strain No. 120
Description of culture	Mature 10-15 day colony in agar/wort; cream, velvety/powdery, button-like elevation in centre, narrow growing margin, finely-fringed, undersurface light-brown with dark-brown centre, diameter of colony 25 to 30 mm	Mature 15 day colony in agar/wort; light-beige, powdery, umbonate, narrow growing margin, undersurface brown, diameter of colony 18 to 20 mm
Morphological characteristics	Septate, branching hyphae 1 to 3 μm wide, numerous pyriform, oval, cylindrical microconidiae measuring 1 to 3 x 3 to 8 μm, few irregular deformed macroconidiae, distorted or fusiform with 2 to 9 septates, measuring 8 to 20 x 25 to 70 μm	Septate, branching hyphae 1 to 3 μm wide, few pyriform, oval, cylindrical microconidiae measuring 1 to 3 x 3 to 8 μm, numerous irregular deformed or fusiform macroconidiae with 2 to 9 septates, measuring 8 to 20 x 25 to 80 μm
Pathogenic characteristics	The result 12 to 15 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	thin necrotic scabs	asbestos-like scabs
	spontaneous recovery following:	
	20 - 25 days	27 - 45 days

TABLE 6 (Continued)

Properties and characteristics of strains	Strain No. VKPGF-728/120	Epizootic Strain No. 120
Reaction response	Results of subcutaneous and intramuscular injection of inactivated corpuscular antigen from cultures:	
	no observed changes in clinical state	inflammation and oedema at point of injection
Antigen response	20 to 25 days after injecting rabbits with corpuscular antigens, antibody titres observed in blood serum:	
	by PHR: 1 : 320 to 1 : 640	1 : 320 to 1 : 640
	by ELISA: 1 : 800 to 1 : 1600	1 : 800 to 1 : 1600
Immunogenic response	Results of immunisation of a group of rabbits with inactivated antigens from cultures (repeated not less than 5 times):	
	establishes immunity	establishes immunity

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TABLE 7

Properties and characteristics of strains	Strain No. VKPGF-729/59	Epizootic Strain No. 59
Description of culture	Mature 10-15 day colony in agar/wort; white, velvety/fluffy, flat with slight elevation in centre of colony, flat growing margin, undersurface brownish, diameter of colony 25 to 30 mm	Mature 15 day colony in agar/wort; cream, velvety/powdery, flat with fluffy white mycelium in centre, thin growing margin, undersurface brownish, diameter of colony 20 to 22 mm
Morphological characteristics	Septate, branching hyphae 2 to 3 μm wide, numerous oval, pyriform, cylindrical microconidiae measuring 2 to 4 x 3 to 6 μm, no or few macroconidiae, elliptical, elongate-oval shape with 2 to 5 septates, measuring 7 to 15 x 25 to 40 μm	Septate, branching hyphae 2 to 5 μm wide, few oval, pyriform, cylindrical microconidiae measuring 2 to 4 x 3 to 7 μm, numerous elliptical, stretched-oval macroconidiae with 2 to 5 septates, measuring 7 to 15 x 25 to 50 μm
Pathogenic characteristics	The result 12 to 15 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	thin necrotic scabs	dense asbestos-like scabs
	spontaneous recovery following:	
	20 - 22 days	25 - 28 days

TABLE 7 (Continued)

Properties and characteristics of strains	Strain No. VKPGF-729/59	Epizootic Strain No. 59
Reaction response	Results of subcutaneous and intramuscular injection of inactivated corpuscular antigen from cultures:	
	no observed changes in clinical state	inflammation at point of injection
Antigen response	20 to 25 days after injecting rabbits with corpuscular antigens, antibody titres observed in blood serum:	
	by PHR:	
	1 : 320 to 1 : 640	1 : 320 to 1 : 640
	by ELISA:	
	1 : 400 to 1 : 1600	1 : 400 to 1 : 1600
Immunogenic response	Results of immunisation of a group of rabbits with inactivated antigens from cultures (repeated not less than 5 times):	
	establishes immunity	establishes immunity

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The vaccine may be prepared using the strain Trichophyton sarkisovii, No. 551/68. It is described for example in USSR Patent No. 1177972 dated 08.05.1985, to which reference is made in its entirety.

This strain was also deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany on .... under reference number .....

In particular, the invention relates to the following:

- a dermatomycosis vaccine, characterised in that it contains antigenic material from at least one of the following dermatophytes:
  - Trichophyton verrucosum, particularly Trichophyton verrucosum Strain No. VKPGF-931/410 and/or
  - Trichophyton mentagrophytes, particularly Trichophyton mentagrophytes Strain No. VKPGF-930/1032 and/or
  - Trichophyton sarkisovii, particularly Trichophyton sarkisovii Strain No. VKPGF-551/68,
  - Microsporum canis, particularly Microsporum canis Strain No. VKPGF-928/1393,
  - Microsporum canis var. obesum, particularly Microsporum canis var. obesum Strain No. VKPGF-727/1311 and/or
  - Microsporum canis var. distortum, particularly Microsporum canis var. distortum Strain No. VKPGF-728/120 and/or
  - Microsporum gypseum, particularly Microsporum gypseum Strain No. VKPGF-729/59,

  and a physiologically acceptable carrier.

