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BIOTECHNOLOGICAL SYSTEM FOR THE SEARCH OF SUBSTANCES WITH POTENTIAL ACTIVITY AGAINST CORONAVIRUSES

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Aim. Development of a biotechnological system based on a non-pathogenic coronavirus strain for humans and a sensitive cell line to the selected strain aimed at identifying compounds with potential antiviral activity.

Methods. The study was conducted on the cell lines CEF, CEFs, and BHK-21, which are sensitive to the avian infectious bronchitis virus (IBV). Cell cultivation was performed in flasks and microplates with adhesive surfaces at 37 °C in a 5% CO_2 atmosphere. To detach the cells from the growth surface, a Versene solution (0.02%) was used, and for trypsinization of CEF, a trypsin solution (0.25%) was applied. Growth media for all cell cultures were prepared based on a mixture of RPMI-1640 and DMEM in a 1:1 ratio, supplemented with 5% fetal serum.

Results. The adaptation of the model virus IBV strain H120 to cultivation in BHK-21 cell cultures was carried out using intermediate CEF and CEFs cultures. In BHK-21 cells, IBV induced a pronounced cytopathic effect and demonstrated high infectious titers, reaching 5.5 lg TCID50/mL. The use of intermediate CEF and CEFs cell cultures facilitated the gradual adaptation of the virus to the new cultivation conditions due to the antigenic affinity between chicken embryo fibroblast cells and avian embryos.

Conclusions: As a result of the conducted research, the vaccine virus IBV H120 was successfully adapted to cultivation conditions in BHK-21 cell cultures, using primary trypsinized chicken embryo fibroblast cells as an intermediate system. The obtained system "BHK-21 cell culture + IBV H120", cultivated at 37 °C in a 5% CO_2 atmosphere, can be recommended for use in further biotechnological and virological studies, particularly for evaluating the antiviral activity of potential drugs against coronaviruses.

Key words: coronavirus, IBV H120, CEF, BHK-21, biotechnological system, infectious titer.

The COVID-19 coronavirus infection has become one of the most pressing issues of modern times for humanity. Such situation is directly related to the emergence and pandemic spread of a novel coronavirus, named SARS-CoV-2, identified as the etiological agent of a new infectious disease—Coronavirus Disease 2019 (COVID-19). To study the pathogenesis of this new infectious disease at the level of the whole organism, animal models remain the only adequate option. Among these, small laboratory animals such as mice, rats, hamsters, and ferrets are the most convenient [1].

The search for such animals was preceded by studies involving the infection of SARS-CoV-2 in transgenic HeLa cells expressing ACE2 receptors derived from various animal species (ranging from mice to monkeys) and humans [2]. It was shown that SARS-CoV-2 could use ACE2 receptors from any source for attachment except those of mice. This led to the development of transgenic mice express-

ing human ACE2 [3]. In these mice, SARS-CoV-2 infection resulted in weight loss, viral replication in the lungs, and interstitial pneumonia. In the search for alternative animal models, molecular docking methods were used to investigate potential interactions between ACE2 from different mammals and the SARS-CoV-2 S-protein. The highest compatibility was found for ACE2 of the Syrian hamster [4]. Moreover, the course of SARS-CoV-2 infection in these hamsters was identical to that in humans, making them an adequate model for studying the pathogenesis and transmission mechanisms and for testing etiotropic therapies for coronavirus infection and disinfectants.

When studying SARS-CoV-2 and other human coronaviruses, it is crucial to understand their biological properties and targets for antiviral drugs, antiseptics, and disinfectants. This requires adequate models capable of replicating coronavirus infection both at the level of susceptible cells and the whole organism [5]. In the search for such models, the use of human-safe coronavirus strains, particularly those originating from birds and their ability to adapt and reproduce in animal cells, deserves attention. Additionally, from a bioethical standpoint, priority should be given to using cell lines readily available to practical laboratories. These cell lines should ensure the production of infectious viruses in high titers and the reproducibility of research results. Thus, in developing a coronavirus cultivation model, the "test virus/susceptible cell culture" system will play a significant role, along with the conditions for virus adaptation to *in vitro* cultivation, standardization of cultivation conditions, and consistent evaluation of research results.

The study aimed to develop a standardized biotechnological *in vitro* system based on a non-pathogenic human coronavirus strain for the screening of substances with potential antiviral activity.

Materials and Methods

The avian infectious bronchitis virus (IBV) vaccine strain «H120,» provided as allantoic fluid with a contagious titer of 6.0 lg EID50/ml (IBV1), was used. The strain was obtained from the "Polymun IBV Multi" vaccine (registration certificate No. BB-00038-02-09) with an infectious titer of 6.0 lg EID50/ml, produced by LLC "BIOTESTLAB".

The BHK-21 cell line was established in 1961 from the kidneys of Syrian hamsters. Since this time, this cell line has been a laboratory standard for the growth of countless viruses and the study of many biological processes. BHR-21 is a fibroblastoid adherent monolayer transformed by cultivation for more than 100 consecutive passages *in vitro* [6].The BHK-21 cell line used in the study was obtained from the cell culture collection of the R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology, NAS of Ukraine. Chicken embryos were supplied by a poultry farm in the city of Poltava and incubated at 37 °C with appropriate humidity for 10 days. After candling, eggs with developed embryos were selected and used to obtain primary cell cultures using the trypsinization method.

