UDC 619:616.98:578.835.2:616-085.371:616-076
DOI: 10.29326/2304-196X-2020-2-33-94-101

Studies on development of early immunity against type O FMD in naturally susceptible animals

D. V. Mikhalishin[1], D. A. Lozovoy[2], V. A. Starikov[3], Yu. S. El'kina[4], M. I. Doronin[5], A. V. Borisov[6]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *ORCID 0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru*
[2] *ORCID 0000-0002-5983-7062, e-mail: lozovoy@arriah.ru*
[3] *ORCID 0000-0002-9960-0887, e-mail: starikov@arriah.ru*
[4] *ORCID 0000-0002-2986-8992, e-mail: elkina_ys@arriah.ru*
[5] *ORCID 0000-0002-4682-6559, e-mail: doronin@arriah.ru*
[6] *ORCID 0000-0001-9880-9657, e-mail: borisov_av@arriah.ru*

SUMMARY
FMD risk in the Russian Federation dictates the need for enhanced measures aiming to prevent the introduction of FMD virus and comprising systematic monitoring research and mass vaccination of susceptible animals in the buffer zone. Research into the development of vaccines for early protection confirm that their use induces the formation of virus-neutralizing antibodies in naturally susceptible animals in the outbreak area, which protects from FMD infection, limits its spread and contains it within the primary outbreak. Taking into account the high speed of the infection spread, such a control measure as using FMD vaccines which induce early protection should be adopted immediately after the occurrence of the outbreak. The article presents the results of the research into the formation of humoral immunity in naturally susceptible animals triggered by administration of inactivated emulsion FMD vaccines capable of ensuring early protection against type O FMD. Culture FMD virus of O/Primorsky/2012, O/Saudi Arabia/08 and O/Mongolia/2017 strains was used for vaccine production. Immunogenic activity of vaccines was tested in cattle, pigs, and sheep. It was found that monovalent emulsion FMD vaccine based on O/Mongolia/2017 strain induced the formation of virus-neutralizing antibodies in the quantity necessary to protect against the homologous strain in seven days after a single administration in the dose of 2 cm^3. Vaccines based on O/Saudi Arabia/08 and O/Primorsky/2012 FMDV strains can protect animals from infection with heterologous O/Mongolia/2017 strain at early stages if a double dose is administered. Vaccines based on the above-mentioned strains induce early immunity formation (seven days after vaccination) against type O FMD. We suggest using the given products in the zones of a higher risk of the virus introduction.

Key words: type O FMD, inactivated monovalent emulsion vaccine, early protection, virus neutralization test.

Acknowledgements: The work was financed under the Governmental Contract 25/19: Research and experimental activities for the development of protective products based on newly isolated currently circulating agents of highly dangerous and exotic animal infections and their testing.

For citation: Mikhalishin D. V., Lozovoy D. A., Starikov V. A., El'kina Yu. S., Doronin M. I., Borisov A. V. Studies on development of early immunity against type O FMD in naturally susceptible animals. *Veterinary Science Today*. 2020; 2 (33): 94–101. DOI: 10.29326/2304-196X-2020-2-33-94-101.

Conflict of interest. The authors declare no conflict of interest.

For correspondence: Maksim I. Doronin, Candidate of Science (Biology), Senior Researcher, Laboratory for FMD Prevention, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: doronin@arriah.ru.*

УДК 619:616.98:578.835.2:616-085.371:616-076

Изучение формирования раннего иммунитета у естественно восприимчивых животных против ящура типа О

Д. В. Михалишин[1], Д. А. Лозовой[2], В. А. Стариков[3], Ю. С. Елькина[4], М. И. Доронин[5], А. В. Борисов[6]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия
[1] *ORCID 0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru*
[2] *ORCID 0000-0002-5983-7062, e-mail: lozovoy@arriah.ru*
[3] *ORCID 0000-0002-9960-0887, e-mail: starikov@arriah.ru*
[4] *ORCID 0000-0002-2986-8992, e-mail: elkina_ys@arriah.ru*
[5] *ORCID 0000-0002-4682-6559, e-mail: doronin@arriah.ru*
[6] *ORCID 0000-0001-9880-9657, e-mail: borisov_av@arriah.ru*

РЕЗЮМЕ
Риск возникновения вспышек ящура на территории Российской Федерации диктует необходимость усиления противоящурных мероприятий, направленных на предупреждение заноса вируса в страну и включающих проведение систематических мониторинговых исследований, а также осуществление

поголовной вакцинации восприимчивых животных в буферной зоне. Исследования по разработке вакцин для ранней защиты подтверждают, что при их использовании происходит выработка вируснейтрализующих антител у естественно восприимчивых животных в зоне вспышки, что служит защитой от заражения ящуром, приводит к сдерживанию инфекции и ее купированию в первичном очаге. Учитывая высокую скорость распространения инфекции, такая мера контроля, как применение вакцин против ящура, обеспечивающих раннюю защиту, должна применяться сразу после вспышки. Представлены результаты исследований по формированию гуморального иммунитета у естественно восприимчивых животных на введение инактивированных эмульсионных противоящурных вакцин, способных обеспечить раннюю защиту против ящура типа О. Для изготовления вакцин использовали культуральный вирус ящура штаммов О/Приморский/2012, О/Саудовская Аравия/08 и О/Монголия/2017. Иммуногенную активность вакцин проверяли на крупном рогатом скоте, свиньях и овцах. Выявлено, что моновалентная эмульсионная противоящурная вакцина из штамма О/Монголия/2017 через 7 сут после однократного введения в дозе 2 см^3 индуцировала выработку вируснейтрализующих антител в количестве, достаточном для защиты от заражения гомологичным штаммом. Вакцины из штаммов вируса ящура О/Саудовская Аравия/08 и О/Приморский/2012 при введении двойной дозы способны защитить животных на ранних сроках от заражения гетерологичным штаммом вируса ящура О/Монголия/2017. Вакцинные препараты из указанных штаммов вызывают формирование иммунитета в ранние сроки (на 7-е сут после вакцинации) против ящура типа О. Данные препараты предлагается использовать в зонах повышенного риска заноса вируса.

