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IDENTIFICATION OF NEW ISOLATES OF THE HORSE STRANGLES CAUSATIVE AGENT IN NORTHERN SIBERIA

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Abstract

Infectious and invasive diseases cause significant damage to the economy and decrease the productivity of horse herd farming. Equine strangles (*Streptococcus equi*) is the most economically damaging. Specific prevention of the disease widespread in Asia, Russia and CIS poses a serious problem. In this work, for the first time in the Far North, we isolated and identified three new *Streptococcus equi* strains prospective for the diagnostics and development of strangles vaccines. The study aimed to culture, identify morphologically, culturally, biochemically, and genotypically new isolates of the equine strangles causative agent for the development of vaccines. A survey of 6-10-month old Yakut and Kazakh foals (*Equus ferus caballus*) was performed in the Republic of Sakha (Yakutia) regions (the farms in Namskiy, Khangalasskiy, Amginskiy, Megino-Kangalarskiy districts and in Yakutsk) and in Kazakhstan in 2015-2017. In total, 63 collected biospecimens included 45 nasal discharges (27 from diseased and 18 from healthy foals), 7 submandibular lymph node abscesses, and 11 parenchymal organs. The infectious agents were isolated and identified by 16S rDNA genotyping using PCR and based on biochemical traits. Morphological and cultural properties were studied using meat peptone broth (MPB) added with 1 % glucose and 10 % horse blood serum and on meat peptone agar (MPA) with 1 % glucose and 10 % horse blood serum or 5 % defibrinated horse blood. Pus swabs and preparations of liquid and agar cultures stained by the Gram procedure were investigated with a light microscope. Biochemical properties were studied by plating on MPA with 40 % bile, 6.5% saline MPA, agar with sodium azide, and Giss's medium with glucose, lactose, mannitol, maltose, sucrose, sorbitol, and dulcite. The isolates were biochemically assigned to species using API 20 Step strips (an API test system, bioMerieux, France). The virulence of the isolates (LD_{50}) was assessed on white mice challenged subcutaneously with 0.2-0.5 cm^3 of 1-day suspensions (1×10^3 to 1×10^9 CFU per mouse). Genotyping was performed with specific primers Seel-F 5′-CGGATACGGTGAT-GTTAAAGA-3′ and Seel-R 5′-TTCCTTCCTCAAAGCCAGA-3′. The *Streptococcus equi* 16S rRNA gene was sequenced for six isolates of strangles streptococcus, of which three we suggest for the development of strangles vaccines. Polymerase chain reaction with specific primers serves as the most reliable and fastest method for identifying strangles streptococcus. Based on genotyping data and the cultural, morphological and biochemical properties, the *Streptococcus equi* H-5/1 isolate belongs to the *Streptococcaceae* family, *Streptococcus* genus, *Streptococcus equi* ssp. *equi* and corresponds to the typical characteristics of the species. The nucleotide sequence of the 16S rRNA gene fragment of the isolate H-5/1 after sequencing was deposited in the NCBI GenBank database (MW486609). The *Streptococcus equi* H-5/1 strain was deposited in the All-Russian State Collection of Microorganism Strains Used in Veterinary Medicine and Animal Husbandry (VGNKI, registration number VKSHM-B-141P, certificate of deposit dated May 22, 2018), and patent for invention No. 2703485 ("A strain of bacteria *Streptococcus equi* used for the production a vaccine against strangles") dated 10/17/2019 was received. The new *Streptococcus equi* strains we described here hold promise in the developing strangles vaccines. Note, *Enterococcus faecales*, *Streptococcus piogenes*, toxigenic and mold fungi *Aspergillus* and *Mucor* genera were also iso-

lated from foals with clinical sings of equine strangles. Our findings attract attention to these microorganisms possibly involved in the development of equine strangles in young horses, which should be accounted in diagnostics of this pathology.

Keywords: equine strangles, streptococcus, *Streptococcus equi*, biochemical traits, genotyping, Yakut horses, Kazakh horses, Siberia, the Far North, bacterial infections

Horse breeding is a key sector of animal husbandry in many countries. However, it is hindered by such factors as horse infections, of which equine strangles (caused by *Streptococcus equi*) is most common [1, 2]. The pathogen is believed to have changed only slightly over the past 700 years, although region-specific traits of strains are not denied [3]. In Russia, the disease has been reported in the Novosibirsk Region, Krasnoyarsk Territory, Altai Territory, Republics of Khakassia, Sakha (Yakutia), and Altai, and Irkutsk Region, as well as in Kazakhstan, Kyrgyzstan, and Mongolia [4–6].

