IMMUNE RESPONSE OF SHEEP VACCINATED WITH 
INACTIVATED COMBINED FOOT AND MOUTH DISEASE, RIFT 
VALLEY FEVER AND SHEEP POX VACCINE 

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Abstract 
A combined vaccine was constructed to contain FMD O1, 
RVF ZH57 and sheep pox Egyptian strain inactivated with Binary 
ethyleneimine (BEl) and adsorbed on aluminium hydroxide gel. 
Comparative studies were conducted to evaluate the prepared 
vaccines (three monovalent FMD, RVF and Sheep Pox separately 
and the combined one). 
It was found that the serum neutralization antibody titer 
of sheep vaccinated with the combined vaccine (FMD and RVF) 
reached its peak earlier than those vaccinated with each as 
monovalent vaccine, separately, while, there was no significant 
difference in efficacy of immune response between combined 
vaccine and sheep pox vaccine. So, it is recommended that animals 
should be boosted at the 4th week post the first dose.

INTRODUCTION 

Combined vaccines are important approach to control the risk of live stock as 
well as human being diseases. Combined vaccines give the ability to use more than 
one antigen at the same time to stimulate the immune response, save time, effort 
and considered more economic. Daoud et al. (2001) found that there was no 
difference in the obtained results of serological tests for monovalent and combined 
Rift Valley Fever (RVF) and Foot and Mouth Disease (FMD). Marcoss (1992) found 
that there was a difference in the immune response between combined RVF / Sheep 
Pox and RVF or Sheep Pox alone. He found that the antibodies started to appear 
earlier in the animals vaccinated with combined vaccine than those vaccinated with 
each vaccine alone. 

Abeer (1996) mentioned that there was significant increase in the immune 
response of FMD in animals simultaneously vaccinated with Sheep Pox and FMD 
vaccine.
So, it is important to produce polyvalent vaccines for sheep and cattle, to enable the veterinary authorities to save time, effort and cost for control such diseases affecting livestock.

Therefore, the aim of this work is to prepare a combined vaccine comprising FMD, RVF and Sheep Pox antigens, and study its effect on immune response of sheep. Also, a comparison will be done with each type of vaccine separately so that one can judge on the validity of this new combined vaccine.

**MATERIALS AND METHODS**

1. ANIMALS
   Twenty-Three adult susceptible sheep free from antibodies against FMD, RVF and Sheep Pox viruses were used.

2. VIRUSES

**A- VIRULENT VIRUSES**

1. FMD VIRUS

   FMD O$_{2}$/93 Egypt, with a titer of $10^8$ MLD$_{50}$/ml was kindly obtained from FMD Vaccine Production Department, Serum & Vaccine Research Institute, Abbassia, Cairo. It was used for challenge and Serum Neutralization Test (SNT).

2. RVF VIRUS

   Rift Valley Fever Zagazig, Human strain (ZH51), isolated from a patient in Zagazig, Sharkiya Governorate (1977) had a titer of $10^{12}$ TCID$_{50}$/ml. It was kindly supplied by RVF Vaccine Production Department, Serum & Vaccine Research Institute, Abbassia, Cairo. It was used for challenge and SNT.

3. SHEEP POX VIRUS

   The Egyptian strain having a titer of $10^5$ SID$_{50}$/ml. It was kindly obtained from Pox Vaccine Production Department, Serum & Vaccine Research Institute, Abbassia, Cairo. It was used for challenge and SNT.

**B- INACTIVATED VIRUSES**

Binary Ethylenedime (BEE) inactivated FMD, RVF and Sheep Pox tissue culture vaccine were obtained kindly respectively from FMD, RVF and Sheep Pox Vaccine Production Departments, Serum & Vaccine Research Institute, Abbassia, Cairo.
3. ANTIGENS FOR ENZYME LINKED IMMUNOSORBANT ASSAY (ELISA)

A- FMD antigen
   It was prepared according to Wagner et al. (1969).

B- RVF antigen
   Lyophilized cell lysate RVF antigen was used for detection of RVF IgG antibodies using ELISA. It was prepared according to Ellen and Botros, (1997).