- a dermatomycosis vaccine, particularly as an agent for treating dogs, cats and horses, characterised

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in that it contains antigenic material from the dermatophyte strains Trichophyton verrucosum No. VKPGF-931/410, Trichophyton mentagrophytes No. VKPGF-930/1032, Trichophyton equinum Strain No. VKPGF-929/381, Trichophyton sarkisovii No. VKPGF-51/68, Microsporum canis No. VKPGF-928/1323, Microsporum canis var. obesum No. VKPGF-727/1311, Microsporum canis var. distortum No. VKPGF-728/120 and Microsporum gypseum No. VKPGF-729/59 together with a physiologically acceptable carrier.

- a dermatomycosis vaccine, more particularly as an agent for treating cattle, characterised in that it contains antigenic material from the dermatophyte strains Trichophyton verrucosum Strain No. VKPGF-931/410, Trichophyton mentagrophytes No. VKPGF-930/1032 and Trichophyton sarkisovii No. VKPGF-51/68 together with a physiologically acceptable carrier,

- a dermatomycosis vaccine as described above, characterised in that it contains 40 to 120 million, preferably 90 million microconidiae,

- a dermatomycosis vaccine as described above, characterised in that it contains thiomersal or formaldehyde or 2-propyolactone as inactivator,

- a dermatomycosis vaccine as described above, characterised in that the physiologically acceptable carrier used is an aqueous solution containing 0.2 to 2.0 percent weight of fermented, hydrolysed muscle protein, 5 to 12 percent weight glucose and 0.1 to 1.2 percent weight yeast extract,

- the dermatophyte strains:

Trichophyton verrucosum Strain No. VKPGF-931/410,
Trichophyton mentagrophytes Strain No. VKPGF-930/1032,
Trichophyton equinum Strain No. VKPGF-929/381,
Microsporum canis Strain No. VKPGF-928/1393,
Microsporum canis var. obesum Strain No. VKPGF-727/1311,
Microsporum canis var. distortum Strain No. VKPGF-728/120 and
Microsporum gypseum Strain No. VKPGF-729/59.

- a process for preparing a vaccine, characterised in that
  a. antigenic material is prepared from at least one of the following strains
     -Trichophyton verrucosum No. VKPGF-931/410,
     -Trichophyton mentagrophytes No. VKPGF-551/68,
     -Trichophyton sarkisovii No. VKPGF-551/68,
     -Microsporum canis No. VKPGF-928/1393,
     -Microsporum canis var. obesum No. VKPGF-727/1311,
     -Microsporum canis var. distortum No. VKPGF-728/120,
     -Microsporum gypseum No. VKPGF-729/59
  and
  b. the antigenic material is mixed with a physiologically acceptable carrier.

- a process as described above, characterised in that an agent, particularly thiomersal, formaldehyde or 2-propyolactone is added to inactivate the dermatophytes.

The invention is illustrated by means of the Examples which follow.

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Examples

Example 1

To produce 1 litre of vaccine, cultures are taken of the strains VKPGF-931/410, 930/1032, 929/381, 551/68, 928/1393, 727/1311, 728/120 and 729/59 and grown in agar/wort at 26°C for 15 days. Each culture is grown in 8 mattress flasks. The fungal mass is then lifted off, homogenised, placed in 200 ml of solution and added to each mixer. The solution used is an aqueous solution containing 1% fermented hydrolyzed muscle protein, 10% glucose and 1% yeast extract. The concentration of microconidiae is brought to 90 million per ml of homogenate. After 2 days 125 ml of each culture in suspension is taken and mixed in a single container. The vaccine may be prepared by mixing together various combinations of the given strains.

To inactivate the homogenate mixture, Timersal is added directly to the cell suspension in the ratio 1:20000. 50 mg of Timersal is added for every litre of homogenate. The cell mixture is allowed to stand at room temperature for 2 days.

The resulting vaccine is bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods, and kept refrigerated at 4°C.

Vaccine produced in this manner was used to immunise animals.

For prophylactic and treatment purposes the vaccine was used in the following doses (see Table 8):