In the Republic of Sakha (Yakutia), with its emphasis on horse breeding in herds, the incidence of the disease in follows is 57.8–62.7%; associated mortality rates reach 4.0% to 22.0% depending on how the epizootic process unfolds [6]. The incidence and mortality rates in the Republic of Kazakhstan are 30.1–46.7% and 16.0–28.3%, respectively [7]. In 2017–2020, the disease was registered in 42–59% of all Yakut foals (unpublished in-house data).

The pathogen needs to be studied in different regions if effective methods for diagnosis, prevention, and treatment of equine strangles are to be developed [8]. In all countries where equine strangles occurs, including Kazakhstan [7], Kyrgyzstan [9], the Netherlands [10], the Arab Republic of Egypt [11], Russia [6, 12], Korea [13], and Brazil [14], isolation and identification of *Streptococcus equi* rely on its morphological, cultural, and biochemical properties. With the advancement of molecular genetics, reports have surfaced that the pathogen can be identified by polymerase chain reaction (PCR) using species-specific genes that are potentially involved in forming the virulent phenotype of *Streptococcus equi* [2, 15–18]. Strains thus obtained are used to diagnose the disease and to manufacture vaccines in the United States, Kazakhstan, and the Netherlands [7, 8, 10].

Strangles pathogen has been reported to cause arthritis in goat kids [19], abortions in mares [6]. It can be isolated in clinically healthy horses, too, since they are its carriers [6, 13, 14]. The *Streptococcus equi* strain H-34 was earlier deposited by the authors hereof at the Russian State Center for Animal Feed and Drug Standardization and Quality (VGNKI, Moscow); it was proposed for the production of equine strangles vaccines and diagnostic streptococcal serum, Serogroup C [6]. The strain has since been un-deposited due to loss of specific antigenicity, i.e., the Russian Federation currently has no vaccine against equine strangles. Other countries produce a variety of vaccines, none of which is registered in Russia [7, 8, 10].

For this research, three new isolates of *Streptococcus equi* have been isolated and identified in the Far North for potential use in the diagnosis of, and development of vaccines against, equine strangles.

The goal hereof was to isolate and study new isolates of *Streptococcus equi* and to identify them in terms of morphological, cultural, biochemical, and molecular genetic properties.

Materials and methods. Biological samples were collected in 2015–2017 at farms in the Republic of Sakha (Yakutia: Namskiy, Khangalasskiy, Amginskiy, Megino-Kangalarskiy Districts, and the City of Yakutsk), as well as in Kazakhstan. In total, 63 collected samples taken from 6-10 months old Yakut and Kazakh horses (*Equus ferus caballus*) included 45 nasal discharge samples (27 from diseased and 18 from healthy foals), 7 samples of submandibular lymph node abscess,

and 11 parenchymal organs (sampled from strangles-claimed foals).

Preseeding treatment was applied before bacteriological studies. Discharge samples on swab probes, pieces of organs, and lymph nodes were submersed for 5 minutes in sterile saline, then treated with 70° alcohol and further washed 2-3 times in saline.

The isolates were studied morphologically and culturally by seeding in meat-peptone broth (MPB): 1% glucose and 10% horse serum; and on meat-peptone agar (MPA): 1% and 10% horse serum or 5% defibrinated horse blood. Pus smears and culture preparations were fixed and Gram-stained. The isolates were studied biochemically by seeding on MPA (40% bile) or on 6.5% salt MPA or on sodium azide agar and Hiss medium with glucose, lactose, mannitol, maltose, sucrose, sorbitol, and dulcite. The cultures were incubated in a TS-1/80 SPU thermostat (JSC Smolenskoye SKTB SPU, Russia) at 37 °C over 18-48 h.

The isolated cultures were identified taxonomically per Bergey's Manual of Systematic Bacteriology [20]; the research team also followed guidelines on laboratory diagnosis of strangles, staphylococcosis, and streptococcosis [21, 22]. To identify the species of the isolates by biochemical indicators, API 20 Step strips in the API test system (bioMerieux, France) were used.

