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OPTIMIZATION AND VISUAL MONITORING OF SERUM-FREE CULTIVATION OF THE GFP-PRODUCING SHEEPPOX VIRUS STRAIN "SSPV (TK-) OMP16" IN VERO CELLS

This study optimized cultivation conditions for a GFP-producing sheeppox virus strain used as a model analogue of the recombinant strain "SSPV (TK-) OMP16". The virus was propagated in continuous Vero cell culture using serum-free OptiPRO medium supplemented with 1% L-glutamine. Cell monolayers were infected with virus doses of 0.01, 0.05, 0.1, and 0.3 $TCID_{50}$/cell and incubated at 37 °C for 120 h. Biological activity was assessed in the culture fluid and in virus-containing suspensions obtained after freeze–thaw treatment of the infected monolayer. Virus accumulation in the culture fluid was limited, whereas freeze–thaw treatment promoted more efficient release of infectious virus and clearer detection of cytopathic effect. The highest titer, 3.5 log $TCID_{50}/cm^3$, was recorded at 0.3 $TCID_{50}$/cell after 72 h of cultivation. These findings confirm the usefulness of GFP for visual monitoring and optimization of serum-free recombinant sheeppox virus cultivation in Vero cells and future veterinary vaccine platform quality control.

Keywords: recombinant sheeppox virus, GFP-producing strain, SSPV (TK-) OMP16, Vero cell culture, biological activity.

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GFP-продукциялаушы штаммды қолдану арқылы «SSPV (TK-) OMP16» рекомбинантты қой шешегі вирусы штамының репликациясын визуалды бақылау

Бұл зерттеуде «SSPV (TK-) OMP16» рекомбинантты штамының модельдік аналогы ретінде қолданылған GFP-продукциялаушы қой шешегі вирусы штамын өсіру жағдайлары оңтайландырылды. Вирус 1% L-глютамин қосылған сарысусыз OptiPRO қоректік ортасын пайдалана отырып, Vero жасушаларының үздіксіз культурасында өсірілді. Жасуша моноқабаты 0,01; 0,05; 0,1 және 0,3 $ТЦД_{50}$/жасуша дозаларымен жұқтырылып, кейін 37 °С температурада 120 сағат бойы инкубацияланды. Биологиялық белсенділік культуралық сұйықтықта және жұқтырылған моноқабатты мұздату-еріту әдісінен кейін алынған вирусқұрамды суспензияда анықталды. Зерттеу нәтижесінде вирус жинақталуы культуралық сұйықтықта шектеулі болғаны, ал мұздату-еріту әдісі инфекциялық вирустың тиімді бөлінуін және цитопатикалық әсердің айқын анықталуын қамтамасыз еткені көрсетілді. Ең жоғары титр 0,3 $ТЦД_{50}$/жасуша дозасында, 72 сағат культивирлеуден кейін 3,5 $lgТЦД_{50}/см^3$ деңгейінде тіркелді. Алынған деректер GFP-маркерінің репликацияны визуалды бақылау, жұқтыру дозасын таңдау және Vero жасушаларында рекомбинантты қой шешегі вирусын сарысусыз өсіру технологиясын жетілдіру үшін тиімділігін дәлелдейді. Жұмыс ветеринариялық мақсаттағы рекомбинантты вакциналық платформаларды әзірлеу, өндірістік үдерістерді стандарттау және вирус биомассасының сапасын бақылау үшін келешек зерттеулерде маңызды практикалық маңызға ие болуы мүмкін.

Түйін сөздер: рекомбинантты қой шешегі вирусы, GFP-продукциялаушы штамм, SSPV (TK-) OMP16, Vero жасуша культурасы, биологиялық белсенділік.

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Оптимизация и визуальный контроль безсывороточного культивирования рекомбинантного штамма «SSPV (TK-) OMP16» вируса оспы овец с использованием GFP-продуцирующего штамма

В настоящем исследовании проведена оптимизация условий культивирования GFP-продуцирующего штамма вируса оспы овец, используемого как модельный аналог рекомбинантного штамма «SSPV (TK-) OMP16». Вирус культивировали в перевиваемой культуре клеток Vero с применением бессывороточной среды OptiPRO, дополненной 1% L-глютамином. Монослой клеток инфицировали различными дозами вируса: 0,01; 0,05; 0,1 и 0,3 $ТЦД_{50}$/кл, с последующим культивированием при 37 °С в течение 120 часов. Биологическую активность определяли в культуральной жидкости и вируссодержащей суспензии после замораживания-оттаивания инфицированного монослоя. Установлено, что накопление вируса в культуральной жидкости было ограниченным, тогда как метод замораживания-оттаивания обеспечивал более эффективное высвобождение инфекционного вируса и выявление цитопатического действия. Наиболее высокий титр, 3,5 $lgТЦД_{50}/см^3$, зарегистрирован при дозе 0,3 $ТЦД_{50}$/кл через 72 часа культивирования. Полученные данные подтверждают эффективность GFP-маркера для визуального контроля репликации, выбора оптимальной дозы инфицирования и совершенствования технологии накопления рекомбинантного вируса оспы овец в клетках Vero при бессывороточных условиях. Работа имеет практическое значение для дальнейшей разработки рекомбинантных вакцинных платформ ветеринарного назначения и контроля качества.