Ключевые слова: ящур типа О, инактивированная моновалентная эмульсионная вакцина, ранняя защита, реакция нейтрализации.

Благодарность: Работа выполнена за счет средств по государственному контракту № 25/19 по теме «Выполнение научно-исследовательских и опытно-конструкторских работ по созданию новых защитных препаратов на основе циркулирующих вновь выделенных изолятов особо опасных и экзотических инфекций животных и их испытание».

Для цитирования: Михалишин Д. В., Лозовой Д. А., Стариков В. А., Елькина Ю. С., Доронин М. И., Борисов А. В. Изучение формирования раннего иммунитета у естественно восприимчивых животных против ящура типа О. *Ветеринария сегодня*. 2020; 2 (33): 94–101. DOI: 10.29326/2304-196X-2020-2-33-94-101.

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Доронин Максим Игоревич, кандидат биологических наук, старший научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: doronin@arriah.ru.*

INTRODUCTION

Foot-and-mouth disease is a viral disease of wild and domesticated cloven-hoofed animals which is economically important due to a large number of naturally susceptible species, significant impact on animal productivity and rapid spread within and between neighbouring geographical regions. Foot-and-mouth disease outbreaks result in high economic losses incurred by huge costs of sanitary measures aimed at eradication of the disease [1–3].

Currently, despite the awareness of cattle owners and trained veterinary staff, FMD outbreaks may remain unnoticed on farms in many countries until the disease spreads beyond the primary outbreak. This situation is due to a lack of identification of FMD clinical signs, delayed or unavailable information on diseased animals, and illegal movement of livestock or contaminated animal products [4, 5].

Between September 2017 and March 2018, about 30 outbreaks of type O FMD in cattle, sheep and goats were registered in several Mongolian aimags bordering Russia in the north and China in the south. The risk of outbreaks in the Russian Federation requires enhanced FMD control measures aimed at preventing the introduction of the virus into the country, including systematic monitoring studies as well as general vaccination of susceptible animals in the buffer zone [4, 6, 7]. Given the high speed of infection spread, FMD vaccines providing early protection of animals should be used immediately after the outbreak has occurred.

Vaccines have been shown to be highly effective and undeniably useful in controlling infectious diseases over many decades. Mono- and polyvalent culture inactivated vaccines are widely used for specific FMD prevention [3]. Studies on the development of vaccines for early protection confirm that their use induces the formation of virus-neutralizing antibodies in naturally susceptible animals in the outbreak area, which protects against FMD infection, limits the infection spread, and contains it within the primary outbreak [1, 2, 7].

The aim was to study humoral and protective immunity during the formation of early protection in naturally susceptible animals after administration of the inactivated emulsion FMD vaccine based on O/Mongolia/2017 strain against the homologous strain as well as vaccines based on O/Saudi Arabia/08 and O/Primorsky/2012 strains against the heterologous strain O/Mongolia/2017.

MATERIALS AND METHODS

Virus. To study humoral immunity, we used O/Mongolia/2017 homologous strain and O/Primorsky/2012 and O/Saudi Arabia/08 heterologous strains of FMD cultural virus.

For challenging of naturally susceptible animals we used aphthous FMD virus of O/Mongolia/2017 strain adapted to these animals.

Cell lines. Monolayer continuous cell lines from the Siberian mountain goat kidney (SMGK30) and Syrian baby hamster kidney (BHK21) were used for propagation of the virus. Primary monolayer cell culture of pig kidney (PK) was used to evaluate antibody titer and antigen innocuity after inactivation.

Animals. In the studies, we used: 1) 26 Holstein-Friesian cows weighing 250–300 kg; 2) 16 pigs of different breeds weighing 30–40 kg; 3) 4 sheep of Romanov breed weighing 20–30 kg.

All animal experiments were conducted in strict accordance with the Interstate Standard for the keeping and

care of laboratory animals (GOST 33216-2014), adopted by the Interstate Council for Standardization, Metrology and Certification, as well as the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22 September 2012 on the protection of animals used for scientific purposes.

Inactivation of the virus, purification and concentration of the antigen. FMD virus was inactivated with aminoethylethyleneimine solution (AEEI). Polyhexamethylene guanidine (PGMG) solution was used to purify the antigen from ballast proteins, including non-structural proteins. Concentration was performed by ultrafiltration in a tangential flow.

Determination of component composition of inactivated suspension. The number of immunogenic components in the inactivated suspension was estimated in accordance with Methodical recommendations for determining the concentration of 146S, 75S, and 12S components of vaccine strains of FMD culture virus in complement fixation test (CFT) [8].

