SELECTION OF HISTOPLASMA FARCIMINOSUM STRAIN TO CREATE A REMEDY FOR EQUINE LYMPHANGOITIS

ANNOTATION

Epizootic lymphangitis is a chronic infectious disease, the causative agent of which is the fungus Histoplasma farciminosum and continues to cause significant outbreaks worldwide, despite several decades of epidemiological surveillance, diagnosis and prevention. Therefore, research on the selection of strains in the composition of anti-lymphangitic agents is very relevant. The following strains of Histoplasma farciminosum fungus were used for selection: 410, T, YH, 5th, 4th, 5th, 17th, 21st, 8ZH. Microbiological studies of the studied strains were carried out with the study of cultural and morphological properties, the isolation of saccharolytic, proteolytic enzymes, the formation of catalase, oxidase, etc. To determine the biological properties of strains, agar and Saburo broth were used. In the course of the work, the cultural and biochemical properties of 9 strains of fungi were studied: 410, T, YH, 5th, 4th, 5th, 17th, 21st, 8ZH, as well as their growth on the Saburo nutrient medium. The validity of choosing a strain to create an anti-lymphangitic agent of horses should include the following parameters: the strain, regardless of the type and genus, should have homogeneous cultural and morphological properties without signs of dissociation and abundant bacterial mass yield, In total, the selected strains will be a biological reserve that can be used in the development of diagnostic tools and prevention of equine lymphangitis.

Key words: equine lymphangitis; fungus; strain; Histoplasma farciminosum; prevention, diagnosis

Кілт сөздер: жылқы лимфангиті; саңырауқұлақ; штамм; Histoplasma farciminosum; алу, диагностика.

Introduction. Epizootic lymphangitis is a chronically occurring infectious disease of ungulates characterized by inflammation of the lymphatic vessels of the skin and subcutaneous tissue with the formation of purulent foci and ulcers. Among herd horses, animals aged from 1 to 4 years are more likely to get sick. Foals are relatively resistant to the disease, especially up to 6 months of age. The disease spreads slowly [1-5]. Recently, the disease has been registered in almost all regions of our republic, which indicates the unreliability of veterinary protection in relation to certain infectious diseases [6-10]. The causative agent of the disease is the fungus Histoplasma farciminosum. The fungus penetrates into the body of horses through damaged skin (scratches, abrasions, wounds, on the minke, etc.) and is
localized in lymphatic vessels, subcutaneous tissue and the skin itself. The incubation period is from 1 to 3 months. Fungi are multicellular or unicellular non-photosynthetic (chlorophyll-free) eukaryotic microorganisms with a thick cell wall [11-18]. (Fig. 1).

Fungal cells are covered with a dense cell membrane consisting of polysaccharides (mannans, glucans, cellulose, chitin), as well as protein, lipids, etc., close to cellulose, and nitrogenous substances similar to chitin. They have a nucleus with a nuclear shell, a cytoplasm with organelles, a membrane. The cytoplasmic membrane contains glycoproteins, phospholipids and ergosterols.

Some mushrooms form a capsule. In fungi, the vegetative body (mycelium) consists of a system of thin branching threads called hyphae. Intertwining, the mycelium forms a mycelium. Hyphae are able to grow in length and develop on the surface or inside the nutrient substrate. Accordingly, the mycelium is substrate (vegetative), growing into the nutrient medium, and air. The ends of the mycelium filaments can be twisted in the form of spirals, curls, etc. [19-20].

The diagnosis was established on the basis of a complex of epizootological, clinical data, pathoanatomic changes, serological studies of blood serum samples and microscopic - exudate from purulent ulcers and the contents of thickened lymph vessels [21].

Prevention is reduced to preventive quarantine, newly imported horse stock, prevention of injuries, equipping horses from safe zones, regular examinations, preventive research, medical examination. In view of this, new trends in the creation of antifungal drugs in the field of veterinary medicine open up real prospects in the fight against this disease of horses [22].

The effectiveness of diagnostics and vaccines depends not only on the quantity and quality of the antigen, but also on the selected strains capable of accelerating the immune response of the animal's body. The strain, regardless of the type and genus, should have homogeneous cultural and morphological properties without signs of dissociation. Despite the common chemical composition, microorganisms may have differences in the nature of growth on nutrient media, the ability to break down carbohydrates, salt resistance, enzyme production, and much more. For this reason, we will study the cultural and morphological properties to select the strain of the fungus.

