Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi
Iğdır University Journal of the Institute of Science and Technology

Productıon of Foot and Mouth Dısease Vırus Vaccıne *(O Type)* on Bhk-21 Cell Line

Ferhat ABBAS[1] Faisal Ameer KHAN[1] Fayyaz AHMAD[1] Azhar HUSSAIN[1] Masroor AHMAD[1]
Mohammed Arif AWAN[1] Mohammed Masood TARIQ[1] Mohammed Azam KAKAR[2]
Abdul WADOOD[1] Mumtaz ALI[1]

ABSTRACT: Footh & Mouth Disease (FMD) "O" type vaccine was produced on Baby Hamster Kidney (BHK)-21 cell line. BHK-21 cells were grown in tissue culture flasks. Monolayers were obtained after 48 hours at 37 ^{o}C. Confluent monolayers were infected with FMD "O" type virus ($TCID_{50}$ $10^{4.5}$/ml). Cytopathic effect (CPE) in the form of clumping and detachment of the cells was observed after 48 hours. Vaccine was found free of any bacterial and mycolplasma contamination. Systemic reaction was observed after injecting cattle, mice and guinea pigs with the virus ($TCID_{50}$ 10^{6}/ml). Nine calves of about 6-8 month of age were vaccinated using the same titer used for safety test and challenged with the virulent virus ($TCID_{50}$ $10^{4.5}$/ml). All the animals withstood the challenge and did not show any reaction. Both of the 2 unvaccinated animals after challenge showed typical FMD lesions. Mouth lesions from ten FMD suspected animals were also collected and adaptation of these field isolates was performed by passages on BHK-21 cell line. No viral CPE was obtained from these field isolates after 3 passages. Suspected antigens were also got typed for FMD. The types O (60%), A (20%), and Asia-1 (10%) were found present using ELISA

Keywords: BHK, vaccine, challenge, virulent, adaptation, cytopethic effect, antigens.

BHK-21 Hücre Hattı üzerinde Şap Hastalığı Virüs Aşısının (O tip) Üretilmesi

Cilt: 1, Sayı: 2, Sayfa: 155-159, 2011
Volume: 1, Issue:2, pp: 155-159, 2011

ÖZET: Şap Hastalığı "0" tipi aşısı, bebek Hamster Böbreğinin (BHK) 21 hücre hattında üretilmiştir. BHK-21 hücreleri doku kültürü şişelerinde dönüştürülmüştür. Otuzyedi ^{0}C' de 48 saatin sonunda mono katmanlar elde edilmiştir. Birleşen mono katmanlara, Şap hastalığı "O"tipi virüsü bulaştırılmıştır. Hücrelerin kümelenmesı ve ayrılması formunda, Sitopatik etki 48 saat sonra gözlenmiştir. Herhangi bir bakteri ve mikoplazma kontaminasyonu için aşı ücretsiz tedarik edilmiştir. Bu virüse ($TCID_{50}$ 10^{6}/ml) maruz kalan sığır, fare ve kobaylarda sistematik reaksiyonlar gözlenmiştir. Yaklaşık 6-9 aylık yaşa sahip 9 buzağı, güvenlik testi için kullanılan aynı titrede aşı yapılmış ve öldürücü virüse ($TCID_{50}$ $10^{4.5}$/ml) meydan okunmuştur. Tüm hayvanlar bu zorluğa dayanmış ve herhangi bir reaksiyon göstermemiştir. Bu zorluktan sonra, iki aşılanmayan hayvanın her ikisi de tipik Şap hastalığı lezyonu göstermiştir. Şap hastalığı şüphesi olan 10 hayvandan alınan ağız lezyonları toplandı ve bu alan izolatlarının adaptasyonu, BHK 21. Hücre hattı üzerinden geçişlerle gerçekleştirilmiştir. Üç geçişten sonra bu alan izolatlarından herhangi bir viral sitopatik etki elde edilmemiştir. Şüpheli antijenler de Şap hastalığı için dikkate alınmıştır. ELISA testi ile O (60%), A (20%), ve Asia-1 (10%) tiplerinin varlık gösterdiği belirlenmiştir.

Anahtar Kelimeler: Adaptasyon, Antijenler, Aşı, BHK, ,direnç, virülent, adaptation, Sitopatik etki.

