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ВЕТЕРИНАРНОЕ ОБЕСПЕЧЕНИЕ ТАБУННОГО КОНЕВОДСТВА: ПРОБЛЕМЫ И ПУТИ РЕШЕНИЯ

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Представлены разработанные иммунобиологические препараты для профилактики инфекционных болезней лошадей табунного содержания. Инфекционные болезни лошадей регистрируют почти во всех странах Азии, Европы и Америки. В России, Казахстане, Киргизии и Монголии наиболее распространенными болезнями являются мыт, ринопневмония и сальмонеллезный аборт, которые наносят значительный экономический ущерб табунному коневодству. В зарубежных странах разрабатывают и производят различные моновакцины, большинство из них применения в России не имеют. Нами установлено одновременное заболевание кобыл ринопневмонией и сальмонеллезом, а также молодняка лошадей ринопневмонией, сальмонеллезом и мытом. В связи с этим актуальной проблемой становится разработка моновакцин и комбинированных иммунобиологических препаратов. Иммуногенность инфекционных вакцин следует усиливать иммуномодуляторами, особенно в экстремальных условиях ведения табунного коневодства. В период проблемы появления антибиотикорезистентных штаммов микроорганизмов следует разрабатывать альтернативные антибактериальные средства: пробиотики и бактериофаги. Выделены, идентифицированы и депонированы во всероссийских коллекциях восемь новых штаммов микроорганизмов. Разработан пробиотик Сахабактисубтил, который используют для профилактики и лечения дисбактериозов, микотоксикозов, мыта, лептоспироза, обеззараживания навоза. Разработаны новые эффективные вакцинные препараты и закваска «Якутская кумысная», которые можно успешно использовать для повышения продуктивности коневодства в других субъектах Российской Федерации и за рубежом. Подготовлена научно-техническая документация на инактивированную вакцину против ринопневмонии, комбинированные двух- и трехвалентные вакцины. Разработанные иммунобиологические препараты защищены 48 патентами на изобретение и могут быть использованы в других странах.

Ключевые слова: лошади, вакцина, пробиотик, кумыс, бактериофаги

VETERINARY SUPPORT OF THE HERD HORSE BREEDING: PROBLEMS AND SOLUTIONS

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Developed immunobiological preparations for the prevention of infectious diseases in herd horses are presented. Infectious diseases of horses are registered in almost all countries in Asia, Europe and America. In Russia, Kazakhstan, Kyrgyzstan, and Mongolia, the most common diseases are strangles

of horses, rhinopneumonia, and salmonella abortion, which cause significant economic damage to herd horse breeding. Various monovaccines are developed and produced in foreign countries, most of them have no use in Russia. We have found a simultaneous disease of mares with rhinopneumonia and salmonellosis, as well as young horses with rhinopneumonia, salmonellosis and strangles. In this regard, the development of monovaccines and combined immunobiological preparations is becoming an urgent problem. Immunogenicity of infectious vaccines should be enhanced by immunomodulators, especially in the extreme conditions of herd horse breeding. At a time when antibiotic-resistant strains of microorganisms are a problem, alternative antibacterial agents should be developed: probiotics and bacteriophages. Eight new microbial strains have been isolated, identified, and deposited in all-Russian collections. Sahabaktisubtil probiotic has been developed, which is used to prevent and treat dysbacteriosis, mycotoxicosis, strangles, leptospirosis, decontamination of manure. New effective vaccine preparations and "Yakutskaya Koumissnaya" starter culture have been developed that can be successfully used to increase the productivity of horse breeding in other regions of the Russian Federation and abroad. Scientific and technical documentation was prepared for inactivated vaccine against rhinopneumonia, combined bivalent and trivalent vaccines. The developed immunobiological preparations are protected by 48 patents for invention and can be used in other countries.

Keywords: horses, vaccine, probiotic, koumiss, bacteriophages

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In Asian and African countries, Europe, the USA [1, 2], the Russian Federation, the Republic of Kazakhstan, Kyrgyzstan and Mongolia [3, 4], the prevention of some mass infectious diseases, including rhinopneumonia (equine viral abortion), salmonella abortion and horse strangles, remains an unsolved problem of herd horse breeding. As a result of these diseases, the yield of foals per 100 breeding mares decreases to 45% [4]. However, in Russia and global practice, there are few effective immunobiological preparations for the prevention and treatment of infectious diseases.