The study utilized cell culture reagents manufactured by Sigma, USA, including RPMI-1640 nutrient medium with L-glutamine and sodium bicarbonate (R8758-500ML) and DMEM medium with 4500 mg/L glucose, L-glutamine, and sodium bicarbonate (D5796-500ML), as well as fetal bovine serum (F7524-500ML). For detaching BHK-21 cells from the growth surface and for enzymatic trypsinization of chicken embryo fibroblast tissues, 0.02% Versene solution and 0.25% trypsin solution from NVO BioTestMed (Kyiv) were used, respectively. Growth media (GM) for all cell types were prepared using a 1:1 ratio of RPMI-1640 and DMEM media with the addition of 5% fetal serum. Antibiotics were added to the press at final concentrations of 100 IU/mL penicillin and 100 µg/mL streptomycin. The seeding concentration was set at 500,000 cells/mL. Cells were cultured in 50 cm^3 flasks with a growth surface of 75 cm^2, as well as in 24-well and 96-well microplates with adhesive surfaces designed for cell cultures (Sarstedt, Germany).

Work with BHK-21 cell cultures is safe for humans since this is a stable cell line derived neither from humans nor primates. According to the European Directive 2000/54/EC, IBV belongs to Biosafety Level 1 (BSL-1), indicating that the likelihood of human infection is extremely low [7]. Viruses in this group pose no individual or public health risk. Therefore, all work with cell cultures and the virus was conducted in a BSL-1 laboratory. All procedures were performed under aseptic conditions in biological safety cabinets. Class 2A biological safety cabinets and personal protective equipment were used during the experiments. Waste disposal was carried out in accordance with the safety requirements for BSL-1 laboratories.

Results and Discussion

In developing an experimental model of respiratory coronavirus infection *in vitro* cell systems for evaluating compounds as potential antiviral agents, the study was divided into three stages:

1. Selection, cultivation, accumulation, and determination of the infectious titer of the model virus strain (test virus).

2. Selection and cultivation of cell cultures sensitive to the model virus strain.

3. Adaptation of the Infectious Bronchitis Virus (IBV) to in vitro cultivation conditions.

The study concluded with a specific example of utilizing the developed experimental model.

At the first stage of the study, the selection of the test virus was guided by the following requirements for the model virus:

- The selected coronavirus had to be adapted for cultivation in cell cultures under the conditions of a general virology laboratory.
- It needed to induce a characteristic cytopathic effect (CPE) in cell culture.
- The virus had to demonstrate a high yield during cultivation in sensitive cell cultures, with an infectious titer of no less than 5–8 lg TCD50/mL.
- The virus had to be safe for laboratory personnel.

According to literature sources, coronaviruses, which are causative agents of avian infectious bronchitis (IBV), are capable of reproducing in chicken kidney (CK) cell cultures, chicken embryos (CE), Syrian hamster kidney (BHK-21) cells, as well as in human epithelial HeLa cell cultures and some others. However, a high viral yield with an infectious titer was not always observed. In particular, coronaviruses that had previously been cultivated in chicken embryos were not always successfully adapted to culture in cell cultures. It was shown that the studied IBV strains grew well at 37 °C in CK, CE, and BHK-21 cultures and exhibited similar growth curves. The exception was HeLa cells. New virions appeared in the culture medium 4–6 hours after injection, and the maximum viral titers of 106.5–108.5 TCID50 (the dose of the virus that infects 50% of cells in culture) in 0.1 mL were recorded after 36 hours. The cytopathic effect (CPE) of IBV was detected within 24 hours of cultivation. Cultivation conditions, particularly temperature, played a significant role. At 30 °C, no apparent CPE or high viral replication was observed, and not all the studied cultures were sensitive to the virus.

Given the above, a promising model for studying potential anti-coronavirus agents can be considered as chicken fibroblast cell lines and BHK-21. As a model test virus, the infectious bronchitis virus (IBV) should be considered, as work on isolating and accumulating infectious material, unlike SARS-CoV-2 and other human-infecting coronaviruses, can be conducted in a general laboratory setting. IBV belongs to the Gammacoronavirus genus, Coronaviridae family, Nidovirales order [8, 9]. Like other coronaviruses, IBV is a positive-sense RNA virus. It has the largest genome among coronaviruses, approximately 27 thousand base pairs in length. Its structure consists of four proteins: spike glycoprotein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) [10]. Infectious bronchitis is an acute respiratory infectious disease caused by IBV. In addition to affecting the respiratory system, IBV can infect bird organs such as the proventriculus, muscles, ovaries, and kidneys, leading to significant economic losses in the poultry industry.