Selection of adjuvant and antigen-adjuvant ratio. Montanide ISA 206 VG was chosen as an adjuvant for the production of monovalent emulsion vaccines against FMD because research literature showed that it induced the formation of antibodies on days 4–7 after immunization [5, 9]. The ratio of antigen to adjuvant was 50:50.

Vaccines. Three samples were produced to study the immunogenic activity of emulsion vaccines against FMD, which were administered in a single dose of 2 cm^3 intramuscularly, and in a double dose – intramuscularly in two sites per 2 cm^3.

The first vaccine was based on O/Mongolia/2017 FMD virus strain. A double dose (4 cm^3) contained 24.22 μg of 146S component and a single dose (2 cm^3) – 12.11 μg.

The second vaccine was based on O/Saudi Arabia/08 FMD strain with 146S component in a dose of 4 cm^3 – 30.1 μg.

The third vaccine was based on O/Primorsky/2012 strain containing 29.2 μg of 146S particles in a double dose (4 cm^3).

Challenging. Seven days after vaccination, animals were challenged with adapted FMD virus of O/Mongolia/2017 strain. Challenging was carried out to the mucosa of the tongue at a dose of 10^4 ID_{50}/0.20 cm^3 (two sites per 0.10 ± 0.05 cm^3).

Blood sampling. Blood sampling was carried out on days 4 and 7 after vaccination.

Determination of VNA titer. The quantity of virus-neutralizing antibodies (VNA) in serum samples was determined by neutralization reaction using a monolayer of PK cell culture according to the generally accepted method [10].

Evaluation of viral antigen innocuity. Innocuity of the inactivated virus was controlled by inoculation into the monolayer PK cell culture.

Evaluation of vaccine immunogenicity. Vaccine immunogenicity was studied in cattle and pigs. The level of humoral immunity in neutralization reaction was determined in sheep. The blood sera obtained on days 4 and 7 after immunization were analyzed by neutralization reaction. Then, cattle and pigs were challenged with O/Mongolia/2017 control strain of FMD virus, 7 days later all animals were euthanized and pathological examination was performed. Animals that had no lesions on their limbs were considered to be protected against FMD. Primary ulcers were not considered.

Statistical data processing. All measurements were made in triplicate. The obtained results were statistically processed to determine arithmetic mean values and the degree of reliability of statistical difference between mean values by Student-Fisher difference method, as well as the determination coefficient [11]. Diagrams were made in Microsoft Excel 2010.

RESULTS AND DISCUSSION

At the first stage of the studies pigs were immunized with monovalent emulsion FMD vaccines based on O/Mongolia/2017, O/Primorsky/2012 and O/Saudi Arabia/08 strains. Subsequent assessment of the formation of early immunity against homologous and heterologous strains was carried out. The results of the study are shown in Figure 1.

Figure 1 shows that in animals immunized with the vaccine based on O/Saudi Arabia/08 strain, antibody titers against the homologous strain increased by 1.3 from day 4 to 7 after vaccination. Titers of antibodies against O/Primorsky/2012 and O/Mongolia/2017 heterologous FMDV strains day 4 after immunization were 2.1 and 1.9 times lower compared to the homologous strain and amounted to 1.95 ± 0.10 and 2.05 ± 0.17 log_2, respectively. On day 7 after the inoculation, VNA titers against O/Primorsky/2012 and O/Mongolia/2017 heterologous strains were 2.5 and 2.1 times lower than those against the homologous strain and corresponded to the values of 2.10 ± 0.10 and 2.35 ± 0.10 log_2, respectively.

In pigs vaccinated with the vaccine based on O/Primorsky/2012 strain, the number of antibodies against the homologous strain on day 4 after immunization was 3.35 ± 0.17 log_2, against O/Saudi Arabia/08 and O/Mongolia/2017 heterologous strains – 2.30 ± 0.22 and 3.25 ± 0.18 log_2. Translating log_2 into natural numbers, it can be noted that on day 4 titers of antibodies against O/Saudi Arabia/08 and O/Mongolia/2017 heterologous strains were 2.1 and 1.1 times lower, respectively, compared to the homologous strain. The difference between the level of humoral immunity against the homologous FMD virus O/Primorsky/2012 and the heterologous strain O/Mongolia/2017 is insignificant. On day 7 after immunization the number of antibodies against the homologous strain was 4.20 ± 0.29 log_2, which is 1.8 times higher than on day 4 after vaccination. Antibody levels on day 7 after vaccination against O/Saudi Arabia/08 and O/Mongolia/2017 heterologous strains were 2.4 and 1.5 times lower respectively.

In pigs immunized with the vaccine based on O/Mongolia/2017 strain on days 4 and 7 after the inoculation, antibodies titers against the homologous strain were 3.00 ± 0.36 and 3.90 ± 0.30 log_2, respectively. On day 7, the number of antibodies against the homologous strain increased by 1.9. The content of antibodies against O/Saudi Arabia/08 and O/Primorsky/2012 heterologous strains on day 4 after vaccination in serum was 1.50 ± 0.18 and 1.75 ± 0.18 log_2, which is 2.8 and 2.4 times lower than against the homologous strain. On day 7 after immunization, the VNA titers against O/Saudi Arabia/08 and O/Primorsky/2012 heterologous strains were 3.7 and 1.6 times lower than those for the homologous strain and amounted to 2.00 ± 0.17 and 3.25 ± 0.20 log_2, respectively.

