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# ALLOCATION METAPNEUMOVIRUS IN CELL CULTURE

**Annotation**

In the results is shown the interaction of metapneumovirus of birds with different types of cell cultures. Picked the most reproductive type cell cultures, depending on the extent of damage and the amount of monolayer viral agent in the titration.

**Keywords:** Metapneumovirus birds, strain «K-32-13», cell culture Vero.

## Introduction

Metapnevmovirus infection of birds (MPIB) was first registered in South Africa in the late 1970s. and for a short period of time covered a number of countries with developed poultry industry. Serological and virological studies indicate widespread MPIB in Kazakhstan, mainly in poultry farms in the central and south-eastern regions of the country [1, 2].

The main difficulties when dealing with MPIB is short period persistence and limited tropism of the virus in birds [3,4]. Interaction of the virus and the cells remains an urgent problem as the solution of which depends on many issues of diagnosis and immunization infection. Reproduction MPIB virus on sensitive cell lines is the main producing specific antigens for the production of diagnostic kits and vaccines.

The aim of our research is to study the reproduction of metapneumovirus (MPIB) birds in homologous and heterologous cell cultures and determination of strain differences in the virus.

## Materials and methods

The experiment performed in the virology laboratory at the Department of «Biosecurity» KazNAU laboratories «Prevention extremely dangerous animal diseases» RSE «Research Institute for Biological Safety Problems» and serological laboratory LLP «UniVet».

In the experiment used the following materials:

Virus: the vaccine strain Nemovac Rhone Merieux (France); PL21 pneumovirus (GP Wilding, 1986), Clone-13 strain PL21 metapneumovirus of birds, K-32-13 highlighted by us from poultry farms KazGER epizootic strain of avian metapneumovirus. Viral strains were maintained in transplantable line Vero cells and stored at -20 ° C.

Diagnosticums: For the detection of antibodies to the pathogen infection metapnevmovirus of birds will use a set BioChek, produced by «Avian Rhinotracheitis Antibody TestKit» (Holland);

Cell culture: primary trypsinized fibroblast culture - FEC and transplantable cell cultures: BHK-21 cells and baby hamster kidney cells Vero-African green monkey kidney.

Nutrient media, serum, and solutions of reagents: culture medium Eagle's MEM and DMEM liquid with L-glutamine; nutrient medium number 199, the liquid, with L-glutamine; DMEM /F12 culture medium with Hepes, liquid, blood serum of cattle, unpreserved for cell cultures, fetal bovine serum;

trypsin solution, 0.25% of the company «US BIO»; versene solution, 0.02%; Hank's solution company «Hyclone». Culture media and solutions with full component mixture of firm «Hyclone» produced by «Biolot».

Titration of virus in cell culture were performed in a sensitive cell culture (the cells Vero) by ten-fold dilutions with appropriate controls. Titer values were calculated by the method of L.J. Reed and H. Muench and expressed lg TD 50 in 1.0 cm3.

Neutralization reaction: reaction set the standard, the classical method described in the manual in virology [5]. The titer of the virus and the neutralization endpoint was calculated according to L.J. Reed & H. Muench based on the CPP in the cell culture after 5-6 days of incubation the culture after inoculation. Neutralizing activity of the serum neutralization is expressed in the index, which is a performance difference of the logarithms of virus titer in the presence of specific and normal sera. In one liter is taken greatest dilution of serum that was capable of inhibiting the activity of the virus made by the indicated dose in 50% of infected cultures.

**Results**

For virus isolation from a biological source material (nasopharyngeal aspirates, tracheal Filter sinuses) suspension was prepared in Hank's solution containing antibiotics accepted concentrations and incubated at room temperature (210 C) for 1.5 hours. The suspension was then centrifuged at 3000 rev / min for 15 minutes.

The results presented in Table 1 demonstrate that MPIB adapted to isolate primary cultures were trypsinized FEC after the 2nd passage.

Table 1 – The results of infection of primary cultures were trypsinized FEC isolate «K-32-13» (n=3)

| № passage | CPP | Time of cultivation, h | Titer of virus lg TD 50/ml. |
|---|---|---|---|
| 1 | + | 120 | 2,10±0,12 |
| 2 | +++ | 96 | 4,42±0,38 |
| 3 | ++++ | 96 | 7,24±0,14 |
| 4 | ++++ | 72 | 7,62±0,13 |
| 5 | ++++ | 48 | 7,86±0,23 |

The experiments on adaptation to isolate MPIB primary trypsinized culture FEC show that this cell line can be used as a system for the reproduction of the virus MPIB. Sensitivity to isolate MPIB transplantable cell cultures Vero, BHK-21 was studied using the method of successive passages, identifying the infectious activity of the virus at every level the transit.