A comparative analysis of the primary structures of the main protease of IBV (2Q6F) and the main protease of SARS-CoV-2 (7C8B) using NCBI Protein BLAST reveals a significant similarity between the main proteases of IBV and SARS-CoV-2 — 41% sequence identity and 55% sequence similarity. Comparison of the active sites of Mpro IBV and SARS-CoV-2 demonstrates not only a high degree of similarity between the studied enzymes but also the structural similarity of their active sites. Thus, it can be stated that there is significant similarity in the primary and secondary structures of the main proteases of IBV and SARS-CoV-2 coronaviruses [11].

Despite the widespread presence of IBV in host epithelial tissues, most of its strains exhibit limited cell tropism [12]: these strains can only replicate in primary chick kidney (CK) cells, chicken embryo kidney (CEK) cells, and chicken embryos, while some isolates infect only chicken embryos [13]. The restricted cell tropism of many IBV strains complicates their use as model test viruses that are non-pathogenic to humans. Therefore, it is essential to develop biotechnological systems that enable the whole conduct of preclinical studies of compounds with potential anti-coronavirus activity. As a possible model virus for creating such an *in vitro* system, the H120 strain, isolated in 1956 after 120 serial passages of the H strain, was chosen. A distinctive feature of this strain is its ability to replicate exclusively in primary cells, such as CEK and CK [12–14].

In the second stage of the research, the task was to **select a sensitive cell** line in which the IBV virus could replicate, resulting in the formation of characteristic tissue cytopathic effects.

It is known that in humans, epithelial cells of the respiratory tract express targets for coronaviruses, including SARS-CoV-2, among which are the attachment receptor — angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) — a receptor used by SARS-CoV-2 for priming the spike glycoprotein S [5]. In addition to damaging lung tissue, SARS-CoV-2 can affect the kidneys, liver, intestines, and the cardiovascular system. The possibility of infecting the Vero E6 cell culture with supernatant fluid obtained from cultivating SARS-CoV-2 in kidney organoid tissues differentiated from human stem cells has been demonstrated. This result indicates the virus's tropism for kidney tissue and the potential use of such organoids as cellular models for reproducing coronavirus infection [16]. Active replication of SARS-CoV-2 in the liver duct, intestinal, and endothelial organoids has been shown by several other authors [17–19].

Thus, the best cell model for studying model coronavirus infection with the aim of substantiating algorithms and evaluating the effects of bioorganic compounds as potential chemotherapeutic agents can be considered the passaged Syrian hamster kidney cell culture, such as BHK-21.

The adaptation of the IBV virus to *in vitro* culture conditions was carried out in three stages, with constant monitoring of the cytopathic effect (CPE) development and determination of the infectious titer at each stage. The virus- contagious titer in the culture medium was determined by titration based on cytopathic effects and calculated using the Karber method. The quality of the cell monolayers in the control wells without virus addition (CC-cell control) and in cultures during the accumulation and titration of the test virus was evaluated under a light microscope, followed by photographic fixation of native and stained micro preparations.

At the initial stage of adaptation, the virus was grown in a primary trypsinized culture of CEF cells. CEF cells were chosen due to their ability to support the growth and replication of specific agents, particularly viruses that typically cannot develop in continuous cell lines. This property is mainly observed during the early passages of cultivation [20]. The second stage involved cultivation in the subculture of CEFs cells, and the third stage was in the passaged BHK-21 cell line for two passages (Fig. 1). The adaptation was considered successful when stable CPE manifestation and consistent viral infectious titers were obtained.

Preparation of FEC. Under sterile conditions, chicken embryos were dissected, separating muscle and connective tissues, followed by washing with an isotonic solution to remove residual proteins and blood. The washed tissues were subjected to mechanical disruption followed by heat treatment with trypsin for 20 minutes. After trypsin exposure, the suspension was filtered through a filter with pores large enough to allow only single cells to pass. Following filtration, cells were separated from the central suspension by centrifugation and then placed in a combined nutrient medium based on DMEM and RPMI-1640, supplemented with 10% fetal bovine serum and antibiotics. The FEC suspen-

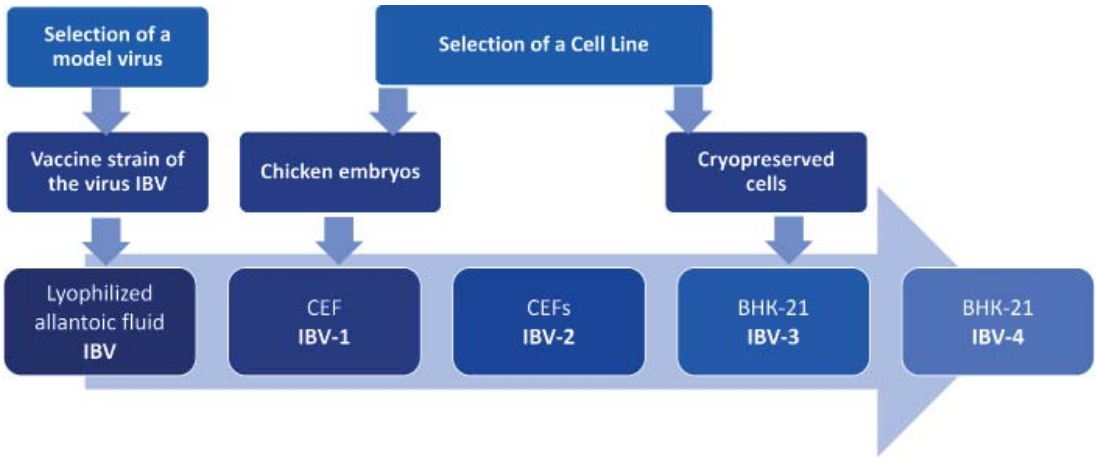


Fig. 1. **Diagram of the adaptation of the vaccine strain of IBV to the conditions of cultivation in the BHK-21 cell culture**

