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EVALUATION OF THE BIOLOGICAL ACTIVITY OF *ORTHOPOXVIRUS* STRAINS IN PRIMARY CHICKEN EMBRYO FIBROBLASTSMyrzakhmetova B.S.¹ , Zhapparova G.A.^{1*} , Kutumbetov L.B.¹ , Tlenchieva T.M.¹ , Zhumabek A.B.¹, Tuyakov A.M.¹ , Azanbekova M.A.¹ , Zhugunissov K. D.¹ ¹ Research Institute for Biological Safety Problems, Gvardeiskiy, 080409, Kazakhstan

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ABSTRACT

Understanding the biological features of the genus, *Orthopoxvirus*, including their replicative activity, is essential for their application in virology and vaccinology. Comparative analysis of biological activity in cell cultures makes it possible to evaluate the replicative potential of strains and the degree of their adaptation to the cellular substrate.

The aim of the study was to standardize a method for obtaining primary chicken embryo fibroblasts (CEF) and to use them as an *in vitro* substrate for assessing the biological activity of various *Orthopoxvirus* strains, with an emphasis on cytopathic effect (CPE) and infectious activity. *Vaccinia virus*, *cowpox virus*, and *camelpox virus* were used in the study. Primary CEF were obtained from 9-11-day embryonated chicken eggs (ECE), the optimal seeding concentration for the formation of a monolayer substrate was determined, and the cultures were infected with the viruses under study; CPE and virus titer were assessed during sequential passages. The results showed that a seeding concentration of 5×10^5 cells/mL ensured the formation of a uniform CEF monolayer within 24 h. Upon infection, all three viruses induced similar CPE, characterized by cell rounding, loss of elongated morphology, and partial destruction of the monolayer, with the main difference being the timing of CPE onset. *Vaccinia virus* strains induced CPE within 24 h, whereas *cowpox virus* and *camelpox virus* induced CPE after 36 and 48 h, respectively. Both *vaccinia virus* strains exhibited high infectious activity and rapid adaptation (titers from 5.50 to 6.58 ± 0.12 lg TCID₅₀/mL), whereas *cowpox* and *camelpox* viruses showed a lower and stable titer (titers from 5.50 to 6.58 ± 0.12 lg TCID₅₀/mL), and delayed CPE development.

The results emphasize the significance of CPE onset time and infectious titer level as key parameters for evaluating replicative capacity and strain adaptation, which is important for laboratory studies and the selection of vaccine candidates.

Keywords: *Orthopoxvirus*, chicken embryo fibroblasts, cytopathic effect, infectious titer

INTRODUCTION

Vaccinia virus (VACV) is a live virus that was used for immunization against smallpox. The use of VACV became a key instrument in the global disease eradication program, and the vaccine based on it remains the only vaccine in history to have completely eradicated a human disease. Despite its long history of use, the origin of VACV and its natural reservoir remain unknown to date, which underscores the need for further study of the biology of *vaccinia* and related *orthopoxviruses* [1-3].

The genus *Orthopoxvirus* of the family *Poxviridae* comprises large, morphologically similar double-stranded DNA viruses with a broad range of susceptible hosts, including humans. Their genomes reach 180-220 thousand base pairs and encode numerous structural and regulatory proteins required for replication, immunomodulation, and adaptation to different host species. Unlike most DNA-containing viruses, *Orthopoxvirus* replication occurs entirely in the cytoplasm of infected cells due to the presence of the virus's own transcription and DNA replication [4-6].

