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Determination of Appropriate BHK-21 Cell Line to Obtain High Infective Titer and 146S FMD Virus Particles

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Abstract: Foot and mouth disease (FMD) is one the most contagious diseases in cloven-hoofed animals and has a great potential for causing severe economic loss in susceptible animals. In the fight against FMD disease, inactive FMD vaccines are widely used. This study was carried out for the detection of suitable BHK-21 cell lines in order to get virus suspensions containing excess amounts of 146S FMD virus serotypes O, A and Asia-1, for FMD vaccine production. For this purpose, in addition to the BHK-21 An30 cell culture currently used in FMD vaccine production, BHK-21 An73 cell lines were passaged 20 times. In every 5 passages, infective titers and 146S virus particle quantities of FMD virus serotypes O, A and Asia-1 were measured. Additionally, karyotype analysis was performed on both cells at the 20th passage levels and the tests revealed that there was no significant change in the morphological features and chromosomal structures of the cells.
It was determined that the infective titer and 146S virus particle quantities of serotypes O, A and Asia-1 of FMD virus were very high in the BHK-21 An73 cell line than they are available in the BHK-21 An30 cell line. The increases of 146S virus particle quantities of serotypes O, A and Asia-1 of FMD virus were statistically significant ($p < 0.05$).
As a result, high titer FMD 146S virus particles can be obtained in the vaccine production process by pre-determining the BHK-21 cell lines that are most sensitive to the FMD virus. Due to the increase in vaccine production capacity, it was concluded that it would make an important economic contribution to the producer.
Keywords: *BHK-21, cell line, FMDV, production, vaccine.*

Yüksek İnfektif Titre ve 146S FMD Virus Partikülü Eldesi İçin Uygun BHK-21 Hücre Kültürü Hatlarının Saptanması

Özet: Şap Hastalığı (FMD), çift tırnaklı hayvanların en bulaşıcı hastalıklarından biri olup duyarlı hayvanlarda ciddi ekonomik kayba neden olma konusunda büyük bir potansiyele sahiptir. FMD hastalığına karşı mücadelede inaktif FMD aşıları yaygın olarak kullanılmaktadır. Bu çalışma, FMD aşı üretiminde fazla miktarda 146S FMD (O, A ve Asia-1) virüs partikülü içeren virüs süspansiyonu elde etmek için uygun BHK-21 hücre hatlarının tespiti amacıyla yapıldı. Bu amaçla şu anda FMD aşı üretiminde kullanılan BHK-21 An30 hücre kültürüne ek olarak, BHK-21 An73 hücre hatları 20 kez pasajlandı. Her 5 pasajda bir, FMD virüsünün O, A ve Asia-1 serotiplerinin enfektif titresi ve 146S virüs partikül miktarları ölçüldü. Ayrıca, her iki hücre hattının 20. pasaj seviyelerinde karyotip analizi yapıldı ve hücrelerin morfolojik özelliklerinde ve kromozom yapılarında önemli bir değişiklik olmadığı saptandı. FMD virüsünün O, A ve Asia-1 serotiplerinin BHK-21 An73 hücre hattında elde edilen enfektif titre ve 146S virüs partikül miktarlarının, BHK-21 An30 hücre hattında saptanan değerlerden yüksek olduğu belirlendi. FMD virüsünün O, A ve Asia-1 serotiplerinin 146S virüs partikül miktarlarındaki artışların istatistiksel olarak anlamlı olduğu saptandı ($p < 0.05$). Sonuç olarak, FMD virusuna karşı en duyarlı BHK-21 hücre hatlarının önceden belirlenmesi ile aşı üretim prosesinde yüksek titrede FMD 146S virus partikülü elde edilebilecektir. Aşı üretim kapasitesinin artmasından dolayı üreticiye ekonomik olarak önemli katkı sağlayacağı kanaatine varıldı.
Anahtar Kelimeler: *BHK-21, hücre hatt,ı FMDV, , üretim, aşı.*

Introduction

Primary and permanent cell cultures are widely used in both laboratory studies, virus isolation and vaccine production studies (FMD, polio, rabies viruses, etc.). Especially in FMD virus isolation and vaccine production studies, BHK (baby hamster kidney) 21 cell lines are used mostly with other cell cultures (Ali et al., 2013; Chapman and Stewart, 1962; Czelleng et al., 1987).

