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THE EFFECT OF SOME ELECTROLYTES ON THE SUSCEPTIBILTY OF BHK TO FMD VIRUS

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ABSTRACT

Three batches of foot and mouth disease virus (FMDV) type O suspension were prepared on 24 hours previously seeded baby hamster kidney (BHK_{21}) cell culture. Each batch was composed of 15 roller cell culture vessels where the first batch represents the blank virus while the second batch was treated with 1ml of 1 mM calcium chloride ($CaCl_2$)/bottle in the inoculum's medium and the third batch received 1gm of mono basic sodium glutamate (MSG) /liter. Titration of individual bottles and pooled harvests of FMDV and complement fixing antigen revealed that $CaCl_2$ and MSG induced higher values than that obtained with the blank control batch where Ca Cl_2 potentially facilitate the adsorption and penetration of FMDV to BHK cells increasing the virus attachment to the cell receptors. On the other side MSG enhanced cell division due to its of amino acid content and accordingly increased the available cell number for virus replication. There will be a need to more research to investigate the effect of $CaCl_2$ and MSG on the immune response of vaccinated animals with FMD vaccine including such materials and their effect on the vaccine stability.

Keywords: FMDV, FMD vaccine , Baby hamster kidney (BHK_{21}), Mono basic sodium glutamate (MSG), Vaccine stability.

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INTRODUCTION

Foot and mouth disease (FMD) is an acute febrile and highly contagious vesicular disease affecting cloven-footed mammals including cattle, sheep, goats, pigs, wild buffaloes and deer. However, severity of infectiousness of the disease is less in sheep but sever in cattle and pigs **(Ferguson *et al.*, 2001).** It is caused by a virus belongs to the genus Aphthovirus in the family Picornaviridae which is a positive RNA virus of 8.4 kb occurs in seven distinct types (A, C, O, Asian-1 and SAT 1-3) **(Mahy and Van Regenmortal, 2010).**

FMD is still endemic in many of African, Asian and South American countries. Several outbreaks of the disease were recorded in most Egyptian governorates **(Farrag *et al.*, 2004 and 2005)** where type O of the virus was consistently isolated. Type A of FMD virus was isolated during the last outbreak in Egypt **(Abd El-Rahman *et al.*, 2006).** It is considered the most important disease of livestock in the world in terms of economic impact **(Janes and Rushton, 2002).** It has been proved that inactivation of FMD virus type O with binary ethylene amine instead of formalin improved the vaccine quality. Such vaccine adjuvanted with aluminum hydroxide gel was successfully used for immunization of cattle and buffaloes in Egypt **(El-Mikkawi, 1980 and OIE, 2004).**

It has been assessed that divalent cations such as calcium and magnesium promote the uptake of DNA into bacterial cells (Transformation) **(Graham and Van der E-b, 1973). Spizizen *et al.* (1986)** found that DNA precipitation with calcium phosphate prior to introduction into cell culture improved the transfection as much as 100 times over the DEAE dextran method. Meanwhile **Kristen and Renaldo (1994)** used $CaCl_2$ as an enhancing agent in isolation of cytomegalovirus in clinical specimens. Moreover, the addition of 1ml M $CaCl_2$ in the outlaying medium of PPR virus elevated its titer on VERO cells comparing with conventional method **(Hussein *et al.*, 2003).** It was also mentioned that addition of mono basic sodium glutamate (MSG) in PPR vaccine was profitable in terms of the virus yields and storage stability. MSG was used as a stabilizing agent in measles,

mumps, rubella, polio, hepatitis and herpes vaccines (**Samia *et al.*, 2000**). The herein study was designed to emphasize the effect of Ca Cl_2 and MSG on FMD virus performance on BHK cells.

MATERIALS & METHODS

1-FMD virus:

Local FMD virus (O_1/3/93) of cattle origin was used in the present work with a titer of 10^9 $TCID_{50}$/ml. It was supplied by the Department of Foot and Mouth Disease Research, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo.

2- Cell culture:

Baby hamster kidney (BHK_{21}) cell culture, colon 13 supplied by The Animal Virus Institute, Birbright, UK was propagated according to **Macpherson** and **Stocker (1962)** and used for virus propagation, titration and preparation of virus antigen.

3- Electrolytes:

3.1- Calcium chloride:

Calcium chloride ($CaCl_2$) was supplied by Adwic Corporation, Cairo, Egypt as fused anhydrous granules of molecular weight 110.99. Three solution with three molarities (1,2&3 mM) of $CaCl_2$ were prepared to assay their cytotoxicity in BHK cell culture.

