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EXPERIMENTAL SUBUNIT VACCINE AGAINST CLASSICAL SWINE FEVER DEVELOPMENT AND TRIAL

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Abstract

Classical swine fever (CSF), the highly contagious viral disease, remains the major threat to pork industry in top ten pork producing countries save the United States. Disease outbreaks and following restrictions in international trade are causing major economic losses worldwide. Wild boars are the natural reservoir of the virus. They represent high danger to pork industry in regions with high density of wild boar population. Live attenuated vaccine has been used in Russia for decades for the total swine population vaccination. Today Russia belongs to world leaders in the pork production, but it still has to be recognized as CSF virus (CSFV) free country (or region) to be incorporated in a global market. The first step in this direction would be the implementation of non-replicating marker vaccines, allowing the differentiation between infected and vaccinated animals (DIVA). Here we first report results of recombinant E2 protein-based vaccine formulations trial in Russian Federation, where optimal administration protocol, adjuvant, dosage of specific component were selected. From the formulations tested, safe and effective vaccine formulation was selected, that needs to be tested for antigen stability and immunity duration in vaccinated animals, and then undergo clinical trial on farm. The aim of our study is the development of the vaccine based on CSFV recombinant surface glycoprotein E2 according to requirements for the country/region free of CSFV. We performed the series of animal trials on *Sus scrofa* Landrace-Duroc breed (total number of pigs 84, divided into 9 experimental and 2 control groups) to assess the vaccination schedule, antigen dosage, and choice of adjuvant. Highly pathogenic CSFV Shi-Men strain was used for challenge in 5×10^5 LD_{50} dose (ARRIAH collection). Double parenteral administration of 10 μg or 30 μg of antigen as well as single administration of 30 or 60 μg of E2 had not provided sufficient level of protection. Oil adjuvant was reactogenic at the inoculation spot even when used once, while polymeric adjuvant has not produced local or systemic reactions after the administration single time or twice. Double administration with the vaccine containing 60 rg of the antigen and polymeric adjuvant has completely protected pigs from the death after the challenge, while in non-vaccinated/challenged control group 5 out of 11 animals died within 14 days post-challenge. Vaccinated animals had less pronounced fever that lasted shorter (rise of the rectal temperature was delayed for 2 days, release of fever 2 days before the control challenge group), frequency and longevity of viremia and virus shedding in nasal swabs were significantly ($p \le 0.05$) reduced as compared to inoculated control piglets. Animals in this vaccinated group gained weight every day after the challenge, being slightly behind non-vaccinated/non-challenged controls. The high levels of antibodies against E2 protein were detected in sera of vaccinated animals before the challenge and they all were negative for antibodies to E^{rns} pro-

tein. After the challenge antibodies to E^{rns} proteins started to raise in sera of all animals save non-vaccinated/non-challenged controls, thus we developed the product that may be implemented as a marker vaccine against CSFV.

Keywords: Classical swine fever, subunit vaccine, CSF E2, adjuvant, vaccine development

Classical swine fever (CSF) is a highly contagious viral disease of domestic and wild pigs, which proceeds in acute, chronic and subclinical forms and causes significant economic damage to many countries with developed swine industry [1-3]. Thus, as the result of the CFS outbreaks in 1993-1998, more than 13 million pigs were eliminated out of necessity in a number of EU countries (Netherlands, Germany, Spain, Belgium, Italy), and the total damage exceeded 5 billion euros [4].

The causative agent of the disease is the RNA-containing virus belonging to the *Pestivirus* genus of the *Flaviviridae* family. The same genus includes the bovine diarrhea virus of I and II types and the border disease virus [5]. The genome of the CSF virus (CSFV) is represented by the single-stranded RNA molecule of positive polarity with the length of 12.3 thousand nucleotides, which encodes 4 structural and 8 non-structural proteins [6, 7]. The CSFV virions are in the form of spherical particles with the diameter of 40-60 nm. They consist of a nucleocapsid and a lipoprotein envelope. The nucleocapsid consists of RNA and C protein, the envelope is formed by three glycoproteins: E^{rns} (gp44/48), E1 (gp33) and E2 (gp55), which owing to disulfide links form the complexes (E^{rns} is homodimer, E1-E2 is heterodimer and E2 is homodimer) [8]. The protein of E^{rns} has a ribonuclease activity [9]. The heterodimeric complex E1-E2 ensures the virus's penetration into a cell [10]. CSFV E2 protein possesses antigenic epitopes on its surface that are involved in cellular and humoral immune responses to the virus: the monoclonal antibodies to this protein have the virus-neutralizing activity [11, 12]. Besides the antibodies to the E2 protein, the antibodies to E^{rns} and non-structural NS3 protein are detected in the infected animals' organisms [13].

