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Features of *in vitro* cultivation of the field strain of canine parvovirus

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Intestinal infections remain one of the most important health care problems. According to WHO, viral diarrhea is the most common infectious disease in the world. A similar situation is registered among the diseases of dogs, where enteritis of infectious etiology occupies a leading place, and the leader is parvovirus enteritis from which dog owners have been suffering for the past forty years. Despite such a long period of time, unfortunately, proper means of treatment and prevention have not been created, and taking into account the persistence of the virus in the external environment and the ability to mutate, the correlation of diagnostic methods is always necessary, which, accordingly, indicates the relevance of studying parvovirus infection in dogs. The use of cell cultures as test objects for the cultivation and accumulation of viral biomass makes it possible to obtain an antigen suitable for use in the manufacture of diagnostic, prophylactic and therapeutic biological preparations. The publication contains research materials on the peculiarities of the cultivation of canine parvovirus isolated on the territory of Ukraine from a 2-month-old purebred puppy that died of parvovirus enteritis. In the biological material isolated from the dead animal by means of laboratory studies, mono-infection and the possibility of its cultivation *in vitro* were established. The possibility of using a transplantable line of rabbit kidney cell culture (RK-13) has been established. Freshly reseeded, after 24 hours of incubation, with at least 70% and no more than 80% cell monolayer and seeding concentration from 1.0×10^5 to 2.0×10^5 cells/ml, as a biological test object for cultivation and accumulation parvovirus biomass. Cultivation was carried out in a thermostat at t = +37.5°C for no more than 10 days, and a mixture of medium 199 and DMEM was used as a supporting medium. The effectiveness of cultivation was determined by the increase in the titer of the infectious activity of the virus, which by the fifth passage was 3.8±0.08 lg TCD_{50/cm^3}.

Key words: canine parvovirus, field isolate, cultural properties, cytopathogenic effect, RK-13

Introduction

Recently, there has been a tendency in the world to increase the population of companion animals, among which dogs occupy a special place. In connection with the increased interest in breeding and keeping small domestic animals and the growth in the number of the latter's population in personal and official use, led to an increase

in the registration of cases of various infectious diseases [11, 17]. But today, knowledge about the diagnosis, pathology of most infectious diseases, the use of effective methods of therapy is not enough. Therefore, the fight against infectious diseases of animals is the most urgent problem for modern veterinary medicine, among which a special place belongs to viral diseases [12, 22], which are characterized by a wide spectrum of distribution with a long-lasting effect on the health of animals, namely, extremely high lability of clinical signs, which complicate diagnosis for a practical veterinarian [2, 10].

The most widespread, highly contagious and key enteropathogen among dogs is parvovirus, which affects the dog population worldwide, causing parvovirus enteritis in the latter, which is one of the main causes of morbidity and mortality in puppies [19, 20]. The lethality of dogs from parvovirus enteritis ranges from 10 to 70%, depending on the form of the disease. The manifestation of parvovirus infection has several pronounced peaks of activity, namely: the beginning, which reached the scale of the pandemic in the eighties and remained at a significant level almost until the nineties; the next, less pronounced but characteristic peak of activity was recorded precisely in the nineties, and the third, the least pronounced, was established at the beginning of the two thousandths [1, 18]. Taking into account the high contagiousness against the background of not always effective vaccination, we get a constant circulation of pathogens, especially in places of significant crowding due to the keeping of dogs. The problem lies in the insufficiently deep study of the biological properties of canine parvovirus, in particular the cultural properties and the possibility of obtaining and accumulating a cultural antigen suitable for improving the diagnosis of this disease in dogs.

The detection of the virus in natural and clinical samples is a complex problem of research and diagnostics, therefore, the reproduction of the virus in laboratory conditions is an important experimental tool in fundamental virological research, which allows studying the peculiarities of the reproduction of the virus, its interaction with the host cell, and knowledge of the pathogenesis of the disease, the etiological factor of which there is a virus. In addition, the study of the features of virus reproduction provides a basis for the diagnosis of viral diseases and the production of vaccines, as well as the creation of recombinant viruses for potential use in gene therapy [5, 17].

Summarizing the above, the aim of our work was to study the *in vitro* cultural properties of a field isolate of canine parvovirus on transplantable lines of cell cultures, with the possibility of obtaining a biological object suitable for the accumulation of viral biomass.