TABLE 8

Animal family	Age	Place of injection	Dosage (ml) Propylactic	Dosage (ml) Treatment
Felidae medium/large cats	1 - 6 months	gluteal muscles	2 to 5	3 to 6
	6 months +	gluteal muscles	3 to 7	4 to 10
Small cats	1 - 5 months	gluteal muscles	1 to 1.5	1 to 1.5
	5 months +	gluteal muscles	1 to 2	1 to 2
Ursidae	1 - 12 months	gluteal muscles	1 to 3	3 to 5
	12 months +	gluteal muscles	3 to 5	5 to 6
Procyonidae	1 - 10 months	gluteal muscles	0.3 to 0.5	0.5
	10 months +	gluteal muscles	0.3 to 0.5	0.5 to 1.0
Viverridae	1 - 12 months	gluteal muscles	0.3 to 0.5	0.5
	12 months +	gluteal muscles	0.5 to 1.0	0.5 to 1.0
Hyaenidae	1 - 12 months	gluteal muscles	1 to 3	1 to 3
	12 months +	gluteal muscles	3 to 5	5 to 6
Canidae	1 - 10 months	gluteal and shoulder muscles	0.3 to 0.5	0.5 to 1.0
	10 months +		0.3 to 1.0	0.5 to 1.0
Equidae	3 - 12 months	neck area	0.3 to 0.5	0.5 to 1.0
	12 months +	neck area	0.5	0.5 to 1.0
Tyropodae	1 - 6 months	shoulder and neck area	3 to 5	5 to 10
	6 months +		5 to 8	7 to 10
Bovidae	1 - 12 months	neck area	3 to 5	5 to 10
	12 months	neck area	5 to 8	7 to 10

Example 2

The vaccine produced by the method described in Example 1 was tested on laboratory animals and various other animals for effectiveness in the prevention and treatment of disease. The results are given in Table 9.

Example 3

The vaccine produced by the method described in Example 1 was also used to treat animals suffering from dermatophytiae. The results are given in Table 10.

TABLE 9

Type of animals	Number	Dosage (cm^3)	Effectiveness
Rabbits	10	1.0	No symptoms of disease after infection with virulent cultures of the fungi T.mentagrophytes, T.verrucosum, T.equinum, M.canis, M.gypseum.
Dogs	5	0.3	
Domestic cats	3	1.0	
Horses	5	0.5	No dermatophytiae linked to the fungi M.canis and T.mentagrophytes after being in direct contact with diseased animals.
Ponies	3	0.3	
Camels	2	5.0	
Bears	2	3.0	
Leopards	2	4.0	
Hyenas	2	2.0	No dermatophytiae linked to the fungi M.canis and T.mentagrophytes after being in direct contact with sources of infection.
Servals	2	3.0	
Ocelots	2	2.0	
Lions	2	3.0	
Tigers	3	7.0	
Nasuas	3	0.5	
Civets	2	1.0	
Rabbits	7	1.5	No symptoms of disease after infection with virulent cultures of the fungi T.sarkisovii and M.gypseum.
Dogs	3	0.5	
Domestic cats	3	1.5	
Black panthers	2	5.0	No dermatophytiae linked to the fungi M.canis, T.mentagrophytes and T.verrucosum after being in direct contact with sources of infection.
Tigers	5	7.0	
Geese	6	3.0	
Bears	3	1.0	
Dogs	8	0.5	
Llamas	2	3.0	

TABLE 10

Type of animals	Number	Dosage (cm^3)	Effectiveness
Black panthers	5	7.0	Affected by microsporosis linked to the fungi M.canis. Recovery took place 12 to 25 days after immunisation.
Black panthers	3	4.0	
Horses	3	1.0	
Ponies	2	0.5	
Lions	3	10	
Tigers	3	10	
Dogs	4	0.5	
Bear	1	5.0	
Hyena	1	5.0	
Domestic cats	15	1.5	Affected by microsporosis linked to the fungi M.canis. Recovery took place 10 to 20 days after immunisation.
Dogs	5	0.5	
Horses	5	0.7	
Black panther	1	6.0	Affected by trichophytosis linked to the fungi T.mentagrophytes. Recovery took place 12 to 15 days after immunisation.
Red foxes	4	1.0	
Bears	2	5.0	
Mountain sheep	1	7.0	
Horses	15	1.0	Affected by microsporosis linked to the fungi M.equinum. Recovery took place 12 to 20 days after immunisation.

Bibliography

(1) Aisenberg, A.A., Noskow, A.I., Kolovatsky, P.P. "Primenenie Yuglona v Veterinarii" in Scientific and Technical Information Bulletin of the State Scientific Control Committee under the Moldavian Council of Ministers (1958), p. 88.

(2) USSR Patent No. 548947 (1976).

* C L M S *

Claims

1. Dermatomycosis vaccine, characterised in that it contains antigenic material from at least one of the following dermatophytes:

- Trichophyton verrucosum, particularly Trichophyton verrucosum Strain No. VKPGF-931/410 and/or
- Trichophyton mentagrophytes, particular Trichophyton mentagrophytes Strain No. VKPGF-930/1032 and/or
- Trichophyton sarkisovii, particularly Trichophyton sarkisovii Strain No. VKPGF-551/68,
- Microsporum canis, particularly Microsporum canis Strain No. VKPGF-928/1393,
- Microsporum canis var. obesum, particularly Microsporum canis var. obesum Strain No. VKPGF-727/1311 and/or
- Microsporum canis var. distortum, particularly Microsporum canis var. distortum Strain No. VKPGF-728/120 and/or
- Microsporum gypseum, particularly Microsporum gypseum Strain No. VKPGF-729/59

together with a suitable physiologically acceptable carrier.