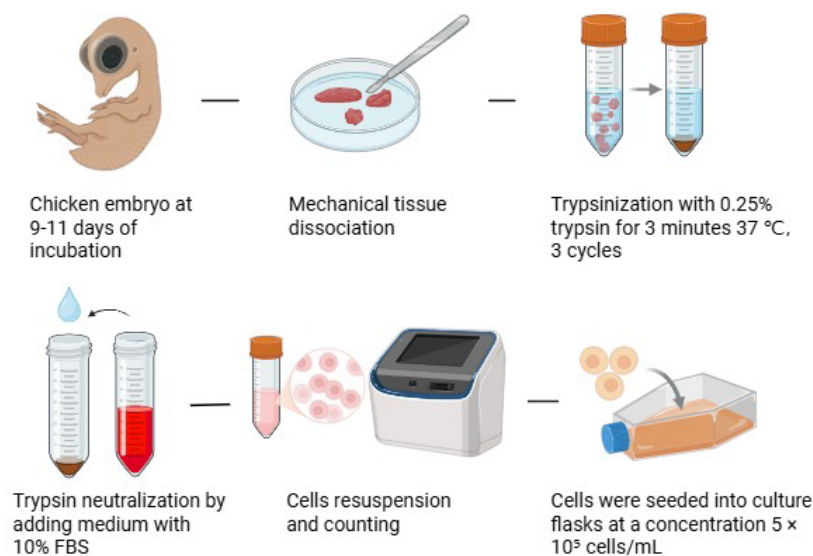
The most extensively studied representatives of *Orthopoxvirus* are *vaccinia virus*, which is widely used as a model system and a platform for the development of recombinant vaccines; *cowpox virus*, which is characterized by a broad range of susceptible hosts; and *horsepox virus*, which has important evolutionary and historical significance [7]. The genus also includes highly pathogenic human viruses, such as *variola virus* and *monkeypox virus*, which remain epidemiologically important. In recent years, the geographic distribution

and frequency of *monkeypox* outbreaks have increased across multiple countries [8]. These changes underscore the need for a detailed study of the biological properties of strains, their virulence, and their mechanisms of interaction with cells of different species.

In addition, representatives of the genus *Orthopoxvirus* have high applied potential: they serve as platforms for the development of recombinant vaccines, oncolytic viruses, and systems for genetic material delivery [9]. Successful application of such technologies requires a deep understanding of the replicative activity, virulence, and cellular tropism of different strains, making research in this area relevant for virology, biotechnology, and biosafety.

To study *Orthopoxvirus* biology, various cellular systems are traditionally used, including continuous and primary cell cultures. Among them, chicken embryo fibroblasts (CEF) are of particular importance and for decades have remained one of the most reliable and physiologically relevant models in virology [10]. CEF are biologically closer to natural tissues, which provides a more accurate reproduction of virus-cell interactions compared with transformed lines [11]. An important advantage of CEF is their high sensitivity to infectious material, which makes them an effective system for isolation and titration of *Orthopoxvirus* viruses, including *vaccinia virus*, *cowpox virus*, and *camelpox virus* [12].

One of the key properties of CEF is the ability to demonstrate a pronounced cytopathic effect characteristic of *orthopoxvirus* infection: cellular degeneration, plaque formation, and cytoplasmic inclusions [13]. Owing to this, CEF



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Figure 1. Schematic representation of the chicken embryo primary cell isolation by trypsinization

are widely used to study replication mechanisms and to assess orthopoxvirus infectivity. In addition, the possibility of obtaining a primary culture immediately before the experiment makes it possible to avoid the genetic instability inherent to continuous lines, which significantly increases the reproducibility of results [14-16]. These properties make CEF a widely used model for studying replicative dynamics, cellular responses to infection, and the virulence of different strains of the family *Poxviridae*. [17-19].

The novelty of this study lies in the standardization of a method for obtaining primary chick embryo fibroblasts (CEF) and their application as an *in vitro* model for the comparative assessment of the replicative activity of several *Orthopoxvirus* strains, including *vaccinia virus*, *cowpox virus*, and *camelpox virus*, based on cytopathic effect dynamics and viral titers. These findings allow the identification of differences in strain adaptation and infectivity that have not been described in detail previously, thereby broadening our understanding of *Orthopoxvirus* biology and their interactions with host cells.

Based on this, the aim of the present work was to standardize the method for obtaining primary chicken embryo fibroblasts and to use them as an *in vitro* model for comparative assessment of the replicative activity of certain representatives of the genus *Orthopoxvirus* based on cytopathic effect dynamics and titer.

MATERIALS AND METHODS

Preparation of a primary chicken embryo cell culture. Isolation of primary cells from chicken embryos was performed in accordance with a standard tissue dissociation protocol. Nine- to eleven-day-old chicken embryos were aseptically removed and mechanically disaggregated to obtain a homogeneous suspension [20-22]. The tissue was then subjected to enzymatic treatment using 0.25% trypsin (Sigma Aldrich) to achieve complete cell dissociation [23]. Enzymatic treatment

was performed three times for 3 min each.