The specific CSF prevention measures in Russia are based on using safe and highly immunogenic live vaccines. The most effective is the CS vaccine, which differs from other similar drugs with the high virus content in one vaccination dose (no less than 10^5 ImD_{50}) [14]. However, classical live vaccines do not allow differentiation of the vaccinated and infected animals [5, 15, 16]. This problem can be solved with using marked vaccines: subunit, chimeric, vector and DNA vaccines, among which only the subunit vaccine is available as commercial drug and the chimeric vaccine is licensed for use [15]. In terms of effectiveness, chimeric vaccines are comparable to traditional live vaccines [15, 16]. Implementation of live vaccines is related to the potential risks of recombination of the vaccine virus with the field one and sometimes is forbidden under the rules of importing animal products, therefore, the subunit recombinant vaccines based on the surface glycoprotein E2 of the CSF virus have been developed as a safe alternative to live vaccines. Subunit vaccines cause shorter immunity, require more time for its formation, are administered 2 times and do not ensure the sterile immunity [16]. Nevertheless, they are safe, ensure the animals protection from the control infection with the virulent virus and make it possible to apply the DIVA (differentiating infected from vaccinated animals) strategy [15, 17, 18]. In vaccinated pigs, by using the method of enzyme-linked immunosorbent assay (ELISA) the antibodies only to E2 protein can be detected, and in animals that were infected with field strains of the CSF virus the antibodies to both E^{rns} and E2 proteins can be detected [19, 20].

Previously, we have obtained and characterized the recombinant E2 en-

velope protein of the Shi-Men strain of the CSF virus [21], and the laboratory sample of the recombinant subunit vaccine against CSF, which protected pigs from infection with the virulent Shi-Men strain of the CSF virus has been developed basing on this protein [22]. In current study, we have improved the technology of obtaining of the CSF virus's recombinant E2 protein, created the construction for the coexpression of the genes of the surface E2 glycoprotein of two genotypes of the CSF virus, circulating in the territory of Russia, and have determined the composition and dosage of the preparation, which is safe and able to protect the experimental animals from infection with the highly pathogenic Shi-Men strain of the CSF virus.

Our objective was the evaluation of optimal composition of preparations, recombinant protein dosage, and administration schedule for the laboratory samples of the recombinant subunit vaccine against the classical swine fever.

Techniques. The laboratory samples of the recombinant subunit vaccine were prepared from the E2 protein of the 8Z, Shi-Men (genotype 1.1) and Alfort-Tübingen strains (genotype 2.3) previously obtained in the baculovirus gene expression system in our laboratory. The sequence encoding the ectodomain of the CSF virus's surface glycoprotein E2 was amplified from the field material (spleen) received by the laboratory in 1997. After determining the primary nucleotide sequence of the amplified fragment, the CSF virus's field isolate (working title 8Z) was classified to the genotype 1.1 along with the highly pathogenic Shi-Men strain (8Z differs from the latter by 8 amino acid substitutions). Then the commercial vector pFastBacHTc (Life Technologies, USA) was modified. On the CpoI and NcoI restriction sites we cut off a fragment between the promoter and the polylinker of the plasmid with the encoded histidine tag and the TEV-protease recognition site, and inserted the sequence encoding the signal peptide of secretion of the melittin bee venom toxin, which has been assembled from three synthetic oligonucleotides. Using the primers containing the NheI and EcoRI restriction sites, we amplified the part of the sequence of the E2 gene of the 8Z isolate (the fragment encoding the 31st amino acid at the C-terminus of the protein forming the transmembrane domain was excluded). As the result of expression of the obtained construct, we observed the production of the E2 surface glycoprotein ectodomain of isolate 8Z secreted into the culture medium. The construct, which simultaneously carries two genes encoding the E2 surface glycoproteins of the Shi-Men (genotype 1.1) and Alfort-Tübingen (genotype 2.3) CSF viruses, has been was obtained synthetically (Eurogen, Russia), both genes are preceded by the sequence of the signal peptide of the melittin secretion and also have been devoid of the region encoding the transmembrane domain. The transfection of insect cells by the recombinant baculovirus genome, infection of the cells, and accumulation of the recombinant protein in the Sf-9 cell culture has been performed according to the technique we described previously [22]. Besides, we used the commercial preparation of recombinant E2 (Prionics AG, Switzerland). The synthetic polyacrylate (Vet-Biohim LLC, Russia), and the incomplete Freund's adjuvant (Sigma Aldrich, USA) have been used as adjuvants. The obtained vaccines have been stored at 4 °C.

The effectiveness of different variants of recombinant subunit vaccine against CSF has been studied at the research facilities of the Lisiy Ostrov (branch of FSC VIEV RAS, Vyshny Volochyok). Piglets (n = 84) of 50-days age (taken from the CSF-free farm in which the prophylactic immunization with the live vaccine against CSF is carried out) have been used for the tests. According to the results of the enzyme immunoassay (ELISA), before the immunization, most piglets did not contain the antibodies to the CSF virus. The animals were distributed into groups depending on the vaccine composition, antigen content

and vaccination schedule.

