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Comparative analysis of VITAVAC oil adjuvants formulated in inactivated foot-and-mouth disease vaccines

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ABSTRACT

Introduction. Foot-and-mouth disease remains one of the most significant transboundary viral infections of livestock, as evidenced by epizootic situation data over the past five years. Culture inactivated vaccines are used for the specific prevention of this disease. For the manufacture of emulsion vaccines, the use of oil adjuvants is essential. Currently, a wide range of these components is available on the global market, including a new product from the Indian company VITAVAC.
Objective. Comparative analysis of VITAVAC oil adjuvants formulated in inactivated foot-and-mouth disease vaccines.
Materials and methods. The following experimental samples of oil adjuvants were tested: VITAVAC 50 (Batch G-221), VITAVAC 70 (Batch G-223), VITAVAC 250 (Batch M-2508). The physicochemical properties of emulsion vaccines manufactured using these adjuvants were analyzed, and their innocuity, safety, and potency were evaluated in accordance with the requirements of the World Organisation for Animal Health.
Results. The most favorable combination of emulsion stability after long-term storage and a pronounced immune response was observed in an experimental vaccine sample formulated with VITAVAC 70 oil adjuvant. Vaccines based on VITAVAC 250 induced virus-neutralizing antibodies with a titer above 1.65 lg SN_{50}, but exhibited lower emulsion stability during storage. The vaccine based on VITAVAC 50 oil adjuvant induced lower levels of specific virus-neutralizing antibodies than the other vaccine variants. The results obtained suggest that oil-based adjuvants of the VITAVAC line, particularly VITAVAC 70, are promising candidates for use in the production of inactivated foot-and-mouth disease vaccines.
Conclusion. The results obtained support the view that VITAVAC 70 is the most promising adjuvant for use in the production of inactivated emulsion vaccines against foot-and-mouth disease for pigs. This product demonstrates comparable performance to the traditionally used oil adjuvants Montanide ISA 206 VG and Montanide ISA 61 VG across several parameters.

Keywords: foot-and-mouth disease, pigs, inactivated emulsion vaccine, oil adjuvants, VITAVAC, Montanide, immune response, micro-neutralization test

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Сравнительный анализ свойств масляных адъювантов компании VITAVAC в составе инактивированных вакцин против ящура

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РЕЗЮМЕ

Введение. Ящур по-прежнему остается одной из наиболее значимых трансграничных вирусных инфекций сельскохозяйственных животных, что демонстрируют данные по эпизоотической ситуации за последние 5 лет. Для специфической профилактики данного заболевания применяют культуральные инактивированные вакцины. Для изготовления эмульсионных вакцинных препаратов требуется использование масляных адъювантов. В настоящее время на мировом рынке представлена широкая линейка данных компонентов, в частности новый продукт индийской компании VITAVAC.
Цель исследования. Проведение сравнительного анализа свойств масляных адъювантов компании VITAVAC в составе инактивированных вакцин против ящура.
Материалы и методы. Исследовали следующие экспериментальные образцы масляных адъювантов: VITAVAC 50 (серия G-221), VITAVAC 70 (серия G-223), VITAVAC 250 (серия M-2508). Осуществляли анализ физико-химических свойств эмульсионных вакцин, изготовленных с применением данных адъювантов, а также определяли авирулентность, безвредность и иммуногенность в соответствии с требованиями Всемирной организации здравоохранения животных.
Результаты. Наиболее оптимальное сочетание стабильности эмульсии при длительном хранении и выраженного иммунного ответа отмечено в экспериментальном образце вакцины, содержащей масляный адъювант VITAVAC 70. Препараты на основе VITAVAC 250 обеспечивали выработку

вируснейтрализующих антител с титром выше 1,65 lg SN_{50}, однако уступали по стабильности эмульсии при хранении. Вакцина с масляным адъювантом VITAVAC 50 по сравнению с другими вариантами индуцировала образование более низкого уровня специфических вируснейтрализующих антител. Полученные данные свидетельствуют о перспективности использования масляных адъювантов линейки VITAVAC, прежде всего VITAVAC 70, для создания инактивированных вакцин против ящура.

Заключение. Совокупность полученных данных позволяет рассматривать VITAVAC 70 как наиболее перспективный адъювант для использования в промышленном производстве инактивированных эмульсионных вакцин против ящура для свиней. Данный препарат сопоставим по ряду показателей с традиционно применяемыми масляными адъювантами Montanide ISA 206 VG и Montanide ISA 61 VG.

Ключевые слова: ящур, свиньи, инактивированная эмульсионная вакцина, масляные адъюванты, VITAVAC, Montanide, иммунный ответ, реакция микронейтрализации

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INTRODUCTION

Foot-and-mouth disease (FMD) remains one of the most significant transboundary viral infections of livestock [1, 2, 3]. In pig breeding, the disease assumes particular epizootological and economic importance, being characterized by high contagiousness, rapid onset of clinical signs, and the introduction of stringent restrictive measures on affected farms [4, 5]. Currently, the risk of foot-and-mouth disease virus (FMDV) entering the territory of the Russian Federation remains high.

