

STUDYING IMMUNOBIOLOGICAL CHARACTERISTICS OF FMDV TYPE O ISOLATES RECOVERED IN THE SOUTH KOREA

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SUMMARY

The paper presents the results of adaptation of FMDV Type O isolates obtained from the Animal and Plant Quarantine Agency to pig cell cultures and organisms as well as studies of their infectivity, antigenic activity as well as stability during passages in the PSGK-30 continuous cell culture. Antigenic matching of FMDV Type O isolates, 2014–2015, obtained from the Animal and Plant Quarantine Agency of the Republic of Korea, with Russian and foreign FMDV Type O production strains was studied. Special attention was given to determination of the inoculation dose for virus cultivation in PSGK-30 for the purpose of subsequent preparation of antigenic preparations and FMD vaccines. The results of strain studies served a basis for their depositing into the FGBI "ARRIAH" strain collection.

Key words: FMD virus, Republic of Korea, cell culture, pigs, antigenic matching.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly dangerous disease of livestock and wild cloven-hooved animals and characterized by significant decrease in performance of diseased animals and high mortality in young animals. The disease danger is accounted for its high contagiousness and potential for rapid spread in the form of epidemics and pan-epidemics, high variability and genetic diversity of strains, multiple routes of infection transmission, serotype-specific immunity in animals [6].

No country even the countries with high-performance veterinary services is safe from FMD introduction; economic losses in agricultural industry in case of the virus introduction and further spread can be huge [4].

In 2015–2016, fifty-nine FMD-affected countries were reported in the world. Most of them (32) were located in the African continent where type O, A, SAT-1, 2, 3 FMD virus was registered. Asian region was the second one where type O, A, Asia-1, SAT-1, 2 FMD virus was reported in 27 countries. From late 2016 till 2017 FMD cases were reported in 13 countries worldwide including the Republic of Korea (South Korea).

The OIE data on FMD occurrence in the world indicate constant presence of the virus in susceptible animal populations in two continents. In Asian region, the disease was reported in cattle, buffaloes, pigs, sheep and goats. In the African continent, the disease was mainly reported in cattle and to lesser extent in buffaloes, sheep and goats.

Map showing FMD epidemic situation in the Asian-Pacific Region in 2014–2016 is given in Figure 1. According to the map type O FMD spread the most widely in the region and followed by type A and type Asia-1 FMD [7, 10].

Thus, FMD epidemic caused by O/SEA/Mya-98 FMDV strain was reported in pigs in the Republic of Korea in July 2014. In December 2014 – April 2015, 185 FMD outbreaks were reported in pigs including five outbreaks where single FMD cases were detected in cattle. FMD outbreaks continued in the country up to March 2016. In early February 2017 eight type O FMD outbreaks were reported; several days later type A FMD outbreak was registered in the region, the epidemic had been eradicated for longer than one month [3, 8, 9]. Considering the said epidemic situation preparation of vaccines and diagnostica based on