Samples of streptococcal isolates were transplanted from semiliquid agar into tryptic soy broth and cultured at 37 °C for 48 hours, then transplanted into dishes with blood agar and onto a dense medium (Colombian agar) with potassium tellurate. The dishes were placed in a thermostat at 37 °C for 24 h. Cultural and morphological properties, the type of hemolysis, the presence of catalase activity were determined, and smears were prepared. Agar cultures were transplanted onto Colombian agar and cultured for 24 h at 37 °C; a suspension in saline was prepared from these cultures. Bacterial suspension in the amount of 0.1-0.2 cm^3 was placed in each well of the plate. Reagents and sterile liquid paraffine were added. The cultures were incubated for 5-24 h at 38 °C. The results were reported in the Reaction Interpretation Table of the test system and processed in Microsoft Excel.

To assess streptococcal virulence, the research team used white outbred mice ($n = 70$) of either gender, 5-8 weeks of age, 18−20 g of weight. The mice were injected subcutaneously with a suspension of live bacterial cells of the streptococci: 0.2-0.5 cm^3 (1×10^3 to 1×10^9 CFUs per specimen). The virulent activity of the LD_{50} isolates was tested by the Kerber method as modified by Ashmarin and Vorobyov [23].

To isolate DNA [24], 1.5 ml of liquid bacterial culture was centrifuged on a multifunctional 5804R unit (Eppendorf, Germany) until a dense precipitate was formed, which was then dissolved in 567 μl of TE buffer; 30 μl of 10 % SDS and 3 μl of proteinase K (20 mg/ml) were added until reaching the final concentration of 100 rg/ml. One-hour incubation at 37 °C would render the solution viscous, a sign of cellular wall destruction. One hundred microliters of 5 M NaCl was added to the solution, followed by thorough stirring and further addition of 80 μl of a CTAB/NaCl solution; 10-min incubation at 65 °C was performed afterwards. Then an approximately equal volume of a 24:1 chloroform-isoamyl alcohol mixture was added to the solution, which was then stirred thoroughly and centrifuged for 5 minutes; the supernatant was transferred into a clean tube, and an equal quantity of a 25:24:1 phenol-chloroform-isoamyl alcohol mixture was admixed; the final mixture was stirred again and centrifuged for 5 minutes.

For DNA precipitation, the supernatant was transferred into a clean tube and isopropanol was added in a quantity equal to ~ 0.6 times the amount of the original supernatant. The DNA precipitate was washed with 70% ethanol, dried

and dissolved in 30 rL of TE buffer. The quality and concentration of the resulting DNA preparation were assessed on a NanoPhotometer™ P330 unit (Implen, Germany).

To genotype the *Streptococcus* isolates, PCR was conducted using strain-specific primers Seel-F 5′-CGGATACGGTGATGTTAAAGA-3′ and Seel-R 5′-TTCCTTCCTCAAAGCCAGA-3′ [17, 18] (CFX-96, amplifier, Bio-Rad, USA) and a qPCRmix-HS LowROX kit (Evrogen, Russia). PCR and reaction medium were configured in accordance with the kit manufacturer's manual. Each reaction mixture (25 μl) contained: 5 μl of qPCRmix-HS LowROX (the master mix), 2 μl of each primer (1.0 μl), and 18 μl of nuclease-free water. PCR parameters: 5 min at 95 °C; 35 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C. PCR products were visualized by electrophoresis in 1.5% agarose gel containing ethidium bromide as an intercalating dye; electric field strength was 6 V/cm. The sensitivity and reliability of the PCR method for identification of *Streptococcus equi* had been confirmed by studies carried out by the Probiotics Quality and Standardization Department, Russian State Center for Animal Feed and Drug Standardization and Quality (Moscow).

The 16S rRNA gene was sequenced at Genomika (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk). A ~ 1500 bps amplicon was produced using specific 16S primers (27F: 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′) for each isolate in a preparative amount, then purified by sorption on magnetic particles (Agencourt AMPure XP, Beckman Coulter, Inc., USA). Sanger sequencing was run on an ABI 3130xl Genetic Analyser (Applied Biosystems, USA) per the manufacturer's standard protocols. Nucleotide sequences were BLAST-analyzed (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify homology with nucleotide sequences deposited in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Fig. 1. Mucopurulent nasal discharge of a Yakut breed foal (*Equus ferus caballus*) showing clinical signs of stranglers (Khangalasski District, village Nemyugyuntsy, 2017).