The results are shown in Table 2. These data indicate that the most susceptible to infection with the virus MPIB cells were Vero.

Table 2 - Study of the properties of the culture isolate "K-32-13" virus MPIB on continuous cell cultures

| № | Culture cells | The biological activity of the virus, lg $TD_{50}$/см$^3$ | | | |
|---|---|---|---|---|---|
| | | Passage 1 | Passage 2 | Passage 3 | Passage 4 |
| 1 | BHK-21 | 2,75±0,21 | 4,12±0,13 | 4,87±0,15 | 4,18±0,14 |
| 2 | VERO | 4,87±0,16 | 6,72±0,00 | 7,73±0,18 | 7,52±0,25 |

The results of titration MPIB strain «K-32-13» in a continuous culture of Vero cells indicate that the adaptation process infectious virus activity gradually increased and reached the level of the transit 4 7,52 ± 0,25 lg TD50/cm3. In the culture of BHK-21 cells the number of cells exposed to the virus in the next CPP cultivation periods, not increased. In determining the ability of the virus to accumulate in the above cultures infectious activity was low and was within 2,75-4,18 lg TD50/cm3. Therefore, as the culture system was chosen for MPIB continuous culture of Vero.

For the primary isolation and subsequent ada ptation isolate MPIB birds used primary cultures were trypsinized cells obtained from 10-12-day-old chicken embryos and FEC Vero, BHK-21 cells were transplanted.

Primary-trypsinized cell culture prepared from the skin and muscle tissue of chicken embryos by dispersing in 0.25% trypsin solution. The culture was grown in stationary conditions for 2-3 days until the formation of a smooth dense monolayer in a nutrient growth medium and Eagle's MEM medium number 199 in a 2:1 ratio with a content of up to 10% of cattle serum and antibiotics.

Before infecting the test tube were released from the cell culture growth medium, washed with maintenance medium without serum were added pathological material of the slurry (or chorioallantoic fluid of infected chicken embryo) in a volume of 0.2 cm $^3$, and the culture was left at (37,5 ± 0,5) ° C for 60 minutes for virus adsorption. Tubes then with infected culture have made 1.0 cm $^3$ supportive environments. Infected cultures were incubated for 7-9 days at a temperature (37,5 ± 0,5) ° C until pronounced cytopathogenic effect of the virus. Isolate the virus causes an acute form of viral infection with a characteristic cytopathic effect: cell rounding and appearance of grain in them in certain areas of the monolayer cells at 2-3 days after infection, culture; disintegration of cell monolayer and a significant part of the occurrence of discontinuities in it; complete degeneration of the monolayer and the formation of syncytial complexes.

As a result of 4-5 consecutive passages of pathological material (nasal discharge, nasal mucosal scrapings, head sinuses and trachea) on Vero cells or chicken embryos, followed by an adaptation of cells to Vero, highlighted one virus isolate K-32-13-inducing cytopathic effect in cell cultures (Figure1).

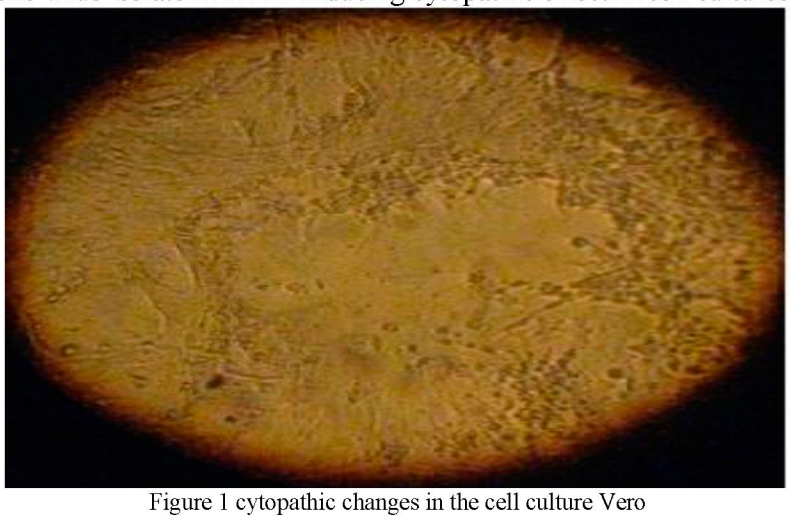

Figure 1 cytopathic changes in the cell culture Vero

For the primary isolation and subsequent adaptation isolate MPIB birds used primary cultures were trypsinized cells obtained from 10-12-day-old FEC chicken embryos and BHK-21 cells transplanted Vero.

