



Biological properties of swine vesicular disease virus strain 2348 Italy/2008

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SUMMARY

Swine vesicular disease (SVD) is a viral infectious disease, which, if acute, is manifested by the clinical pattern similar to a number of vesicular diseases including foot-and-mouth disease. In case of subclinical disease, there are no evident clinical signs, therefore the diagnosis is problematic, and there can be the risk of the disease introduction into the Russian Federation with the infected pigs. The key measure for the prevention of SVD introduction involves control diagnostic testing of all animals imported in the country that makes it necessary to keep updated the currently used methods and tools for the disease laboratory diagnosis. The paper demonstrates data on experimental infection of pigs with SVDV strain 2348 Italy/2008 that belongs to the most recent one of the four known phylogenetic groups. The virus was kindly provided by the World Reference Laboratory for Foot-and-Mouth Disease (Pirbright, Great Britain), and it was adapted to the monolayer continuous cell cultures of porcine origin (IB-RS-2 and PSGK-30). The pigs were intradermally infected with concentrated cultured virus at a dose of 10^3 TCID₅₀. The infected animals demonstrated clinical signs typical for the acute disease. There was evidence that the virus was not transmitted to the intact animal in case husbandry conditions were met that allowed to avoid the infection transmission by the fecal-oral and contact mechanisms. As a result of the experiment, reference sera were collected at different time intervals post infection and their activity was determined using virus microneutralization test in cell culture and ELISA. Aphthae collected from the infected animals were deposited into the Strain collection of the Reference Laboratory for Foot-and-Mouth Disease, FGBI "ARRIAH".

Keywords: swine vesicular disease, laboratory diagnosis, experimental infection, enzyme-linked immunosorbent assay, virus microneutralization test in cell culture

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Изучение биологических свойств штамма «№ 2348 Италия/2008» вируса везикулярной болезни свиней

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РЕЗЮМЕ

Везикулярная болезнь свиней – инфекционная вирусная болезнь, сопровождающаяся при остром течении клинической картиной, общей для ряда заболеваний с везикулярным синдромом, включая ящур. При субклиническом течении явные признаки болезни отсутствуют, что затрудняет постановку диагноза и может создавать угрозу заноса инфекционного заболевания на территорию Российской Федерации с инфицированными свиньями. Основной мерой предотвращения заноса везикулярной болезни свиней является проведение контрольных диагностических исследований всех ввозимых на территорию страны животных, что требует постоянного поддержания в актуальном состоянии используемых методов и средств лабораторной диагностики

заболевания. В статье представлены данные по экспериментальному заражению естественно восприимчивых животных штаммом «№ 2348 Италия/2008» вируса везикулярной болезни свиней, принадлежащего к наиболее поздней из четырех известных филогенетических групп. Вирус получен из Всемирной справочной лаборатории по ящуру (Пирбрайт, Великобритания) и адаптирован к перевиваемым монослойным культурам клеток свиного происхождения IB-RS-2 и ПСГК-30. Свиней заражали интрадермально концентрированным культуральным вирусом в дозе 10^9 ТЦД₅₀. У зараженных животных наблюдали клинические признаки, характерные для острого течения болезни. Показано отсутствие передачи вируса интактному животному при соблюдении условий содержания, предотвращающих фекально-оральный и контактный механизмы передачи инфекции. В результате опыта получены референтные образцы сыворотки крови свиней в разные сроки после инфицирования, определена их активность в реакции микронеutralизации вируса в культуре клеток и в иммуноферментном анализе. Отобранный от зараженных животных афтозный материал заложен на хранение в рабочую коллекцию штаммов микроорганизмов референтной лаборатории диагностики ящура ФГБУ «ВНИИЗЖ».

Ключевые слова: везикулярная болезнь свиней, лабораторная диагностика, экспериментальное заражение, иммуноферментный анализ, реакция микронеutralизации вируса в культуре клеток

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INTRODUCTION

Swine vesicular disease (SVD) is a contagious viral disease associated with a complex of symptoms common for a number of diseases with vesicular syndrome, and, first of all, for foot-and-mouth disease (FMD) being one of the economically significant animal diseases. Due to the similar clinical manifestations, the differential diagnosis of the vesicular diseases is impossible without a number of virus-specific tools for laboratory tests [1–5].