Results. Forty cultures similar to *Streptococcus equi* in cultural, enzymatic, and hemolytic properties were sampled in 2015-2016 from horses showing clinical signs of stranglers, see Fig. 1. Eleven samples were found to be contaminated with toxigenic and mold fungi of the genera *Aspergillus* and *Mucor*.

Pre-treatment was very effective in suppressing the growth of concomitant microflora, resulting in easier and faster isolation of a pure culture. Intravital diagnosis of equine stranglers remains challenging, especially in the Far North. Poor low-quality diets were found to contribute to the fungal infection of the respiratory tract in animals, which is concurrent with equine stranglers.

All isolated cultures were preliminarily classified as *Streptococci*. They grew well in 10% horse serum MPB, as well as on 10% horse serum 1% MPA. Culturing

in 1% glucose serum MPB exhibited uniform clouding of the medium with white sediment that would go up and form pigtail-like pieces, which is typical of *Streptococcus equi*. On 1% glucose serum agar, some cultures produced tiny dewdrop-like colonies; convex shiny white colonies that had smooth edges and matte merged convex colonies were observed as well.

Subsequently, 7 isolates grew on 1% glucose, 40% bovine bile MPA, as well as on 6.5% sodium chloride MPA; they would ferment glucose, lactose, maltose, mannitol, sorbitol, and dulcite, whereby they produced acid without gas. These isolates exhibited near-bottom growth of dense white sediment in broths. On agars, they would form mucous white colonies. Black colonies grew on the potassium tellurate medium. No catalase activity. Optical microscopy revealed short cocci chains in broth cultures, grape cluster-like cocci in Gram-stained preparations of agar-grown cultures.

Identified species of streptococci isolated from equine strangles-affected Yakut and Kazakh foals (*Equus ferus caballus*) (Republic of Sakha—Yakutia, Republic of Kazakhstan, 2015-2017)

Isolate	Identification	
	PCR genotyping	biochemical traits
P1	*Streptococcus equi*	–
N-1 kaz	*Streptococcus equi*	*Streptococcus equi*
N-5/1	*Streptococcus equi*	*Streptococcus equi*
N-12-3	*Streptococcus equi*	*Streptococcus equi*
7-3	*Streptococcus equi*	–
1-3	*Streptococcus equi*	–
H-34	–	*Enterococcus faecalis*
Khatas-3	–	*Streptococcus pyogenes*
ChG	–	*Enterococcus faecalis*
4g	–	*Enterococcus faecalis*
MK 1/1	–	*Enterococcus faecalis*
YuG	–	*Enterococcus faecalis*
SM	–	*Enterococcus faecalis*
M	–	*Enterococcus faecalis*

N o t e. Dashes denote isolates not studied by the corresponding method.

Biochemically, culturally, and morphologically, the isolates 4g, MK 1/1, YuG, SM, H-34, and M were classified as *Enterococcus faecalis*, whereas Khatas-3 was classified as *Streptococcus piogenes*, see Table. At this point, it was impossible to isolate *Streptococcus equi,* as the samples were highly contaminated with toxigenic and mold fungi, as well as with an association of *Streptococci*. These findings bring attention to the role of *Enterococcus faecales* and *Streptococcus piogenes* in the progression of the respiratory infectious process.

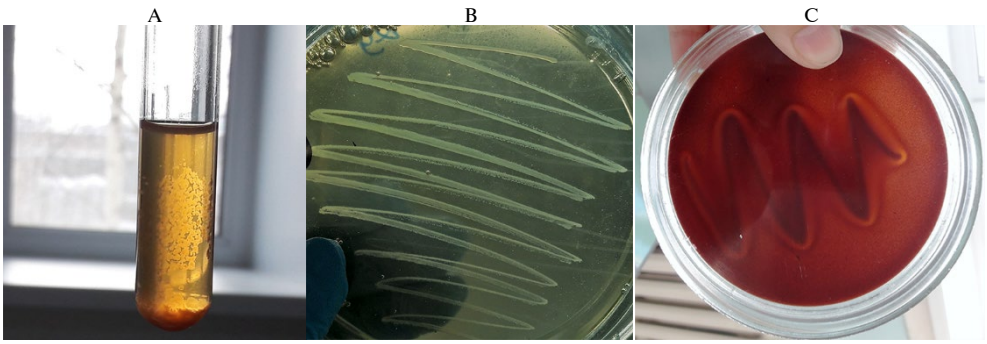

Fig. 2. Growth of *Streptococcus equi* isolated from the nasal cavity of stranglers-affected Yakut foals (*Equus ferus caballus*) in meat-peptone broth (A), **on meat-peptone agar** (B), **on blood agar (β-hemolysis)** (C) (Republic of Sakha—Yakutia, Republic of Kazakhstan, 2017)