The vaccine preparations (2 ml) were injected intramuscularly once or 2 times. In case of 2-times administration, the first vaccination of piglets was carried out at 50-days age, the second one in 21 days. In case of one-time administration, the animals were vaccinated 21 days before the challenge. 14 days after the second vaccination (or 21 days after the one-time vaccination), the animals of all groups, except for the X group, were intranasally and intramuscularly injected with 2 ml of the Shi-Men strain (1.25×10^5 LD_{50}/ml) (All-Russian Research Institute for Animal Health, Vladimir, Russia). Before the challenge, the animals of the X group (negative control) were transferred to other building in order to exclude the possibility of accidental infection with the CSF virus. After the control challenge, every second day during 2 weeks, the piglets' body temperature was measured, nasal swabs and blood samples were taken and weighing was performed. On day 14 after the challenge, all piglets were euthanized; the samples of spleen, tonsils and mesenteric lymph nodes were taken from each animal.

The authors confirm that the permission to conduct the experiments from the Committee on Ethics and Humane Treatment of Animals of the FSC VIEV RAS has been received, and all the requirements for working with animals have been met.

The blood sera have been investigated for the presence of the antibodies to the E2 protein of the CSF virus by the ELISA method in the commercial test systems (CSF-SEROTEST, Vetbiochem, Russia) and the antibodies to E^{rns} protein (Priochek CSFV E^{rns}, Thermo Fisher Scientific, USA) according to the attached instructions. For registering the reaction results, the iMark plan-table photometer with the 450 nm filter (Bio-Rad, USA) has been used. The results have been expressed as the coupling ratio ($C_{rat.}$) which has been calculated according to the instruction of the test system's manufacturer. The data have been interpreted (positive or negative serum) according to the criteria of the test system's manufacturer. The CSF virus RNA in the nasal swabs and sera were detected by the method of polymerase chain reaction (PCR) using the commercial test system for the detection of classical swine fever virus by the PCR method (Vetbiochem, Russia); the results were designated as "+" or "–".

The obtained data have been processed by the analysis of variance (ANOVA). The mean values (*M*) and standard errors of the mean (±SEM) have been calculated. In the Results section, the differences between the indices for the group I (the vaccine preparation recommended by us for further tests) and group IX (challenge control) were statistically significant at $p \le 0.05$.

Results. Animal assignment to groups, vaccine composition, antigen content and vaccination schedules are described in Table 1.

1. Design of the experiment on comparison of different vaccination schedules and subunit vaccines against the classical swine fever (CSF) (research facilities of Lisiy Ostrov, FSC VIEV RAS, Vyshny Volochyok)

Group	Vaccines	Piglets number per group	
		total	E2 seropositive on day of the 1st or single immunization
	Double vaccination		
I	E2 protein of8Z, 60 μg, synthetic polyacrylate	15	6
II	E2 protein of 8Z, 30μg, synthetic polyacrylate	10	4
III	E2 protein of 8Z, 10 μg, synthetic polyacrylate	5	2
IV	E2 protein of Shi-Men + Alfort-Tübingen, 30 μg, Freund's incomplete adjuvant	5	2
V	E2 protein of 8Z, 30 μg, Freund's incomplete adjuvant	15	5
VI	Commercial E2 preparation, 60 μg, synthetic polyacrylate	5	0

Continued Table 1

	Single vaccination		
VII	E2 protein of 8Z, 60 μg, synthetic polyacrylate	4	2
VIII	E2 protein of 8Z, 30 μg, Freund's incomplete adjuvant	5	2
	Controls		
IX	Challenge control (unvaccinated animals infected at the 84-days age)	11	4
X	Negative control (unvaccinated and uninfected animals)	8	5

Note. Under double vaccination of piglets, the 1st one was performed at the age of 50 days, the 2nd one — 21 days after the 1st vaccination; the control challenge was made 14 days after the second vaccination. Single vaccination was performed at the age of 64 days, the control challenge — 21 days after the vaccination.

2. Effectiveness of different vaccination schedules and CSF subunit vaccines (research facilities of Lisiy Ostrov, FSC VIEV RAS, Vyshny Volochyok)

Group	Vaccines	Piglets			
		infected	with fever above 41 °C	sick	dead
I (n = 15)	E2 protein of 8Z, 60 μg, synthetic polyacrylate	15	6	0	0
II (n = 10)	E2 protein of 8Z, 30μg, synthetic polyacrylate	10	3	1	0
III (n = 5)	E2 protein of 8Z, 10 μg, synthetic polyacrylate	5	5	5	1
IV (n = 5)	E2 protein of Shi-Men + Alfort-Tübingen, 30 μg, Freund's incomplete adjuvant	5	0	0	0
V (n = 15)	E2 protein of 8Z, 30 μg, Freund's incomplete adjuvant	15	4	2	2
VI (n = 5)	Commercial E2 preparation, 60 μg, synthetic polyacrylate	5	3	0	0
VII (n = 4)	E2 protein of 8Z, 60 μg, synthetic polyacrylate, single vaccination	4	4	4	3
VIII (n = 5)	E2 protein of 8Z, 30 μg, Freund's incomplete adjuvant, single vaccination	5	5	1	0
IX (n = 11)	Challenge control (unvaccinated animals infected at the 84-days age)	11	11	11	5
X (n = 8)	Negative control (unvaccinated and uninfected animals)	0	0	0	0