As it follows from Figure 1, the level of humoral immunity in pigs vaccinated with monovalent early protection vaccines against FMD increases by day 7 post-vaccination.

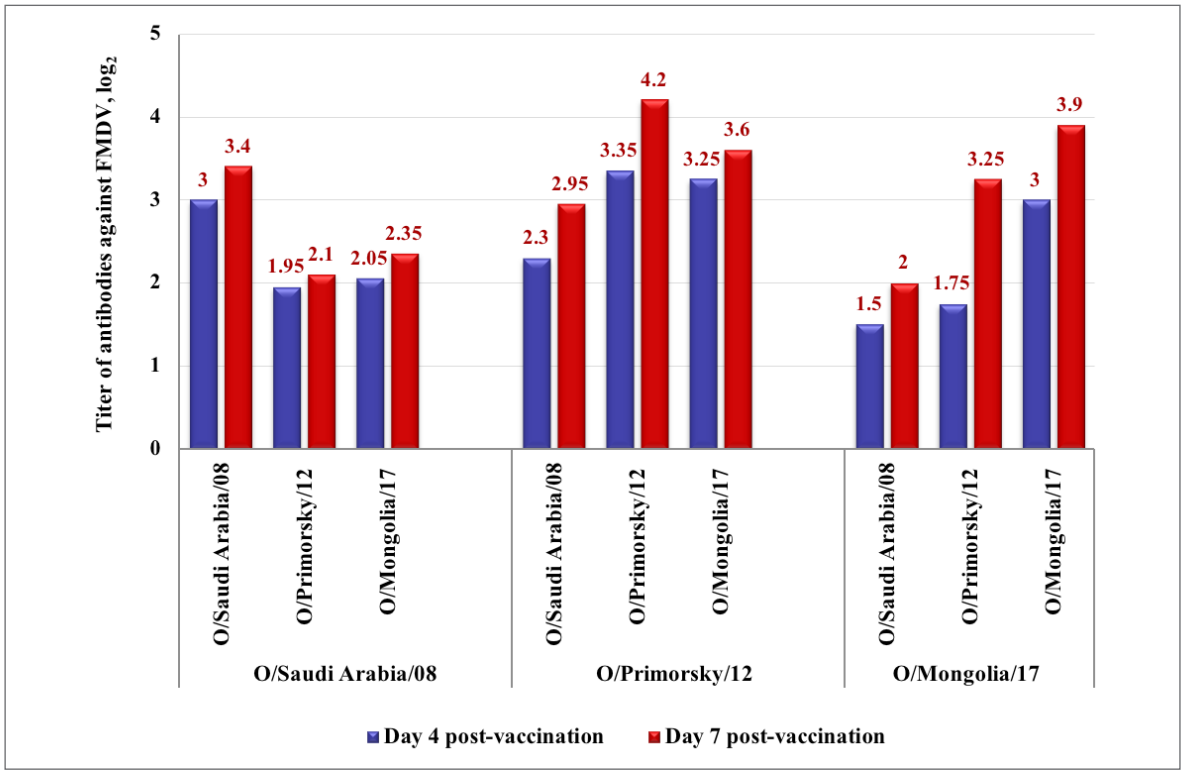

Fig. 1. Dynamics of VNA formation in pigs after use of vaccines based on O/Saudi Arabia/08, O/Primorsky/2012 and O/Mongolia/2017 strains in a double dose (4 cm³) against homologous and heterologous FMDV strains, day 4 and 7 post-vaccination

Рис. 1. Динамика образования ВНА у свиней после применения вакцин из штаммов О/Саудовская Аравия/08, О/Приморский/2012 и О/Монголия/2017 в двойной дозе (4 см³) против гомологичных и гетерологичных штаммов вируса ящура на 4-е и 7-е сут после вакцинации

The products manufactured have the best immunogenic activity mainly in relation to homologous strains. In further experiments, pigs were infected with O/Mongolia/2017 strain 7 days after vaccination to assess their protective immunity. One non-vaccinated animal was used for control. The results of the challenging are presented in Table 1.

Table 1 shows that animals immunized with vaccines based on O/Primorsky/2012 and O/Mongolia/2017 strains did not manifest FMD signs after challenging. The emulsion FMD vaccine based on O/Saudi Arabia/08 strain 7 days after administration and subsequent challenging did not protect 1 out of 5 pigs. VNA titre in the diseased animal was 2.00 $\log_2$. The control animal also contracted FMD.

The next stage of the work was devoted to comparative analysis of formation of early immunity in cattle after administration of a single and double dose of monovalent emulsion FMD vaccines. Antibody levels in blood of immunized animals against homologous and heterologous strains of FMD virus were estimated. The results of the study are shown in Figure 2.

The data presented in Figure 2 demonstrates that when the cattle was administered the monovalent emulsion FMD vaccine based on O/Mongolia/2017 strain at a dose of 2 cm³ on the 4th day the antibody titers against the homologous strain amounted to 2.83 ± 0.33 $\log_2$, and with immunization at a dose of 4 cm³ – to 3.46 ± 0.24 $\log_2$. When the vaccine was administered at a dose of 2 cm³ on day 4 post-vaccination, the content of antibodies against the homologous strain was 1.6 times lower compared to the case when a double dose was inoculated.