In October and November 2017, new streptococcal isolates were sampled

from stranglers-affected foals; in broth, these exhibited near-wall growth and formed white flaky sediment, see Fig. 2, A, whereas on agar, they formed tiny dewdrop-like translucent colonies, see Fig. 2, B. β-hemolysis was observed on blood agar, see Fig. 2, B. Isolates would not grow on media containing bile or sodium chloride; they fermented glucose and lactose, whereby acid would be produced without gas; however, they would not ferment mannitol, sorbitol, or dulcite. Microscopy of Gram-stained preparations revealed long convoluted chains of gram-positive cocci in a one-day broth culture, short chains, paired or singular cocci in agar-grown colonies. Morphologically, culturally, and biochemically, these isolates were identical to *Streptococcus equi*.

Testing of 40 isolates identified the nucleotide sequence of the *Streptococcus equi* rRNA S16 gene in six of them (P1, H-1 kaz, H-5/1, H-12-3, 1-3, 7-3), all sampled from foals with clinical signs of equine stranglers. Three (H-1 kaz, H-5/1, and H-12-3) were further selected for vaccine development. The H-34 strain, deposited earlier as *Streptococcus equi* (All-Russian Collection of Cell Cultures, Virus Strains, Microbes and Micropathogens, VGNKI, Moscow; no longer deposited) was no longer compliant with the requirements and specifications of the test system (see Table) due to multiple repeated inoculations in long-term storage. Molecular genetic typing confirmed the analyzed nucleotide sequence of the H-5/1, H-12-3, H-1 kaz strains to be 100% identical to the nucleotide sequence of a *Streptococcus equi* 16S rRNA gene fragment. *Streptococcus equi* H-5/1, being the most promising strain, passed certification testing at the Probiotics Quality and Standardization Department of VGNKI (All-Russian Collection of Microorganism Strains).

Streptococcus equi H-5/1 produced β-glucosidase, β-glucuronidase, and leucinaryl amidase; it fermented esculin, starch, and glycogen producing acid without gas; it did not ferment ribose, arabinose, mannitol, sorbitol, trehalose, inulin, or raffinose, nor would it produce acetoin or hydrolyzed hippurate.

For white mice, LD_{50} of *Streptococcus equi* H-5/1 was 1×10^2 CFU/specimen in parenteral administration.

Sequencing of the 16S rRNA gene for the H-5/1 isolate yielded the following nucleotide sequence:

TGCAAGTGGAACGCACAGATGATACGTAGCTTGCTACAATTATCTGTGAGTCGCGAACG
GGTGAGTAACGCGTAGGTAACCTAGCTTATAGCGGGGGATAACTATTGGAAACGATAGC
TAATACCGCATAAAAGTGGTTGACCCATGTTAACCATTTAAAAGGAGCAACAGCTCCACT
ATGAGATGGACCTGCGTTGTATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCGAC
GATACATAGCCGACCTGAGAGGGTGAACGGCCACACTGGGACTGAGACACGGCCCAGA
CTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGGAACCCTGACCGAACA
ACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGAGAAGAACAG
TGATGGGAGTGGAAAGTCCATCATGTGACGGTAACTAACCAGAAAGGGACGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGT
AAAGCGAGCGCAGGCGGTTTGATAAGTCTGAAGTTAAAGGCAGTGGCTTAACCATTGTA
TGCTTTGGAAACTGTTAAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCG
GTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTA
ACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGCTGAGTGCTAGGTGTTAGGCCCTTTCCGGGGCTTAGTGCCGGAGCT
AACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACC
TTACCAGGTCTTGACATCCCGATGCTATTCTTAGAGATAAGAAGTTACTTCGGTACATTG
GAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC
GCAACGAGCGCAACCCTTATTGTTAGTTGCCATCATTAAGTTGGGCACTCTAGCGAGACT
GCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACC
TGGGCTACACACGTGCTACAATGGTTGGTACAACGAGTCGCAAGCCGGTGACGGCAAG
CTAATCTCTGAAAGCCAATCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTC
GGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTA
CACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCGTTA
AGGAGCCAGCCGC

BLAST analysis showed this sequence to match the 16S rRNA genes in two *Streptococcus equi* subsp. *equi* strains: NCTC9682 (MW486609) and ATCC 39506 (MW486609).