After termination of the enzymatic activity by adding medium containing 10% serum, the resulting cell suspension was filtered and gently mixed. Cell density was assessed using an automatic cell counter (Counter II). The prepared cells were seeded into culture flasks and 96-well plates at a concentration of 5×10^5 cells/mL and incubated at 37 °C in 5% CO₂ [24]. A schematic representation of the cell isolation procedure is shown in Figure 1.

After obtaining the CEF culture, a study was conducted to determine the optimal seeding concentration that ensured formation of a cell monolayer within the shortest cultivation time. Cells were seeded into culture flasks at seeding concentrations of 3×10^5 , 4×10^5 , and 5×10^5 cells/mL under standard cultivation conditions. Adhesion and the density of cell monolayer formation were assessed visually using an inverted microscope after 24, 48, and 72 h of cultivation.

Viral strains. Strains of the genus *Orthopoxvirus* (family *Poxviridae*) were used in this work. The study included the following viral strains: strain KM-40 (*Camelpox virus*), strain SR-65/K (*Cowpox virus*), and strains Vaccinia -1 and Vaccinia -2 (*Vaccinia virus*).

All viral strains were obtained from the “Collection of Microorganisms” laboratory of the Research Institute for Biological Safety Problems (RIBSBP). Prior to transfer to the laboratory, the strains were maintained by passaging in embryonated chicken eggs (ECE) and stored in a frozen state for at least 10 years. Upon receipt in the laboratory, the viral preparations were used directly to infect primary CEF cultures without prior adaptation to cell culture. The main characteristics of the viral strains used are provided in Table 1. Work with the viral strains was performed in compliance with biosafety requirements in the “Especially Dangerous Infectious Diseases” laboratory at biosafety level BSL-3.

Table 1. Characteristics of Orthopoxvirus strains used in the study

№	Strain name	Virus species	Passport data		
			Susceptible biosystem	Number of passages	Passport titer
1	KM-40 (vaccine)	<i>Camelpox</i>	ECE	41	7,17 lg TCID ₅₀ /mL
2	SR-65/K (vaccine)	<i>Cowpox</i>	Lamb kidney cells (LK)	65	6,75 lg TCID ₅₀ /mL
3	Vaccinia -1 (vaccine)	<i>Vaccinia</i>	ECE	2	6,08 lg TCID ₅₀ /mL
4	Vaccinia -2 (vaccine)	<i>Vaccinia</i>	ECE	ND	ND

Note: ND - not determined.

Cell culture infection procedure. Prior to infection, cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (penicillin 100 U/mL and streptomycin 100 µg/mL) at 37 °C in an atmosphere of 5% CO₂ until an 80-90% cell monolayer was achieved.

For infection, the culture medium was removed, after which the cells were washed three times with Hanks' solution. Then, 100 µL of viral inoculum was added to a 25 cm² culture flask containing 5 mL of maintenance medium (DMEM + 2% FBS). The cultures were incubated for 1 h at 37 °C (for adsorption), with periodic gentle rocking of the flask. The cells were then rinsed three times again with Hanks' solution and maintenance medium was added. The cells were incubated at 37 °C in the presence of 5% CO₂.

At specified time intervals (e.g., 24, 48, 72, and 96 h post-infection), cytopathic effect development was evaluated visually under a microscope, and the viral titer was determined by the 50% tissue culture infectious dose (TCID₅₀/mL) assay.

Each experiment was performed in triplicate to ensure statistical reliability of the results.

Assessment of cytopathic effect. The cytopathic effect of the viruses was assessed visually using an inverted microscope on primary CEF cultures for 96 h after infection. Observations were performed to identify morphological changes characteristic of the infectious process, such as cell rounding and detachment from the surface; formation of multinucleated giant cells (syncytia); and lysis and destruction of the cell monolayer.