C- Sheep Pox antigen
   It was prepared according to House, et al. (1990).

4. PREPARATION OF THE COMBINED VACCINE
   The inactivated FMD virus was mixed together with inactivated RVF virus and inactivated Sheep Pox virus. This combined vaccine was mixed equally with aluminium hydroxide gel. The dose of combined vaccine (3ml) adjusted to contain $10^7$ TCID$_{50}$ of RVF, $10^{6.2}$ TCID$_{50}$ Sheep Pox and $10^9$ TCID$_{50}$ FMD.

5. EVALUATION OF THE PREPARED VACCINE
   a- Sterility test: The vaccine should be free from any fungal, bacterial or micoplasma contamination.
   b- Safety and potency tests: were done for FMD, RVF and Sheep Pox vaccines according to Henderson (1970), EI Nimr (1980) and OIE Manual (2000), respectively.

6. EXPERIMENTAL DESIGN
   Twenty-three adult sheep were classified as follows:
   Group A. Three sheep vaccinated subcutaneously (S/C) with 3ml combined vaccine and non-challenged.
   Group B. Two sheep vaccinated (S/C) with 3ml combined vaccine and then, challenged intradermal (I/D) with virulent FMD virus $10^6$ TCID$_{50}$/dose.
   Group C. Two sheep vaccinated (S/C) with 3ml combined vaccine and then, challenged (S/C) with virulent RVF virus $10^7$ TCID$_{50}$/sheep.
   Group D. Two sheep vaccinated (S/C) with 3ml combined vaccine and then, challenged intradermal (I/D) with virulent Sheep Pox virus $10^8$ SID$_{50}$/sheep.
   Group E. Two sheep were vaccinated (S/C) with 1ml of inactivated FMD vaccine containing $10^9$ TCID$_{50}$/sheep.
   Group F. Two sheep were vaccinated (S/C) with 1ml of inactivated RVF vaccine containing $10^6$ TCID$_{50}$/sheep.
   Group G. Two sheep were vaccinated (S/C) with 1ml of inactivated Sheep Pox vaccine containing $10^{6.2}$ TCID$_{50}$/sheep.
**Group H.** Two sheep were used as control non-vaccinated non-challenged.

**Group I.** Two sheep were challenged intra-dermohlingual with $10^4$ TCID$_{50}$/ml FMD virulent virus.

**Group J.** Two sheep were challenged (S/C) with $10^5$ TCID$_{50}$/ml RVF virulent virus.

**Group K.** Two sheep were challenged (I/D) with $10^3$ SJD$_{50}$/ml Sheep Pox virulent virus.

**SEROLOGICAL TESTS**

1- **SERUM NEUTRALIZATION TEST (SNT)**

Specific neutralizing antibodies against FMD virus were detected following Ferrier (1976). Antibodies against RVF virus were detected following Pini et al. (1973) and specific antibodies against sheep pox virus were detected following OIE Manual (2000).