2. Vaccine according to claim 1, characterised in that it contains antigenic material of the dermatophyte strains Trichophyton verrucosum No. VKPGF-931/410, Trichophyton mentagrophytes No. VKPGF-930/1032, Trichophyton equinum Strain No. VKPGF-929/381, Trichophyton sarkisovii No. VKPGF-51/68, Microsporum canis No. VKPGF-928/1323, Microsporum canis var. obesum No. VKPGF-727/1311, Microsporum canis var. distortum No. VKPGF-728/120 and Microsporum gypseum No. VKPGF-729/59.

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3. Vaccine according to claim 1, characterised in that it contains antigenic material from Trichophyton verrucosum Strain No. VKPGF-931/410, Trichophyton mentagrophytes No. VKPGF-930/1032 and Trichophyton sarkisovii No. VKPGF-51/68.

4. Vaccine according to one of claims 1 to 3, characterised in that it contains 40 to 120 million microconidiae per cc.

5. Vaccine according to one of claims 1 to 3, characterised in that it contains Thiomersal or formaldehyde or 2-propyolactone as inactivator.

6. Vaccine according to one of claims 1 to 3, characterised in that the physiologically acceptable carrier used is an aqueous solution containing 0.2 to 2 percent weight of fermented hydrolysed muscle protein, 5 to 12 percent weight of glucose and 0.1 to 1.2 percent weight of yeast extract.

7. Trichophyton verrucosum Strain No. VKPGF-931/410.

8. Trichophyton mentagrophytes Strain No. VKPGF-930/1032.

9. Trichophyton equinum Strain No. VKPGF-929/381.

10. Microsporum canis Strain No. VKPGF-928/1393.

11. Microsporum canis var. obesum Strain No. VKPGF-727/1311.

12. Microsporum canis var. distortum Strain No. VKPGF-728/120.

13. Microsporum gypseum Strain No. VKPGF-729/59.

* C L M S *

14. Use of the vaccine according to one of claims 1 to 6 in the preparation of a pharmaceutical composition for the prophylaxis and treatment of dermatomycosis.

15. Use of the vaccine according to one of claims 1 to 6 for preparing a pharmaceutical preparation for building up immunity and/or resistance to infection by dermatophytes.

16. Process for preparing a vaccine, characterised in that

a. antigenic material is prepared from at least one of the following strains
-Trichophyton verrucosum No. VKPGF-931/410,
-Trichophyton mentagrophytes No. VKPGF-551/68,
-Trichophyton sarkisovii No. VKPGF-551/68,
-Microsporum canis No. VKPGF-928/1393,
-Microsporum canis var. obesum No. VKPGF-727/1311,
-Microsporum canis var. distortum No. VKPGF-728/120,
-Microsporum gypseum No. VKPGF-729/59

and

b. the antigenic material is mixed with a physiologically acceptable carrier.

17. Process according to claim 13, characterised in that an agent, particularly Thiomersal, formaldehyde or 2-propyolactone is added in order to inactivate the dermatophytes.

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ABSTRACT

DERMATOMYCOSIS VACCINE

The present invention relates to the preparation of vaccines and their use in preparing compositions for specifically preventing and treating dermatomycoses.

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It has also been noted that the areas where a live vaccine is injected may become a specific focus in which cultures of vaccinal strains may at certain times be produced. Vaccinal strains have residual virulence. Given that some species of domestic animals come into frequent contact with humans, the occurrence of such specific foci in these animals is unacceptable.

This invention now provides universal dead vaccines for the specific treatment and prevention of dermatomycosis in animals and corresponding immunogenic fungal strains.

This aim has been achieved by using the following fungal strains as vaccinal strains: Trichophyton verrucosum, (especially VKPGF-931/410), T. mentagrophytes (especially No. VKPGF-930/1032), T. equinum (especially No. VKPGF-929/381), Microsporum canis (especially No. VKPGF-928/1393), M. canis var.obesum (especially No. VKPGF-727/1311), M. canis var. distortum (especially No. VKPGF-728/120), M. gypseum (especially No. VKPGF-729/59). Vaccines can be produced by using various combinations of antigenic material from the above strains together with a suitable carrier.

A preferred combination consists of Trichophyton verrucosum No. VKPGF-931/410, Trichophyton mentagrophytes No. VKPGF-930/1032, Trichophyton equinum No. VKPGF-551/68, Microsporum canis No. VKPGF-928/1393, Microsporum canis var. obesum No. VKPGF-727/1311, Microsporum canis var. distortum No. VKPGF-728/120 and Microsporum gypseum No. VKPGF-729/59, particularly for use in dogs, cats and horses.

Another preferred combination of vaccine strains consists of Trichophyton verrucosum No. VKPGF-931/410,

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DATE OF RECEIPT 11/12/98

Mascheroder Weg 1B, W-3300 Braunschweig, Germany.

Their characteristics are set out below:

TRICHOPHYTON VERRUCOSUM, No. VKPGF-931/410

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on 1.10.92 under the Serial No. DSM 7277.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic strain No. 410, which was identified on a deer in 1978. The strain was identified using the Rebell-Taplin key (Rebell, G., Taplin, D.: Dermatophytes, their recognition and identification, 1978) and according to Kashkin, P.N. et al. (Opredelitel patogennykh, toksigenykh vrednykh dlya cheloveka gribov, 1979).