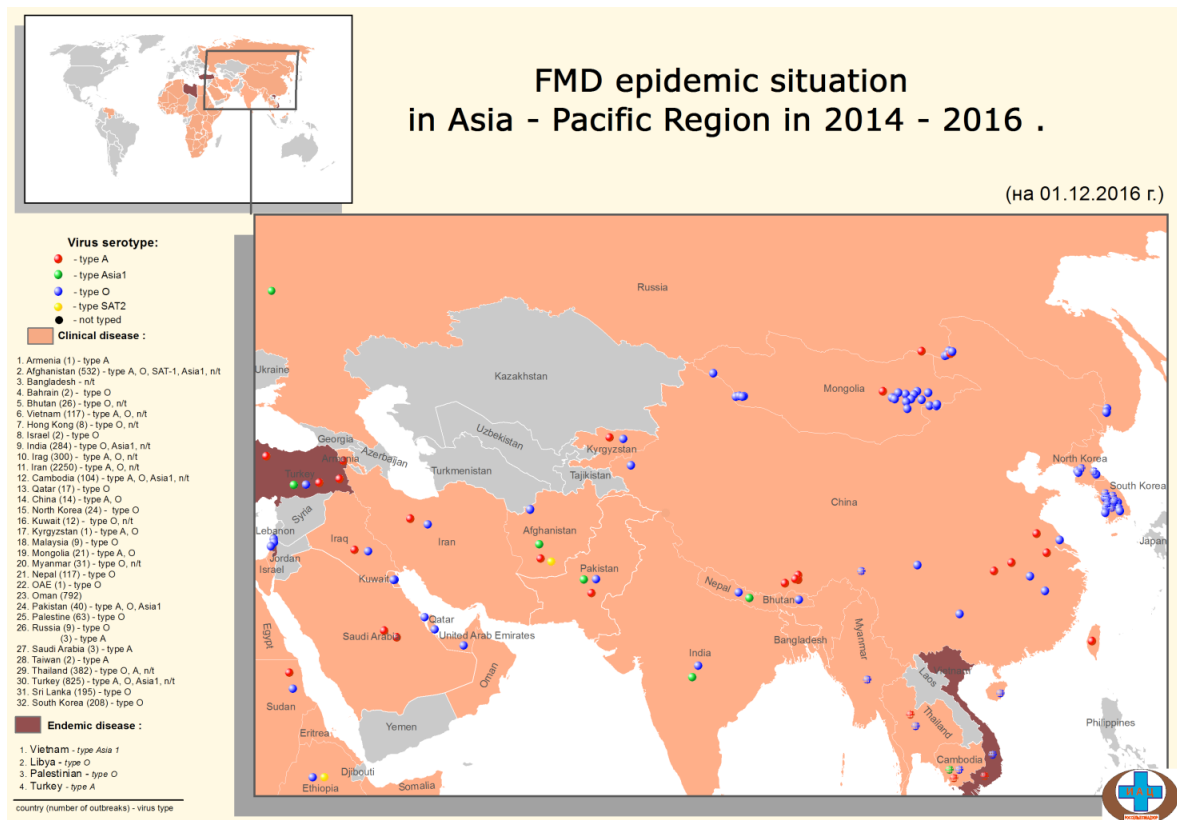


Fig. 1. FMD epidemic situation in the Asian-Pacific Region in 2014–2016 (as of 03.10.2016)
URL: http://www.fsvps.ru/fsvps-docs/ru/iac/foreign/2016/september/yashur_asia.pdf

currently epidemic virus isolates recovered in the Republic of Korea has become highly important.

Since type O FMD virus is widely spread in the Asian-Pacific Region there is a risk of the virus introduction to the Russian Federation. Tests of the isolates recovered in the Republic of Korea for their matching to production type O FMDV strains used by the FGBI "ARRIAH" are the most important for the RF Subjects located in the Far East region.

Scientific community has discussed aspects of FMD virus cultivation for a long time but multiple studies do not address all virus reproduction peculiarities. The virus never accumulates similarly in cell cultures and its accumulation rate depends on many factors affecting adsorption [1].

Currently, cell lines derived from domestic pig, PK, IB-RS-2, PSGK-30 and others, are proposed for the virus isolation, titration and cultivation [2]. All mentioned cell cultures demonstrate different susceptibility to FMD virus so it is impossible to prepare virus material with both high infectivity and high virus-specific protein levels in all cell cultures. Hence, virtually every FMDV strain requires individual approach to its cultivation.

The study was aimed at examination of FMDV isolates submitted from the Republic of Korea with virological and serological methods. Due to cultivation peculiarities of the isolate selected for detailed examination (relatively low viral antigen accumulation and sufficiently high infectivity levels) one of the study's goals was to determine optimal infectious dose inducing the highest viral material accumulation in cell cultures for the specified period.

The next goal was to optimize conditions for production FMDV O/KOR/JC02D1/2014 strain cultivation for further use for large-scale reproduction for diagnosticum and vaccine preparation.

