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THE USE OF CELL SYSTEMS FOR THE MANUFACTURE OF VACCINE AGAINST FOWL POX

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Abstract. The work demonstrates the possibility of growing and propagating the fowl pox virus of the birds of the Baku strain in the primary culture of Japanese quail embryo cells. It was found that the fowl pox virus of the bird strain "Baku" is adapted to both cell systems, an increase in the titer of fowl pox virus was observed both in the culture of Japanese quail embryo cells and in the culture of chicken fibroblast embryos. It was found that the culture of the cells of the Japanese quail embryo compared with the culture of the cells of the chicken embryo is a profitable tissue culture for the production of vaccines because of its simplicity, economic availability, lack of extraneous pollution and the stability of biological properties. It has been established that the culture of cells of Japanese quail embryos is a promising system for creating highly immunogenic, specific prophylaxis against fowl pox.

Keywords: *fowl poxvirus, primary cell culture, strains viruses, vaccine.*

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1. Introduction

Birds Fowl pox is quite widespread disease and creates large problems for industrial poultry, causing significant economic damage to poultry farms. Clinical signs may vary according to host susceptibility, virulence of the virus strain, and lesion distribution The disease has been described in chickens, turkeys, pigeons, ostriches, quails, pheasants, and canaries, and the emergence of outbreaks is associated to environmental conditions, vector populations, and the habits of the affected species (Da Silva *et al.,* 2009). In addition to commercial poultry production, small farmers are affected. Despite the increase in the number of backyard chicken farms in the last few years, little attention has been given to the health care and diseases of these birds (Da Silva *et al*., 2009).

To protect birds from fowl pox viruses, timely vaccination is important. Fowl Pox Vaccine is for vaccination of healthy chickens. When used as indicated, it will aid in preventing the clinical signs caused by the virulent field strains of fowl pox virus. For example, the known data, about However, quail, chickens, and turkeys vaccinated with quail pox virus were protected against quail pox virus challenge. An isolate of psittacine pox virus, applied as a vaccine, protected chickens against challenge with the same virus isolate and also against challenge with two other psittacine pox virus isolates, confirming a close or identical antigenic relationship with each other. When combined in a multivalent vaccine, quail, psittacine, and fowl pox viruses induced excellent protection in chickens against challenge with the three respective viruses (Winterfield & Reed, 1985).

The large size of the genome fowl pox virus makes it a useful vector for recombinant DNA technology. Although the disease has been described in both commercial and indigenous chickens in Nigeria, data are limited on seroprevalence in free range chickens. Such data are, however, important in the design and implementation of fowl pox virus vector vaccine (Adebajo *et al*., 2012). Scientific studies are known, the oral vaccination against Fowlpox was investigated via drinking water containing the F132-c strain of fowl pox virus, per single drinking water vaccination, 90% or more of chickens were not protected, however, they were protected when vaccinated twice via drinking water. A weak immune response occurred by a slight infection after the first vaccination, and due to memory cells, a booster could work well after the second vaccination. These results suggest the possibility of reducing both the amount of virus required for a vaccine and the labor cost in the field (Ariyoshi *et al*., 2003). For example "*Israeli*" vaccine strain of fowl poxvirus grows efficiently in chicken embryo fibroblasts but not in cell lines derived from monkey kidney or human fibroblasts. In assays for the titration of the infectivity of this virus in secondary cultures of chicken embryo fibroblasts, a good correlation of the infectivity titers of the virus was obtained (Hashavya *et al*., 2002).

Fowl Pox Vaccine produced in most countries is a chicken embryo propagated, freeze-dried, live virus vaccine for chicken. But in modern veterinary medicine, vaccines based on cell cultures have found widespread use which is important.

Designed by in Azerbaijan VCRI by F.B. Shirinov and A.N. Gojaev in 1985, the embryonic vaccine against fowl pox of chickens from the strain "Baku", grown on chicken embryos, caused the insufficiently perfect technology of its production (Yusifova, 2018). It is already known that cultivation of fowl pox virus in chicken embryo cell cultures is common in preparing biomass for vaccine production. It is also known that the fowl pox virus causes a cytopathic effect in cell culture, and with prolonged passage it loses contagiousness to the bird, but retains immunogenic properties. Last year's cultural vaccines against fowl pox of birds, did not inferior in immunogenicity to embryonic preparations. The disadvantage of embryonic viral vaccines against birds fowl pox is a low level of infectious activity in chickens (Yusifova, 2018). In this regard, the search for fowl pox virus strains in birds adapted to a cell culture with high infectious and immunogenic properties is relevant for the production of modern drugs. Determining the sensitivity of primary cell cultures to fowl pox virus, monitoring the dynamics of accumulation of poxvirus in cell culture is relevant in modern veterinary medicine. Researchers use different cell culture and different strain of viruses to improve vaccines (Yusifova, 2017). This is important to increase the effectiveness of vaccination against infected birds. In our article it is described at a cultivation of the strain "Baku" of fowl pox virus in the culture of quail chicken embryo cells and fibroblast cell culture chicken in order to obtain highly pathogenic antigen. We carried out successive passages of fowl pox virus on primary cell systems to preserve and increase its antigenic properties. Similar study we can observe in works of some researchers as a part of vaccines against different infected animals and fowl pox of birds.

