Trial for production of FMD cell culture vaccine grown in medium containing serum replacement

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The present study was designed in a trial to use serum replacement instead of the newborn calf serum in propagation of BHK-21 cell cultures with subsequent reducing the cost of foot and mouth disease (FMD) vaccine production.

Two batches of BHK-21 cell culture were prepared where the medium of the first batch was supplemented with newborn calf serum while the medium of the second batch was supplemented with serum replacement.

FMD virus was propagated 7 passages using BHK-21 cell culture. Both virus titration and complement fixation titer (CF) revealed that propagation of FMD virus in cell cultures supplemented with newborn calf serum yields a titre higher than that in case of cells supplemented with serum replacement. Also two batches of FMD inactivated vaccine were prepared from the virus propagated in the two-mentioned cell culture batches. Two groups of susceptible calves were vaccinated with these vaccines. Both of virus neutralization test (VNT) and enzyme linked immunosorbent assay (ELISA) revealed that higher antibody levels were induced in calves vaccinated with the vaccine prepared from cells supplemented with calf serum than those vaccinated with vaccine prepared from cells grown with serum replacement. BHK-21 cell culture supplement with newborn calf serum is most susceptible for FMD virus propagation yielding higher titer of the virus. Moreover, the growth pattern of the used cell culture was much better when the newborn calf serum was used.

Foot and Mouth disease is a highly infectious disease of ungulates primarily cattle, sheep, goats and pigs. It also affects wild animals such as buffaloes and deer and causes disease in hedghogs (Donaldson and Alexanderson, 2002). There are seven types of FMD virus have been identified as; O, A, C, SAT_1, SAT_2, SAT_3 and Asia1 (Franki *et al.,* 1991). It was stated that vaccine could be prepared from virus grown in tissue cultures of surviving fragments of epithelium from cattle tongues, monolayer calf kidney cells, or suspension of BHK-21 cells (Ubertini *et al.,* 1967 and Capstick, 1963). The choice of virus strain for vaccine preparation is of great importance. The vaccine should be immunogenic and produces a good protection level so; good culture host must be available (Moussa *et al.,* 1974 and Girard *et al.,* 1964).

There are many factors affecting cell culture growth. One of these important factors is the serum supplement (5-10%) for growth medium (Mizrahi and Shahar, 1977). Serum contains proteins, polypeptides, hormones, nutrients metabolites, inhibitors and minerals (Ian-Freshney, 1994).

Few proteins may be required as carriers for minerals, fatty acids, and hormones. Albumin, globulins and fibronectin (cold-insoluble globulin) are beneficial proteins that promote cell attachment while, macroglobulin inhibits trypsin. Fentin in foetal serum enhances cell attachment, transferring bind iron and making it less toxic but available.

There are other growth factor as fibroblast growth factor (FGF), epidermal growth factor (EGF) and insulin like growth factor IGF-1, IGF-2.

Hormones may exhibit a variety of different effects on cells and it is often difficult to recognize the key pathway while insulin promotes the uptake of glucose and amino acids. On the other hand, serum may contain substances inhibiting cell proliferation. Some of these may be artifacts as bacterial toxins from contamination prior to filtration or the γ-globulin factor which may contain antibodies that cross react with the culture. It was found that inactivation of serum removes complement and reduces the cytotoxic action of immunoglobulin without damage of polypeptide growth factors (Sefton and Rubin, 1971).

For acceptability of production of inactivated tissue culture foot and mouth disease vaccine in Egypt, there is a need to minimize the cost requirements for vaccine production. Successful

growth of tissue culture requires the presence of serum that provides essential factors for cell growth but the use of imported newborn calf serum (Ahmed, 1998) raises the cost of vaccine productions.

After the appearance of bovine spongiform encephalopathy (BSE) in cattle and human in Europe, the importation of bovine serum had been restricted by General Organization of Veterinary Services in Egypt to prevent the introduction of BSE in Egypt. Consequently, substitutes of bovine sera are required.

BHK-21 cell strains are susceptible to FMD virus (Cowan *et al.,* 1974) but varied in their susceptibility to infection (Clarke and Spier, 1980).