[1] Center for Advanced Studies in Vaccinology & Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan

[2] Livestock and Dairy Development Department Quetta, Balochistan, Pakistan

Correspondence author:Ferhat ABBAS, ferhatcasvab@yahoo.com

Geliş tarihi / *Received:* 24.11.2010
Kabul tarihi / *Accepted:* 30.04.2011

INTRODUCTION

Foot and mouth disease (FMD) is a contagious disease of mammals and causes severe economic losses in susceptible cloven-hoofed animals. The direct and indirect losses due to FMD in Pakistan are reported (Zulfiqar, 2003). The disease is characterized by a vesicular condition of the feet, buccal mucosa and, in females, the mammary glands. The severity of clinical signs varies with the strain of virus, the exposure dose, the age and breed of animal, the host species and its degree of immunity (Ministry of Agriculture Fisheries and Food, 1986). Of the Domesticated species such as, cattle, pigs, sheep, goats and buffaloes are susceptible to FMD, in addition, many species of cloven-hoofed wildlife, such as deer, antelope and wild pigs may become infected (F.A.O., 1984).

FMD virus a positive sense single-stranded RNA virus belongs to picornaviridae family occurring in seven serotypes that is O, A, C, Asia-1, SAT1, SAT2, SAT3, with a wide diversity (Domingo et al., 2002). Due to highly contagious nature and economic importance of FMD, the laboratory diagnosis and serotyping of the virus is very important (Kitching and Donaldson, 1987). Confirmation of the virus is very important for its diagnosis. Different tests such as Enzyme Linked Immunosorbent Assay (ELISA) (Ferris and Dawson, 1988; Roeder and Le Blanc Smith, 1987., Bergmann et al., 2003), PCR (Amarel- Doel et al., 1993; Bastos, 1998), Hybridisation (Woodbury et al., 1995) and sequencing are done for its typing, subtyping and confirmation. A portable real-time reverse transcriptase-polymerase chain reaction assay is also reported for the rapid detection of foot and mouth disease virus (Callahan et al., 2002). A solid-phase competition ELISA for measuring antibody to foot and mouth disease virus is also reported (Mackay et al., 2001). The tissue of choice for laboratory diagnosis is infected epithilium. Established cell lines, such as BHK-21, continuous pig kidney cell, and primary cells such as bovine thyroid cell, and lamb kidney cell can be used for virus propagation (Clarke and Spier, 1980). Un-weaned mice are an alterative to cell culture. Some field viruses may require several passages before they become adapted to mice (Skinner, 1960). The control of FMD is usually a national responsibility. Routine vaccination against FMD is used in many countries. Some countries prefer the use of strict movement controls and slaughter of infected animals. Because of the presence of multiple serotypes of the virus, FMD vaccine should be multivalent to ensure antigenic coverage against prevailing viruses. Selection of viral strains for the production of vaccine is very important (Paton et al., 2005).

The objective of this study was to prepare a vaccine against "O" type FMD on BHK-21 cell line as most of the cases are found caused by this type not only in Balochistan but also in Pakistan. The objective of this study was also to collect FMD field samples from different areas of Balochistan, and to make attempts to get them adapted on BHK-21 cell line and to prepare a vaccine from these field isolates after the typing and sub-typing is done. This vaccine will be very effective to control the disease as it will be produced from the strains causing the disease in the province or even in the country.

MATERIALS AND METHODS

Virus Source: The FMD virus "O" type was obtained from Veterinary Research Institute, Lahore. The virus was stored at -20^{o} C.

Propagation of virus in cell culture: Seed virus (FMD "O" type) stored at –20^{o}C was first checked to make sure there is no contamination in the virus. Sterility was checked with slight modification according to the method described (European Pharmacopeia, 2008). Briefly, the seed virus was mixed in 10 ml of growth medium and filtered through a 0.2 micron filter. The content from the filter was streaked on nutrient agar plate and in PPLO broth and was incubated for 10 days at 37^{o} C.

