Study of immunogenic properties of associated inactivated vaccine against horse influenza and petanus

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Abstract An associated inactivated vaccine has been developed for the specific prophylaxis of tetanus and equine influenza caused by various influenza viruses of the H3N8 serotype. The strain composition of the associated vaccine was determined considering the recommendations of the International Epizootic Bureau, as well as the virus circulating in Russia, isolated in 2007 and therefore posing a certain danger to horse breeding in the Russian Federation. The immunogenic properties of the new associated vaccine were studied in a laboratory model and horses. The results of studies of the associated vaccine FFE Kurskaya Biofabrika showed that the investigated vaccine preparation has high immunogenic activity and can cause a long-term intense immune response against influenza and tetanus in laboratory animals and horses.

1 Introduction

Influenza infection remains a serious problem for horse breeding, causing massive outbreaks of respiratory diseases in horses around the world [2,5,7,13,20,21]. The disease causes significant damage to horse breeding due to a decrease in the breeding and sporting value of recovered animals, treatment costs, quarantine measures, disruption of sports competition plans, and other economically significant reasons [3,10,14,16,19,24,31]. Currently, equine influenza epizootics are mainly caused by the 2nd subtype virus - A / horse 2 / (H3N8) [6,9,8,11,17,22,26,30].

In addition to combating diseases of viral etiology in horse breeding, the prevention of infectious bacterial diseases, especially tetanus, is of particular importance, given the degree of its danger to humans and animals [1]. The most effective way to fight both flu and tetanus infections is vaccination. Specific prevention of influenza and equine tetanus is based on systematic annual immunization [1,12,15,16,18,23,27,29]. To solve this problem, an associated inactivated vaccine was developed at the Kursk Biofactory, intended for the specific prevention of tetanus and equine influenza caused by various influenza viruses of the H3N8 serotype. The strain composition of the associated vaccine was determined considering the recommendations of the International Epizootic Bureau, as well as the virus circulating in Russia, isolated in 2007 and therefore posing a certain danger to horse breeding in the Russian Federation. [4,16,25,28].

This study aimed to examine the immunogenic properties of a new associated vaccine in a laboratory model and horses in comparison with one of the commercial drugs currently used for the prevention of equine influenza and tetanus in the Russian Federation.

2 Materials and methods

The vaccine against influenza and equine tetanus associated inactivated developed by FFE Kurskaya Biofabrika is a mixture of purified antigens of the equine influenza virus strains A / horse2 / France98 (NZN8-European line) and A / horse2 / Bitza-2007 (NZN8-American line) isolated from extraembryonic fluid of SPF-chicken embryos, as well as purified and concentrated tetanus toxoid (strain Cl. tetani Kolle-8) with the addition of aluminum hydroxide as an adjuvant.

Comparison drug. A commercial associated inactivated vaccine for the prevention of influenza and equine tetanus Fluequin - T manufactured by Biovetéa Czech Republic was used as a reference drug.

Animals. The study of the immunogenic properties of the associated vaccine against equine influenza and tetanus was carried out on 100 guinea pigs weighing 400-500 g, which do not have specific antibodies to the causative agents of these diseases. To assess the titer of anti-tetanus antibodies, 950 white mice weighing 15-17 g were used. Also, the research was carried out on 45

horses of different ages from horse breeding farms in the Moscow region.

Reaction of Neutralization. The presence of neutralizing antibodies against tetanus infection was carried out in the test of neutralization of the working dose of tetanus toxin according to the Ehrlich method and was expressed in IU / ml. The toxin was standardized using a control antitoxic serum to C. tetani toxin produced by FGBU NTSESMP (Russia).

Hemagglutination inhibition reaction. Assessment of the antigenic and immunogenic activity of the associated vaccine to the equine influenza H3N8 virus was carried out according to the generally accepted method of hemagglutination inhibition reaction using the Equine Influenza Diagnostic Kit manufactured by FFE Kurskaya Biofabrika according to its instructions for use.

Statistical processing of the obtained data. Statistical analysis was performed using the Microsoft Office XP Professional, Statistica 5.0, Statsoft Inc software package for Windows.