According to global FMD situation reports, numerous outbreaks of the disease were registered in the countries adjacent to the Russian Federation from 2020 to the third quarter of 2025. For example, in 2020 two outbreaks were reported in China and 156 in Turkey. In 2021, there were 2 outbreaks in China and 103 in Mongolia. In 2022, one FMD outbreak was again notified by China; three by Mongolia; one by Kazakhstan. In 2023, four outbreaks were reported in China; eleven in South Korea. All these cases were associated with FMDV serotype O, the genetic lineages of which are the most prevalent worldwide. In 2023, Turkey experienced more than 200 FMD outbreaks involving serotypes O and SAT 2 [6, 7]. In 2024, 3 outbreaks were reported in China (serotype O); in Turkey – 104 (serotypes A, O, SAT 2). In the first three quarters of 2025, FMD cases were reported not only in Asia, but also in Europe. On the Asian continent two outbreaks of the disease were recorded (serotype O) in China; 1 (serotype O) – in Mongolia; 19 (serotype O) – in South Korea. Turkey experienced 4 FMD outbreaks caused by serotype A, 222 by serotype O, 12 by serotype SAT 1, 91 by serotype SAT 2. In recent years, cases of this disease have again been recorded in Europe, following decades in which many European countries had maintained FMD-free status. For example, Germany reported one FMD outbreak caused by O/ME-SA/SA-2018 genetic lineage, which previously circulated in Turkey. O/ME-SA/PanAsia2^{ANT-10} genetic lineage that originated in Pakistan in 2017–2018 [8, 9, 10] was responsible for more than 10 outbreaks in Hungary and Slovakia.

Vaccination is the primary means of specific prevention of foot-and-mouth disease [11]. The effectiveness and duration of post-vaccination immunity depend not only on the antigen concentration in the dose and its valency, but also on the type of adjuvant used [12, 13]. Adjuvants are compounds of both inorganic and organic origin that non-specifically enhance the immune response to specific antigens, thereby increasing their immunogenicity by up to tenfold [14, 15, 16].

In veterinary practice, culture inactivated adsorbed FMD vaccines are used, containing adjuvants such as aluminum hydroxide and saponin [14, 16]. At the same time, despite their widespread use, these vaccines have several disadvantages: the induced immune response is often short-lived, necessitating more frequent revaccinations; reactogenicity and allergenicity are elevated due to the inclusion of saponin as an immunostimulant; protective efficacy in pigs remains insufficient and unsatisfactory even when the dose and frequency of administration are increased; and the lack of standardized quality criteria for saponin may lead to variability between vaccine batches [17, 18].

Culture inactivated emulsion vaccines are also used in farms to prevent foot-and-mouth disease. The use of oil adjuvants formulated in such vaccines has a number of advantages. Firstly, it promotes a more intense and prolonged immune response, since the oil phase forms a depot at the injection site that slowly releases antigen over time. This ensures sustained stimulation of the immune system, which leads to a prolonged maintenance of high-level specific antibody titres. Secondly, the vaccine stimulates both the humoral response (Th2-type) and cell-mediated immunity (Th1-type), contributing to the formation of complex, multifaceted protection [19, 20]. Thirdly, these vaccines demonstrate high efficacy in cattle as well as, critically, in pigs, effectively eliminating the main drawback of conventional adsorbed vaccines. Fourthly, emulsion vaccines are characterized by high safety and low reactivity owing to their good biocompatibility, as

evidenced by the lack of pronounced local reactions at the injection site. Fifthly, industrially manufactured oil adjuvants have a stable and standardized composition, which ensures reproducibility between vaccine batches [21, 22].

For many years in the Russian Federation, Seppic (France) oil adjuvants marketed as Montanide, which exhibit enhanced properties, have been used in the production of emulsion vaccines for cattle, pigs, goats, sheep, poultry, and aquaculture species. However, the relatively high cost of these adjuvants posed a significant constraint on the large-scale manufacturing of veterinary vaccines.

In the manufacture of culture inactivated emulsion FMD vaccines, oil adjuvants based on mineral and synthetic oils are most commonly used, forming emulsions of the water-in-oil (W/O) and water-in-oil-in-water (W/O/W) types. At the same time, stringent requirements are applied to the composition and properties of oil adjuvants: they must guarantee emulsion stability during storage and handling, be safe and well tolerated by the target animals, and induce the necessary level of virus-neutralizing antibodies (≥ 1.65 lg SN_{50}) [23].

In 2023 to expand the range of adjuvants used in manufacture the Federal Center for Animal Health started cooperating with VITAVAC Company (India), which produces oil adjuvants. Experimental samples of VITAVAC 50, VITAVAC 70, and VITAVAC 250 were submitted by the company for the production of emulsion FMD vaccines. Of special interest is a comparative analysis of these adjuvants with Montanide ISA oil adjuvants extensively used internationally, with regard to modeling the production and testing of FMD vaccines.

The objective of the study is the comparative analysis of VITAVAC oil adjuvants as components of inactivated foot-and-mouth disease vaccines.