For an objective assessment of CPE severity, a scale including the following categories was used:

- 0 - no visible changes;
- 1+ - mild cytopathic effect (up to 25% of cells altered);
- 2+ - moderate CPE (25-50% of cells showing signs of damage);
- 3+ - marked CPE (50-75% of cells affected);
- 4+ - complete monolayer lysis (more than 75% of cells

affected).

The observation results were recorded for each strain separately at 24, 48, 72, and 96 h, which allowed comparison of viral virulence and determination of optimal reproduction conditions [25].

Determination of viral infectious activity. Viral infectious activity was determined by titration in cell culture. For this purpose, 10-fold serial dilutions of the virus from 10⁻¹ to 10⁻⁸ in maintenance medium containing 2% FBS were used to infect a cell culture prepared in a 96-well plate by adding 100 µL of each virus dilution into 4 wells of the plate in triplicate. The cells were incubated at 37 °C in an atmosphere of 5% CO₂ for 5-7 days. The presence of CPE was determined by microscopy. Virus titers were calculated using the Reed and Muench method and expressed as lg TCID₅₀/mL.

Statistical analysis. All experiments were performed in three independent replicates. Data are presented as mean ± standard deviation (SD). Statistical processing of results was performed using GraphPad Prism software. One-way analysis of variance (ANOVA) was used to assess differences between groups. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Determination of the optimal CEF seeding concentration. As part of standardizing the method for obtaining and culturing CEF, we conducted a study to determine the optimal seeding density that ensures the formation of a continuous cell monolayer in the shortest possible cultivation time. The effectiveness of different seeding concentrations was assessed based on the morphological characteristics of the cell culture and the time required for monolayer formation. The results of the study are presented in Table 2.

As shown in Table 2, at a seeding concentration of 5×10⁵ cells/mL, the cells were evenly distributed over the surface of the culture flask and formed a cell monolayer within the first day. At a lower concentration (3-4×10⁵ cells/mL), a sparse cell monolayer was observed; despite maintenance of cell viability and adhesion, a complete monolayer did not form either

Table 2. Results of determining the optimal CEF seeding concentration

Seeding concentration (cells/mL)	Characteristics of the cell monolayer		
	Day 1 of cultivation	Day 2 of cultivation	Day 3 of cultivation
3×10 ⁵	Adhesion of individual cells	Sparse monolayer	Monolayer not formed
4×10 ⁵	Sparse monolayer	Sparse monolayer	Monolayer not formed
5×10 ⁵	Confluent monolayer	Confluent monolayer	Monolayer maintained

Table 3. Degree of CPE development of different viral strains in CEF cells

Viral strain	24 h	48 h	72 h	96 h
Vaccinia -1 (Vaccinia virus)	4+	4+	-	-
Vaccinia -2 (Vaccinia virus)	4+	4+	-	-
KM-40 (Camelpox virus)	0	3+	4+	-
CP-65/K (Cowpox virus)	0	3+	4+	-

Note: «-» indicates complete destruction and detachment of cells from the adhesion surface.

on the second or on the third day of cultivation. Thus, a concentration of 5×10^5 cells/mL was determined to be optimal for obtaining a monolayer CEF culture within 24 h.

Results of CPE assessment. CPE in viruses on primary CEF cultures was assessed using the semi-quantitative scale (0-4+) described in Section 2.4. For each strain, the final CPE categories were recorded at 24, 48, 72, and 96 h post-infection. The results are presented in Table 3.

As shown in Table 3, different strains exhibited differences in virulence and in the rate of CPE development. *Vaccinia virus* strains showed pronounced CPE as early as 24 h post-infection and maintained a high level of cell monolayer damage at 48 h, indicating high virulence and rapid replication in CEF cells. In contrast, camelpox and cowpox strains showed delayed CPE dynamics: the first morphological changes were

observed by 36 h, and complete monolayer lysis by 72 h, reflecting a slower replication cycle and comparatively lower virulence.

Cytopathic effect of Orthopoxvirus strains in primary CEF cultures. Infection of primary CEF cultures with the studied viral strains led to the development of a similar cytopathic effect, differing only in the time of onset.

In the control, cells retained the typical elongated morphology and formed a dense monolayer (Fig. 2a).