3 Research results

At the initial stage, the assessment of the immunogenic activity of the associated vaccine was carried out on laboratory animals in parallel with the reference drug. For this, four groups of 25 guinea pigs were formed. The animals of the first, second and third groups were injected with the associated inactivated vaccine produced by FFE Kurskaya Biofabrika in a volume of 1.0 cm3 (one dose). Animals of the fourth (control) group were immunized with one dose of the reference drug. Periodically, before and after vaccination, blood was obtained from all animals for the study of sera for the presence of anti-influenza and anti-tetanus antibodies. The results of studying the dynamics of the humoral immune response in laboratory animals to influenza infection of horses (H3N8) are presented in Table 1. The indices of the amount of neutralizing antibodies in the blood serum of laboratory animals against tetanus infection are presented in Table 2.

The data presented in tables 1 and 2 indicate that the associated vaccines induce the formation of specific antibodies against influenza and tetanus in immunized guinea pigs. At the same time, the average titer of antibodies to the equine influenza virus (H3N8) in animals immunized with the associated FFE vaccine Kurskaya Biofabrika on the 15th day after vaccination was slightly higher than in animals vaccinated with the associated vaccine Fluequin-T and amounted to 4.8 log2 and 4, 2 log2, respectively. The level of neutralizing antibodies against tetanus infection after the first vaccination in this period in animals immunized with the test vaccines was at the same level and amounted to 6.0-6.5 IU/ml.

Table 1. The level of specific antibodies to the H3N8 equine influenza virus in blood serum samples from guinea pigs after immunization with associated vaccines

Group name	**Number of days after primary vaccination**									
	15	30	**Re-vaccination**	60	90	120	150	180	240	360
				The level of antibodies in the reaction of inhibition of hemagglutination, log_2 (M ± m) at P <0.05						
Group 1	4,7±0,28	5,2±0,33		8,9±0,2	9,0±0,27	9,0±0,3	8,2±0,33	7,8±0,3	6,7±0,28	5,5±0,28
Group 2	4,5±0,31	4,7±0,24		8,6±0,3	8,9±0,33	8,7±0,28	8,5±0,25	7,5±0,31	6,2±0,33	5,0±0,33
Group 3	5,2±0,3	5,8±0,25		9,2±0,3	9,5±0,33	9,3±0,24	8,8±0,3	7,9±0,33	7,0±0,28	6,1±0,28
The group immunized with the reference drug	4,2±0,24	4,7±0,28		7,3±0,28	7,8±0,41	7,6±0,2	7,2±0,1	6,7±0,3	6.,0±0,41	4,5±0,41

Table 2. The level of neutralizing antibodies to tetanus toxin in blood serum samples of guinea pigs after immunization with associated vaccines

Group name	**Number of days after primary vaccination**									
	15	30	**Re-vaccination**	60	90	120	150	180	240	360
				The level of antibodies in the neutralization reaction IU / ml (M ± m) at P <0.05						
Group 1	6,5±0,22	7,5±0,14		43,0±0,12	45,5±0,24	45,0±0,16	45,0±0,25	42,5±0,2	38,5±0,18	32,5±0,22
Group 2	6,0±0,12	6,5±0,2		41,5±0,22	43,0±0,2	43,5±0,18	42.5±0,3	41,0±0,25	36,0±0,22	29,0±0,18
Group 3	6,0±0,24	7,5±0,18		45,0±0,2	46,5±0,22	46.0±0,24	45,0±0,22	44,0±0,2	38,5±0,16	32,0±0,24
The group immunized with the reference drug	6,5±0,28	7,5±0,22		46,0±0,18	47,5±0,28	47,0±0,2	46,5, ±0,3	45,0±0,18	41,0±0,28	34,0±0,2

When studying the immunogenic activity of vaccines, the dynamics of the formation of the humoral immune response against equine influenza virus and tetanus over a long period after immunization with associated vaccines is of great interest. To study the duration and intensity of immunity, periodically, throughout the year, the serum of vaccinated animals was examined for the presence of antibodies against equine influenza and tetanus. The results of studies of blood sera of animals on day 60 after repeated vaccination showed a slowdown in the growth of antibody titers against influenza and tetanus infections in groups of animals vaccinated with the studied vaccines. At the same time, the average titer of antibodies against equine influenza H3N8 after immunization with the FFE Kurskaya Biofabrika vaccine was higher than those of the reference drug and amounted to 9.1 log2 and 7.8 log2, respectively. The titers of neutralizing antibodies against tetanus infection in this period in animals immunized with the Kurskaya Biofabrika FFE vaccine increased significantly and corresponded to 45.0 IU/ml, which was significantly equal to those of the reference drug, the titer of which was 47.5 IU/ml.