MATERIALS AND METHODS

Foot-and-mouth disease virus (FMDV). In 2016 six isolates of type O FMDV belonging to SEA topotype recovered from pigs and cattle during outbreaks in the Republic of Korea in 2014–2015 were sent to the FGBI "ARRIAH" by the Animal and Plant Quarantine Agency of the Republic of Korea. Five successive passages in primary calf kidney cell culture and then five successive passages in continuous baby hamster kidney cell culture (BHK-21) were carried out for the virus adaptation. Infectivity titres in BHK-21 cell culture was 6.0–7.16 Ig TCID₅₀/cm³.

Virus reproduction in cell cultures. Continuous Siberian ibex kidney (PSGK-30) and porcine kidney (IB-RS-2) cell cultures were used for the isolate reproduction. Maintenance semi-synthetic medium and Eagle's medium, respectively, and initial virus suspension at ratio of 1:10 were added to plastic flasks with 25 cm² growth area and completely formed monolayer. Infected cultures were incubated at 37 °C until cytopathic effect (CPE) became evident. Axio Vert.A1 light laboratory inverted microscope (Carl Zeiss) was used for recording and photographing of the virus-infected cells. Presence of CPE in 90–95% of the monolayer surface within 18–24 hours evidenced the isolate adaptation to cell culture.

FMDV titration in cell culture. The cell culture-adapted isolates were titrated with micromethod. To do this, 10-fold dilutions of each virus suspension were made in nutrient Eagle's medium and added in triplicate to wells of 96-well microplate; then, continuous IB-RS-2 cell culture suspension was added to the wells. Titration results were recorded 72 hours after based on the presence of virus-specific CPE and expressed as Ig TCID₅₀/cm³.

Table 1
Infectivity and antigenic activity of Korean FMDV isolates adapted to PSGK-30 cell culture (n = 3)

Parameter	Isolate					
	O/KOR/JC35/2015	O/KOR/JC71/2015	O/KOR/JC84/2015	O/KOR/JC02D1/2014 (02 D1-11)	O/KOR/JC02D1/2014 (02 D1-2)	O/KOR/JC02D6/2014
Infectivity titre in IB-RS-2 cell culture (lg TCID ₅₀ /cm ³)	6.58 ± 0.14	6,67 ± 0.14	7.17 ± 0.28	7.5 ± 0.17	7.25 ± 0.25	6.75 ± 0.25
ELISA antigenic activity titre (dilutions)	1:16	1:32	1:32	1:16	1:64	1:32

Antigenic properties of the isolates examined with microneutralization test. Microneutralization test with reference sera derived from naturally susceptible animals vaccinated against production FMDV strains available in the FGBI "ARRIAH": O₁ Manisa (European vaccine strain), O Taiwan 3/97, O₁ Campos (South American vaccine strain), O PanAsia-2 (Saudi Arabia/2008), O No. 2102/Zabaikalsky/2010 (Russia/2010) and O No. 2212/Primorsky/2014 was used for antigenic properties examination.

The isolates were tested for their antigenic properties in accordance with Methodical recommendations for determination of antigenic matching between FMDV epidemic isolates and production strains with microneutralization test (FGBI "ARRIAH", 16.11.2012). Titres of reference sera against 100 TCID₅₀ of homologous and 100 TCID₅₀ of heterologous virus were determined with microneutralization test using cross titration with five virus doses, calculated using linear regression equation and expressed as lg. Antigenic relationship value (r_1 -value) was calculated as anti-logarithm of difference of serum titres against homologous and heterologous virus.

Microneutralization test results were interpreted as follows:

when $r_1 \geq 0.3$ – field isolate and production strain are closely related and vaccine based on the said production strain will protect from the epidemic virus;

when $r_1 < 0.3$ – field isolate differs from production strain, vaccine based on the said production strain will not be able to protect from the epidemic virus;

when $r_1 = 0.28...0.32$ – borderline value.

Re-isolation of the virus in pigs. Three-four month-old animals were infected with FMD virus by intradermal in-

jections in 4 sites of coronet, 0.1 cm³ per site; 10% virus suspension prepared from aphthous material was used for subsequent passages. Clinical manifestations were recorded every 12 hours as aphthae developed at the site of the virus material injection.