Research Material and Methodology

During the research, the basic rules of good laboratory practice GLP were followed (*Good laboratory practice*), provisions of the "General Ethical Principles of Animal Experiments", adopted by the First National Congress of Bioethics (Kyiv, 2001). The experimental part of the research was carried out in accordance with the requirements of international principles "European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Purposes" (Strasbourg, 1986), "Rules for conducting work with the use of experimental animals", approved by the order of the Ministry of Health no. 281 of November 1, 2000 "On measures to further improve organizational forms of work with the use of experimental animals" and the corresponding Law of Ukraine "On protection of animals from cruel treatment" (no. 3447-IV dated February 21, 2006, Kyiv). Euthanasia was performed using drugs that ensure rapid, painless death, in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes" (conclusion on the procedure for carrying out scientific research in accordance with bioethical norms, approved at the meeting of the Department of Epizootology, Microbiology and Virology of the National University of Life and Environmental Sciences of Ukraine protocol no. 4 from 16.04.2024) [3, 4, 6, 13].

Diagnostic studies for the confirmation of parvovirus were carried out using *VetExpert* express test systems in dogs (CPV/CCV Ag, solid-phase immunochromatographic analysis for qualitative detection of Canine Parvovirus/Coronavirus antigen), manufactured in Poland. Diagnostic studies on the presence of mono-infection with only parvovirus antigen in dogs were carried out on the basis of the private veterinary laboratory of LLC "Bald" (Kyiv) using enzyme-linked immunosorbent assay and polymerase chain reaction. For isolation of parvovirus antigen from pathological material and accumulation of viral material, transplantable lines of cell cultures SPEV (pig embryo kidneys), BHK-21 (Syrian hamster kidney fibroblasts), RK-13 (rabbit kidneys) were used. Virological studies, namely the isolation and identification of viruses, were carried out using cell cultures, by infecting laboratory animals. Cells were grown in glass and plastic mattresses with a volume of 25, 50 and 100 ml. stationary method, as well as in the wells of polystyrene plates with a seed concentration from 1.0×10^5 to 2.0×10^5 cells/ml. Reproduction of field isolates of parvovirus was carried out on cell culture with the formation of a monolayer in the range of 70–80%. At the end of the incubation period, the infected culture was frozen at a temperature –30°C and subjected to thawing in order to destroy the structure of cell integrity due to temperature "stress" and ensure the complete release of the virus. Microbiological studies to determine bacterial contamination and antibiotic sensitivity of detected microorganisms were carried out by sowing pathological material on nutrient media. Calculation of the infectious titer was calculated according to the method of Reed and Mench. The cultured virus-containing liquid was used to perform RGA to detect the agglutinating properties of parvovirus and coronavirus to erythrocytes of animals of various species. A suspension of erythrocytes and RGA was obtained according to generally accepted methods [16, 21].

Statistical processing of data was carried out using the *Statistica* program, all experiments were performed in 3 repetitions. average values were considered significant at P<0.05 [15].

Research Results and Their Discussion

The work was carried out at the department of epizootology, microbiology and virology in the conditions of the problematic research laboratory "Microbiology, virology and immunobiotechnology" of the Ukrainian National Institute of Biotechnology. A fragment of the dog's intestine was used to isolate the virus after it died from parvovirus enteritis. The intestine with its contents was removed during the post-mortem examination, placed in a sterile dish with physiological saline and frozen. After thawing, the material was crushed by grinding in a mortar with sterile quartz sand, diluting with Hanks' solution (1:10). Large particles were removed by centrifugation for 20–30 min at 2500 rpm./min (g = 800). Next, the supernatant liquid was collected, antibiotics were added to the sediment (1000 units of penicillin and streptomycin per 1 ml), and placed in a refrigerator at 4°C for 60 min. Control of the effectiveness of such treatment was carried out by means of sowing on nutrient media for aerobes, anaerobes and fungi. After receiving a negative result of bacteriological control, the virus-containing material was used to infect cell cultures.