Ключевые слова: SSPV (TK-) OMP16, GFP-продуцирующий штамм, вирус оспы овец, клетки Vero, биологическая активность.

Introduction

Sheeppox is a highly contagious viral disease of small ruminants with significant veterinary and economic importance. The causative agent of the disease is sheeppox virus (SPPV), which belongs to the genus Capripoxvirus of the family Poxviridae. The disease is characterized by fever, lesions of the skin and mucous membranes, and the formation of papules and nodules. In severe cases, it may be accompanied by lesions of internal organs and animal mortality. Sheep pox and goat pox are considered transboundary infections and remain relevant in countries of Asia, the Middle East, Africa, and certain regions of Europe (Babiuk et al., 2008; Tuppurainen et al., 2017; World Organisation for Animal Health, 2024).

Due to the high epizootiological significance of capripoxvirus infections, special attention is given to the development of effective specific prophylactic measures and improvement of technologies for obtaining viral biomass. Representatives of the genus Capripoxvirus are considered not only as pathogens of animal diseases but also as promising viral vectors for the development of recombinant vaccines. Their restricted host range and ability to express foreign antigens make capripoxviruses a convenient platform for the construction of veterinary vector vaccines (Boshra et al., 2013; Teffera, 2019). Previous studies have also demonstrated the practical relevance of capripoxvirus-based vaccines for veterinary prophylaxis and protection against related capripoxvirus infections (Hamdi et al., 2020; Zhugunissov et al., 2020).

Recombinant sheeppox virus strains can be used for the expression of foreign antigens and the development of vaccine candidates with defined immunobiological properties. It has previously been shown that the sheeppox virus genome can be modified using genomic regions, including the thymidine kinase gene, for the insertion of foreign genes without substantially impairing viral replication in vitro (Chervyakova et al., 2021). This provides a basis for the development of recombinant strains that can simultaneously serve as models for studying viral replication and as platforms for producing vaccine preparations.

One of the promising antigens for inclusion in recombinant vaccines is the outer membrane protein Omp16 of Brucella spp. This protein is considered an immunologically significant component capable of inducing specific cellular and humoral immune

responses. Experimental studies have shown that recombinant Omp16 and Omp19 proteins induce protective immune responses against Brucella abortus, confirming the potential use of Omp16 as a target antigen in the development of vaccine constructs (Huy et al., 2021; Pasquevich et al., 2009).

Model strains containing reporter genes are of great importance for optimizing the cultivation technology of recombinant viruses. One of the most widely used reporter markers is green fluorescent protein (GFP). Its expression allows visual detection of infected cells and monitoring of viral replication processes in cell culture. The advantage of GFP lies in the possibility of direct fluorescence observation without the use of additional substrates, making this marker a convenient tool for assessing the dynamics of viral reproduction (Chalfie et al., 1994; Soboleski et al., 2005).

Virological studies and technological accumulation of viral biomass largely depend on the choice of the cell system. Vero cell culture is widely used for the production of viral vaccines and viral vectors due to its stable growth, high susceptibility to various viruses, and suitability for scaling up biotechnological processes (Kiesslich & Kamen, 2020). At the same time, the use of serum-free media is also important, as they reduce the risk of contamination with animal-derived components, increase the reproducibility of cultivation, and simplify subsequent standardization of the production process. In particular, Vero cells can be successfully cultivated in OptiPRO medium, which is used for cell growth and viral production (Lee et al., 2020).

In the present study, a GFP-producing strain was used to optimize the accumulation technology of the recombinant sheeppox virus strain "SSPV (TK-) OMP16". This strain was applied as a model analogue of the recombinant virus, allowing visual monitoring of viral replication and viral biomass accumulation in Vero cell culture. The aim of this study was to optimize the parameters for the accumulation of viral biomass of the GFP-producing sheeppox virus strain in Vero cell culture using serum-free OptiPRO medium.

Materials and methods

Virus. The study used a sheeppox virus strain producing the green fluorescent protein GFP. The strain was cultivated in a continuous Vero cell line using the serum-free OptiPRO medium. The biological activity of the strain was 4.0 log $TCID_{50}/cm^3$. The strain was obtained from the microorganism collection of the Research Institute for Biological Safety Problems.