CPE manifested as cell rounding, loss of the elongated fibroblast morphology, disruption of intercellular contacts, and partial destruction of the monolayer. In vaccinia virus strains, pronounced morphological changes were observed 24 h after infection (Fig. 2b and Fig. 2c), whereas in camelpox and cowpox strains, similar manifestations were recorded after 36-47

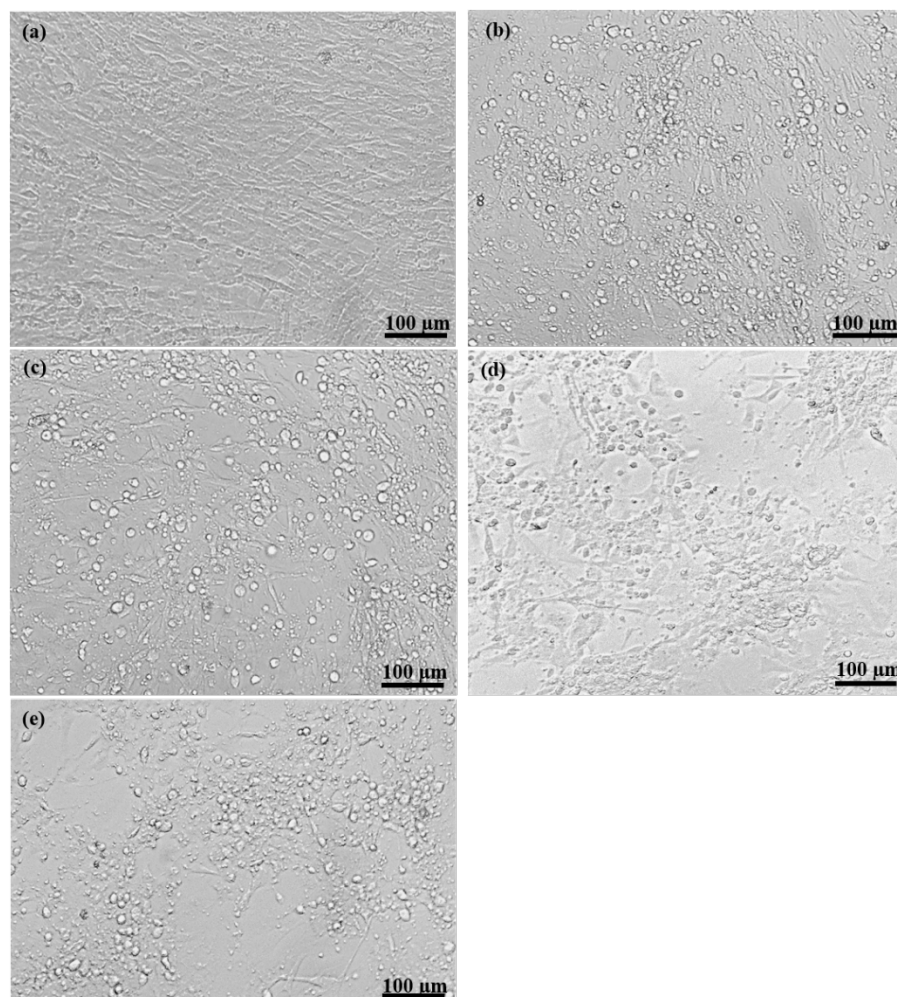


Figure 2. Cytopathic effect of *Orthopoxvirus* strains in primary CEF cell cultures: (a) Control; (b) Vaccinia -1 (24 h); (c) Vaccinia -2 (24 h); (d) Camelpox (36 h); (e) Cowpox (48 h). Images were obtained using an inverted microscope (Biomed-3) at $\times 100$ magnification. Brightness and contrast were adjusted to improve visualization.

h (Fig. 2d and Fig. 2e).

Thus, all studied strains induced a similar cytopathic effect in primary CEF culture, differing predominantly in the rate of its development.

Infectious activity of Orthopoxvirus strains in CEF culture. The infectious activity of the studied strains in primary CEF culture was evaluated based on virus titer values and the dynamics of CPE development during sequential passaging (Figs. 3 and 4).