The present work was designed to evaluate the use of serum replacement on quality culture medium, in addetion to the effects of serum replacement on the antigenic and immunogenic properties of FMD virus propagated on BHK-21 cultures in comparison with other batches supplemented with newborn calf serum were also evaluated.

Material and Methods

Animals. Fourteen healthy calves' 6-9 months-old free from antibodies against FMDV were used.

Virus.

Tongue epithelium originated FMD virus. (Titre 10^9 MLD_{50}/ml) It was supplied by the Dept. of FMD Vaccine Research, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The virus was passed 7 times in BHK-21 cells using both calf serum and serum replacement.

BHK-21 cell adapted FMD virus. The local strain of FMD virus (O1/93/Agga) was adapted to BHK-21 cells and had a titer of 10^9 $TCID_{50}$/ml. It was used in VNT and ELISA.

Baby hamster kidney cells BHK-21. BHK-21 cells (Animal Virus Research, UK) were propagated using Eagle minimum essential medium (MEM) with Earl's salt according to the technique described by (Macpherson and Stocker, 1962).

Serum replacement 1 (50X). (Sigma, S0638). It was supplied in 10ml bottle and used as 10% in modified MEM Eagle plus 1% newborn calf serum to compensate the lack of adhesion factor in serum replacement. It was stored at 2-8^{o}C till used. Serum replacement contains highly purified heat- treated bovine serum albumin but does not contain growth factors, steroid hormones or mutagens.

Newborn calf serum. (Multiser). It was used as 10% in MEM Eagle and stored at -20^{o}C.

Cell viability and cell counting. Cells count and viability were determined by using 1% trypan blue According to (Soliman, 1997). The dead cells were stained blue while living ones remained unstained. The virus was inoculated as multiplicility of infection of 0.01 and incubated at 37^{o}C for 24 h. with MEM of Hank's salt. The harvested virus was freezed and thawed and kept at -20^{o}C till be used.

Virus titration. Virus titration test was carried out to estimate the infectivity titre of FMD virus, which was harvested from the inoculated tissue culture as described by (Mahy and Kangaro, 1996).

Complement fixation test (CFT). Complement fixation test was carried out to estimate the antigenicity of FMD, which harvested from the inoculated tissue culture as described by (Bradish *et al.,* 1966).

Vaccine preparation. FMD virus was titrated using BHK-21 cell line. The virus was inactivated by binary ethylemimine (BEI) and adjuvenated with aluminum hydroxide gel 2% according to (Sen and Rao, 1990).

Vaccine evaluation. The prepared vaccines were evaluated according to (OIE, 2004).

Sterility test. Vaccine samples were cultured on thioglycolate broth, Sabauroud's and nutrient agar.

Safety. Safety test of the prepared vaccine was done by intradermolingual inoculation of 1ml in 4 sites of susceptible calf as described by (Henderson, 1970).

Vaccination trial. Calves were grouped into 3 groups. The first two groups contained 12 calves (6/ group) and the 3rd group contained 2 calves and remained as control. The first group received 2ml of inactivated vaccine prepared from FMD virus using newborn calf serum, while the second group received 2ml of inactivated vaccine prepared from FMD virus using serum replacement. Antibody titers in vaccinated groups were estimated weekly for 24 weeks using ELISA and virus neutralization test. Challenge test. Evaluation of vaccine potency was performed using challenge test in cattle. After 3 weeks of vaccination, 3 animals of all vaccinated groups

Table (1): Titers of FMD virus propagated in BHK-21 cell cultures supplemented with newborn calf serum or serum replacement.

BHK cell passage number	Log_{10} ($TCID_{50}$/ml)	
	Cells supplement with newborn calf serum	Cells supplemented with serum replacement
1	7.55	6.8
2	7.8	6.8
3	7.8	7.05
4	8.3	7.3
5	8.55	7.3
6	8.55	7.3
7	8.8	7.55

Table (2): Complement fixation test of FMD virus propagated in BHK-21 cell cultures supplemented with newborn calf serum or serum replacement.