MATERIALS AND METHODS

Oil adjuvants. The following experimental samples of Indian oil adjuvants were used in the study: VITAVAC 50 (Batch G-221), VITAVAC 70 (Batch G-223), VITAVAC 250 (Batch M-2508). Oil adjuvants Montanide ISA 206 VG and Montanide ISA 61 VG (Seppic, France) served as controls.

Experimental vaccine samples. Culture inactivated FMDV strains – SAT-1/Kenya/2017 (SAT-1/I genetic lineage) and SAT-2/LIB/39/2012 (SAT-2/VII/Lib-12 genetic lineage) were used as antigens. The FMDV was cultured in a growth medium containing whole blood-protein hydrolysate and Hottinger broth. The virus was inactivated for vaccine manufacture in accordance with the recommendations of previous publications [24]. Based on these oil adjuvants, five variants of culture inactivated emulsion FMD vaccines were prepared. The compositions of the vaccines are given in Table 1.

The ratio of the aqueous (antigen) phase to the oil phase, as well as the emulsification parameters, were selected in accordance with technological regulations and adjuvant manufacturers' recommendations to obtain stable, finely dispersed emulsions. VITAVAC 50 and 250 oil adjuvants are analogous to Montanide ISA 61 VG and ISA 206 VG, respectively, with regard to the amounts added to the antigen. VITAVAC 70 oil adjuvant is analogous to Montanide ISA 70 VG, but it has not previously been used for the manufacture of FMD vaccines. In this study, it was decided to produce an experimental sample of an emulsion vaccine with the addition of VITAVAC 70, since it is a new product.

Testing of the emulsion physico-chemical properties. The type of emulsion was determined by the drop-test, that is, by applying a drop of the emulsion to the surface of water and visually evaluating whether it dispersed or remained as a discrete drop [23]. Dynamic viscosity was measured using a rotary viscometer at an emulsion temperature of (20 ± 2) °C.

Table 1
Characteristics of experimental samples of culture inactivated emulsion vaccines against FMDV SAT 1 and SAT 2 serotypes

Emulsion sample	146S concentration, μg/dose		Adjuvant	Adjuvant/antigen ratio (w/w) by weight
	SAT-1/Kenya/2017	SAT-2/LIB/39/2012		
Control 1	7.5	7.5	Montanide ISA 206 VG	50/50
Control 2	7.5	7.5	Montanide ISA 61 VG	60/40
Experiment 1	7.5	7.5	VITAVAC 250	50/50
Experiment 2	7.5	7.5	VITAVAC 50	60/40
Experiment 3	7.5	7.5	VITAVAC 70	70/30

The emulsion stability was tested by two methods. In the accelerated stability test, a 10 mL vaccine sample was transferred into a centrifuge tube and, 24 hours after preparation and storage at (4 ± 2) °C, was centrifuged at 3,000 rpm for 30 minutes at (20 ± 2) °C. The resulting separation into distinct fractions was then analyzed to assess emulsion stability.

The following fractions may be observed after centrifugation of the emulsion:

A – oil fraction
B – opalescent (or creamy) layer;
C – emulsion layer;
D – coalescent layer;
E – aqueous fraction (antigen component).

The second method involved storing experimental emulsion samples for the entire shelf life of the vaccine (18 months) at temperatures of (4 ± 2), (20 ± 2), and (37.0 ± 0.1) °C, with periodic visual inspection once per month for phase separation and changes in consistency.

Safety testing of experimental vaccine samples. Ten clinically healthy pigs weighing 30–40 kg were used to assess vaccine reactogenicity. The animals were immunized intramuscularly with a triple dose of the vaccine (6.0 mL) for each of the 5 sample groups. The animals were observed for 10 days after immunization; their general condition, appetite, and behavior were assessed, and body temperature and local reactions at the injection site were recorded.

Evaluation of humoral immunity. Twenty clinically healthy pigs weighing 30–40 kg were divided into 5 groups of 4 animals each, according to the vaccine variant. In each group, 2 mL samples were injected intramuscularly to the animals. Blood was collected from all animals before immunization and at 21 day post vaccination (dpv). Prepared sera were tested to determine the level of virus neutralizing antibodies by micro-neutralization test (MNT) using the porcine kidney cell line (IB-RS-2). In accordance with the requirements of the World Organisation for Animal Health (WOAH), a serum with a virus-neutralizing antibody titer of at least 1.65 lg SN_{50} is considered positive in the MNT.

All animals were handled in compliance with ethical and international standards. The testing was approved by the Federal Centre for Animal Health Bioethics Commission.

Statistical data were processed using generally accepted methods of variational statistics with the aid of the statistical software packages SPSS, Microsoft Excel, and Statistica. The results are expressed as mean values ± standard deviation [25, 26].

RESULTS AND DISCUSSION

For comparative analysis of oil adjuvants, five experimental samples of culture inactivated emulsion vaccines were prepared using: VITAVAC 50 (G-221 Batch), VITAVAC 70 (G-223 Batch), VITAVAC 250 (M-2508 Batch), and Montanide ISA 206 VG and Montanide ISA 61 VG.

