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Optimization of cultivation parameters for bovine respiratory syncytial virus strain Vologda/2019

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SUMMARY

There are currently many controversial issues in the study of bovine respiratory syncytial infection. In this regard, it is relevant to study the biological properties of the virus, optimize the methods of its cultivation and select the most technologically advanced methods of designing diagnostic and prevention tools for this disease. The aim of this work was to select sensitive cell systems and to optimize the cultivation parameters in selected cell cultures. The Vologda/2019 strain of the bovine respiratory syncytial infection virus isolated from biological material obtained from a calf with respiratory symptoms was used in the experiment. The strain was adapted to the continuous cell culture derived from bovine turbinate tissue (BT) and deposited in the State collection of microorganism strains at FGBI "ARRIAH". It was established that the continuous cell lines of fetal bovine trachea (FBT) and calf kidney (RBT) are the most sensitive cell systems for the reproduction of the bovine respiratory syncytial virus strain Vologda/2019, the maximum accumulation of the virus was observed in these cell cultures. The cytopathic activity of the virus in the FBT cell culture ranged from 4.78 ± 0.18 to 5.50 ± 0.16 lg $TCID_{50}/cm^3$, and in the RBT cell culture – from 4.00 ± 0.23 to 4.75 ± 0.20 lg $TCID_{50}/cm^3$ at days 4–5 of cultivation. It was determined that in case of multiplicity of inoculation of FBT and RBT cell cultures with the virus at 0.1 lg TCD_{50}/cell and the use of 2% glutamine in the maintenance nutrient medium, as well as 2% horse or cattle blood serum, it is possible to obtain virus material with high cytopathic activity.

Key words: bovine respiratory syncytial virus, Vologda/2019 strain, cultivation, cytopathic activity, virus titer.

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Оптимизация параметров культивирования вируса респираторно-синцитиальной инфекции крупного рогатого скота штамма «Вологда/2019»

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РЕЗЮМЕ

В настоящее время в изучении респираторно-синцитиальной инфекции крупного рогатого скота есть множество спорных вопросов. В связи с этим актуальным является изучение биологических свойств вируса, оптимизация методов его культивирования и подбор наиболее технологичных приемов конструирования средств диагностики и профилактики данного заболевания. Целью настоящей работы являлись выбор чувствительных клеточных систем и оптимизация параметров культивирования в подобранных культурах клеток. В опытах использовали штамм «Вологда/2019» вируса респираторно-синцитиальной инфекции крупного рогатого скота, выделенный из биологического материала, полученного от теленка с признаками респираторной патологии. Штамм адаптирован к перевиваемой культуре клеток слизистой носовых перегородок крупного рогатого скота (ВТ) и депонирован в Государственную коллекцию штаммов микроорганизмов ФГБУ «ВНИИЗЖ». Установлено, что перевиваемые линии клеток трахеи эмбриона крупного рогатого скота (FBT) и почки теленка (RBT) являются наиболее чувствительными клеточными системами для репродукции респираторно-синцитиального вируса крупного рогатого скота штамма «Вологда/2019», в данных культурах клеток отмечалось максимальное накопление вируса. Цитопатическая активность вируса в культуре клеток FBT на 4–5 сут культивирования составила от 4,78 ± 0,18 до 5,50 ± 0,16 lg $ТЦД_{50}$/$см^3$, а в клеточной системе RBT – от 4,00 ± 0,23 до 4,75 ± 0,20 lg $ТЦД_{50}$/$см^3$. Определено, что при множественности заражения культур клеток FBT и RBT вирусом в 0,1 lg $ТЦД_{50}$/кл, использовании в составе поддерживающей питательной среды 2% глютамина, а также 2% сыворотки крови лошади либо крупного рогатого скота удается получить вирусный материал с высокой цитопатической активностью.

Ключевые слова: респираторно-синцитиальный вирус крупного рогатого скота, штамм «Вологда/2019», культивирование, цитопатическая активность, титр вируса.

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INTRODUCTION

Implementation of commercial methods in livestock farming, on the one hand, led to increase in productivity, and on the other hand, caused such serious problems as favorable conditions for massive spread of infectious diseases. Respiratory infections take the leading position among most common bovine diseases [1]. They can emerge both independently (parainfluenza-3, infectious rhinotracheitis, viral diarrhea, adenoviruses, respiratory syncytial infection) and in various associations of viral and bacterial etiology, causing enormous economic losses to livestock industry [2–4].

To date bovine respiratory syncytial virus (BRSV) infection has been registered in many countries across the globe (Japan, Germany, USA, Croatia, Belgium), as well as, since 1975, in Russia [4–6].

