Determination of Growth Characteristics of the Foot and Mouth Viruses (A, O, Asia-1) in Bhk-21 An_{30} and Bhk-21 An_{73} Cell Cultures

Research Article

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Abstract

In this study, it was aimed to investigate BHK-21An_{73} and BHK-21An_{30} growth rate of cell cultures and the effects on foot and mouth disease (FMD) vaccine virus strains titers. For this purpose, growth rate of suspended cell cultures of BHK-21An_{73} and BHK-21An_{30} were determined. A TUR 11, O TUR 07 and Asia-1/11 strains of foot and mouth disease (FMD) vaccine virus strains were produced separately on BHK-21An_{30} and BHK-21$An_{73.}$ BHK-21An_{30} cell amounts during seven incubation days of reached to peak level in monolayer form at the 4th day with $1,3x10^6$/ml cell numbers, in suspension form at the 4th day with $4,1x10^5$ cell numbers. BHK-21An_{73} reached to peak level in monolayer form at the 6th day $2,2x10^6$/ml cell numbers while in suspension form at the 3rd day with $2,8\ x10^5$ cell numbers. After the production of A, O and Asia-1 vaccine viruses, in suspension BHK-21An_{30} cell cultures average of 146S values respectively; 0,51 µg/ml, 0,18 µg/ml and 0,16 µg/ml were detected while infective titers were determined as average 6,87 (Plaque Forming Units) pfu/ml, 6,22 pfu/ml and 6,49 pfu/ml. After the production of A, O and Asia-1 vaccine viruses, in suspended BHK-21An_{73} cell cultures average of 146S values respectively; 2,11 µg/ml, 2,59 µg/ml and 0,53 µg/ml were detected while infective titers were determined as average 6,99 pfu/ml, 7,82 pfu/ml and 6,37 pfu/ml. As a result, in the production of A, O and Asia-1 vaccine viruses in BHK-21$An_{73,}$ resulted in higher 146S values and higher infective titers than BHK-21$An_{30.}$ It is concluded that for FMD vaccine production the using of BHK-21An_{73} cell culture is more appropriate for both of cost of manufacture and productive time.

Key Words: Foot and Mouth Disease, FMDV vaccine strains, BHK-21 cell culture.

Introduction

Foot and Mouth Disease (FMD) is the most highly contagious diseases of animals and Foot and Mouth Disease Virus (FMDV) quickly replicates and spreads within the infected animal, among incompact susceptible animals and by aerosol (Grubman et al., 2004).

FMD is categorized by OIE (World Organization for Animal Health) as an OIE List A disease, which by definition, means that it has the potential for rapid and widespread within and between countries and can cause severe economic impact. (Alexandersen et al., 2003; Radwan et al.2016). Therefore in countries where the disease is endemic, cattle are regularly vaccinated against FMD (Barteling & Vreesvijk, 1991). Seven distinct serotypes of FMDV with indistinguishable clinical effects have been defined, namely types O, A, C, Southern African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1. One serotype will not protect against subsequent infection with another (Alexandersen et al., 2003; Radwan et al., 2016).

Today, most vaccine, in monolayer or suspended BHK-21 cells, is produced in laboratories with suitable bio-safety standards. Vaccination has an important role in the fight against foot and mouth disease and millions of doses of vaccine are produced in the world. The mail goal in vaccine manufacturing industry is to produce the most economic, the most practical and the maximum amount antigenic component (Bartelling, 2002).

In this study, it was aimed to investigate BHK-21 An_{73} and BHK-21 An_{30} growth rate of cell cultures and the effects on foot and mouth disease vaccine virus strains (FMDV) titers.

Materials and Methods

Cell Culture

Baby Hamster Kidney Cells (BHK-21 cell line) were used by subclonning the BHK-21 cell culture various cell lines clones with different sensitivities to the FMDV were determined. In our country these lines BHK-21 An_{30}, is used for the production of foot and mouth virus. BHK-21 cell line which brought to our cell bank from Institute of Brescia in Italy and designated as BHK-21An_{73} Cells were grown in Glasgow Minimum Essential Medium containing 10% fetal calf serum (FCS) and 0,01% penicillin-streptomycine-neomysinesulphate at 37 ^{0} C in a CO_2 incubator for 48 h.

Viruses

A tur 11, O tur 07 and Asia-1-11 strains were used in the study.

Determination of the cell number of BHK-21An_{73} and BHK-21An_{30} cells and calculate the growth kinetics of cell cultures

Monolayer cells were subcultured three times and $4x10^5$ h/ml cell were provided. Cell numbers and morphology were examined in every 24 hours. Growth kinetics of cells was determined with cell count by using BürkerChamber.

Virus seeding

Virus seeding was performed after monolayer cells are adapted to the suspended cells. A, O and Asia-1 cultures that were produced were freezed/thawed 3 times at -70 ºC and centrifuged at 3000 rpm.

Identifying infectious titer

Infective titers were determined by plaque test (Institute of FMD Protocol, 2010).

