Immunogenicity of Inactivated Vaccine against Rhinopneumonia, Salmonella Abortion and Horse Strangles

M.P. Neustroev1,2, K.P. Yurov3, S.V. Alexeyenkov3, N.P. Tarabukina1, S.G. Petrova1
1 Federal State Budgetary Scientific Institution Yakut Scientific Research Institute of Agriculture named after M.G. Safronov, Laboratory of Veterinary Biotechnology and Laboratory for the Development of Microbial Preparations, Scientific-Production Center "Hotu-Bact" Ltd., 677001, Republic of Sakha (Yakutia), Yakutsk, Bestuzheva-Marlinskovo, Str.23/1
2 Yakut State Agricultural Academy, Sergelyakh highway 3km, 3, Yakutsk, Republic of Sakha (Yakutia), 677007, Yakutia
3 Federal State Budgetary Scientific Institution All-Russian Scientific Research Institute of Experimental Veterinary Medicine named after Kovalenko Y.R., Federal Agency of Scientific Organizations of Russia, Laboratory of Virology and Reference Laboratory of the International Epizootic Bureau for Rhinopneumonia and Dourine of Horses, 109428, Moscow, Ryazansky Avenue, 24/1

Abstract
The aim of the work is to assess the immunogenicity of inactivated associated vaccine against rhinopneumonia, salmonella abortion and horse strangles. Methods: A laboratory test was performed on BALB/c mice aged 10-14 days weighing 5-7 grams and outbred white mice weighing 18-20 grams. An experimental vaccine series was used, a culture fluid (fugate) of the bacterial strain Bacillus subtilis TNP-3 was used as the immunomodulator. Two series of experiments were carried out. The level of antibodies was determined in ELISA. To evaluate the immunogenic properties of the preparation against rhinopneumonia, a control virus was administered with the adapted horse rhinopneumonia virus at a dose of 0.02x6,0 lg TCD50/ml intracerebrally 14 days after the primary and 14 days after the second vaccination. To assess the immunogenic properties of the preparation against salmonella abortion, infection with virulent strains in a lethal dose was administered subcutaneously 14 days after vaccination. The effectiveness of immunization was determined by the number of mice resistant to morbidity and mortality to infection, as well as by the change in mass in comparison with animals of negative (n = 4) and positive (n = 9) control groups. Repeated vaccination of the vaccine with an interval of 14 days provided on average 1,5-2 fold antibody growth. A two-fold vaccination of the vaccine protected 88,8 % of the lethal outcome. Infection with virulent strains of salmonella and streptococcus among the immunized mice did not cause disease and mortality. Consequently, the vaccine has antigenic activity that provides the synthesis of specific antibodies, and is characterized by a sufficiently high immunogenic activity.

Key words -Immunogenicity, vaccine, rhinopneumonia, salmonella abortion, horse strangles.

INTRODUCTION:
The prevention of some common infectious diseases of horses, including rhinopneumonia (viral abortion of horses), salmonella abortion, and horse strangles, remains an unresolved problem in the Republic of Kazakhstan, in the Russian Federation, primarily of horse herd breeding [1, 2]. As a result of losses from these diseases, the yield of foals decreases by 100 % to 45 % [3, 4].

According to the veterinary service of the Republic of Sakha (Yakutia) and our studies in 2002-2016, outbreaks of horse rhinopneumonia were recorded in 96 locations in 15 districts, and salmonella abortion in 139 locations in 20 districts.
The significant spread of infectious abortions in recent years is largely due to the insufficiency of routine diagnostic studies, the lack or low effectiveness of vaccine prevention; uncontrolled delivery of horses of breeds from other regions of Russia and the exchange between different regions of the republic, extreme conditions of winter pasture, peculiarities of conducting the industry (the concentration of a significant number of horses during the weaning of foals), a decrease in natural resistance in the winter-spring period, a prolonged persistence of pathogens in permafrost conditions of Eastern Siberia [5, 6].
The system of anti-epizootic measures against rhinopneumonia developed for horse farms in the European part of the Russian Federation, under the conditions of horse herd in Yakutia showed insufficient effectiveness, in particular due to the certain reactogenicity of the viral vaccine [13]. Based on this, we developed a harmless and immunogenic inactivated vaccine against rhinopneumonia. We evaluated it for immunogenicity and harmlessness on laboratory animals. It was established its effectiveness in the conditions of disadvantaged farms [7, 8]. In 2010-2012 we have developed, tested and approved by the Rosselkhoznadzor an inactivated vaccine against salmonella abortion of horses. Currently, the preparation is used in the subjects of the Russian Federation and Kazakhstan.

An inactivated vaccine against horse strangles was proposed in 2000. Due to the expiry of the validity period of the permits, the vaccine is not produced. Currently, epizootically topical strains of streptococcus are isolated, promising for the development of a new vaccine. Vaccines against horse strangles, salmonella abortion and rhinopneumonia have been developed in the Republic of Kazakhstan, which are introduced separately. These vaccines are not registered in the Russian Federation [3, 4].
As the results of our studies indicate, rhinopneumonia in the condition of herd horse maintenance is recorded as a mixed viral-bacterial infection. In addition, about 20-30 % of the mares are carriers of a horse strangles streptococcus, thereby serving as sources of infection for foals, in which the horse strangles is often observed as a mixed infection.
with rhinopneumonia, salmonellosis [9-13]. The virus of the rhinopneumonia possesses immunosuppressive properties and aggravates the course of salmonellosis and horse strangles. At the same time, the mortality rate can reach 22 %, [13].