For isolation, adaptation and accumulation of parvovirus, primary trypsinized and transplanted cell cultures are used. Successful cultivation of viruses is carried out in cell cultures obtained from animals sensitive to this virus. The most effective reproduction of canine parvovirus occurs in cells obtained from the organs of dogs and cats, including: in cultures of cat or dog kidney cells, mink lungs, without manifestation of a cytopathogenic effect. But, according to other researchers, canine parvovirus is able to detect the cytopathogenic effect of the virus in the cat kidney cell line [7, 8]. In order to expand the list of the use of biological objects suitable for the cultivation and accumulation of parvovirus, we conducted an experimental study on the suitability such transplantable lines of cell cultures as hamster kidney (BHK-21), rabbit kidney (RK-13) and pig kidney (SPEV) (fig. 1).

In the body of animals, most viruses show a tropism towards young, rapidly dividing cells, so we tested the infection of cell culture at different time periods after reseeding and cell population of cell cultures. It was established that newly transplanted cells with at least 70% and no more than 80% monolayer of cells after 24 hours of incubation were the most sensitive to the incubation of the virus-containing isolate. Control of the number of introduced cells during reseeding was carried out using a camera with a Goryaev grid. The most optimal seeding concentration was from 1.0×10^5 to 2.0×10^5 cells/ml. For the cultivation of transplantable lines of cell cultures, a nutrient medium was used, which included media 199 and DMEM — 45% each, fetal bovine serum — 10% and antibiotics.

Incubation of cell cultures was carried out in a thermostat at t = +37.5°C for no more than 10 days. For intensive accumulation of culture material that can be used for infection and accumulation of virus-containing material, cell cultures were reseeded every 72 h, this also stimulated cell growth at a more intensive rate, which is necessary for better contact of the virus with the cell. A solution of trypsin and versene in a ratio of 1:6 to 1:3 and an antibiotic was used for transplanting the culture. The concentration was adjusted depending on the state of "seeding" of the mattress with cells.

Infection was carried out as follows: the growth medium was drained from the mattress and 2.5–3 cm^3 of viral material was introduced into it. The mattress was placed in a thermostat for an hour at a temperature of +37.5°C. At the same time, every 15 min, the monolayer of cells was slowly washed with viral material 4–5 times, after which the latter was drained and a supporting medium was introduced into the mattress, which for the infected cell culture had the same composition as the nutrient medium, only without serum. Cultivation was carried out according to generally accepted methods, evaluating the cytopathogenic effect (CPD) according to the four-plus system, under an inverted microscope at low magnification, relative to the control.

The simplest signs that indicated the multiplication of the virus were degenerative changes in the cells, that is, the manifestation of the virus's CDP — the formation of visible morphological changes in the cell is called a cytopathogenic effect or action. Cytopathogenic changes in infected cell cultures depend on the biological properties and dose of the virus under study (fig. 2).

The cytopathogenic effect of parvovirus was manifested in the following sequence: inhibition of cell division processes, partial shrinkage of cells, partial darkening in the form of cell agglutination. Subsequently, the development of classical changes, namely the destruction of cells by their stretching and acquiring the shape of a "star", which was followed by the rupture of the cell wall. Incubation of viruses on cell cultures was carried out for no more than 10 days at t = +37.5°C, monitoring the state of the cell monolayer every 24 h under a microscope at low magnification (×56) (fig. 2).

As a result of our experiment, it was established that the most sensitive cell culture line for cultivating a field isolate of parvovirus was rabbit kidney and slightly less sensitive hamster kidney after the third passage, and pig kidney cell culture was more sensitive in the first three passages. The reproduction of parvovirus in cell culture was controlled by the time of manifestation of CPD, which directly depended on the density of the monolayer of cells: after 70% — after 48 h, 80% — after 72 h, and if more than 90% of the monolayer — the destruction of cells occurred rather slowly, it was necessary to incubate the culture up to 10 days At the end of the incubation period, the infected culture was frozen at a temperature –30°C and subjected to thawing in order to destroy the structure of cell integrity due to temperature "stress" and ensure the complete release of the virus.