Determination of amount of 146S

The amount of 146S was determined with Sucrose Density Gradient test (Institute of FMD Protocol, 2010).

Each analysis was performed three times, and the mean values were calculated.

Also BHK-21An$_{30}$ and BHK-21An$_{73}$ were compared.

Results

Cell numbers of monolayer BHK-21An$_{30}$ cell cultures that passaged during 7 days were investigated. The cell numbers at 4th day reached peak level. Numbers of cells were decreased after 6th day as a result of cell degeneration (Table 1).

When the cell numbers of monolayer BHK-21An$_{73}$ cell cultures that passaged during 7 days were investigated, the cell numbers at 6th reached peak level. On the 7th day of culturing, cell numbers were decreased because of cell degeneration (Table 1).

Cell numbers of suspended BHK-21An$_{30}$ cell cultures that passaged during 6 days were investigated. The cell numbers at 4^{rth} day reached peak level. Numbers of cells were decreased after 5th day as a result of cell degeneration (Table 2).

Cell numbers of suspended BHK-21An$_{73}$ cell cultures that passaged during 6 days were investigated. The cell numbers at 3rd day reached peak level. Numbers of cells were decreased after 4th day as a result of cell degeneration (Table 2).

Table 1. Daily cell increasing kinetics of monolayer BHK-21An$_{30}$ and BHK-21An$_{73}$

Number of Cells	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
An$_{30\ average}$	1,1 x10^5	2,5 x10^5	9,3 x10^5	1,3 x10^6	1,3 x10^6	1 x10^6	6 x10^5
An$_{73\ average}$	1,1 x10^5	5,4 x10^5	1,4 x10^6	1,7 x10^6	1,9 x10^6	2,2 x10^6	1 x10^6

Table 2. Daily cell increasing kinetics of suspended BHK-21An$_{30}$ and BHK-21An$_{73}$

Number of Cells	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day
An$_{30\ average}$	1,4 x10^5	2,6 x10^5	3,1 x10^5	4,1 x10^5	3,4 x10^5	2,4 x10^5
An$_{73\ average}$	1,4 x10^5	2,8 x10^5	2,8 x10^5	2,7 x10^5	1,3 x10^5	1,1 x10^5

100% cytopathogenic effects (CPE) were observed in A tur 11, O tur 07 virus strains inoculated BHK-21An$_{73}$ cell cultures were completed their growth cycle in 17 hour. 100% CPE were observed in Asia-1-11 virus strain inoculated in suspended BHK-21An$_{73}$ culture was completed own growth cycle in 22 hour. A, O and Asia-1viruses inoculated in suspended BHK-21An$_{30}$ cultures were completed their growth cycle in 41hour.

The average amount of viral particules, 146S, in suspended BHK-21 An$_{30}$ cell cultures were 0.51 µg/ml for type A, 0,18µg/ml for type O and 0,16 µg/ml for Asia-1. The average amount of viral particules, 146S, for same viruses in BHK-21 An$_{73}$ cell culture were 2,11 µg/ml for type A, 2,59 µg/ml for type O and 0,53 µg/ml Asia-1 (Table 3).

The average of infective titers were determined in suspended BHK-21An$_{30}$, as average; A, 6,87 pfu/ml, O,

6,22 pfu/ml and Asia-1, 6,49 pfu/ml. After the production of A, O and Asia-1 vaccine viruses, in suspended BHK-21An$_{73}$ cell cultures average of infective titers respectively; were determined as average 6,99 pfu/ml, 7,82 pfu/ml and 6,37 pfu/ml (Table 4).

Table 3. Daily cell increasing kinetics of suspended BHK-21An$_{30}$ and BHK-21An$_{73}$

Results of 146S/µg/ml	Average An$_{30}$	Average An$_{73}$
A	0,51 µg/ml	2,11 µg/ml
O	0,18 µg/ml	2,59 µg/ml
Asia-1	0,16 µg/ml	0,53 µg/ml

Table 4. Results of Infective Titers in suspended BHK-21An$_{30}$ andBHK-21An$_{73}$ virus production

Results of Infective titer	Average An$_{30}$	Average An$_{73}$
A	6,87 pfu/ml	6,99 pfu/ml
O	6,22 pfu/ml	7,82 pfu/ml
Asia-1	6,49 pfu/ml	6,37 pfu/ml

Discussion and Conclusions

Suspended and monolayer cultures of BHK-21 cells are used in production of vaccine for food and mouth disease. Different cell clones were obtained in BHK-21 cell culture cloning studies. These obtained BHK-21 clone cells are used in different names in different laboratories.

In our country, BHK-21An$_{30}$ and BHK-21An$_{31}$ cell lines are used in the FMD virus isolation and FMD vaccine production.

This study was performed to show the possible usage of these two clones, BHK-21An$_{30}$ and BHK-21An$_{31}$ cells, which are used currently in practice (Harmsen et al. 2011; Rahman et al., 2007; Abbas et al., 2011; Shirai et al., 1990) and BHK-21An$_{73}$ cell obtained from the Berscia Institute in Italy, for obtaining the most efficient virus in both isolation of the viruses and in production of vaccine.