Evaluation of dynamic viscosity. The emulsion vaccine samples manufactured using VITAVAC adjuvants had a dynamic viscosity comparable to that of the control vaccines. Compared to the control, VITAVAC emulsions exhibited a slight decrease in viscosity (averaging 10–25%), potentially easing vaccine administration.

Notably, during the pre-emulsion preparation stage, the viscosity of emulsions produced with VITAVAC oil adjuvants was quite high, as visually observed during mixing on a multifunctional laboratory mixer. As shown in Table 2, the dynamic viscosity of the tested samples of the experimental emulsion vaccines No. 1, 2, and 3 was (0.081 ± 0.001), (0.093 ± 0.001), and (0.112 ± 0.001) Pa×s, respectively. These values

Table 2
Dynamic viscosity and type of emulsions prepared using VITAVAC 50, 70, 250 oil adjuvants ($n = 3$, Mean ± SD, $p < 0.01$)

Emulsion sample	Adjuvant	Dynamic viscosity, Pa×s	Type of emulsion (according to drop-test results)
Control 1	Montanide ISA 206 VG	0.091 ± 0.001	W/O/W
Control 2	Montanide ISA 61 VG	0.124 ± 0.001	W/O
Experiment 1	VITAVAC 250	0.081 ± 0.001	W/O/W
Experiment 2	VITAVAC 50	0.093 ± 0.001	W/O
Experiment 3	VITAVAC 70	0.112 ± 0.001	W/O

W/O/W – complex emulsion, W/O – simple reverse emulsion.

fell within the standard ranges: based on many years of observations by Federal Centre for Animal Health specialists, the dynamic viscosity for complex emulsions prepared with Montanide ISA 206 VG is typically 0.090–0.110 Pa×s, whereas for a simple reverse emulsion with Montanide ISA 61 VG, it ranges from 0.010 to 0.150 Pa×s.

Overall, testing of the finished product revealed that for emulsions prepared using VITAVAC 50, 70, and 250 oil adjuvants, the dynamic viscosity was 25, 10, and 11% lower, respectively, compared to the control values for the two emulsion types.

Determination of the emulsion type. The drop test confirmed that vaccines formulated with VITAVAC 50 and VITAVAC 70 are W/O emulsions, whereas those containing VITAVAC 250 and Montanide ISA 206 VG are W/O/W emulsions (Table 2). This finding aligned with the declared characteristics of the adjuvants and enabled direct comparisons among them.

Evaluation of emulsion stability. The stability of prepared experimental vaccine samples was tested by accelerated method. As shown in Table 3, centrifugation of experimental samples No. 1, 2, and 3 resulted in a small amount (5%) of oil phase separation, which is acceptable for oil emulsions. No separation of the antigenic (aqueous) phase was observed in the vaccine containing VITAVAC 70. Vaccines containing VITAVAC 50 and VITAVAC 250 showed a greater tendency toward opalescent oil layer formation and partial aqueous phase separation, possibly due to the adjuvants' composition.

The emulsion stability was also evaluated during storage of experimental vaccine samples at (4 ± 2), (20 ± 2) and (37.0 ± 0.1) °C for 18 months. Sample No. 1, prepared with VITAVAC 250 oil adjuvant and stored for one month at (4 ± 2) °C, remained stable with 5% A-phase separation. At a temperature of (20 ± 2) °C, separation into an oily phase (5%) and an opalescent layer (20%) was observed, while the antigenic phase did not separate. After one month of storage at (37.0 ± 0.1) °C, this emulsion separated into the A and B phases in the same amounts, along with an additional 7% antigenic component (E-phase). After 18 months of storage at (4 ± 2) °C, 30% antigenic phase separation was observed, which was critical for the emulsion stability.

At (4 ± 2) and (20 ± 2) °C, after the entire observation period, sample No. 2 (containing VITAVAC 50 oil adjuvant) exhibited 5% oil fraction separation. The E-phase did not separate. At a temperature of (37.0 ± 0.1) °C, the appearance of the A-phase at 5% and separation of the antigenic component (30%) were also noted, which was not critical, as this temperature is not intended for vaccine storage.

Sample No. 3, containing VITAVAC 70, remained stable after 18 months of storage at all the above-mentioned temperatures, exhibiting only 5% A-phase separation – a level that is entirely non-critical. The antigenic phase did not separate, and the emulsion remained stable.

Thus, during long-term storage (18 months) under various temperature conditions, the vaccine containing VITAVAC 70 demonstrated the best emulsion stability: it remained homogeneous, oil phase separation did not exceed ~5%, and no visual separation of the antigenic phase was observed.

Emulsions formulated with VITAVAC 50 and VITAVAC 250 occasionally showed more pronounced separation, including significant aqueous phase separation at elevated storage temperatures – a limitation that may require further optimization of processing parameters before use. In the tested FMD vaccines based on SAT 1 and SAT 2 serotypes, VITAVAC 70 oil adjuvant demonstrated an optimal combination of rheological properties and emulsion stability across a wide range of storage temperatures.