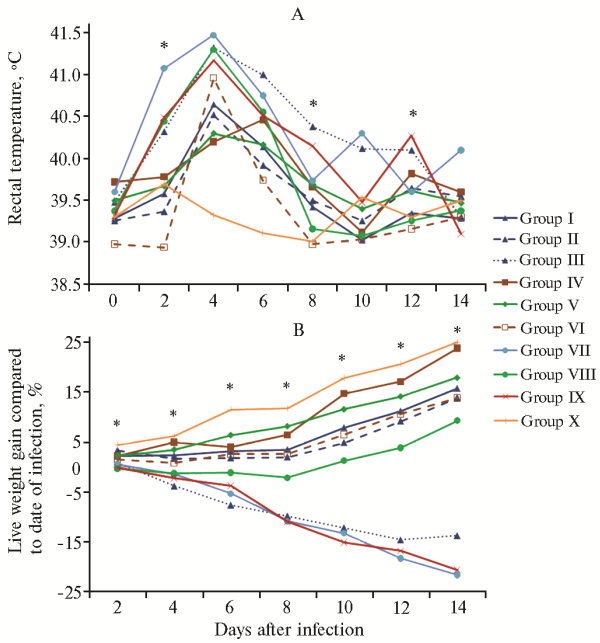

Fig. 1. Rectal temperature (A) **and increase in body weight** (B) **in piglets upon different vaccination schedules and CSF subunit vaccines** (research facilities of Lisiy Ostrov, FSC VIEV RAS, Vyshny Volochyok). See groups' descriptions in Table 1. The asterisks indicate the points for which the differences between groups I and IX are statistically significant at $p < 0.05$.

The experiment results showed (Table 2) that the injection of all samples of the vaccine against the CSF has not followed by general suppression of the condition and the increase in body temperature of the piglets. The control challenge of the unvaccinated animals (group IX) led to the development of the typical clinical manifestations. On day 14 after the challenge, 5 of 11 piglets in this group died (mortality 45.5%), the rest ones showed a significant decrease in body weight. In groups III and VII, the mortality rate was 20.0 and 70.0% respectively. Up to 8th day after the challenge, a fever was found in the animals

of group VIII, after that the temperature returned to normal value. In groups I, II, IV, V and VI, a moderate and short-term increase in temperature occurred, and only in some animals the temperature of 41°C was registered (Fig. 1, A, see Table 2).

The maximum decrease in body weight was observed in piglets of groups III and VII: the registered values were identical to those in group IX (challenge control) – the uniform decrease of these indices was observed from the 1st to the 14th day after the challenge (see Fig. 1, B). In groups I, II, IV, V, VI, and VIII, either there was no any decrease in the animals' body weight, or it manifested in a significantly lesser extent ($p \leq 0.05$). Moreover, from days 4-8 after the challenge, in groups I, IV, V and VI the complete restoration of the dynamics of increase in body weight occurred that is characteristic for the control (uninfected) piglets.

3. Blood level of antibodies to CSFV proteins E^{rns} and E2 ($C_{bind.}$ as per instructions of test kit manufacturers) **in piglets upon different vaccination schedules and CSF subunit vaccines** (research facilities of Lisiy Ostrov, FSC VIEV RAS, Vyshny Volochyok)

Group	Vaccines	Vaccination		Challenge with		Day 14 after the challenge		
		1st/single	2nd	E2	E^{rns}	E2	E^{rns}	
							$C_{bind.}$	"+", %
I	E2 protein of 8Z, 60 μg, synthetic polyacrylate	60.72 (+)	67.10 (+)	104.89 (+)	18.59 (–)	107.35 (+)	47.42 (+)	73.3
II	E2 protein of 8Z, 30μg, synthetic polyacrylate	53.17 (+)	57.05 (+)	105.29 (+)	17.94 (–)	108.37 (+)	45.95 (+)	70.0
III	E2 protein of 8Z, 10 μg, synthetic polyacrylate	70.00 (+)	57.90 (+)	81.44 (+)	23.14 (–)	108.40 (+)	52.98 (+)	80.0
IV	E2 protein of Shi-Men + Alfort-Tübingen, 30 μg, Freund's incomplete adjuvant	69.66 (+)	67.50 (+)	71.96 (+)	27.88 (–)	105.28 (+)	50.84 (+)	60.0
V	E2 protein of 8Z, 30 μg, Freund's incomplete adjuvant	62.95 (+)	71.11 (+)	86.51 (+)	22.48 (–)	103.65 (+)	31.88 (–)	53.8
VI	Commercial E2 preparation, 60 μg, synthetic polyacrylate	43.94 (–)	40.36 (–)	101.78 (+)	–0.12 (–)	112.26 (+)	35.10 (–)	60.0
VII	E2 protein of 8Z, 60 μg, synthetic polyacrylate, single vaccination	62.83 (+)	nd	47.93 (–)	24.78 (–)	107.40 (+)	55.70 (+)	100
VIII	E2 protein of 8Z, 30 μg, Freund's incomplete adjuvant, single vaccination	60.26 (+)	nd	81.64 (+)	25.10 (–)	108.84 (+)	44.50 (+)	60.0
IX	Challenge control (unvaccinated animals infected at the 84-days age)	51.00 (+)	41.15 (–)	39.89 (–)	14.25 (–)	51.07 (+)	30.30 (–)	33.3
X	Negative control (unvaccinated and uninfected animals)	63.64 (+)	50.17 (+)	47.58 (–)	5.51 (–)	41.38 (–)	9.70 (–)	0

N o t e. nd — no data; "+"/"–"— presence/absence of the relevant antibodies in ELISA test.