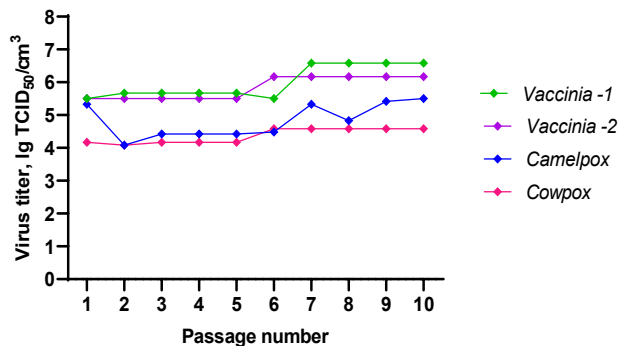


Figure 3. Changes in infectious titers of Orthopoxvirus strains during sequential passaging in CEF culture

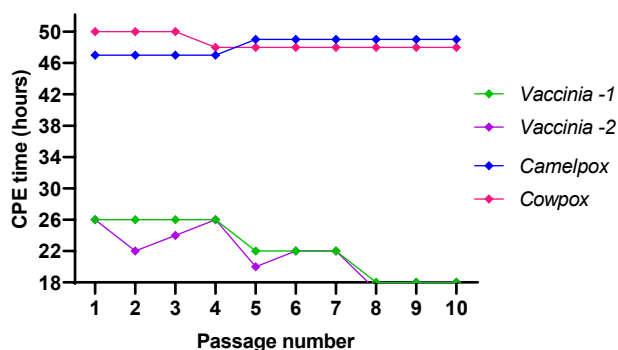


Figure 4. Time to CPE development across passages

The *vaccinia virus* strain Vaccinia -1 was characterized by high infectious activity. Infectious titer values at early passages were 5.50 lg TCID₅₀/mL, followed by an increase to 6.58±0.12 lg TCID₅₀/mL at passages 7-10. At the same time, a reduction in the time to CPE onset from 26 to 18 h was observed, indicating efficient viral adaptation in cell culture during serial passaging.

A similar trend was observed for the *vaccinia virus* strain Vaccinia -2. Here, the infectious titer remained at 5.50 lg TCID₅₀/mL during the first passages and increased to 6.17±0.19 lg TCID₅₀/mL starting from the sixth passage. The time to CPE onset gradually decreased from 26 to 17-18 h.

The camelpox virus strain demonstrated moderate infectious activity. Infectious titer values varied in the range from 4.08±0.13 to 5.50 lg TCID₅₀/mL across all passages, while the time to complete CPE development remained relatively stable at 36-72 h.

The lowest infectious activity was recorded for the

cowpox virus strain. The infectious titer remained within 4.17±0.12-4.58±0.12 lg TCID₅₀/mL without a pronounced trend during sequential passaging, and CPE was observed 48-72 h after infection.

As a result of the study, it was established that all investigated orthopoxvirus strains differed substantially in the level of infectious activity in primary CEF culture. The highest infectious titers and a pronounced adaptation dynamic were characteristic of vaccinia viruses, whereas the camelpox and cowpox strains were characterized by lower and more stable infectious activity.

Statistical analysis using one-way ANOVA showed that the differences in viral titer between the vaccinia virus and the other strains were statistically significant ($p < 0.05$), whereas the differences between camelpox and cowpox were not significant ($p < 0.05$).

DISCUSSION

Standardization of CEF isolation and cultivation ensures reproducible monolayer formation, which is essential for the reliable assessment of the morphological and functional properties of the culture. In the present study, a seeding density of 5×10^5 cells/mL allowed the formation of a uniform monolayer within 24 h, whereas lower densities (3×10^5 and 4×10^5 cells/mL) produced sparse or incomplete monolayers even after 72 h. This methodological standardization reduces inter-experimental variability and improves the reliability of downstream analyses, thereby establishing a basis for a unified protocol for the evaluation of cell morphology, proliferation, and functional characteristics.

All *Orthopoxvirus* strains tested in primary CEFs induced similar cytopathic effects (CPE), characterized by morphological alterations and partial monolayer destruction. The principal difference was the rate of CPE development: vaccinia virus strains induced visible changes within 24 h post-infection, whereas cowpox virus and camelpox virus strains exhibited CPE only after 36 h. These observations suggest that variation in the timing of CPE onset may reflect strain-specific differences in replicative potential and adaptation to cell culture, in agreement with previous reports on *Orthopoxvirus* tropism [26].