For the prevention of equine rhinopneumonitis, a live virus vaccine and an inactivated one with an immunomodulator have been developed [4]. In various countries, live and inactivated monovaccines against strangles are developed and applied [5-7].

As our research and other authors' results show, mares often simultaneously suffer from rhinopneumonitis and salmonella abortion. It is known that about 20-30% of mares are carriers of the strangles-causing streptococcus. They serve as sources of infection and facilitate the spread of strangles among foals [8, 9]. Among young horses, there are instances of simultaneous illness from rhinopneumonitis, salmonellosis, and strangles. With such complications, mortality can reach up to 22% [3].

The purpose of the study is to introduce the developed immunobiological preparations for the prevention of infectious diseases in horses of herd breeding.

The genus and species identification of microorganisms were conducted according to the "Reference for Microbiological and Virological Studies" (1982), the "Identifier of Zoopathogenic Microorganisms" (1995), and the "Bergey's Manual of Determinative Bacteriology" (1997). The survivability of infectious disease pathogens was studied according to commonly accepted veterinary sanitation methods.

Preclinical and clinical trials, and the development of dossiers were conducted according to the Federal Law of 12.04.2010 (№ 61-FL),

orders of the Ministry of Agriculture of the Russian Federation from 06.03.2018 (№ 101), from 22.08.2017 (№ 430). The strains are deposited in the Russian State Center for Animal Feed and Drug Standardization and Quality (VGNKI).

To study the antagonistic properties of bacteria, passported strains were tested: *Staphylococcus aureus* (strain 209 R) obtained in the VGNKI, *Staphylococcus equi* H-34, *Salmonella abortus equi* BN-12 - YSRIA, *Escherichia coli* (strain 1257) - ARRIVSHE; *Salmonella pullorum* (strain 10b), *Brucella abortus* (in-82) - ARRIEVM, as well as local strains: *St. aureus* K-1, *Sal. typhymurium, Sal. dublin, Pseudomones, Micobacterium, Rajendra soloni, Streptomyces, Fusarium.*

We have isolated, identified, and deposited the following strains in the All-Russian State Collection of Strains of Microorganisms used in Veterinary Medicine and Animal Husbandry (VGNKI): *Sal. abortus*. equi BN-12, *Str. equi* H 5/1, *Bacillus subtilis* TNP-3, *Bacillus subtilis* TNP-5.

Deposits have been made in the State Collection of Microorganisms of Normal Microflora (GKNM) " MRIEM " named after G.N. Gabrichevsky of the Russian Consumer Protection Agency: *Lactobacillus acidophilus* K 1901, *L. acidophilus* K 1902, *L. delbrueckii* subsp. *Bulgaricus* K 1903. The strain *Clavispora lusitaniae* 1D (U-4861) is deposited in the Bioresource Center of the All-Russian Collection of Industrial Microorganisms (BRC ARCIM), SRC "Kurchatov Institute" (GosNIIGenetika). Molecular-genetic identification of microorganisms was carried out by SKC "Genomics" and VGNKI [3].

The studies conducted showed that the survival rates of some microorganisms on external environment objects in permafrost conditions are 2-3 times longer than the life preservation periods of similar microorganisms in the southern and European territories of Russia and abroad. During the study of microbial contamination and survival rates of microorganisms, a significant content of aerobic spore-forming bacteria (more than 2 × 106 CFU/g) was found in the permafrost soils of Central Yakutia. The isolation of viable *Bacillus* bacteria from the representatives of mammoth fauna preserved in permafrost soils (aged 30-40 thousand years) proves the role of permafrost in preserving Pleistocene-era bacteria. Permafrost contributes to the prolonged preservation of foci and transmission factors of infectious disease agents. The isolation of pathogens from glacier surfaces and from wild animals indicates the danger of food contamination during storage and consumption. The circulation of viral disease agents among northern reindeer and horses suggests the role of migratory birds in spreading infectious diseases. The study of epizootology of anthrax remains relevant. Knowledge of microorganism survival rates in extreme conditions of the Far North and the study of the microbiota of wild animals and migratory birds is essential for optimizing anti-epizootic and epidemiological measures to ensure biological safety. The problem is of global significance.