Each country uses its local vaccine strains to produce vaccines. The "*Hitchner*", "*Calnek*" and "*Cutter*" strains are used in the production of "Pox blen" associated vaccines. So, company Avipro for vaccines use "*P", "C", "Chicken PV*" strains viruses. The composition of the Russian vaccines against fowl pox birds includes strains "*K", "Ospovak", "27-ASh*", "*ND*" (Safarov *et al*., 2016). In Azerbaijan and other

countries were registered many vaccines against fowl pox. For example, in 2002 company "Nobilis" (Holland) registered attenuated vaccines against fowl pox. For the vaccines company "Nobilis", used the strain viruses "FPC". 2009 - "Avipro" (German) vaccines is made from chicken embryos infected with fowl pox viruses, strain viruses "*HP-B*" and the strain "FPC", 2011 – "*Gallivac*" (France) the strain "*Cutter*" is widely used in the production of associated vaccines. Strain viruse "*Cutter*" - used in the production of associated vaccines "Cevac". 2014 Company "Avivak" (Russia) used the embryo vaccine against fowl pox which is made from a live attenuated strain of chicken pox virus strain "*K*" (Yusifova, 2017, 2019).

The vaccines described above are prepared on an embryonic basis, but it is important to note that vaccines prepared on the basis of cell cultures are more profitable from an economic point of view. Therefore, many manufacturers began to produce vaccines based on cell systems. Considering what was said in our studies, we cultivated a local strain of the birdpox virus in strain "Baku" in a cell culture.

Currently researchers use mostly local strains to improve vaccines. This is important to increase the effectiveness of vaccination against fowl pox. The study in this article is aimed at a comparative analysis of the strain "Baku" of fowl pox virus adapted to the culture of quail chicken embryo cells and fibroblast cell culture chicken in order to obtain highly pathogenic antigen.

To this end we carried out successive passages of fowl pox virus on primary cell systems to preserve and increase its antigenic properties.

2. Materials and methods

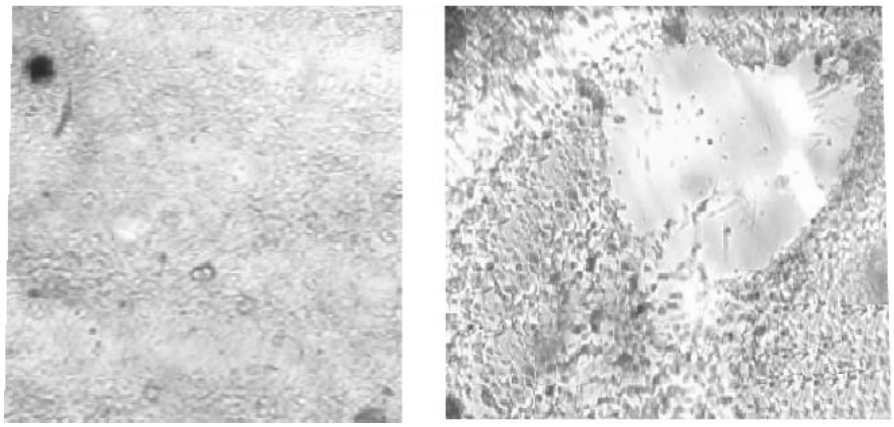
Cell cultures were prepared by the standard trypsinization method. The sensitivity of the cell culture was determined by infecting it with the fowl pox virus of birds in dilutions of up to 10^{-4}. Changes in cell culture were detected by microscopy. During the cultivation, the timing of the onset and the nature of cytopathic effect were determined. The titer was determined by the method of Reed and Mench, ($EID_{50/ml}$), and in the RHA reaction, the virus titer was respectively expressed in $HAU_{50/0,5мл}$. (Yusifova, 2019)

3. Results and discussion

We conducted several passages of fowl pox virus on primary cell cultures of Japanese quail embryos and chicken fibroblasts.

The biological properties (the time of adsorption of the fowl pox virus in the primary cell systems of Japanese quail embryos and chicken fibroblasts) of the virus were studied. Optimal conditions for the propagation of fow pox virus were determined in the primary cell systems of quail embryos and in the cellular system of chicken embryos. The cytopathogenic effect of fowl pox virus in cell systems was observed under a microscope. Studies have shown that the cytopathic effect in cell cultures of chicken fibroblast embryos can occur on the first 3 days after fowl pox virus infection.

The cytopathic effect in the culture of chicken fibroblast embryo cells can be observed at passage 3-5, in the culture of Japanese quail embryo cells can be detected at passage 10 on day 5 after infection. In both cell cultures, cell destruction was observed for the first 48 hours. In the next 72 hours, the cytopathogenic effect is evaluated as "+ +" and "+ + +".



a b

Fig 1. Culture of quail embryo cells.
a) Not infected the poxviruse. b) Are infected the poxviruse

The cytopathic effect of viruses is equally evident in both chicken embryo cell culture and quail embryo cell culture. But the time of onset of the cytopathic effect in cell cultures was not the same. The cytopathic effect of the "*Baku*" strain of fowl pox virus in the chicken embryo cell culture was observed 96 hours after infection, and in the quail embryo cell culture, 144 hours after infection.

4. Conclusion

The research results showed that the fowl pox virus of the strain "*Baku*" has adapted to both cell systems. We observed an increase in the titer of fowl pox virus both in the culture of Japanese quail embryo cells and in the culture of chicken fibroblast embryos. In both cultures, the virus titer was 256-512 GAE / 0.5 ml. It should be noted that quail embryos are not contaminated by any infections, which cannot be said about chicken embryos. For the most part, chicken embryos are infected with various infections, which complicates the production of cell systems, and in turn, the production of vaccines. As a result of this, we gave preference to the culture of the coyotes for quail embryos, which are less contaminated by any infections.

As a result of studies, it was found that the cell culture of the Japanese quail embryo compared with the chicken embryo cell culture is a profitable tissue culture for vaccine production because of its simplicity, economy, lack of extraneous contaminants and the stability of biological properties. It was established that the cell culture of Japanese quail embryos is a promising system for creating highly immunogenic specific prophylaxis against fowl pox.

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