sion at a concentration of 1×10^6 cells/mL was inoculated into culture flasks and cultivated for 48–72 hours to form a dense monolayer of cells, the condition of which was monitored using light microscopy.

To remove residual growth medium, the formed cell monolayer of the FEC culture was washed with phosphate-buffered saline, after which a 0.25% trypsin solution was applied to detach the cells from the growth surface and to dissociate the cells. A suspension of these cells in a growth medium was prepared to reach a seeding concentration of 0.8×10^6 cells/mL, which was then added at 1 mL per well in a 24-well plate and cultured at 37 °C in a 5% CO_2 atmosphere for 24 hours. The quality of the cell monolayer was monitored under an inverted microscope.

The BHK-21 cell line was stored as a cryopreserved sample in liquid nitrogen at a temperature of −196 °C. The cells were revived at 37 °C, and the percentage of viable cells in the suspension was determined using a vital stain. After that, the cells were adapted to culture conditions in a growth medium supplemented with 10% bovine embryo serum throughout 3 consecutive passages, with mandatory microscopy to monitor the cell condition.

Before the start of the study, the BHK-21 cell culture was standardized to a seeding concentration of $0.8–1.0\times10^6$ cells/mL, then added to the wells of a 24-well culture plate and cultured at 37 °C in a 5% CO_2 atmosphere for 24 hours. The formation and quality of the cell monolayers were monitored microscopically under an inverted microscope. For the experiments, cultures were used in which no areas of degeneration or disruption of the monolayer integrity were present.

The studies used the infectious bronchitis virus (IBV) from the IBK-Multi vaccine (registration certificate No. 88-0038-02-13, batch 1519). This live lyophilized vaccine from the allantoic fluid of chicken embryos (CE) specifically contained the H120 coronavirus strains with an infectivity level of more than 3.5 lg EID50 (embryotoxic dose). This virus was sequentially adapted for cultivation in the BHK-21 cell culture, using the first-passage chicken embryo fibroblast (CEF) culture as an intermediate.

For the preparation of IBV H120 for cultivation, the lyophilized vaccine in the vial was completely dissolved in 5 mL of phosphate-buffered saline (PBS). Then, 1 mL of the resulting suspension was applied to fully formed and washed cell monolayers of the CEF culture. The infected cells were cultured at 37 °C for 24–72 hours. The presence of the cytopathic effect (CPE) of the IBV H120 virus was evaluated both in native cultures and after staining the cells using the Romanowsky-Giemsa method. For the preparation of IBV H120 for cultivation, the lyophilized vaccine in the vial was completely dissolved in 5 mL of phosphate-buffered saline (PBS). Then, 1 mL of the resulting suspension was applied to fully formed and washed cell monolayers of the CEF culture. The infected cells were cultured at 37 °C for 24–72 hours. The presence of the cytopathic effect (CPE) of the IBV H120 virus was evaluated both in native cultures and after staining the cells using the Romanowsky-Giemsa method.

After the cultivation was completed, the virus-containing material was collected and labeled as IBV-1, with its infectious titer determined in TCID50/mL. The obtained IBV-1 virus pool was subsequently used to infect the CEFs culture. After cultivating IBV-1 in the CEFs culture under similar conditions, a new virus pool, IBV-2, was obtained. Following two consecutive passages of the virus in the BHK-21 cell culture, the virus pools IBV-3 and IBV-4 were obtained.

It was found that after 24–72 hours of cultivation, the IBV-1 virus exhibited pronounced cytopathic effects in the primary trypsinized CEF cell culture, with its infectious titer ranging from 2.0 to 2.5 lg TCID50/mL. When transitioning to the CEFs subculture, the virus titer increased by 2 lg TCID50/mL (to 4.0 lg TCID50/mL), demonstrating a 100-fold increase in infectious activity.

During the virus adaptation in the passaged BHK-21 cell culture, conducted over two consecutive passages, samples IBV-3 and IBV-4 were obtained. In these samples, the infectious virus titer increased by 3.5 lg TCID50/mL, reaching a maximum level of 5.5 lg TCID50/mL (Fig. 2). Due to its stability and high infectious activity, this strain was selected for further studies of the antiviral activity of test samples in vitro in BHK-21 cell culture.

