Immune responses to inactivated oil adjuvanted Equine Herpes Virus-1 using different emulsifiers in horses

B K Singh*, S N Tandon and N Virmani
National Research Centre on Equines, Hisar 125 001, India

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Immune responses to formalin inactivated, oil adjuvanted equine herpes virus-1 (EHV-1) using Tween-80 (OET-80) and mannide monooleate (OEMM) emulsifiers were compared with a killed EHV-1 commercial vaccine in the three groups of EHV-1 seronegative Kathiawari horses. Each group of horses (n=3) was immunized with three doses each of the OET-80, OEMM and commercial vaccine. The complement fixation test (CFT) and virus neutralization test (VNT) were used to measure antibody levels in these horses. Mild complement-fixing (CF) antibody responses were observed after primary immunization with all the three products. Nearly 4-fold rise in CF antibody response after first booster in horses was seen up to 5, 6 and 9 weeks with OET-80, commercial vaccine and OEMM, respectively. Second booster CF antibody response was also seen in all the three groups. After primary immunization, VN antibody response was noticed in all the three groups. However, first booster injection of commercial vaccine, OET-80 and OEMM increased nearly 4-fold rise in VN antibody titre; which lasted up to 7, 9, and 12 weeks, respectively. VN antibody titre obtained after first booster injection of OEMM in horses was significantly higher (P ≤ 0.05) as compared to OET-80 and commercial vaccine. After second booster injection rise in VN antibody titre was also observed in all the three groups of immunized horses. Control group of horses did not show any CF and VN antibodies responses. OEMM produced better CF and VN antibody responses than OET-80 and commercial vaccine.

Keywords: booster effect, CFT, EHV-1 immunogen, emulsifiers, horses, immunization, VNT

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Introduction

Commercial oil adjuvanted inactivated equine herpes virus-1 vaccine is being imported for immunoprophylaxis of brood mares against (EHV-1) infection in India. This commercial vaccine contains army 183 EHV-1 strain (exotic). Although, this vaccine is considered safe1, but, little is known about its protective efficacy. Live viral vaccines may induce better immunity but may cause vaccination illness, persisting infection and reversion of virulence2. Moreover, there is also scope of import of new exotic EHV-1 strain if live vaccine is imported, because modified live virus vaccine retained the ability to cause disease3. Humoral and cell-mediated immune responses are essential for affording protection against EHV-1 infection4. For many years, involvements of EHV-1 in causing clinical cases in equines were carried out in India5-8 and nearly 13.5% (349/2573) horses were found seropositive9. But, an attempt towards developing a killed vaccine incorporating Indian EHV-1 strain (Hisar-90-7) for immunoprophylaxis of brood mares against EHV-1 infection in this country was initiated recently10. It has been reported that 100 µg EHV-1, inactivated with formaldehyde, adjuvanted with mineral oil, emulsified with Tween -80 produced good immune response. This report deals with the immune responses to inactivated oil adjuvanted equine herpes virus-1 using different emulsifiers in horses.

Materials and Methods

Preparation of Immunogens

Indian strain of EHV-1 (Hisar-90-7) was cultivated in equine dermal cells (ED), partially purified and pelleted. The virus titre of the seed virus was determined and it was diluted in such a way that per dose of immunogen contained quantified virus particles. Formalin was added to inactivate the virus10. This formed the aqueous phase of the immunogen. Residual infectivity of the virus was tested by inoculating the formalin treated virus on the tissue culture monolayer. Aqueous phase of the viral

*Author for correspondence:
Tel: 91-1662-276151; Fax: 91-1662-276217
E-mail: bksinghmrec@yahoo.co.in
antigen was blended with mineral oil followed by its
emulsification with polyoxyethylene sorbiton mono-
oleate (Tween-80) for first type of immunogen
product (OET-80). For the preparation of second type
of immunogen product, the aqueous phase was
blended with mineral oil followed by its
emulsification with mannide monooleate (OEMM).
The immunogen thus prepared using 2 different
emulsifiers was dispensed in such a way that 2 mL of
it may contain quantified inactivated virus particles.
Both products were then tested for sterility, safety and
immunogenic potency. Sterility testing of these
products was carried out for bacteria, fungi and
mycoplasma contamination. Safety testing of each
product was carried out by inoculating 0.5 mL of each
product intraperitoneally in 5 BALB/C mice.

