Efficacy of inactivated bivalent formo-hydroxide aluminum vaccine in streptococcus of pigs

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Abstract. An inactivated bivalent formol-aluminum hydroxide vaccine (IBFAHAO) against swine streptococcosis has been developed on the basis of strains of two serogroups (D and C). It has been established that it is harmless, creates tense immunity in white mice and piglets, and prevents streptococcosis in conditions of a dysfunctional economy. Immunological restructuring of the piglets' body is accompanied by the activation of cell-humoral resistance indicators and the synthesis of specific antibodies, which persist for at least 6 months after vaccination.

Keywords: antibodies, vaccine, immunity, prevention, pigs, streptococci, streptococcosis.

Pig breeding occupies a large share in the meat balance of the Russian Federation [1,2,10,11,16, 17]. Despite the high rates in industrial and small-scale pig breeding, viral and bacterial infections are still recorded, causing great damage to pig breeding [3,4,5]. Most often, infections are recorded in the pathology of young animals, in the etiology of which opportunistic microorganisms play an important role [6,7]. Among them, a significant place is occupied by streptococcosis, which occur in various forms, affecting different sex and age groups of pigs and pose a threat to human health [8, 9].

Due to the fact that in some pig farms streptococcosis are massive, affecting the broodstock and piglets, against the background of violations of the conditions of feeding and maintenance, there is a need for vaccination. However, the available vaccines do not always create intense immunity due to the circulation of a wide range of streptococcal serovars, as well as the lack of toxigenic components of the corresponding streptococcal serogroups in their composition.

The aim of the research was the design and assessment of the activity and immunogenic properties of the inactivated bivalent formol-aluminum hydroxide vaccine IBFAHAO we created.

Materials and methods. For the manufacture of IBFAHAO vaccine, we used strains of Streptococcus suis, serovars D and C, isolated from dead newborn piglets with a septic form of streptococcosis.

The biomass of streptococci was obtained by cultivation in Hottinger's broth with 0.4% glucose with amine nitrogen 200 mg% at a temperature of 37-38°C for 18-20 hours. Cultivation was carried out at the beginning in incubation (within 3-4 hours), stationary "rest" mode. Then on a rocking chair and at the end again at rest. Grown cultures of streptococci containing a bacterial mass with a concentration of 4 billion m.k. in cm³, toxins and capsule components were inactivated with formalin at a final concentration of 0.3% and sorbed on aluminum hydroxide

(10%), pH was stabilized at 7.2 ... 7.4. The culture inactivation time and sorbent were selected empirically.

To determine the completeness of inactivation of streptococci and the quality of the vaccine preparation, inoculations were made on nutrient media. The safety and immunogenicity of samples of inactivated bivalent formol-aluminum hydroxide vaccine was tested on white mice weighing 18 ... 20 g. Experiments were carried out with one of the experimental vaccine samples on 14-day-old piglets, which were immunized with an experimental batch of IBFAHAO vaccine against swine streptococcosis, obtained according to our laboratory regulations. The vaccine was injected intramuscularly twice with an interval of 10 days at doses of 2 and 3 cm³. One month after the second vaccination, three vaccinated and two similar non-immunized piglets were infected with a culture of streptococci of the LSP strain at a dose of 2 billion m.k. intraperitoneally. In the future, the effectiveness of the vaccine was assessed in the conditions of the farm on piglets.

To assess the resistance of pigs and the state of the immune response of vaccinated pigs, hematological studies were carried out and individual cell-humoral indicators of the organism's resistance were determined according to the methods described in the relevant sources [12,13,14,15].

The digital material was subjected to statistical processing using Microsoft Excel 10 programs with the derivation of the Student's t-test.

Research results. The vaccine produced according to the IBFAHAO regulation developed by us was sterile. After administration to white mice, it turned out to be harmless, did not cause deviations in their physiological status. When challenged with a virulent culture of streptococci, all twice vaccinated white mice were resistant (immunogenicity 100%).

To determine the immunogenicity of the IBFAHAO vaccine, experiments were carried out on 3 piglets at the age of 14 days. Post-vaccination complications were not observed, except for the appearance of a small swelling at the injection site, 5-8 mm in diameter, which disappeared completely after 7-10 days. All vaccinated piglets after infection remained without visible clinical changes during the observation period (1.5 months). Unvaccinated piglets (2 animals) fell ill and died on the 8th and 12th days after infection. During bacteriological studies of the pathological material obtained from them, streptococci were isolated. The results of the experiment with the control infection with streptococci testify to the high immunogenicity of the tested drug.

