Standardization of vaccinal dose of FMD bivalent vaccine for cattle

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SUMMARY
An inactivated bivalent foot and mouth disease (FMD) vaccine was prepared from the vaccine strains O1/3/93 and the outbreak strain A/1/EGY/2006. Three formula were prepared each contains different ratio from the two viruses, the first was (50% of each virus type), the second was (60% of type O and 40% of type A) and the last one was (40% of type O and 60% type A). The three formulas are sterile, safe and potent. Groups of calves were vaccinated with the three formula using two different doses of each vaccine (2ml and 3ml). The vaccine induced antibody titer was monitored using SNT and ELISA. The obtained serological results revealed that the vaccine formula containing 50% of each virus type induced the best immune response with no significance difference between the two different doses.

INTRODUCTION
Foot and mouth disease (FMD) is one of the most important viral disease facing dramatically the cloven hoofed animals and resulted in devastating economic losses and assumes enzootic form in Egypt (Moussa et al., 1984; Daoud, et al., 1988 and Abd El-Rahman, et al., 2006). Since 1970 up to January 2006 serotype O1 is the predominant type in Egypt (Abd El-Rahman et al., 2006 and Ismail, 1994). Abd El-Rahman et al. (2006) reported that an outbreak of FMDV/ type A started in bulls imported from Ethiopia and kept in quarantine station at Ismallia Governorate then spread among local cattle, buffaloes and dairy farms in most governorates in upper and lower Egypt. The virus was identified as FMDV/A/Egypt/2006 by FMD department-VSVRI and World Reference Laboratory for FMD (UK). In Egypt control of FMD depends mainly on well designed vaccination programs using high potent specific vaccine. Nowadays a new bivalent inactivated FMD vaccine was flourished up containing both of the local vaccine strain O1/3/1993 and the outbreak strain A/1/EGY/2006). So, it was of importance to standardize such vaccine in an intelligent manner and that is the aim of the present work.
MATERIAL AND METHODS

Animals:

Laboratory animals:

Twenty Albino Guinea pigs were divided into 4 groups (5 animals/group). Three groups were used to test the potency of each of the prepared inactivated FMD vaccine formula while the 4th group was kept as un-vaccinated control.

Calves:

17 apparently healthy cross breed frazien calves of about 8—12 months of old free from antibody against strain O1/3/93 and A/1/Egypt/2006 were used. Three of them were used for the safety of the prepared vaccines. The rest of animals (14 calves) were divided into 7 groups as follow:

Group-1 vaccinated subcutaneously with 2 ml of the first formula of the vaccine (50% of each virus strain) whereas, group 2 inoculated with 3 ml.

Group-3 vaccinated with 2 ml of the second vaccine formula (60% of virus strain O1/3/93 and 40% of strain A) whereas, group 4 inoculated with 3 ml.

Group-5 vaccinated with 2 ml of the vaccine formula contains 40% of strain O1/3/93 and 60% of strain A) whereas, group 6 inoculated with 3ml.

Group-7 was kept without vaccination as test control.

All animals were housed under hygienic measures in separate isolates receiving balanced ration and adequate water and subjected to daily clinical examination. Serum samples were obtained from all animal groups weekly post vaccination for 4 weeks then monthly up to 4 months later to estimate the induced FMD antibodies.

FMD viruses:

Cell culture adapted FMD local strains (type-O1/3/1993 and type-A/EGY/1/2006) of a titer $10^8$ TCID$_{50}$/ml were used in the purpose of vaccine preparation; serum neutralization test and ELISA. These virus types were supplied by the Department of FMD research, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

Preparation of an inactivated bivalent FMD vaccine:

Three formula of an inactivated bivalent FMD vaccine were prepared containing different ratios of the two viruses as follow:

1- Contains 50% of each virus type.

2- Contains 60% of type O+40% of type A.

3- Contains 40% of type O+60% type A). The two virus types were inactivated using binary ethyleneimine (BEI) and 30% of aluminum hydroxide gel was added as adjuvant to each form according to Roshdy (1992).
Quality control tests:
The three prepared FMD vaccine forms were subjected to quality control tests including the following tests:

Sterility:
Random samples of each vaccine form were cultured on thioglycolate broth; Sabaroud's and nutrient agar and mycoplasma medium and observed for 14 days.

Safety test:
It was carried out according to Henderson (1970) where 1ml of each vaccine form was inoculated intradermolingual in 3 sites of the tongue of a susceptible calf. The vaccine was considered safe if no rise in the animal body temperature and no local or general lesions were detected.

Potency test:
It was carried out according to Terpestra (1974) where each vaccine form was tested in a separate group of 5 albino Guinea pigs. Three weeks post vaccination; these animals were challenged against \(10^4\) MLD\(_{50}\) of only type O1/3/93 of virulent FMD virus under complete strict hygienic measures in separate isolates to avoid any probable hazard.