In spite of high contagiousness and increased virus resistance to the environmental factors, the morbidity of the susceptible animals varies substantially. The direct losses due to SVD are not significant, because in case of the disease outbreak the animals demonstrate only temporal loss of productivity and low mortality. The indirect damage due to the disease eradication measures are however significant [3, 4, 6–8]. The factor aiding to the spread of the infection involves the SVD's capacity for subclinical development being long undetected. During the latest officially reported epidemics the asymptomatic disease resulted in the mass SVD spread in susceptible animals, and the disease was reported only based on the serological monitoring data [9, 10]. Therefore, the largest World Reference Laboratories involved in examination and diagnosis of the diseases with vesicular syndrome developed, implemented and maintain up-to-date tools for SVD laboratory diagnosis [5].

The SVD virus, as well as many members of *Picornaviridae* family, is characterized by relatively high antigenic variability. There are four separate phylogenetic groups in the singular serotype, which developed successively at different time points and significantly differ antigeni-

cally [5, 10]. In the Russian Federation, SVD is considered to be an exotic disease and it has never been reported, but it was observed in the USSR (Odessa Oblast) in 1972. Strain 463 Odessa/1972 isolated and identified during the epidemics was placed into SVD phylogenetic group II, the isolates from which are antigenically different from the subsequent isolates of genetic groups III and IV [5, 10–12]. Hence, of specific academic and practical interest for SVD diagnosis is the virus strain 2348 Italy/2008 that belongs to the latest of the known groups, i.e. to group IV; and according to the published reports it causes mainly asymptomatic and subclinical disease on the pig farms [7].

The goal of the work was representation of the SVD clinical pattern in naturally susceptible animals following experimental infection with SVDV strain 2348 Italy/2008 and examination of the dynamics of the antibody formation in the infected pigs. The experiment poses a unique opportunity for the collection of sera from the experimental pigs at different, precisely known time points following infection, and such sera are of some value as reference controls that can be used for diagnostic purposes.

MATERIAL AND METHODS

Virus. In the experiment we used the SVDV provided by the World Reference Laboratory for Foot-and-Mouth Disease (Pirbright, Great Britain). The virus was adapted to monolayer continuous cell cultures and deposited National Collection of Microorganism Strains of the FGBI "ARRIAH" as 2348 Italy/2008 strain. The infectivity titer was determined in IB-RS-2 cell culture-containing 96-well culture plates using micromethod. The passage VII virus cultured in IB-RS-2 cell culture and having infectivity ti-

ter (7.73 ± 0.60) Ig TCID₅₀/50 µl was used for experimental infection and hyperimmunization of the naturally susceptible animals.

Cell culture. In order to adapt SVDV strain 2348 Italy/2008 the following porcine cell cultures produced in the cell cultivation sector of the Innovation Department of the FGBI "ARRIAH" were used: PKC (primary porcine kidney cell culture), PSGK-30 (continuous porcine cell culture) and IB-RS-2 (continuous porcine kidney cell culture). The cell sensitivity to the virus was determined by a series of "blind" passages in 25 cm³ plastic culture flasks containing maintenance medium. The blind passages were carried out until apparent cytopathic effect (CPE). The infected cell culture was incubated at (37.0 ± 0.5) °C for at least 72 hours. The duration of each consecutive passage was reduced as the time necessary for the development of 80–100% CPE reduced.

Animals. Three 35–40 kg Yorkshire and Landrace gilts at the age of 4 months old were used for the experimental infection, and they had been delivered from the infectious disease-free farms of the Vladimir Oblast.

All animal experiments were performed in strict compliance with interstate standard on laboratory animal keeping and handling GOST 33215-2014, adopted by Interstate Council on Standardization, Metrology and Certification and pursuant to the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Experimental infection of pigs. The animals were kept isolated at the animal keeping facilities of the FGBI "ARRIAH" for 29 days, and they had free access to water and feed. Pigs 1 and 2 were intradermally infected with the concentrated virus at a dose of 10^9 TCID₅₀ inoculated in the snout, coronary bands of both forelegs and one hind leg. For hyperimmune serum production the convalescent animals were additionally immunized on day 21 post infection (dpi), i.e. 100-fold concentrated virus antigen supplemented with incomplete Freund's adjuvant in 1:1 ratio was intramuscularly inoculated. Intact animal 3 was transferred to the isolator with the infected gilts on 4 dpi and they were further kept together. Before and during the experiment, blood samples were regularly collected from all animals at 2–6 day intervals. The blood samples were used for serological tests.