According to our data, the canonical schemes of anti-epizootic measures for rhinopneumonia, salmonellosis or horse strangles, including vaccination of commercial vaccines, do not provide the necessary effect in horse farms in the Northern regions of Eastern Siberia for a number of reasons:

- a double vaccination of the viral vaccine is a negative factor in the conditions of Yakutia, since the second administration of the vaccine falls on January-February, the most difficult period in terms of climatic conditions. The bringing of herds at this time is extremely undesirable due to the laboriousness and danger of abortion of a traumatic nature;
- vaccination of the viral vaccine is undesirable due to low air temperature to minus 43-50 °C, dysbacteriosis of the gastrointestinal tract in horses; deficiency of fodder by protein and mineral-vitamin components;
- the immunization period against the rhinopneumonia, salmonella abortion and horse strangles coincides with vaccination against anthrax. Therefore, it is impossible to combine vaccination of different vaccines in the period after weaning foals.

Thus, the development of vaccines against viral-bacterial infections, including effective immunomodulators, remains topical. Moreover, there are no analogues of the associated infections, including effective immunomodulators, remains topical. Moreover, there are no analogues of the associated infections, including effective immunomodulators, remains topical. Moreover, there are no analogues of the associated infections, including effective immunomodulators, remains topical. Moreover, there are no analogues of the associated infections, including effective immunomodulators, remains topical. Moreover, there are no analogues of the associated infections, including effective immunomodulators, remains topical. Moreover, there are no analogues of the associated infections, including effective immunomodulators, remains topical.
- repeated introduction of the vaccine provided an increase in immunity and protected 88.8% of the death (Figure 3);
- after infection with a virulent strain of Sal. abortus equi BN-12, the disease and mortality were not marked among the immunized mice; in the control group, after the injection of the causative agent out of 10, all fell ill and 8 died;
- infection of vaccinated mice with a virulent strain of a horse strangles streptococcus did not cause disease of all mice, in the group of non-immunized mice all fell ill and 6 (60%) died.

In previous laboratory tests of the immunogenicity of the inactivated vaccine against rhinopneumonia, it was established that the protective effect of experimental infection of HVH-1 on the incidence of linear mice in single and double-entry vaccine is 60 %, on lethality – with a single and double injection of the vaccine – 100 % [8].

Immunization of pregnant mares in November (the period of weaning foals) by inactivated vaccine causes the production of specific hemagglutinating antibodies in high titers, stimulates immunobiological reactivity (increase of phagocytic, bactericidal and lysozyme activity, content of β-globulin fraction of protein and lymphocytes). As a result, immunity develops relatively high tension and it increases the business yield of foals.

The inactivated vaccine against rhinopneumonia with a culture fluid of the bacterial strain Bacillus subtilis TNP-3 with single administration is not inferior in effectiveness to live viral vaccine.

Immunization of pregnant mares in disadvantaged settlements by rinopneumonia increases the business yield of foals by 10.9 ... 33.3 % [7].

Laboratory monitoring of immunogenicity in linear and outbred white mice of an experimental sample of vaccine inactivated against rhinopneumonia and salmonella abortion was carried out. Associated divalent vaccine prevents from experimental infection with the virus of rinopneumonia 87.5 %, and from the injection of the causative agent of salmonella abortion – 100 % of immunized animals. The relatively high immunogenicity of the inactivated vaccine against rhinopneumonia and salmonella abortion is due to the antigenic activity of vaccine strains that provides the synthesis of specific antibodies in high titers and an immunomodulating component that stimulates the immunobiological reactivity of the organism [14].

It should be noted that monovalent vaccine against rhinopneumonia and divalent vaccine against rhinopneumonia and salmonella abortion have a low reactivity for linear mice, which manifests itself in a decrease in the live weight of vaccines compared to control groups.

The trivalent vaccine against rhinopneumonia, salmonella abortion and horse strangles, containing an additional inactivated horse strangles of streptococcus has increased toxicity on linear mice at the age of 2 weeks, which negatively affects the immunity to the rhinopneumonia virus. On outbred mice at the age of 2-3 months toxicity of vaccines was not shown. Bacterial components of the vaccine have a toxic effect on linear mice, and as a result, further control techniques need to be improved by optimizing the dosage of the preparation and the vaccination schedule.
The high efficiency of the inactivated trivalent associated vaccine, in our opinion, can be explained by the antigenic activity of the vaccine strains and the immunomodulating component - the culture fluid (fugate) of the bacterial strain Bacillus subtilis TNP-3, which, according to our previous studies, can induce interferon synthesis and stimulate the immunobiological reactivity of the organism, strengthen immunogenicity of inactivated bacterial and viral vaccines [15, 16].

Thus, the inactivated vaccine against rhinopneumonia, salmonella abortion and horse strangles with the culture liquid of the bacterial strain Bacillus subtilis TNP-3, is characterized by a sufficiently high immunogenic activity when tested on laboratory mice.

REFERENCES: