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Biological properties of foot-and-mouth disease virus A 2205/G-IV strain

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ABSTRACT

According to the World Organisation for Animal Health, foot-and-mouth disease (FMD) is regularly reported in domestic and wild cloven-hoofed animals in Africa. G-I, G-IV, G-VI, G-VII, ASIA/Iran-05 genetic lineages of serotype A FMD virus are considered to be the most widespread on the African continent. Given the close economic and trade relations maintained by the Russian Federation with the countries of North Africa, of particular interest for us is studying the FMD virus of serotype A G-IV genetic lineage, which has been responsible for the infection outbreaks in the naturally susceptible animal population of the said region every year since 2012, and there is a risk of introduction of this virus genotype into the Russian Federation. Therefore, the issues of FMD introduction risk assessment and timely diagnosis are relevant for the Veterinary Service of Russia. FMD virus A 2205/G-IV strain tested for its biological and antigenic properties in cell cultures and naturally susceptible animals (cattle and pigs) was adapted for its reproduction in initially trypsinized porcine kidney (PK) cell culture, continuous monolayer cell cultures (IB-RS-2, PSGK-30, YaDK-04, BHK-21) by five serial passages. The virus was considered to be adapted when 90–95% cytopathic effect developed within 14–19 hours after the cell culture infection. The virus adapted to the cell cultures was tested for its infectivity with microtitration in IB-RS-2 cell culture. The virus strain tested for vaccine matching with microneutralization test (MNT) demonstrated significant difference from production A/Turkey/06, A_{22} No. 550/Azerbaijan/64, A_{22}/Iraq/64, A/Iran/97, A No. 2155/Zabaikalsky/2013, A No. 2166/Krasnodarsky/2013, A No. 2269/ARRIAH/2015 strains of FMD virus.

Keywords: foot-and-mouth disease virus, genotype, cell culture, vaccine matching, Africa

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Биологические свойства штамма A 2205/G-IV вируса ящура

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РЕЗЮМЕ

По данным Всемирной организации здравоохранения животных (ВОЗЖ), в странах Африки регулярно регистрируют ящур среди домашних и диких парнокопытных животных. Наиболее распространенными на Африканском континенте считаются генетические линии G-I, G-IV, G-VI, G-VII, ASIA/Iran-05 вируса ящура серотипа А. Поскольку Российская Федерация поддерживает тесные торгово-экономические отношения со странами Северной Африки, для нас особый интерес представляет изучение вируса ящура серотипа А генетической линии G-IV, который начиная с 2012 г. ежегодно является причиной вспышек инфекции в популяции естественно восприимчивых животных данного региона, при этом существует риск заноса вируса данного генотипа на территорию Российской Федерации. В связи с этим вопросы оценки риска заноса и своевременной диагностики ящура являются актуальными для ветеринарной службы России. В ходе исследований по изучению биологических и антигенных свойств штамма А 2205/G-IV вируса ящура в культурах клеток и организме естественно восприимчивых животных (крупный рогатый скот и свиньи) вирус адаптировали к репродукции в первично трипсинизированной культуре клеток свиной почки (СП), перевиваемых монослойных культурах клеток (IB-RS-2, ПСГК-30, ЯДК-04, ВНК-21) в течение пяти последовательных пассажей. При наступлении 90–95%-го цитопатического действия в течение 14–19 ч после инфицирования культуры клеток вирус ящура считали адаптированным. Инфекционную активность адаптированного к культурам клеток вируса изучали титрованием микрометодом в культуре клеток IB-RS-2. Оценка антигенного соответствия в реакции микронейтрализации показала значительное отличие изучаемого штамма от производственных вакцинных штаммов А/Турция/06, A_{22} № 550/Азербайджан/64, A_{22}/Ирак/64, А/Иран/97, А № 2155/Забайкальский/2013, А № 2166/Краснодарский/2013, А № 2269/ВНИИЗЖ/2015 вируса ящура.

Ключевые слова: вирус ящура, генотип, культура клеток, антигенное соответствие штаммов, Африка

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INTRODUCTION

Foot-and-mouth disease (*Aphthae epizooticae)* is an acute highly contagious infectious disease of cloven-hoofed animals, which is caused by an epitheliothropic RNA virus of the family *Picornaviridae*, genus *Aphthovirus*. The disease is characterized by fever, hypersalivation, aphthous lesions of oral mucosa, muzzle, interdigital cleft and coronary band skin, reduced milk and meat performance of livestock [1, 2, 3]. The disease was first described in the mid-XVI century, and, to this day, it continues to adversely impact the development of global trade and economy, as well as food security of the countries, since it is associated with enormous losses in the animal husbandry sector of agriculture [4, 5]. According to the current classification of the World Organisation for Animal Health (WOAH), foot-and-mouth disease belongs to the group of transboundary infections [6].

FMD virus has 7 serotypes significantly different from one another: A, O, C (not reported since 2004), SAT-1 (South Africa Territories-1), SAT-2, SAT-3, Asia-1 [7, 8]. The genetic diversity of field isolates and lack of cross-resistance to different FMDV serotypes in animals contribute to its wide spread all over the world. However, strict compliance with restrictions aimed at excluding the entry of livestock susceptible to foot-and-mouth disease from FMD infected regions into the countries free from the infection, as well as the application of state-of-the-art diagnosis and prevention tools help to prevent the occurrence of new FMD outbreaks. However, wild cloven-hoofed animals that are also susceptible to the virus can migrate at long distances and continuously maintain its persistence in a herd [9, 10, 11, 12]. Wild cloven-hoofed animals sharing the same territory with livestock act as a source of infection, and this is one of the causes of numerous new FMD outbreaks [13, 14, 15].

No country, not even the one with a highly effective animal disease prevention and control system in place, is shielded from FMD virus introduction, and FMD occurrence and further spread may cause a huge economic damage for agriculture [16]. The WOAH data on FMD infected countries of the world show that the virus is present in the susceptible animal population of three continents, with the highest number of FMD endemic countries being reported in Africa [17, 18, 19, 20]. Given the close trade and economic partnership maintained by the Russian Federation with the countries of this geographical region, studying the FMD virus circulating in Africa continues to be an urgent matter.

The aim of the study is to investigate the biological, antigenic and reproduction properties of foot-and-mouth disease virus A 2205/G-IV strain.

MATERIALS AND METHODS

FMDV A 2205/G-IV isolate (AFRICA topotype of G-IV genetic lineage) was provided to the FGBI "ARRIAH" by the World Reference Laboratory for Foot-and-Mouth Disease (Pirbright, Great Britain) for research purposes. The virus had been isolated from the pathological material collected from cattle in the Arab Republic of Egypt during FMD outbreaks in February 2018. During the study, the virus was adapted for its reproduction in initially trypsinized and continuous monolayer cell cultures.

Cell culture. The following cell lines were used for FMD virus A 2205/G-IV strain adaptation to cell cultures: PK (initially trypsinized porcine kidney cell culture), IB-RS-2 (continuous porcine kidney cell culture), PSGK-30 (continuous cell line of porcine origin), BHK-21 (continuous newborn Syrian hamster cell culture) and YaDK-04 (continuous domestic goat gonad cell culture). The cell cultures were tested for their susceptibility to FMDV A 2205/G-IV strain by serial passages in 25 cm^3 plastic culture flasks with a completely formed monolayer supplemented with maintenance nutrient medium. The infected cell monolayer was incubated at a temperature of (37.0 ± 0.2) °C until the development of apparent cytopathic effect (CPE). The time required for the development of 90–95% CPE reduced gradually with each subsequent passage.

Tests of the strain for biological activity in cell culture. The virus adapted to the cell cultures was tested for its biological activity by microtitration in 96-well culture plates. For this purpose, 4-fold serial dilutions of the virus were prepared in duplicate using sterile Eagle's medium (pH 7.6) containing kanamycin, an antibiotic, at a concentration of 20 IU/cm^3. Freshly prepared IB-RS-2 cell suspension with a concentration of (0.8–1.0) × 10^6 cells/cm^3 demonstrating no signs of contamination with foreign microorganisms was used as a biological activity indicator. The plates containing the reaction components were covered and placed into a carbon dioxide (CO_2) incubator with CO_2 concentration of 5% at a temperature of (37.0 ± 0.2) °C for 48 hours. The test results were recorded based on the specific CPE caused by the virus in the cell culture using an inverted microscope. The virus biological activity titre was calculated according to the Karber method and expressed as lg TCID/cm^3.

Animals. Six 8–10-month-old Russian Black Pied calves with a weight of 260–295 kg and six 4-month-old Large White gilts with a weight of 35–40 kg originating from infectious disease free farms of the Vladimir Oblast were used for FMDV A 2205/G-IV strain adaptation and infectivity titre determination in naturally susceptible animals.

Animal experiments conducted as part of the FMDV strain tests for its biological properties were carried out in compliance with interstate standard GOST 33215-2014 "Guidelines for accommodation and care of animals" adopted by the Interstate Council for Standardization, Metrology and Certification (Protocol No. 73-P of 22 December 2014).

FMD virus adaptation in cattle. For the virus adaptation, the virus culture suspension was administered to the animals intradermalingually in 4 sites at a volume of 0.1 cm^3. At passage 2, a 10% virus suspension prepared from passage 1 aphthous material was administered. Every 12 hours, the animals were examined for FMD clinical manifestations based on the presence of aphthous lesions.

Tests of FMD virus for infectivity in cattle. The infectivity titre of the studied FMD virus strain was determined according to the Henderson method. For this purpose, 10-fold dilutions of a 10% aphthous suspension of passage 2 FMD virus were prepared using phosphate buffer solution (PBS). The prepared virus dilutions were administered intradermalingually to two calves. The titration results were recorded after 24 hours based on the presence of aphthae at the virus-containing material inoculation site. The virus infectivity titre in cattle was expressed as lg ID_{50}/0.1 cm^3.

FMD virus adaptation in pigs was carried out by infecting 4-month-old piglets (2 piglets per passage) with the virus culture suspension administered intradermally in the coronary band. For passage 2, a 10% suspension prepared from passage 1 aphthous material with PBS was used. FMD clinical signs (aphthous lesions) were recorded every 12 hours.

Tests of FMD virus for infectivity in pigs were carried out by administration of the virus suspension (10-fold dilutions of a 10% suspension of passage 2 aphthae with PBS) to two gilts intradermally in the coronary band according to the Graves and Cunliffe method. The virus titration results in pigs were recorded after 24 hours based on the presence of aphthae at the virus-containing material dilution inoculation site. The virus infectivity titre in pigs was expressed as lg ID_{50}/0.1 cm^3.

Microneutralization tests of the isolate for antigenic properties. For determination of antigenic relationship (r_1 value) between A 2205/G-IV strain and production strains of serotype A FMD virus with microneutralization test (MNT), reference bovine sera from animals immunized with monovalent vaccines based on the following FMD virus strains were used: A/Turkey/06, A_{22} No. 550/Azerbaijan/64, A_{22}/Iraq/64, A/Iran/97, A No. 2155/Zabaikalsky/2013, A No. 2166/Krasnodarsky/2013, A No. 2269/ARRIAH/2015. The tests were carried out in accordance with "Methodical guidelines for determination of antigenic relationship between field isolates and production strains of foot-and-mouth disease virus using cross microneutralization test"[1], the test results were interpreted according to M. Rweyemamu [21].

Reference serum titres against 100 $TCID_{50}$ of homologous and heterologous viruses were determined with MNT by the serum cross-titration with five doses of the virus, calculated using linear regression equation and expressed as lg. Antigenic relationship coefficient (r_1 value) was calculated as the antilog of difference between serum titre (lg) against the heterologous virus and serum titre (lg) against the homologous virus.

The test results were interpreted as follows: $r_1 \geq 0.3$ suggests that there is an antigenic relationship between the field isolate and the production strain and that the vaccine based on the production strain will confer protection against the field virus; $r_1 < 0.3$ indicates that the field isolate is different from the production strain and that the vaccine based on this strain will not confer protection against the field virus.

RESULTS AND DISCUSSION

FMDV A 2205/G-IV strain adaptation to cell cultures. FMD virus A 2205/G-IV strain was adapted by five serial passages in IB-RS-2, PSGK-30, BHK-21, YaDK-04 and PK cell cultures. Test results are presented in Table 1.

Table 1
Results of FMDV A 2205/G-IV strain adaptation in cell cultures ($n = 3$)

Cell culture	Passage No.	CPE development period, hours	Virus titre, lg $TCID_{50}$/cm^3
IB-RS-2	1	18	5.50 ± 0.13
	2	15	6.73 ± 0.22
	3	15	7.03 ± 0.07
	4	11	7.60 ± 0.07
	5	7	6.59 ± 0.30
PSGK-30	1	20	6.80 ± 0.12
	2	15	7.25 ± 0.15
	3	15	6.81 ± 0.23
	4	9	7.70 ± 0.24
	5	9	7.28 ± 0.16
BHK-21	1	22	7.28 ± 0.24
	2	23	7.03 ± 0.25
	3	20	7.13 ± 0.37
	4	15	7.25 ± 0.25
	5	11	7.22 ± 0.22
YaDK-04	1	12	6.64 ± 0.11
	2	11	7.01 ± 0.40
	3	10	6.69 ± 0.02
	4	10	7.60 ± 0.07
	5	11	7.27 ± 0.37
PK	1	10	7.28 ± 0.17
	2	7	6.88 ± 0.12
	3	10	6.85 ± 0.03
	4	9	7.00 ± 0.05
	5	7	6.94 ± 0.80

[1] MU 76-12 Methodical guidelines for determination of antigenic relationship between field isolates and production strains of foot-and-mouth disease virus using cross microneutralization test: approved by the Rosselkhoznadzor on 13.09.2017. Vladimir: FGBI "ARRIAH"; 2017. 24 p.

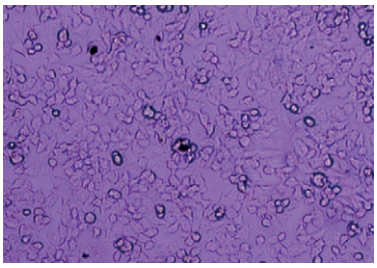

Fig. 1. Negative control BHK-21 cell culture 72 hours after the start of cultivation (200× magnification)

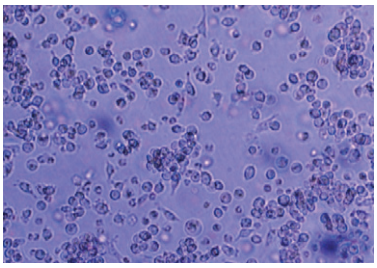

Fig. 2. FMDV A 2205/G-IV strain CPE in BHK-21 cell culture 20 hours after inoculation (200× magnification)

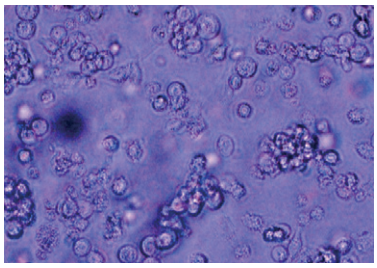

Fig. 3. Degenerative changes in BHK-21 cells as a result of FMDV A 2205/G-IV strain replication (400× magnification)

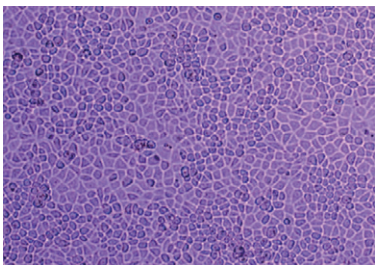

Fig. 4. Negative control IB-RS-2 cell culture 72 hours after the start of cultivation (200× magnification)

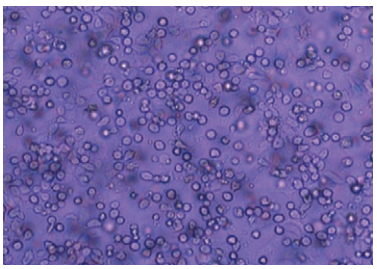

Fig. 5. FMDV A 2205/G-IV strain CPE in IB-RS-2 cell culture 15 hours after inoculation (200× magnification)

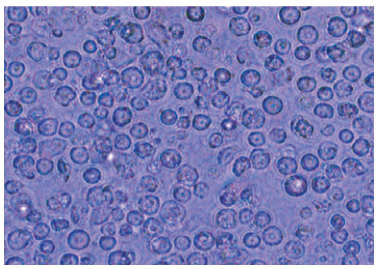

Fig. 6. Degenerative changes in IB-RS-2 cells as a result of FMDV A 2205/G-IV strain replication (400× magnification)

The negative control cell culture remained unchanged during 72 hours of observation (Fig. 1, 4). The formation of groups of rounding vacuolated cells (Fig. 2, 5) subsequently demonstrating degenerative changes of nuclei such as karyolysis and karyorrhexis (Fig. 3, 6) was interpreted as specific CPE characteristic of FMD virus. Then the cells became wrinkled and decreased in size, and that eventually led to the monolayer destruction. The above changes in the cells were indicative of the active replication of the virus.

The findings allow to conclude that FMD virus was successfully adapted to PSGK-30 and BHK-21 cell cultures. The most stable virus infectivity titre (between 7.03 ± 0.25 and 7.28 ± 0.24 lg $TCID_{50}/cm^3$) was recorded during the virus adaptation in BHK-21 cell culture. The highest infectivity of FMD virus A 2205/G-IV strain was 7.70 ± 0.24 lg $TCID_{50}/cm^3$ at passage 4 in PSGK-30 cell culture monolayer. The virus infectivity titre in IB-RS-2 cell culture increased gradually with every passage: from 5.50 ± 0.13 lg $TCID_{50}/cm^3$ at passage 1 to 7.60 ± 0.07 lg $TCID_{50}/cm^3$ at passage 4, but then began to decrease at passage 5. By contrast, the virus titre decreased during serial passages in PK cell culture. In YaDK-04 cell culture, CPE was steadily observed on average 11 hours after the virus inoculation. The infectivity titre increased in an undulating manner from 6.64 ± 0.11 lg $TCID_{50}/cm^3$ at passage 1 to 7.27 ± 0.37 lg $TCID_{50}/cm^3$ at passage 5.

FMDV A 2205/G-IV strain adaptation to naturally susceptible animals. To adapt FMD virus A 2205/G-IV strain to naturally susceptible animals, the virus containing material with activity of 6.81 ± 0.23 lg $TCID_{50}/cm^3$ prepared by passage 3 in PSGK-30 cell culture was used.

During the experiment, the animals were daily observed, first and foremost for their general health state, for lameness (piglets) and salivation (calves), for the severity and number of aphthous lesions at the virus suspension inoculation sites, their body temperature was measured.

At passage 1, apparent and well formed primary aphthae characteristic of foot-and-mouth-disease were found in the oral cavity (cattle) and on the coronary bands (pigs) 28 hours after the inoculation. The virus adaptation in naturally susceptible animals was considered to be successful based on the development of pronounced FMD clinical signs. The resulting aphthous material was used to prepare a 10% suspension for the virus infectivity tests in cattle and pigs, as well as in initially trypsinized PK cell culture. The results of the virus titration in animals and in PK cell culture are shown in Table 2.

Table 2
Results of infectivity titre determination for 10% aphthous suspension during FMDV A 2205/G-IV strain adaptation

Description of material	FMD virus infectivity titre in biological systems		
	in cattle, lg $ID_{50}/0.1\ cm^3$ ($n = 1$)	in pigs, lg $ID_{50}/0.1\ cm^3$ ($n = 1$)	in PK cell culture, lg $TCID_{50}/0.1\ cm^3$ ($n = 3, p < 0.01$)
10% aphthous suspension passage 1 in cattle	4.00	–	4.67 ± 0.30
10% aphthous suspension passage 1 in pigs	–	3.25	4.33 ± 0.17
10% aphthous suspension passage 2 in cattle	5.50	–	6.00 ± 0.14
10% aphthous suspension passage 2 in pigs	–	5.00	5.92 ± 0.22