On day 7 post-vaccination with a single dose, the level of VNA against O/Mongolia/2017 strain was 4.67 ± 0.23 $\log_2$, and with a double dose – 5.00 ± 0.21 $\log_2$, which is an insignificant difference. After the administration of the vaccine, the VNA against the homologous strain on day 7 increased by 3.6 times compared to the data obtained on day 4 post-vaccination. When a double dose of the vaccine was used from the 4th to 7th days after immunization, there was a 2.9-fold increase in the titre of antibodies against O/Mongolia/2017 strain.

When the vaccine based on O/Mongolia/2017 strain was administered at a dose of 2 cm³ on the 4th day after immunization of cattle, the level of VNA against heterologous strains O/Saudi Arabia/08 and O/Primorsky/2012 was 2.21 ± 0.41 and 2.46 ± 0.29 $\log_2$, respectively. The number of antibodies against the homologous strain was 2.83 ± 0.24 $\log_2$, which is 1.5 and 1.3 times higher than against the heterologous strains O/Saudi Arabia/08 and O/Primorsky/2012, respectively.

It was found that on day 4 after the administration of a double dose of monovalent emulsion vaccine based on O/Mongolia/2017 strain, the level of VNA against the homologous strain was 3.46 ± 0.19 $\log_2$, against heterologous strains O/Saudi Arabia/08 and O/Primorsky/2012 – 3.21 ± 0.33 and 3.21 ± 0.22 $\log_2$, respectively. In other words, the values of antibody after administration of this vaccine at a dose of 4 cm³ against the homologous strain were 1.2 times higher than against the heterologous strain.

On day 7 after the administration of a single dose of the vaccine based on O/Mongolia/2017 strain, the level

Table 1
Study of humoral and protective immunity in pigs vaccinated with a double dose of inactivated emulsion vaccines against FMD

Таблица 1
Исследование гуморального и протективного иммунитета свиней, вакцинированных двойной дозой инактивированных эмульсионных вакцин против ящура

Vaccine strain	Titers of antibodies against FMDV, $\log_2$ ($M \pm m$)						Results of challenging with O/Mongolia/17 strain day 7 post-vaccination (challenged/ protected)
	Day 4 post-vaccination			Day 7 post-vaccination			
	O/Saudi Arabia/08	O/Primorsky/12	O/Mongolia/17	O/Saudi Arabia/08	O/Primorsky/12	O/Mongolia/17	
O/Saudi Arabia/08	3.00 ± 0.38	1.95 ± 0.10	2.05 ± 0.17	3.40 ± 0.36	2.10 ± 0.10	2.35 ± 0.35	5/4
O/Primorsky/12	2.30 ± 0.22	3.35 ± 0.17	3.25 ± 0.18	2.95 ± 0.15	4.20 ± 0.29	3.60 ± 0.27	5/5
O/Mongolia/17	1.50 ± 0.18	1.75 ± 0.18	3.00 ± 0.36	2.00 ± 0.33	3.25 ± 0.26	3.90 ± 0.30	5/5
Control							1/0

of VNA against the homologous strain was 4.67 ± 0.23 $\log_2$, and against the heterologous strains O/Saudi Arabia/08 and O/Primorsky/2012 corresponded to the values of 3.58 ± 0.12 and 3.92 ± 0.19 $\log_2$. Thus, on day 7 after immunization, the number of antibodies against the homologous strain exceeded the VNA content against the heterologous strains O/Saudi Arabia/08 and O/Primorsky/2012 by 2.1 and 1.7 times, respectively.

After the administration of the vaccine at a dose of 4 cm^3 on day 7, the titer of antibodies against homologous strain O/Mongolia/2017 was 5.00 ± 0.22 $\log_2$, against heterologous strains O/Saudi Arabia/08 and O/Primorsky/2012 – 4.46 ± 0.33 and 4.33 ± 0.22 $\log_2$, respectively. That is, on the 7th day after the administration of a double dose of the vaccine based on O/Mongolia/2017 strain, the level of antibodies against heterologous strains practically did not