Virus titration in pigs. Titration of 10% virus suspension from aphthae with glycerol (ãã) was carried out in accordance with STO 00495527-0130-2009 "Control FMD virus strains from pigs".

Results of the virus titration in pigs were recorded 24–48 hours after as presence of aphthae at the site of the virus-containing material injection. Infectivity titre was expressed as lg ID₅₀/0.1 cm³.

Enzyme-linked immunosorbent assay (ELISA). FMD virus was tested for its antigenic activity in cultural and aphthous suspensions with indirect double sandwich ELISA using ELISA test-kit for detection of FMD virus antigens in accordance with its instruction approved by Director of the FGBI "ARRIAH" on April 4, 2015.

Statistical processing of results. Results obtained during tests were statistically processed using Microsoft Office Excel programme.

RESULTS AND DISCUSSION

Virus materials received from the Republic of Korea induced CPE in PSGK-30 and IB-RS-2 cell cultures within 15–24 hours starting with the first passage.

Results of tests of 2d-passage PSGK-3-adapted isolates for their infectivity and antigenic activity are given in Table 1.

Infectivity titres of the isolates reproduced in PSGK-30 cell culture were within (6.58 ± 0.14) – (7.5 ± 0.17) lg TCID₅₀/cm³, and their antigenic activity tested

Table 2
Antigenic matching between Korean isolate and production type O FMDV strains (n = 2)

Isolates	Production strains					
	O ₁ Manisa	O Taiwan 3/97	O ₁ Campos	O PanAsia-2	O No. 2102/Zabaikalsky/2010	O No. 2212/Primorsky/2014
O/KOR/JC02D1/2014 (02 D1-2) from pigs	0.03	0.06	0.01	0.02	0.33	0.64
O/KOR/JC02D6/2014 from pigs	0.05	0.04	0.02	0.01	0.35	0.41
O/KOR/JC84/2015 from pigs	0.18	0.09	0.12	0.17	0.54	0.69
O/KOR/JC02D1/2014 (02 D1-11) from pigs	0.04	0.07	0.18	0.02	0.55	0.76
O/KOR/JC71/2015 from cattle	0.23	0.37	0.15	0.1	0.62	0.45
O/KOR/JC85/2015 VR1500129 from cattle	0.32	0.6	0.09	0.17	> 1.0	0.73

Table 3
O/KOR/JC02D1/2014 FMDV infectivity and antigenic activity in 10% aphthous suspension ($n = 3$)

Parameter	1 st passage	2 ^d passage	3 ^d passage
Infectivity titre in IB-RS-2 cell culture (lg TCID ₅₀ /cm ³) (± 0.25)	6.58 \pm 0.14	7.58 \pm 0.14	7/08 \pm 0/14
Infectivity titre in pigs (lg ID ₅₀ /0.1 cm ³)	n/t	n/t	5/5 \pm 0/25
Antigenic activity titre determined with ELISA (dilutions)	n/t	n/t	1:256

n/t – not tested.

with ELISA was 1:16–1:64. Therewith, O/KOR/JC02D1/2014 (O2 D1-2) isolate demonstrated the highest antigenic activity whereas its infectivity titre was 7.25 ± 0.25 lg TCID₅₀/cm³. Therefore, this isolate was selected for further testings.

Results of testing of the isolates for their antigenic matching (r_1 -value) to Russian production type O FMDV strains and foreign strains being a part of the vaccines used in the Republic of Korea with microneutralization tests are given in Table 2.

Korean isolates recovered from pigs antigenically differ from production O₁ Manisa, O Taiwan 3/97, O₁ Campos, O PanAsia-2 strains ($r_1 = 0.01 \dots 0.18$) but antigenically related to Russian production O No. 2102/Zabaikalsky/2010 and O No. 2212/Primorsky/2014 strains belonging to SEA topotype ($r_1 = 0.33 \dots 0.76$). Isolates recovered from cattle demonstrated close relationship within SEA topotype

($r_1 = 0.45 \dots > 1.0$) as well as antigenic relationship with O Taiwan 3/97 isolate ($r_1 = 0.37 \dots 0.6$).