Culture media and supplements. Serum-free OptiPRO medium supplemented with 1% L-glutamine was used as both the growth and maintenance medium for the continuous Vero cell line derived from African green monkey kidney epithelial cells.

Virus cultivation. Infection of the monolayer of the continuous Vero cell line in plastic cell culture flasks with surface areas of 150 and 300 cm^2 (TPP) was performed by adding 0.5 cm^3 of viral suspension. The infected cells were incubated at 37-38°C for 60 min, after which the inoculum was removed. The monolayer was washed several times with culture medium, maintenance OptiPRO medium was added, and the cells were further cultivated at 37-38°C for 4-5 days. During this period, the cell monolayer was examined daily by microscopy. The cytopathic effect of sheeppox virus on the cell monolayer was characterized by cell rounding, formation of grape-like cell clusters, followed by disruption of intercellular contacts and complete destruction of the cell monolayer.

Multiplicity of infection and cultivation period. Virus accumulation in Vero cell culture at different doses of viral suspension was assessed by infecting the cell monolayer at doses of 0.01, 0.05, 0.1, and 0.3 $TCID_{50}$/cell, followed by incubation at 37°C. At 12 h post-infection and subsequently every 6 h, four cell culture flasks were collected from each group over a total cultivation period of 120 h. The culture fluid was removed from the selected flasks, and the infected monolayer was covered with phosphate-buffered saline and frozen at −20°C. After 24 h, the flasks were thawed, and biological activity was determined in both the culture fluid and the thawed virus-containing suspension by titration on a Vero cell monolayer.

Determination of the biological activity of the viral strain. The virus titer was determined by the method of serial tenfold dilutions on a Vero cell monolayer in 96-well microplates. The experiments were performed in four replicates. The virus titer was calculated using the Reed–Muench method (Reed & Muench, 1938).

Results and their discussion

Multiplicity of infection

The multiplicity of infection and the optimal cultivation period required for the accumulation of viral biomass with high infectious activity were determined using different infection doses of the studied

virus and cultivation periods ranging from 12 to 120 h at 37°C. For this purpose, Vero cell monolayers were infected with the virus at doses of 0.01, 0.05, 0.1, and 0.3 $TCID_{50}$/cell and incubated at 37°C for 120 h, with replacement of the serum-free OptiPRO medium after 2-3 days.

At the beginning of the experiment, 12 h after infection, and subsequently every 6 h, cell culture flasks were collected from each group. The culture fluid was removed, the monolayer was covered with phosphate-buffered saline, and the flasks were frozen at -20°C. After thawing, biological activity (BA) was determined in the collected culture fluid (CF) and in the thawed cell suspension in phosphate-buffered saline (PBS) by titration in Vero cell culture. The results of the study are presented in Table 1.

Table 1

Multiplicity of infection and optimal cultivation time for the GFP-producing sheeppox virus strain

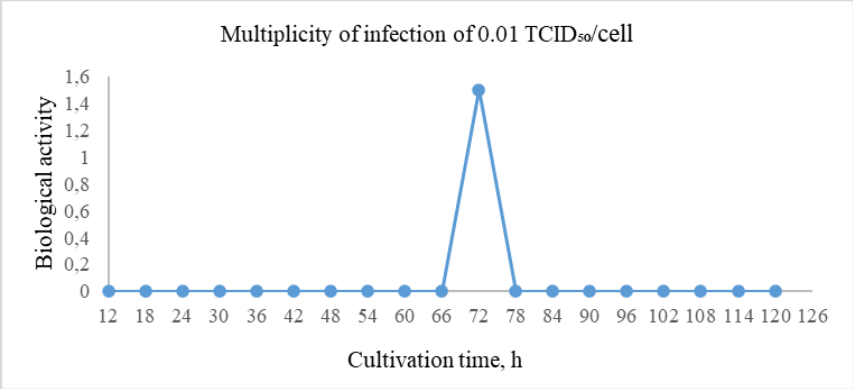
Cultivation time, h	Biological activity, log $TCID_{50}/cm^3$							
	Culture fluid (CF)				Phosphate-buffered saline (PBS)			
	Infection dose, $TCID_{50}$/cell							
	0.01 $TCID_{50}$/cell	0.05 $TCID_{50}$/cell	0.1 $TCID_{50}$/cell	0.3 $TCID_{50}$/cell	0.01 $TCID_{50}$/cell	0.05 $TCID_{50}$/cell	0.1 $TCID_{50}$/cell	0.3 $TCID_{50}$/cell
12	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	1.5
30	0	0	0	0	0	0	1.5	1.5
36	0	0	0	0	0	0	0	1.5
42	0	0	0	0	0	0	1.5	1.5
48	0	0	0	0	0	0	1.5	1.5
54	0	0	0	1.5	0	0	0	1.5
60	0	0	0	0	0	0	1.5	0
66	0	0	0	0	0	1.5	0	2.5
72	0	0	0	0	1.5	0	2.5	3.5
78	0	0	0	0	0	0	2.5	1.5
84	0	0	0	0	0	0	1.5	0
90	0	0	0	0	0	0	1.5	1.5
96	0	0	0	0	0	0	2.5	2.5
102	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0

Analysis of the dynamics of biological activity (log $TCID_{50}/cm^3$) of the GFP strain of sheeppox virus, depending on the cultivation period (hours) on a Vero cell monolayer and the infecting virus dose, revealed substantial differences between the studied systems: culture fluid (CF) and infected cells in PBS after thawing.