This virus isolate after the 3rd passage cause severe viral infection with a characteristic cytopathic effect: cell rounding and appearance of grain in them in certain areas of the monolayer cells at 2-3 days after infection, culture; disintegration of cell monolayer and a significant part of the occurrence of discontinuities in it; complete degeneration of the monolayer and the formation of syncytial complexes.

**Conclusion**

As a result, 4-5 successive passages of pathological material (nasal discharge, nasal mucosal scrapings, sinuses of the head and the trachea), and on cells of FEC and transplantable cells Vero, one isolated virus isolate K-32-13, causing a cytopathic effect in cell cultures.

REFERENSES

1. Assanov N.G., Sansyzbay A.R., Mussoev A.M (2012) International Conference pp. 153-156 Uralsk.

2. Assanov N.G., Sansyzbay A.R., Mussoev A.M (2013) Serological aspects of avian metapneumovirus infection in Kazakhstan Annual 19th International Scientific Conference Research for Rural Development 2013. Latvia University of Agriculture, Jelgava, Latvia 15 - 17 May 2013 pp.147-150.

3. Borisova I.A. Metapnevmovirusnaya infection of birds / Borisova I.A., Borisov A.B // RatsVetInform. 2008. - № 12. - pp. 9 - 11.

4. Sensitivity of cell cultures to metapneumovirus birds / B.B.Trefilov, LY Danko // Questions regulatory veterinary medicine. - 2010. - № 1. - pp. 44-46.

5. Galushin V.M., Marfenin N.N. Russian and international recommendations on principles sobledeniyu Biotec 2011.

ЛИТЕРАТУРА

1 Асанов Н.Г., Сансызбай А.Р., Мусоев А.М (2012) Құстың инфекциялық ринотрахеиті Халықаралық ғылыми конф. материалдары Орал қ. б.153-156.

2 Асанов Н.Г., Сансызбай А.Р., Мусоев А.М (2013) Серологические аспекты инфекции птичьего метапневмовирус в Казахстане ежегодной 19-й Международной научной конференции исследованиям в целях развития сельских районов 2013 Латвийский сельскохозяйственный университет, Елгава, Латвия 15 -. 17 мая 2013 рр.147-150.

3 Борисова И.А. Метапневмовирусная инфекция птиц /И.А.Борисова, А.В. Борисов // РацВетИнформ. 2008. - №12. - С.9 - 11.

4 Чувствительность клеточных культур к метапневмовирусу птиц / Б.Б.Трефилов,

Л.Ю. Данко// Вопросы нормативно-правового регулирования в ветеринарии. – 2010.- №1.- С.44-46.

5 Галушкин В.М., Морфенин Н.Н. Международные и российские рекомендации по соблюдению принципов биотэки 2011 г.

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МЕТАПНЕВМОВИРУСТЫ ЖАСУША ӨСІНДІЛЕРІНДЕ БӨЛІП АЛУ

**Резюме**

Құстың метапневмовирусының әр-түрлі жасуша өсінділеріндегі өсу нәтижелері келтірілген. Жасуша өсіндісінің дара қабатының бұзылу сатысы және вирусты титрлеу, жасушаның өсінділік көрсетуі анықталған.

**Кілт сөздер:** Құстың метапневмовирусты инфекциясы, «К-32-13» штамы, Vero жасуша өсіндісі.

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ВЫДЕЛЕНИЯ МЕТАПНЕВМОВИРУСА НА КЛЕТОЧНЫХ КУЛЬТУРАХ

**Резюме**

Представлены результаты взаимодействия метапневмовируса птиц с различными типами культур клеток. Определена наиболее репродуктивная тип культур клеток в зависмости от степени поражения монослоя и количеством вирусного агента при титровании.

**Ключевые слова:** Метапневмовирус птиц, штамм «К-32-13», культура клеток Vero.

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