BHK cell passage number	50% haemolysis of CF activity	
	Cells supplemented with newborn calf serum	Cells supplemented with serum replacement
1	1/2	Undiluted
2	1/2	Undiluted
3	1/4	Undiluted
4	1/4	1/2
5	1/8	1/2
6	1/16	1/4
7	1/16	1/8

Table (3): Mean FMD neutralizing antibody titers vaccinated calves.

Animal group	Log $_{10}$ per weeks post vaccination														
	0	**1**	**2**	**3**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**
Group (1)	0.3	0.6	0.9	1.2	1.5	1.65	1.8	1.95	1.65	1.35	1.2	1.05	0.75	0.6	0.3
Group (2)	0.3	0.45	0.75	1.2	1.35	1.35	1.5	1.35	1.2	1.2	0.9	0.75	0.45	0.3	0.3

Group (1): calves vaccinated with the vaccine prepared from the virus propagated on BHK-21 using newborn calf serum.

Group (2): calves vaccinated with the vaccine prepared from the virus propagated on BHK-21 using serum replacement

Table (4): Mean FMD-ELISA antibody titers (log_{10}) in vaccinated calves.

Animal group	Mean ELISA antibody titer (log_{10}) per weeks post vaccination														
	0	**1**	**2**	**3**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**
Group (1)	0.3	1.05	1.35	1.65	1.8	1.8	1.95	2.1	1.8	1.8	1.65	1.35	1.2	0.75	0.6
Group (2)	0.3	0.9	1.2	1.5	1.65	1.65	1.8	1.8	1.65	1.5	1.2	0.9	0.45	0.6	0.45

Group (1): calves vaccinated with the vaccine prepared from the virus propagated on BHK-21 using newborn calf serum.

Group (2): calves vaccinated with the vaccine prepared from the virus propagated on BHK-21 using serum replacement

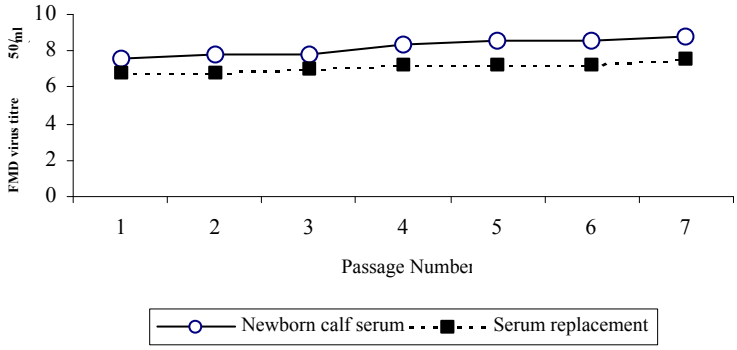

Fig. (1): Titre of FMD virus propagated in BHK-21 cell cultures supplement with newborn calf serum and serum replacement.

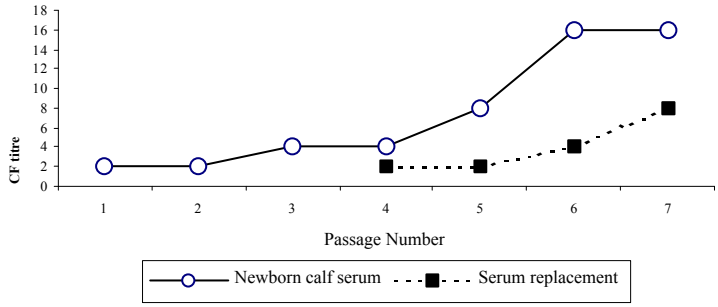

Fig. (2): Titration of antigenicity of FMD virus propagated in BHK-21 cell cultures suplement with newborn calf serum and serum replacement using CFT.

