Effect of Different Inactivators on the Efficacy of Egyptian Foot and Mouth Disease SAT2 Vaccine

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Abstract

In 2012, Foot and Mouth Disease Virus (FMDV) of serotype SAT2 caused a devastating FMD epidemic in Egypt. In order to control it, a vaccine was produced, based on the Egyptian SAT2 field isolate. As it is often difficult to produce good vaccines from FMDV isolates of the SAT serotypes, a study was carried out to determine the optimal inactivation protocol for the Egyptian SAT2 strain. In comparison to the usual FMDV inactivation protocol with binary ethyleneimine (BEI) alone, an alternative protocol was employed which used formalin as an additional inactivating agent and possible antigen stabilizer in combination with BEI. Inactivation kinetics was recorded for both protocols and the preservation of the antigens were determined by complement fixation test (CFT). From both antigens trial vaccines were formulated with Montanide ISA 206 and tested in guinea pigs. While inactivation with 0.1 M BEI alone resulted in an inactivation rate of 0.53 log10 TCID50/hour, BEI combined with formalin (BEI-FA) resulted in a significantly higher inactivation rate of 2 log10 TCID50/hour. While BEI inactivation of the antigen led to a drop in CFT titer from 1/32 to 1/16, no such decline was found with the BEI-FA inactivation protocol. The BEI-FA protocol also resulted in a trial vaccine with higher immunogenicity in guinea pigs. For 28 days post vaccination (dpv) sera, SNT and ELISA titers of 1.8 and 2.05, respectively, were recorded for the BEI inactivated antigen while for BEI-FA inactivated antigen, SNT and ELISA titers of 2.1 and 2.3, respectively, were recorded. Finally it was clear that, the use of BEI-FA as an inactivator for FMDV (SAT2) reduces the time of inactivation and provides good and safe antigen content consequently higher post vaccinal antibody titer.

Keywords: FMDV SAT-2, inactivation, binary ethyleneamine, binary ethyleneamine and formaldehyde, vaccine preparation, G. pigs.

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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 Paton et al., (2009). 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, with high morbidity but low mortality Depa et al., (2012). There are seven types of FMD virus (FMDV) have been identified as; O, A, C, SAT1, SAT2, SAT3 and Asia1 OIE (2010).

In order to prevent or control the spread of FMD, vaccination of susceptible animals is a method of choice.

Virus inactivation and safety tests are critical steps in FMD vaccine production. Foot-and-Mouth disease (FMD) vaccines in particular, guaranteed safety is essential because any occurrence of the disease will have great economic consequences Barteling and Cassim (2004).

Viral inactivation methods for vaccines production, include chemical treatments e.g., formaldehyde Barteling and Woortmeizer (2002), binary ethyleneimine Miguel et al., (2011), beta-propiolactone Culbertson et al., (1956), sodium chloride (NaCl) or phosphate Joris et al., (2012) physical treatments e.g., heat Somjai et al., (2007), and ultraviolet (UV) irradiation Suphachai et al., (2008).

Classical FMD vaccines were inactivated by formaldehyde (FA) which is still was resided in use in several vaccine producing companies. A residual remained or the preparation lost immunizing potency by using formalin inactivation. It has been recognized early that formalised vaccines, although innocuous, might contain ineffective virus Ali et al., (2009).

Several studies (with non-adsorbed virus) showed that at the FA concentration presribed by Waldmann et al., (1941), inactivation plots were not linear and often showed “tailing off”, which may cause incomplete inactivation Wesslen and Dinter (1957), Graves (1963), Barteling et al., (1983) and Barteling and Woortmeizer (1984). Consequently, large vaccine batches may contain some surviving virus, however, in the field the vaccines performed quite well, without causing outbreaks that clearly could be associated with vaccination campaigns. However, in 1981 it was shown by King et al., (1981) that an outbreak strain isolated in the UK, originating from Brittany (France), could well be vaccine related. By comparing nucleotide sequences of outbreak strains and vaccine strains it was also shown by Beck and Strohmaier (1987) that the sequences of European outbreak strains were very similar to those of vaccine strains suggesting that most of the outbreaks in Europe very likely were caused by improperly inactivated vaccines or by viruses that had been escaped from vaccine production laboratories.