It was found that viral reproduction in the culture fluid was characterized by limited intensity and a delayed increase in infectious activity. Detectable biological activity of the strain was recorded after 54 h of cultivation. It should be noted that, after 54 h of cultivation, biological activity of 1.5 log $TCID_{50}/cm^3$ was detected only at the infecting virus dose of 0.3 $TCID_{50}$/cell. Other time intervals did not support effective viral reproduction in this system.

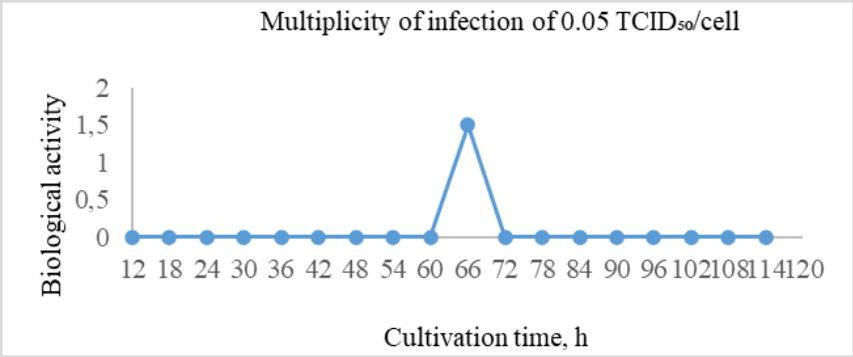
The accumulation dynamics of the fluorescent sheeppox virus strain depending on the infection dose are presented in Figures 1-4.

Figure 1
Accumulation of the fluorescent sheeppox virus strain in Vero cell culture at a multiplicity of infection of 0.01 $TCID_{50}$/cell



The data presented in the diagram show that, at a multiplicity of infection of 0.01 $TCID_{50}$/cell, the biological activity of the virus reached 1.5 log $TCID_{50}$/cm³ at 72 h of cultivation. Further cultivation up to 120 h inhibited the replication of the GFP strain of sheeppox virus in the Vero cell monolayer.

Figure 2
Accumulation of the GFP strain of sheeppox virus in Vero cell culture at a multiplicity of infection of 0.05 $TCID_{50}$/cell



Similar to the previous case, virus accumulation was observed after 66 h of cultivation, followed by a subsequent decrease in its biological activity.

Analysis of the diagram shows that, at a multiplicity of infection of 0.1 $TCID_{50}$/cell, the biological activity of the virus reached 1.5 log $TCID_{50}$/cm³ at 30, 42, 48, and 60 h of cultivation. Further cultivation up to 72 and 78 h increased the biological activity of the virus to 2.5 log $TCID_{50}$/cm³, followed by a subsequent decrease in infectious activity to 1.5 log $TCID_{50}$/cm³. After 96 h of cultivation, the infectivity of the virus increased again and reached 2.5 log $TCID_{50}$/cm³.

The results presented in the diagram show that viral biological activity was detected from 24 to 54 h of cultivation at a level of 1.5 log $TCID_{50}$/cm³. An increase in viral infectivity to 2.5 log $TCID_{50}$/cm³ was observed at 66 and 96 h. The highest biological activity of the GFP strain of sheeppox virus, 3.5 log $TCID_{50}$/cm³, was recorded after 72 h of cultivation at a multiplicity of infection of 0.3 $TCID_{50}$/cell.

Figure 3
Accumulation of the GFP strain of sheeppox virus in Vero cell culture at a multiplicity of infection of 0.1 $TCID_{50}$/cell