Analysis of infectious titer dynamics during serial passaging showed that *vaccinia virus* strains possess a greater replicative capacity in CEF culture, as demonstrated by a steady increase in titer up to 6.58 ± 0.12 lg TCID₅₀/mL at later passages. By contrast, cowpox virus and camelpox virus strains maintained lower and more stable titers, suggesting a more limited capacity for adaptation in primary culture. These differences may be attributable to intrinsic genetic features of the strains as well as to the extent of their prior adaptation to cell culture systems [27,28].

Taken together, these findings indicate that the rapid CPE development and titer increase observed for vaccinia virus strains make them particularly suitable for cultivation and further experimental use, including vaccine platform development [1]. In contrast, *cowpox virus* and *camelpox virus* strains require additional investigation to clarify the factors restricting their replication in primary CEF cultures, which may involve differences in cell tropism, host cell responses, and

strain-specific genetic differences.

These results are consistent with previous observations showing that zoonotic *Orthopoxvirus* species differ in their interspecies transmission potential and that their replicative behavior in cell culture is closely associated with epidemiological patterns and population vaccination history [7,29].

Thus, standardized primary cell systems represent an important model for the comparative analysis of viral replication dynamics, which is of critical importance for virology, biotechnology, and the development of vaccine strategies.

CONCLUSION

The experiments showed that the rate of CPE onset and progression, as well as the level of infectious titer, are key parameters for assessing viral activity and the degree of adaptation of *Orthopoxvirus* strains in a cell system. These indicators can be used as criteria for selecting strains for laboratory studies and the development of vaccine candidates.

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AUTHOR CONTRIBUTIONS

Myrzakhmetova B.Sh.- study planning, conducting experiments, article design; Zhapparova G.A. - conducting experiments; Kutumbetov L.B.- study planning, statistical processing of results, assistance in design of the article; Tlenchiyeva T.M. - conducting experiments; Zhumabek A.B. - conducting experiments, translation of the article into English; Tuyakov A.M. - conducting experiments, translation of the article into English; Azanbekova M.A. - conducting experiments, statistical processing of results; Zhugunissov K.D.- conducting experiments, statistical processing of results.

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ОЦЕНКА БИОЛОГИЧЕСКОЙ АКТИВНОСТИ ВИРУСОВ РОДА ORTHOROXVIRUS НА ПЕРВИЧНЫХ КУРИНЫХ ЭМБРИОНАЛЬНЫХ ФИБРОБЛАСТАХ

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АННОТАЦИЯ

Использование представителей рода Orthoroxvirus в вирусологии и вакцинологии требует углубленного изучения их биологических свойств, в том числе их репликационной активности. Сравнительный анализ биологической активности в клеточных культурах позволяет оценивать репликативный потенциал штаммов и степень их адаптации к клеточному субстрату.

Цель исследования заключалась в стандартизации метода получения первичных куриных эмбриональных фибробластов (ФКЭ) и использовании их в качестве субстрата *in vitro* для оценки биологической активности различных штаммов Orthoroxvirus с акцентом на цитопатическое действие (ЦПД) и инфекционную активность. В исследованиях использовали вирусы оспавакцины, оспы коров и оспы верблюдов. Первичные клетки ФКЭ получали из 9-11 суточных развивающихся куриных эмбрионов (РКЭ), определяли оптимальную посевную концентрацию для формирования монослойного субстрата и инфицировали исследуемыми вирусами, оценивали ЦПД и титр вируса при последовательных пассажах. Результаты показали, что посевная концентрация 5×10^5 кл/мл обеспечивает формирование равномерного клеточного монослоя ФКЭ уже через 24 ч. При заражении все три вируса вызывали однотипное ЦПД - округление клеток, утрату вытянутой формы и частичную деструкцию монослоя, различаясь главным образом по времени проявления ЦПД. Штаммы оспавакцины вызывали ЦПД через 24 ч, вирусы оспы коров и оспы верблюдов через 36 и 48 ч., соответственно. Высокую инфекционную активность и быструю адаптацию показали оба штамма вируса оспавакцины (титры от $5,50$ до $6,58 \pm 0,12$ lg ТЦД₅₀/мл), тогда как вирусы оспы коров и верблюдов имели более низкий и стабильный титр (от $4,58 \pm 0,12$ до $5,50$ lg ТЦД₅₀/мл) и замедленное формирование ЦПД.