The strain of bacteria *B. subtilis* TNP-3 has more pronounced antagonistic activity against pathogenic microorganisms for humans and animals, *B. subtilis* TNP-5 – against plant disease agents (rhizoctonia, fusarium, and potato scab). Bacterial strains do not have pathogenic properties for laboratory and agricultural animals.

As a result of the studies conducted, the probiotic Sahabactisubtil has been developed and introduced for the prevention of dysbiosis, increasing the immunobiological reactivity of agricultural animals. Instructions for use have been approved (from 06.06.2012). The drug has been registered (71-111.12-0850 № SLA-1.6/01632).

It has been found that in the conditions of Yakutia, composting of poultry litter, manure with various substrates, sawdust, sapropel, peat, straw, zeolite does not always produce a positive sterilization result. The most reliable sterilization method is adding an antagonist microbe *B. subtilis* TNP-3 in the form of a 1 billion suspension or B. subtilis adsorbed on zeolite (0.5% of the compost mass) with an exposure of 80 days during the summer period.

Studies of the gut microbiota of young horses showed a decrease in the content of lactobacilli, bifidobacteria, lactopositive Escherichia, and fungi in the winter period (December – March). During this period, an increase in the number of opportunistic microflora – lactose-negative

Escherichia and staphylococci was noted. The results obtained indicate the development of dysbiosis in young horses. As is known, when the ratio of opportunistic and normal microbiota is disturbed, the digestibility of feeds and the immunobiological reactivity of the body decrease.

It should be noted that the intestines of young horses are colonized by spore-forming aerobic bacteria from the first days of life. The dominance of these microorganisms in the gut microbiota of horses persisted throughout the study period. The obtained data confirm the results of previous studies showing that during the formation of the normal flora of foals, calves, piglets, birds, and cell animals, spore-forming aerobic bacteria perform an active protective function against pathogenic and opportunistic microorganisms and are representatives of the normal microbiota of the animal's body in the conditions of the Far North.

The development of intestinal dysbiosis, reduced immunobiological reactivity of the body, and the spread of helminthiasis, rhinopneumonia, strangles, and salmonellosis are facilitated by weaning foals from their mothers, inadequate and insufficient feeding, prolonged low temperatures (–43 … 45 °C), and absence of planned preventive measures.

We have found that the use of the probiotic Sahabactisubtil with zeolite when feeding young horses in winter corrects the disturbed gut microbiota, stimulates immunobiological reactivity, and increases average daily gains. The positive effect of the probiotic on the body of the young is explained by the ability of bacterial strains *B. subtilis* TNP-3 and *B. subtilis* TNP-5 to stimulate the development of normal microflora, suppress the development of toxin-producing mold fungi, produce enzymes. Proteolytic, gelatinase, amylolytic, cellulolytic, β-glucanase, fructosyltransferase, and xylanase activity of bacterial strains contributes to increased digestibility and assimilation of nutrients.

Dehelminthization of young animals of 8-9 months of age after weaning from their mothers, practiced in almost all horse breeding farms of the Republic of Sakha (Yakutia), exacerbates the phenomenon of dysbiosis, leading to decreased growth and development rates. The drug Sahabactisubtil should be prescribed for the treatment and prevention of parasitic diseases of horses with any anthelmintic drugs.

Feed supplement based on local raw materials with the drug Sahabactisubtil, when fed to in-foal mares, increases the digestibility of dry matter by 5.14%, organic matter by 2.29%, raw protein by 26.05%, raw fiber by 3.95%, and raw fat by 4.74%. The metabolic energy level of the mares in the control group was lower by 6.30 MJ or 6.54%. The probiotic also has a positive effect on the business output of foals compared to the control group of animals (increased by 10%).