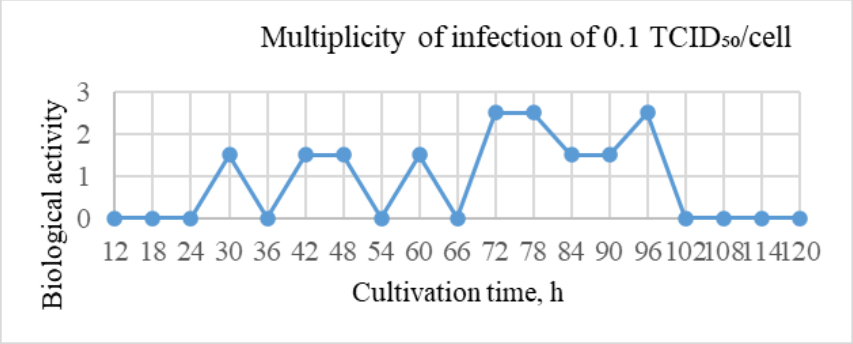
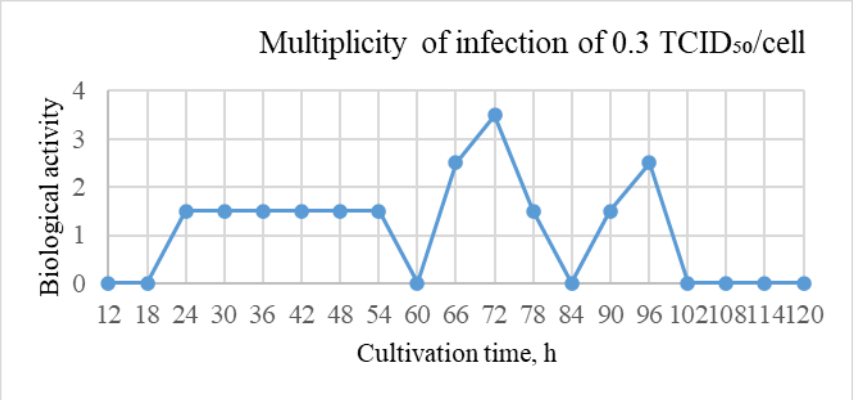


Figure 4.
Accumulation of the GFP strain of sheeppox virus in Vero cell culture at a multiplicity of infection of 0.3 $TCID_{50}$/cell



Biological activity of the virus

After the required incubation period at 37°C, the culture fluid was removed from the flasks, and the biological activity of the virus was determined. Viral reproduction in the culture fluid is shown in Figure 5.

In contrast, when phosphate-buffered saline was added to the cell monolayer followed by freezing and thawing after 24 h, an earlier onset of viral replication was observed, with infectious activity detected as early as 24 h of cultivation. The subsequent dynamics were characterized by a steady increase in biological activity, reaching maximum values of 2.5-3.5 log $TCID_{50}/cm^3$ within 66-96 h, predominantly at infection doses of 0.1-0.3 $TCID_{50}$/cell.

The cytopathic effect of the fluorescent sheeppox virus strain in Vero cell culture during determination of biological activity (BA) at different cultivation times and virus doses is presented in Figures 6–8.

Figure 5
Cytopathic effect of the GFP strain of sheeppox virus on Vero cells during determination of biological activity in the culture fluid: multiplicity of infection 0.3 $TCID_{50}$/cell, virus dilution 10^{-1}, cultivation time 54 h.

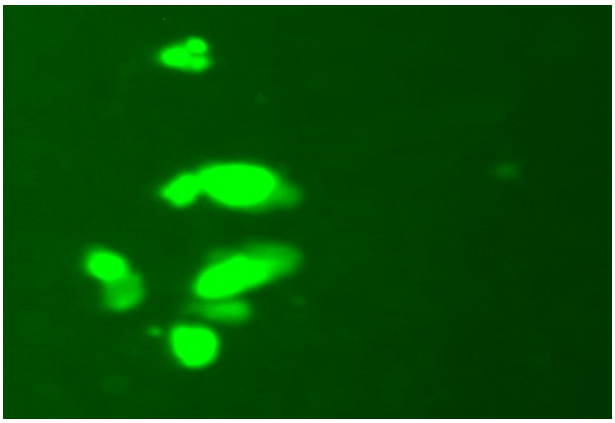
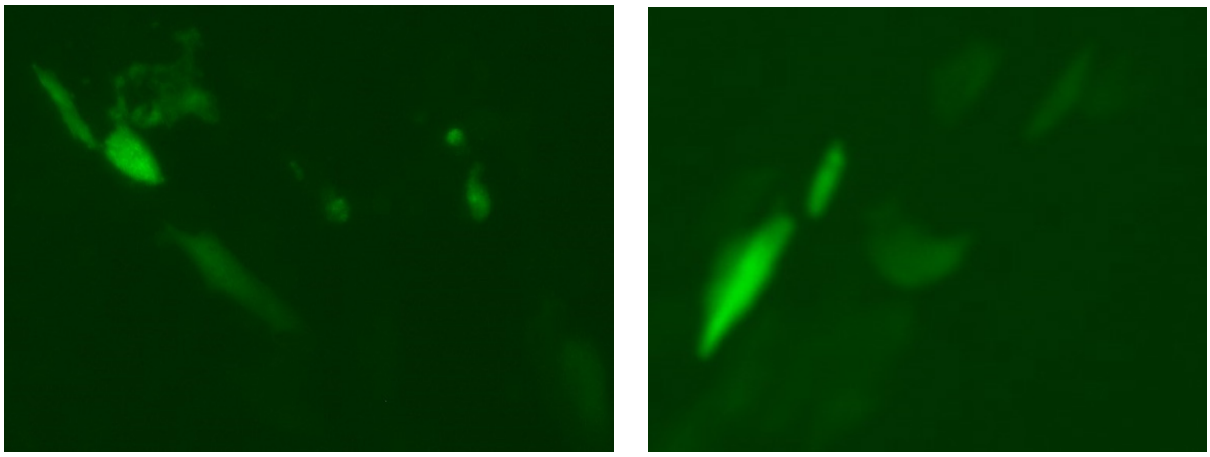