2- **ELISA TEST**

This test was used to detect IgG antibodies in serum samples against FMD virus according to Hamblin, et al. (1986), against RVF virus according to Meegan et al. (1987), and against sheep pox virus according to Carn (1993).
Table 1. The mean Neutralizing Index (NI) at the following weeks post-vaccination.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Types of Vaccine</th>
<th>Weeks post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (FMD)</td>
<td>3</td>
<td>Combined FMD, RVF, &amp; Sheep Pox Vaccine</td>
<td>0.4 1.1 1.35 1.5 1.8 1.95 2.1 2.4 2.4 1.95 1.95 1.8 1.6 1.5 1.5</td>
</tr>
<tr>
<td>GA (RVF)</td>
<td>3</td>
<td>FMD</td>
<td>0.5 0.8 1.4 1.8 2.3 2.5 2.6 2.8 2.7 2.5 2.4 2.2 2.0 1.9 1.9</td>
</tr>
<tr>
<td>GA (Sheep Pox)</td>
<td>3</td>
<td>RVF</td>
<td>0.35 0.9 1.6 2.3 1.8 1.7 1.7 1.6 1.5 1.4 1.2 1.0 0.9 0.6 0.6</td>
</tr>
<tr>
<td>GE</td>
<td>2</td>
<td>Sheep Pox</td>
<td>0.3 0.8 1.12 1.35 1.65 1.8 1.8 1.87 1.95 1.8 1.5 1.2 1.2 1.1 0.6</td>
</tr>
<tr>
<td>GF</td>
<td>2</td>
<td>FMD</td>
<td>0.5 0.6 1.2 1.5 1.7 2.2 2.4 2.5 2.5 2.2 2.0 1.8 1.5 1.4 1.2</td>
</tr>
<tr>
<td>GG</td>
<td>2</td>
<td>RVF</td>
<td>0.2 0.7 1.5 2.0 1.7 1.6 1.6 1.5 1.5 1.3 0.9 0.7 0.5 0.3 0.3</td>
</tr>
<tr>
<td>GH (FMD)</td>
<td>2</td>
<td>Sheep Pox</td>
<td>0.3 0.6 0.4 0.6 0.4 0.3 0.3 0.6 0.4 0.6 0.5 0.4 0.4 0.3 0.5</td>
</tr>
<tr>
<td>GH (RVF)</td>
<td>2</td>
<td>Control Non Vaccinated Sheep</td>
<td>0.5 0.4 0.7 0.4 0.5 0.5 0.4 0.4 0.5 0.7 0.7 0.4 0.5 0.4 0.4</td>
</tr>
<tr>
<td>GH (Sheep Pox)</td>
<td>2</td>
<td>Control Non Vaccinated Sheep</td>
<td>0.2 0.5 0.4 0.3 0.6 0.6 0.3 0.5 0.3 0.2 0.6 0.5 0.3 0.4 0.4</td>
</tr>
</tbody>
</table>

GA = combined FMD, RVF and Sheep Pox vaccinated animals. GE = FMD vaccinated animals. GG = Sheep Pox vaccinated animals. GF = RVF vaccinated animals. GH = control non vaccinated animals.
Table 2. The mean results of ELISA test of vaccinated sheep with combined or single vaccine as well as control sheep.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Types of Vaccine</th>
<th>Weeks post - vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>GA (FMD)</td>
<td>3</td>
<td>Combined FMD, RVF</td>
<td>0.038</td>
</tr>
<tr>
<td>GA (RVF)</td>
<td>2</td>
<td>RVF sheep Pox vaccine</td>
<td>0.181</td>
</tr>
<tr>
<td>GA (Sheep Pox)</td>
<td>2</td>
<td>Sheep Pox</td>
<td>0.043</td>
</tr>
<tr>
<td>GE</td>
<td>2</td>
<td>FMD</td>
<td>0.028</td>
</tr>
<tr>
<td>GF</td>
<td>2</td>
<td>RVF</td>
<td>0.175</td>
</tr>
<tr>
<td>GG</td>
<td>2</td>
<td>Sheep Pox</td>
<td>0.040</td>
</tr>
<tr>
<td>GH (FMD)</td>
<td>2</td>
<td>Control Non Vacciated Sheep</td>
<td>0.027</td>
</tr>
<tr>
<td>GH (RVF)</td>
<td>2</td>
<td>Sheep Pox</td>
<td>0.182</td>
</tr>
<tr>
<td>GH (Sheep Pox)</td>
<td>2</td>
<td>Sheep Pox</td>
<td>0.031</td>
</tr>
</tbody>
</table>