3.2- Mono Basic Sodium Glutamate (MSG):

MSG ($HOOC\text{-}CH_2CH_2\text{-}CHNH_2$) $COO\text{-}NH_2O$ (**Zello *et al.*, 1995**) is a white crystalline powder of molecular weight 187.13. Its cytotoxicity was tested in BHK using different concentration prepared deionized distilled water as 0.1; 1 and 10gm/liter.

4- Virus titration:

It was carried out using the micro titer technique to the individual and pooled virus yields according to **Florence *et al.* (1992)** and the virus titer was calculated according to **Reed and Muench (1938).**

5-FMDV type-O antigen:

It was prepared in BHK cell culture according to **Lefevre and Diallo (1990)** from each batch of the prepared virus suspensions and titrated by complement fixation test.

6- Complement fixation test (CFT):

CFT was carried out according to **Traub and Manso (1944)** to estimate the obtained FMD virus antigens using reference FMD type O hyper immune serum supplied by The Department of Foot and Mouth Disease Research.

RESULTS & DISCUSSION

FMD is an OIE list-A disease that still threatens animal wealth in developing countries. The disease is rampant in most African and Asian countries in spite of the tremendous efforts for vaccination and control **(Daoud *et al.*, 1988).**

The present work was a suggestion to improve the performance of FMD virus in BHK cell cultures so as to procure the favorable conditions to produce high quality vaccine. The obtained results revealed that augmentation of the virus inoculum's medium of FMD virus with 1mM $CaCl_2$ (which was found to be non toxic to BHK cells) improved the virus titers of individual and pooled harvests of bottles of batch number 1comparing with the blank harvests as shown in **Table (3&4).** These finding could be attributed to the eligibility of calcium ions in improving the cell uptake of RNA of FMD virus. The exact mechanism of the enhancing impact of $CaCl_2$ on virus-cell attachment and penetration is not fully understood. However, **Hussein *et al.* (2003)** emphasized on the potentiality of calcium ions on concentrating PPR virus RNA on the receptors of Vero cells. Similar motions were also ascertained by **Elliot *et al.* (2007).** Other workers found that $CaCl_2$ had an enhancing effect on cytomegalovirus isolation from clinical specimens **(Kristen and Rinaldo, 1994).** $CaCl_2$ does not only improve virus infectivity but also it has a stabilizing action on the virus **(Quinn *et al.*, 2002).**

MSG with FMD virus inoculum's medium showed detectable increase in the individual and pooled virus yields **(Table-3&4)** where it augmented the virus-cell interaction leading to exaltation of the virus titer. **Samia *et al.* (2000)** found that MSG potentiates the cell growth through supporting amino acid uptake by the cells, glucose consumption and protein turnover in addition to its being a source of energy and a donor to amides and amino acids. These factors help together in improving the virus performance in the cell culture.

Further studies may be in need to envisage the exact action of $CaCl_2$ and MSG in maximizing FMD virus titer and other actions as stabilizing effect and their impact on the immune response of vaccinated animals with FMD vaccine incorporated with such materials.

Table 1. Cytotoxicity of Ca Cl_2 on BHK_{21} cell culture.

Tested molarity	Cytotoxicity
1mM	-
2mM	±
3mM	+

N.B.: 1mM of Ca Cl_2=1/1000 molar ±= mild cytotoxicity

Table 2. Cytotoxicity of MSG on BHK_{21} cell culture.

Tested concentration	Cytotoxicity
0.1gm/liter	-
1.0gm/liter	-
10gm/liter	+

Table 3. Titer of FMD virus (log_{10} $TCID_{50}$/ml).

Batch number	Tested virus	FMD virus titer in bottles number														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	IVY*	8.1	8.3	8.5	8.0	8.2	8.4	8.6	8.1	8.3	8.4	8.2	8.5	8.3	8.0	8.6
	PVY**	8.3														
2	IVY	8.9	8.7	8.8	8.6	9.0	9.1	8.8	8.9	8.7	9.0	8.6	8.7	8.9	8.8	9.0
	PVY	8.8														
3	IVY	8.7	8.6	8.5	8.8	8.7	8.4	8.5	8.8	8.6	8.9	8.7	8.5	8.7	8.8	8.6
	PVY	8.6														

Batch number 1= Control untreated infected BHK cell culture virus yield.
Batch number 2= Infected BHK cell culture treated with $CaCl_2$ virus yield.
Batch number 3= Infected BHK cell culture treated with MSG virus yield.
*IVY= Individual virus yield.
**PVY= Pooled virus yield.

Table 4. Titer of FMD virus antigen using CFT.

Tested antigen	Hemolysis of CF activity
Batch-1	1/8
Batch-2	1/16
Batch-3	1/16

N.B.: 1/8= 50% hemolysis of CF activity

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