The average values of the relative content of antibodies ($C_{bind.}$, %) to E2 and E^{rns} proteins in the piglets' blood serum are given in Table 3. 2 weeks after the second immunization, the seroconversion to E2 protein was found in all vaccinated animals, while in unvaccinated animals by this time the status was seronegative. After the control challenge, in vaccinated animals, the content of antibodies to E2 continued to increase. As of the moment of the challenge, the antibodies to E^{rns} protein have not been detected in all piglets, however, on the 14th day after the challenge such antibodies were found in most infected animals. The minimum share of the animals which manifested the positive response to E^{rns} has been found in groups IV, V, VI, VIII and in the challenge control group. It is necessary to remember that seronegative animals should be vaccinated (in our case, most animals reached this status by the age of 50 days), because

a high level of maternal antibodies may adversely affect the development of the immune response after the vaccination [23, 24].

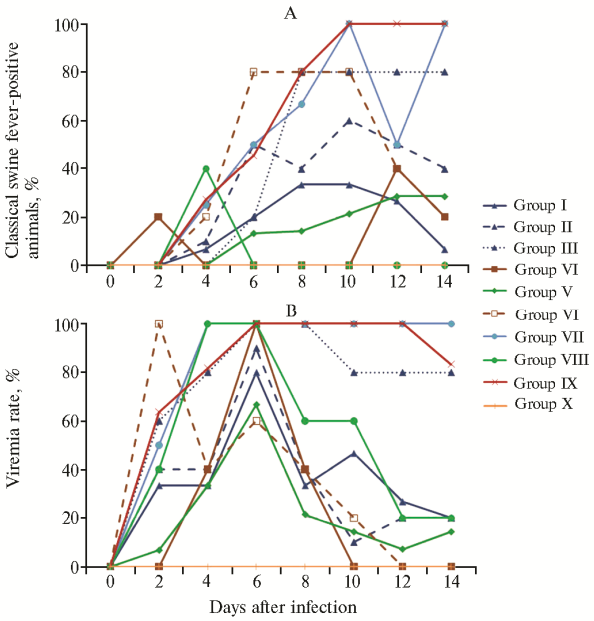

Fig. 2. Detection of CSF virus RNA in nasal swabs (A) **and viremia** (B) **in piglets upon different vaccination schedules and CSF subunit vaccines** (research facilities of Lisiy Ostrov, FSC VIEV RAS, Vyshny Volochyok). For PCR analysis; see the description in Table 1; the differences of the patterns when detecting the virus in the nasal swabs and blood of animal groups I and IX are statistically significant at $p < 0.05$.

The data on viremia and detection of CSF virus in nasal swabs after the control challenge of the piglets are given in Figure 2. The vaccine preparations have not ensured the sterile immunity: viremia and presence of CSF virus in nasal swabs were detected in all experimental groups. The highest values were in groups II, III, and VII, and the lowest in groups I, IV and V (see Fig. 2, A). It is noteworthy that the data on virus isolation are positively correlated with the clinical manifestations of CSF. In the blood serum of the animals of groups III, VII and IX, the CSF virus was detected on the 6th and 8th day, then the viremia was found in at least 80% of the animals of these groups. At the same time, in the groups I, II, IV, V and VI, the peak of viremia occurred on the 6th day, and already from the 8th day less than half of the animals contained the virus in the blood, after that the share of positively reacting animals continued to decrease (see Fig. 2, B). It is known that the absence of sterile immunity is characteristic of both live and subunit vaccines against CSF, however, a significant decrease in viral load allows us to conclude that this vaccine is highly effective [25].

The analysis of the obtained data shows that the vaccine preparations used in groups I, IV, and VI have ensured a sufficient degree of protection after the control challenge. However, the emulsified preparation with the antigen dose of 30 μg, having demonstrated good results in average, has not prevented the lethal development of the disease in two piglets. Moreover, even a single application of this preparation was followed by the development of reactogenicity (Fig. 3, A, B) that excludes the possibility of using such preparations in production conditions. The comparison of the efficiency of the application schedules with 1-time and 2-time vaccination testifies about the advantage of the latter one that is consistent with the data previously obtained from other researchers [1, 26].

Some authors report that single application of the E2-based recombinant subunit vaccine showed itself to be effective [27, 28] that may be due to the successful choice of the adjuvant, the introduction in the vaccine composition of additional immunostimulating molecules and using the piglets being completely naive to the CSF virus.