Thus, the use of an intermediate CEFs cell culture during the adaptation of IBV, initially cultured in a natural biological system such as avian embryos, to another biological system — BHK-21 cell culture — is justified. This approach allows for a gradual alleviation of the differences in cultivation conditions by initially using an antigenically related fibroblast cell culture from

chicken embryos, which is much closer in antigenic composition to that of the chicken embryo, where the virus was adapted in ovo. Later, the virus can be gradually adapted to in vitro cultivation in the BHK-21 Syrian hamster kidney cell culture — a biological system with cells that differ in antigenic composition and growth factors from chicken embryo cells. This adaptation scheme allowed the test virus, IBV H120, to be quickly (within 4 passages) adapted to cultivation conditions in epithelial cells, ensuring the production of the virus with a high infectious titer and standardizing the cultivation conditions.

The cytopathic effect of the IBV-1 and IBV-2 viruses in CEF and CEFs cell cultures, compared to the control culture, is shown in Fig. 3. It is evident that in the samples with CPE, degenerative changes such as cell rounding, detachment from the surface, and lysis are observed, confirming the pathogenic effect of the virus.

The assessment of CPE caused by IBV-3 and IBV-4 in BHK-21 cells showed degenerative changes in the cell monolayer (Fig. 4). For IBV-3, after 24 hours (Fig. 4, *b, g*), satisfactory granular degeneration of cells was observed, manifested by the disruption of intercellular contacts and partial destruction of the monolayer. For IBV-4, after 48 hours (Fig. 4, *e, z*), a similar pattern was noted: the cells lost their defined shape, and a fine cell structure, typical of more severe cell damage, was observed.

The conducted study demonstrated a successful approach to adapting the IBV H120 strain to cultivation conditions in BHK-21 cells using intermediate chicken embryonic fibroblasts (CEF). This ensured a high viral infectious titer of 5.5 lg TCID50/mL and a cytopathic effect in cell cultures. Thus, the "BHK-21 cell line + IBV H120" system can be recommended for further research on potential antiviral agents due to the stability of viral reproduction and the availability of the utilized cell lines.

Comparing our results with other studies, we notable that the presented methodology is faster and less labor-intensive than previously published approaches. For instance, in the study by Jiang et al. (2023), an adaptation method involving 80 virus passages in Vero cells was described to achieve the extended tropism of the IBV HV80 strain [21]. Our proposed use of intermediate CEF cells enabled faster adaptation of the virus to new cultivation conditions while maintaining its infectious activity.

A similar approach was employed by Otsuki et al. (1979), who also used chicken embryonic cells for the adaptation of IBV strains [8]. However, their results demonstrated limited viral reproduction in mammalian cell lines such as BHK-21. Comparing our work with the study by Casais et al. (2003), where IBV was adapted to BHK-21 cells through prolonged passages, we achieved a similar level of viral reproduction with fewer adaptation steps [22]. This result may be attribut-

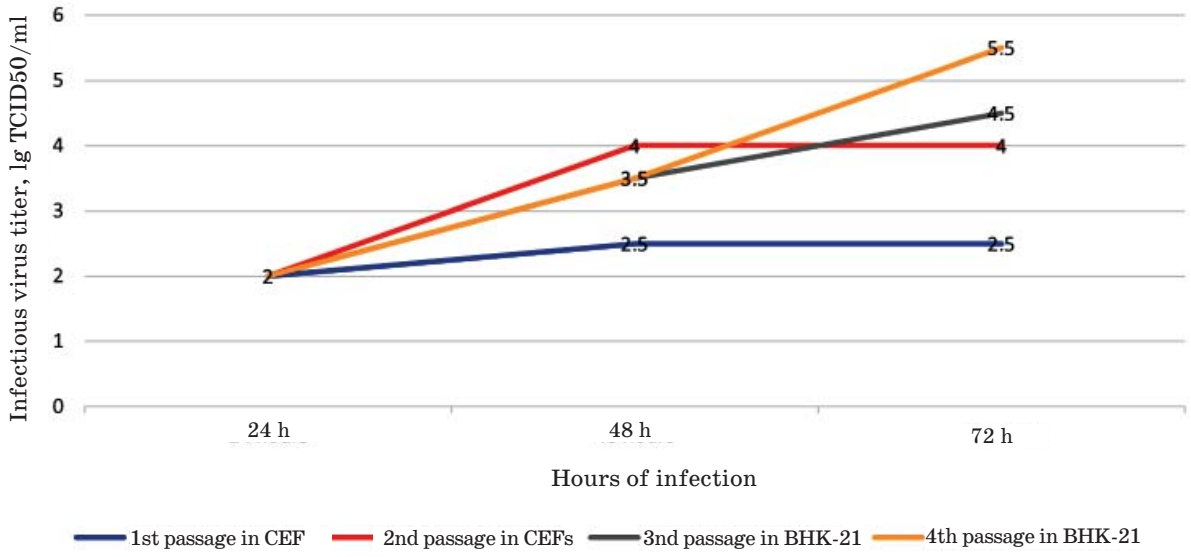
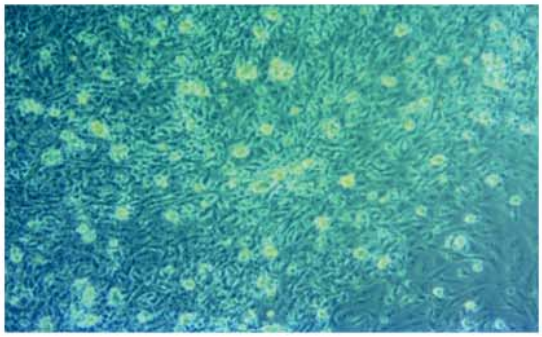
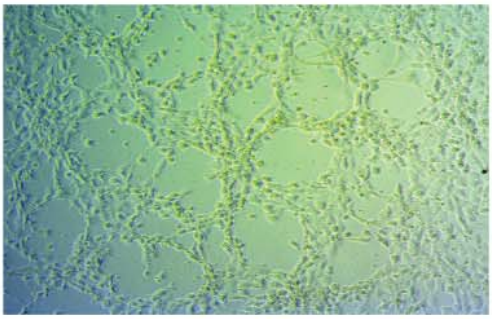