BRSV belongs to the family *Pneumoviridae*, genus *Orthopneumovirus*, species *Bovine orthopneumovirus*. All cattle breeds, as well as buffaloes, aurochs, yaks, bison, zebu, etc. are susceptible to the virus. According to the majority of domestic and foreign authors, the most BRSV-susceptible cattle are calves aged 1–12 months. However, the disease is less common in young animals up to 4 weeks of age; this fact may be associated with a more responsible attitude and proper care of newborn calves. There is evidence of outbreak occurrence among adult cattle, which may be associated with the mechanical penetration of the pathogen into the herd, where no measures were applied to prevent BRSV entry. Such cases include purchase of infected livestock, use of non-authorized cattle movement areas for transportation, etc. The incubation period lasts 2–5 days. There are three forms of the disease: subclinical, acute and hyperacute [7].

Diseased or convalescent animals often appear the source of infection. The most likely way of the virus transmission is via respiratory aerosols or from contact, through secretions from eyes, nose and tracheal mucosa. A number of authors have shown that intrauterine infection facilitates circulation of the respiratory syncytial virus in the herd. There is no data on transmission of the BRSV pathogen in cattle with semen; this issue needs further study [6, 8, 9].

At present, many issues related to the course of infection also remain insufficiently studied. It is known that BRSV replicates in respiratory tract cells, however, there are data of some Russian researchers on the virus adaptation to bovine kidney and testicular cell cultures [2, 7, 10].

BRSV diagnosis is made on the basis of epizootological and clinical data, pathological changes and laboratory test results. Laboratory diagnosis includes detection of specific antibodies using enzyme-linked immunosorbent assay (ELISA) or neutralization test; identification of the respiratory syncytial virus genome using real-time reverse transcription polymerase chain reaction (real-time RT-PCR); virus isolation from biological material derived from animals using culture techniques [2, 3, 8]. Blood sera, nasal discharge samples, tracheal and bronchial exudates, lung and bronchi fragments are commonly used as test material [5, 10, 11].

BRSV is often difficult to isolate in cell culture due to its lability and instability in the environment [7, 8]. The result of virus isolation in cell culture and its further identification using PCR or ELISA largely depend on the proper selection, storage, and transportation of biomaterial samples from diseased animals [10]. According to the data of domestic and foreign authors primary trypsinized cultures of bovine embryonic cells (bovine embryonic kidney (BEK) cells, bovine embryonic lung (BEL) cells, etc.) are used for virus isolation [7–9].

Real-time RT-PCR along with retrospective serological methods are the most promising methods for BRSV diagnosis [9]. It should be noted that virological methods play a significant role in the development of diagnostic systems and means of specific disease prevention. It is extremely important to obtain highly potent cultural virus material for production of high-quality test systems, kits, vaccines and specific sera [2, 3, 12].

Thus, the study of the viral biological properties, optimization of cultivation and selection of components are of great relevance for developing diagnostic and prevention tools for this disease.

The aim of this work was to optimize the cultivation parameters for BRSV strain Vologda/2019 in previously selected sensitive cell systems.

MATERIALS AND METHODS

BRSV strain Vologda/2019 isolated from biological material obtained from a calf with respiratory symptoms and adapted to the continuous bovine turbinate tissue (BT) cell culture with the infectivity titer of 4.0 lg $TCID_{50}/cm^3$ was used in the experiment. In 2019 the obtained BRSV strain Vologda/2019 was deposited in the State collection of microorganism strains at FGBI "ARRIAH".

In order to study the cultural properties of the indicated BRSV strain and its adaptation to high-performance cell lines, the following animal cell culture systems were used: bovine calf kidney (RBT), mucous membranes of fetal bovine nasal septum (FBN), fetal bovine trachea (FBT), rhesus monkey kidney (MA-104), goat gonads (YaDK-04) [13].

A 24-hour cell monolayer grown in 25–175 cm^3 plastic flasks was used for BRSV cultivation in continuous cell lines. The initial cell concentration in the cell suspension was 100–300 ths/cm^3. BRSV strain Vologda/2019 was inoculated into the cell culture at a dose of 0.1 $TCID_{50}$/cell. The cultivation time was 6–8 days provided that the monolayer integrity was preserved.

The sensitivity of continuous cell culture to BRSV strain Vologda/2019 was determined by successive passages [12]. For that, the culture inoculation was preceded by virus adsorption in monolayer cells, and no maintenance nutrient medium was added.

The virus was inoculated after removal of growth nutrient medium, then it was allowed to contact with the cell monolayer in CO_2 incubator at 37 °C for 1.5 h and after that maintenance semi synthetic nutrient medium (SSM) supplemented with 2% bovine or horse serum and glutamine was added. The virus material was collected when cytopathic effect (CPE) was demonstrated in 70–80% cell monolayer surface. The obtained virus was stored at minus 80 °C. The virus cytopathic effect was determined by virus microtitration assay [12] in BT or FBT cell cultures. A distinctive feature of this study was that BRSV strain Vologda/2019 was inoculated onto cell monolayer previously grown for 24 hours.