Therefore, the cultural, morphological, and biochemical properties, as well as the genetic markers identified the H-5/1 isolate as *Streptococcus equi* ssp. *equi* (fam. *Streptococcaceae*, genus *Streptococcus*); the isolate matched the type characteristics of this species. Nucleotide sequence of the 16S rRNA gene fragment, H-5/1 isolate, was deposited in NCBI GenBank after sequencing (MW486609). Based on the findings, the *Streptococcus equi* H-5/1 was deposited in the All-Russian State Collection of Microorganism Strains Used in Veterinary Medicine and Animal Husbandry (VGNKI, registration number VKSHM-B-141P, certificate of deposit dated May 22, 2018); novelty confirmed by Invention Patent No. 2703485 ("A strain of bacteria *Streptococcus equi* used for the production a vaccine against strangles") dated Oct 17, 2019.

Streptococcus equi H-5/1 is now used as a production strain to make immunobiologicals for the prevention of equine strangles in the Russian Federation. *Streptococcus equi* H-1 kaz isolated from a sick Kazakhstani horse could be used to develop a vaccine for use in herd breeding in Kazakhstan. Many researchers note the need to isolate region- or country-specific *Streptococcus equi* strains to make vaccines [1, 2, 8].

The presence of toxigenic and mold fungi in the nasal discharge of respiratory disease-affected fowls could be due to the prevalence of microscopic fungi (genera *Aspergillus and Mucor*) in the vegetation of the winter-grazing pastures in Yakutia, especially in rainy years [25, 26].

The findings presented herein are in line with what Dauvillier *et al.* reported [27], where they noted the presence of various fungi in equine respiratory tracts and pointed out the need to study their role in the etiology of respiratory diseases in horses and humans. Alarming is the fact that *Streptococcus zooepidemicus*, which causes severe illness in humans, is excreted from the nasal cavity in healthy and sick horses alike [28], although the present study did not detect these microorganisms. Any effort to diagnose and prevent strangles must be adjusted for the possibility of the horse contracting rhinopneumonia or influenza. It is crucial to investigate the relations between bacterial, viral, and fungal infections [6, 29, 30], which could be the line of future research.

Our data suggests Sakhabaktisubtil, a probiotic developed by the Yakutsk Agricultural Research Institute, could effectively treat mycotoxicosis [25]. Newly devised stranglers prevention methods must provide room for using antifungals and immunomodulators to improve immunological reactivity.

The findings reported herein confirm that isolating *Streptococcus equi* from sick or infected animals by standard culturing methods (and developing a vaccine) could be challenging due to low sensitivity coupled with the complexity of the process [31], especially under the extreme conditions of herd breeding. PCR is the fastest, most sensitive and specific method for diagnosis of equine stranglers and identifying *Streptococcus equi* [15, 16, 31].

Thus, the research team was able to isolate, identify by their cultural, enzymatic, and hemolytic properties, and genotype new isolates of *Streptococcus equi*, which could be used to diagnose this pathology, as well as to develop a stranglers vaccine. The *Streptococcus equi* H-5/1 strain has been deposited in the All-Russian State Collection of Microorganism Strains Used in Veterinary Medicine and Animal Husbandry (VGNKI, registration *number VKSHM-B-141P*, certificate of deposit dated May 22, 2018). Invention Patent No. 2703485 ("A strain of bacteria *Streptococcus equi* used for the production a vaccine against strangles") dd. Oct

17, 2019 was thereby granted. *Enterococcus faecales*, *Streptococcus piogenes* cultures, toxigenic and mold fungi of the genera *Aspergillus* and *Mucor*. were isolated from foals exhibiting clinical signs of stranglers. Efforts to diagnose and prevent stranglers and other respiratory diseases in foals should be adjusted for the presence of these microorganisms as possible pathoflora.

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