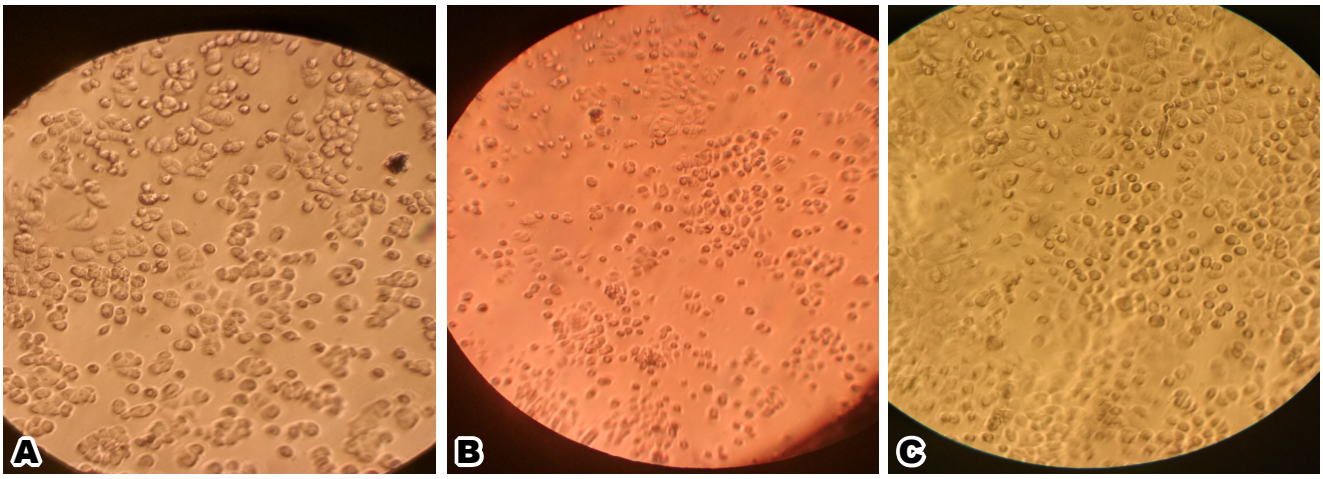

Fig. 1. Cell cultures, 12 hours after transplanting: A — RK-13; B — BHK-21; C — SPEV. ×56

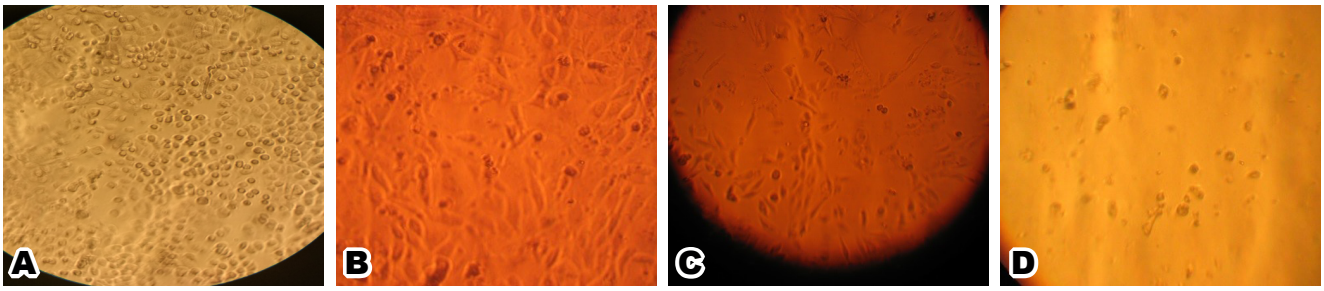

Fig. 2. Effect of parvovirus on RK-13 cell culture (×56). A — before infection; B — state of cell culture 48–72 hours after infection; B — state of cell culture 4–5 days after infection; D — state of cell culture 7–8 days after infection

The sensitivity of the cell lines was determined not only by the expression of the CPD of the virus on the cells, but also by the level of accumulation of the virus in the cell suspension. The titer of infectious activity of the material was determined by titration of viral material in cell culture on sterile 96-well microplates. Calculation of the infectious titer was performed according to the method of Reed and Mench. In the course of the experiment, the following results were obtained: at the beginning of the experiment, the titer of infectious activity on the culture of rabbit kidney cells averaged 1.5 lg TCD_{50/cm^3}, after the third passage — up to 2.7±0.06 lg TCD_{50/cm^3}, and after 1st — increased to 3.8±0.08 lg TCD_{50/cm^3}.

Therefore, analyzing literary sources, it was established that today most parvoviruses isolated from dogs are cultivated *in vitro* on cell cultures obtained from the organs of dogs and cats [9, 14]. We have established the suitability for cultivation and accumulation of a field isolate of parvovirus on the culture of rabbit kidney cells (RK-13), where after adaptation of the virus, the cytopathogenic effect was noted after 72 h. incubation, and on the 5th–7th day — 90–100% cytopathogenic effect, while the titer of infectious activity increased with each new passage of viral material and by the fifth was 3.8±0.08 lg TCD_{50/cm^3}.