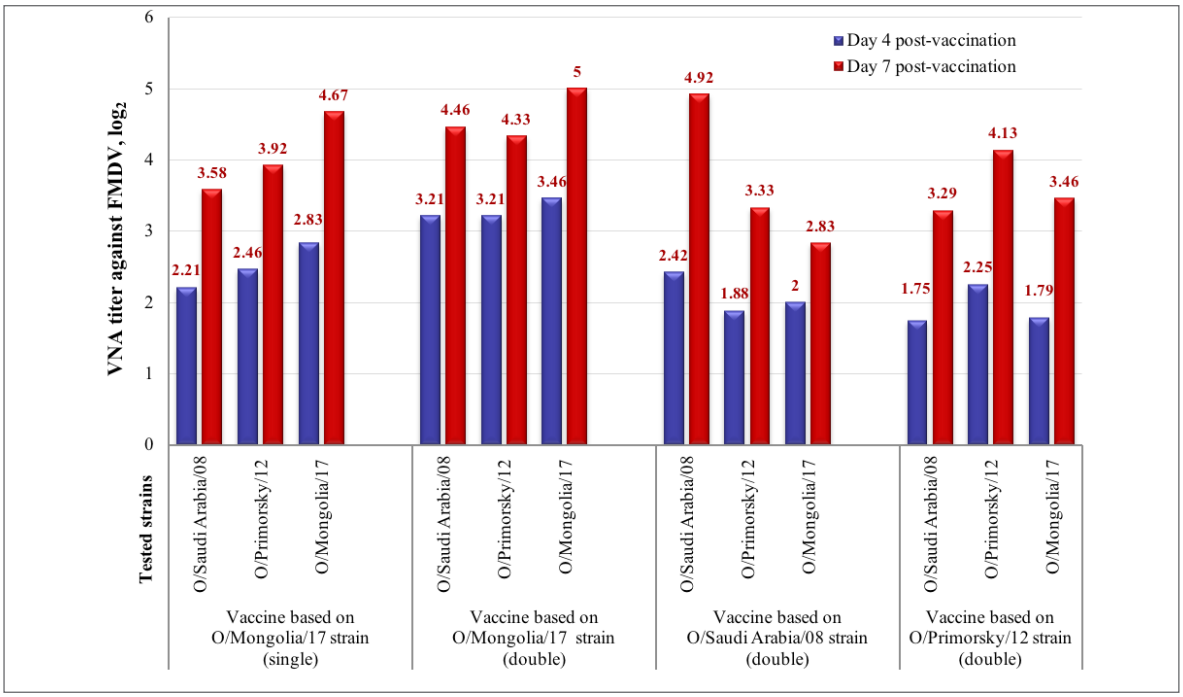

Fig. 2. Dynamics of VNA formation in cattle after administration of a single or double dose of the vaccine based on O/Mongolia/2017 strain, double dose of vaccines based on O/Saudi Arabia/08 and O/Primorsky/2012 strains against homologous and heterologous FMDV strains, day 4 and 7 post-vaccination

Рис. 2. Динамика образования ВНА у КРС после применения одной и двойной дозы вакцины из штамма О/Монголия/2017, двойной дозы вакцин из штаммов О/Саудовская Аравия/08 и О/Приморский/2012 против гомологичного и гетерологичных штаммов вируса ящура на 4-е и 7-е сут после вакцинации

differ from the VNA titre against the homologous strain. It should be noted that the immunization of cattle using a double dose on day 7 if compared to the administration of a single dose of the vaccine allowed increasing the number of antibodies against strains O/Saudi Arabia/08 and O/Primorsky/2012 by 1.8 and 1.3 times, respectively.

From the data presented in Figure 2, it follows that on day 4 and 7 after the inoculation, antibody levels were 2.42 ± 0.14 and 4.92 ± 0.15, respectively, in cattle vaccinated with a double dose of emulsion FMD vaccine based on O/Saudi Arabia/08 strain. The values of antibody titers on days 4 and 7 after immunization increased by 5.7.

On day 4 after the vaccination, the antibody titer values against heterologous strains O/Primorsky/2012 and O/Mongolia/2017 were 1.88 ± 0.15 and 2.00 ± 0.35 $\log_2$, respectively. The difference between antibody titers against homologous and heterologous strains was 1.5 and 1.3, respectively.

On day 7 after the vaccination with the same vaccine, VNA against heterologous strains O/Primorsky/2012 and O/Mongolia/2017 corresponded to the values of 3.33 ± 0.19 and 2.83 ± 0.12 $\log_2$, which is 3.0 and 4.3 times lower than the antibody titer against the homologous strain.

In animals vaccinated with monovalent emulsion vaccine based on O/Primorsky/2012 strain at a dose of 4 cm^3, the antibody titers against homologous strain on days 4 and 7 after immunization were 2.25 ± 0.14 and 4.13 ± 0.11 $\log_2$, respectively. The activity of cattle's humoral immunity against the homologous strain increased by 3.7 on days 4 to 7 after immunization.

On day 4 after the vaccination, VNA against the heterologous strains O/Saudi Arabia/08 and O/Mongolia/2017 was 1.75 ± 0.13 and 1.79 ± 0.10 $\log_2$, respectively, which is 1.4 times lower than against the homologous strain. On day 7 after immunization, antibody titers against heterologous strains corresponded to the values of 3.29 ± 0.12 and 3.46 ± 0.10 $\log_2$. Thus, the number of antibodies against heterologous strains O/Saudi Arabia/08 and O/Mongolia/2017 decreased by 1.8 and 1.6 times compared to the data for the homologous strain.

The results of the study of protective immunity on day 7 post-vaccination in cattle vaccinated with monovalent FMD vaccines are presented in Table 2. Two non-vaccinated animals were used as controls.

According to Table 2, after the administration of the monovalent emulsion vaccine based on O/Mongolia/2017 strain at a dose of 2 cm^3 we observed the formation of VNA in titers sufficient to protect against direct challenging with the homologous strain. The results of challenging with O/Mongolia/2017 strain 7 days after vaccination showed that all six animals immunized with a double dose of vaccine based on O/Primorsky/2012 and O/Mongolia/2017 strains had no FMD signs. The emulsion FMD vaccine based on O/Saudi Arabia/08 strain 7 days after administration did not provide protection against challenging of one of the six animals. The diseased animal on day 7 after the vaccination had VNA titre equal to 2.75 $\log_2$. FMD signs were also observed in two control animals.