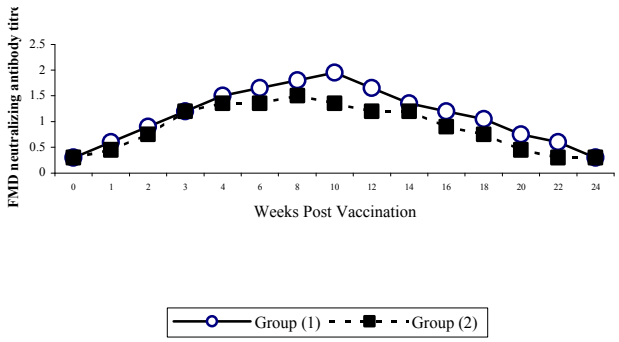

Fig. (3): Mean FMD neutralizing antibody titer in vaccinated calves

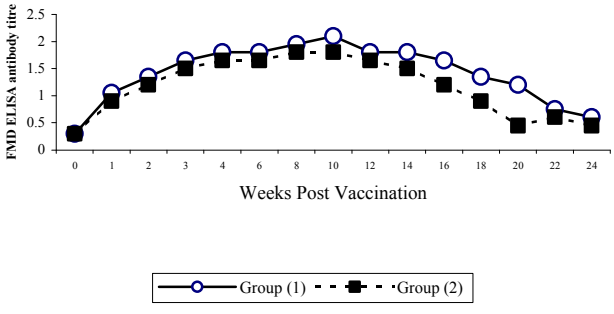

Fig. (4): Mean FMD ELISA antibody titre (log10) in vaccinated calves

and 2 non-vaccinated calves were challenged with 10,000 MLD_{50}, (Bartling and Swan, 1998).

Serological evaluation.

Virus neutralization test (VNT). The neutralizing antibody titer of FMD was determined according to (Ferriera, 1976).

Enzyme Linked Immunosorbent Assay (ELISA). The test was performed according to (Hamblin *et al.,* 1986).

Discussion

Cell cultures especially cell line are widely used for production of animal viral vaccines where they often provide high virus titer yield of large quantities and good immunogenicity. Cell culture preparation, maintenance and passaging require the availability of normal healthy growth requirements. One of the most important ingredients of such requirements is the newborn calf serum, which must be of high quality. Serum must possess no toxic effect on cultured cell, virus replication and accordingly virus immunogenicity.

In addition, serum price showed a progressed increasing in the last few years, so light was spotted on serum replacements in trials to decrease the final cost of cell culture vaccines and to avoid probability of animal sera be contaminated with the causative agent of bovine spongiform encephalitis (BSE).

The present work could be considered a beam of such light directed to study the effect of serum replacement on the immunogenicity of FMD virus propagated in BHK-21 culture and passed using serum replacement.

The obtained results revealed that the titer of 7^{th} passage of FMD virus in BHK-21 cell cultures supplemented with newborn calf serum was 8.8 $TCID_{50}$/ ml which was higher than that obtained from cell cultures supplemented with serum replacement (7.55 $TCID_{50}$ /ml) (Table 1). CF haemolysis activity of the FMD virus yield from cells with newborn calf serum possessed higher complement fixing unit (1/16) than that obtained from cells with serum replacement (1/8) (Table 2). These findings may be attributed to the presence of transferring factors which binds iron in cell culture media making it less toxic but reduces its bio-available (Guilbert and Iscove, 1976). However, obtained virus titers appeared to be satisfactory as (Moussa *et al.,* 1974) FMD virus titers used in vaccine production must be not less than $10^{7.7}$ $TCID_{50}$/ml with complement fixing activity 1:4 (100% complement activity) (Girard *et al.,* 1964; Mousa *et al.,* 1974 and Fawzy *et al.,* 2004).

Regarding the immunogenicity of 2 batches of FMD inactivated vaccine prepared from BHK-21 cells supplemented with calf serum and serum replacement, virus neutralization test (Table 3) and ELISA (Table 4) indicated that calves vaccinated with the first vaccine exhibited antibody titres of higher levels than those exhibited in calves vaccinated with the second vaccine. These results could be discussed on the bases of the fact that BHK-21 strain could affect replication immunogenicity of FMD virus (Ahmed, 1998).

On the other hand, serum replacement has some advantages as it originated from non-animal material so it is free from suspected animal viruses or antibodies. In addition, it possesses less ability to contamination with bacteria, fungal or mycoplasma contaminants (Hynes, 1992).

From the obtained results, it could be concluded that, although newborn calf serum is of higher cost than serum replacement, it is preferable for FMD vaccine production. Further studies are needed to screen the effect of serum replacement on FMD virus structure and cell vitality.

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