Figure 6
Cytopathic effect of the fluorescent sheeppox virus strain on Vero cells during determination of biological activity in thawed cells: (a) multiplicity of infection 0.01 $TCID_{50}$/cell, cultivation time 72 h; (b) multiplicity of infection 0.05 $TCID_{50}$/cell, cultivation time 66 h

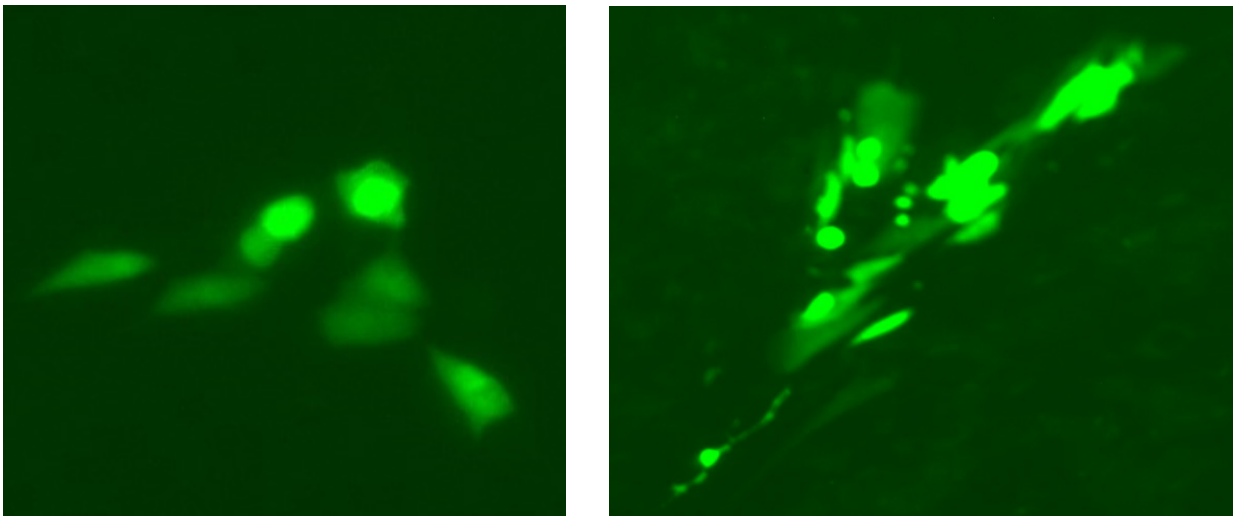


a **b**

At low infection doses, namely 0.01 and 0.05 $TCID_{50}$/cell, the cytopathic effect of the virus was manifested by weak fluorescence in the Vero cell monolayer. It should also be noted that the infectious activity of the virus at a dose of 0.01 $TCID_{50}$/cell developed more slowly and became detectable on the third day of cultivation, whereas the virus at a dose of 0.05 $TCID_{50}$/cell infected the cells 6 h earlier than the lower dose.

As shown in Figures 7 and 8, after the freeze–thaw process in phosphate-buffered saline, the cytopathic effect of the GFP strain on Vero cells at 24 and 48 h of cultivation, at an infection dose of 0.1 $TCID_{50}$/cell and a tenfold dilution of the viral suspension, was characterized by multiple fluorescent foci in the cell monolayer. At a higher infection dose of 0.3 $TCID_{50}$/cell, viral CPE was detected at higher dilutions of the viral suspension: at 72 h of cultivation, the cytopathic effect was observed at a dilution of 10^{-3}, whereas at 96 h, it was detected at a dilution of 10^{-2}.