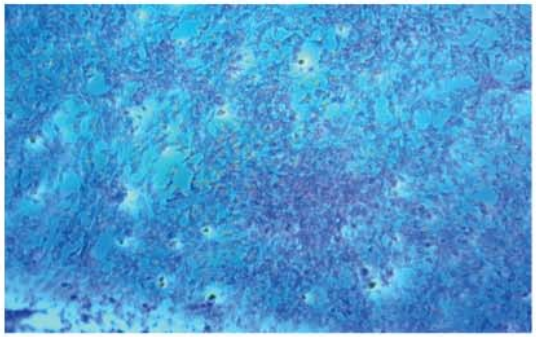
Fig. 2. **Infectious titer of IBV in cell cultures during the adaptation process to *in vitro* cultivation conditions**



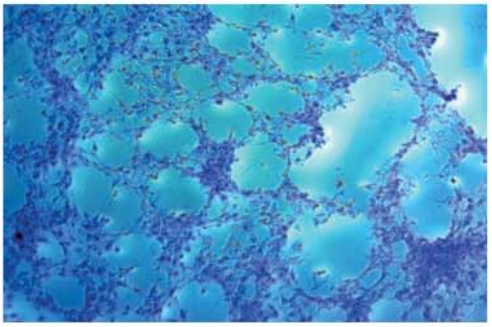
a. CEF cell culture, native preparation (×10)



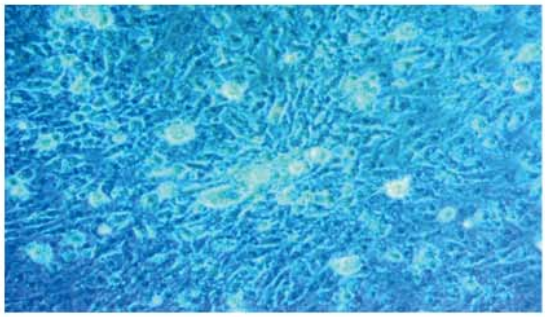
b. IBV-I CPE in CEF cell culture, native preparation (×10)



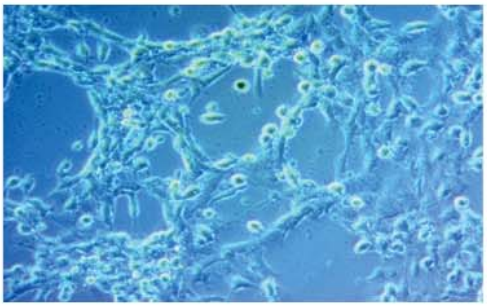
c. CEF cell culture, Romanowsky-Giemsa stained preparation (×10)



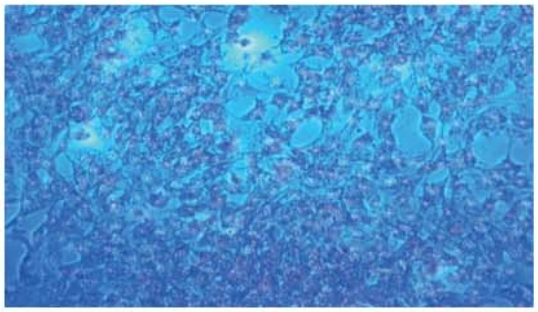
d. IBV-I CPE in CEF cell culture, Romanowsky-Giemsa stained preparation (×10)



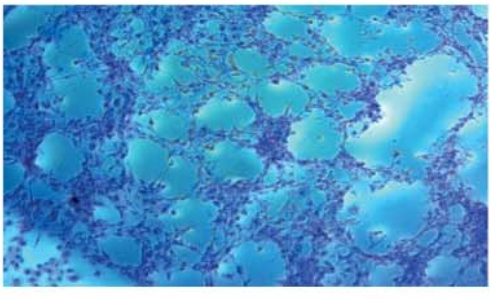
e. CEF cell culture, native preparation (×20)



f. IBV-I CPE in CEF cell culture, native preparation (×20)

