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Development of a differential diagnostic nutrient medium for the express diagnosis of animal dermatophytosis

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Abstract. The aim of the study was to develop a differential diagnostic nutrient medium for the rapid diagnosis of animal dermatophytosis. Abroad, for these purposes, environments such as Dermatophyte Test Medium (DTM) are successfully used, but in Russia such diagnostics has not been developed. As a result of our research, we selected the optimal composition of the nutrient medium, including growth and selective supplements. DTM-Expert, a developed domestic environment for growth, indicator and selective properties, was not inferior to foreign analogues, while it was much cheaper and more ergonomic in use. The widespread introduction of the developed DTM-Expert environment will significantly simplify the diagnosis of dermatophytosis and increase its effectiveness. Also, DTM-Expert can be used to monitor the latent micronization in animals that have no clinical signs of dermatophytosis.

1. Introduction

Infections caused by dermatophyte fungi are widespread in Russia and abroad, but their diagnosis causes difficulties for both practicing veterinary specialists and employees of diagnostic laboratories.

Diagnostics of animals dermatophytosis directly depends on a number of factors: on the choice of research method, laboratory or clinic equipment, and, most importantly, on personnel qualifications. Most often, only Wood's lamp is used in veterinary clinics - the method is simple and cheap, but with low efficiency of about 30-60 % [1, 2]. PCR diagnostics is a fast and accurate method of research, but the high cost of equipment and the necessary qualifications when working with a PCR analyzer make the method less common, especially in small cities [3-5]. Mycological seeding is considered the "gold standard" for the diagnosis of dermatophytosis, but such a study can only be carried out in a laboratory [6]. The prolonged growth of dermatophytes makes it possible to make a conclusion not earlier than in 14-16 days, which significantly influences the course of treatment of a sick animal. In this regard, it is necessary to find a more reliable, cheap and fast way to diagnose dermatophytosis, which can be carried out directly at the site of patient care.

In 1969 D. Taplinetal (1969) proposed DTM medium (Dermatophyte Test Medium) [7]. Identification of dermatophytes is based on a change in pH - as the mushrooms grow, they absorb proteins and secrete alkaline metabolites, while the indicator, phenol red, changes the color of the medium from yellow to red. The environment is widely used in the practice of doctors in the diagnosis of dermatophytosis in humans. Its application does not require special conditions and skills - the environment belongs to the category of "point-of-caretest", which allows its use everywhere [8]. Following the medicine, DTM-environment began to be widely used in veterinary medicine. Today,

there are a number of foreign companies that produce ready-to-use media. Despite the advantages of imported DTM-environments, they have significant drawbacks - first of all, the high price of approximately $ 5-6 per bottle. In addition to the price, there are cases of false positives on the growth of opportunistic fungi on Wednesday.

These facts suggest that there is a need to develop a domestic nutrient medium for the rapid diagnosis of animal dermatophytosis, which is the ultimate goal of our study.

Meanwhile, this environment must meet the following requirements:

- provide growth needs of dermatophyte fungi;
- have selective properties against opportunistic fungi and bacteria;
- have indicator properties;
- have ergonomic packaging;
- be comfortable to use.

2. Materials and methods

The studies were conducted in the laboratory of mycology and antibiotics named after A Kh Sarkisov of Federal Research Center "All-Russian Research Institute of Experimental Veterinary Medicine named after K. I. Skryabin and Ya. R. Kovalenko of the Russian Academy of Sciences".

When performing the study, experimental samples of the DTM-Expert medium were used, as well as Saburo's agar, Muller-Hinton agar and, as a control sample, we used an imported commercial diagnostic DTM medium.

To create a sample of the DTM-Expert environment, we used the components described in the classical formulation of D. Taplin et al. (1969) - soy hydrolyzate, glucose, agar, phenol red, selective additives for inhibiting the growth of bacteria and mold fungi, as well as growth supplements to improve the growth energy of dermatophytes.