BRSV microtitration for each passage was performed in sterile 96-well flat-bottom microtitration plates at 0.2 cm^3 per well. Dilutions of virus-containing material (VCM) in SSM (10^{-1}–10^{-8}) were prepared in sterile Eppendorf tubes for that pupose [12]. The prepared virus dilutions were transferred with a single-channel mechanical pipette into the wells of a culture plate with a grown monolayer of FBT or BT cell cultures at 0.1 cm^3 per well starting with the highest dilution. The plate was placed in a CO_2-incubator with 5% carbon dioxide at 37 °C for 1.5 h for virus adsorption in the monolayer cells. After virus and cell monolayer contacted, 0.1 cm^3 of SSM supplemented with 2% horse blood serum and glutamine was added. Observation was carried out using Olympus CKX53 inverted microscope (40×–400× magnification) and color phase-contrast sliders of the warm and cold spectrum to increase the image sharpness. The final reading of the virus titration results were performed after 10 days of incubation, provided that the cell monolayer integrity in control wells was preserved [12].

The infectivity level was calculated according to Reed and Muench method and expressed as lg $TCID_{50}/cm^3$ [12].

BRSV antigen titer in inactivated preparations was performed using the 'ELISA kit for antigenic diagnosis of Bovine Respiratory Syncytial Virus (BRSV)' (Bio-X Diagnostics, Belgium) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Five successive passages were performed in RBT, FBN, FBT, MA-104 and YaDK-04 culture systems to study sensitivity of various cell cultures to BRSV strain Vologda/2019.

The virus-induced CPE varied in all cell cultures. At day 3 local changes in individual cells and/or cell groups were observed in RBT cell culture (Fig. 1, 2).

After 4–5 days, local CPE foci were formed in RBT cell culture as a result of BRSV strain Vologda/2019 replication, the main "pattern" of the monolayer became smooth due to cell deformation, and detachment of a large number of cells and their structural elements in suspension was observed (Fig. 3). Figure 4 clearly shows the difference between a supposedly pure and inoculated culture, that is, practically no cell detachment is observed in suspension, there are no changes, the monolayer "pattern" is distinct and individual cells are clearly visible.

At day 7–8 the CPE induced by BRSV strain Vologda/2019 reached 70–80% in RBT cell culture. The monolayer was dispersed, the cells were destroyed, "holes" were formed (Fig. 5). When a similar situation was observed, the virus-containing material was frozen at minus 80 °C and placed for storage. Control test results with no virus inoculation are shown in Figure 6.

Cell changes involving accumulation of destroyed or deformed cells could be observed in FBT cell culture at

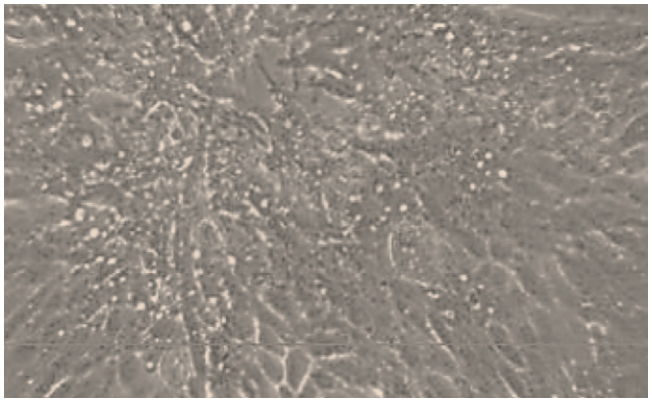

Fig. 1. BRSV-inoculated RBT cell culture (day 3, ×400 magnification)

Рис. 1. Культура клеток RBT, инокулированная вирусом РСИ КРС (3-и сут, увеличение ×400)

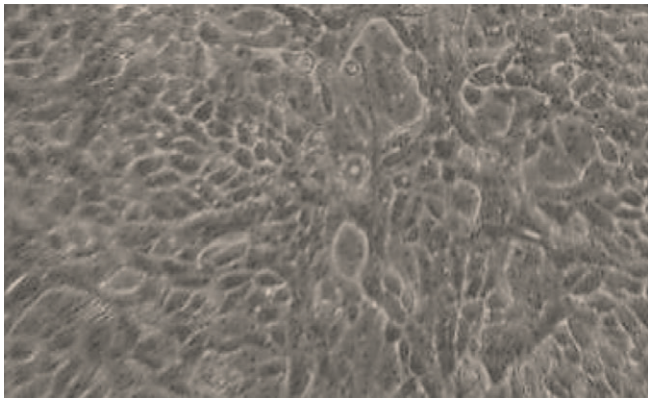

Fig. 2. Non-BRSV-inoculated RBT cell culture (day 3, ×400 magnification)

Рис. 2. Культура клеток RBT без инокуляции вируса РСИ КРС (3-и сут, увеличение ×400)