Virus microneutralization test in cell culture. Virus microneutralization test (MNT) in 2348 Italy/2008 SVD virus-based cell culture and sensitive continuous cell culture IB-RS-2 was used for pig serum testing for virus-neutralizing antibodies. The pig serum samples were tested in 96-cell culture plate by serial dilutions with the virus suspension inoculated at pre-calculated working dose. The plate was kept in CO₂-incubator with 5% CO₂ at (37.0 ± 0.5) °C for 1 hour in order the virus neutralizing antibodies contained in the tested sera could interact with the virus. Then the cell culture of $(0.8-1.0) \times 10^6$ cells/cm³ was inoculated. The plate was again transferred to the CO₂-incubator at (37.0 ± 0.5) °C for 60–72 hours. The reaction was examined using the inverted microscope and the number of cells with the preserved monolayer was calculated. The antibody titer was calculated using Karber's method.

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay (ELISA) was used for the determi-

nation of SVDV antibodies in animal sera. The assay was performed according to the 'Methodical Guidance on Detection of SVDV Antibodies Using Competitive Sandwich ELISA with Monoclonal Antibodies 5B7' (CS-ELISA), and using ELISA kit for SVDV antibody detection (FGBI "ARRIAH", Russia). Commercial PrioCHECK SVDV Ab Kit (Prionics Lelystad B.V., Netherlands) was used as a reference diagnostic test-kit. The test-kits were used according to the manufacturer's instructions.

RESULTS AND DISCUSSION

SVDV adaptation to cell cultures. SVDV strain 2348 Italy/2008 was adapted by means of blind passages in PKC cell culture (3 passages), PSGK-30 (5 passages) and IB-RS-2 (8 passages). For further work the cell culture was selected, to which the virus adapted most rapidly and which required less time for 80–100% CPE development. Herewith, the infectivity titers remained constant during at least five successive passages. The data in Table 1 demonstrate that SVDV strain 2348 Italy/2008 better adapted to the continuous cell lines PSGK-30 and IB-RS-2.

There were no signs of the virus reproduction in PKC cell culture during three passages. During cultivation in PSGK-30 and IB-RS-2 cultures, the virus induced 100% CPE in 48 hours. By passage 3, the time period necessary

Table 1
Adaptive capacity of SVDV strain 2348 Italy/2008 in cell cultures of porcine origin

Cell culture	Passage	Time for 80–100% CPE development, hours	Infectivity titer determined using MNT cell culture, Ig TCID ₅₀ /50 µl
PKC	I	none	–
PSGK-30	I	48	n/t
IB-RS-2	I	48	n/t
PKC	II	none	–
PSGK-30	II	48	n/t
IB-RS-2	II	24	n/t
PKC	III	none	–
PSGK-30	III	21	n/t
IB-RS-2	III	18	7.80 ± 0.60
PSGK-30	IV	18	n/t
IB-RS-2	IV	18	7.80 ± 0.60
PSGK-30	V	18	n/t
IB-RS-2	V	18	7.73 ± 0.60
IB-RS-2	VI	18	6.98 ± 0.60
IB-RS-2	VII	18	7.73 ± 0.60
IB-RS-2	VIII	18	6.75 ± 0.60

n/t – not tested.

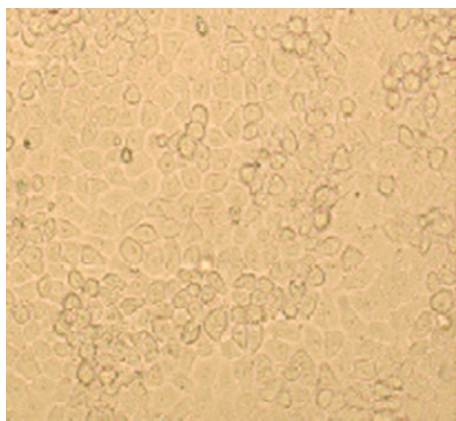


Fig. 1. IB-RS-2 cell culture after 96 hours of cultivation (magnification 200×)

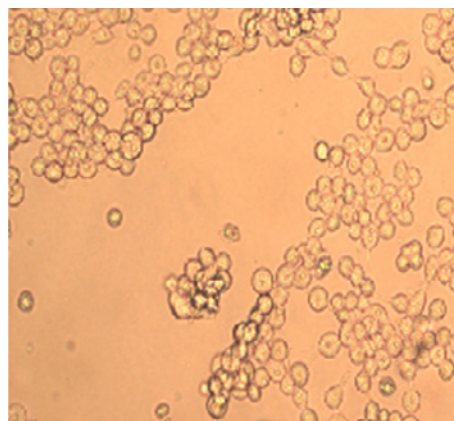


Fig. 2. CPE of SVDV in IB-RS-2 cell culture in 15 hours post inoculation (magnification 200×)