The biological properties of the strain are described in Table 1.

Strain No. VKPGF-931/410 differs from the epizootic strain in its faster growth in nutrient medium, the enormous production of microconidiae, lower virulence and the absence of any reaction with its antigens.

TRICHOPHYTON MENTAGROPHYTES, No. VKPGF-930/1032

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on 1.10.92 under the Serial No. DSM 7279.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain

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No. 1032 which was found on a horse in 1985. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 2.

The Strain No. VKPGF-930/1032 differs from the epizootic strain by its faster growth in nutrient medium, the enormous production of microconidiae, its lower virulence and the absence of any reaction with its antigens.

TRICHOPHYTON EQUINUM, No. VKPGF-929/381

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on 1.10.92 under the Serial No. DSM 7276.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 381 which was found on a horse in 1986. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 3.

Strain No. VKPGF-929/381 differs from the epizootic strain by its faster growth in nutrient medium, lower virulence and the absence of any reaction with its antigens.

MICROSPORUM CANIS, No. VKPGF-928/1393

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on 1.10.92 under the Serial No. DSM 7281.

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The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 1393 which was found on a cat in 1988. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 4.

Strain No. VKPGF-928/1393 differs from the epizootic strain by its faster growth in nutrient medium, its enormous capacity to carry spores, lower virulence and the absence of any reaction with its antigens.

MICROSPORUM CANIS VAR. OBESUM, No. VKPGF-727/1311

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on 1.10.92 under the Serial No. DSM 7280.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 1311 which was found on a tiger in 1986. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 5.

Strain No. VKPGF-727/1311 differs from the epizootic strain by its faster growth in nutrient medium, its enormous capacity to carry spores, its lower virulence and the absence of any reaction with its antigens.

MICROSPORUM CANIS VAR. DISTORTUM, No. VKPGF-728/120

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on 1.10.92 under the Serial No. DSM 7275.

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The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 120 which was found on a black panther in 1987. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 6.

Strain No. VKPGF-728/120 differs from the epizootic strain by its faster growth in nutrient medium, the enormous production of microconidiae, its lower virulence and the absence of any reaction with its antigens.

MICROSPORUM GYPSEUM, No. VKPGF-729/59

The strain was deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany, on 1.10.92 under the Serial No. DSM 7274.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic Strain No. 59 which was found on a horse in 1985. The strain was identified as described above (Rebell, Taplin, loc. cit. and Kashkin, loc. cit.). The biological properties are described in Table 7.

Strain No. VKPGF-729/59 differs from the epizootic strain by its faster growth in nutrient medium, the enormous production of microconidiae, the lower virulence and the absence of any reaction with its antigens.

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TABLE 1

Properties and characteristics of strains	Strain No. VKPGF-931/410	Epizootic Strain No. 410
Description of culture	Mature 10-15 day single-spore colony in agar/wort; white, velvety, convex, narrow growing margin undersurface colourless, diameter of colony 10-15 mm	Mature 25-30 day colony in agar/wort; cream, leathery/velvety, folded, undersurface colourless, diameter of colony 9-13 mm
Morphological characteristics	Mature 10-15 day culture with septate branching hyphae 1 to 3 mm wide, numerous oval, pyriform microconidiae measuring 1.5 to 3 x 3 to 5 mm, no macroconidiae	Mature 25-30 day culture with septate branching mycelium 1 to 3 mm wide, few oval, pyriform, cylindrical microconidiae measuring 1 to 3 x 3 to 7 mm, single elongate irregular shape macroconidiae with 2-5 septates measuring 3 to 5 x 25 to 30 mm, numerous arthrospores in chains 6 to 8 mm in diameter, chlamydospores 10 to 12 mm in diameter
Pathogenic characteristics	The result 12 to 15 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	Thin necrotic scabs	Dense asbestos-like scabs, possible suppuration
	spontaneous recovery after:	
	19 - 20 days	25 - 30 days

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TABLE 2

Properties and characteristics of strains	Strain No. VKPGF-930/1032	Epizootic Strain No. 1032
Description of culture	Mature 10-15 day single-spore colony in agar/wort; cream, velvety/powdered, flat with slight flat elevation in centre, narrow growing margin, fringed, undersurface light brown, diameter of colony 25 to 30 mm	Mature 25-30 day colony in agar/wort; white, flat, narrow growing margin, undersurface reddish-brown, diameter of colony 15 to 20 mm
Morphological characteristics	Septate, branching hyphae 1 to 3 mm wide, numerous pyriform, oval microconidiae measuring 1 to 3 x 2 to 6 mm, no macroconidiae	Septate, branching straight and spiral hyphae 1 to 3 mm wide, round, flattened pyriform micronconidiae measuring 1 to 3 x 2 to 6 mm, few elongate-oval macroconidiae with 2 to 5 septates, measuring 2 to 6 x 15 to 25 mm
Pathogenic characteristics	The result 9 to 10 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	necrotic scabs	dense, asbestos-like scabs, possible supparation
	spontaneous recovery after:	
	22 - 25 days	30 - 35 days