According to the instructions, when leptospirosis is detected, infected animals are isolated and treated with antibiotics, and then vaccinated. However, it is known that the use of antibiotics causes intestinal dysbiosis, decreases immunobiological reactivity and vaccine immunogenicity, and promotes the emergence of antibiotic-resistant strains of leptospira. We have found that the drug Sahabactisubtil in laboratory conditions causes lysis of leptospires serogroups *Pomona, Tarassovi, Grippotyphosa, Hebdomadis, Sejroe, Icterohaemorrhagiae, Canicola.* Laboratory studies have shown the resistance of bacterial strains *B. subtilis* TNP-3 and *B. subtilis* TNP-5 to the effects of a wide range of antibiotics (kanamycin, rifampicin, levomycetin, furadoxin, ampicillin, neomycin, eryprim, and kinoex), low resistance to cefuroxime, cefatoxin, ciroperazine, gentamycin, sensitivity to ciprofloxacin. The probiotic Sahabactisubtil is recommended to be used in combination with antibiotics to suppress the causative agent of leptospirosis, increase immunobiological reactivity, eliminate dysbiosis, and disinfect the external environment.

One of the promising measures to combat mycotoxicosis is considered to be the biological method using aerobic spore-forming bacteria of the genus *Bacillus*. We have found that bacterial strains *B. subtilis* TNP-3 and *B. subtilis* TNP-5 have a pronounced fungicidal effect against microscopic fungi *Aspergillus niger, Mucor ramosissimus, Candida albicans, Fusarium semitectum*. The probiotic Sahabactisubtil, adsorbed on oats, can be used as a remedy to combat mold fungi in feeds. Treating oats with the Sahabactisubtil probiotic reduces the number of mold fun-

gi by 40 times, normalizes intestinal microbiocenosis, stimulates immunobiological activity, and increases the live weight of the young.

In the system of measures for the treatment and prevention of strangles in horses, conducting therapeutic measures is of great importance. In laboratory conditions, a pronounced antagonistic activity of the *B. subtilis* TNP-3 strain against the causative agent of horse strangles was established. The possibility of using a suspension of the *B. subtilis* TNP-3 bacterial strain for the treatment of horse strangles has been proven, with its relatively high therapeutic effectiveness, especially in mixed viral-bacterial diseases (strangles and rhinopneumonia). Adding strains of bacteria *B. subtilis* TNP-3 to the composition of inactivated vaccines against strangles and salmonella abortion in horses enhances their immunogenicity. The immunomodulatory ability is due to the immune-stimulating and interferon-inducing activity of bacterial strains *B. subtilis* TNP-3 and *B. subtilis* TNP-5.

For the specific prevention of salmonella abortion in horses, an inactivated vaccine has been developed from the *Sal. abortus equi* BN-12 strain with the fugate of the *B. subtilis* TNP-3 strain as an immunomodulator. Preclinical trials were conducted on laboratory white mice, clinical trials on horses. The absence of toxicity of the drug was established. The immunogenicity in white mice was 90%, in mares - 100%. Production tests of the vaccine showed that after immunization, the business yield of foals increased by 13.8%. The economic effectiveness of using the vaccine with the fugate of the *B. subtilis* TNP-3 strain for every 1 ruble of costs amounted to 14.1 rubles. Instructions for the use of the vaccine were approved and a registration certificate was obtained (71-1-10.19-4495 No. SLA -1-1.6/01631 dated 10.06.2019).

We have developed a new inactivated vaccine against horse strangles with an immunomodulator made from the bacterial strain *Str. equi* H-5/1 with the fugate of the bacterial strain *B. subtilis* TNP-3 added as an immunomodulator. Preclinical trials on laboratory animals (white mice, rabbits) showed that the vaccine protects against experimental infection with a pathogenic strain of the strangles streptococcus in up to 90% of white mice and does not have a toxic effect on the organism of laboratory animals.

Clinical trials were conducted on 6-8-month-old foals. The effectiveness of immunization is 97.6%. The economic effect per head amounted to 3.99 thousand rubles, and the profit for every 1 ruble of costs was 6.65 rubles. The vaccine is registered in the registry of medicinal products for veterinary use (Rosselkhoznadzor RF) under the registration number 71-1-27.21-4828 No. SLA -1-27.21/03691 dated 08.12.2021.