Working seed virus was given 3 passages on BHK-21 cell line before infecting the final cell culture. The BHK-21 cells were grown in tissue culture flasks at 37^{o}C using Hank's growth medium according to the method described (Villegas, 1989) with slight modification. Monolayer from one tissue culture flask was transferred into 4 other flasks for the purpose of cell propagation. A 0.25% trypsin solution was added in each flask and incubated at 37^{o} C for 5 minutes to separate the individual cells and then the cells were transferred to other flasks and fed with growth medium and incubated at 37^{o} C for growth. The BHK-21 cells were also kept in liquid nitrogen in freezing media. Once the monolayers were completed, the media was removed from each flask and the monolayers were washed

2-3 times with PBS. Each flask was infected with the "O" type of FMD virus ($TCID_{50}$ $10^{4.5}$/ml) by adding 3-4 drops of the virus suspension and incubated for 45 minutes at 37°C for virus attachment. The flasks were then fed with the growth medium. There were some uninfected flasks kept as well in the same incubator as controls. Flasks were examined next day for viral CPE and compared with the control flasks. Flasks showing CPE were marked and culture fluid from these flasks was harvested and pooled together. Virus titration was conducted (Cunningham, 1973) in 96-well tissue culture plates before using the culture fluid for vaccine preparartion. The BHK-21 cell line was grown in 96-well tissue culture plate and infected with ten fold dilutions of the virus. The plate was examined daily for CPE and virus titer was calculated.

Dispensing of vaccine: Vaccine was made using the method described (Doel and Staple, 1982; Barteling and Vreeswijk, 1991) with slight modification, briefly 0.1% aluminium hydroxide gel was made and glycocol buffer (NaCl 10.4g, NaOH 5.4g, Glycine 15.8g, Distilled water 90 ml) was added at the rate of 1% to the gel. The gel and the glycocol buffer both were autoclaved separately. The FMD Virus "O" type ($TCID_{50}$ 10^6/ml) was inactivated by adding formalin at the rate of 0.1% and kept overnight. Equal amount of inactivated virus and gel was mixed and churned for 36-48 hours. The vaccine was bottled at the rate of 50 ml per bottle (Dose: 5ml/large animal) and labeled with instructions. The vaccine was stored at 4^0 C.

Safety & Sterility tests: Safety tests were performed on the final product of virus (European Pharmacopeia, 1993). Unvaccinated for FMD, with no history of previous FMD, two calves 6-8 month of age were selected at Government Dairy Farm, Livestock Department Quetta. In calves intradermally on the dorsal surface of the tongue 0.1 ml of vaccine was injected at 10 sites and the animals were observed for 4 days to see for any reaction. The 5th day 3 full bovine dose was given subcutaneously to each and the animals were observed for another 7 days. Guinea pigs and mice were also used to test the safety of the vaccine. Two guinea pigs and 5 mice were given 2 ml and 0.5 ml of vaccine (European Pharmacopeia, 1986) subcutaneously, respectively. These laboratory animals were kept under observation for 7 days for any death or any local or systemic reaction. Sterility was also checked as discussed before to see for the presence of any contamination.

Challenge & Protection test: About 6-8 month of age, 9 calves out of 11 were vaccinated and challenged (Vianna et al., 1993) and for this purpose unvaccinated calves with previous no history of disease were selected. Two calves were kept unvaccinated and only challenged. Three groups (3 in each group) were made and were given 5 ml, 1 ml, and 0.5 ml of the vaccine respectively. Three weeks later the vaccinated and non-vaccinated animals were challenged with 0.1 ml of the FMD "O" type virus ($TCID_{50}10^{4.5}$/ml) intradermally on the dorsal surface of the tongue at two sites. The animals were observed for 15 days for any local or systemic reaction.

FMD field isolates adaptation and typing: Mouth lesions from 10 FMD suspected animals were collected in a sterile screw capped tube in 5 ml of buffer glycerin (0.04 M phosphate buffer with equal amount of glycerol, 1% Gentamycin, pH 7.2). The adaptation of field isolates was carried out using BHK-21 cells according to the method described with slight modification (Clarke and Spier, 1980). The epithelium sample from each animal was blotted dry on absorbent paper to reduce the glycerol content and then chopped with a scissors in a beaker containing PBS. The suspected epithelium was ground in a sterile pestle and mortar using sterile sand and 2-3 ml of tissue culture media containing antibiotics. The suspension was clarified by centrifugation at 2000 rpm for 10 minutes. The supernatant was filtered by a 0.2 micron filter and used to inoculate BHK-21 cells. BHK-21 cells were incubated at 37°C for 72 hours and examined daily for viral CPE. Three passages were given for each sample.