The BHK-21 cell production process can be generally divided into two. Namely, single layer cell cultures (monolayer) and suspended cell cultures. The BHK-21 cell is a fibroblast cell isolated from one

day old baby hamster's kidney cell by Mc Pherson and Stocker in 1961. BHK-21 C13 cell was obtained by cloning this cell. After Mowat and Chapman reported that the BHK-21 C13 cell was susceptible to foot and mouth disease in 1962, the suspended production process of this cell was developed in the same year (Barteling, 2002).

Foot and mouth disease (FMD) is caused by a virus of the genus *Aphthovirus*, family *Picornaviridae*. There are seven serotypes of FMD virus (FMDV), namely O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1, that infect cloven-hoofed animals (Callens and De cereq, 1997; OIE, 2017). Infection with any one serotype does not confer immunity against another. Within serotypes, many strains can be identified by biochemical and immunological tests of the domesticated species. Cattle, pigs, sheep, goats and water buffalo (*Bubalus bubalis*) are susceptible to FMD. Many species of cloven-hoofed wildlife may become infected, and the virus has occasionally been recovered from other species as well (Grubman and Baxt, 2004; OIE, 2017). The foot and mouth disease is included in the list of notifiable terrestrial and aquatic animal diseases listed by the International Committee and recommendations issued by the Regional Commissions instructed the OIE Headquarters as it has the potential to cause rapid and intensive spread within the country and across countries, and serious economic losses (OIE, 2017). There are multiple transmission routes, such as a wide host distribution of the foot and mouth disease, ability to infect with small doses, rapid replication rate, high level of viral expression and aerial spread. These features make foot and mouth disease a difficult and costly disease in terms of control and eradication. Countries free from the disease take major measures to prevent the entry of the virus. Therefore, foot and mouth disease imposes major restrictions on international trade of livestock and animal products (Alexandersen et al., 2003; Archetti et al., 1995; Nawaz et al., 2019). Vaccination takes an important place in the fight against FMD (Fransis et al., 1987; OIE, 2017). For this purpose, vaccination campaigns are organized in FMD-infected countries, and cattle and other susceptible animals are vaccinated. A large amount of vaccines are needed in the fight against FMD disease and FMD vaccines are produced in some countries (Rweyemamu et al., 1989; Xiao et al., 2016). After understanding that it is necessary to develop *in vitro* methods for large scale production of the FMD virus in the production of the vaccine, the advantages of monolayer and suspended cell lines have been noticed after the use of cattle tongue epithelial cells in the wide production of the virus, and pig kidney (PK) and BHK cell cultures started to be used in its reproduction. Other cell cultures in which FMD virus is produced *in vitro* are primary cell cultures such as calf thyroid, calf testicle, calf kidney, lamb kidney, pig kidney and bovine tongue epithelium, Mengeling-Vaughn pig kidney line (IBRS), and hamster lung cell line called HmLu (Aktas and Samuel, 2000). Today, most of the foot and mouth disease vaccines are produced in laboratories with appropriate biosafety standards using BHK-21 cells produced as monolayer or suspended. In order to meet the amount of vaccine needed to be used against FMD, intensive production activities are carried out in vaccine production facilities (Çokçalışkan et al., 2016; Sareyyüpoğlu et al., 2019). BHK-21 cell clones used in vaccine production have an important effect in obtaining FMD vaccine virus with high titer, infective titer and 146S virus particles. 146S FMD virus particles in different titers are obtained from BHK-21 cell clones with the same cell number. Obtaining low levels of FMD 146S virus particles in each series of vaccine production depending on BHK-21 cell clones has a great impact on both the vaccine amount and the vaccine production cost.

This study conducted by us provides the high dose of FMD 146S virus particles needed by providing high levels of FMD 146S virus particles in the unit vaccine suspension required for the production of FMD vaccine for the use of BHK-21 An73 cell clones instead of BHK-21 An30, which is still used in routine FMD vaccine virus production. It was carried out in a short time and at low cost in order to obtain the required FMD vaccine dose.

Material and Methods

Penicillin (Applichem-A1837) and Streptomycin (Applichem-A1852): Antibiotics were sterilized by filtering through 0.22 µm porous filter after preparing 10.000 ppm stock solutions by dissolving with distilled water. Penicillin 100 IU/ml and Streptomycin 50 µg/ml were used in cell and virus cultures.