At the next stage of the work the formation of early immunity in sheep after immunization with monovalent emulsion FMD vaccine based on O/Mongolia/2017 strain in a dose of 4 cm^3 was studied. Estimation of the sheep's immune status was difficult due to the lack of a virus adapted to this type of animal, for this reason, no challenging was carried out. The results of the determination of VNA titre on day 4 after immunization of the animals are presented in Table 3.

Table 3 shows that on day 4 after immunization the level of antibodies against the homologous strain was

Table 2
Study of humoral and protective immunity in cattle vaccinated with monovalent emulsion vaccines against FMD

Таблица 2
Исследование гуморального и протективного иммунитета у КРС, привитых моновалентными эмульсионными вакцинами против ящура

Vaccine strain, dose	Titers of antibodies against FMDV, $\log_2$ ($M \pm m$)						Results of challenging with O/Mongolia/17 strain day 7 post-vaccination (challenged/protected)
	Day 4 post-vaccination			Day 7 post-vaccination			
	O/Saudi Arabia/08	O/Primorsky/12	O/Mongolia/17	O/Saudi Arabia/08	O/Primorsky/12	O/Mongolia/17	
O/Mongolia/17 2 cm^3	2.21 ± 0.41	2.46 ± 0.29	2.83 ± 0.33	3.58 ± 0.12	3.92 ± 0.19	4.67 ± 0.23	6/6
O/Mongolia/17 4 cm^3	3.21 ± 0.33	3.21 ± 0.22	3.46 ± 0.24	4.46 ± 0.12	4.33 ± 0.11	5.00 ± 0.21	6/6
O/Saudi Arabia/08 4 cm^3	2.42 ± 0.14	1.88 ± 0.15	2.00 ± 0.22	4.92 ± 0.15	3.33 ± 0.19	2.83 ± 0.12	6/5
O/Primorsky/12 4 cm^3	1.75 ± 0.13	2.25 ± 0.14	1.79 ± 0.10	3.29 ± 0.12	4.13 ± 0.11	3.46 ± 0.10	6/6
Control 1							1/0
Control 2							1/0

Table 3
Study of humoral and protective immunity in sheep vaccinated with inactivated emulsion vaccine against FMD based on O/Mongolia/2017 strain

Таблица 3
Исследование гуморального иммунитета у овец, привитых противоящурной инактивированной эмульсионной вакциной из штамма O/Монголия/2017

Vaccine strain	Dose	No. of an animal	VNA titers day 4 post-vaccination (log_2) against FMDV strains		
			O/Saudi Arabia/08	O/Primorsky/12	O/Mongolia/17
O/Mongolia/17	4 cm^3	1	2.50	3.00	3.75
		2	3.25	2.75	3.75
		3	3.00	3.50	3.50
		4	3.00	3.75	4.00
		M ± *m*	2.94 ± 0.38	3.25 ± 0.38	3.75 ± 0.14

3.75 ± 0.14 log_2. Titers of VNA against the heterologous strains O/Saudi Arabia/08 and O/Primorsky/2012 corresponded to the values of 2.94 ± 0.38 and 3.25 ± 0.38 log_2, respectively. Thus, on the 4th day after the administration of the vaccine, sheep formed early immunity, the activity of which against the heterologous strains O/Saudi Arabia/08 and O/Primorsky/2012 was 1.8 and 1.4 times lower compared to the homologous strain. In other words, the use of FMD inactivated emulsion vaccine based on O/Mongolia/2017 strain contributes to the formation of early immunity in sheep after administration of the vaccine.

CONCLUSION

Studies on the formation of early immunity in naturally susceptible animals against FMD type O indicate that FMD emulsion vaccine based on O/Mongolia/2017 strain on day 7 after the administration of a double dose to pigs induced the formation of VNA in the amount of 3.90 ± 0.30 log_2. On day 7 after immunization of cattle with this vaccine using a dose of 2 cm^3 VNA titer against homologous strain corresponded to the value of 4.67 ± 0.23 log_2, a dose of 4 cm^3 – 5.00 ± 0.21 log_2. Results of challenging of cattle and pigs with O/Mongolia/2017 strain 7 days after immunization showed that all animals vaccinated with homologous virus had no generalized FMD signs.

When a double dose of FMD emulsion vaccine based on O/Primorsky/2012 strain was administered, on day 7 after immunization, the VNA titer in pigs against the heterologous strain O/Mongolia/2017 was 3.60 ± 0.27 log_2 and in cattle – 3.46 ± 0.10 log_2. According to the results of the challenging, all animals vaccinated with FMD vaccine based on O/Primorsky/2012 strain were protected against O/Mongolia/2017 FMDV strain.

FMD inactivated emulsion vaccine based on O/Saudi Arabia/08 strain 7 days after administration at a dose of 4 cm^3 contributed to the formation of VNA against O/Mongolia/2017 strain in pigs in the amount of 2.35 ± 0.35 log_2, in cattle – 2.83 ± 0.12 log_2. At the same time, after challenging with O/Mongolia/2017 FMDV strain, immunization with this vaccine using a double dose did not protect one out of five gilts and one of six cows. In diseased animals on day 7 post-vaccination, the VNA titers were 2.75 log_2 (cattle) and 2.00 log_2 (pig).