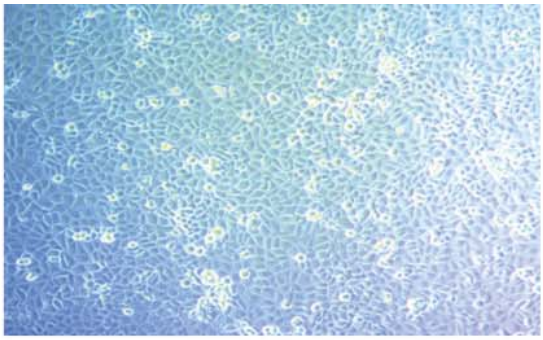


g. CEF cell culture, Romanowsky-Giemsa stained preparation (×20)

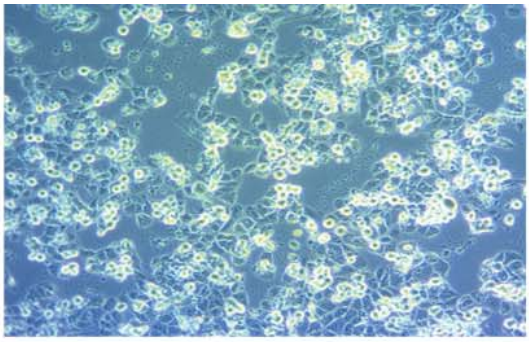


h. IBV-2 CPE in CEF cell culture, Romanowsky-Giemsa stained preparation (×20)

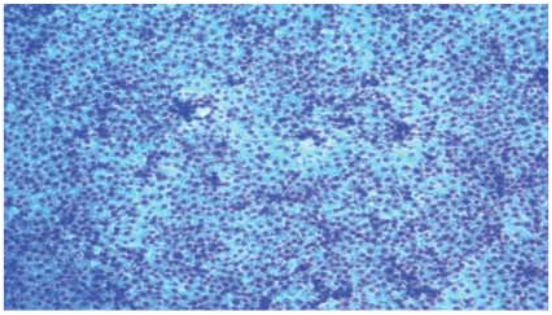
Fig. 3. **CEF cell culture in the control and CPE of the studied virus**



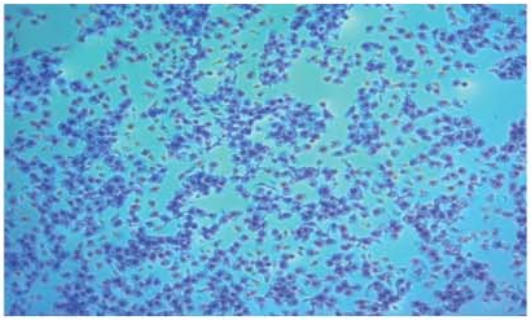
a. BHK-21 cell culture, native preparation (×10)



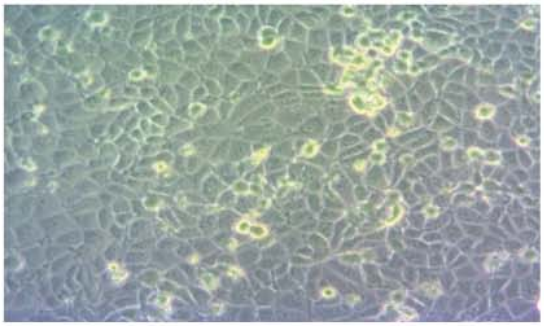
b. CPE of IBV-3 in BHK-21 cell culture, native preparation (×10)



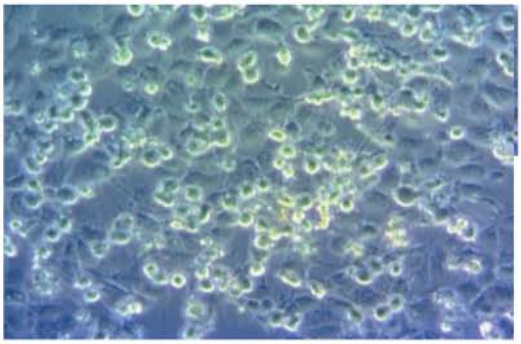
c. BHK-21 cell culture, Romanowsky-Giemsa stained preparation (×10)



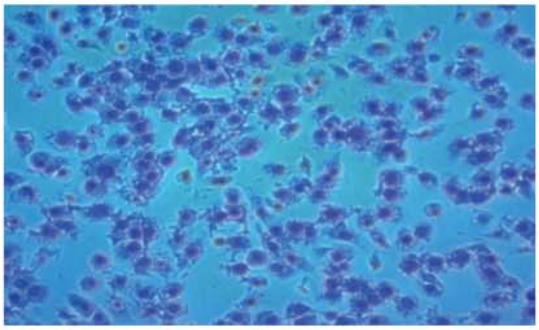
d. CPE of IBV-3 in BHK-21 cell culture, Romanowsky-Giemsa stained preparation (×10)