Immunization of Horses
Kathiawari breed of adult horses of both sexes, tested sero-negative for EHV-1 antibody by CFT and
VNT were used for immune response study. Animals
were maintained in isolation in stables during the
whole immunization studies. OET-80, OEMM and
commercial EHV-1 vaccines were inoculated in 3
groups of horses (each group having 3 horses).
Control group-4, had 6 horses (2 horses for each type
of product) and was inoculated with oil adjuvant
emulsified with Tween-80/mannide monooleate and
cell lysates. An imported commercial vaccine was
inoculated by intramuscular route (I/M) in all three
immunizations. The compatibility and safety of the
immunization material was examined by observing
the injected spots and general condition of the animals
for 7 days after primary and booster immunization.
Temperature was taken twice daily. The horses were
observed carefully for any abnormality including
nasal discharge. Primary immunization studies were
of 8 weeks period.
The first boosters with OET-80 and OEMM were
given by S/C route on 9th week and immune
responses were studied up to 20th week. The
commercial vaccine (imported) was injected by I/M
route on 9th week after primary immunization as per
manufacturer’s instruction. Serum samples of these
horses were collected at weekly intervals up to 30th week. The
animals after each immunization were observed for
thermal reaction, nasal discharge and any reaction at
the site where immunogen was injected. The second
booster studies were of 10 weeks period.

Serological Tests
Virus Neutralization Test (VNT)
A microtitre VNT conducted in rabbit kidney (RK-
13) cells was similar to that followed by Singh et al
11. The VN antibody titre of 1:8 (0.9 in log10) was
considered as positive.

Complement Fixation Test (CFT)
The CF test using polyethylene glycol (PEG)
concentrated EHV-1 antigen was performed as per
Singh 10. CF antibody titres were expressed as the
reciprocal of the highest dilution of the serum
reducing the haemolysis to 50% or less. The CF
antibody titre of 1:8 (0.9 in log10) was considered as
positive.
The correlation co-efficient between the results
obtained of CF and VN tests in various immunization
schedules were calculated using Chi-square test 12.

Results
Residual infectivity of the killed virus when tested
by inoculating on tissue culture monolayer was found
negative for infectious agent. It was safe as it did not
produce any adverse reaction either in BALB/C mice
or in immunized horses up to 7 d of critical
observation. Primary and first booster immunization
of horses by S/C route with OET-80 led to the
development of soft swelling on 24 h post
immunization in 2 out of 3 horses at the site of
inoculated spot which completely subsided after 48 h.
Commercial vaccine did not produce swelling at the
site of inoculation. None of the horses showed either
nasal discharge or thermal reaction post-
immunization. When OET-80 was inoculated by I/M
route during second booster, it did not produce any
reaction at the site of inoculation.

CF antibody responses to inactivated oil adjuvanted
EHV-1 using different emulsifiers after primary
immunization in horses are shown in Table 1. On
primary immunization of horses with OET-80 and
commercial vaccine immune response by CFT was
detected on week 1 while at 2 weeks post-
immunization in OEMM group. After first booster
injection in horses with OET-80, OEMM and
commercial vaccine, nearly 4-fold rise in CF antibody
titres were recorded for weeks 5, 9 and 6, respectively.
The correlation co-efficient between the results using Chi-square test showed that CF antibody titre in the horses immunized with OEMM induced significantly higher (P ≤ 0.05) in the horses after the first booster compared to OET-80 and commercial immunogen. After second booster injection of OET-80, commercial vaccine and OEMM, the CF antibody lasted for 7, 8 and 9 weeks, respectively (Table 3). VN antibody responses to inactivated oil adjuvanted EHV-1 using different emulsifiers after primary immunization in horses are shown in Table 1. Primary immunization of horses did not show any significant difference in VN antibody responses with OET-80, OEMM and commercial vaccine. After first booster injection in horses with commercial vaccine, OET-80 and OEMM and, nearly 4 fold rise in CF antibody titres were recorded for 7, 9 and 12 weeks, respectively (Table 2). The correlation co-efficient between the results of VN antibody titre using Chi-square test showed that the horses immunized with OEMM induced significantly higher VN antibody titre (P ≤ 0.05) in the horses after the first booster compared to OET-80 and commercial immunogen. After second booster injection, VN antibody responses in groups 2 and 3 immunized horses lasted for 12 weeks while it lasted for 7 weeks in group 1 (Table 3). Horses of control group-4 were seronegative by CFT and VNT to EHV-1 during the entire period of study.

**Discussion**

Several emulsifiers including Tween-80 and mannide monooleate containing mineral oil have been used in experimental vaccines. In this study, Tween-80 and mannide monooleate were used for emulsification of oil adjuvanted EHV-1 antigen with the aim that there should not be local reaction at injected spot and for getting good serological responses.
responses. However, OET-80 produced local swelling at the site of inoculated spot. Local swelling at the site of inoculated spot of oil adjuvanted vaccine is possible. In earlier study, Thomson et al\textsuperscript{16} used Tween-80 with hope that it would lessen the severity of local swelling but in present study, it could not be achieved. It might be due to S/C injection of OET-80. OEMM did not produce any swelling at the inoculated spot. When OET-80 and OEMM were inoculated by I/M route during second booster injection, there was no reaction at the inoculated spot. The commercial vaccine developed by Bryans\textsuperscript{17} inoculated by I/M route also did not show any reaction at the inoculated spot.