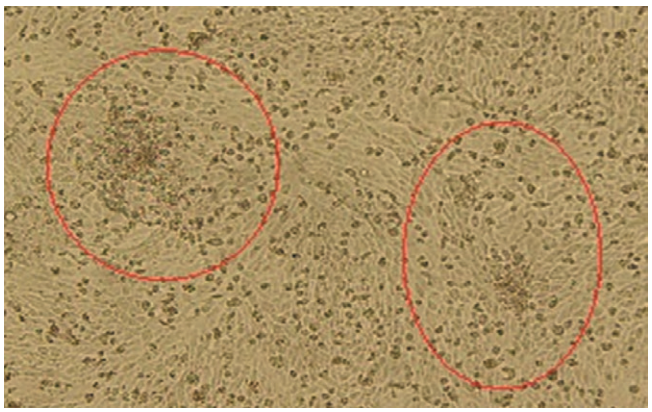

Fig. 3. RBT cell culture inoculated with BRSV (day 5, ×200 magnification)

Рис. 3. Культура клеток RBT, инокулированная вирусом РСИ КРС (5-е сут, увеличение ×200)

Рис. 4. Культура клеток RBT без инокуляции вируса РСИ КРС (5-е сут, увеличение ×200)

Fig. 4. RBT cell culture not inoculated with BRSV (day 5, ×200 magnification)

day 4 of virus cultivation (Fig. 7). Control test results with no virus inoculation are shown in Figure 8.

Further studies showed more pronounced CPE caused by BRSV strain Vologda/2019. By a third passage 80% monolayer destruction and formation of conglomerates and syncytia were observed on day 7 of cultivation in FBT cell culture (Fig. 9). When a similar pattern was observed, the virus containing-material was frozen at minus 80 °C and placed for storage. Control test results with no virus inoculation are shown in Figure 10.

The virus CPE was not observed in MA-104 and FBN cell cultures; the virus titers measured by ELISA confirmed that BRSV strain Vologda/2019 is not replicated in these cell systems (titer decline in percentage equivalence). At passages 4 and 5 no changes were observed in the cell monolayer of MA-104 and FBN cultures.

For YaDK-04 cell culture, the viral CPE was manifested by occasional cell rounding and monolayer thinning. The microtitration test showed that the virus consistently replicates in this cell system, but the levels of virus accumulation differ from those in RBT and FBT cell cultures. This fact indicates the need to optimize the cultivation parameters to achieve the best result.

BRSV strain Vologda/2019 titers were measured for each passage in BT cell culture by microtitration method, and the specificity of the obtained BRSV preparations was confirmed using ELISA (Table 1).

The studies showed that during BRSV strain Vologda/2019 cultivation in FBT cell culture typical CPE was observed on days 4–5 after passages 3–5. By passage 5 the viral CPE level gradually amounted to 5.50 ± 0.16 lg $TCID_{50}/cm^3$. BRSV titer was 1:2 at passage 1 and 1:64 at passage 5 in ELISA. A similar trend was observed during BRSV cultivation in RBT cell culture: on day 4–5 of cultivation (passage 5) the virus titer was 4.75 ± 0.20 lg $TCID_{50}/cm^3$, and it increased to 1:32 in ELISA.

Inoculation of BRSV strain Vologda/2019 in MA-104 and FBN cell systems led to a decrease in its activity to 1.50 ± 0.17 lg $TCID_{50}/cm^3$, and the virus was not detected at passages 4–5. A similar decrease in the virus titer was confirmed by ELISA.

During BRSV strain Vologda/2019 cultivation in YaDK-04 cell culture stable virus activity was noted throughout all 5 passages (2.50 ± 0.17 – 3.33 ± 0.18 lg $TCID_{50}/cm^3$). It is possible that by optimizing the cultivation parameters in YaDK-04 cell system, a higher cytopathic effect of the virus

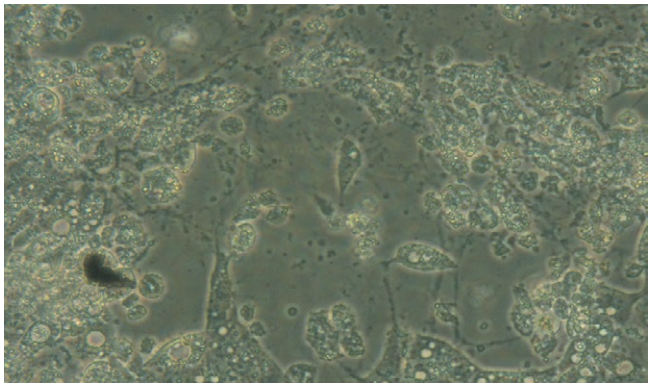

Fig. 5. BRSV-inoculated RBT cell culture (day 7, ×200 magnification)

Рис. 5. Культура клеток RBT, инокулированная вирусом РСИ КРС (7-е сут, увеличение ×200)