Also the using of formalin alone as inactivator may alter the structure of the virion Brown (1968). For all these reasons, on the less confidence in the formaldehyde treatment forced many production laboratories changed to Aziridine group inactivators like Acetyleneimine(AEI) as inactivator Brown and Newmann (1963) and Brown et al., (1963). Linear inactivation plots were obtained with acetyl ethylenimine (AEI) and other aziridines Brown et al., (1963) and Bahnemann (1975). Although it was shown that, under proper conditions, FA-inactivation plots - of Al(OH)3-adsorbed virus were linear as well Barteling and Woortmeizer (1984), inactivation with aziridins became the method of choice. Binary ethylenimine (BEI) is used in particular, because this method developed by Bahneman (1975) and Bahneman (1990) circumvents the direct handling of the very toxic other aziridins. Aziridines lack the cross-linking and fixation activity of formaldehyde that made the old-type-Frenkel vaccines stable for five years and more (non-published observations).

Inactivation with BEI often results in a loss of some 10-30% of the 146S antigen (observation at Ov1). Therefore, for labile vaccine strains (such as SAT2 Zim 7/83), the antigen is first inactivated with BEI and then fixed with formaldehyde Rweyemamu (1989).

The aim of this study was carried out to determine the optimal inactivation time of FMD virus Type SAT2 isolated in Egypt using
formaldehyde (FA) in combination with binary ethyleneimine (BEA) as inactivators at the same time to studying inactivation kinetics of them.

**Materials and Methods**

**FMD Virus (Types SAT-2)**

The virus strain used in this study was Type SAT-2/2012 isolated from 2012 outbreak Gharbia province which was typed and subtyped in VSVRI and confirmed by pirbright laboratories. The virus was serially passaged 7 times in baby hamster kidney (BHK21) monolayer cell cultures. The virus harvested and stored at –70° c.

**Tissue Cultures**

**Baby Hamster Kidney Cell Line (BHK 21)**

It was obtained from the World Reference Lab. Pirbright Surrey, U.K. these cells were used for virus propagation and also to confirm complete inactivation process for BEI only and BEI-formaldehyde where the tissue cultures were observed after 24 hrs and 48 hrs for the pathognomic cytopathic effect (CPE) of FMDV according to Neeta et al., (2011).

**Chemical Inactivators**

**Bromoethyamine Hydrobromide (BEA)**

95% (molecular weight = 204.9);- It was obtained from Aldrich Chemical Company Limited Gillinham, Dorest, U.K.

**Sodium Hydroxide (Analar) NaOH** (Molecular weight =40);- It was obtained from PRATAP chemical industries PVT.LTD (INDIA) was used in concentration of 0.2 Normal for dissolving (BEA) in cyclization process according to Bahneman (1975).

**Formaldehyde 40%**

It was obtained from BDH Chemicals Likited Poole, U.K. It's molecular weight was 30.03, it was used in a concentration 0.04% in inactivation process according to Farid et al., (1979).

**Sodium Thiosulphate (Na₂S₂O₅ .5H₂O)**

20% solution in double distilled water, was prepared, sterilized by autoclaving. The chemical obtained from Merk Company, Germany. It was used in a final concentration of 2% to neutralize the excess of BEA after the inactivation process its molecular weight was 248.18 as described by Girard et al., (1977).

**Sodium Bisulphite (Na₂S₂O₅)**

It was prepared as 20% solution. Its molecular weight was (190.10) obtained from Merck Company, Germany, sterilized by autoclaving, it was used in a final concentration of 2% to neutralize the excess of formaldehyde after the inactivation process according to Farid et al., (1979).

**Titeration of FMDV Virus Infectivity of (Types SAT-2)**

It was carried out in tissue culture; the infectivity titer was calculated according to Karber (1931).

**Complement Fixation Test (CFT)**

It was carried out according to Health protection Agency (2009).