Cell cultures: BHK-21 An30 and BHK-21 An73 cell cultures were obtained from FMD (SAP) institute cell bank laboratory. The cells were propagated with Glasgow Minimum Essential Medium (GMEM) (Applichem-A-1321) containing inactivated fetal calf serum (FCS)(Biochrom) by adding 10%.

Viruses: FMD serotype A, O and Asia-1 viruses (A/NEP/84, O/TUR/07, Asia-1/TUR/11) were obtained from the FMD (SAP) institute virus bank laboratory**.**

Gum Tragacanth (Sigma 9000-65-1): Gum was used as a covering medium in determining infective titer. It was dissolved 1.3% in distilled water. After

autoclaving at 121°C for 20 minutes, it was mixed 50%+50% (v/v) with 2XGMEM medium.

Colchicine stock solution: (2,5x10^{-3} molar) 100mg colchicine + 100 ml PBS (pH 6.8, not containing calcium and magnesium) stir until dissolved.

Hypotonic solution: (0.56% KCl). Dissolve 0.56 gm of KCl in 100 ml of sterile distilled water, prepare it just before the use (pH 7.2 to 7.4).

Fixative: Three-part of methanol and 1 part of glacial acetic acid were mixed at the time of use and chilled properly at -20°C.

Giemsa stain solution: 1gr of Giemsa was dissolved in 66 ml of glycerol using mortar and pestle for 45 minutes. It was transferred into an aluminum foil wrapped flask and heated for 45 minutes to 1 hour. Later it was placed at room temperature for some time and 84ml of methanol was added. Again it was placed for room temperature for some time and transfer to the dark amber bottle. The 5ml of stock solution of Giemsa was mixed with 45ml of distilled water before use.

Trypan blue solution (Biochrom, H350): 0.5% (w/v) was used as dye solution in cell counts.

Cell count: The Bürker chamber has 9 large squares (1 mm2 each), divided by double lines (0.05 mm apart) into 16 group squares. The double lines form small 0.0025 mm^2 squares. The chamber depth is 0.1 mm. The cells were counted. Briefly, both operators take 10 µl of cell suspension with a micropipette and put them in the cell count chamber and then count the cells in each of the 4 large squares. At the end of the procedure the operators calculate the average of the 4 readings (from 4 large squares) and calculate the cell concentration as follows: (Gunetti et al., 2012).

$$\left[\frac{\text{Cell}}{\text{ml}}\right] = \left[\frac{\sum\text{cell counted 4 large squares}}{4}\right] \text{ x (dilution factor) x } 1 \text{ x } 10^4)$$

Preparation of BHK-21 An30 and An73 cell cultures. BHK-21 An30 and An73 cells were produced as a monolayer with GMEM containing 10% FCS in 25 cm^2 flasks at 4x10^5 cells/ml. After staining the cells with 10% trypan blue solution 2 days after each passage, live cell counts were performed and cell morphologies were examined for 20 days (With Bürker slide and Muse cell counting device). A, O and Asia-1 serotypes of FMD virus were cultivated in monolayer BHK-21 An30 and An73 cells, where morphological properties, reproductive kinetics, and chromosomal changes were examined in every 5 passages, and amounts of 146S virus particles FMD A, O and Asia-1 serotypes and their infective titers were determined (Van Wezej, 1967).

Karyotype analysis of BHK-21 An30 and BHK-21 An73 cell lines: In the karyogram study of BHK-21 An 30 and An 73 cell lines, chromosomes of a total of 50 metaphase plaques were examined at the level of passage 20. For this purpose, the media of BHK-21 An30 and BHK-21 An73 monolayer cell cultures produced in 25 cm2 flasks at the 20th passage level were discarded, trypinized and reconstituted to be 2-5x10^4 cells per ml /. 2 ml. Cells were added to the petri dishes containing 35 mm diameter coverslips. The petri dishes were incubated at 37 ºC in a CO_2 incubator for 24 hours. At the end of the incubation period, the final concentration of freshly prepared colchicine stock solution was added to the monolayer cells in the coverslips at 4 ºC and left for 4-6 hours incubation at 37 ºC in a CO2 incubator. The petri dishes were then removed from the incubator and their media was discarded. 1 ml from the hypotonic solution (KCl at 0.075 M, 37 ºC) was added dropwise onto the coverslips in the petri dishes by pipette and incubated for 30 minutes at 37 ºC in an incubator with CO_2. At the end of the period, 2 ml of cold cornoy's fixative solution was added dropwise to the coverslips without removing the hypotonic solution. Plates were kept at room temperature for 10 minutes. At the end of the period, fixative and hypotonic solutions were aspirated from petri dishes. Coverslips were removed with the aid of a forceps and the cells were stained for 10-20 minutes by adding giemsa dye to the working solution. The stained coverslips were carefully washed 3 times with tap water and dried at room temperature. Cells on coverslips were examined with an immersion lens (100X lens) under a light microscope. (Freshney, 2010).