Test strains were M. canis V916 and T. mentagrophytes V516 dermatophyte fungi from the collection of the laboratory of mycology and antibiotics named after A Kh Sarkisov. When studying the selective properties of the medium, we used the strains of P. aeruginosa, E. coli, S. epidermidis, K. pneumoniae bacteria. Mold fungi cultures were used to test the indicator properties: Penicillium spp., Scopulariopsis spp., Cladosporum spp., Aspergillus spp. Clinical material selected from animals suspicious of dermatoftoz disease was used to test the environment. When collecting material, the method of Mackenzie was also used.

During the work, bacteriological, mycological, clinical research methods were used.

3. Research results

To assess the growth and selective properties of the diagnosticum under development, samples of four experimental series of DTM media were made; and a comparative study of their properties was carried out. The evaluation criteria were the severity and growth rate of the cultures of T. Mentagrophytes and M. canis fungi in comparison with a similar commercial medium.

The first sample of the DTM-Expert nutrient medium was made according to the classical formulation described by D. Taplin et al. Sowing test cultures for the obtained samples of the nutrient medium showed that it possesses growth-supporting and indicator properties, especially with respect to the fungus T. mentagrophytes. 5 days after sowing, a large colony was formed, while the medium acquired a pronounced red color. However, the growth of the dermatophyte fungus of M. canis species was less pronounced, the staining of the medium for 5 days was practically absent. The result obtained testified to the need to modify the classical composition of the medium and optimize the ratio of components to ensure the growth of M. canis, which is currently the most common and pathogenic species.

In connection with the results obtained, four variants of the DTM-Expert medium were made, enriched with various growth supplements: Series 1 - cysteine (the amino acid is contained in the hair and skin keratin, assimilated by keratinophilic dermatophytes); Series 2 - a complex of vitamins; Series

3 - protein-peptide complex (additional source of organic nitrogen); Series 4 - a complex of mono-and polysaccharides, as an additional source of carbon.

All samples obtained were seeded with the cultures of these fungi, taking into account the results of the growth of colonies and the severity of the colour of the medium was recorded daily for 12 days. DTM commercial medium was used as a control medium.

According to the results of the experiment, it was noted that the Series 4 enriched with a complex of mono- and polysaccharides possesses the best properties. The growth of fungi, including M. canis, was manifested for 3-4 days, clear staining appeared already for 5 days, while the size of the colonies reached 16-18 mm in diameter.

The results of a comparative study of the growth properties of the DTM-Expert nutrient medium of the four experimental series are presented in figures 1 and 2.

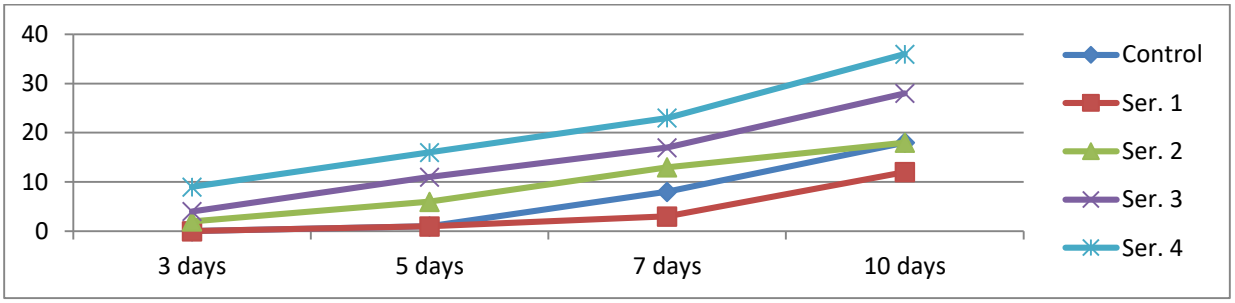

Figure 1. Growth energy of M. canis on DTM environments with various growth supplements. Control - DTM environment according to the classic no-add-ons. Axis Y - diameter of mushroom colonies, mm.