References

1. Agnihotri D, Singh Y, Maan S, Jain VK, Kumar A, Sindhu N, Jhamb R, Goel P, Kumar A. Molecular detection and clinico-hematological study of viral gastroenteritis in dogs. *Haryana Vet*. 2017; 56 (1): 72–76. Available at: https://www.luvas.edu.in/haryana-veterinarian/download/harvet2017/19.pdf
2. Borland S, Gracieux P, Jones M, Mallet F, Yugueros-Marcos J. Influenza A virus infection in cats and dogs: A literature review in the light of the "One Health" concept. *Front Publ Health*. 2020; 8 (83): 83. DOI: 10.3389/fpubh.2020.00083.
3. Companion Animals. *Animal Welfare Institute*. Available at: https://awionline.org/content/companion-animals (last access 21.06.2024).
4. European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. Strasbourg, 18. III. 1986. Available at: http://zakon4.rada.gov.ua/laws/show/994_137 (in Ukrainian)
5. Faisst S. Propagation of viruses. Animal. In: Granoff A, Webster RG (eds). *Encyclopedia of Virology*. A reference book. 2nd ed. 1999: 1408–1413. DOI: 10.1006/rwvi.1999.0236.
6. Good Laboratory Practice (GLP). In: On the approval of the Procedure for medicinal products preclinical study and medicinal products preclinical study materials examination. The order no. 944 from 14.12.2009. Aailable at: https://ips.ligazakon.net/document/re17348?an=48 (in Ukrainian)
7. Hafenstein S, Palermo LM, Kostyuchenko VA, Xiao C, Morais MC, Nelson CDS, Bowman VD, Battisti AJ, Chipman PR, Parrish CR, Rossmann MG. Asymmetric binding of transferrin receptor to parvovirus capsids. *Proc Natl Acad Sci USA*. 2007; 104 (16): 6585–6589. DOI: 10.1073/pnas.0701574104.