Detection of FMD 146S virus particles and infective titers 146S virus particles: The determination of the 146S virus particle amount was done by the SDG (Sucrose Density Gradient) method in FMD (SAP) Institute Protocol (2010). With the help of the gradient maker, a linear gradient was obtained in the ultracentrifuge tube by layering sucrose concentrations starting with 45% sucrose at the bottom and 15% sucrose at the top. 0.5 ml of virus sample was added slowly over the gradients and the tubes were placed in the ultracentrifuge godet. Godet was placed in the rotor and centrifuged at 41.000 rpm for 2 hours at +4°C temperature in a vacuum condition. At the end of the centrifuge, to ensure that the samples pass through a computer-connected UV detector (254 nm), the device was started to be dispensed from the bottom of the tube and at a suitable flow rate

of 60% sucrose base. When the sample started to pass through the detector, the appropriate absorbance width was set and the computer program (Chroma Simple) was started. After the peak occurred in the program, the starting and ending points of the peak are drawn; the program automatically calculated the amount of 146S particles (µg/ml) measured as the area of the peak (Özbilge et al., 2020; Shirai et al., 1990).

Plaque test (Infective titer): Monolayer BHK-21 An30 and An73 cells were prepared in 6-well plates 48 hours before the test. 100 µl 10^{-1} to 10^{-6} dilution of the virus inoculated to the cells. The plates were allowed to adsorb at 37 °C in a 5% CO_2 incubator for 1 hour, and 3ml medium (50% Gum + 50% GMEM 2X) was added to each well at the end of this period. Plates were incubated again for 48 hours at 37 °C and 5% CO_2 incubator. At the end of the period, 2ml staining solution prepared with crystal violet was added to each well after discarding the media. Finally, the dye was discarded and the plates were washed with tap water. In the evaluation, the last dilution in which plaques were counted was read and infectious titer pfu / ml was calculated (Baer and Kehn-Hall, 2014; Berg et al., 1963).

Statistical Analysis: All data were analyzed with Shapiro-Wilk and Levene Statistical tests. According to these two statistical results, independent samples t-Test was performed to detect the differences in amounts of FMD 146 S virus particles and infective titers of FMD virus that reproduced in BHK-21 An30 and An73 cell cultures at the 5, 10, 15, and 20th passage levels. The statistical results were evaluated on the 95% confidence interval. For the statistical analyses SPSS 22.0 (Inc., Chicago II, USA) software was used.

Ethics committee approval is not required for this article

Results

Karyotype properties of BHK-21 An30 and An73 cell cultures: In the karyogram study of BHK-21 An30 cell with 2n: 44 chromosome number, at the end of the 20th passage, the total number of 50 metaphase (MF) plates examined in the chromosome number 41 (2 MF), 42 (1 MF), 43 (1 MF), 44 (While 31 MF) is detected as 45 (6 MF) and 46 (at 3 MF), the BHK-21 An73 cell has the following number of chromosomes in 50 metaphase plates, 40 (at 3 MF), 41 (1 MF), 43 (1 Mf), 44 (29 MF), 45 (4 MF), 46 (2 MF) 47 (3 MF) and 49 (1 MF)(see Figure 1,2,3,4).