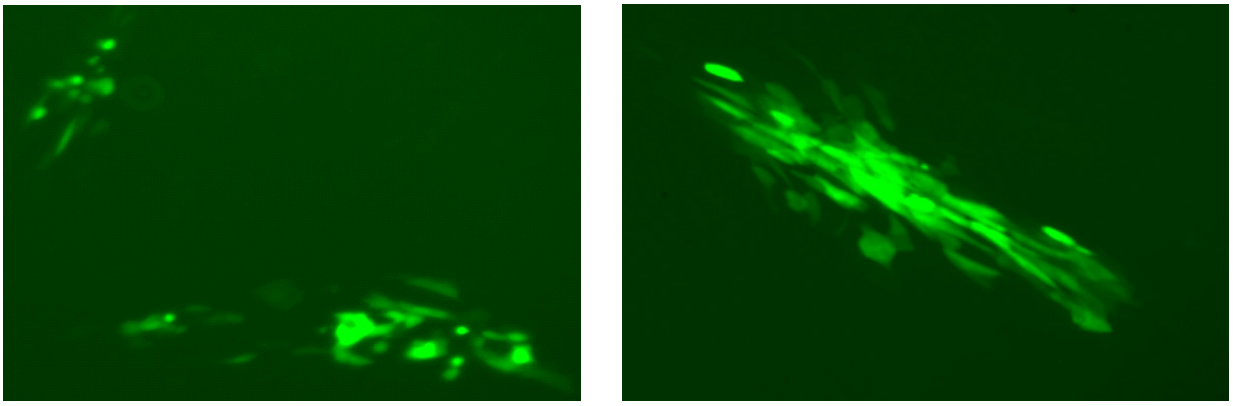
Figure 7
Cytopathic effect of the fluorescent sheeppox virus strain on Vero cells during determination of biological activity in thawed cells: (c) multiplicity of infection 0.1 $TCID_{50}$/cell, virus dilution 10^{-1}, cultivation time 24 h; (d) multiplicity of infection 0.1 $TCID_{50}$/cell, virus dilution 10^{-1}, cultivation time 48 h.



c **d**

Figure 8

Cytopathic effect of the fluorescent sheeppox virus strain on Vero cells during determination of biological activity: (e) multiplicity of infection 0.3 $TCID_{50}$/cell, virus dilution 10^{-3}, cultivation time 72 h; (f) multiplicity of infection 0.3 $TCID_{50}$/cell, virus dilution 10^{-2}, cultivation time 96 h.



e **f**

Thus, the obtained results demonstrate that the biological activity of the virus in the culture fluid collected after a defined period of incubation on the Vero cell monolayer was low and was detected only after 54 h of cultivation. In contrast, when phosphate-buffered saline was used, a clear dose-dependent pattern was observed: an increase in the infection dose was accompanied by enhanced viral replication and earlier achievement of maximum titers. At the minimum dose of 0.01 $TCID_{50}$/cell, virus accumulation was delayed and less pronounced in both systems; however, it remained more intensive after freeze–thaw treatment of the monolayer with phosphate-buffered saline.

This indicates that ice crystals formed during freezing damage the cell membranes. During thawing, the disrupted membrane can no longer retain the cellular contents, allowing viral particles accumulated in the cytoplasm or nucleus to be released. Therefore, the freeze–thaw method applied to the infected monolayer using PBS demonstrated a clear advantage over virus accumulation in the culture fluid and ensured more efficient replication of the pathogen in this system.

The obtained results show that the GFP-producing sheeppox virus strain can be effectively used as a model system for visual monitoring of viral replication and optimization of viral biomass accumulation conditions in Vero cell culture. The use of this approach is justified, since sheeppox virus belongs to the genus Capripoxvirus and is of considerable interest both for veterinary prophylaxis and as a potential platform for the development of recombinant viral vaccines (Babiuk et al., 2008; Boshra et al., 2013; Teffera, 2019; Tuppurainen et al., 2017; World Organisation for Animal Health, 2024). Previous studies have also demonstrated the practical relevance of capripoxvirus-based vaccines and their potential application in veterinary prophylaxis (Hamdi et al., 2020; Zhugunissov et al., 2020). The use of a model strain carrying a fluorescent marker makes it possible to assess the dynamics of viral replication without additional complex visualization procedures, which is particularly important when optimizing technological cultivation parameters.

In the present study, virus accumulation in the culture fluid was characterized by low intensity and limited biological activity. Infectious activity in the culture fluid was detected only under specific conditions, which may indicate the predominantly cell-associated nature of virus accumulation in the studied system. This is consistent with the replication features of poxviruses, in which a considerable proportion of viral particles may remain associated with infected cells during the early and intermediate stages of the reproductive cycle (Boulter & Appleyard, 1973; Chervyakova et al., 2021; World Organisation for Animal Health, 2024). Therefore, analysis of the culture fluid alone does not always reflect the actual level of infectious virus accumulation in cell culture.