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TABLE 3

Properties and characteristics of strains	Strain No. VKPGF-929/381	Epizootic Strain No. 381
Description and culture	Mature 10-15 day single-spore colony in agar/wort; white, velvety/powdery, flat with slight elevation in centre, narrow growing margin, fringed, undersurface light-brown, diameter of colony 15 to 20 mm	Mature 15 day colony in agar/wort; white, velvety, slightly creased centre, narrow growing margin, undersurface reddish-brown, diameter of colony 13 to 15 mm
Morphological characteristics	Septate, branching hyphae 1 to 3 mm wide, numerous oval pyriform microconidiae measuring 2 to 3 x 3 to 6 mm, no macroconidiae	Septate, branching hyphae with coil end, 1 to 4 mm wide, few oval, pyriform microconidiae measuring 2 to 3 x 3 to 7 mm, lobar macroconidiae measuring 4 to 7 x 15 to 25 mm
Pathogenic characteristics	The result 10 to 12 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	necrotic scabs	asbestos-like scabs, possible supparation
	spontaneous recovery after:	
	20 - 22 days	25 - 30 days

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TABLE 4

Properties and characteristics of strains	Strain No. VKPGF-928/1393	Epizootic Strain No. 1393
Description of culture	Mature 10-15 day single-spore colony in agar/wort; white, fluffy, convex, narrow growing margin, arachnoid, undersurface brown, diameter of colony 30 to 35 mm	Mature 15 day colony in agar/wort; greyish-beige, arachnoid, powdery in centre, growing margin fringed, undersurface yellowish, diameter of colony 20 to 25 mm
Morphological characteristics	Septate, branching hyphae 1 to 4 mm wide, numerous pyriform, cylindrical microconidiae, few fusiform macroconidiae with 3 to 11 septates, measuring 10 to 20 x 40 to 75 mm	Septate, branching hyphae 2 to 6 mm wide, few pyriform, cylindrical microconidiae measuring 1 to 3 x 3 to 7 mm, numerous fusiform macroconidiae with 3 to 11 septates, measuring 10 to 20 x 45 to 85 mm
Pathogenic characteristics	The result 9 to 11 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	necrotic scabs	dense asbestos-like scabs
	spontaneous recovery following:	
	20 - 24 days	25 - 45 days

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TABLE 5

Properties and characteristics of strains	Strain No. VKPGF-727/1311	Epizootic Strain No. 1311
Description of culture	Mature 10-15 day colony in agar/wort; white, fluffy, flat with a denser central dome-like elevation, narrow growing margin, fringed, undersurface colourless with brown centre, diameter of colony 30 to 35 mm	Mature 15 day colony in agar/wort; greyish, fasciculate/arachnoid with pieces of cottony white mycelium; growing margin fringed, undersurface brownish, diameter of colony 23 to 28 mm
Morphological characteristics	Sepatate, branching hyphae 1 to 3 mm wide, numerous pyriform, oval and cylindrical microconidiae measuring 1 to 3 x 3 to 7 mm, few short, elliptical, fusiform, elongate-oval macroconidiae, some irregular shapes, less frequently "beaked", with 2 to 5 septates, measuring 11 to 20 x 25 to 50 mm	Septate, branching hyphae 1 to 5 μm wide, few oval, cylindrical microconidiae measuring 1 to 3 x 3 to 8 mm, numerous elliptical, fusiform, elongate-oval or irregularly-shaped macroconidiae with 2 to 5 septates, measuring 11 to 20 x 25 to 55 mm
Pathogenic characteristics	The result 12 to 15 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	thin necrotic scabs	dense asbestos-like scabs
	spontaneous recovery following:	
	10 - 25 days	25 - 30 days

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TABLE 6

Properties and characteristics of strains	Strain No. VKPGF-728/120	Epizootic Strain No. 120
Description of culture	Mature 10-15 day colony in agar/wort; cream, velvety/powdery, button-like elevation in centre, narrow growing margin, finely-fringed, undersurface light-brown with dark-brown centre, diameter of colony 25 to 30 mm	Mature 15 day colony in agar/wort; light-beige, powdery, umbonate, narrow growing margin, undersurface brown, diameter of colony 18 to 20 mm
Morphological characteristics	Septate, branching hyphae 1 to 3 mm wide, numerous pyriform, oval, cylindrical microconidiae measuring 1 to 3 x 3 to 8 mm, few irregular deformed macroconidiae, distorted or fusiform with 2 to 9 septates, measuring 8 to 20 x 25 to 70 mm	Septate, branching hyphae 1 to 3 µm wide, few pyriform, oval, cylindrical microconidiae measuring 1 to 3 x 3 to 8 mm, numerous irregular deformed or fusiform macroconidiae with 2 to 9 septates, measuring 8 to 20 x 25 to 80 mm
Pathogenic characteristics	The result 12 to 15 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	thin necrotic scabs	asbestos-like scabs
	spontaneous recovery following:	
	20 - 25 days	27 - 45 days