Amount of FMD 146S virus particles: Following the propagation of FMD virus serotypes A, O and Asia-1 on BHK 21 An30 and An73 cell cultures at the 5, 10, 15, and 20th passage levels, the amount of mean FMD 146S virus particles was observed as 0.48, 0.37, 0.29, 0.53, and 1.58, 1.72, 1.69, 1.68 µg/ml for A/NEP/84; 1.59, 1.75, 0.96, 0.80, and 2.59, 2.12, 2.83, 2.37 µg/ml for O/TUR/07; 0.44, 0.48, 0.81, 0.75, and 1.38, 0.89, 2.01 ve 2.08 for Asia-1/TUR/11 produced in the BHK 21 An30 and An73 cell cultures respectively (see Figure 5, 6, 7), (see Table 1).

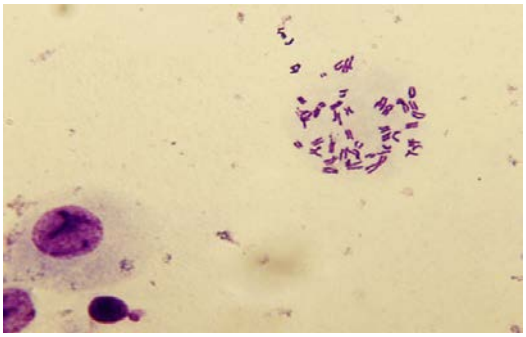

Figure 1. Karyotype of BHK-21 An30 monolayer cells.