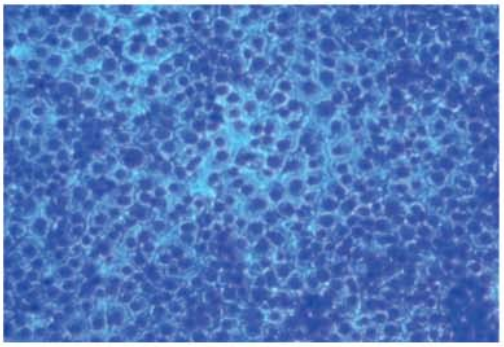
e. BHK-21 cell culture, native preparation (×20)



f. BHK-21 cell culture, Romanowsky-Giemsa stained preparation (×20)



g. CPE of IBV-4 in BHK-21 cell culture, native preparation (×20)



h. CPE of IBV-4 in BHK-21 cell culture, Romanowsky-Giemsa stained preparation (×20)

Fig. 4. **BHK-21 cell culture in control and CPE (cytopathic effect) induced by the studied virus**

ed to the optimization of the nutrient media used (a mixture of RPMI-1640 and DMEM) and the application of an intermediate cell line.

In the study by Bickerton et al. (2018), the role of the S protein in determining the cell tropism of IBV was explored [13]. They identified that mutations in the S protein contribute to the extended tropism of the virus to mammalian cells. Our study did not include genetic analysis of the adapted strain; however, further examination of genomic changes in IBV H120 could provide additional insights into the mechanisms of its adaptation.

Finally, the study by Shen et al. (2004) demonstrated that temperature conditions significantly affect IBV replication in cell cultures [23]. Similarly, our study showed that an optimal temperature of 37 °C and a 5% CO_2 atmosphere are critical for ensuring the stability of viral reproduction.

CONCLUSIONS

As a result of the conducted research, the IBV H-120 virus was successfully adapted to cultivation conditions in the subcultured BHK-21 cell culture, using a primary trypsinized chicken embryo fibroblast culture as an intermediate system. It was established that the IBV H120 strain consistently induces cytopathic effects in BHK-21 cells, manifested as satisfactory granular degeneration of the cell monolayer and the formation of syncytia, and demonstrates high infectious activity. The obtained "BHK-21 cell culture + IBV H120" system, cultivated at 37 °C in a 5% CO_2 atmosphere, can be recommended for use in determining the antiviral activity of substances against coronaviruses.

Author Contributions

Smetiukh M.P. — planning and conducting the research, analyzing the results, and writing the article.

Soloviov S.O. — contributed to the development of the research concept, writing certain sections of the article.

Trokhimenko O.P. — conducting the research, writing certain sections of the article.

Dziublyk I.V. — development of the research concept.

Kamatsky O.A. —design of graphic materials.

Savchuk I.V. — final editing of the article.

Bobyr N.A. — writing certain sections of the manuscript.

All authors agree with the final version of the manuscript.

The authors declare that there are no conflicts of interest.

This article does not contain studies involving humans or animals conducted by any of the authors.

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БІОТЕХНОЛОГІЧНА СИСТЕМА ДЛЯ ПОШУКУ РЕЧОВИН З ПОТЕНЦІЙНОЮ ДІЄЮ ПРОТИ КОРОНАВІРУСІВ

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Мета. Розроблення біотехнологічної системи, в основі якої є штам коронавірусу, який не є патогенним для людини, та чутлива клітинна лінія до обраного штаму, для виявлення сполук із потенційною противірусною активністю.

Методи. Дослідження проводилося на клітинних лініях ФЕК, ФЕКс та ВНК-21, які характеризуються чутливістю до вірусу інфекційного бронхіту курей (IBV). Культивування клітин здійснювали у флаконах та мікропланшетах із адгезивною поверхнею при температурі 37 °С в атмосфері 5% CO_2. Для відділення клітин від поверхні росту застосовували розчин Версена (0,02%), а для трипсинізації ФЕК — розчин трипсину (0,25%). Ростові середовища для всіх культур клітин готували на основі суміші живильних середовищ RPMI-1640 і DMEM у співвідношенні 1:1 з додаванням 5% фетальної сироватки.

Результати. Адаптацію модельного вірусу IBV штаму H120 до культивування в клітинних культурах ВНК-21 із застосуванням проміжних культур ФЕК і ФЕКс. У клітинах ВНК-21 IBV спричиняв виражену цитопатичну дію і демонстрував високі інфекційні титри, які досягали 5,5 lg ТЦД50/мл. Використання проміжних клітинних культур ФЕК та ФЕКс забезпечило поступову адаптацію вірусу до нових умов культивування завдяки антигенній спорідненості клітин фібробластів ембріонів курки з курячими ембріонами.

Висновки. Вакцинний вірус IBV H120 було успішно адаптовано до умов культивування в перещеплювальних клітинах ВНК-21 за рахунок використання як проміжної системи первинно-трипсинізованих клітин фібробластів ембріону курки. Отримана система «культура клітин ВНК-21 + IBV H120», за умов культивування при 37 °С в атмосфері 5% CO_2, може бути рекомендована для використання в подальших біотехнологічних, вірусологічних дослідженнях, зокрема для визначення противірусної активності потенційних лікарських засобів по відношенню до коронавірусів.

Ключові слова: коронавірус, IBV H120, ФЕК, ВНК-21, біотехнологічна система, інфекційний титр.