**Samples of Virus**

samples were taken prior and after inactivation for residual virus infectivity and CFT.

**Inactivation of FMD Virus (Type SAT-2) by Using BEI only**

BEI was prepared by adding 2% of the salt (exactly 0.1 M) in prewarmed 0.2 N NaOH and keeping the solution for 1 hour at 37°C, and used at a final concentration of 0.001 M (1mM of BEI) kept at 37° c for 24 hours at ph 8.0 according to Bahnemann (1990). Sodium thiosulphate 20% was added to treat virus after inactivation in final concentration of 2% (during 24 hrs.) to neutralize the effect of BEI.

**Inactivation of FMD Virus (Types SAT-2) by Using Combination of BEI 1mM and 0.04% FA (BEI-FA)**

According to the method described by Barteling and Cassim (2004) sodium thiosulphate 20% was added to virus after the inactivation in final concentration of 2% (up to 24 hours), also sodium bisulphite 20% was added after inactivation process to neutralize the excess of formaldehyde in final concentration of 2% also.
EFFECT OF DIFFERENT INACTIVATORS ON THE EFFICACY ...

**Baby-Suckling Mice**

The inactivated virus either by BEI alone or BEI-FA was inoculated into Swiss suckling mice 2-4 days old to confirm the complete inactivation of the FMDV. Five swiss albino baby-sucking mice for each inactivation method sample were inoculated I/P with 100μl. Death of baby mice after 24 hours of inoculation was considered non-specific, while paralysis of the hind limbs or death on the 2nd to 7th day was considered specific positive to FMDV El-Sayed et al., (2012).

**Guinea Pigs**

Thirty Albino male apparently healthy adult Guinea pigs of approximately 500 grams body weight were used for testing of FMD SAT-2 oil vaccines.

**Preparation of Inactivated FMD Vaccines**

The vaccine formulation was carried out according to the method described by Gamil (2010), El-Sayed et al., (2012a) and Tom et al., (2012) where the oil phase consisted of Montanide ISA 206 mixed with the inactivated viruses as equal parts of an aqueous and oil phase (weight/weight) and mixed thoroughly. The vaccine was prepared on the base that (2 ml) of vaccine contains not less 10^8 TCID50/dose of virus type SAT-2.

**Testing of FMDV SAT-2 Prepared Vaccines in Guinea Pigs**

Thirty adult male guinea pigs approximately 500 gm weight from VSVRI, Foot and Mouth Disease Department were randomly divided in to 3 groups (10 guinea pigs). Two groups were inoculated S/C by either inactivated BEI alone or BEI-FA vaccine and one group was kept as control without vaccination.

All groups of guinea pigs were bleeding weekly kept for 21 days and then blood samples were collected for antibody detection against FMD virus SAT2 strain using serum neutralization test (SNT) and ELISA.

**Serum Neutralization Test (SNT)**

It was performed using the micro titer technique as described by Ferreira (1976) to estimate antibodies against FMD in sera of guinea pigs.

**Indirect enzyme linked immune sorbent assay ELISA**

ELISA and its reagents were prepared according to Hamblin et al., (1986) and used to follow up the immune response in animals.

**Results and Discussion**

The tabulated results in table (1) and figure (1) showed that the inactivation of FMDV-type SAT2 with BEI only was completed at 16 hours post inactivation with no residual virus as detected in tissue cultures or in baby mice. But virus inactivation with combination of BEI-FA at the same conditions showed complete inactivation of the virus at 4 hours post inactivation process. These results agree with those of Ali et al., (2009) who mentioned that only with Binary ethyleneimine (BEI) in concentration of 0.1M, FMD type A virus was completely inactivated after 15 hours, and FMDV type O1 was completely inactivated after 14 hours, also agree with Bahnemann (1990), Hassanin et al., (2013) and Aarthi et al., (2004) who showed that the complete inactivation of FMDV type SAT2 with no residual virus was detected after 16hr at 37ºC using BEI of morailities 0.1mM.