The combined vaccine against rhinopneumonia and strangles in young horses is made from inactivated strains of the rhinopneumonia virus CV/69, the bacteria *Str. equi* H-5/1, and an immunomodulator from the strain of bacteria *B. subtilis* TNP-3. Preclinical testing showed no acute toxicity, allergenic, or pyrogenic properties. The combined vaccine protects against experimental infection by the rhinopneumonia virus in 75% of vaccinated animals, and against the strangles agent in 80%. Production trials confirmed the results of preclinical research.

Preclinical and clinical trials of the inactivated vaccine against rhinopneumonia have been completed. The protective effect from experimental VGL-1 infection regarding morbidity in linear mice with one and two administrations of the vaccine was 60%, and regarding mortality was 100%. The inactivated vaccine against rhinopneumonia, with the culture fluid of the bacterial strain *B. subtilis* TNP-3, is as effective as the live virus vaccine with a single administration. Immunizing breeding mares in areas affected by rhinopneumonia increases the business yield of foals by 10.9-33.3%.

The combined vaccine is made from the CV/69 strain of the rhinopneumonia virus and the bacterial strain *Sal. abortus equi* BN-12, with the addition of an immunomodulator – a culture fluid (fugate) from the bacterial strain *B. subtilis* TNP-3. The vaccine protects against experimental infection by the rhinopneumonia virus in 87.5% of vaccinated horses and from salmonella abortion infection in 100%. Immunization with the vaccine in affected areas increases the business yield of young horses up to 24.1%.

We have developed a trivalent vaccine, made from the CV/69 strain of the rhinopneumonia virus (ARRIEVM), strains of bacteria *Sal. abortus equi* BN-12 causing salmonella abortion, and *Str. equi* H-34. The drug protects against experimental infection with the rhinopneumonia virus in 88.8% of laboratory mice, and against salmonella abortion and strangles in 100%.

The trivalent vaccine stimulates immunobiological reactivity, induces the synthesis of specific antibodies in high titers, and increases the business yield of foals from 18 to 38%. Clinical trials of the combined trivalent vaccine in young horses (657 heads) demonstrated high effectiveness: it protects 94-100% of the animals vaccinated from the disease.

The developed vaccines in terms of immunogenicity, harmlessness, and eco-friendliness not only match but also surpass known global vaccines [2, 10]. It should be noted that antibiotics in the composition of the vaccine disrupt the development of humoral immunity [11].

High efficacy of the inactivated vaccines can be explained by the antigenic activity of the vaccine strains and the immunomodulatory component – the culture fluid (fugate) of the bacterial strain *B. subtilis* TNP-3. According to our previous research results, it can induce interferon synthesis and stimulate the immunobiological reactivity of the organism, enhancing the immunogenicity of inactivated bacterial and viral vaccines [3, 4].

Kumys – a traditional fermented milk product of the Turkic peoples – has been known since ancient times. However, in the production of kumys, as in any fermented milk products, a starter culture is necessary. For the first time in Yakutia, we developed a starter culture called "Yakut Kumys" using epy strains of bacteria *Lactobacillus acidophilus* K 1901, *L. acidophilus* K 1902, L. *delbrueckii subsp. bulgaricus* K 1903, and the yeast culture *Clavispora lusitaniae* 1 D, intended for mass production.

The search for new strains of microorganisms - producers of biologically active substances used in the development of immunobiological preparations continues. As an alternative to antibiotics, new probiotic preparations and bacteriophages are being developed.

To commercialize the developments, a licensed enterprise "Khoto-Bakt" Scientific Production Center was established and operates. Vaccines are marketed in the regions of Russia and Kazakhstan. The plan includes entering the Mongolian market.

CONCLUSIONS

1. Eight new strains of microorganisms have been isolated, identified, and deposited in the all-Russian collections. The long survivability of microorganisms, conditioned by the permafrost of the region, complicates the epidemiological and epizootic situation, but it is of interest to microbiologists.
2. A probiotic called "Sahabaktisubtil" has been developed, which is used for the prevention and treatment of dysbacteriosis, mycotoxicosis, strangles, leptospirosis, and for disinfecting manure.
3. New effective vaccine preparations and the "Yakut Kumys" starter culture have been developed, which can successfully be used to increase horse breeding productivity in other regions of the Russian Federation and abroad. The scientific novelty of the developments is confirmed by 48 invention patents.

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