The vital activity of any organism, including fungi, is expressed by metabolism. This process is not feasible without the participation of enzymes. Pathogenic microorganisms produce many enzymes that freely penetrate into tissues, where they interact with extracellular and intracellular molecules. Some so-called aggression enzymes destroy the tissues and cells of the macroorganism, thereby causing the spread of pathogenic organisms and their toxins in infected tissues. Assessment of the biochemical activity of the pathogen will make it possible to correctly differentiate the species and, possibly, to identify pathogenicity factors of the studied microorganism.

In this regard, studies on the selection of strains in the composition of anti-lymphangitic agents are very relevant.

**Research material and method.** The following strains of the Histoplasma farciminosum fungus obtained from the RIBSP "collections of microorganisms" laboratory were used in the work: Histoplasma farciminosum: 410, T, YUCH, 5YU, 4YU, 5C, 17YU, 21YU, 8ZH. The appearance of the colonies was evaluated visually and microscopically according to the generally accepted method.

**Nutrient media.** To cultivate strains of Histoplasma farciminosum fungi, nutrient media were used: Saburo agar and Saburo broth.

**Mushroom cultivation.** Cultivation of H. farciminosum fungus was carried out on nutrient media in 2 ways - 1) cultivation on a dense nutrient medium Saburo agar and 2) cultivation of H. farciminosum fungus in liquid nutrient media

**Light microscopy.** To prepare the preparations, a drop of water was applied to the glass, into which the test material was placed and fixed in the flame of the burner. The preparation of dyes and staining
were carried out according to generally accepted methods. After staining, the preparations were washed and microscoped using an MBI-3 microscope using oil immersion.

*Electron microscopy.* During the electron microscopic analysis of Histoplasma farciminosum mushroom strains on the JEM 100 CX, JEOL electron microscope, their shape was determined and photographs were taken with an increase of x10000 for morphometric calculation of the size of the bacteriophage. The sizes of Histoplasma farciminosum mushroom strains were taken on the negative plates with a magnifying glass, with a division scale of 0.1 mm.

*Determination of the biochemical activity of the fungus.* Catalase test Drops of hydrogen peroxide were applied directly to the colony with a loop. When gas was formed, it was noted as a positive result.

Oxidase test. 1 drop of the reagent was applied directly to the colony under study and the appearance of dark purple staining was observed. With a positive reaction at the place of application of the culture, the tampon turns blue for 1 min, with a negative reaction, its color does not change.

The urease activity of the mushroom strains was determined by the alkalinization of a nutrient medium containing urea in the presence of an acidity indicator.

**Research results.** In the studies, a comparative study of the cultural and morphological biochemical properties of 9 strains of the Histoplasma farciminosum fungus was carried out. To study the morphology at the macro and micro levels, strains of the Histoplasma farciminosum fungus were grown on broth and Saburo agar for 7-10 days. Then the appearance of the colonies, the growth pattern and thickness of the mycelium, the size of the spores were determined.

Cultural and morphological characteristics of mushrooms "17YU", "21YU", "8ZH" Histoplasma farciminosum are shown in Figures 2-7.

Figure 2 – Cultural and morphological characteristics of the fungus "T" Histoplasma farciminosum

a) Strain "410" of Histoplasma farciminosum fungus

b) Strain "4YU" of the fungus Histoplasma farciminosum

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As can be seen from Figure 2 (a), the "T" strain has velvety towering folded colonies with gray-white grooves. On top of these colonies, the growth of white rounded colonies resembling a ball of cotton wool was noted, as well as colonies of gray-white color in the center of a yellowish color with a hairy surface, tightly adjacent to the medium. The underside is painted dark yellow. The edges of the colonies are smooth, branching filaments of mycelium form a loose plexus 2.0 - 4.0 microns thick. The spores are rounded 2.0-4.0 microns in diameter.

b) Strain "410" of the fungus Histoplasma farciminosum in a dense environment grew in the form of trapezoidal colonies of gray-white color separated by grooves. In the central part, colonies of different sizes and shapes, light brown color with a shaggy surface. The size of the mycelium is 2.0 – 3.0 microns thick, diameter 5.0 – 7.0 microns.

c) Strain "4YU" developed in the form of round colonies towering above the dense environment of Saburo ellipsoid shape, velvety, folded with small grooves of gray-white color. The edges of the colonies have a cobwebby appearance, and the underside of the colony is dark yellow, with a diameter of 2.0-3.5 microns. Young hyphae are thinner, their size is 1.2-1.3 microns. The spores are rounded 2.5-3.5 microns in diameter with a two-contour shell.