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TABLE 7

Properties and characteristics of strains	Strain No. VKPGF-729/59	Epizootic Strain No. 59
Description of culture	Mature 10-15 day colony in agar/wort; white, velvety/fluffy, flat with slight elevation in centre of colony, flat growing margin, undersurface brownish, diameter of colony 25 to 30 mm	Mature 15 day colony in agar/wort; cream, velvety/powdery, flat with fluffy white mycelium in centre, thin growing margin, undersurface brownish, diameter of colony 20 to 22 mm
Morphological characteristics	Septate, branching hyphae 2 to 3 mm wide, numerous oval, pyriform, cylindrical microconidiae measuring 2 to 4 x 3 to 6 mm, no or few macroconidiae, elliptical, elongate-oval shape with 2 to 5 septates, measuring 7 to 15 x 25 to 40 mm	Septate, branching hyphae 2 to 5 mm wide, few oval, pyriform, cylindrical microconidiae measuring 2 to 4 x 3 to 7 mm, numerous elliptical, stretched-oval macroconidiae with 2 to 5 septates, measuring 7 to 15 x 25 to 50 mm
Pathogenic characteristics	The result 12 to 15 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit:	
	thin necrotic scabs	dense asbestos-like scabs
	spontaneous recovery following:	
	20 - 22 days	25 - 28 days

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The vaccine may be prepared using the strain Trichophyton sarkisovii, No. 551/68. It is described for example in USSR Patent No. 1177972 dated 08.05.1985, to which reference is made in its entirety.

This strain was also deposited at the DSM - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, W-3300 Braunschweig, Germany on 01.10.1992 under reference number DSM 7278.

In particular, the invention relates to the following:

- a dermatomycosis vaccine, characterised in that it contains antigenic material from at least one of the following dermatophytes:
  - Trichophyton verrucosum, particularly Trichophyton verrucosum Strain No. VKPGF-931/410 and/or
  - Trichophyton mentagrophytes, particularly Trichophyton mentagrophytes Strain No. VKPGF-930/1032 and/or
  - Microsporum canis, particularly Microsporum canis Strain No. VKPGF-928/1393,
  - Microsporum canis var. obesum, particularly Microsporum canis var. obesum Strain No. VKPGF-727/1311 and/or
  - Microsporum canis var. distortum, particularly Microsporum canis var. distortum Strain No. VKPGF-728/120 and/or
  - Microsporum gypseum, particularly Microsporum gypseum Strain No. VKPGF-729/59,

  and a physiologically acceptable carrier.

- a dermatomycosis vaccine, particularly as an agent for treating dogs, cats and horses, characterised

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in that it contains antigenic material from the dermatophyte strains Trichophyton verrucosum No. VKPGF-931/410, Trichophyton mentagrophytes No. VKPGF-930/1032, Trichophyton equinum Strain No. VKPGF-929/381, Trichophyton sarkisovii No. VKPGF-551/68, Microsporum canis No. VKPGF-928/1393, Microsporum canis var. obesum No. VKPGF-727/1311, Microsporum canis var. distortum No. VKPGF-728/120 and Microsporum gypseum No. VKPGF-729/59 together with a physiologically acceptable carrier.

- a dermatomycosis vaccine, more particularly as an agent for treating cattle, characterised in that it contains antigenic material from the dermatophyte strains Trichophyton verrucosum Strain No. VKPGF-931/410, Trichophyton mentagrophytes No. VKPGF-930/1032 and Trichophyton sarkisovii No. VKPGF-551/68 together with a physiologically acceptable carrier,

- a dermatomycosis vaccine as described above, characterised in that it contains 40 to 120 million, preferably 90 million microconidiae,

- a dermatomycosis vaccine as described above, characterised in that it contains thiomersal or formaldehyde or 2-propyolactone as inactivator,

- a dermatomycosis vaccine as described above, characterised in that the physiologically acceptable carrier used is an aqueous solution containing 0.2 to 2.0 percent weight of fermented, hydrolysed muscle protein, 5 to 12 percent weight glucose and 0.1 to 1.2 percent weight yeast extract,

- the dermatophyte strains:

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Examples

Example 1

To produce 1 litre of vaccine, cultures are taken of the strains VKPGF-931/410, 930/1032, 929/381, 551/68, 928/1393, 727/1311, 728/120 and 729/59 and grown in agar/wort at 26°C for 15 days. Each culture is grown in 8 mattress flasks. The fungal mass is then lifted off, homogenised, placed in 200 ml of solution and added to each mixer. The solution used is an aqueous solution containing 1% fermented hydrolyzed muscle protein, 10% glucose and 1% yeast extract. The concentration of microconidiae is brought to 90 million per ml of homogenate. After 2 days 125 ml of each culture in suspension is taken and mixed in a single container. The vaccine may be prepared by mixing together various combinations of the given strains.

To inactivate the homogenate mixture, Thiomersal is added directly to the cell suspension in the ratio 1:20000. 50 mg of Thiomersal is added for every litre of homogenate. The cell mixture is allowed to stand at room temperature for 2 days.

The resulting vaccine is bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods, and kept refrigerated at 4°C.