Table 3
Stability of experimental FMD vaccine samples formulated with VITAVAC 50, 70, 250 oil adjuvants (accelerated aging method)

Sample	Adjuvant	Fraction, %				
		A	B	C	D	E
Control 1	Montanide ISA 206 VG	5	0	95	0	0
Control 2	Montanide ISA 61 VG	0	0	100	0	0
Experiment 1	VITAVAC 250	5	20	65	0	10
Experiment 2	VITAVAC 50	5	5	80	0	10
Experiment 3	VITAVAC 70	5	0	95	0	0

Potential fractions observed after centrifugation: A – oil, B – opalescent layer (creamy oil), C – emulsion, D – coalescent layer (dense emulsion), E – antigenic component (aqueous fraction).

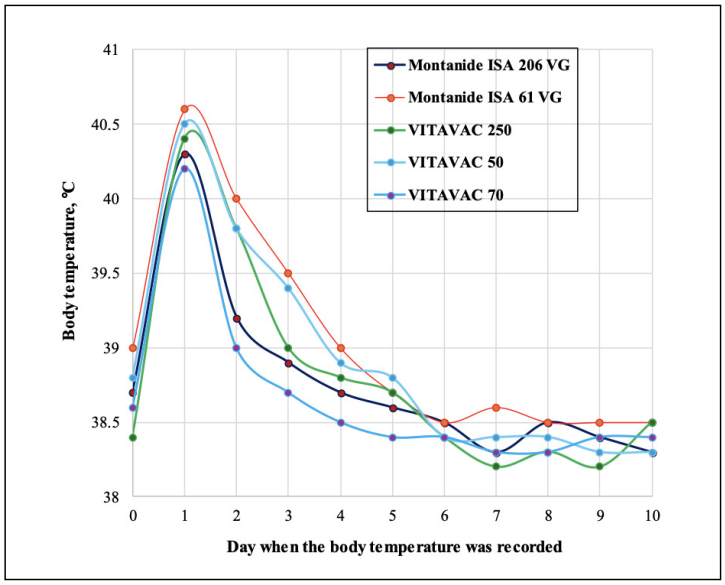

Fig. 1. Body temperature of pigs after administration of experimental FMD emulsion vaccine samples, formulated using various oil adjuvants (n = 3, Mean ± SD, p < 0.01)

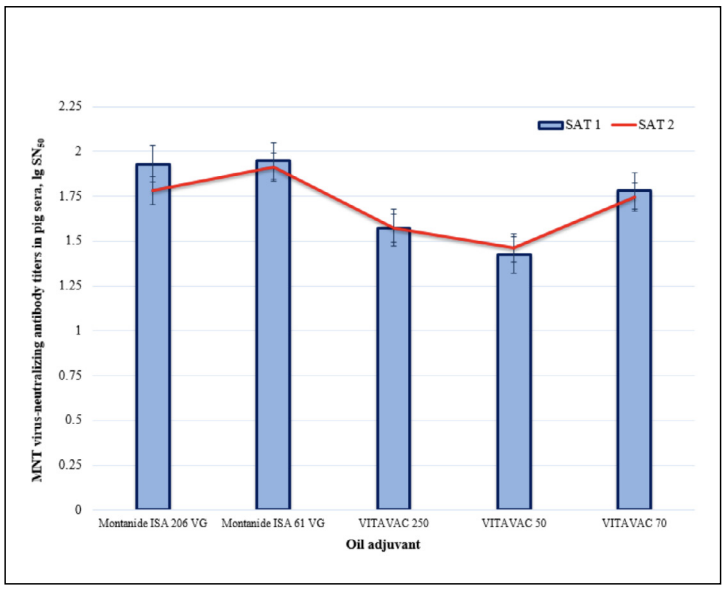

Fig. 2. Virus-neutralizing antibody levels against FMDV serotypes SAT 1 and SAT 2 at 21 dpv in pigs immunized with experimental emulsion vaccines formulated with various oil adjuvants (n = 3, Mean ± SD, p < 0.01)

Safety testing of experimental vaccine samples. The reactogenicity of experimental vaccine samples formulated with the above-mentioned oil adjuvants was studied in pigs. The animals were observed for 10 days after immunization, their general condition, appetite, behavior, body temperature and local reactions at the injection site were evaluated.

After a single injection of vaccines at both standard and triple doses, pigs in all groups did not develop oleogranulomas or other pronounced local reactions at the injection site. At 1 dpv, the animals' body temperature briefly increased to (40.4 ± 0.2) °C. This was regarded as a physiological reaction to the administration of the inactivated vaccine and was observed in all groups, including the controls. Subsequently, body temperature remained within physiological norms, and the behavior and appetite of the animals continued to be satisfactory (Fig. 1).

Thus, based on the results of the conducted studies, all vaccine variants formulated with VITAVAC and Montanide ISA oil adjuvants can be characterized as safe for pigs following a single injection.

Evaluation of humoral immunity. The humoral immunity of pigs was studied following the administration of five experimental emulsion vaccine samples formulated using the above-mentioned oil adjuvants.

The MNT results are shown in Table 4 and Figure 2. The results demonstrate that at 21 dpv, specific virus-neutralizing antibodies against FMDV strains SAT-1/Kenya/2017 and SAT-2/LIB/39/2012 were observed in all experimental and control groups. At the same time, the levels of virus-neutralizing antibodies and their distribution across groups differed.