Результаты подчеркивают значимость времени проявления ЦПД и уровня инфекционного титра как ключевых параметров для оценки репликативной способности и адаптации штаммов, что важно для лабораторных исследований и отбора кандидатов вакцин.

Ключевые слова: Orthoroxvirus, куриные эмбриональные фибробласты, цитопатическое действие, инфекционный титр.

ORTHOROXVIRUS ВИРУС ШТАММАДАРЫНЫҢ БИОЛОГИЯЛЫҚ БЕЛСЕНДІЛІГІН АЛҒАШҚЫ ТАУЫҚ ЭМБРИОНЫ ФИБРОБЛАСТТАРЫНДА БАҒАЛАУ

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АНДАТПА

Вирусология мен вакцинологияда Orthoroxvirus түріне жататын вирустарды қолдану олардың биологиялық қасиеттерін, соның ішінде репликациялық белсенділігін терең зерттеуді талап етеді. Жасуша өсінділеріндегі биологиялық белсенділікті салыстырмалы талдау штамдардың репликациялық әлеуетін және олардың жасушалық субстратқа бейімделуін бағалауға мүмкіндік береді.

Бұл зерттеудің мақсаты алғашқы тауық эмбрионы фибробласттарын (БЭФ) алу әдісін стандарттау және оларды әртүрлі Orthoroxvirus вирустары штамдарының биологиялық белсенділігін бағалау үшін *in vitro* субстрат ретінде пайдалану болды, олардың цитопатогенді әсеріне (ЦПӘ) және инфекциялық белсенділігіне назар аударылды. Зерттеуде оспавакцина, сиыр шешегі және түйе шешегі вирустары қолданылды. Алғашқы жасуша түрлері 9-11 күндік дамып келе жатқан тауық эмбриондарынан (БЭФ) алынды. Моноқабатты субстратты қалыптастыру үшін оңтайлы себу концентрациясы анықталды және жасушалар зерттелген вирустармен жұқтырылды. БЭФ және вирустық титр сатылы пассаждау арқылы бағаланды. Нәтижелер 5×10^5 жасуша/мл инокуляция концентрациясы 24 сағат ішінде біркелкі жасуша моноқабатының түзілуін қамтамасыз ететінін көрсетті. Инфекция кезінде үш вирус та бірдей цитопатогенді әсер (ЦПӘ) тудырды, ол жасушалардың дөңгелектенуі, созылған пішінінің жоғалуы және моноқабаттың ішінара жойылуы көрінісі ретінде ерекшеленді. Оспавакцина штамдары цитопатогенді әсерді 24 сағат ішінде тудырды, ал сиыр шешегі мен түйе шешегі вирустары сәйкесінше 36 және 48 сағат ішінде ЦПӘ тудырды. Оспавакцина вирусының екі

штаммы да жоғары инфекциялық белсенділік пен тез бейімделуді көрсетті (титрлері 5,50-ден $6,58 \pm 0,12$ lg TCID₅₀/мл-ге дейін), ал сиыр шешегі мен түйе шешегі вирустары титрі төмен және тұрақтырақ ($4,58 \pm 0,12$ -ден 5,50 lg TCID₅₀/мл-ге дейін) және ЦПӨ түзілуін кешіктірді. Алынған нәтижелер штамдардың репликациялық қабілеті мен бейімделуін бағалаудың негізгі параметрлері ретінде ЦПӨ басталу уақытының және инфекциялық титр деңгейінің маңыздылығын көрсетеді, бұл алынған нәтижелер зертханалық зерттеулер мен вакцина кандидаттарын таңдау үшін маңызды кезең болып табылады.

Түйінді сөздер: *Orthopoxvirus*, тауық эмбрионы фибробласттары, инфекциялық титр, цитопатогенді әсер.