On day 4 after vaccination of sheep with FMD inactivated emulsion vaccine based on O/Mongolia/2017 strain at a dose of 4 cm^3 VNA titer against the homologous strain was 3.75 ± 0.14 log_2, which attested to the fact that this preparation could form early immunity. The level of humoral immunity in sheep vaccinated with this vaccine against strains O/Saudi Arabia/08 and O/Primorsky/2012 was 1.8 and 1.4 times lower compared to the homologous strain. Studies conducted in cattle suggest that FMD inactivated emulsion vaccine based on O/Mongolia/2017 strain can provide protective immunity in sheep as well.

REFERENCES

1. Barnett P., Garland J. M., Kitching R. P., Schermbrucker C. G. Aspects of emergency vaccination against foot-and-mouth disease. *Comp. Immunol. Microbiol. Infect. Dis*. 2002; 25 (5–6): 345–364. DOI: 10.1016/s0147-9571(02)00031-0.

2. Quattrocchi V., Pappalardo J. S., Langellotti C., Smitsaart E., Fondevila N., Zamorano P. Early protection against foot-and-mouth disease virus in cattle using an inactivated vaccine formulated with Montanide ESSAI IMS D 12802 VG PR adjuvant. *Vaccine*. 2014; 32 (19): 2167–2172. DOI: 10.1016/j.vaccine.2014.02.061.

3. Salt J. S., Barnett P. V., Dani P., Williams L. Emergency vaccination of pigs against foot-and-mouth disease: protection against disease and reduction in contact transmission. *Vaccine*. 1998; 16 (7): 746–754. DOI: 10.1016/s0264-410x(97)86180-4.

4. Dudnikov A. I. Early protection following FMD immunization [Rannyaya zashchita posle protivoyashchurnoj immunizacii]. *Veterinarna medizina: mizhvid. tem. nauk. zb*. Kharkiv; 2008; 91: 189–191. (in Russian)

5. Golde W. T., Pacheco J. M., Duque H., Doel T., Penfold B., Ferman G. S., et al. Vaccination against foot-and-mouth disease virus confers complete clinical protection in 7 days and partial protection in 4 days: Use in emergency outbreak response. *Vaccine*. 2005; 23 (50): 5775–5782. DOI: 10.1016/j.vaccine.2005.07.043.

6. Mikhalishin D. V., Lyozova T. N., Khodakova N. N., Borisov A. V., Klyukina N. D., Starikov V. A., et al. Dynamics of anti-FMD humoral immunity in cattle immunized with trivalent type A, O, Asia-1 sorbated vaccine. *Proceedings of the Federal Centre for Animal Health*. 2007; 5: 75–82. eLIBRARY ID: 14454047. (in Russian)

7. Horsington J., Zhang Z., Bittner H., Hole K., Singanallur N. B., Alexandersen S., Vosloo W. Early protection in sheep against intratypic heterologous challenge with serotype O foot-and-mouth disease virus using high-potency, emergency vaccine. *Vaccine*. 2015; 33 (3): 422–429. DOI: 10.1016/j.vaccine.2014.11.043.

8. Lozovoy D. A., Mikhalishin D. V., Starikov V. A., Shishkova A. A., Doronin M. I., Medvedeva N. N., et al. Methodical recommendations for determination of concentration of 146S, 75S, 12S components of vaccine strains of culture FMD virus in complement fixation test (CFT) [Metodicheskie rekomendacii po opredeleniyu koncentracii 146S, 75S, 12S komponentov

vakcinnyh shtammov kul'tural'nogo virusa yashchura v reakcii svyazyvaniya komplementa (RSK)]: adopted by FGBI "ARRIAH", 21.09.2017 (No. 3917). Vladimir: FGBI "ARRIAH"; 2017. 51 p. (in Russian)

9. Cox S. J., Barnett P. V. Experimental evaluation of foot-and-mouth disease vaccines for emergency use in ruminants and pigs: a review. *Vet. Res*. 2009; 40 (3):13. DOI: 10.1051/vetres:2008051.

10. Methodical guidelines for neutralization test for determination of animals' status in case of FMD [Metodicheskie ukazaniya po postanovke reakcii nejtralizacii dlya opredeleniya statusa zhivotnyh pri yashchure]: adopted by General Veterinary Administration of MoA USSR 26.12.83 115-6a. (in Russian)

11. Lakin G. F. Biometrics [Biometriya]: Study guide. 4th ed. updated and revised. M.: Higher School; 1990. 352 p. (in Russian)

Received on 19.12.2019
Approved for publication on 02.03.2020

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Dmitry V. Mikhalishin, Candidate of Science (Veterinary Medicine), Head of Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia.

Dmitry A. Lozovoy, Doctor of Science (Veterinary Medicine), Associate Professor, Deputy Director for Research and Development, FGBI "ARRIAH", Vladimir, Russia.

Vyacheslav A. Starikov, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia.

Yulia S. El'kina, Post-Graduate Student, Technologist, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia.

Maksim I. Doronin, Candidate of Science (Biology), Senior Researcher, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia.

Alexey V. Borisov, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia.

Михалишин Дмитрий Валерьевич, кандидат ветеринарных наук, заведующий лабораторией профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Лозовой Дмитрий Анатольевич, доктор ветеринарных наук, доцент, заместитель директора по НИР и развитию ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Стариков Вячеслав Алексеевич, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Елькина Юлия Сергеевна, аспирант, технолог лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Доронин Максим Игоревич, кандидат биологических наук, старший научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Борисов Алексей Валерьевич, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.