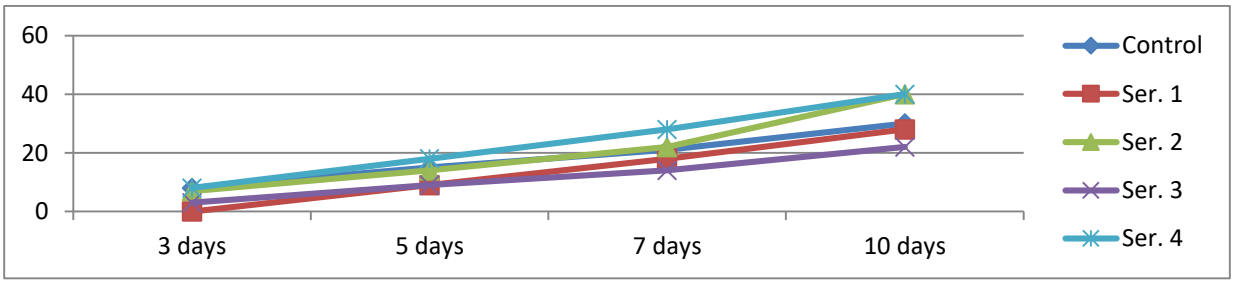

Figure 2. Growth energy of T. Mentagrophytes on DTM media with various growth supplements. Control - DTM environment according to the classic no-add-ons. Axis Y - diameter of mushroom colonies, mm.

In the study of growth properties, it was noted that the complex of mono- and polysaccharides showed itself better than others in relation to indicator properties, showing a clear change in the colour of the medium already on day 5 with the growth of M. canis, and on day 3 with growth of Tr. mentagrophytes. Due to better growth, alkalization was much more active, and the colour change was more intense. When using other additives, the growth was weak or completely absent, and the indicator properties were characterized by the absence or very slight colour change.

At the second stage of the study, it was required to confirm the specificity of the diagnostic environment only by planting mushroom museum cultures, and also to prove the possibility of using it when seeding clinical material. To do this, we carried out comparative tests by planting a freshly isolated strain of M. canis and a museum strain of M. canis V916. In the first 5 days, the strains grew almost equally. The indication appeared simultaneously on the 3rd day. On the 10th day, a faster growth was observed for the collection M. canis V916 strain. The DTM medium supplemented with D4 well supports the growth of the freshly isolated M. canis V19-18 strain (table 1).

Table 1. Comparison of growth rate of freshly isolated strain and collection M. Canis strain.

Strain	3rd day	5th day	10th day
M. canis V916	9 mm, redness of the environment	15 mm, redness of the environment	36 mm
M. canis V19-18	9 mm, redness of the environment	15 mm, redness of the environment	23 mm

The next stage of our work was the confirmation of the selective properties of DTM-Expert diagnostic environment. As selective additives, various antimicrobial preparations were introduced into the medium, designed to prevent the growth of bacteria contaminants most frequently present in the clinical material. After selecting the optimal composition of antibiotics, we conducted an experiment to study the ability of the medium to inhibit the growth of bacteria using the following strains: Ps. aeruginosa, E. coli, S. epidermidis, Kl. pneumoniae. Müller-Hinton medium served as control. It was established that all the studied bacteria were inhibited, except for Ps. aeruginosa on the DTM-Expert medium; in this case, there is a weak growth of culture and red staining of the medium, but this does not affect the diagnosis of dermatophytes.

At the final stage, the diagnostic effectiveness of the developed environment was studied by seeding clinical material from animals.