8. Kapil S, Cooper E, Lamm C, Murray B, Rezabek G, Johnston L, Campbell G, Johnson B. Canine parvovirus types 2c and 2b circulating in north american dogs in 2006 and 2007. *J Clin Microbiol.* 2008; 45 (12): 4044–4047. DOI: 10.1128/JCM.01300-07.
9. Licitra BN, Whittaker GR, Dubovi EJ, Duhamel GE. Genotypic characterization of canine coronaviruses associated with fatal canine neonatal enteritis in the United States. *J Clin Microbiol*. 2014; 12 (52): 4230–4238. DOI: 10.1128/JCM.02158-14.
10. Moore DP, Cantón GJ, Louge Uriarte EL. Editorial: Infectious diseases affecting reproduction and the neonatal period in cattle. *Front Vet Sci*. 2021; 8: 679007. DOI: 10.3389/fvets.2021.679007.
11. Nichiporuk S, Radzikhovskyi M. Big hearts of little friends. *Scientific and Tech Bull SSRCIVMPFA IAB*. 2022; 23 (1): 105–110. DOI: 10.36359/scivp.2022-23-1.15. (in Ukrainian)
12. Ohshima T, Mochizuki M. Evidence for recombination between feline panleukopenia virus and canine parvovirus type 2. *J Vet Med Sci.* 2009; 71 (4)*:* 403–408. DOI: 10.1292/jvms.71.403.
13. On the Protection of Animals from Cruelty. Law of Ukraine no. 3447-IV.2006; no. 27: 230. Available at: https://zakon.rada.gov.ua/laws/show/3447-15#Text (in Ukrainian)
14. Pakkanen K, Nykky J, Vuento M. Late steps of parvoviral infection induce changes in cell morphology. *Virus Res*. 2008; 137 (2): 271–274. DOI: 10.1016/j.virusres.2008.07.019.
15. Petrovska I, Salyha Y, Vudmaska I. *Statistical Methods in Biological Research*. An educational and methodological manual. Kyiv, Agrarian science; 2022: 172 p. (in Ukrainian)
16. Polishchuk VP, Budzanivska IG, Shevchenko TP. *Practical Training Manual for the Course "General Virology"*. Kyiv, Phytosocial Center, 2005: 204 p. (in Ukrainian)
17. Radzykhovsky ML, Dyshkant OV. *Fundamentals of Veterinary Virology*. A manual. Kyiv, NUBiP of Ukraine. 2022: 180 p. Available at: https://dglib.nubip.edu.ua/handle/123456789/9464 (in Ukrainian)
18. Radzykhovskyi M, Sokulskiy I, Dyshkant O, Antoniuk A, Gutyj B, Sachuk R. Experimental study of tropism of cultivated canine parvovirus in the immunogenesis organs of puppies. *Reg Mech Biosys*. 2022; 13 (3): 241–246. DOI: 10.15421/022231.
19. Santana WO, Silveira VP, Wolf JM, Kipper D, Echeverrigaray S, Canal CW, Truyen U, Lunge VR, Streck AF. Molecular phylogenetic assessment of the canine parvovirus 2 worldwide and analysis of the genetic diversity and temporal spreading in Brazil. *Infect Genet Evol*. 2022; 98: 105225. DOI: 10.1016/j.meegid.2022.105225.
20. Sato-Takada K, Flemming AM, Voordouw MJ, Carr AP. Parvovirus enteritis and other risk factors associated with persistent gastrointestinal signs in dogs later in life: a retrospective cohort study. *BMC Vet Res.* 2022; 18 (1): 96. DOI: 10.1186/s12917-022-03187-7.
21. Shirokobokova VP. *Medical Microbiology, Virology and Immunology*. Vinnytsia, Nova Knyha, 2011: 952 p. (in Ukrainian)
22. Summa M, von Bonsdorff CH, Maunula L. Pet dogs — a transmission route for human noroviruses? *J Clinical Virol.* 2012; 53 (3): 244–247. DOI: 10.1016/j.jcv.2011.12.014.
23. Venn EC, Preisner K, Boscan PL, Twedt DC, Sullivan LA. Evaluation of an outpatient protocol in the treatment of canine parvoviral enteritis. *J Vet Emerg Crit Care*. 2017; 27 (1): 52–65. DOI: 10.1111/vec.12561.

Особливості культивування *in vitro* польового штаму парвовірусу собак

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Кишкові інфекції залишаються однією із найважливіших проблем охорони здоров'я. За даними ВООЗ, найпоширенішими у світі серед інфекційних хвороб є вірусні діареї. Подібна ситуація реєструється і серед хвороб собак, де ентерити заразної етіології посідають провідне місце, а лідером є парвовірусний ентерит, від якого власники собак потерпають останні сорок років. Незважаючи на такий тривалий термін часу, на жаль, не створено належних засобів лікування і профілактики, а враховуючи стійкість вірусу у зовнішньому середовищі і здатність до мутації, завжди необхідною є кореляція діагностичних методів, що, відповідно, свідчить про актуальність вивчення парвовірусної інфекції у собак. Використання культур клітин як тест-об'єктів для культивування і накопичення вірусної біомаси дає можливість отримання антигена, придатного для використання у виготовлені діагностичних, профілактичних і лікувальних біопрепаратів. У публікації наведено матеріали досліджень особливостей культивування парвовірусу собак, виділеного на території України від безпородного цуценяти віком 2 місяці, який загинув від парвовірусного ентериту. В отриманому від загиблої тварини біологічному матеріалі лабораторними дослідженнями встановлено моноінфікування і можливість його культивування і*n vitro*. Встановлено можливість використання перещеплювальної лінії культури клітин нирки кроля (RK-13), щойно пересіяної, після 24 годинної інкубації, з не менше 70% і не більше 80% моношаром клітин і посівною концентрацією від $1{,}0\times10^5$ до $2{,}0\times10^5$ кл./мл як біологічного тест об'єкта для культивування і накопичення парвовірусної біомаси. Культивування проводили в умовах термостату за t = +37,5°С не більше 10 діб., а як підтримуюче середовище використовували суміш середовища 199 і ДМЕМ. Ефективність культивування визначали за зростанням титру інфекційної активності вірусу, який до п'ятого пасажу становив 3,8±0,08 lg $ТЦД_{50/см^3}$.

Ключові слова: парвовірус собак, польовий ізолят, культуральні властивості, цитопатогенна дія, RK-13

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