It is worth noting individually that it is necessary to include in the vaccine composition of the E2 proteins of both genotypes (group IV). Only one of

the four antigenic epitopes of E2 in the main genotypes of the CSFV is conservative [1, 29, 30] that may lead to lowered efficiency of the recombinant vaccine when infection with heterologous strains of the CSFV [31]. Considering the fact that in the territory of Russia the circulation of: 1 (historical) and 2 [32, 33] CSFV genotypes are shown, the necessity to include in the vaccine composition of the E2 proteins of the both CSFV genotypes is not in doubt. The researches we performed have shown that such vaccine by its efficiency is not inferior to the preparation based on the E2 protein of the genotype 1 of the CSF virus. However, the oil adjuvant used in the manufacture of this preparation caused a reactogenicity, therefore, it is necessary to compare the efficiency of the preparations based on synthetic polyacrylate including those when inoculation of the animals with highly virulent strains of different CSFV genotypes.

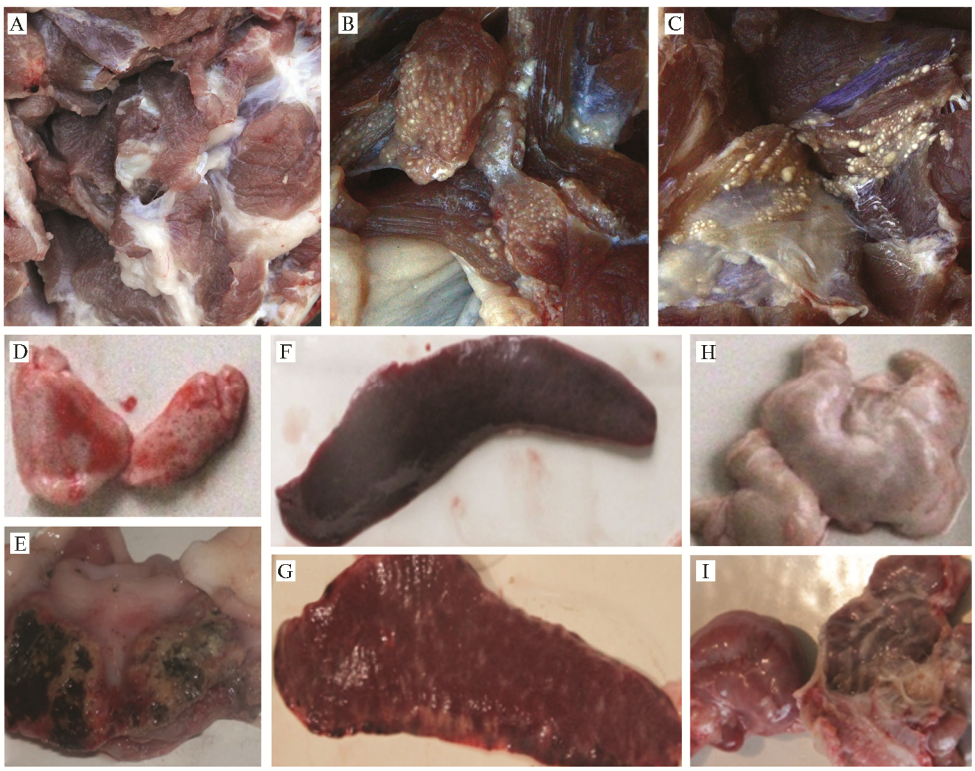

Fig. 3. Reactogenicity upon immunization and pathological changes in piglets challenged with CSF virus upon different vaccination schedules and CSF subunit oil adjuvant vaccines (research facilities of Lisiy Ostrov, FSC VIEV RAS, Vyshny Volochyok; see the description in Table 1).

A: The muscle tissue is unchanged, the animal of group VIII.

B: Muscle tissue, the injection site, the animal of group VIII. Diffusely spaced white milliary nodules are visible.

C: Muscle tissue, the injection site, the animal of group V. Diffusely spaced white diffuse miliary nodules are visible.

D: Tonsil of the animal of group I. Without changes.

E: Tonsil of the animal of group IX. Erosive-ulcerative lesion of the mucous coat with the area of necrosis (black) and the strongly pronounced inflammatory reaction around the pathological region.

F: Spleen of the animal of group I. Elastic consistency. The edges are sharp.

G: Spleen of the animal of group IX. From the parietal and visceral surface of the necrosis area of gray-white color, local regions of hemorrhages located mainly along the organ's edge.

H: Mesenteric lymph node of the animal of group I. Without changes.

I: Mesenteric lymph node of the animal of group IX. The capsule is sharply tense, the parenchyma at the section has extensive hemorrhages.

So, as the result of the researches, the preparation based on the recom-

binant vaccine against the classical swine fever (CSF), which combines safety and high efficiency, has been found. The two-time immunization with the vaccine containing E2 (60 μg/dose) of the 8Z strain of genotype 1 of CSFV and based on synthetic polyacrylate as an adjuvant ensured the apparent humoral immune response with the high degree of defense against the clinical manifestations of CSF and has not followed by the manifestation of reactogenicity. The application of the recombinant vaccine against the CSF has made it possible to differentiate the vaccinated and infected animals.

