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Studies on Growth Kinetics of the FMDV Serotype SAT-2 Egyptian Strain in Cell Culture

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Abstract

Foot and mouth disease virus (FMDV) Type SAT-2/Egypt/2012 isolated from El-Gharbiya Governorate was propagated six serial passages into monolayer BHK21 cell line. Growth curve of the infected virus, development of complement fixing (CF) antigens and 146s content in infected BHK21 cells incubated at 2 different temperature (35ºC and 37ºC) were studied using the 6th virus passage. Maximum infectivity titer with 100% CPE of the infected cells was detected 21 hours post inoculation at both incubation temperatures. The highest complement fixing antigens titer and 146S protein content of the same strain was recorded 18 hours post infection at both different incubation temperatures. So the best time of choice for virus harvest is 18hrs. post inoculation at 37ºC incubation.

Key words: Foot and mouth disease virus (FMDV), BHK21, virus passage.
**Introduction**

Foot and Mouth disease (FMD) is a highly infectious disease of ungulates primarily cattle, sheep, goats and pigs. It also affects wild animals such as buffaloes and deer (Donaldson and Alexanderson, 2002). Foot-and-mouth disease virus (FMDV) is the etiologic agent of one of the most devastating diseases that can affect cloven-hoofed livestock. Infection with FMDV causes an acute disease that spreads very rapidly and is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats (Grubman and Baxt, 2004). Although FMD has a low mortality figure, its high morbidity and contagiousness can lead to enormous economic consequences (Guzman et al., 2008). Seven types of FMDV have been recognized including O, A, C, SAT1, SAT2, SAT3 and Asia 1 (Franki et al., 1991).

Persistent infection with FMDV O (Akesu/58/2002) was established successfully with the aid of ammonium chloride which can not be selected and established merely by growth of BHK-21 cells survived cytolytic infection (Xuan et al., 2011). Classic study of this kind defined the one-step growth curve, in which cells in a culture are infected simultaneously using a high multiplicity of infection and the increase in infection virus over time is followed by sequential sampling and titration. Virus that is free in the medium can be titrated separately from viruses that remain cell associated (Frederick et al., 1999).

FMDV grow well on BHK-21 cell line enabling large-scale production of antigen with good complement fixing properties. The BHK-21 cell line culture provides better growth for FMDV than the suspension culture. It has also been reported that with subsequent passage in BHK-21 clone 13 cell line, the titre of FMDV increased significantly. Also the susceptibility and infectivity titers of IBRS-2 and MDPK cell lines were less as compared to BHK-21 cells, and thus had no advantage over BHK-21 cell line for vaccine production. The field isolates of FMDV could be passaged in BHK21 clone 13 monolayer cell culture, which showed characteristic CPE and were readily adapted between 3rd and 5th passage. The CPE usually develops within 48 hours, if no CPE is detected the cells should be frozen and thawed, used to inoculate fresh cultures and examined for CPE for another 48 hours. An alternative to the virus isolation is cell suspension plaque test which also quantify the virus present in sample. However, the cell culture system is laborious, time consuming, and relatively low sensitive. It also requires careful handling of specimens and a biosafety laboratory (Neeta et al., 2011).

Production of (FMDV) vaccine in tissue culture is widely used all over the world. In order to increase the potency of the vaccine, the virus has to be harvested at the proper time following cell infection (Moussa et al., 1984). To determine such optimum time, the growth curve of the virus in infected tissue culture, the development of complement fixing antigen and I46S content has to be studied.

In February 2012 is an exceptional situation and a serious development in Egypt with an important live stock sector, by the confirmation of SAT2 in Egypt, beside that Egypt also endemic country for types O and A. previous SAT2 incursions were limited to Saudi Arabia (2001) and Libya (2003) and were apparently self limiting, as they did not spread across international borders (FAO, 2012).

This work was designed to study the growth curve of (FMDV) type SAT-2 virus isolated and typed in Veterinary Serum and Vaccine research Institute (VSVRI), Cairo, Egypt, and designated as Type SAT-2/ Egypt 2012 isolated from El- Garbia province, in addition to study the development of it's infectivity and antigenicity in BHK_{21} cells.

**Materials and Methods**

**Virus**

Foot and mouth disease virus SAT-2/ Egypt 2012 isolated from 2012 outbreak El- Grbia Province, and confirmed by Animal Virus Research Institute, Pirbright, U.K, it was propagated twice in primary monolayer of bovine kidney cells and then in BHK_{21} monolayer cells 6 serially passages.

**Tissue cultures**
Baby hamster kidney cell cultures (BHK<sub>21</sub>) were obtained from the World Reference Lab. Pirbright Surrey, U.K. The cells were serially passaged and maintained in the FMD Research department, Veterinary Serum and Vaccine Research Institute, abbasia, Cairo (Huang et al., 2011).

**Media**

Minimum Essential Medium (MEM) modified with Hank’s salt (Morton, 1970) was supplied by Flow Laboratories, U.K. It was used as maintenance medium with 1-2 % horse serum added and was adjusted to pH 7.6-7.8 for the infected cell cultures.

**Growth curve design**

The 6<sup>th</sup> BHK<sub>21</sub> passage type SAT-2 /Egypt 2012 was inoculated on BHK21 cells in 24 roller bottles using Multiplicity of Infection (MOI) as 1:100 according to Community Coordinating Institute (CCI) Netherlands. Every 3 hrs intervals till 24 hrs after examination of cytopathic effect (CPE) 3 bottles were collected as follow:

a- From the first bottle: a sample for the cell free virus was obtained and kept at -70°C.

b- From the Second bottle: Collect the cell associated virus in the bottle and kept at -70°C after adding the same amount of the removed media.

c- Third bottle: collect all the content of the bottle (free & cell) associated virus and kept at -70°C (representing the yield total virus).

