Study of cell culture strains sensitivity to sacbrood virus

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Abstract. A huge number of cell culture strains obtained from one organ (tissue) of an animal, sensitive to causative agents of various infections, are known. However, a comparative characterization of various cell culture strains sensitivity to such bee viruses as SBV has not yet been carried out. This work was carried out in the Department of Cell Biotechnology and the Laboratory of Bee Diseases of the Federal State Budget Scientific Institution “Federal Scientific Centre VIEV”. For the study, we took cell cultures of the African green monkey kidneys (Vero (3 strains) - Vero (strain from the FSC VIEV Collection)), Vero K (strain from the FSC VIEV Collection), Vero (strain from Germany). We have shown that all 3 of the Vero cell cultures above strains are sensitive to SBV, however, the cell model in which the most pronounced signs of CPD were revealed is the Vero K cell line. The first signs of CPD manifestation virus in the culture of Vero K cells were observed at first passage. After 6 days, s screeds were found in the monolayer after infection. At the second passage of the virus after 5 days, culturing CPD manifested itself as the appearance of voids, cell strands, and rounding of the cells part.

1. Introduction
Nowadays, animal cell cultures have become an integral part of biotechnology. They are used to solve scientific problems of general biology, cytology, genetics, virology, immunology and infectious pathology, as well as in solving many problems of our time [1-10], however, in vitro research is not widely used in the study of pathogens of viral infections in bees to understand the mechanism of their action.

A huge number of cell culture strains obtained from one organ (tissue) of an animal, sensitive to causative agents of various infections, are known. So, there are various strains of calf kidney cells: RBT (sensitive to rinderpest viruses, infectious rhinotracheitis, coronavirus diarrhea, pestivirus diarrhea of calves), PT-80 (parainfluenza virus, infectious rhinotracheitis, viral diarrhea, bovine parvovirus), MDBK (infectious rhinotracheitis virus), parainfluenza-3, viral diarrhea of cattle), Taurus-1 (influenza virus, parainfluenza, cattle adenovirus, infectious rhinotracheitis, adenovirus, diarrhea virus) [3,6]. However, a comparative characterization of various cell culture strains sensitivity to such bee viruses as sacbrood virus (SBV) has not yet been carried out.

SBV is one of the most common pathogens of brood and adult bees’ infectious pathology, which caused serious losses of bee colonies in Thailand, India, Vietnam, China and other countries [11-15]. Scientists have proposed various methods of this disease treatment and prevention [16-19].

For many years, sacbrood virus (SBV) has been one of the most serious problems of modern beekeeping and the economy of the Russian Federation, and measures are being taken in the country to eliminate this infectious disease [1, 2, 20-24].

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We set the task of studying differences in the sensitivity of cell cultures within the same species and tissue to SBV.

2. Materials and methods
The work was carried out in the Department of Cell Biotechnology and the Laboratory of Bee Diseases of the Federal State Budget Scientific Institution “Federal Scientific Centre VIEV”. For the study, from the Specialized collection of transplanted somatic cell cultures of agricultural and commercial animals of the RKKK (P), (SHG) and the cryobank of the FSC VIEV, cultures of the African green monkey kidney (Vero (3 strains) - Vero (strain from the FSC VIEV Collection) Vero K (strain from the FSC VIEV Collection), Vero (strain from Germany) Cultures were maintained using growth medium - IGLA MEM + 5% fetal cattle serum. To remove cells during subculture, a mixture of 0.02% versene solution with 0.25% trypsin solution in a ratio of 9: 1 was used.

To prepare a virus contained suspension, we used dead larvae from a colony of bees with classic signs of sacbrood virus manifestation. The material was ground in a porcelain mortar with saline. The resulting suspension was centrifuged at 10,000 rpm for 10 minutes. After filtration through filters with a pore size of 0.45 µm, the supernatant was used to infect the cell cultures.

Cell cultures were grown to obtain a confluent monolayer. The growth medium was removed from the culture flasks, and infectious material was applied to the cells. After contact of the virus and the monolayer for 1-2 hours, the infecting suspension was removed and a supporting nutrient medium identical to the growth medium was added to the vials. The cultivation was carried out at a temperature of 34 °C. The material was collected after 6-14 days of cultivation. In the obtained samples, SBV was diagnosed by RT-PCR. The material from the previous passage was used for further passage of the virus.

3. Research results
To accomplish this task, we carried out the cultivation of SBV in three strains of the Vero cell line to identify differences in cell cultures of the same species and tissue origin in the ability to manifest signs of cytopathogenic defect (CPD).

1) Vero (strain from the FSC VIEV Collection);
2) Vero K (strain from the FSC VIEV Collection);
3) Vero (strain from Germany).

We found that all 3 of the above-mentioned Vero cell culture strains are sensitive to SBV (figure 1), however, the cell model in which the most pronounced signs of CPD were identified is the Vero K cell line.

Figure 1. Cell culture Vero K. Control culture, 7 days. (n = 3).
The first manifestation signs of the CPD virus in the cell culture Vero K were observed at the first passage. After 6 days, screeds were found in the monolayer after infection. At the second passage of the virus after 5 days, culturing CPD was manifested in the form of voids, cell strands, rounding of a part of the cells (figure 2).

![Figure 2. Cell culture Vero K. 2nd passage SBV, 7 days. Many screeds, destruction of part of a monolayer, many floating cells, pyknosis of nuclei (n = 3).](image)

In Vero cell cultures (strain from the FSC VIEV Collection); and Vero (strain from Germany) showed no evidence of CPD. The presence of the virus was confirmed by RT-PCR.

4. Conclusion
For the first time, a screening of various cell culture strains sensitivity to SBV was carried out. We have determined a comparative characteristic of the Vero culture strains sensitivity. The obtained data can help to better understand the differences among cell cultures of the same species and tissue origin. Dyakonov L.P. et al. (2011) believed that cell culture strains may differ in sensitivity to different groups of viruses. It is necessary to prove the possibility of differentiating cell subpopulations in their manifestation of CPD signs during the cultivation of one or another virus. For this reason, we have conducted a similar study.

During the experiment, it was proved that all three Vero cell cultures were susceptible to SBV, however, signs of CPD were detected in the Vero K strain. In their work, Kweon C. et al. (2015) found that their Vero culture strain was sensitive to SBV without showing signs of CPD. Dyakonov L.P. et al. (2011) found that cell sublines obtained from one organ of different animals are susceptible to different groups of viruses. It can be concluded that cell cultures of the same species and tissue origin are distinguishable in their sensitivity to various viruses [6,25].

Considering the fact that the Vero K cell culture is capable of showing signs of cytopathogenic defects during cultivation of the sacbrood virus, this strain can be recommended for determining its virulence.

The obtained data are not only of interest in the study of the sensitivity of cell cultures to SBV, but also open up opportunities for in-depth study of the sensitivity of cell culture strains to various viruses of vertebrates and invertebrates.

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