The study of electron microscopy showed that all the studied strains of spherical and round fungi with a diameter of different sizes: strain "T" of the fungus 5.0 – 6.0 microns, strain “410” – have a size of 2.0 - 4.0 microns, and strain “4YU” – 5.0 – 7.0 microns.

Figure 4 – Cultural and morphological characteristics of the fungus "5YU" Histoplasma farciminosum

a) Strain "5C" of the fungus Histoplasma farciminosum

b) Strain "YUCH" of the fungus Histoplasma farciminosum
As can be seen from Figure 3 (a), the strain "5YU" of the fungus, folded white colonies, the edges of the colony have a cobwebby appearance, other colonies are rarely located on the surface, having the appearance of cotton lumps consisting of a septic, branched ascending mycelium. Colonies on the underside are yellow. The thickness of the mycelium averaged 2.0-4.0 microns. The spores are rounded with a diameter of 2.0-4.0 microns.

b) strain "5C" of the fungus on Saburo agar grew in the form of powdery colonies of whitish-gray color on the upper side and dark yellow on the lower side, during aging the colonies acquired a brownish-red color due to sporulation. The edges of the colony are smooth, the peripheral zone is wide, with grains. The colonies of the fungus consisted of a rising septic mycelium and chains of spores, into which the filaments of the mycelium disintegrated. The thickness of the mycelium averaged 4.0 – 5.0 microns. The brownish-red color of the colonies was given by rounded spores with a diameter of 6-8 microns.

The strain "YUCH" of the fungus grew dark gray colonies, on the lower side in the passing color isolated colonies had a transparent honey color. The continuous growth of the colony is dark brown. The thickness of the mycelium is 2.0 – 4.0 microns. The spores are oval with a length of 5.0 -6.0 microns and a width of 4.0 - 4.5 microns, concave on one side.

The study of electron microscopy showed that the above strains of fungi have an oval shape and a diameter of different sizes: the strain of the fungus is “5YU” – 4.0-4.5 microns, 5C – 2.0-4.0 microns and “4YU” – 5.0-7.0 microns.
Figure 7 – Cultural and morphological characteristics of mushrooms "21YU", "8ZH" Histoplasma farciminosum

Strain "17YU" of the fungus on the nutrient medium of the colony looks velvety, towering, folded with shallow grooves of gray-white color. The edges of the column are smooth, the peripheral zone is not wide, thin branching filaments of mycelium form a loose plexus of gray color with a size of 3.0 – 5.0 microns, a diameter of 5.0 – 6.0 microns.

The strain "21YU" of the fungus has grown as a continuous growth of small, medium and granular colonies of yellowish-matte color in the upper part, and in the lower part in a passing amber-cloudy color, 5.0 – 6.0 microns thick and 2.0 – 4.0 microns in diameter.

The strain "8ZH" of the fungus consists of numerous branching septic hyphae of various thicknesses. There are various forms of thickening at the ends of the mycelial filaments. Loose velvety colonies are yellowish in the center and white along the edge, the underside of the colonies is yellow. Colonies with grooves. The edges of the colonies are smooth, the peripheral zone is narrow, thin branching filaments of mycelium form a loose plexus, the thickness of the mycelium averaged 2.5–3.7 microns. The spores are rounded with a diameter of 5.0-6.2 microns.

The study of electron microscopy showed that all the studied strains of spherical and pear–shaped fungi with a diameter of different sizes: 17u - 5.0–6.0, 21yuch - 2.0–4.0 microns and Strain "8ZH" of the fungus from 4-6 microns.

The enzymes catalase and oxidase were used to determine the biochemical activity of fungi. Catalase was determined using hydrogen peroxide as a substrate. In the presence of catalase, there was an intense release of oxygen bubbles. Strains of the Histoplasma farciminosum fungus were grown on Saburo agar for 7 days. The test results are shown in Figures 8-10, and in Table 1.