REFERENCES

1. Chander V., Nandi S., Ravishankar C., Upmanyu V., Verma R. Classical swine fever in pigs: recent developments and future perspectives. *Animal Health Research Reviews*, 2014, 15(1): 87-101 (doi: 10.1017/S1466252314000024).
2. Beltran-Alcrudo D., Falco J.R., Raizman E., Dietze K. Transboundary spread of pig diseases: the role of international trade and travel. *BMC Veterinary Research*, 2019, 15(1): 64 (doi: 10.1186/s12917-019-1800-5).
3. Zhou B. Classical swine fever in China — an update minireview. *Frontiers in Veterinary Science*, 2019, 6: 187 (doi: 10.3389/fvets.2019.00187).
4. Sergeev V.A., Orlyankin B.G., Alekseev K.P., Zaberezhnyi A.D., Aliper T.I., Nepoklonov E.A. *Veterinariya*, 2018, 4: 3-11.
5. Kirkland P.D., Le Potier M.-F., Vannier P., Finlaison D. *Pestiviruses*. In: *Diseases of swine*. J.J. Zimmerman, L.A. Karriker, A. Ramirez, K.J. Schwartz, G.W. Stevenson (eds.). Wiley-Blackwell, 2012: 538-553.
6. Rümenapf T., Meyers G., Stark R., Thiel H.J. Molecular characterization of hog cholera virus. In: *Ruminant Pestivirus infections. Archives of Virology (Supplementum 3), vol. 3*. B. Liess, V. Moennig, J. Pohlenz, G. Trautwein (eds.). Springer, Vienna, 1991: 7-18 (doi: 10.1007/978-3-7091-9153-8_2).
7. Lindenbach B.D., Muttay C.L., Thiel H.J. *Flaviviridae*. In: *Fields virology*. D.M. Knipe, P.M. Howley (eds.). Lippincott Williams and Wilkins, Philadelphia, 2013: 712-746.
8. Thiel H.J., Stark R., Weiland E., Rümenapf T., Meyers G. Hog cholera virus: molecular composition of virions from a pestivirus. *Journal of Virology*, 1991, 65: 4705-4712.
9. Wang F.I., Deng M.C., Huang Y.L., Chang C.Y. Structures and Functions of Pestivirus Glycoproteins: Not Simply Surface Matters. Viruses, 2015, 7(7): 3506-3529 (doi: 10.3390/v7072783)
10. Weiland E., Stark R., Haas B., Rьmenapf T., Meyers G., Thiel H.J. Pestivirus glycoprotein which induces neutralizing antibodies forms part of a disulfide-linked heterodimer. *Journal of Virology*, 1990, 64(8): 3563-3569.
11. Nepoklonov E.A., Aliper T.I., Leneva I.A. *Voprosy virusologii*, 1999, 2: 54-60 (in Russ.).
12. Chang C.Y., Huang C.C., Lin Y.J., Deng M.C., Chen H.C., Tsai C.H., Chang W.M., Wang F.I. Antigenic domains analysis of classical swine fever virus E2 glycoprotein by mutagenesis and conformation-dependent monoclonal antibodies. *Virus Research*, 2010, 149(2): 183-189 (doi: 10.1016/j.virusres.2010.01.016).
13. Schroeder S., von Rosen T., Blome S., Loeffen W., Haegeman A., Koenen F., Uttenthal A. Evaluation of classical swine fever virus antibody detection assays with an emphasis on the differentiation of infected from vaccinated animals. *Revue Scientifique et Technique (International Office of Epizootics)*, 2012, 31(3): 997-1010 (doi: 10.20506/rst.31.3.2173).
14. Nepoklonov E.A. *Klassicheskaya chuma svinei: razrabotka metodov laboratornoi diagnostiki i sredstv spetsificheskoi profilaktiki. Doktorskaya dissertatsiya* [Classical swine fever: the development of laboratory diagnostic methods and means of specific prevention. DSc Thesis]. Moscow, 2000 (in Russ.).
15. Blome S., Mos C., Reimann I., König P., Beer M. Classical swine fever vaccines — State-of-the-art. *Veterinary Microbiology*, 2017, 206: 10-20 (doi: 10.1016/j.vetmic.2017.01.001).
16. Larson K.L., Zaabel P., Spickler A.R., Roth J.A. *NAHEMS Guidelines: Vaccination for Contagious Diseases, Appendix B: Vaccination for Classical Swine Fever*. USDA, 2017.
17. Hulst M.M., Westra D.F., Wensvoort G., Moormann R.J. Glycoprotein E1 of hog cholera virus expressed in insect cells protects swine from hog cholera. *Journal of Virology*, 1993, 67: 5435-5442.
18. König M., Lengsfeld T., Pauly T., Stark R., Thiel H.-J. Classical swine fevervirus: independent induction of protective immunity by two structural glycoproteins. *Journal of Virology*, 1995, 69: 6479-6486.
19. Huang Y.-L., Denga M.-C., Fun-In Wang F.-I., Huang C.-C., Chang C.-Y. The challenges of classical swine fever control: Modified live and E2 subunit vaccines. *Virus Research*, 2014, 179: 1-11 (doi: 10.1016/j.virusres.2013.10.025).
20. Dong X.N., Chen Y.H. Marker vaccine strategies and candidate CSFV marker vaccines. *Vaccine*, 2007, 25(2): 205-230 (doi: 10.1016/j.vaccine.2006.07.033).
21. Krivonos A.V., Zaberezhnyi A.D., Grebennikova T.V., Aliper T.I., Musienko M.I., Gibadulin