ELISA represented as optical density. FMD cut off = 0.082  RVF cut off = 0.185  Sheep Pox cut off = 0.085
Cut off according to Edward (1985).
Table 3. The mean results of serological tests of challenged sheep as well as control ones.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type Vaccine</th>
<th>No.</th>
<th>Weeks post-vaccination</th>
<th>Days post challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0  1  2  3  4</td>
<td>1  3  5  7  10  15</td>
</tr>
<tr>
<td>GB (FMD)</td>
<td>Combined</td>
<td>2</td>
<td>0.5 1.2 1.5 1.8 1.95</td>
<td>1.8 1.85 1.95 2.1 2.5 2.5</td>
</tr>
<tr>
<td>GB (FMD)</td>
<td>FMD, ELISA</td>
<td></td>
<td>0.043 0.116 0.145 0.196 0.194</td>
<td>0.182 0.154 0.197 0.219 0.250 0.292</td>
</tr>
<tr>
<td>GB (FMD)</td>
<td>ELISA</td>
<td></td>
<td>0.5 0.8 1.2 1.5 1.8</td>
<td>2.2 1.8 2.2 2.5 2.5 2.8</td>
</tr>
<tr>
<td>GC (RFP)</td>
<td>RFP</td>
<td>2</td>
<td>0.182 0.196 0.230 0.252 0.266</td>
<td>0.270 0.252 0.272 0.297 0.310 0.308</td>
</tr>
<tr>
<td>GD (FMD)</td>
<td>Sheep Pox</td>
<td></td>
<td>0.4 0.6 1.0 1.9 2.2</td>
<td>1.8 1.7 1.5 1.8 2.5 2.6 2.8</td>
</tr>
<tr>
<td>GD (FMD)</td>
<td>Vaccine</td>
<td>2</td>
<td>0.045 0.083 0.172 0.200 0.170</td>
<td>0.162 0.134 0.176 0.220 0.231 0.243</td>
</tr>
</tbody>
</table>

ELISA represented as optical density
FMD cut off = 0.082  RFP cut off = 0.185  Sheep Pox cut off = 0.085
No. = Number of animals
RESULTS AND DISCUSSION

The intervention for vaccinating animals by each vaccine singly is troublesome and time consuming. Therefore, since some years, many vaccine producing factories succeeded in combining more than one vaccine to be inoculated in the same dose and at the same time (Taha et al., 1991).

The present study aimed to carry experimental trials for preparing a combined vaccine including foot and mouth antigen, Rift Valley Fever and sheep pox antigens together, and comparing the sero-conversion of sheep vaccinated with this combined vaccine, as well as animals vaccinated with each monovalent.

Regarding serum neutralization test, Table 1 revealed the neutralizing antibodies for FMD vaccine started to appear from 1<sup>st</sup> week being (0.8) neutralizing index (NI) and reaching its peak at the 12<sup>th</sup> week (1.95). In those vaccinated with the combined vaccine, the FMD antibodies at the 1<sup>st</sup> week were (1.1) and reaching their peak at the 10<sup>th</sup> week (2.4) and still within the protective level up till the end of the experiment (24 weeks) (1.5), as recorded by Ferreira (1976) who found that the level of protection was (1.2). Regarding neutralizing antibodies for RVF vaccine, they started to appear from the 1<sup>st</sup> week, in animals vaccinated with RVF vaccine alone. The mean of neutralizing index was (0.8) and reached its peak at the 10<sup>th</sup> week being (2.5). In those vaccinated with the combined vaccine at the 1<sup>st</sup> week, the neutralizing index was (0.8) and reached its peak at 10<sup>th</sup> week being (2.8) and still being protective (1.9) up till the end of the experiment (24 weeks) as mentioned by Pini et al. (1973) who found that the protective level of antibody against RVF was (1.5) log<sub>10</sub>TCD<sub>50</sub>. In animals vaccinated with either FMD or RVF as monovalent vaccine, it was found that the protective level was up till the 18<sup>th</sup> week in FMD (1.2), and for RVF in the 20<sup>th</sup> week was (1.5) and then, began to decrease below the protective level.

