DOI: 10.29326/2304-196X-2023-12-2-164-170

Inactivation of foot and mouth disease virus for vaccine production

D. V. Mikhalishin, V. V. Mikhalishin, Y. M. Gochmuradov, Yu. S. El'kina
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY

Inactivation is the loss by a virus of ability to reproduce and infect susceptible animals while retaining its antigenic properties. In this paper, the effectiveness of aminoethylethanolamine when used as an FMDV inactivant is shown. The inactivation rates under selected parameters, effect of aminoethylethanolamine on virus stability during inactivation and on vaccine immunogenicity after storage at 2–8 °C were determined. The method for calculation of 50% aminoethylethanolamine inactivating concentration (IC_{50}) which enables to determine quality parameters of the virus-containing suspension, to compare the inactivating agent activities and their ability to ensure the vaccine innocuity within the given period of time is presented. It was established that IC_{50} for purified and non-purified virus-containing suspensions was identical (0.0045%), and its safety after 12 hours of inactivation was one $TCID_{50}$ per 10^9–10^{11} L of the virus containing suspension. It was also found that double increase in inactivation time increased the virucidal activity of aminoethylethanolamine by a factor of 1.8 for serotype O and 2.4 for serotype A. At the same time, the removal of cell debris had no significant effect on the inactivation process. Aminoethylethanolamine does not destroy 146S virus particles and it was confirmed by immunogenicity testing of the vaccines during storage. This means that 15% aqueous solution of aminoethylethanolamine, manufactured by Russian Company OOO "Biokhimresurs" (Vladimir) complies with high quality standards. Immunogenicity test of bivalent FMD vaccine for cattle by challenging demonstrated that its potency was 10.08 protective doses per 2 cm^3 of the vaccination dose.

Keywords: foot and mouth disease virus, virucidal activity, inactivating agent, vaccine

Acknowledgements: The study was funded by the FGBI "ARRIAH" within the framework of "Veterinary Welfare" research work.

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

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

For correspondence: Dmitry V. Mikhalishin, Doctor of Science (Veterinary Medicine), Head of Laboratory for FMD Prevention, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: mihalishindv@arriah.ru.*

УДК 619:616:98:578.835.2:615.371

Инактивация вируса ящура для изготовления вакцин

Д. В. Михалишин, В. В. Михалишин, Ы. М. Гочмурадов, Ю. С. Елькина
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ

Инактивация – это потеря вирусом способности к репродукции и инфицированию восприимчивых животных при сохранении антигенных свойств. В работе показана эффективность применения аминоэтилэтиленимина в качестве инактиванта вируса ящура. Определены скорость снижения титра инфекционности при выбранных параметрах процесса инактивации, влияние аминоэтилэтиленимина на стабильность вируса в процессе инактивации и на иммуногенность вакцин после хранения при температуре 2–8 °С. Представлен метод вычисления пятидесятипроцентной инактивирующей концентрации аминоэтилэтиленимина ($К_{50}$), позволяющей определять качественную характеристику вируссодержащей суспензии, сравнивать активность инактивантов и их способность обеспечивать авирулентность препарата в заданный промежуток времени. В результате проведенных исследований было установлено, что значение $К_{50}$ для неочищенной и очищенной вируссодержащих суспензий было одинаковым – 0,0045%, а уровень безопасности после 12 ч инактивации составил одну $ТЦД_{50}$ в 10^9–10^{11} л вируссодержащей суспензии. Также было выявлено, что увеличение времени инактивации в два раза повысило вирулицидную активность аминоэтилэтиленимина: для типа О – в 1,8 раза, для типа А – в 2,4 раза. В то же время очистка от клеточного дебриса на процесс инактивации не оказывала существенного влияния. Аминоэтилэтиленимин не разрушает 146S частицы вируса, что и было подтверждено при исследовании иммуногенной активности вакцин в процессе хранения. Таким образом, аминоэтилэтиленимин, выпускаемый в виде 15%-го водного раствора российской фирмой ООО «Биохимресурс» (г. Владимир), соответствует высоким стандартам качества. При исследовании иммуногенности эмульсионной бивалентной противоящурной вакцины для крупного рогатого скота в остром опыте установили, что активность препарата составила 10,08 защитных доз в прививном объеме 2 $см^3$.

Ключевые слова: вирус ящура, вирулицидная активность, инактивант, вакцина

Благодарности: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Ветеринарное благополучие».

Для цитирования: Михалишин Д. В., Михалишин В. В., Гочмурадов Ы. М., Елькина Ю. С. Инактивация вируса ящура для изготовления вакцин. *Ветеринария сегодня*. 2023; 12 (2): 164–170. DOI: 10.29326/2304-196X-2023-12-2-164-170.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Михалишин Дмитрий Валерьевич, доктор ветеринарных наук, заведующий лабораторией профилактики ящура ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: mihalishindv@arriah.ru.*

INTRODUCTION

The production of whole-virion vaccines against foot and mouth disease is a complex and multi-stage process. One of the mandatory steps in the vaccine technology is the inactivation of the foot and mouth disease virus (FMDV). Inactivation is the loss by a virus of the ability to reproduce and infect susceptible animals while retaining its antigenic properties [1].

The main requirement for the inactivant is that it must change the viral nucleic acid but must not significantly diminish the properties of the protein responsible for immunogenicity. Formaldehyde was the first chemical agent used to inactivate the virus for the vaccine production [2]. An innocuous vaccine suitable for cattle immunization was obtained using this substance.

Formaldehyde as an inactivant has a number of drawbacks: narrow range of concentrations that inactivate viral infectivity and reduce vaccine immunogenicity; the multicomponent nature of the inactivation curve [3–5], which does not allow to determine the endpoint of inactivation and the innocuity of the vaccine [6]; products of formaldehyde reaction with non-functional proteins are allergenic [7]; vaccine shelf life is compromised by higher temperatures. Hydroxylamine, methylglyoxal, and ethylene oxide have been tested as inactivants, but they are not widely used in the manufacture of antiviral vaccines.

Studies by F. Brown et al. [8, 9] have discovered a new class of chemicals (aziridines) for inactivation, which are superior in inactivation rate and in preservation of FMDV immunogenic properties.

Bahnemann H. G. [10, 11] proposed using a mixture prepared by synthesis of ethylenimine from 2-bromoethylamine hydrobromide for inactivation of FMD virus. This drug was called binary ethylenimine, which is widely used in the manufacture of FMD vaccines.

Aziridines inactivate the viral infectivity in a first-order reaction without significant reduction in immunogenicity. Linear inactivation kinetic makes it possible to calculate the innocuity level of the vaccine [12–16].

The interaction rate of the inactivant with the viral protein envelope and the nucleic acid is directly proportional to the concentration of the reacting groups. Beside the concentration of the inactivant, the inactivation rate is influenced by temperature, incubation time, pH of the medium and components of the virus-containing suspension. During vaccine manufacture, special significance is given not to the virus inactivation rate, but to the ability of the chemical agent to ensure its complete inactivation [17].

The complete inactivation of FMD virus is a critical requirement in the production of FMD vaccine to ensure the safety of the product. The vaccine innocuity can be controlled more effectively by the analysis of inactivation kinetics used to determine the theoretical time of inactivation, as well as the procedure of the virus inactivation. The logarithmic graph of the residual infectivity, depending on the inactivation time, should be linear. This straight line allows extrapolation to the endpoint of the inactivation. Validation of the inactivation process is an essential part of quality assurance [18, 19]. Aminoethylethanolamine (AEEA) in the studies of N. A. Ulupov et al. was identified as most suitable inactivant, which is currently used in the manufacture of FMD vaccines [20].

Advantages and prospects of AEEA use in the production of FMD vaccines has been noted by many authors in their works [21–24]. The World Organization for Animal Health recommends using binary ethylenimine to inactivate FMDV for vaccine production [25]. The mechanism of viral inactivation by ethylenimine and its oligomers consists in alkylation of nucleic acid or protein due to the ring opening reaction [6, 20].

This article presents some results of studies of the FMDV infectivity inactivation using AEEA. A method for evaluation of the inactivant virucidal activity and its effect on vaccine immunogenicity and antigen stability during storage is demonstrated.

MATERIALS AND METHODS

Virus. Production strains of FMDV types A, O, C, Asia-1, SAT-1, SAT-2, SAT-3 adapted to newborn rabbits and BHK-21/2-17 cell suspension culture.

Animals: 18–20 g white mice, 3–5 day-old suckling mice, 250–300 kg cattle.

Cell culture: trypsinized primary porcine kidney culture (PK), BHK-21/2-17 suspension cells.

Inactivant: 15% aqueous solution of 1-(2-aminoethyl)-aziridine (OOO "Biokhimresurs", Russia) and anhydrous AEEA stored on dry NaOH.

Determination of IC_{50} of inactivants. The determination of AEEA virucidal activity is based on the determination of inactivant concentration, which reduces the infectivity of the virus-containing suspension to 10^0 LD_{50} or $TCID_{50}$ at the specified parameters of time, temperature and pH. By analogy with potency test by calculation of 50% protective dose, it is possible to calculate the concentration of an inactivant that protects 50% of infected animals or culture flasks from the virus (IC_{50}).

The IC_{50} was determined using Kerber – Ashmarin formula:

$$\lg IC_{50} = \lg D_{max} - \lg d\ (\Sigma L_i - 0.5),$$

where D_{max} is the maximum concentration of the inactivant at which all test targets are not infected with FMD;

d – multiplicity of tested inactivant concentrations;

ΣL_i is the sum of ratios between the number of non-infected suckling mice or PK monolayer flasks free from CPE (cytopathic effect) with the number of infected suckling mice or flasks.

The result of the IC_{50} determination is the percentage concentration of the inactivant in the FMDV suspension, which reduces its infectivity to one LD_{50} or $TCID_{50}$ in the tested suspension volume.

Example:

An inactivant is added to the precisely measured volumes of the tested virus-containing suspension, making two-fold serial dilutions. The same pH and temperature values are adjusted in the samples. After a defined incubation period, the residual infectivity is determined using suckling mice or cell culture. For each concentration of the inactivant, 10 animals or flasks are used. The mice are observed for 7 days, and the cell culture is monitored for 3–4 days. Number of affected and unaffected test targets are recorded.

Concentration of the inactivant	Number of test animals	Number of non-affected animals	Ratio between the numbers of non-infected and infected animals
0.005	10	0	0/10
0.010	10	3	3/10
0.020	10	7	7/10
0.040	10	10	10/10
0.080	10	10	10/10

Calculation of IC_{50} based on the data in the table: $\lg IC_{50} = \lg 0.08 - \lg 2\ (0/10 + 3/10 + 7/10 + 10/10 + 10/10 - 0.5) = 2.9 - 0.3 \times 2.5 = 2.9 - 0.75 = 2.15$.

The antilogarithm of 2.15 is 0.014, hence $IC_{50} = 0.014\%$.

The calculated concentration of the inactivant in the viral preparation, equal to 0.014%, reduces the infectivity to the level of one infectious unit during a specified time, at specified temperature and pH. The advantage of the method is the rapid determination of the viral inactivation mode for vaccine production. The calculated concentration of the inactivant, which protects 50% of test subjects, reflects the qualitative characteristics of any component of the virus-containing suspension, the role of temperature, pH in the AEEA inactivation and its virucidal activity for a different batch and during storage [17].

Determination of the FMDV inactivation rate. The inactivation rate was determined after calculation of IC_{50}. An inactivant was introduced into the viral suspension to obtain a concentration several times higher than IC_{50}, the mixture was incubated at a given temperature, samples of the suspension were taken every hour and stored frozen until testing for infectivity. The constructed graph of the infectivity titer reduction described the inactivation rate and the vaccine innocuity level for a certain duration of inactivation.

Experimental vaccines based on lapinized FMD virus were prepared on acetate, ammonia and phosphate buffer solutions. Viral suspensions containing 10% rabbit tissue were purified with chloroform, AEEA solution was added to a concentration of 0.03%, pH was adjusted within 7.2–7.8 and then incubated at a temperature of 26–27 °C for 24 hours. Vaccines containing 59.5% of the suspension, 30% of aluminum hydroxide with 3% Al_2O_3, 10% of glycerin and 0.5% of 10% saponin solution were formulated from innocuous suspensions.

A bivalent emulsion vaccine was formulated containing using inactivated suspension of cultured FMDV types A and O and adjuvant Montanide™ ISA 206 in a ratio of 1:1 and containing 4 μg of FMDV 146+75S component of each type per inoculation dose.

The immunogenicity of the vaccines was tested on adult white mice, which were vaccinated subcutaneously with 0.4 cm^3 of whole vaccine and of the vaccine diluted 2, 4, 8, 16 times using phosphate buffer. After 21 days, mice were infected with a homologous virus adapted to them at a dose of 10^4 LD_{50}/cm^3. After an 8-day observation, a 50% immunization dose of the vaccine (ImD_{50}) was determined using the Kerber – Ashmarin formula.

A bivalent water-in oil-in water complex emulsion vaccine was tested on cattle using quantitative method. The vaccine was injected intramuscularly to three groups of animals (five animals per each group), each subsequent dose was decreased by a factor of 4 (2.0, 0.5 and 0.12 cm^3). Animals were challenged on Day 28 after immunization by inoculation of 10^4 $ID_{50}/0.2$ cm^3 of homologous virus into the mucous membrane of the tongue. After 7 days, the animals were examined and a protective dose (PD_{50}) per vaccination volume for each type of virus included in the vaccine was established.

All animal experiments were conducted in strict accordance with the GOST 33215-2014 and GOST 33216-2014 interstate standards for laboratory animal keeping and handling, adopted by the Interstate Council for Standardization, Metrology and Certification, as well as in accordance with the requirements of Directive 2010/63/EU of the European Parliament and the Council of the European Union, September 22, 2010 on the protection of animals used for scientific purposes.

RESULTS AND DISCUSSION

The results of the studies aimed at determination of the AEEA concentrations in a viral suspension, which reduce the virulence of samples to one LD_{50}, after challenge of 3–5 day suckling mice are shown in Table 1.

As we can see, 0.0025% AEEA concentration in suspensions prepared using acetate and ammonia buffer solutions and 0.0031% AEEA concentration in suspension

Table 1
Effect of medium used for lapinized FMDV type O suspension on AEEA virucidal activity ($n = 7$)

Acetate buffer solution	Ammonia buffer solution	Phosphate buffer solution
IC_{50} value after virus inactivation for 24 hours at 26–27 °C and pH 7.2–7.8		
0.0025 ± 0.00029 $P < 0.005$	0.0025 ± 0.00029 $P < 0.005$	0.0031 ± 0.00031 $P < 0.001$

prepared using phosphate buffer solution reduced infectivity to one LD_{50} after 24 hours incubation at 26 °C.

Taking into account the published data that aziridines inactivate FMDV infectivity in a first-order reaction and the inactivation rate is proportional to the concentration of the inactivant used, graphs were constructed to reduce the infectivity of type O in the phosphate buffer at 26–27 °C for 0.01% and 0.02% AEEA concentrations. These concentrations were 3.22 and 6.45 times higher than the IC_{50} value, which should have accelerated inactivation by a corresponding factor. However, the average inactivation rate after 3 and 6 hours of incubation was 4 and 8 times higher than when 0.0031 ± 0.00031% concentration was used.

The average values obtained by three experiments to study the inactivation kinetics of FMDV types A, O, C, Asia-1, SAT-1, SAT-2, SAT-3 using a phosphate buffer solution, AEEA at the concentration of 0.03% and at 26–27 °C and pH 7.2–7.8, showed that, despite the differences in the infectivity titers before the start of inactivation, infectivity was lost in all 7 serotypes after 2 hours. If inactivation lasted 8 hours, the innocuity level corresponded to one LD_{50} in 0.1 L (3 h) and in 10^9 L (6 h). Extrapolation of the inactivation curves on 8 hour duration suggested that the innocuity level of the suspension corresponded to one LD_{50} in $10^{15.3}$–$10^{15.8}$ L for serotypes A, O, C, Asia-1, SAT-1, SAT-2, SAT-3. The average inactivation rate of all 7 serotypes of the FMD virus was $10^{3.4}$ LD_{50} per hour.

Since the ethylenimine dimer is toxic, it is used as aqueous solutions (for example, a 15% AEEA aqueous solution), and stability of the active substance in the solution decreases during storage. Therefore, the next step was to study the virucidal activity of 15% AEEA solution at 2–8 °C after 0, 2, 4, 6 months of storage against cultural FMDV type O. The results obtained are presented in Table 2.

As we can see, the virucidal activity of the inactivant did not decrease after 6 month storage at 2–8 °C, since the 50% inactivating concentration (IC_{50}) did not change significantly after 2, 4, 6 months compared to the original concentration.

It was also found that 15 and 1% AEEA solutions prepared using demineralized water remained active for 6 months (observation period) at 2–8 °C. Anhydrous AEEA when stored over dry alkali (NaOH) remained active for 20 years (observation period) at a storage temperature of minus 10–20 °C (unpublished data).

The effect of AEEA on the immunogenicity of lapinized FMDV in a vaccine containing aluminum hydroxide, glycerin and saponin was studied for serotype O, which is the most labile one.

After purification with chloroform, AEEA solution was added to the suspension samples of the lapinized virus to a concentration of 0.03%. After 24 hours of the virus inactivation at 26–27 °C and pH 7.2–7.8, innocuity was tested and vaccines were formulated. To assess the immunogenicity of the vaccines, the method of ImD_{50} quantification was used on 18–20 g white mice. The animals were immunized subcutaneously with the vaccine diluted in 1/15 M phosphate buffer solution and challenged 21 days after vaccination. The immunogenicity of the vaccines was determined after manufacture, after a year and 8 years of storage at 2–8 °C. The results of the experiments are presented in Table 3.

Table 2
Effect of storage time on AEEA virucidal activity

Inactivant storage time (months)	IC_{50} (%)
0	0.0036 0.0040 0.0047
$M \pm m$	0.0041 ± 0.0003
2	0.0050 0.0047 0.0043
$M \pm m$	0.0047 ± 0.0002
4	0.0029 0.0038 0.0047
$M \pm m$	0.0038 ± 0.0005
6	0.0050 0.0040 0.0052
$M \pm m$	0.0047 ± 0.0005

Table 3
Dependence of FMDV type O immunogenic component stability on suspension medium and storage time

No.	Virus suspension medium	AEEA concentration in suspension (%)	Immunogenicity of vaccines for mice (ImD_{50} in 1 cm^3)		
			after manufacturing	after 1 year of storage	after 8 years of storage
1	Acetate buffer solution	0.03	0.16 0.17 0.16 0.15	0.15 0.19 0.18 0.15	0.18 0.21 0.16 0.17
$M \pm m$			0.16 ± 0.008	0.17 ± 0.021	0.18 ± 0.022
2	Ammonia buffer solution	0.03	0.19 0.19 0.10 0.11	0.29 0.19 0.23 0.11	0.30 0.25 0.24 0.14
$M \pm m$			0.15 ± 0.049	0.21 ± 0.076	0.23 ± 0.067
3	Phosphate buffer solution	0.03	0.12 0.13 0.13 0.14	0.16 0.18 0.21 0.20	0.21 0.19 0.20 0.23
$M \pm m$			0.13 ± 0.008	0.19 ± 0.022	0.21 ± 0.017

It was found that the AEEA optimal safe concentration made it possible to formulate vaccines identical in immunogenicity, which did not decrease their activity after 8 years of storage. However, this worked with those vaccines in which the virus was suspended in an acetate buffer solution. A tendency to decreased activities were found in the vaccines from the virus suspended in ammonia and phosphate buffer solutions.

In the process of FMDV inactivation, an inactivant obtained by two methods of synthesis was used: the first – anhydrous AEEA was obtained by polymerization of ethylenimine, and AEEA aqueous solution containing piperazine was obtained by cyclization of the AEEA sulfate ester. The latter is still used in the manufacture of FMD vaccines

Table 4
Effect of inactivation time on AEEA virucidal activity

Inactivation time (hours)	IC_{50} (%)	
	FMDV type O	FMDV type A
12	0.0027 0.0030 0.0025	0.0031 0.0030 0.0025
$M \pm m$	0.0027 ± 0.0001	0.0029 ± 0.0001
24	0.0015 0.0008 0.0011	0.0014 0.0009 0.0012
$M \pm m$	0.0011 ± 0.0001	0.0012 ± 0.0001

Table 5
Effect of purification on FMDV type O inactivation

Number of trials	IC_{50} (%)	
	purified suspension	unpurified suspension
1	0.0050	0.0048
2	0.0036	0.0040
3	0.0043	0.0040
4	0.0051	0.0052
$M \pm m$	0.0045 ± 0.0007	0.0045 ± 0.0006

Table 6
Immunogenicity of bivalent emulsion FMD vaccine for cattle

Vaccine description	Vaccination dose (cm^3)	Generalised foot infection		PD_{50}	
		A	O	A	O
Emulsion vaccine based on adjuvant Montanide™ ISA 206; Antigen: Type O – 4 μg, Type A – 4 μg	2.0	– – – – –	– – – – –	10.08	10.08
	0.5	– – – – –	– – – – +		
	0.12	+ + + + +	– + + + +		
Control		+	+		

"–" no podal generalization;
"+" podal generalization.

from a virus reproduced in BHK-21/2-17 cell suspension culture.

At the next stage, the kinetics of FMDV type O infectivity reduction, reproduced in a BHK-21/2-17 cell suspension culture in a 2,000 L bioreactor with 0.02% AEEA concentration and at 37 °C was analyzed. It was found that the inactivation was significantly lower than the inactivation rate of the lapinized virus, despite the fact that the inactivation temperature of the culture virus was 10 °C higher. This can be explained by the fact that the FMDV inactivation rate decreases with an increase in the salt concentration in the suspension and pH increase. In our experiments, the decrease in the inactivation rate is caused by the presence of the entire mass of whole BHK-21 cells and cell debris and a higher concentration of salts and other organic substances in the suspension. However, the innocuity level of the suspension after 12 hours of inactivation was one $TCID_{50}$ in 10^9–10^{11} L of suspension.

Taking into account the fact that an increase in the temperature of the virus-containing suspension contributes to an increase in AEEA virucidal activity, the inactivation process was started immediately after the virus finished its reproduction in BHK-21/2-17 cell suspension culture at 37 °C. Effect of inactivation time at 37 °C on the inactivant virucidal activity was tested after 12 and 24 hours.

The results presented in Table 4 demonstrate that an increase in incubation time at a constant temperature contributed to an increase in AEEA virucidal effect for type O by 1.8 times, for type A by 2.4 times.

Since a finely dispersed matter is formed during inactivation, it is technologically more convenient to perform inactivation before the purification of the virus-containing suspension. It was therefore necessary to prove that such an inactivation mode is sufficient to obtain an innocuous suspension of both purified and unpurefied cultural FMDV (Table 5).

It was found that IC_{50} for unpurified and purified suspensions was the same and amounted to 0.0045%. This fact confirmed that the cellular debris contained in the unpurified suspension did not have a negative effect on the inactivation process.

Complement fixation testing of cultural FMD virus of serotypes A, O, C and Asia-1 before and after inactivation showed that 146S component of the virus was not destroyed.

Using the developed inactivation mode of the FMD culture virus of types O and A, a bivalent emulsion vaccine was formulated, which was tested for immunogenicity by quantitation in cattle (Table 6).

The data in Table 6 demonstrate that the vaccine potency for both types was 10.08 PD_{50} in a vaccination dose of 2 cm^3.

CONCLUSION

FMDV adapted to 2–3 day old rabbits and to BHK-21/2-17 cell suspension culture was used to test the virucidal activity of the ethylenimine oligomer – N-aminoethylethanolamine (AEEA). The first stage of inactivant evaluation was the determination of the concentration that gives a non-virulent viral suspension at the specified parameters of the inactivation process (temperature, virus concentration, duration, pH), and the calculation of IC_{50}. IC_{50} for unpurified and purified suspensions was the same and amounted to 0.0045%. This fact confirmed that the cellular debris and lapinized non-functional proteins contained in the unpurified suspension did not have a negative effect on the inactivation process.

The constructed graph of infectivity titer reduction with the selected inactivation parameters (0.02% AEEA concentration at 37 °C) made it possible to determine the innocuity after 12 hours of inactivation, which amounted

to one $TCID_{50}$ in 10^9–10^{11} L of a virus-containing suspension intended for vaccine production.

The analysis of the dependence of the AEEA concentration and the vaccine storage duration on the FMDV immunogenicity showed that after inactivation 146S antigen did not break down.

Immunogenicity of bivalent FMDV vaccine for types A and O was 10.08 PD_{50} in 2 cm^3 vaccination dose. Virucidal activity, stability, high inactivation rate, minimal damage to 146S antigen indicates that AEEA has a number of advantages over other aziridines, used for FMDV inactivation. Thus, 15% aqueous solution of aminoethylethanolamine, produced by the Russian company OOO "Biokhimresurs" (Vladimir) meets all high quality standards.

REFERENCES

1. Sergeev V. A., Nepoklonov E. A., Aliper T. I. Viruses and viral vaccines. Moscow: Biblionika; 2007. 524 p. (in Russ.)

2. Vallée H., Carré H., Rinjard P. Sur l'immunisation antiaphteuse par le virus formolé. *Rev. gén. Méd. vét*. 1926; 35: 129–134. (in French)

3. Barteling S. J., Woortmeyer R. Formaldehyde inactivation of foot-and-mouth disease virus. Conditions for the preparation of safe vaccine. *Arch. Virol*. 1984; 80 (2–3): 103–117. DOI: 10.1007/BF01310652.

4. Gard S. Theoretical considerations in the inactivation of viruses by chemical means. *Ann. N. Y. Acad. Sci*. 1960; 83: 638–648. DOI: 10.1111/j.1749-6632.1960.tb40934.x.

5. Melvin P. A., Pryce J.-M. G. Production of viral antigens with N-acetyl-ethyleneimine. Patent No. US3318775A United States, Int. C12N7/00. Imperial Chemical Industries Ltd. Date of filing: 29.01.1963. Date of publication: 09.05.1967. Available at: https://patents.google.com/patent/US3318775A/en.

6. Ulupov N. A., Mikhalishin V. V., Gusev A. A., Lyozova T. N. Aminoetiletilenimin – sredstvo inaktivatsii infektsionnosti virusa yashchura = Aminoethylethanolamine is an inactivating agent of foot and mouth disease virus infectivity. *Sovremennye aspekty veterinarnoi patologii zhivotnykh: materialy konferentsii, posvyashchennoi 40-letiyu so dnya osnovaniya VNIIZZh (23–25 noyabrya 1998 g.) = Modern aspects of animal pathology: proceedings of the conference, devoted to 40th anniversary of ARRIAH (November 23–25, 1998)*. Vladimir; 1998; 53–62. (in Russ.)

7. Pay T. W. F., Telling R. C., Kitchener B. L., Southern J. Some observations of FMD virus inactivation with acetylethyleneimine. *In: European Commission for the Control of Foot-and-Mouth Disease. Report of the Meeting of the Research Group of the Standing Technical Committee.* Rome: FAO; 1972; 81–86. Available at: https://www.fao.org/3/ca9381en/ca9381en.pdf.

8. Brown F., Crick J. Application of agar-gel diffusion analysis to a study of the antigenic structure of inactivated vaccines prepared from the virus of foot-and-mouth disease. *J. Immunol*. 1959; 82 (5): 444–447. PMID: 13641679.

9. Brown F., Hyslop N. St G., Crick J., Morrow A. W. The use of acetylethyleneimine in the production of inactivated foot-and-mouth disease vaccines. *J. Hyg. (Lond)*. 1963; 61 (3): 337–344. DOI: 10.1017/s0022172400039620.

10. Bahnemann H. G. Inactivation of viral antigens for vaccine preparation with particular reference to the application of binary ethylenimine. *Vaccine*. 1990; 8 (4): 299–303. DOI: 10.1016/0264-410x(90)90083-x.

11. Bahnemann H. G. Binary ethylenimine as an inactivant for foot-and-mouth disease virus and its application for vaccine production. *Arch. Virol*. 1975; 47 (1): 47–56. DOI: 10.1007/BF01315592.

12. Mikhalishin D. V. Development of technology for manufacture of FMD emulsion vaccine: Author's Abstract of Thesis for degree of Candidate of Science (Veterinary Medicine). Vladimir; 2021. 39 p. (in Russ.)

13. Ulupov N. A. Vliyanie bufernogo rastvora na inaktivatsiyu virusa yashchura formal'degidom = Effect of buffer solution on foot and mouth disease virus inactivation with formaldehyde. *Veterinariya*. 1974; 9: 38–39. (in Russ.)

14. Ulupov N. A., Dudnikov A. I. Izuchenie aktivnosti khimicheskikh preparatov pri inaktivatsii virusa yashchura = Study of chemical activity for foot and mouth disease virus inactivation. *Aktual'nye problemy veterinarnoi virusologii: tezisy dokladov nauchnoi konferentsii VNIYaI = Topical issues of veterinary virology: conference paper abstracts of All-Russian FMD Research Institute scientific conference*. Vladimir; 1983; 34–35. (in Russ.)

15. Ulupova T. N., Ulupov N. A., Rybakov S. S., Koropov V. N., Dudnikov A. I. Sravnitel'noe izuchenie inaktiviruyushchei aktivnosti aziridinov po otnosheniyu k virusu yashchura = Comparative study of aziridine inactivating activities in relation to foot and mouth disease virus. *Aktual'nye problemy veterinarnoi virusologii: tezisy dokladov nauchnoi konferentsii VNIYaI = Topical issues of veterinary virology: conference paper abstracts of All-Russian FMD Research Institute scientific conference*. Vladimir; 1983; 37–39. (in Russ.)

16. Warrington R. E., Cunliffe H. R., Bachrach H. L. Derivatives of aziridine as inactivants for foot-and-mouth disease virus vaccines. *Am. J. Vet. Res*. 1973; 34 (8): 1087–1091. PMID: 4356910.

17. Ulupov N. A. Evaluation technique of chemical inactivation properties. Vladimir; 1980. 4 p. (in Russ.)

18. Barteling S. J. Development and performance of inactivated vaccines against foot and mouth disease. *Rev. Sci. Tech*. 2002; 21 (3): 577–588. DOI: 10.20506/rst.21.3.1361.

19. Aarthi D., Ananda Rao K., Robinson R., Srinivasan V. A. Validation of binary ethyleneimine (BEI) used as an inactivant for foot and mouth disease tissue culture vaccine. *Biologicals*. 2004; 32 (3): 153–156. DOI: 10.1016/j.biologicals.2004.09.001.

20. Ulupov N. A., Dudnikov A. I., Mikhalishin V. V. Lapinized foot-and-mouth disease vaccine of the FGI "ARRIAH" origin. *Proceedings of the Federal Centre for Animal Health*. 2008; 6: 323–340. eLIBRARY ID: 14933111. (in Russ.)

21. El'kina Yu. S. Vaccines against foot and mouth disease types O, Asia-1, A to induce early immunity in animals: Author's Abstract of Thesis for degree of Candidate of Science (Veterinary Medicine). Vladimir; 2021. 21 p. (in Russ.)

22. Eremets V. I., Bol'shakova M. V., Fomina G. A., et al. Izyskanie effektivnykh inaktivantov pri krupnomasshtabnom proizvodstve protivoyashchurnykh vaktsin = Search for effective inactivating agents in large-scale production of foot and mouth disease vaccines. *Nauchnye osnovy proizvodstva veterinarnykh biologicheskikh preparatov: tezisy dokladov Vserossiiskoi nauchno-prakticheskoi konferentsii, posvyashchennoi 30-letiyu instituta (8–9 iyunya 2000 g.) = Scientific basis for production of veterinary biological products: conference paper abstracts of All-Russian Scientific and Practical Conference devoted to 30th Anniversary*

of the Institute (June 8–9, 2000). Shchelkovo; 2000; 52–54. (in Russ.)

23. 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. (in Russ.)

24. Lozovoy D. A., Mikhalishin D. V., Lyozova T. N., Mikhalishin V. V., Konstantinov A. V., Starikov V. A., Borisov A. V. Laboratory studying efficacy of sorbate vaccine against heterologous strains of type A FMD virus. *Veterinariya*. 2016; 2: 16–19. eLIBRARY ID: 25522294. (in Russ.)

25. Foot and mouth disease (infection with foot and mouth disease virus). *In: WOAH. Terrestrial Animal Health Code*. 2022; Chapter 3.1.8. Available at: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.08_FMD.pdf.

Received 25.01.2023
Revised 28.02.2023
Accepted 20.03.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Dmitry V. Mikhalishin, Doctor of Science (Veterinary Medicine), Head of Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru.*

Valery V. Mikhalishin, Doctor of Science (Veterinary Medicine), Professor, Chief Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *AuthorID: 768290, Scopus Author ID: 14027142200, e-mail: mihalishin@arriah.ru.*

Ykhlas M. Gochmuradov, Postgraduate Student, Veterinarian, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-2508-8411, e-mail: gochmuradov@arriah.ru.*

Yulia S. El'kina, Candidate of Science (Veterinary Medicine), Junior Researcher, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia; *https/orcid.org/0000-0002-2986-8992, e-mail: elkina_ys@arriah.ru.*

Михалишин Дмитрий Валерьевич, доктор ветеринарных наук, заведующий лабораторией профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru.*

Михалишин Валерий Васильевич, доктор ветеринарных наук, профессор, главный научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ» г. Владимир, Россия; *AuthorID: 768290, Scopus Author ID: 14027142200, e-mail: mihalishin@arriah.ru.*

Гочмурадов Ыхлас Мурадович, аспирант, ветеринарный врач лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-2508-8411, e-mail: gochmuradov@arriah.ru.*

Елькина Юлия Сергеевна, кандидат ветеринарных наук, младший научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https/orcid.org/0000-0002-2986-8992, e-mail: elkina_ys@arriah.ru.*