![Preparation for the experiment](image)

Table 1 – Oxidoreductase activity of Histoplasma farciminosum mushroom strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxidase</td>
</tr>
<tr>
<td>Histoplasma farciminosum “410”</td>
<td>+</td>
</tr>
<tr>
<td>Histoplasma farciminosum “T”</td>
<td>–</td>
</tr>
<tr>
<td>Histoplasma farciminosum “YUCH”</td>
<td>+</td>
</tr>
<tr>
<td>Histoplasma farciminosum “5YU”</td>
<td>+</td>
</tr>
</tbody>
</table>
Histoplasma farciminosum “4YU”  
Histoplasma farciminosum “5C”  
Histoplasma farciminosum “17YU”  
Histoplasma farciminosum “21YU”  
Histoplasma farciminosum “8ZH:”  

Notes: “+” - the presence of an enzyme  
“-” - absence of enzymatic activity

It can be seen from the data in Table 1 that all the studied strains produce oxidase and catalase under these cultivation conditions. The ability of the fungus to produce oxidase and catalase should be attributed to pathogenicity factors that allow avoiding the oxidative response of the macroorganism, which consists in the release of toxic substances: peroxide and free radicals. The visualization of the reaction is reflected in Figures 6 and 7.

Catalase activity
Strain “T”  Strain “410”  Strain “4YO”  Strain “5YU”  Strain “5C”

Figure 9 – Determination of catalase activity of Histoplasma farciminosum fungi

Oxidase activity
Strain “T”  Strain “410”  Strain “4YU”  Strain “YU”  Strain “5C”

Figure 10 – Determination of oxidase activity of Histoplasma farciminosum fungi
As can be seen from Figures 9 and 10, all the Strains studied had catalase and oxidase activity, except for Strains "T" and "21YU", oxidase negatively.

The urease activity of Strain fungi was determined by the alkalinization of a nutrient medium containing urea in the presence of an acidity indicator.

Note.: 1–"Т"; 2–“410”; 3–“4YU”; 4–“5YU”; 5–“5С”; 6–“YUCH”; 7–“17YU”; 8–“21YU”; 9–“8ZH”

Figure 11 – Urease activity of Strains of the fungus H. farciminosum

As a result of experiments, it was found that during cultivation for 1-2 days, the medium acquired a pink hue due to a change in pH.

As can be seen from Figure 11, the urease activity of Strains was different. High urease activity was noted for Strains “410”, “4YU”, “5С”, “17YU” и “21YUCH” - the leaching of the nutrient medium occurred already on the 1st day of cultivation, and by the end of the second day the entire nutrient medium was stained crimson.

The ability to ferment sugars by pathogen was studied using carbohydrate-containing media. The results of the studies showed that this microorganism does not emit carbon dioxide during the fermentation of sugars, fermentation was judged by the acidification of the medium. The results are shown in table 9.

Table 9 – Assimilation of carbohydrates by Strains by Histoplasma farciminosum mushroom

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbohydrates</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>glucose</td>
</tr>
<tr>
<td>«4YU»</td>
<td>+</td>
</tr>
<tr>
<td>«5YU»</td>
<td>+</td>
</tr>
<tr>
<td>«5C»</td>
<td>+</td>
</tr>
<tr>
<td>«YUCH»</td>
<td>+</td>
</tr>
<tr>
<td>«T»</td>
<td>+</td>
</tr>
<tr>
<td>«410»</td>
<td>+</td>
</tr>
<tr>
<td>«17YU»</td>
<td>+</td>
</tr>
<tr>
<td>«21YU»</td>
<td>+</td>
</tr>
<tr>
<td>«8ZH»</td>
<td>+</td>
</tr>
</tbody>
</table>

Notes: “+” - assimilation of carbohydrate; “-” - absence of assimilation of carbohydrate

From the data in Table 9, it can be seen that all the Strains studied fermented glucose and galactose. None of the Strains studied used lactose. The absorption of carbohydrates such as sucrose, maltose and arabinose varied depending on the Strain. The Histoplasma farciminosum fungus assimilated monosaccharides well, whereas for the fermentation of disaccharides, apparently, not all Strains studied under these cultivation conditions produced the appropriate enzymes. Strains "5C" and "YUCH" did not assimilate any of the disaccharides used in the experiment, Strains "4YU", "5YU" "T" and "8ZH" fermented sucrose and maltose. The ability of Histoplasma farciminosum fungus to ferment glucose can
also be attributed to factors indirectly determining pathogenicity, since glucose is the main energy substance of the body.

Thus, as a result of the conducted research, the actual collectible Strain Histoplasma farciminosum "T" and "8ZH" were selected as a potential candidate for the manufacture of antifungal agents, as the most viable, fast-growing, accumulating a large mushroom mass, and their biological properties were studied.

Conclusion. The development of highly effective means of prevention and diagnostics is currently one of the main tasks to ensure the biological and epizootological safety of the Republic of Kazakhstan.