Obtained data on absence of antigenic relationship of Korean isolates (2014–2015) with O₁ Manisa strain and their antigenic relationship with the strains belonging to SEA topotype (O/SKR/7/10) correlate with the World Reference Laboratory (Pirbright) data [9].

Re-isolation of PSGK-30 cell culture-adapted O/KOR/JC02D1/2014 virus (infectivity titre of 7,5 lg TCID₅₀/cm³ in IB-RS-2 cell culture) was attempted in pigs in the FGBI "ARRIAH" animal facilities. To do this, 2d-passaged cultural virus was administered to the animals, three successive passages were made. At the 1st passage aphthae were observed only 52 hours after infection therewith there were no primary aphthae in animals but systemic infection and presence of aphthae on hoof bulb and around the edges of lips were recorded. Systemic weakness, rise in body temperature and anorexia were observed in the animals. At the 2^d passage aphthae were detected 36 hours after infection pigs demonstrated the same signs of systemic infection as at the 1st passage. At the 3^d passage aphthae developed at the site of the virus injection 24 hours after infection and their significant enlargement with abundant aphthous lymph was observed. 10% suspension was prepared from the virus material collected at each passage and used for subsequent infectivity determination in IB-RS-2 cell culture. 3^d-passage aphthous material was also tested for its infectivity in pigs and antigenic activity with ELISA. Table 3 shows results of tests of 10% aphthous suspensions at the 1–3-passages for their infectivity and antigenic activity titre determination.

Presented data indicate that infectivity titre of the virus did not significantly changed during the virus passaging in pigs and was within (6.58 ± 0.14) – (7.58 ± 0.14) lg TCID₅₀/cm³. Infectivity titre of aphthous material of the 3^d passage in pigs was 5.5 ± 0.25 lg ID₅₀/0.1 cm³. The virus was ELISA type-specific and ELISA active at a dilution of 1:256.

Infectious dose selection method was chosen from the methods described in the papers on successful FMD virus adaptation in cell cultures for its reproduction [2, 5]. The virus dilutions ($10^{-1.0}$ to $10^{-6.0}$ dilutions made with maintenance semi-synthetic medium) were added to 6 plastic flasks containing monolayer PSGK-30 cell culture and incubated at 37 °C. Data on ELISA testing of virus stocks prepared during infectious dose selection for their antigenic activity are given in Table 4.

Complete CPE (95–100%) was observed 15–18 hours after infection with the virus at a dilution of $10^{-1.0}$; 21–27 hours after inoculation with $10^{-2.0}$ – $10^{-4.0}$ virus dilutions and 30 hours after inoculation with $10^{-5.0}$ – $10^{-6.0}$ virus dilutions. When infectious dose of $10^{-3.0}$ was used time of CPE onset increased up to 24 hours due to infectious dose de-

Fig. 2. CPE dynamics in PSGK-30 cell culture infected with O/KOR/JC02D1/2014 FMDV at a dilution of 10^{-3}

- 1 – monolayer before infection with the virus material;
 2–5 – monolayer 2, 6, 9, 12 hours after infection;
 6 – CPE onset, accumulation of round dead cells 15 hours after infection;
 7–8 – CPE development 18–21 hours after infection with the virus;
 9 – complete monolayer disruption 24 hours after infection.