The material from animals was sown on three media - DTM-Expert was used as a test. To compare effectiveness, we used the imported DTM-environment, and as a control - Saburo environment with chloramphenicol. Sowing material was carried out by the classical method, including Mackenzie method - brushes were applied to the agar in three or four places throughout the Petri dish. Then the crops were incubated in a thermostat at a temperature of 27-29 ° C for 21 days, the control was carried out every day. On 12-16 days, when the culture reached a sufficiently large size, microscopy of the colony was performed to confirm affiliation with dermatophytes. A negative result was the lack of growth of dermatophytes on all 3 test media.

A total of 40 wool samples were examined, of which 14 were positive for dermatophytosis. The medium of the isolated dermatofite fungi was found to be one belonging to Tr. mentagrophytes species, the rest - M. canis. Acceleration of dermatophytes on all three media was 35%. On Saburo's medium, the growth of dermatophytes began on average 4-6 days after sowing, by 8-10 days the diameter of the colony was, on average, 4-6 cm. Due to the absence of inhibitors in the Saburo medium, abundant growth of contaminant fungi often occurred. Of the 14 positive samples, 4 were highly contaminated, making it difficult for dermatophytes to grow. Most commonly, contaminant fungi belonged to Penicillium, Alternaria, Scopulariopsis, Mucor genera, less commonly to Aspergillus and Cladosporum.

The effectiveness of the imported DTM-environment was 32.5%, the beginning of the growth of colonies was noted, on average, at 6-8 days, the colour change occurred on 8-9 day, sometimes on 10 days. It is worth noting that the negative aspect is the size and shape of the bottle, which makes it difficult to differentiate dermatophytes by morphology. Contaminants do not allow dermatophytes to form a visible colony, the culture itself has poor growth, and macroconidia were formed no earlier than 18 days or were not detected at all. In addition, often there was a false positive color change of the medium due to a pronounced increase in contaminants, which significantly reduces the efficiency of diagnosis of dermatophytosis.

As for the experimental “DTM-Expert” environment, its efficiency is practically not inferior to the imported environment and constitutes 30%. The growth of dermatophytes was observed on 7–9 day, and the change in the colour of the medium occurred on 8–10 day (figure 3). The number of opportunistic fungi is significantly less than in the imported medium, and there have been no cases of false positive colour change (table 2).

Table 2. Comparative results of mycological studies of clinical material using different nutrient media.

	Samples seeded	Dermatophytes selected,%	Visible growth, day	Color change, day	The pronounced growth of fungi-contaminants, %	False positive reaction, %
DTM-expert	40	30	7-9	8-10	15	0
Imported medium		32.5	6-8	8-9	22.5	23.5
Saburo		22.5	4-6	-	25	-

Figure 3. M. canis growth on DTM-Expert on the 8th day.

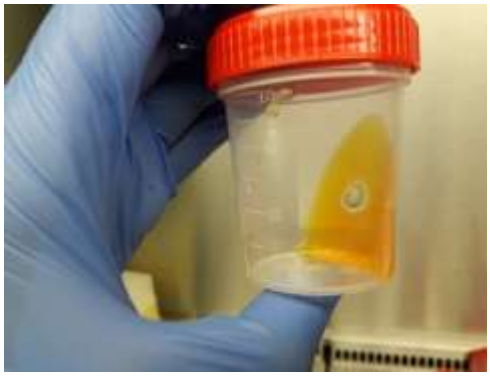

Figure 4. The growth of mold fungus on "DTM-Expert" environment on the 8th day without changing the colour of the medium.

4. Conclusions

"DTM-Expert" experimental environment is practically not inferior to foreign analogues, showing high results of detecting dermatophytes in clinical material, the effectiveness of the environment is 30%. The indicator qualities of the environment allow determining the causative agent on average on the 7-9th day of growth, while in the experiment there was not a single false positive response of the environment to the growth of extraneous microflora, which distinguishes it from the imported analogue. The developed environment has ergonomic packaging, convenient to use; and its price is at least two times lower than imported counterparts. The medium used in the above experiment is not the final option, the study to improve it will be carried out in the future. It is also planned to create detailed instructions and test the final environment with a large number of clinical material.

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