Vaccine produced in this manner was used to immunise animals.

For prophylactic and treatment purposes the vaccine was used in the following doses (see Table 8):

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Claims

1. Vaccine, characterised in that it contains antigenic material from at least one of the following inactivated (killed) dermatophyte strains:
Trichophyton verrucosum No. VKPGF 931/410 (DSM No. 7277) or
Trichophyton mentagrophytes No. VKPGF 930/1032 (DSM No. 7279) or
Trichophyton equinum No. VKPGF 929/381 (DSM No. 7276) or
Microsporum canis No. VKPGF 928/1393 (DSM No. 7281) or
Microsporum canis var. obesum No. VKPGF 727/1311 (DSM No. 7280) or
Microsporum canis var. distortum No. VKPGF 728/120 (DSM No. 7275) or
Microsporum gypseum No. VKPGF 729/59 (DSM No. 7274).

2. Vaccine according to claim 1, characterised in that it contains antigenic material from a combination of the dermatophyte strains recited in claim 1.

3. Vaccine according to claim 1 or 2, characterised in that it additionally contains antigenic material from Trichophyton sarkisovii No. VKPGF 551/68 (DSM No. 7278).

4. Vaccine according to one of claims 1 to 3, characterised in that it contains antigenic material from the following dermatophyte strains:
Trichophyton verrucosum No. VKPGF 931/410 (DSM No. 7277) and
Trichophyton mentagrophytes No. VKPGF 930/1032 (DSM No. 7279) and
Trichophyton sarkisovii No. VKPGF 551/68 (DSM No. 7278).

5. Vaccine according to one of claims 1 to 3, characterised in that it contains antigenic material from the following dermatophyte strain:

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Trichophyton verrucosum No. VKPGF 931/410 (DSM No. 7277) and
Trichophyton mentagrophytes No. VKPGF 930/1032 (DSM No. 7279) and
Trichophyton equinum No. VKPGF 929/381 (DSM No. 7276) and
Microsporum canis No. VKPGF 928/1393 (DSM No. 7281) and
Microsporum canis var. obesum No. VKPGF 727/1311 (DSM No. 7280) and
Microsporum canis var. distortum No. VKPGF 728/120 (DSM No. 7275) and
Microsporum gypseum No. VKPGF 729/59 (DSM No. 7274).

6. Vaccine according to one of claims 1 to 5, characterised in that the antigenic material consists of inactivated pure dermatophyte cultures.

7. Vaccine according to one of claims 1 to 6, characterised in that the dermatophyte culture has been inactivated with thiomersal, 2-propyolactone or formaldehyde.

8. Vaccine according to one of claims 1 to 7, characterised in that the dermatophyte culture has been homogenised.

9. Vaccine according to one of claims 1 to 8, characterised in that it contains 40 to 120 million microconidia per ml.

10. Vaccine according to one of claims 1 to 9, characterised in that it contains an adjuvant.

11. Vaccine according to one of claims 1 to 10, characterised in that it contains a physiologically acceptable carrier.

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12. Vaccine according to one of claims 1 to 11, characterised in that it contains, as physiologically acceptable carrier, an aqueous solution containing 0.2 to 2% by weight of fermented hydrolysed muscle protein, 5 to 12% by weight of glucose and 0.1 to 1.2% by weight of yeast extract.

13. Trichophyton verrucosum No. VKPGF 931/410 (DSM No. 7277).

14. Trichophyton mentagrophytes No. VKPGF 930/1032 (DSM No. 7279).

15. Trichophyton equinum No. VKPGF 929/381 (DSM No. 7276).

16. Microsporum canis No. VKPGF 928/1393 (DSM No. 7281).

17. Microsporum canis var. obesum No. VKPGF 727/1311 (DSM No. 7280).

18. Microsporum canis var. distortum No. VKPGF 728/120 (DSM No. 7275).

19. Microsporum gypseum No. VKPGF 729/59 (DSM No. 7274).

20. Use of a vaccine according to one of claims 1 to 12 for preparing a pharmaceutical composition for the prevention and/or treatment of dermatomycoses.

21. Use of the vaccine according to one of claims 1 to 12 for preparing a pharmaceutical composition for building up immunity and/or resistance against infections by dermatophytes.

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22. Process for preparing a vaccine according to one of claims 1 to 12, characterised in that the dermatophyte culture is homogenised in an aqueous solution, incubated for about two days and then inactivated.

23. Process according to claim 22, characterised in that, after homogenisation, the mixture is adjusted to 40 to 120 million microconidia per ml.

24. Process according to claim 22 or 23, characterised in that after homogenisation the mixture is adjusted to about 90 million microconidia per ml.

25. Process according to one of claims 22 to 24, characterised in that the inactivation is carried out using thiomersal, 2-propyolactone or formaldehyde.

26. Process according to one of claims 22 to 25, characterised in that, before the homogenisation step, the culture is grown on a solid nutrient base.

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ABSTRACT

DERMATOMYCOSIS VACCINE

The present invention relates to the preparation of vaccines and their use in preparing compositions for specifically preventing and treating dermatomycoses.

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