Table (2) represented that the virus inactivation rate was 0.53 log10/hour using BEI only, and 2.0 log10/hour using combination of BEI-FA in agreement with those of Ali et al., (2009) who mentioned that by using BEI only the FMDV inactivation rate was 0.4 log10 TCID50/hour. But with combination of BEI-FA the inactivation rate was 2.5 log10 TCID50 /hour with FMDV type A but with FMDV type O1 the inactivation rate using BEI only was 0.5 log10 /hour. But with combination of BEI-FA the inactivation rate was 3 log10 TCID50/hour, and also agreed with Barteling and Cassim (2004) who mentioned the BEI-FA plots obtained with the 5 vaccine strains that are routinely produced at Onderstepoort Veterinary Institute (OVI) are given. The inactivation rates were varying from 2.0 (SAT1-SAR9/81) to more than 3 logs per hour (SAT2-ZIM 7/83 and SAT2 KNP-19/89/2) Thus, sometimes inactivation is over a 100-fold faster if FA is added, also agreed with Bahnemann (1990), Hassanin et al., (2013) and Aarthi et al., (2004) they mentioned that the
inactivation rate ranged from 0.53 -1.15 log10 TCID50.

Table (3) demonstrated the results obtained by estimation of CFT (Types SAT-2) before and after inactivation process showing that reduction in antigenic content was 1/16 after inactivation using BEI only while no change in CFT content using combination of BEI-formalin. These results were agreed with those of Ali et al., (2009) who mentioned that the CFT for the inactivated virus (type O) with BEI only is reduced from 1/32 to become 1/16 after inactivation while virus (type A) reduced from 1/16 to 1/8, but on the use combination of BEI-formalin there was no change in CFT with the 2 virus types. Our results disagree with Bahman (1975) who mentioned that the complement fixation test of 2 types of virus (O1 and A/1) using BEI only as inactivator were not changed before and after inactivation process but come in agreement with results obtained by Ali et al., (2009) and Hassanin et al., (2013) who mentioned that the complement fixation test of FMDV type SAT2 using BEI at 37°C, showed that antigen titers were changed from 1/32 to 1/16.

From table (4), Figure (2,3) it was shown that the serum neutralizing antibody titers in serum of G. pigs after 28 days post vaccination with both types of vaccine using BEI only or BEI-formaldehyde as inactivator were (1.8, 2.1 log10 respectively) and ELISA results were (2.05, 2.3 log10 respectively). These results come parallel to those of El-Sayed et al., (2012a), Hassanin et al., (2013) and Eman (2012) they mentioned that vaccination of different groups of Guinea Pigs with such preparations showed that the highest antibody titer appeared after 28 days post vaccination was 1.8 and 2.1 log10 using SNT and ELISA respectively using inactivated virus with 0.1M BEI at 37°C.

In conclusion, it was clear that inactivation of FMDV type SAT-2 by BEI-FA together has provided a very fast inactivation process without affecting the antigenic structure of the virus which will limit proteolytic destruction of antigen and increase antigen yields.

It is expected that by cross-linking activity of FA the stability of the antigen (and of vaccines) and the endurance of the immune response will be favorably influenced.
**Table 4:** Mean FMD SAT2 antibody titers in Guinea pigs vaccinated with the prepared FMDV inactivated vaccine.

<table>
<thead>
<tr>
<th>Weeks post vaccination</th>
<th>Antibody titer to FMDV inactivated with</th>
<th>Antibody titer to FMDV inactivated with</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>BEI</td>
<td>BEI-FA</td>
</tr>
<tr>
<td></td>
<td>SNT</td>
<td>ELISA</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>0.45</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>1.05</td>
<td>1.38</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>1.75</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Antibody titers expressed in log_{10}.

**Figure (1):** Inactivation Kinetics of FMD virus Type SAT2/2012 either BEI only or BEI-FA at 37°C at pH 8.

**Figure (2):** Mean FMD SAT2 serum neutralizing antibody titers in Guinea pigs vaccinated with the prepared FMDV inactivated vaccine with BEI or BEI-FA.
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