Ten field samples (mouth epithelium) of the suspected animals were also sent to Institute for Animal Health, Pirbright, Surrey, UK., through National Veterinary Laboratory, NARC, Islamabad for typing . Samples were typed by ELISA.

RESULTS AND DISCUSION

No contamination was observed in nutrient agar plates and PPLO broth streaked with the FMD "O" type virus after 10 days of incubation at 37° C. This virus was further used to infect BHK-21 cell line to produce vaccine.

Confluent monolayers were obtained after 48 hours in the tissue culture flasks incubated at 37° C. Vi-

ral CPE was observed after 48 hours in infected flasks. There was clumping, aggregation and detachment of the cells in the infected flasks. Cells were collected, pooled together and saved at -20° C. Uninfected flasks were observed for comparison and were found with intact monolayers. Virus titer for the pool culture was 10^{-6} $TCID_{50}$/ml

Both the calves did not show any unwanted reaction except there was redness observed at the surface of the tongue where needle was pricked. The final product was found safe as no death, local or systemic reaction was observed in guineapigs and mice. The vaccine was found free of bacterial and mycoplasma contamination.

The vaccine was found potent and safe as no feet lesions and vesicles in oral cavity were observed in any of the vaccinated animals after challenge, except for one of the calves among 3 given 0.5 ml of the vaccine which showed some mild laceration at the dorsal surface of the tongue. There was slight redness on the surface of the tongue 0f all animals due to the prick of the needle but no typical mouth lesions which are characteristic of FMD observed in any of the animals. Almost similar results have been reported by other workers as well (Vianna et al., 1993). Among 2 unvaccinated animals one showed very typical lesions such as vesicles on the feet and in the oral cavity while lesions in the other unvaccinated animal were present but were not that severe. This severity of the lesion could be due to the degree of immunity as reported by others as well (Ministry of Agriculture, Fisheries and Food, 1986).

None of the field viral strain got adapted from suspected epithelium on BHK-21 cell line because no CPE was observed even after 3 passages. Similar results have been reported (Clarke and Spier, 1980) as BHK-21 cells are less sensitive than primary cells for detecting low amount of antigens. Also there are reports that some field viruses need several passages before they become adapted (Skinner, 1960). Lamb kidney cell could be a good option for adaptation of virus. In addition, passages of suspected suspension in mice for the concentration of the virus before inoculating cell cultures can be tried to obtain good results.

The suspected materials from 10 animals were typed through ELISA. Out of 10 six were found type "O" (60%), two were Asia-1 (20%), and 1 was found type A (10%), while one of the samples was negative. This could be due to very low or no antigen presence. Being "O" the aggressive type (Kitching, 2005), In Pakistan O A and Asia 1 are reported with almost similar results (Zulfiqar, 2003). Typing of this vaccine is of great importance before preparing an effective vaccine because there is no cross immunity among the different types.

REFERENCES

Amarel-Doel, C.M.F., Owen, N. E., Feris, N. P.,. Kitching R. P and. Doel, T. R., 1993. Detection of foot-and-month disease viral sequences in clinical specimens and ethyleneimine-inactivated preparations by the polymerase chain reaction. Vaccine, 11: 415-421.

Barteling, S. Z. and Vreeswijk, Z., 1991. Development in foot-and-mouth disease vaccine. Vaccine, 9: 75-88.

Bastos, A.D.S., 1998. Detection and characterization of foot-and –mouth disease virus in sub-Saharan Africa. J. Vet. Res., 65: 37-47.

Bergmann, I. E., Neitzert, B., Malirat, V., Ortiz, S., Colling, A., Sanchez C. and Correa Melo, E., 2003. Rapid serological profiling by enzyme-linked immunosorbent assay and its use in an epidemiological indicator of foot-and- mouth disease viral activity. Arch. Virol., 148: 891-901.

Callahan, J. D., Brown, F.,. Csorio, J F. A., Sur, H., Kramer, E., Long, G. W., Lubroth, J., Ellis, S. J., Shoulers, K. S.,. Gaffney, K. L.,. Rock D. L.and Nelson, W. M., 2002. Use of a portable real-time transcriptase polymerase chain reaction assay for rapid detection for foot-and-mouth disease virus . J. Am. Vet. Med., 220 (11): 1636-1642.