The most pronounced virus accumulation was observed after treatment of the infected monolayer by the freeze–thaw method in phosphate-buffered saline. This approach promoted the release of viral particles from disrupted cells and allowed higher levels of biological activity to be detected compared with the culture fluid. These findings indicate that,

during cultivation of the GFP strain of sheeppox virus, a significant proportion of the infectious material accumulates intracellularly or remains associated with the cell monolayer. Therefore, inclusion of the freeze–thaw step is technologically important for obtaining a virus-containing suspension with a higher infectious titer (Boulter & Appleyard, 1973).

Analysis of the effect of multiplicity of infection revealed a dose-dependent pattern. At low infection doses, such as 0.01 and 0.05 $TCID_{50}$/cell, viral replication developed more slowly and was accompanied by weaker fluorescence in the Vero cell monolayer. When the infection dose was increased to 0.1 and 0.3 $TCID_{50}$/cell, earlier manifestation of the cytopathic effect and more pronounced accumulation of viral biomass were observed. This indicates that the initial infectious load is one of the key factors determining the rate of virus spread through the cell monolayer and the achievement of the maximum infectious titer.

The best results were obtained at a multiplicity of infection of 0.3 $TCID_{50}$/cell and a cultivation period of 72 h, when the infectious titer reached 3.5 log $TCID_{50}/cm^3$. This regime can be considered the most optimal among the tested variants, as it provided a high level of biological activity without excessive prolongation of the cultivation period. Extension of the cultivation period to 96-120 h did not lead to a stable further increase in infectious activity, which may be associated with progressive destruction of the cell monolayer, reduced cell viability, and depletion of the culture medium.

The use of the GFP marker is of particular importance in this study. Green fluorescent protein is widely used as a reporter of gene expression and allows infected cells to be visually detected by microscopy (Chalfie et al., 1994; Soboleski et al., 2005). In the present work, the presence of GFP enabled observation of fluorescent foci formation in the Vero cell monolayer, which made it possible to assess the intensity of viral replication at different cultivation times. This approach may be useful during the initial optimization of recombinant virus cultivation conditions, as it combines classical infectious titer determination with visual assessment of virus spread in cell culture.

The obtained data are also important for the further development of the recombinant sheeppox virus strain “SSPV (TK-) OMP16”. Previous studies have shown that the sheeppox virus genome can be used for the insertion and expression of foreign antigens, including recombinant constructs based on capripoxviruses (Boshra et al., 2013; Chervyakova et al., 2021; Teffera, 2019). The Omp16 protein of Brucella spp. is considered an immunologically significant antigen capable of inducing a specific immune response (Huy et al., 2021; Pasquevich et al., 2009). In this regard, optimization of accumulation parameters for the model GFP-producing strain represents an important stage for subsequent development of cultivation technology for the recombinant strain expressing OMP16.

The use of Vero cell culture and serum-free OptiPRO medium is also technologically justified. Vero cells are widely used in the production of viral vaccines and viral vectors due to their stable growth, susceptibility to a broad range of viruses, and suitability for scale-up (Kiesslich & Kamen, 2020). Serum-free media improve the reproducibility of cultivation, reduce the risk of introducing undesirable animal-derived components, and simplify standardization of the production process. It has previously been shown that Vero cells can be successfully cultivated in OptiPRO medium and used for viral production (Lee et al., 2020). Therefore, the selected cultivation system is promising for further technological adaptation.

At the same time, it should be noted that the present study was aimed at optimizing the in vitro accumulation conditions of a model GFP-producing strain. The obtained results characterize the replication features of the virus in Vero cell culture but do not reflect the immunogenic properties of the recombinant strain “SSPV (TK-) OMP16”. Further development of the vaccine candidate requires additional studies, including assessment of recombinant insert stability, expression level of the target OMP16 protein, genetic stability of the strain during passaging, as well as evaluation of immunogenicity and safety in vivo.

Thus, the conducted studies demonstrated that the GFP-producing sheeppox virus strain is a convenient model for visual monitoring of viral replication and optimization of viral biomass accumulation conditions. The most effective cultivation regime involved infection of the Vero cell monolayer at a dose of 0.3 $TCID_{50}$/cell, followed by cultivation for 72 h and application of the freeze-thaw method to the infected monolayer in phosphate-buffered saline. The obtained data can be used as a basis for further optimization of the accumulation technology for the recombinant sheeppox virus strain “SSPV (TK-) OMP16”.

Conclusion

As a result of the conducted studies, the technology for accumulating viral biomass with a high level

of infectious activity was optimized for the fluorescent sheeppox virus strain using different infection doses of 0.01, 0.05, 0.1, and 0.3 $TCID_{50}$/cell and cultivation periods ranging from 12 to 120 h at 6 h intervals.

The best results were obtained at a multiplicity of infection of 0.3 $TCID_{50}$/cell and a virus incubation period of 72 h. Under these conditions, the infectious titer reached 3.5 log $TCID_{50}/cm^3$ in the Vero cell monolayer using the serum-free OptiPRO maintenance medium.

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