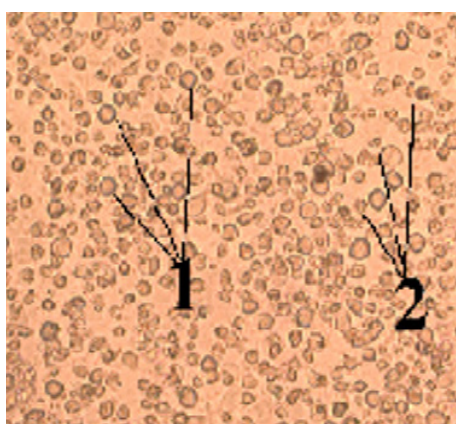


Fig. 3. CPE of SVDV in IB-RS-2 cell culture in 18 hours post inoculation (magnification 200×):
1 – rounded cells, 2 – in the process of degradation

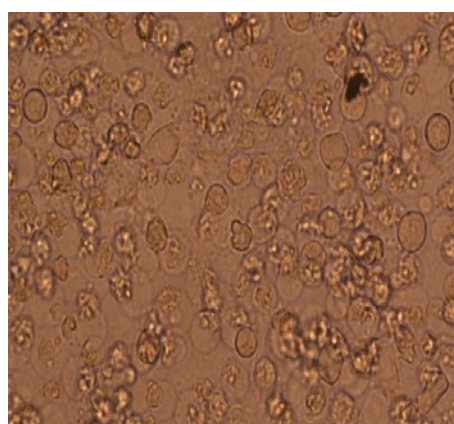


Fig. 4. CPE of SVDV in IB-RS-2 cell culture in 18 hours post inoculation (magnification 400×)

for the complete destruction of the cell monolayer reduced to 21 hours for PSGK-30 and to 18 hours for IB-RS-2. IB-RS-2 cell culture was selected for further work due to its sensitivity to SVDV. The pattern of the cellular degenerative changes caused by 2348 Italy/2008 was typical for SVD agent. In 12–15 hours post inoculation, initially irregular polygon-shaped monolayer cells of IB-RS-2 culture (Fig. 1) slightly stretched under the effect of the accumulated virus and took the shape of a spindle (Fig. 2). Hereafter, the cells rounded, detached from the plastic walls of the culture flask (Fig. 3) and finally shrunk and degraded (Fig. 4).

During six passages in monolayer continuous IB-RS-2 cell culture the SVDV strain 2348 Italy/2008 demonstrated relatively constant infectivity ranging from (6.98 ± 0.60) to (7.80 ± 0.60) $\lg \text{TCID}_{50}/50 \mu\text{l}$. Seventh passage 100-fold concentrated virus cultured in IB-RS-2 cell culture and having the infectivity titer (7.73 ± 0.60) $\lg \text{TCID}_{50}/50 \mu\text{l}$ was used for the infection of pigs. Cell suspension containing sixth passage SVDV strain 2348 Italy/2008 and having the infectivity titer (6.98 ± 0.60) $\lg \text{TCID}_{50}/50 \mu\text{l}$ was used for microneutralization test.

Infection of naturally susceptible animals. The pigs were observed daily during the whole experiment. The observation involved clinical examination with thermo-

metry, assessment of the general condition of the animals, pattern of movements and neurological signs.

In 2 dpi, animals 1 and 2 infected with the concentrated cultured SVDV strain 2348 Italy/2008 demonstrated clinical signs typical for the acute disease. Reduced motion activity and hyperthermia were reported in both pigs. Initial aphthae were formed at the sites of intradermal inoculation: in pig 1 – on the snout and coronary bands, in pig 2 – on coronary band of three limbs. Generalized infection and formation of secondary aphthae were reported on 5 dpi. Significant improvement of the pigs' health conditions was reported only after the formation of the secondary aphthae and before 7 dpi. Diarrhea was reported in animal 2 on 4 dpi and starting from 5 dpi the animal demonstrated lameness due to hoof inflammation. Herewith, the animal mostly remained laying and laboriously rose on carpal joints. On 11 dpi the animals demonstrated pododermatitis complicated with purulent process formed due to secondary infection. In pig 1 diarrhea was reported and lameness was demonstrated from 7 dpi; they were not so expressed but lasted longer, until 11 dpi, due to pododermatitis formation. During the whole experiment, none of the animals demonstrated any nervous signs, and they did not refuse from feed. Starting

from 5 dpi, both animals demonstrated papular eruption on the belly and in the pelvic area that was later accompanied with itching.

Animal 3 demonstrated no clinic signs of SVD, hyperthermia or behavioral changes.