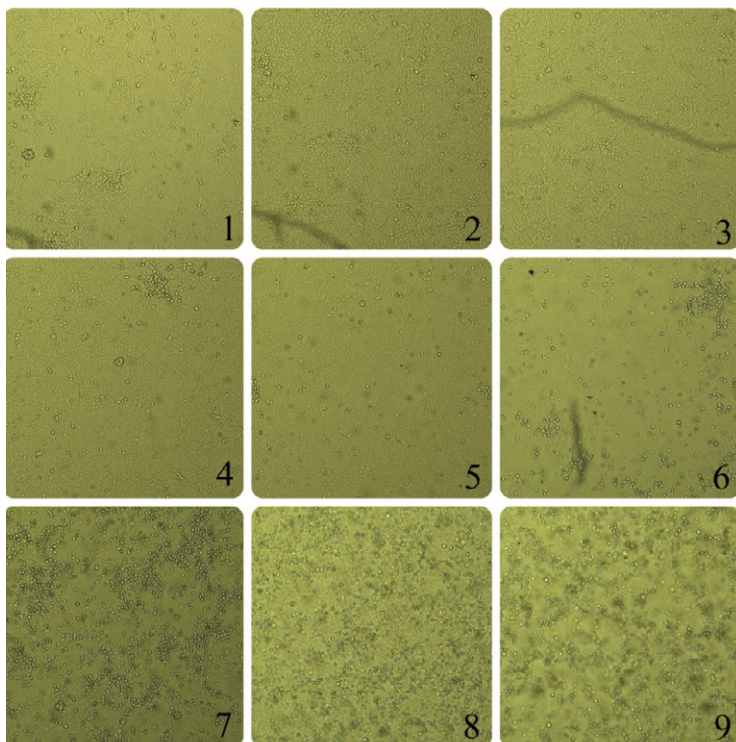


Table 4
Antigenic activity of O/KOR/JC02D1/2014 FMDV at different dilutions used for infection of PSGK-30 cell culture

Parameter	Virus dilution					
	10 ^{-1.0}	10 ^{-2.0}	10 ^{-3.0}	10 ^{-4.0}	10 ^{-5.0}	10 ^{-6.0}
Time of CPE onset (hours)	15–18	21	24	27	30	30
ELISA antigenic activity titre (dilution)	1:64	1:64	1:128	1:64	1:64	1:32

creasing (Fig. 2) but ELISA virus antigenic activity increased up to 1:128; that was the maximum for various infectious doses. Therefore, infectious dose of 10^{-3.0} was selected for FMDV cultivation in PSGK-30 cell cultures in 1,500 cm³ flasks for the purpose of antigen preparation.

O/KOR/JC02D1/2014 strain (02 D1-2) was tested for its stability by 5 passages in continuous PSGK-30 cell culture to be further used for biologicals production (Table 5). For this purpose, virus at each successive passage was titrated in continuous IB-RS-2 cell culture.

O/KOR/JC02D1/2014 strain demonstrated stable high titres of biological activity (6.00 ± 0.25) – (7.25 ± 0.25) lg TCID₅₀/cm³ throughout all passages while time of CPE onset decreased from 24 to 20–18 hours throughout 3–5 passages.

Based on test results the selected FMDV strain was deposited to the FGBI "ARRIAH" Collection of Microorganism Strains in the form of production strain and porcine control strain under accession number O No. 2271/KOR/JC02D1/2014 for diagnosticum and vaccine preparation and their control.

CONCLUSION

Type O FMDV KOR/JC02D1/2014 strain was adapted to continuous porcine cell cultures as well as to pig body. Infectious dose was selected for the virus reproduction in PSGK-30 cell culture as well as for infection of monolayer cell cultures and further scaling up of the virus material for diagnosticum and vaccine preparation. The virus was tested for its stability by five successive passages in continuous PSGK-30 cell culture.

FMDV isolates recovered from pigs and cattle in the Republic of Korea were closely related to production type O No. 2102/Zabaikalsky/2010 and O No. 2212/Primorsky/2014 strains belonging to SEA topotype based on microneutralization test results. Therefore, vaccines prepared from the said production strains will protect vaccinated animals from FMD virus circulating in the Republic of Korea.

FMDV strain under accession number O № 2271/KOR/JC02D1/2014 was deposited in the FGBI "ARRIAH" Collection of Microorganism Strains for diagnosticum and vaccine preparation and their control.

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Table 5
Biological activity of O/KOR/JC02D1/2014 strain reproduced in PSGK-30 cell culture by successive passaging (n = 3)

Passage No.	Time of CPE onset, hours	Virus titre, lg TCID ₅₀ /cm ³
1	24	6.33 ± 0.14
2	24	7.25 ± 0.25
3	20	7.08 ± 0.28
4	18	6.00 ± 0.25
5	18	6.41 ± 0.14

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