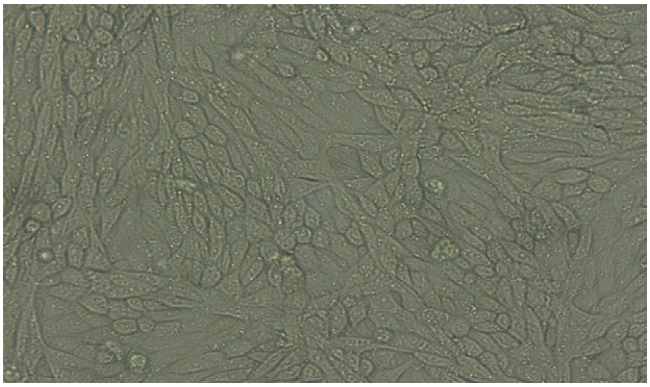

Fig. 6. Non-BRSV-inoculated RBT cell culture (day 7, ×400 magnification)

Рис. 6. Культура клеток RBT без инокуляции вируса РСИ КРС (7-е сут, увеличение ×400)

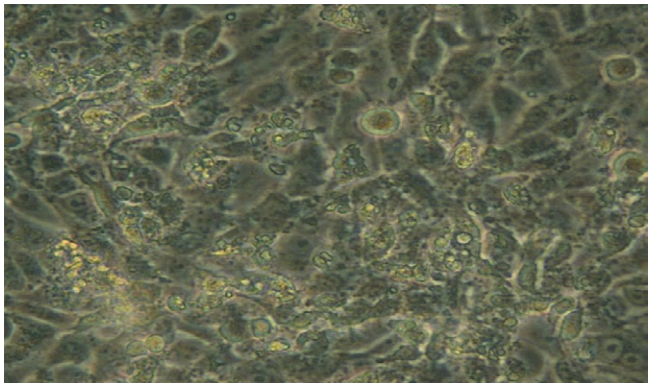

Fig. 7. BRSV-inoculated FBT cell culture (day 4, ×400 magnification)

Рис. 7. Культура клеток FBT, инокулированная вирусом РСИ КРС (4-е сут, увеличение ×400)

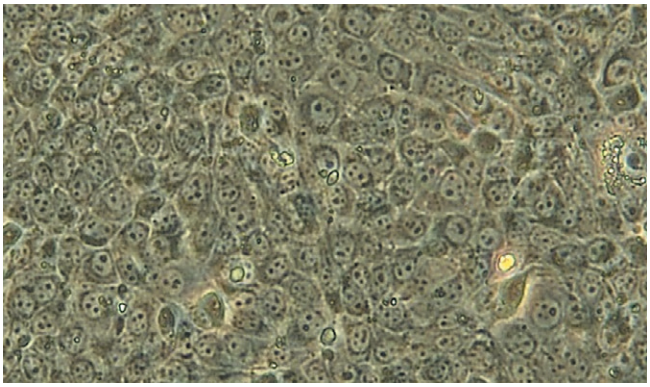

Fig. 8. Non-BRSV-inoculated FBT cell culture (day 4, ×400 magnification)

Рис. 8. Культура клеток FBT без инокуляции вируса РСИ КРС (4-е сут, увеличение ×400)

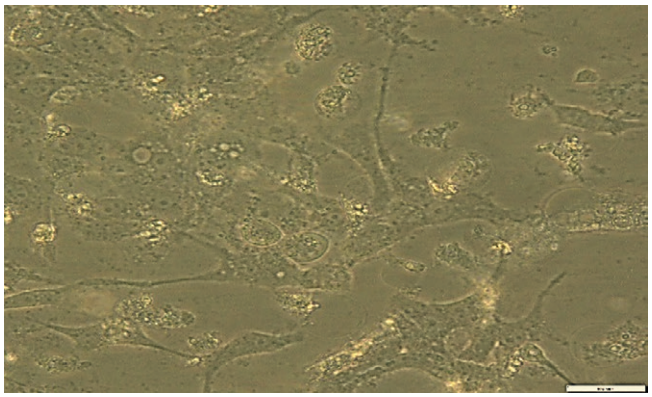

Fig. 9. BRSV-inoculated FBT cell culture (day 7, ×400 magnification)

Рис. 9. Культура клеток FBT, инокулированная вирусом РСИ КРС (7-е сут, увеличение ×400)

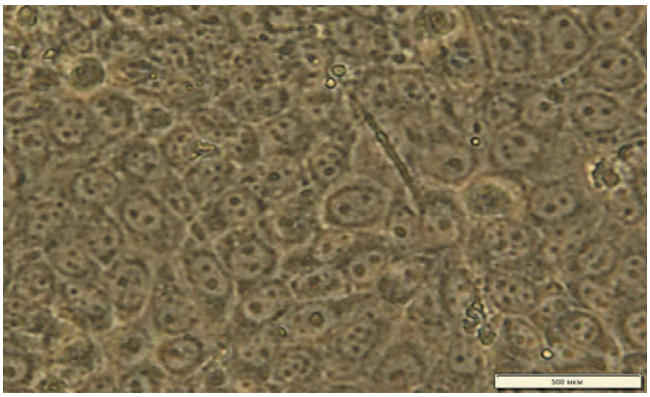

Fig. 10. Non-BRSV-inoculated FBT cell culture (day 7, ×400 magnification)