The infectivity titres of 10% aphthous suspensions of the virus prepared using the material collected from cattle and pigs were found to be as follows: at passage 1 in animals – 4.00 and 3.25 lg ID_{50}/0.1 cm^3; at passage 2 – 5.50 and 5.00 lg ID_{50}/0.1 cm^3, respectively. In initially trypsinized PK cell culture, the infectivity titres of 10% aphthous suspensions of the virus prepared using the aphthae collected from cattle and pigs were found to be as follows: at passage 1 – 4.67 ± 0.30 and 4.33 ± 0.17 lg $TCID_{50}$/0.1 cm^3; at passage 2 – 6.00 ± 0.14 and 5.92 ± 0.22 lg $TCID_{50}$/0.1 cm^3, respectively, and this is indicative of FMDV A 2205/G-IV strain adaptation to naturally susceptible animals.

Microneutralization tests of the strain for antigenic properties. High variability of FMDV within one serotype leads to the emergence of new isolates that may differ from the previously recovered strains of the virus as regards their virulence, immunogenicity and antigenic properties. Among FMDV serotypes, serotype A FMD virus demonstrates the most pronounced antigenic variability, and this may result in strain-specific diagnosis problems. In view of this, the determination of antigenic relationship between the newly recovered FMDV isolates and the characterized and production strains of heterologous genotypes is of particular interest when studying FMD virus. The results of FMDV A 2205/G-IV strain tests for antigenic relationship are presented in Table 3.

FMD virus A 2205/G-IV strain was found to be antigenically different from production FMD virus A/Turkey/06, A_{22} No. 550/Azerbaijan/64, A_{22}/Iraq/64, A/Iran/97, A No. 2155/Zabaikalsky/2013, A No. 2166/Krasnodarsky/2013, A No. 2269/ARRIAH/2015 strains and not related to them. The findings are consistent with the data of the World Reference Laboratory for Foot-and-Mouth Disease (Pirbright, Great Britain) [22].

Table 3
Antigenic relationship (r_1) between A 2205/G-IV strain and production strains of serotype A FMD virus ($n = 3$) in MNT

FMD virus strains (genotypes)	r_1 value
A_{22} No. 550/Azerbaijan/64 (A/ASIA/Iraq-64)	0.22
A_{22}/Iraq/64 (A/ASIA/Iraq-64)	0.15
A/Iran/97 (A/ASIA/Iran-97)	0.21
A/Turkey/06 (A/ASIA/Iran-05)	0.08
A No. 2155/Zabaikalsky/2013 (A/ASIA/Sea-97)	0.06
A No. 2166/Krasnodarsky/2013 (A/ASIA/Iran-05SIS-10)	0.19
A No. 2269/ARRIAH/2015 (A/ASIA/G-VII)	0.25

CONCLUSION

The results of FMDV A 2205/G-IV strain tests for its biological and infectious properties are indicative of its high stability in cattle and pigs, and this may pose a significant risk in case of genotype A/AFRICA/G-IV FMD virus introduction into FMD free countries.

The tests of FMD virus A 2205/G-IV strain for its antigenic properties revealed its significant difference from production serotype A FMDV strains included in the vaccines used for the preventive immunization of naturally susceptible animals in the Russian Federation and neighbouring countries (r_1 values ranged from 0.06 to 0.25). The test results show that emergency response measures to be implemented in case of occurrence of foot-and-mouth disease caused by the virus of A/AFRICA/G-IV genetic lineage require the development and production of diagnostica and vaccines based on FMDV strains that are homologous or closely related to genotype A/AFRICA/G-IV FMD virus.

To ensure the economic stability and food security, as well as FMD freedom of the Russian Federation, to minimize the economic damage in case of possible FMD outbreak occurrence, we consider it appropriate to continuously monitor the global FMD situation in order to assess the risk of the disease agent introduction into Russia, to develop tools for timely FMD diagnosis based on heterogeneous field isolates of FMD virus that are not typical for our geographical region.

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