Administration of the emulsion vaccine containing VITAVAC 250 (Sample No. 1) stimulated the production of virus-neutralizing antibodies at 21 dpv, with titers exceeding the thresholds recommended by WOAH. The average titers of virus-neutralizing antibodies against the FMDV SAT-1/Kenya/2017 and SAT-2/LIB/39/2012 strains were (1.575 ± 0.087) lg SN_{50}, which made it possible to consider this variant as providing a satisfactory level of specific protection. However, virus-neutralizing antibody titers against SAT-1/Kenya/2017 and SAT-2/LIB/39/2012 were 2.3-fold and 1.6-fold lower, respectively, compared to control No. 1.

The emulsion vaccine containing VITAVAC 50 provided a positive immune response; however, the mean titers of virus-neutralizing antibodies were lower than those induced by the emulsion vaccine containing Montanide ISA 61 VG and the other VITAVAC-based vaccine variants. For example, VITAVAC 50 stimulated the production of antibodies against SAT-1/Kenya/2017 and SAT-2/LIB/39/2012 strains that were, respectively, 3.3 and 2.8 times lower than those of control No. 2; 1.4 and 1.3 times lower than those of VITAVAC 250; and 2.3 and 1.9 times lower than those of VITAVAC 70.

The highest level of antibodies was detected in animals of experimental group No. 3, which were immunized with an experimental vaccine sample containing VITAVAC 70.

In all groups, administration of a triple dose of the emulsion vaccine resulted in higher levels of virus-neutralizing antibodies compared to the standard dose, which is consistent with the general pattern of dose-dependent immune stimulation by vaccines.

Based on a comparison of emulsion stability, safety, and immunogenicity, VITAVAC 70 exhibited the most favorable overall characteristics. Among the inactivated culture emulsion vaccines analyzed, VITAVAC 250 and VITAVAC 50 adjuvants were inferior to VITAVAC 70 in terms of both emulsion stability and the production of virus-neutralizing antibodies. All tested adjuvants produced emulsion vaccines that matched the declared emulsion type and were safe for pigs. It should be noted that VITAVAC 250 and 50 require additional optimization of the formulation and emulsification parameters to achieve high emulsion stability and vaccine immunogenicity.

Table 4
Specific antibody levels (MNT) following immunization of pigs with FMD vaccines containing various oil adjuvants ($n = 3$, Mean ± SD, $p < 0.01$)

Sample	Adjuvant	Animal No.	Specific antibody levels (MNT), Ig SN_{50}			
			0 dpv		21 dpv	
			SAT 1	SAT 2	SAT 1	SAT 2
Control 1	Montanide ISA 206 VG	1	< 0.5	< 0.5	1.875	1.650
		2	< 0.5	< 0.5	1.875	1.650
		3	< 0.5	< 0.5	2.025	1.950
		4	< 0.5	< 0.5	1.950	1.875
		Mean ± SD	< 0.5	< 0.5	1.931 ± 0.072	1.781 ± 0.155
		5*	< 0.5	< 0.5	2.550	2.550
		6*	< 0.5	< 0.5	2.625	2.550
Control 2	Montanide ISA 61 VG	7	< 0.5	< 0.5	1.725	1.650
		8	< 0.5	< 0.5	2.025	1.950
		9	< 0.5	< 0.5	2.025	2.025
		10	< 0.5	< 0.5	2.025	2.025
		Mean ± SD	< 0.5	< 0.5	1.950 ± 0.150	1.913 ± 0.179
		11*	< 0.5	< 0.5	3.000	2.625
		12*	< 0.5	< 0.5	3.000	2.625
Experiment 1	VITAVAC 250	13	< 0.5	< 0.5	1.650	1.500
		14	< 0.5	< 0.5	1.650	1.650
		15	< 0.5	< 0.5	1.500	1.500
		16	< 0.5	< 0.5	1.500	1.650
		Mean ± SD	< 0.5	< 0.5	1.575 ± 0.087	1.575 ± 0.087
		17*	< 0.5	< 0.5	2.475	2.475
		18*	< 0.5	< 0.5	2.550	2.475
Experiment 2	VITAVAC 50	19	< 0.5	< 0.5	1.350	1.500
		20	< 0.5	< 0.5	1.500	1.500
		21	< 0.5	< 0.5	1.350	1.350
		22	< 0.5	< 0.5	1.500	1.500
		Mean ± SD	< 0.5	< 0.5	1.425 ± 0.087	1.463 ± 0.075
		23*	< 0.5	< 0.5	2.100	2.250
		24*	< 0.5	< 0.5	2.250	2.250
Experiment 3	VITAVAC 70	25	< 0.5	< 0.5	1.725	1.725
		26	< 0.5	< 0.5	1.800	1.800
		27	< 0.5	< 0.5	1.800	1.725
		28	< 0.5	< 0.5	1.800	1.725
		Mean ± SD	< 0.5	< 0.5	1.781 ± 0.038	1.744 ± 0.038
		29*	< 0.5	< 0.5	2.550	2.625
		30*	< 0.5	< 0.5	2.625	2.400

dpv – day post vaccination, SD – standard deviation, *p* – significance level, MNT – micro-neutralization test, SAT 1 – SAT-1/Kenya/2017 strain, SAT 2 – SAT-2/LIB/39/2012 strain;
* vaccine safety testing.