In this study, monolayer BHK-21An$_{73}$ cell culture showed bigger growth rate than BHK-21An$_{30}$ cells on 4th day of seven day incubation and reach the highest growth rate on the 6th day of incubation. But BHK-21An$_{30}$ cells reach the peak cell count on the 4th day, and reduced on the 6th day. On the other hand, when compare the suspended BHK-21An$_{30}$ and BHK-21An$_{73}$ cell culture, BHK-21An$_{30}$ cell cultures show faster and higher growth. These results indicated that BHK-21 An$_{30}$ cell cultures adapted suspended form and therefore BHK-21An$_{73}$ cell cultures should also be adapted to suspended form.

When compared to reproductive characteristics of A, O and Asia-1 vaccine seed strains in BHK-21An$_{30}$ and BHK-21An$_{73}$ cell culture, 146S values of FMD virus in BHK-21An$_{73}$ cell cultures were 4.13 times for type A, 14.4 times for O type and 3.3 times for Asia-1 type (Table 3).

In addition, high level of infectious titer in BHK-21An$_{73}$ cell cultures indicates that BHK-21An$_{73}$ cell was more sensitive to the production of A, O and Asia- 1 seed of vaccine virus.

Infectious titer obtained from BHK-21An$_{73}$ cell culture virus infectious titer is related to other studies (Ali 2013; Abbas, 2011). But 146S values obtained from BHK-21An$_{73}$ cell culture were high compared to other studies (Ali et al., 2013; Rweyemamu et al., 1989). These results reveal the difference among BHK-21 cells clone.

As a result, in the production of A, O and Asia-1 vaccine viruses in BHK-21 An$_{73,}$ resulted in higher 146S values and higher infective titers than BHK-21 An$_{30.}$ Therefore, it is concluded that for FMD vaccine production the using of BHK-21 An$_{73}$ cell culture is more appropriate for both of cost of manufacture and productive time.

References

Abbas, F., Khan, F.A., Ahmad, F., Hussain, A., Ahmad, M., Awan, M.A., Tariq, M.M., Kakar, M.A., Wadood, A., & Ali, M. (2011). Production of Foot and Mouth Disease Virus Vaccine (O Type) on BHK-21 Cell Line. *IğdırUniv J InsSci Tech,* 1(2), 155-159.

Ali, S.M., Ismail, A.H., Soliman, E.M., & Hanaa, A.M. (2013). Studies on Growth Kinetics of the FMDV Serotype SAT-2 Egyptian Strain in Cell Culture. *J Vet Adv,* 3(2), 92-97.

Alexandersen, S., Zhang, Z., Donaldson, A.I., & Garland, A.J.M. (2003). The Pathogenesis and Diagnosis of Foot and Mouth Disease. *J Comp Path*, 129, 1-36.

Bartelling, S.J., & Vreeswijk, J. (1991). Developments in foot- and-mouth disease vaccines. Central Veterinary Institute, Lelystad, The Netherlands. *Vaccine,* Feb 9(2), 75-88.

Bartelling, S.J. (2002). Development and performance of inactivated vaccines against foot and mouth disease. *Rev Sci Tech Off intEpiz*, 21, 577-588.

Grubman, M.J., & Baxt, B. (2004). Foot-and-Mouth Disease. *ClinMicrobiol Rev*, 17 (2), 465–493.

Harmsen, M.M., Fitjen, H.P.D., Westra, D.F., & Cocomartin, J.M. (2011). Effect of thiomersal on dissociation of intact (146S) foot-and-mouth disease virions into 12S particles as assessed by novel ELISAs specific for either 146S or 12S particles. *Vaccine,* 29(15), 2682-2690.

Institute of Foot and Mouth Disease Protocol. (2010).

Radwan, M.E.I., Khalifa, N.O., & Fahmy, H.A. (2016). Molecular epidemiology of foot and mouth disease virus during 2014 with references to biochemical changes in Egyptian buffaloes. *World J BiolMedScience*, 3(1), 68-81.

Rahman, S.U., Rabbani, M., Sahidullah, K., & Muhammed, Z. (2007). Studies on In Vitro Culture Characteristics of Adherent Baby Hamster Kidney-21(BHK-21) Cell Line. *Int J of Agri-Biol*, 1560-8530, 821-826.

Rweyeamu, M.M., Umehara, O., Giorci, W., Medeiros, R., Lucca, D.N., & Balzatar, M. (1989). Effect of formaldehyde and binary ethyleneimine (BEI) on the Integrity of Foot and Mouth Disease Virus Capsid. *Rev Sci Tec Off IntEpiz*, 8(3), 747-764.

Shirai, J., Chatchawanchonteera, A., Sinsuwongwat, W., Makarasen, P., & Sugimura, T. (1990). Estimation of 146S Particles in Foot and Mouth Disease Virus (FMDV) Vaccine by Using Computer Analysing System. *Jpn J Vet Sci*, 52(3), 621-630.