The maximum titer of antibodies against influenza infection in the group of animals immunized with the FFE Kurskaya Biofabrika vaccine was determined within 60-90 days after the repeated vaccination and was 9.0 log2. A similar dynamic was observed in the group of animals vaccinated with the reference drug, while the titer against influenza virus was lower and amounted to 7.6 log2. It should be noted that the maximum level of antibodies against tetanus infection in the group of animals immunized with the FFE Kurskaya Biofabrika vaccine was determined within 60-120 days after the second vaccination, which was 44.8 IU/ml.

In the period from 120 to 360 days after vaccination, the level of antibodies against influenza and tetanus infections in all studied groups decreased. In general, after immunization with the FFE Kurskaya Biofabrika vaccine, the average titer of antibodies against H3N8 influenza decreased by 3 log2 and amounted to 5.5 log2. In the group of animals immunized with the Fluequin-T vaccine, during this period, the titer of antibodies against influenza decreased by 2.5 log2 and amounted to 4.5 log2. The level of neutralizing antibodies against tetanus infection in animals immunized with the test vaccines decreased by the 360th day of the study and amounted to 31.0 IU/ml in the groups of animals immunized with the Kurskaya Biofabrika FFE vaccine and 34.0 IU/ml in the group of animals immunized with the Fluequin-T vaccine.

The results of the immunogenicity of the studied vaccines in laboratory models indicate high rates of immunogenic activity against influenza and tetanus infections. The results of using the associated vaccine in a production environment may differ from the data obtained in an experiment on model animals, due to the presence of the immune background, different reactivity of the immune system of organisms, and other reasons. In this regard, a study of the associated inactivated vaccine FFE Kurskaya Biofabrika was carried out on 45 horses of various ages. The vaccine was administered intramuscularly, twice with an interval of 30 days, in a volume of 1 cm3 per animal. Before immunization and at various times after it, blood was taken from horses, sera were obtained and tested for the presence of anti-influenza and anti-tetanus antibodies. The research results are shown in Fig. No. 1 and No. 2.

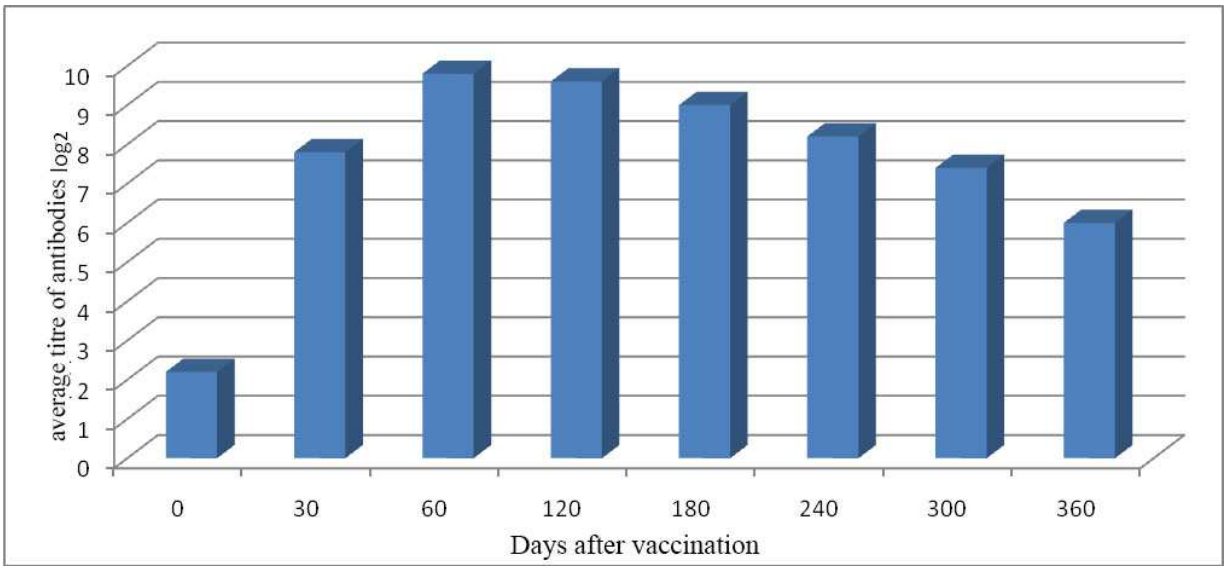

Figure №1 - Dynamics of the formation of post-vaccination humoral immune response in horses to the H3N8 influenza virus after vaccination with the associated FFE vaccine Kurskaya Biofabrika