CONCLUSION

Evaluation of the physicochemical properties of experimental inactivated culture emulsion vaccines formulated with VITAVAC 50, 70, and 250 oil adjuvants (India) showed that the vaccine containing VITAVAC 70 exhibited the best storage stability across a wide temperature range. No antigenic phase separation or significant phase separation occurred during testing.

VITAVAC 50, 70, and 250 oil adjuvants are suitable for manufacturing inactivated culture emulsion FMD vaccines, producing emulsions with satisfactory rheological properties and ensuring vaccine safety for pigs after a single injection of a triple dose.

It was revealed that vaccines containing VITAVAC 70 and VITAVAC 250 oil adjuvants provided the formation of virus neutralizing antibodies against FMDV SAT-1/Kenya/2017 and SAT-2/LIB/39/2012 strains with a titre above 1.65 lg SN_{50}. However, the experimental group that received VITAVAC 70-based vaccine showed the highest MNT antibody titers among all VITAVAC adjuvants tested.

It was found that the vaccine containing VITAVAC 50 did not induce virus-neutralizing antibodies in sufficient quantity to protect against FMD compared with the other studied variants, and it likely requires optimization of its application parameters (e.g., concentration, emulsification conditions, etc.).

Taken together, these data indicate that VITAVAC 70 is the most promising adjuvant for the industrial production of inactivated emulsion FMD vaccines for pigs, being comparable in several parameters to the traditionally used oil adjuvants Montanide ISA 206 VG and ISA 61 VG.

REFERENCES

1. Arzt J., Sanderson M. W., Stenfeldt C. Foot-and-mouth disease. *Veterinary Clinics of North America: Food Animal Practice*. 2024; 40 (2): 191–203. https://doi.org/10.1016/j.cvfa.2024.01.001

2. Rodina E. V., Rodin V. N., Boryaeva Y. A. Regionalisation and the current epizootic situation regarding foot-and-mouth disease in animals. *International Research Journal*. 2025; (11). https://doi.org/10.60797/IRJ.2025.161.67 (in Russ.)

3. Bouguedour R., Ripani A. Review of the foot and mouth disease situation in North Africa and the risk of introducing the disease into Europe. *Revue Scientifique et Technique*. 2016; 35 (3): 757–768. https://doi.org/10.20506/rst.35.3.2566

4. Dhikusooka M. T., Ayebazibwe C., Namatovu A., Belsham G. J., Siegismund H. R., Wekesa S. N., et al. Unrecognized circulation of SAT 1 foot-and-mouth disease virus in cattle herds around Queen Elizabeth National Park in Uganda. *BMC Veterinary Research*. 2016; 12:5. https://doi.org/10.1186/s12917-015-0616-1

5. Fana E. M., Mpoloka S. W., Leteane M., Seoke L., Masoba K., Mokopasetso M., et al. A five-year retrospective study of foot-and-mouth disease outbreaks in Southern Africa, 2014 to 2018. *Veterinary Medicine International*. 2021; 2021:7438809. https://doi.org/10.1155/2021/7438809

6. Jo H.-E., You S.-H., Choi J.-H., Ko M.-K., Shin S. H., Song J., et al. Evaluation of novel inactivated vaccines for the SAT 1, SAT 2 and SAT 3 serotypes of foot-and-mouth disease in pigs. *Virology Journal*. 2019; 16 (1):156. https://doi.org/10.1186/s12985-019-1262-1

7. Ularamu H. G., Lefebvre D. J., Haegeman A., Wungak Y. S., Ehizibolo D. O., Lazarus D. D., et al. Complex circulation of foot-and-mouth disease virus in cattle in Nigeria. *Frontiers in Veterinary Science*. 2020; (7):466. https://doi.org/10.3389/fvets.2020.00466

8. Kabelo T. I., Fana E. M., Hyera J. M., Lebani K. A review of foot-and-mouth disease status and control measures in Botswana. *Tropical Animal Health and Production*. 2023; 55 (4):278. https://doi.org/10.1007/s11250-023-03674-5

9. Wasfy M., Bazid A. H., Nayel M., Ata E. B., Elfeil W. K., Attia M., Elsayed M. Immunogenicity of a foot-and-mouth disease (FMD) vaccine against serotypes O, A, SAT-2, and Asia-1 in the Middle East and many parts of Africa, Southeast Asia and Europe. *Virology Journal*. 2025; 22 (1):98. https://doi.org/10.1186/s12985-025-02698-7

10. Kardjadj M. History of foot-and-mouth disease in North African countries. *Veterinaria Italiana*. 2018; 54 (1): 1–12. https://doi.org/10.12834/vetit.928.4711.2