Рис. 10. Культура клеток FBT без инокуляции вируса РСИ КРС (7-е сут, увеличение ×400)

can be obtained. The virus titer for this viral raw material in ELISA was 1:2 at the 1st passage and 1:4 at the subsequent ones, which is considered a stable result, but it is insufficient for the production of a high-quality virus-containing preparation.

The tests showed that RBT and FBT cell cultures are the most suitable cell systems for BRSV strain Vologda/2019 propagation. MA-104 and FBN cell systems turned out to be unsuitable for BRSV strain Vologda/2019 accumulation and reproduction.

Table 1
Titers of BRSV strain Vologda/2019 in various cell cultures ($n = 3$)

Таблица 1
Титр вируса РСИ КРС штамма «Вологда/2019» в различных культурах клеток ($n = 3$)

Cell culture	Passage No.	Cultivation period, days	Virus titer, lg $TCID_{50}/cm^3$	Virus titer by ELISA, dilution
RBT	1	8–10	3.0 ± 0.25	1:2
	2	8–10	3.63 ± 0.07	1:4
	3	7–10	3.56 ± 0.06	1:4
	4	4–5	4.0 ± 0.23	1:16
	5	4–5	4.75 ± 0.20	1:32
FBT	1	8–10	3.63 ± 0.15	1:2
	2	8–10	4.0 ± 0.22	1:4
	3	7–10	4.33 ± 0.23	1:4
	4	4–5	4.78 ± 0.18	1:32
	5	4–5	5.5 ± 0.16	1:64
MA-104	1	10	1.83 ± 0.16	1:2
	2	10	1.67 ± 0.16	1:2
	3	10	1.50 ± 0.17	1:2
	4	10	n/d	–
	5	10	n/d	–
FBN	1	10	2.23 ± 0.23	1:2
	2	10	1.67 ± 0.10	1:2
	3	10	1.50 ± 0.17	1:2
	4	10	n/d	–
	5	10	n/d	–
YaDK-04	1	9	2.50 ± 0.17	1:2
	2	10	3.25 ± 0.25	1:4
	3	9	3.12 ± 0.18	1:4
	4	8	3.16 ± 0.17	1:4
	5	9	3.33 ± 0.18	1:4

n/d – not detected (не обнаружено);
«–» – negative result (отрицательный результат).

In order to increase the virus titer it was necessary to select the optimal dose for cell culture infection. Highly efficient RBT and FBT cell systems were chosen. To inoculate BRSV strain Vologda/2019 strain into the selected cell cultures, the virus-containing material was diluted to 0.001–0.1 $TCID_{50}$/cell (Fig. 11).

As it is demonstrated in Figure 11, at multiplicity of infection 0.001 $TCID_{50}$/cell, the CPE levels of BRSV strain Vologda/2019 in FBT and RBT cell cultures were 2.50 and 2.75 lg $TCID_{50}/cm^3$, respectively. At multiplicity of infection 0.01 $TCID_{50}$/cell the virus CPE in FBT and RBT cell systems was 4.00 and 3.25 lg $TCID_{50}/cm^3$, respectively. Also, slow virus accumulation was noted in RBT and FBT cell cultures at a given infection dose; the cultivation time to achieve 80% monolayer destruction induced by BRSV was 9 and 12 days, respectively. Given the resistance of cell systems to aging