**Infectivity titration**

Thawing, centrifugation and titration of the obtained virus samples were carried out, and the virus infectivity titer was calculated after 10 fold serial dilutions according to Karber (1931).

**Complement fixation test (CFT)**

It was carried out according to Traub and Manso (1944).

**146S content of the FMD SAT-2 virus was estimated (intact virion)**

It was characterized according to Barteling and Melon (1974) to determine the virus antigenecity.

**Results and Discussion**

It was of interest to follow up the growth curve of FMDV serotype SAT-2/Egypt/ 2012 to determine the proper time at which the harvested virus could be contained the required amount of (146S) of intact virus to produced a good and potent vaccine.

### Table 1: Development of infectivity, complement fixing activity and 146 S content of Foot and Mouth disease virus type SAT-2/Egypt 2012 on BHK<sub>21</sub> cell culture incubated at 37°C

<table>
<thead>
<tr>
<th>Time elapsed after Cell infection</th>
<th>Virus Titer Log&lt;sub&gt;10&lt;/sub&gt; (TCID&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
<th>Complement fixing activity titer(log&lt;sub&gt;10&lt;/sub&gt;)</th>
<th>146S Content Intact Virion (µg/ml)</th>
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<tbody>
<tr>
<td>*** HPI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3hrs.</td>
<td>4.2</td>
<td>-</td>
<td>4.5</td>
</tr>
<tr>
<td>6hrs.</td>
<td>5.3</td>
<td>1.55</td>
<td>7.05</td>
</tr>
<tr>
<td>9hrs.</td>
<td>5.8</td>
<td>1.5</td>
<td>7.2</td>
</tr>
<tr>
<td>12hrs.</td>
<td>6.3</td>
<td>1.8</td>
<td>7.7</td>
</tr>
<tr>
<td>15hrs.</td>
<td>6.8</td>
<td>1.55</td>
<td>8.3</td>
</tr>
<tr>
<td>18hrs.</td>
<td>7.05</td>
<td>1.7</td>
<td>8.5</td>
</tr>
<tr>
<td>21hrs.</td>
<td>7.55</td>
<td>1.9</td>
<td>9.05</td>
</tr>
<tr>
<td>24hrs.</td>
<td>6.55</td>
<td>1.55</td>
<td>8.3</td>
</tr>
</tbody>
</table>

*** T.V.Y.: Total virus yield. **** HPI: Hours Post Infection
Table 2: Development of infectivity, complement fixing activity and 14S content of Foot and Mouth disease virus type SAT-2 /Egypt 2012 in BHK cell culture incubated at 35°C.

<table>
<thead>
<tr>
<th>Time elapsed after Cell infection</th>
<th>Virus Titer Log_{10} (TCID_{50}/ml)</th>
<th>Complement fixing activity titer(log_{10})</th>
<th>14S Content Intact Virion (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3hrs.</td>
<td>3.3</td>
<td>-</td>
<td>4.3</td>
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<tr>
<td>6hrs.</td>
<td>5.3</td>
<td>1.2</td>
<td>6.55</td>
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<td>9hrs.</td>
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<tr>
<td>24hrs.</td>
<td>6.55</td>
<td>1.55</td>
<td>8.3</td>
</tr>
</tbody>
</table>

* C.F.V.: Cell free Virus; ** C.A.V.: Cell associated Virus; *** T.V.Y.: Total virus yield; **** HPI: Hours Post Infection.

Fig. 1: Development of infectivity titer of FMDV Type SAT-2 Egypt 2012.

Fig. 2: Development of complement fixing activity of FMDV type SAT-2 Egypt 2012.
According to the short eclipse phase of family Picornaviridae (2 hours) as reported by Frederick et al., (1999) FMDV Type SAT-2 Egypt 2012 was detected in BHK$_{21}$ cells, 3 hours post virus infection. Our results were in agreement with Draginas and pappous (1969) who found that the virus could be detected 90 minutes after cell infection with FMD virus.

Virus infectivity of serotype SAT-2/Egypt/2012 virus was also detected 3 hours post inoculation for both cell free and total virus yield, whereas cell associated virus could not be detected. These results were supported by those of Polatnick and Bachrach (1960) who found that the (FMDV type A) could be detected 100- 110 minutes after cell infection.

Virus infectivity titers increased by time and reached their maximum titers 21 hours for the total virus yield. Also CPE appeared soon after 3 hours (Pi) and reached 100% 21 hours post infection. These results disagreed with what reported by Moussa et.al (1972) who found that after 24 hours 100% CPE was achieved with maximum antigenicity for serotype O virus but agreed with Ali (2009) who found that after 21 hours 100% CPE was achieved with maximum antigenicity for serotype A virus.

The antigenicity detected by complement fixing titers after 3 hours (Pi) for cell free and total virus yield, reached their maximum by 18 hours (Pi).

The obtained results revealed that 146S content reached its maximum level by 18 hours (Pi).

Depending on the fact that FMDV antigenicity is the protective capacity of FMD vaccine; the best time for virus harvestation was found to be 18 hours (Pi) where the high CF titer and highest 140S amount were obtained. These finding were accepted by Girard et.al (1964)

Accordingly, serotype SAT-2/ Egypt 2012 of FMDV could be harvested 18 hours post inoculation at 37°C with maximum complement fixation titer 1.5, and high 146S content in the total virus yield providing high quality virus for vaccine production.

**References**


STUDIES ON GROWTH KINETICS OF THE FMDV SEROTYPE SAT-2 ...