11. Lozovoy D. A., Rakhmanov A. M., Mikhalishin D. V., Mikhalishin V. V., Gulenkin V. M. Ekonomicheskaya tselesoobraznost' ispol'zovaniya emul'sionnykh vaktsin protiv yashchura v Rossii v sovremennykh usloviyakh = Economic feasibility of emulsion-based FMD vaccine application in Russia under current conditions. *Nauchnye osnovy proizvodstva i obespecheniya kachestva biologicheskikh preparatov dlya APK: materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii, posvyashchennoi 50-letiyu instituta (Shchyolkovo, 25–27 sentyabrya 2019 g.). = Scientific grounds for biological product manufacture and quality assurance in agribusiness: proceedings of the International Scientific and Practical Conference devoted to 50th Anniversary of the Institute (Schyolkovo, September 25–27, 2019)*. Moscow: All-Russian Research and Technological Institute of Biological Industry; 2019; 59–64. https://elibrary.ru/glunot (in Russ.)

12. Pigoleva I. V., Sharypov A. S., Shabalina T. N., Zaglyadova S. V., Kitova M. V., Antonov S. A. et al. Testing of emulsion vaccines based on white oils obtained as a result of hydrocatalytic processing of oilstock. *Veterinary Science Today*. 2017; (4): 42–48. https://veterinary.arriah.ru/jour/article/view/332 (in Russ.)

13. Akache B., Stark F. C., Agbayani G., Renner T. M., McCluskie M. J. Adjuvants: engineering protective immune responses in human and veterinary vaccines. *Methods in Molecular Biology*. 2022; 2412: 179–231. https://doi.org/10.1007/978-1-0716-1892-9_9

14. Mikhalishin D. V., Elkina Yu. S., Gochmuradov Yk. M., Doronin M. I., Guseva M. N. An effective way to eliminate foot-and-mouth disease. *Veterinariya*. 2025; (1): 10–14. https://doi.org/10.30896/0042-4846.2025.28.1.10-14 (in Russ.)

15. Zhou Y., Yin W., Teng Z., Zhao Y., Lu Y., Qian Y., Deng B. Enhanced immunogenicity of foot-and-mouth disease virus-like particles using a water-in-oil-in-water adjuvant. *Vaccines*. 2024; 13 (1):24. https://doi.org/10.3390/vaccines13010024

16. Jones L. S., Peek L. J., Power J., Markham A., Yazzie B., Middaugh C. R. Effects of adsorption to aluminum salt adjuvants on the structure and stability of model protein antigens. *Journal of Biological Chemistry*. 2005; 280 (14): 13406–13414. https://doi.org/10.1074/jbc.M500687200

17. Aucouturier J., Dupuis L., Ganne V. Adjuvants designed for veterinary and human vaccines. *Vaccine*. 2001. 19 (17–19): 2666–2672. https://doi.org/10.1016/s0264-410x(00)00498-9

18. Starikov V. A., Mikhalishin D. V., Lyozova T. N., Mikhalishin V. V. Comparative examination of immunogenicity of sorbate and emulsion vaccines for cattle. *Veterinariya*. 2015; (3): 21–26. https://elibrary.ru/rppxsc (in Russ.)

19. Brito L. A., Malyala P., O'Hagan D. T. Vaccine adjuvant formulations: a pharmaceutical perspective. *Seminars in Immunology*. 2013; 25 (2): 130–145. https://doi.org/10.1016/j.smim.2013.05.007

20. Shi S., Zhu H., Xia X., Liang Z., Ma X., Sun B. Vaccine adjuvants: understanding the structure and mechanism of adjuvanticity. *Vaccine*. 2019; 37 (24): 3167–3178. https://doi.org/10.1016/j.vaccine.2019.04.055

21. Singh R. K., Sharma G. K., Mahajan S., Dhama K., Basagoudanavar S. H., Hosamani M., et al. Foot-and-mouth disease virus: immunobiology, advances in vaccines and vaccination strategies addressing vaccine failures – an Indian perspective. *Vaccines*. 2019; 7 (3):90. https://doi.org/10.3390/vaccines7030090

22. Charerntantanakul W. Adjuvants for swine vaccines: mechanisms of actions and adjuvant effects. *Vaccine*. 2020; 38 (43): 6659–6681. https://doi.org/10.1016/j.vaccine.2020.08.054

23. Foot and mouth disease (infection with foot and mouth disease virus). *In: WOAH. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* Chapter 3.1.8. https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.01.08_FMD.pdf

24. Mikhalishin D. V., Mikhalishin V. V., Gochmuradov Y. M., El'kina Yu. S. Inactivation of foot and mouth disease virus for vaccine. *Veterinary Science Today*. 2023; 12 (2): 164–170. https://doi.org/10.29326/2304-196X-2023-12-2-164-170

25. Stumpf M. P. H. Biology challenging statistics. *Statistical Applications in Genetics and Molecular Biology*. 2018; 17 (4):20180048. https://doi.org/10.1515/sagmb-2018-0048

26. Viari A. Big data in biology. *Médecine Sciences*. 2012; 28 (12): 1027–1028. https://doi.org/10.1051/medsci/20122812001 (in French)

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