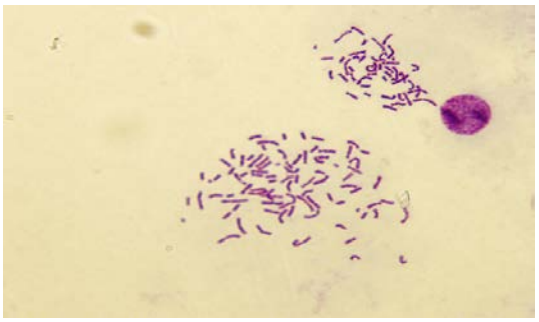

Figure 2. Karyotype of BHK-21 An73 monolayer cells

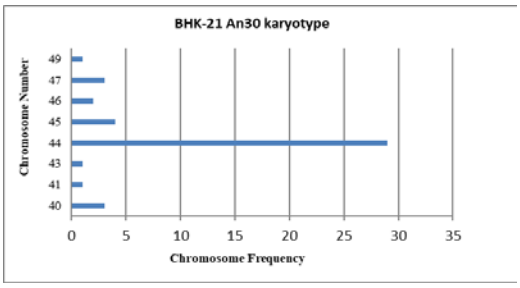

Figure 3. Chromosome of BHK-21 An30 cell line

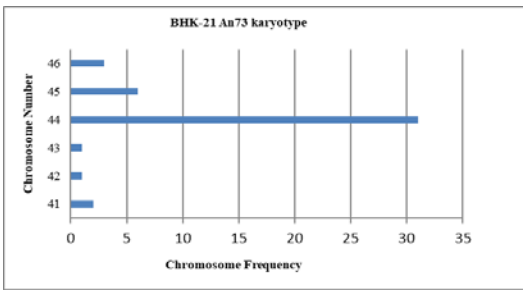

Figure 4. Chromosome of BHK-21 An73 cell line

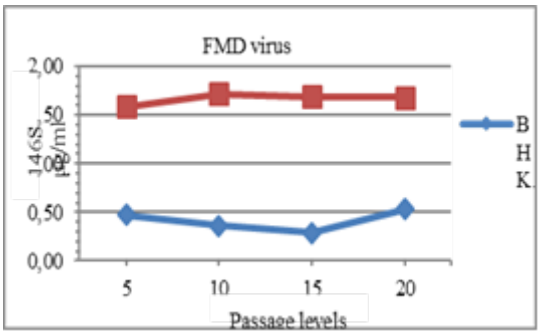

Figure 5: Comparison of 146S virus particle of the FMDV serotype A/NEP/84

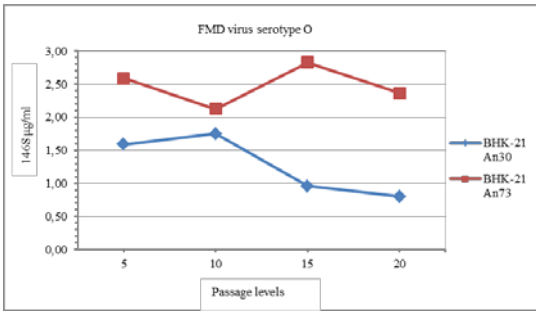

Figure 6: Comparison of 146S particle of the FMDV serotype O/TUR /07

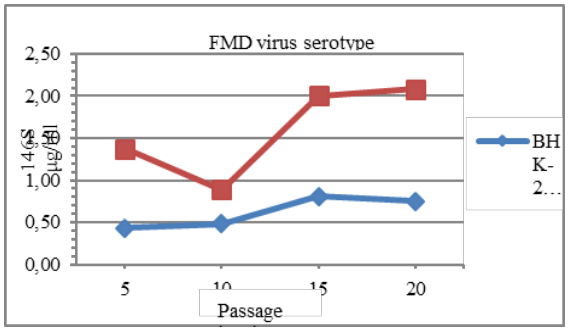

Figure 7: Comparison of 146S particle of FMDV serotype Asia-1/TUR/11

FMD infective virus titers on BHK 21 An30 and An73 cell cultures: The mean infective titers of FMD virus for serotype A/NEP/84, O/TUR /07 and Asia-1/TUR/11 produced at the 5, 10, 15, and 20th passage level of BHK 21 An30 cell culture were determined to be $10^{5.08}$, $10^{5.08}$, $10^{6.20}$, $10^{2.30}$; $10^{4.82}$, $10^{4.82}$, $10^{5.10}$, $10^{3.50}$ and $10^{6.94}$, $10^{5.10}$, $10^{5.70}$, $10^{5.90}$ pfu/ml respectively (see Table 2). Infective titers of FMD serotype A/NEP/84, O/TUR/07 and Asia-1/TUR/11 viruses produced in BHK 21 An73 cell cultures at 5, 10, 15 and 20th passage levels, were determined as $10^{7.67}$ $10^{6.70}$, $10^{8.00}$, $10^{7.70}$ (for A strain); $10^{8.07}$, $10^{7.60}$, $10^{8.00}$, $10^{6.90}$ (for O strain) and $10^{8.07}$, $10^{6.70}$, $10^{8.00}$, $10^{8.10}$ (for Asia-1) pfu/ml respectively (see Figure 8, 9, 10) (see Table 2).

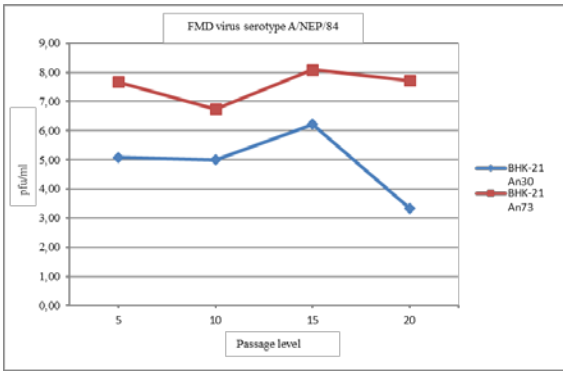

Figure 8: Comparison of infective titer of FMD serotype A/NEP/84

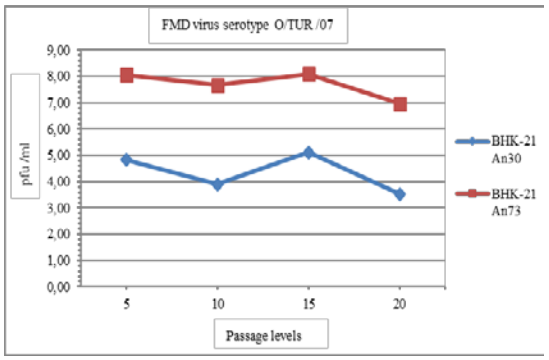

Figure 9: Comparison of infective titer of FMD serotype O/TUR /07

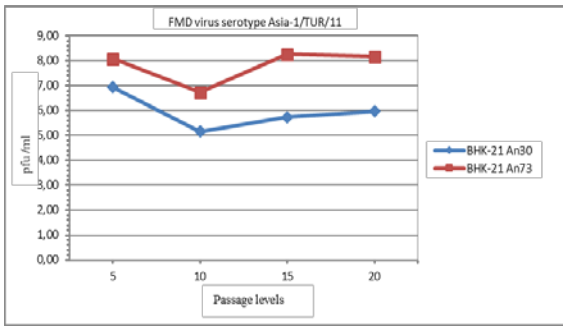

Figure 10: Comparison of infective titer of FMD serotype Asia-1/TUR/11

Statistical Analysis: 146S values of all three serotypes pertaining to FMD viruses were shown to be higher in BHK-21 An73 cell cultures compared to BHK An30 cell cultures, and these increases were statistically significant ($p < 0.05$) (see Table 1).