During the experimental infection samples of biological materials were collected from the diseased animals: aphthous epithelium and lymph. Molecular and genetic tests of the biomaterials confirmed presence of SVDV genome that was similar to the genome of SVDV strain 2348 Italy/2008. The obtained epithelium sample was deposited to the collection of the strains of microorganisms at the Laboratory for FMD Diagnosis, FGBI "ARRIAH".

Serological tests. Blood samples used for serum production were collected from pigs before the experiment and during the whole experiment starting from 4 dpi. Total of 26 samples with different serological activity were collected. Studies showed correlation between the SVDV virus antibody test results obtained using various test methods. The results are demonstrated in Table 2.

The obtained results demonstrated that the diseased pigs had virus-specific antibodies that were detected by all test-kits: in animal 1 – starting from 7 dpi; in animal 2 – starting from 4 dpi. The antibody titers increased until 11 dpi, and they further gradually decreased [8]. Herewith, animal 2 demonstrated higher virus-specific antibody titer as compared to animal 1. For the production of the hyper-immune sera against SVDV the convalescent animals were additionally immunized on 21 dpi. Additional immunization intensified the immune response as expected, and antibody titers in the sera of both pigs reached significant values that was demonstrated by all test results.

Pig 3 did not demonstrate any antibodies by any of the tests that led to the conclusion that the animal was not diseased with SVD, although it was housed with the diseased pigs. It could be supposed that the animal was not infected because of the housing conditions that minimized the virus transmission: the pigs were housed in dry and daily cleaned spacious room. This excluded the contact route of the infection transmission. In spite of the fact that SVD infected animals typically shed the virus with the feces for a long time and the fecal-oral transmission route remains the major one, the concentration of the virus particles in the feces is relatively low as a number of researchers reported [3, 6]. Feeding and watering of the pigs was arranged in such a way as to allow the animals to consume the combined feed in short order and to supply the water in the drinking bowls on an ongoing basis thus continuously renewing it. That excluded contamination of both feed and water by the excrements and therefore prevented the infection transmission from the diseased animals to the contact one either with water or with feed.

CONCLUSION

The research results demonstrated that swine vesicular disease induced by SVDV strain 2348 Italy/2008 was characterized by typical clinical signs and accompanied with the expressed immune response. Early virus-specific antibody response was reported in the infected pigs on 4 dpi. Length of the log and stationary phases of the productive stage of antibody formation following the primary contact with SVDV amounted to about two weeks: the level of virus-specific antibodies reached its maximum on 11–15 dpi

and steadily decreased. Following repeated virus inoculation the animals demonstrated evident dynamics in the increase of the virus-specific antibodies that was maintained until the experiment completion. Since SVD is an

Table 2

Activity of sera collected from pigs at different time intervals post disease and immunization with 2348 Italy/2008 SVDV strain and tested using different test-kits ($n > 3$)

Animal	dpi	ELISA results						MNT results	
		ARRIAH test-kit		CS-ELISA		PrioCHECK SVDV Ab Kit			
		Ig	result	Ig	result	Ig	result	Ig	result
1	0	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	4	< 1	neg.	< 1	neg.	1.00	pos.	1.81	pos.
	7	2.20	pos.	2.08	pos.	1.90	pos.	2.58	pos.
	11	2.68	pos.	2.08	pos.	2.08	pos.	2.71	pos.
	15	2.51	pos.	2.08	pos.	1.78	pos.	2.58	pos.
	21*	2.34	pos.	1.78	pos.	1.48	pos.	2.41	pos.
	25	2.68	pos.	2.34	pos.	1.90	pos.	3.01	pos.
	29	3.71	pos.	3.28	pos.	2.98	pos.	3.79	pos.
	31	4.19	pos.	3.71	pos.	3.89	pos.	3.79	pos.
2	0	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	4	1.78	pos.	1.30	pos.	1.30	pos.	2.11	pos.
	7	2.68	pos.	2.98	pos.	2.98	pos.	2.71	pos.
	11	2.98	pos.	2.81	pos.	2.98	pos.	2.89	pos.
	15	2.68	pos.	2.68	pos.	2.81	pos.	2.71	pos.
	21*	2.68	pos.	2.08	pos.	2.51	pos.	2.71	pos.
	25	2.98	pos.	2.68	pos.	2.51	pos.	3.49	pos.
	29	4.01	pos.	3.58	pos.	3.11	pos.	3.91	pos.
	31	4.19	pos.	3.58	pos.	3.71	pos.	3.91	pos.
3	0	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	4	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	7	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	11	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	17	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	21	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	24	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	27	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.

* booster immunization.

exotic disease for the Russian Federation, the sera of the convalescent experimental animals are of definite value as they can be used as reference sera during the disease laboratory diagnosis.

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