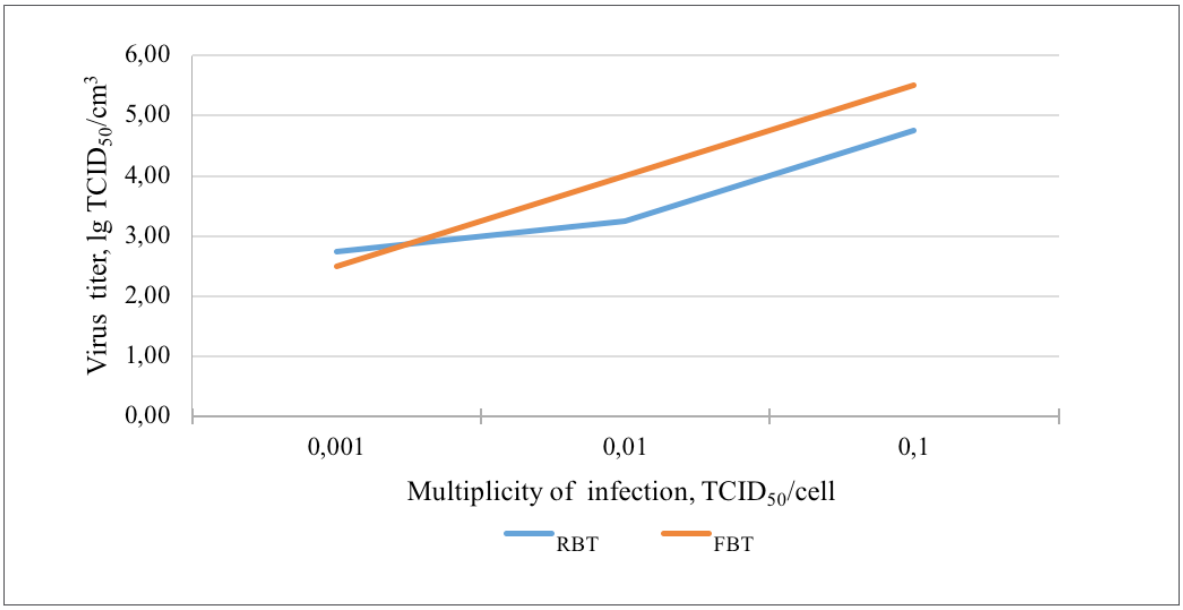

Fig. 11. Effects of inoculation multiplicity on the BRSV strain Vologda/2019 activity (n = 3)

Рис. 11. Влияние множественности заражения на активность вируса РСИ КРС штамма «Вологда/2019» (n = 3)

and death (18 days before the onset of cell degradation for FBT and 16 days for RBT), this result can be considered acceptable. However, the study results showed that with an increase in the multiplicity of infection to 0.1 $TCID_{50}/cm^3$, 80% monolayer destruction and virus accumulation in RBT and FBT cell cultures proceeded faster (4 and 5 days, respectively), while the CPE titer of BRSV strain Vologda/2019 was 4.75 ± 0.16 and 5.50 ± 0.80 lg $TCID_{50}/cm^3$, respectively. Therefore, this level of multiplicity of infection is optimal.

In order to optimize the composition of the semi synthetic nutrient medium used as a maintenance one for inoculation of BRSV strain Vologda/2019, it was decided to assess the effect of supplementation with glutamine at different concentrations of the original tested substance that had been previously shown by T. Yu. Kochish [1] (Table 2).

According to Table 2, the glutamine supplementation of the nutrient medium resulted in gradual increase of BRSV strain Vologda/2019 CPE. When 0.1 and 0.5% glutamine were added to the nutrient medium, the virus titer in RBT cell culture increased from 3.00 ± 0.25 to 3.56 ± 0.06 lg $TCID_{50}/cm^3$, which is a relatively low infectivity level for this virus. When a medium containing 0.1 and 0.5% glutamine was added at passage 3 in FBT cell culture, it was possible to obtain a virus with the infectivity titer 3.63 ± 0.15 and 4.00 ± 0.12 lg $TCID_{50}/cm^3$, respectively. It was found that the most optimal concentration of glutamine in SSM is 2%, where it was possible to achieve the maximal infectivity titer of BRSV by passage 3: 4.75 ± 0.20 lg $TCID_{50}/cm^3$ (RBT) and 5.50 ± 0.08 lg $TCID_{50}/cm^3$ (FBT). A further increase in the concentration of glutamine

Table 2
BRSV strain Vologda/2019 activity in case of glutamine-enriched nutrient medium (*n* = 3)

Таблица 2
Активность вируса РСИ КРС штамма «Вологда/2019» при обогащении питательной среды глютамином (*n* = 3)

Glutamine amount in maintenance medium, %	Virus cytopathic effect, lg $TCID_{50}/cm^3$					
	RBT			FBT		
	Passage 1	Passage 2	Passage 3	Passage 1	Passage 2	Passage 3
0.1	3.00 ± 0.25	3.25 ± 0.25	3.37 ± 0.20	3.12 ± 0.25	3.33 ± 0.22	3.63 ± 0.15
0.5	3.00 ± 0.25	3.37 ± 0.20	3.56 ± 0.06	3.33 ± 0.22	3.63 ± 0.17	4.00 ± 0.12
1	3.56 ± 0.06	3.56 ± 0.17	4.00 ± 0.23	3.63 ± 0.16	4.00 ± 0.22	4.00 ± 0.25
2	4.00 ± 0.23	4.30 ± 0.23	4.75 ± 0.20	4.33 ± 0.23	4.78 ± 0.18	5.50 ± 0.08
3	3.56 ± 0.17	4.00 ± 0.23	3.56 ± 0.06	3.83 ± 0.20	3.83 ± 0.20	3.73 ± 0.12
No-glutamine medium	3.37 ± 0.20	3.00 ± 0.25	3.56 ± 0.06	3.33 ± 0.27	3.63 ± 0.12	4.00 ± 0.17