The antibodies started to appear in the 1<sup>st</sup> week in sheep vaccinated with sheep pox vaccine. The mean of neutralizing index was (0.7) and reached its peak at the 3<sup>rd</sup> week (2.0), then, gradually decreased till the 12<sup>th</sup> week (1.5) as recorded by Cotral (1978) who determined that the NI ≥ 1.5 was considered protective for sheep pox virus. The antibodies in these animals decreased to be (0.3) at the end of the experimental period (24 weeks), while, in those vaccinated with the combined vaccine, the antibodies started to appear in the 1<sup>st</sup> week being (0.9) and reached their peak at the 3<sup>rd</sup> week (2.3) and still within the protective level up till the 12<sup>th</sup> week (1.5), then, gradually decreased up till the end of the experiment being (0.6) at the 24<sup>th</sup> week.
From the previous results, it is clear that sheep pox vaccine acted as an immuno-potentiating agent, and the use of the combined vaccine is of much benefit for increasing the duration of the immuno-response of the animals. This agrees with Taha et al. (1991) who found that the NI in sheep vaccinated with combined vaccine reached its peak earlier than in those vaccinated with each vaccine alone. Also this agrees with Abeer (1996) who mentioned that there was significant increase in the immune response of FMD in animals simultaneously vaccinated with sheep pox and FMD vaccines.

It is also clear that, in animals vaccinated with sheep pox vaccine, the antibody level decreased from the 14th week below the protective level. It is recommended that these animals should be boostered with sheep pox vaccine at the 4th week from the first dose as said by Manal et al. (2003) to prolong the period of immunity. On the other side, in animals non-vaccinated (Group H), the NI did not exceed (0.7).

From Table 3, it is clear that the combined vaccine can protect animals against the three viruses when challenged four weeks post-vaccination with virulent FMD virus (10^6 MLD50/ml), RVF virus (10^5 TCID50/ml) and sheep pox virus (10^3 SID50/ml). These results agreed with the data obtained by Marcoos (1992) who prepared RVF/sheep pox combined vaccine and also protected sheep when challenged with both virulent viruses (RVF and sheep pox virus). Also, Daoud et al. (2001) found that combined vaccine (RVF/FMD) could protect animals against both viruses when challenged with virulent RVF and FMD viruses.

In non-vaccinated challenged sheep, there was no rise in the NI before challenge (Table 3). Then, it began to be detectable on the 7th day for FMD, RVF and sheep pox, respectively, and reached its peak on the 15th days post-challenge for FMD, RVF and sheep pox viruses, respectively.

The results of SNT and ELISA tests were in parallel to each other and equally sensitive. This agreed with Niklasson et al., (1984). Finally, from the previous mentioned data, it is clear that FMD/RVF and sheep pox combined vaccine could be used safely for protection of sheep against these diseases.
REFERENCES


الأستجابة المناعية للأغذام المحصنة بلقاح مركب ثلاثي لفيروسات الحمى القلاعية وحمي الوادي المصدع وجدري الأغذام

عبير أحمد طلعت، مرتبة محمد علي، إلى صبيح سلامة

مختبر بحوث الأسنان واللقاحات لمركز بحوث الزراعة والرئاسة العامة - جيزة - مصر

تم تحضير وتقييم لقاح مركب يحتوي على ثلاث عشات من فيروس الحمى القلاعية الحاوية (0) و فيروس حمي الوادي المصدع الحاوية (ZHB0) و فيروس جدري الأغذام الحاوية المصرية والذين تم تطبيقهما بواسطة مادة البيئية ذات الامن والمخلطة مع مادة الألومنيوم هيدروكسيد جيل، وقد أجريت عدة تجارب لقياس الاستجابة المناعية للحيوانات المحصنة باللقاحات المنفردة ومقارنة نتائجها مع نتائج التلال المركب. ووجد أن الأنسج المناعية المعطاة في أسلاك الحيوانات المحصنة باللقاح المركب وصل إلى مستوى أعلى في فترة أقل من الحيوانات المحصنة باللقاحات المنفردة (الحمى القلاعية وحمي الوادي المصدع) بينما لم يوجد فرق معنوي واضح بين الحيوانات المحصنة باللقاح المركب ولقاح جدري الأغذام المنفرد، مما يصبح بإمكان جرعة منخفضة ماعة من الأسراب الرابع من بداية التحصين.