In comparison to infected titers of all three serotypes, A/NEP/84, O/TUR/07 and Asia-TUR /11 of FMD viruses in 5, 10, 15 and 20th passages in terms of BHK-21 An 30 and An73 cell cultures; an increase was found in infective titers in BHK-21 An73 cell culture compared to BHK-21 An 30, and these increases are statistically significant ($p < 0.05$).

Table 1. The comparison of amounts of FMD 146S virus particles produced in BHK-21 An30 and An73 cell cultures

Cell Cultures	Passage Level	Amounts of FMD 146 S virus particles (µg/ml)											
		A/NEP/84				O/TUR/07				Asia-TUR/11			
		Mean Value (X)	SX	SEM	P value	Mean Value (X)	SX	SEM	P value	Mean Value (X)	SX	SEM	P value
BHK-21 An30	5	0,48	0,14	0,05	0,001	1,59	0,15	0,05	0,001	0,44	0,05	0,02	0,001
BHK-21 An73	5	1,58	0,04	0,01		2,59	0,11	0,04		1,38	0,09	0,03	
BHK-21 An30	10	0,37	0,09	0,03	0,001	1,75	0,09	0,03	0,004	0,48	0,12	0,04	0,001
BHK-21 An73	10	1,72	0,1	0,03		2,12	0,25	0,09		0,89	0,12	0,04	
BHK-21 An30	15	0,29	0,08	0,03	0,001	0,96	0,11	0,04	0,001	0,81	0,13	0,05	0,001
BHK-21 An73	15	1,69	0,15	0,05		2,83	0,16	0,06		2,01	0,09	0,03	
BHK-21 An30	20	0,53	0,11	0,04	0,001	0,80	0,08	0,03	0,001	0,75	0,16	0,06	0,001
BHK-21 An73	20	1,68	0,17	0,06		2,37	0,12	0,04		2,08	0,12	0,04	

Table 2. The comparison of FMD infective virus titers on BHK-21 An30 and An73 cell cultures

Cell Cultures	Passage Level	**FMD infective virus titers** (pfu/ml)											
		A/NEP/84				O/Tur/07				Asia-TUR/11			
		Mean Value (X)	SX	SEM	P value	Mean Value (X)	SX	SEM	P value	Mean Value (X)	SX	SEM	P value
BHK-21 An30	5	5,08	0,54	0,20	0,001	4,82	0,98	0,37	0,001	6,94	0,12	0,05	0,001
BHK-21 An73	5	7,67	0,61	0,23		8,07	0,45	0,17		8,07	0,42	0,16	
BHK-21 An30	10	5,08	0,17	0,06	0,001	4,82	0,21	0,08	0,001	5,10	0,15	0,06	0,003
BHK-21 An73	10	6,70	0,84	0,31		7,60	0,25	0,09		6,70	1,14	0,43	
BHK-21 An30	15	6,20	0,96	0,36	0,001	5,10	1,49	0,56	0,001	5,70	1,38	0,52	0,002
BHK-21 An73	15	8,00	0,49	0,18		8,00	0,49	0,18		8,00	0,69	0,26	
BHK-21 An30	20	2,30	0,48	0,18	0,001	3,5	0,52	0,20	0,001	5,90	0,96	0,36	0,001
BHK-21 An73	20	7,70	0,73	0,27		6,9	0,80	0,30		8,10	0,69	0,26	

Numerically, the highest infective titer increase in BHK-21 An 30 and An73 cell culture was found in the 15th passage for FMD A/NEP/84, O/TUR/07, and it was observed to decrease in the 20th passage. For the Asia-TUR/11 serotype, the highest infective titer increase was obtained in the 5th passage for BHK-21 An 30 in the 20th passage for BHK-21 An73.

In general, it was concluded that the infective titer tends to decrease after the 15th passage for FMD A/NEP/84, O/TUR/07 in BHK-21 An 30 and An73 cell culture.