Subsequently, experiments were carried out in a pig farm, unfavorable for streptococcosis. In the conditions of the farm, 14-18 day old piglets were vaccinated with an experimental series of IBFAHAO vaccine.

The research results are summarized in table 1.

N⁰	Number of pigs in a	Vaccine dose, cm ³	Safety		Antibody titers	
	group		%	death (animals)	60 days	180 days
1	70	2,0	97,1	2	1:32 -1:64	1:16-1:32
		3,0			(av.51)	(av. 25)
2	10	5,0	70	3	1:16-1:32	1:8 - 1:16
		5,0			(av. 25)	(av. 14)

Table 1 – Antibodygenesis and safety of vaccinated piglets

Immunological restructuring in vaccinated piglets was accompanied by the production of specific antibodies, titers of which after 2 months were 1:32 ... 1:64 (51 on average). Antibodies

remained at a fairly high level for 180 days after vaccination at an average of 1:25. In piglets vaccinated with a commercial vaccine (associated polyvalent against salmonellosis, pasteurellosis and streptococcosis), antibody titers were on average 2 times lower.

The death of piglets in both groups was not due to streptococcosis, which was confirmed by laboratory tests.

The results of hematological studies are presented in table 2. The content of erythrocytes, hemoglobin, total protein after vaccination did not undergo significant changes.

Table 2 – Piglet hematological parameters (n=3)

Indicators	Before and after (14 days) vaccination			
Erythrocytes(10 ¹² /l)	7.2±0.22	7.7±0.21		
Hemoglobin(g/l)	104.6±2.32	108.8 ± 2.51		
Leukocytes(10 ⁹ /l)	27.1±1.41	28.6±1.13		
Total protein (g/l)	55.1±0.31	56.1±0.25		
Albumin(g/l)	21.8±1.34	22.3±1.41		
Globulins(g/l)	73.7±1.23	79.2±1.12		

The total number of leukocytes increased by 5.5% and globulins - by 7.4%. 14 days after vaccination, certain changes were noted in the piglet leukoformula (table 3). After vaccination, the number of stab neutrophils increased 1.4 times, against the background of a decrease in segmented neutrophils and monocytes by 2.8 and 2.5%, respectively. The content of lymphocytes after vaccination increased by 6.6%.

Indicators	Units	Before and after (14 days) vaccination		
Neutrophils: stab	%	3.4±1.12	4.8±1.12	
segmented		8.1±0.72	5.3±0.66	
Eosinophils	%	6.3±0.81	5.1±1.08	
Basophils	%	0.1±0.02	0.11 ± 0.01	
Monocytes	%	7.1±0.81	4.6 ± 0.07	
Lymphocytes	%	75.1±4.13	80.1±3.15	

Table 3- Piglet blood leukoformula

Certain changes were observed in piglets in terms of natural resistance, characteristic of the immune response (table 4).

Table 4 – Indices of natural resistance of piglets before and after two-fold immunization with IBFAHAO vaccine.

Indicators	Units	Before and after vaccination	
Lysozyme activity	%	12.3±0.67	14.6±0.78
Phagocytic activity	%	47.3±1.65	55.3±1.83
Phagocytic number	c.u.	4.1±0.3	5.1±0.3
HCT test (CB)	%	7.8±0.4	10.2±0.6
HCT test (AB)	%	62.8±2.36	66.8±2.13

In particular, after vaccination there was an increase in lysozyme activity of blood serum (by 1.8%), phagocytic activity (FA) and phagocytic number (FP) by 17 and 24%, respectively. An increase in the functional activity of blood neutrophils was observed in spontaneous (SV) (by 30%) and stimulated (AB) (10%) variants of reactions.

Conclusion. As a result of the studies, it was established that the inactivated formolaluminum hydroxide (IBFAHAO) vaccine prepared against swine streptococcosis is harmless and creates intense immunity in laboratory animals and piglets. Immunological restructuring of the piglets' organism after double vaccination is accompanied by antitelogenesis and a certain restructuring of the cellular-humoral parameters of the organism's resistance, characteristic of the vaccine process. The vaccine produced according to the developed regulations can be recommended for use in dysfunctional farms for the prevention of swine streptococcosis.

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