Table 3
Effect of blood sera concentration in the nutrient medium on BRSV strain Vologda/2019 activity (*n* = 5)

Таблица 3
Влияние концентрации сыворотки крови в питательной среде на активность вируса РСИ КРС штамма «Вологда/2019» (*n* = 5)

Sera concentration in nutrient medium, %	Virus cytopathic effect, lg $TCID_{50}/cm^3$			
	RBT		FBT	
	bovine sera	horse sera	bovine sera	horse sera
0.1	3.25 ± 0.25	3.50 ± 0.15	2.75 ± 0.25	3.63 ± 0.15
0.5	3.37 ± 0.20	3.00 ± 0.25	3.25 ± 0.08	4.00 ± 0.12
1	4.30 ± 0.23	4.00 ± 0.23	3.56 ± 0.19	4.00 ± 0.25
2	4.33 ± 0.12	4.50 ± 0.23	4.75 ± 0.25	5.50 ± 0.16
3	4.00 ± 0.23	3.56 ± 0.17	4.00 ± 0.23	3.73 ± 0.12
Non-sera supplemented medium	3.00 ± 0.25	3.37 ± 0.20	3.50 ± 0.33	4.00 ± 0.17

in the semi synthetic culture medium led to a decrease in the cytopathic effect of the virus in the considered cell systems. When using the medium not supplemented with glutamine in the control samples, the virus titer was at the level from 3.00 ± 0.25 to 4.00 ± 0.17 lg $TCID_{50}/cm^3$.

Based on the data obtained, it can be concluded that 2% glutamine supplementation of the nutrient medium results in increase in the infectivity titer of the virus, and also reduces the time of the virus's maximal accumulation in FBT and RBT cell cultures.

The next stage of the experiment was to study the effect of horse and bovine sera-containing SSM on the virus reproduction.

As it is known, bovine serum is often contaminated with various viral agents, including bovine viral diarrhea virus, which can impact virus accumulation in the cell culture and reduce the cytopathic effect of the virus under study [14]. The use of horse serum could exclude contamination of the viral suspension with this pestivirus and thereby allow obtaining the virus with high biological activity.

Five consecutive passages were performed in RBT and FBT cell cultures to study impact of the tested animal sera concentration on the virus infectivity. The data are presented in Table 3.

Table 3 shows that when SSM was supplemented with 0.1 and 0.5% bovine sera, the virus infectivity levels in RBT cell culture were 3.25 ± 0.25 and 3.37 ± 0.20 lg $TCID_{50}/cm^3$, respectively, and in FBT cell culture – 2.75 ± 0.25 and 3.25 ± 0.08 lg $TCID_{50}/cm^3$, respectively. Gradual increase of bovine serum concentration from 1 to 2% in the nutrient medium resulted in increase in the infectivity titer both in RBT (4.33 ± 0.12 lg $TCID_{50}/cm^3$) and in FBT (4.75 ± 0.25 lg $TCID_{50}/cm^3$). Further increase in the concentration of bovine blood serum caused loss of infectivity in both cultures, which may be associated with the virus lability and its high sensitivity to the nutrient medium components.

When studying the effect of horse serum concentration in a nutrient medium for BRSV strain Vologda/2019 propagation, it was found that the optimal dose was 2%. When this amount of serum was added to the medium, the virus titer reached 4.50 ± 0.23 lg $TCID_{50}/cm^3$ in RBT and 5.50 ± 0.16 lg $TCID_{50}/cm^3$ in FBT, which demonstrated the best result in this study. Addition of a small (0.1%) or large (3%) dose of horse serum to SSM caused decrease in BRSV strain Vologda/2019 infectivity titer.

Thus, we can conclude that the addition of horse or bovine sera has a beneficial effect on replication of BRSV strain Vologda/2019. The optimal concentration of bovine or horse sera in the nutrient medium is 2%. Maximal virus CPE was achieved by adding the indicated amount of serum.

CONCLUSION

The study of sensitivity of continuous cell cultures to BRSV strain Vologda/2019 showed that RBT and FBT cell cultures are effective for obtaining a highly potent virus suspension. These cell culture lines can be used to obtain virus material in order to develop tools for diagnosis and specific prevention of this disease.

The optimal cell culture infection dose is 0.1 $TCID_{50}/cm^3$, thereat the virus titers were 4.75 ± 0.16 lg $TCID_{50}/cm^3$ and 5.50 ± 0.80 lg $TCID_{50}/cm^3$ for RBT and FBT, respectively.

The enrichment of the nutrient medium with 2% glutamine increased the titer of the virus cultivated in these cell systems.

The addition of horse or bovine sera has a positive impact on BRSV strain Vologda/2019 CPE. It has been established that the optimal concentration of serum in the nutrient medium is 2%. BRSV strain Vologda/2019 CPE reached maximal when this amount of serum was added.

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