R.A., Tsibezov V.V., Bogdanova V.S., Kal'nov S.L., Aliper T.I., Nepoklonov E.A. *Voprosy virusologii*, 2000, 2: 29-36 (in Russ.).
22. Tsibezov V.V., Bogdanova V.S., Zaberezhnyi A.D., Grebennikova T.V., Krivonos A.V., Dudnikov L.A., Kal'nov S.L., Aliper T.I., Nepoklonov E.A. *Voprosy virusologii*, 2000, 2: 36-41 (in Russ.).
23. Klinkenberg D., Moormann R.J.M., de Smit A.J., Bouma A., de Jong M.C.M. Influence of maternal antibodies on efficacy of a subunit vaccine: transmission of classical swine fever virus between pigs vaccinated at 2 weeks of age. *Vaccine*, 2002, 20: 3005-3013 (doi: 10.1016/S0042-207X(02)00283-X).
24. Lipowski A., Drexler C., Pejsak Z. Safety and efficacy of a classical swine fever subunit vaccine in pregnant sows and their offspring. *Veterinary Microbiology*, 2000, 77(1-2): 99-108 (doi: 10.1016/S0378-1135(00)00266-2).
25. van Oirschot J.T. Vaccinology of classical swine fever: from lab to field. *Veterinary Microbiology*, 2003, 96(4): 367-384 (doi: 10.1016/j.vetmic.2003.09.008).
26. Bouma A., de Smit A.J., de Kluijver E.P., Terpstra C., Moormann R.J. Efficacy and stability of a subunit vaccine based on glycoprotein E2 of classical swine fever virus. *Veterinary Microbiology*, 1999, 66(2): 101-114 (doi: 10.1016/S0378-1135(99)00003-6).
27. Madera R., Gong W., Wang L., Burakova Y., Lleellish K., Galliher-Beckley A., Nietfeld J., Henningson J., Jia K., Li P., Bai J., Schlup J., McVey S., Tu C., Shi J. Pigs immunized with a novel E2 subunit vaccine are protected from subgenotype heterologous classical swine fever virus challenge. *BMC Veterinary Research*, 2016, 12(1): 197 (doi: 10.1186/s12917-016-0823-4).
28. Suárez M., Sordo Y., Prieto Y., Rodríguez M.P., Méndez L., Rodríguez E.M., Rodríguez-Mallon A., Lorenzo E., Santana E., González N., Naranjo P., Frías M.T., Carpio Y., Estrada M.P. A single dose of the novel chimeric subunit vaccine E2-CD154 confers early full protection against classical swine fever virus. *Vaccine*, 2017, 35(34): 4437-4443 (doi: 10.1016/j.vaccine.2017.05.028).
29. van Rijn P.A., Miedema G.K., Wensvoort G., van Gennip H.G., Moormann R.J. Antigenic structure of envelope glycoprotein E1 of hog cholera virus. *Journal of Virology*, 1994, 68(6): 3934-3942.
30. Yu M., Wang L.F., Shiell B.J., Morrissy C.J., Westbury H.A. Fine mapping of a C-terminal linear epitope highly conserved among the major envelope glycoprotein E2 (gp51 to gp54) of different pestiviruses. *Virology*, 1996, 222(1): 289-292 (doi: 10.1006/viro.1996.0423).
31. Huang Y.L., Deng M.C., Wang F.I., Huang C.C., Chang C.Y. The challenges of classical swine fever control: modified live and E2 subunit vaccines. *Virus Research*, 2014, 179: 1-11 (doi: 10.1016/j.virusres.2013.10.025).
32. Vlasova A., Grebennikova T., Zaberezhny A., Greiser-Wilke I., Floegel-Niesmann G., Kurinnov V., Aliper T., Nepoklonov E. Molecular epidemiology of classical swine fever in the Russian Federation. *Journal of Veterinary Medicine and Infectious Disease Veterinary Public Health*, 2003, 50(8): 363-367 (doi: 10.1046/j.1439-0450.2003.00695.x).
33. Titov I., Tsybanov S., Malogolovkin A. Genotyping of classical swine fever virus using high-resolution melt analysis. *Journal of Virological Methods*, 2015, 224: 53-57 (doi: 10.1016/j.jviromet.2015.08.012).