Serum neutralization test:
Neutralizing FMD (type-O and type-A/Egypt/2006) antibodies were monitored in the sera of vaccinated calves using the micro-titer technique as described by Golding et al. (1976) following the directions of the King (2002).

Enzyme linked immunosorbant assay (ELISA):
ELISA was carried out for estimation of FMD (type-O and type-A/Egypt/2006) antibodies in the sera of vaccinated calves according to the method described by McCullough and Butcher (1985) and modified by Chenard et al. (2003).

RESULTS AND DISCUSSION
Foot and mouth disease (FMD) and its related research aspects represent a wide; interesting and progressable field for Veterinary Scientists. It was found that Egypt is usually threatened by FMD outbreaks which were; at most cases; caused by the virus type-O but the last one was found to be caused by the virus type-A (Abd El-Rahman et al., 2006). So it was of intelligent to prepare a specific bivalent vaccine that could be able to protect the Egyptian animals against the old established and the new arisen FMD virus strains. The present study was planned as an essential step in vaccine production that means the standardization of the required dose of the inactivated bivalent FMD vaccine on immunological bases. Through out the present work Three formula were prepared each contains different ratio from the two viruses, the first was (50% of each
virus type), the second was (60% of type O and 40% of type A) and the last one was (40% of type O and 60% type A). The experiment results showed that all of these forms were free from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma); safe and potent where vaccinated Guinea pigs did not show any signs of illness after vaccination and they were able to withstand the challenge with virulent virus type O₁ / 3/ 93. These findings appear to be supported by the directions of Henderson (1970), Terpestra (1974) and Rozas et al. (1993) who considered the vaccine is safe and potent if no rise in the animal body temperature and no local or general lesions were detected with an ability to overcome the challenge with virulent virus.

Tables (1 & 2) showed that antibodies to both FMD V type O, and A were detected by the 4th week post vaccination with titers of 2.5log₁₀ for type-O and 2.10log₁₀ for type-A by SNT and 2.7 for type-O and 2.1 for type-A by ELISA. These findings agree with those obtained by Moussa et al. (1976), Bengelsdorff (1989), Lorenz and Wittmann (1983), Hamblin et al. (1986), Halima et al. (2003) and Cheard et al. (2003) who stated that these SNT and ELISA titers were considered of good values and able to protect vaccinated cattle against virulent virus infection. In addition, these levels of cattle immune response to the different forms and different doses of the bivalent FMD vaccine; were obtained without any apparent difference between the different vaccinated animal groups reflecting a clear fact that the presence of more than one serotype of FMD virus in a vaccine dose not interfere with the induction of antibodies against another serotype and the correlation of antibody titer with protection coming in complete agreement with what suggested by Anon (2000).

From the demonstrated results it could be concluded that; from the immunological and economical aspects; the most suitable dose of the locally produced inactivated bivalent FMD vaccine is 2ml from the vaccine form containing 50% of each virus type.
Table 1 Mean neutralizing antibody titres against serotype O1/3/93 and A/Egypt/2006 in calves vaccinated with different forms of the bivalent inactivated FMD vaccine.

<table>
<thead>
<tr>
<th>Form 1</th>
<th>O1</th>
<th>A</th>
<th>Form 2</th>
<th>O1</th>
<th>A</th>
<th>Form 3</th>
<th>O1</th>
<th>A</th>
<th>control</th>
<th>O1</th>
<th>A</th>
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<td>0.35</td>
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<td>1.50</td>
<td>2.95</td>
<td>2.35</td>
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<td>1.2</td>
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<tr>
<td>3 ml</td>
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<td>0.40</td>
<td>1.0</td>
<td>0.75</td>
<td>2.4</td>
<td>2.1</td>
<td>2.7</td>
<td>2.1</td>
<td>0.30</td>
<td>1.35</td>
<td>0.95</td>
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<td>2.5</td>
<td>1.4</td>
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<td>1.50</td>
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<td>2.7</td>
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<td>2 ml</td>
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<td>2.1</td>
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<td>3 ml</td>
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</tbody>
</table>

*WPV = week post vaccination  ** MPV = Month post vaccination

Form 1 contains 50% of each virus type  Form 2 contains 60% of type O1 and 40% of type A
Form 3 contains 40% O1 and 60% A
Table 2 Mean ELISA antibody titres against serotype O1/3/93 and A/Egypt/2006 in calves vaccinated with different forms of the bivalent inactivated FMD vaccine.

<table>
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<th>FMD virus type O1- and A ELISA antibody titers /periods post vaccination</th>
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<td></td>
<td>O1</td>
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<tr>
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<td>2 ml</td>
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<tr>
<td>Form 3</td>
<td>3 ml</td>
</tr>
<tr>
<td>Control</td>
<td>0.65</td>
</tr>
</tbody>
</table>

*WPV = week post vaccination
** MPV = Month post vaccination
Form 1 contains 50% of each virus type
Form 2 contains 60% of type O1 and 40% of type A
Form 3 contains 40% O1 and 60% A
REFERENCES


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