Consequently, new trends in the creation of diagnostic and prophylactic drugs and new directions of research in the field of veterinary medicine open up real prospects for the development of intensive technology for the manufacture of antifungal drugs with technical and economic efficiency.

Selection of a viable, up-to-date collectible Strain Histoplasma farciminosum and study of its biological properties for the manufacture of an inactivated vaccine.

When selecting Strings as potential candidates for the preparation of antifungal agents, preference was given to certified Strains of the fungus that are relevant for Kazakhstan, that is, isolated directly from sick animals from disadvantaged farms of the republic.

For the successful cultivation of a particular microorganism, nutrient media should be close to the natural conditions of its habitat by their properties. Nutrient media rich in nutrients (pancreatic hydrolysate of fish meal; pancreatic hydrolysate of casein; yeast extract; monosubstituted sodium phosphate, glucose, microbiological agar) were used for mushroom cultivation. Saburo agar is a medium with a low pH (5.6) for cultivation.

Urease secretion is characteristic of almost all Strains. This criterion serves as a systematic feature in the identification of the species. Ureases indirectly contribute to the pathogenic action of microorganisms in the infectious process. Given the fact that the studied microorganism develops in the lymphatic and circulatory system, it was important to study its ability to ferment carbohydrates.

Thus, the growth pattern and morphology of the Strains studied are different and this criterion cannot always be used when identifying a species. It should be noted that the Strains isolated in the South Kazakhstan region ("YUCH", "4YU", "5YU") in different years differ in their morphological characteristics from each other and from the Strains isolated in other areas.

As a result of the conducted research, the actual collectible Strain Histoplasma farciminosum "T" and "8ZH" were selected as a potential candidate for the manufacture of antifungal agents, as the most viable, fast-growing, accumulating a large mushroom mass, and their biological properties were studied.

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REFERENCES


ТУЙНІН


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зерттеумен, сахаролитикалық, протеолитикалық ферменттерді оқшаулау, каталаза, оксидаза және т.б. штаммдардың биологиялық қасиеттерін анықтау үшін агар мен Сабуро сорпасының қоректік орталары пайдаланылады. Жұмыс барысында санырауқұлақтардың 9 штаммының өсіндісі және биохимиялық қасиеттері зерттелді: 410, Т, ЮЧ, 5Ю, 4Ю, 5С, 17Ю, 21Ю, 8ZH, сондай-ақ олардың Сабуро коректік ортасына қосу. Жылқылардың лимфангоитке қарсы құралын жасау үшін штаммды таңдаудың негізідігі өзге параметрлерді қамтыйын тіс: штамм түрі мен тұқымна карамастан, диссоциация белгілері және бактериялық массаның мол шығымы, біртекті морфологиялық -өсінді қасиеттеріне ие болуы тіс. Нәтижесінде таңдалған штамдар жылқы лимфангоитпен диагностикалау және алдын алу құралдарын әзірлеу кезінде пайдаланулыу мүмкін биологиялық резерв болып табылады.

РЕЗЮМЕ

Эпизоотический лимфангоит – хроническое инфекционное заболевание, возбудителем которого является гриб Histoplasma farciminosum и по-прежнему вызывающего значительные вспышки во всем мире, несмотря на несколько десятилетий эпидемиологического надзора, диагностики и профилактики. Следовательно, исследование по выбору штаммов в составе противолимфангоитных средств являются весьма актуальны. Для выбора были использованы следующие штаммы гриба Histoplasma farciminosum: 410, Т, ЮЧ, 5Ю, 4Ю, 5С, 17Ю, 21Ю, 8ZH. Микробиологические исследования изучаемых штаммов проведены с изучением культурально-морфологических свойств, по выделению сахаролитических, протеолитических ферментов, образанию каталазы, оксидазы и т.д. Для определения биологических свойств штаммов использованы питательные среды агар и бульон Сабуро. В ходе работы были изучены культуральные и биохимические свойства 9 штаммов грибов: 410, Т, ЮЧ, 5Ю, 4Ю, 5С, 17Ю, 21Ю, 8ZH, а также их рост на питательной среде Сабуро. Обоснованность выбора штамма для создания противолимфангоитного средства лошадей должна включать следующее параметры: штамм, независимо от вида и рода, должен обладать однородными культурально-морфологическими свойствами без признаков диссоциации и обильным выходом бактериальной массы, В итого выбранные штаммы, будут являться биологическим резервом, который может быть использован при разработке средств диагностики и профилактики лимфангоита лошадей.