Clarke, J.B. and Spier, R. E., 1980. Variation in the susceptibility of BHK populations and cloned cell lines to three strains of foot- and –mouth disease virus. Arch. Virol., 63:1-9.

Cunningham, C. H., 1973. A laboratory guide in virology. 7th ed. Burgess Publishing Co., Minneapolis, MN.

Doel, T. R. and Staple, R. F., 1982. The elution of FMD from vaccines adjuvanted with aluminium hydroxide and with saponin. J. Biol. Stand., 10: 185-195.

Domingo, E., Baranowski, E., Escarmis C.,and Sobrino, F., 2002. Foot-and-mouth disease virus. Comp. Immunol. Microbiol. Infect. Dis., 25: 297-308.

European Pharmacopeia, 1986. Second Edition. Editions of the Council of Europe, Strasbourg, France.

European Pharmacopeia, 1993. Second Edition. Monograph No. 63. Editions of the Council of Europe, Strasbourg, France.

European Pharmacopeia, 2008. Version 6.1. Monograph No. 63. Editions of the Council of Europe, Strasbourg, France.

Ferris, N.P. and Dawsonm, M., 1988. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular disease. Vet. Microbial., 16: 201-209.

Food and Agriculture Organization of the United Nations (FAO), 1984. Emerging Diseases of Livestock. Vol. 1. The Diseases

and their Diagnosis, Geering W. A, ed. FAO, Rome, Italy. 1: 43-51.

Kitching, R.P. and Donaldson, A. I., 1987. Collection and transportation of specimens for vesicular virus investigation. Rev. Sci. Tech. Off. Int. Epiz., 6:263-272

Kitching, R.P., 2005. Global epidemiology and prospects for control of foot-and- mouth disease. Curr. Top. Microbiol. Immunol., 288: 133-148.

Mackay, D. K., Bulut, A. N., Rendle, T., Davidson F. and Ferris, N. P., 2001. A solid-phase competition ELISA for measuring antibody to foot-and-mouth disease virus. J. Virol. Methods., 97 (1-2): 33-48.

Ministry of Agriculture, Fisheries and Food, 1986. Foot-and-mouth disease. Ageing of lesions. Her majesty's Stationery Office, London, UK.

Paton, D. J., Valarchar, J. F., Bergmann, I., Matlho, O. G., Zakharov, V. M., Palma E. L. and Thomson, G. R., 2005. Selection of foot-and- mouth disease vaccine strains-a review. Rev. Sci. Tech. Off . Int. Epiz., 24: 981-993.

Roeder, P.L. and Le Blanc Smith, P. M., 1987. The detection and typing of foot-and-mouth disease virus by enzyme-linked immunosorbent assay: a sensitive, rapid and reliable technique for primary diagnosis. Res. Vet. Sci., 43: 225-232.

Skinner, H.H., 1960. Some techniques for producing and studying attenuated strains of the virus of foot-and-mouth disease. OIE., 53: 634-650.

Vianna Filho, Y.L., Astudillo, V.I., Gomes, G., Fernandez, C.E. Rozas, E., Ravison J. A. and Alanso, A., 1993. Potency control of foot-and-mouth disease vaccine in cattle. Comparison of the 50% protective dose and protection against generalization. Vaccine, 11(14): 1424-1428.

Villegas, P., 1989. Procedure for primary chicken embryo liver cells in tissue culture. In: Lab. Manual, avian virus diseases (AM 805). College of Vet. Medicine, University of Georgia, Athens, Ga, 7p.

Woodbury, E.L., Ilott, M.C., Brown C.C. and Salt, J.S., 1995. Optimization of an In situ hybridization technique for the detection of foot-and-mouth disease virus in bovine tissues using the digoxigenin system. J. Virol. Methods., 51: 89-94.

Zulfiqar, M., 2003. Draft report for development of national disease control policy for foot and mouth disease in Pakistan under the FAO project "Support for Emergency Prevention and Control of Main Transboundry Animal Diseases in Pakistan Rinderpest, FMD, PPR".