According to these results, it was concluded that BHK-21 An73 cell culture and BHK-21 An30 cell culture compared to 146S values in the same passage number of FMD vaccine serotypes and infective titer values were more positive ($p < 0.05$) than BHK-21 An30 in BHK-21 An73 cell culture.

Discussion and Conclusion

Suspended and monolayer-style BHK-21 cell cultures are widely used in both isolation of FMD virus and in the production of foot and mouth disease vaccine against FMD disease. Different cell clones were obtained in cloning studies in BHK-21 cell cultures. These BHK-21 clone cells obtained are used under different names and in different laboratories. (Abbas et al., 2011; Ali et al., 2013; Czellenge et al., 1987).

Both BHK-21 An30 and BHK-21 An31 cell lines of BHK-21 cells pertaining to different clones are

used in FMD virus isolation or vaccines production processes in Turkey. These two clones are still in use (Abbas et al., 2011, Harmsen et al., 2011, Jerome and Howard, 1964, Rahman et al., 2007, Shirai et al., 1990), provided by the Brescia Institute in Italy. This project was carried out to demonstrate the usability of cells called BHK-21 An73 in order to obtain high efficiency virus both in isolation of foot and mouth virus isolation and in the production of foot and mouth vaccine by passaging in the institute. In the production of vaccines used in the fight against FMD disease, the dose amount to be given to each animal is determined according to the 146S virus particles obtained in milligrams after production (Bartelling, 1991; OIE, 2017). In this study, when comparing monolayer BHK-21 An30 and An73 cell cultures, 146S virus particles in BHK-21 An73 of A, O and Asia-1 foot and mouth vaccine strains cell cultures were much higher than BHK An30 cell cultures, with 146S value in both cell cultures, in the 15th passage it made a peak, it was approximately 6 times higher for A type, 3 times higher for O type and 2.5 times higher for Asia-1, and that the infective titers were high in BHK-21 An73 cell culture therefore BHK-21 An73 cell A, O and Asia-1 vaccine has proven to be more suitable for the production of seed viruses.

In studies for determining the reproductive characteristics of FMD virus in different BHK-21 cell culture lines in different laboratories, infective titers of FMD virus have been reported to be $10^{4.5}$ to $10^{8.3}$ log10 and 146S virus particle amount is 0.44 µg/ml (Abbas et al., 2011; Czelleng et al., 1987). In this study, virus infective titers obtained from BHK-21 An73 cell cultures were close to the values obtained in other studies (Abbas et al., 2011; Ali et al, 2013), while other infectious virus particles obtained in BHK-21 An73 cell cultures are higher with respect to other studies, it is thought that these high rates are created due to deviation from BHK-21 cell clones used in other studies.

The morphological structural changes were determined in the monolayer both BHK-21 An30 and An73 cell cultures after 17 passage levels in the 20-day passaging period and 146S measurements and infective titers decreased. Czelleng et al. (1987), reported that chromosome numbers of BHK cell lines were found to be 42 for AC-9 and Tubingen and Torino BHK-21 cell lines; 46 for Razi II and 82 for Brescia-derived BHK-21 cells, and there was a correlation between the chromosome numbers in BHK-21 cells and FMD virus titers produced in these cells. In this study conducted by us, with respect to the karyotype analysis performed at the end of every 5 passages, peak chromosome numbers found in BHK-21 An30 and An73 cell lines (n=44) were determined to be quite close to results reported by other study regarding chromosome numbers found in BHK-21 Tubingen and Torino (n=42) and AC-9 and Razi II (n=46) cell lines (Czelleng et al. 1987). However, in these studies, it was found that chromosomal numbers did not change significantly in BHK-21 An30 and AN73 cell lines after 15th passage although cell aging and morphological changes occur.

Consequently; BHK-21 An73 cell cultures, which will be used both in isolation of foot and mouth disease virus and in the production of foot and mouth disease vaccine, should be selected before use, and BHK-21 An73 cell culture used in this study should be selected both in terms of cost in vaccination production and in the increase of annual vaccine dose amount. It was concluded that it is more suitable than cell culture and that the number of cell passages should not exceed 20 passages during production.

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Disclosure

Author Contributions: VG and AKK designed the experiments, AKK, VG, BBÖ, NT and YG carried out the experiments, VG, MH and GÖ analysed the data and wrote the paper.

Conflict of interest: The authors declare that they have no conflict of interest.

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