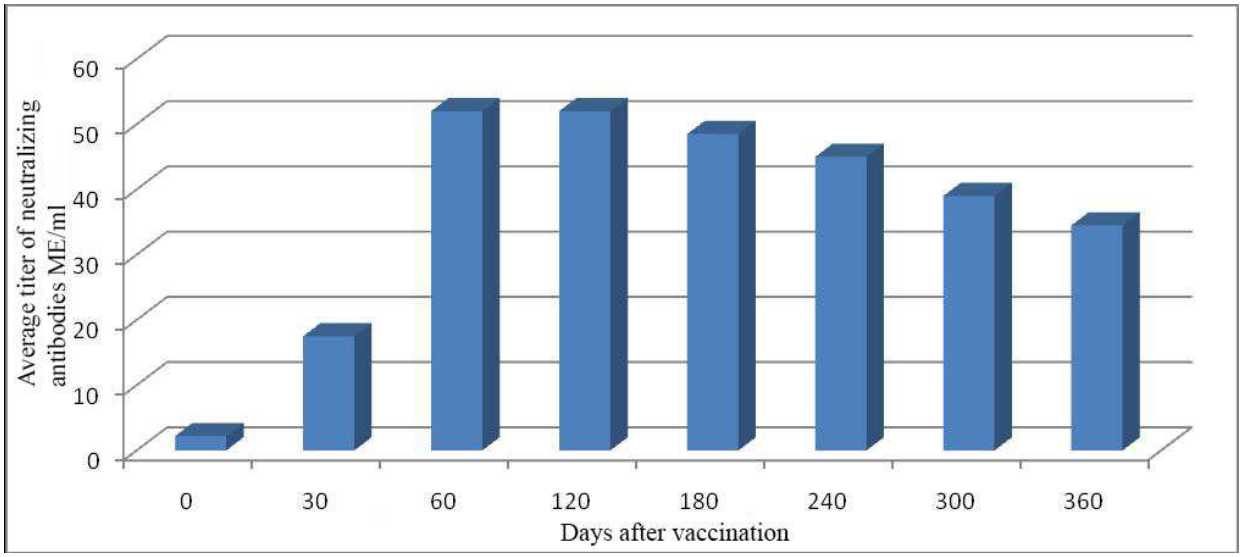

Figure №2 - Dynamics of the formation of post-vaccination humoral immune response in horses to tetanus toxoid after vaccination with the associated FFE vaccine Kurskaya Biofabrika

The results of the study indicate that by the time of immunization, antibodies to influenza virus of the second type (H3N8) in a titer of 2.2 log2 were found in the blood serum of horses. The presence of specific antibodies is probably due to previous vaccination. From the data presented in Figures 1 and 2, the associated vaccine induces the formation of specific antibodies against influenza and tetanus in immunized horses. On the 30th day after the primary immunization in the blood serum of horses, an increase in the average titer of specific antibodies to the equine influenza virus, the second subtype, to 7.8 log2 and tetanus to 17.0 IU/ml was found. The horses were also boosted with the associated vaccine.

30 days after repeated vaccination, the level of specific antibodies increased significantly and was 9.9 log2 for influenza infection and 52.0 IU/ml for tetanus infection. The results of studies of blood sera of horses by 90 days after repeated vaccination showed that the growth of antibody titers against influenza and tetanus infections stopped. The most pronounced humoral immune response in horses to influenza infection of the second subtype was observed in the period 30-90 days after repeated vaccination and amounted to 9.9 log2. The maximum level of antibodies against tetanus infection was determined within 60-120 days after repeated vaccination and was 48.0-52.0 IU/ml. Subsequently, a decrease in the titers of specific antibodies was observed,

both against influenza and against tetanus infections. In general, on the 360th day after vaccination, the serum antibody titer decreased slightly and amounted to 6.0 log2 against influenza infection and 34.5 IU/ml against tetanus infection.

4 Conclusion

Thus, the results of studies of the associated FFE vaccine Kurskaya Biofabrika showed that the investigated vaccine preparation has high immunogenic activity and can cause a long-term intense immune response against influenza and tetanus in laboratory animals and horses.

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