After primary immunization of horses with OET-80 and commercial vaccine CF antibody was detected in the first week post-immunization while it was detected in the second week post-immunization with OEMM. Moderate VN antibody titre appeared in second week post-immunization with OET-80 and OEMM, which increased in week 4-5 post immunization. The CF and VN antibodies responses after primary immunization with all three immunogens were moderate. If horses are not exposed earlier to EHV-1 vaccination/infection then there may be possibility of mild CF and VN antibody response after primary vaccination. However, it has been reported that 13.5% adult Indian horses are found seropositive to EHV-1\textsuperscript{19}; hence, good serological response may be expected. Both first and second booster injections showed good rise in CF and VN antibodies. Good serological responses in horses after immunization with EHV-1 killed vaccine\textsuperscript{18} were also reported in earlier studies. In the present study, booster immunization showed significant seroconversion of CF and VN antibody which are as per line of earlier report\textsuperscript{2} that vaccination of mature and young horses with inactivated EHV-1 whole virus containing conventional mineral oil to the immunizing antigen leads to development of rapid and higher titre of VN and CF antibodies. The significant seroconversion (CF and VN antibodies) after first and second booster injection justifies the parameter of potency testing with some extent where animal challenge is not permissible as per OIE Manual\textsuperscript{19}.

The CF and VN antibody persistence after first booster injection with OEMM remained for longer duration compared to those immunized with OET-80. It indicated that EHV-1 killed antigen blended in mineral oil, emulsified with mannide monooleate helped in improving the quality of the immunogen in slow releasing of antigen particle from adjuvant to the injected spot and stimulating immune system slowly giving better CF and VN antibody response than OET-80. Subcutaneous route used for immunization of horses during primary and first booster injection of OET-80 and OEMM might have also influenced immune responses. It has been reported earlier that the immunization route used to deliver conventional viral vaccines may also influence the type and magnitude of the resulting immune responses as observed in case of subunit vaccine\textsuperscript{20}. But, S/C inoculation of oil emulsion vaccine may cause local reaction resulting swelling at the inoculated spot as noticed with OET-80; hence in subsequent injection

Table 3—Complement-fixing and virus-neutralizing serum antibody responses to inactivated oil adjuvanted equine herpes virus-1 using different emulsifiers after second booster injection in horses

<table>
<thead>
<tr>
<th>Weeks after first booster injection (weeks after primary immunization)</th>
<th>Complement-fixing antibodies titre log\textsubscript{10} (GM±SD)</th>
<th>Virus-neutralizing antibodies titre log\textsubscript{10} (GM±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OET-80</td>
<td>OEMM</td>
<td>Com.Vac</td>
</tr>
<tr>
<td>1(21)</td>
<td>1.4±0.173</td>
<td>1.3±0.173</td>
</tr>
<tr>
<td>2(22)</td>
<td>1.4±0.173</td>
<td>1.5±0.0</td>
</tr>
<tr>
<td>3(23)</td>
<td>1.4±0.173</td>
<td>1.7±0.173</td>
</tr>
<tr>
<td>4(24)</td>
<td>1.5±0.3</td>
<td>1.8±0.0</td>
</tr>
<tr>
<td>5(25)</td>
<td>1.4±0.458</td>
<td>1.7±0.173</td>
</tr>
<tr>
<td>6(26)</td>
<td>1.3±0.346</td>
<td>1.6±0.346</td>
</tr>
<tr>
<td>7(27)</td>
<td>1.0±0.458</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>8(28)</td>
<td>-</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>9(29)</td>
<td>-</td>
<td>1.1±0.346</td>
</tr>
<tr>
<td>10(30)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

GM= Geometric mean titre; Com.Vac=Commercial vaccine; - = Negative; Control group-4 of horses remained seronegative to EHV-1 by CFT and VNT

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(second booster injection) both OET-80 and OEMM were injected through I/M route.

Current efforts were to test safe and effective emulsifiers (viz Tween-80 a non-ionic emulsifier and mannide monooleate a non-ionic but surface active emulsifier) in combination with inactivated EHV-1 antigen blended in mineral oil. Formulation of EHV-1 inactivated antigen containing oil emulsion and emulsifying agent, Tween-80 and mannide monooleate have produced good serological responses. In earlier experimental vaccines also formulation containing oil emulsion and Tween-80 has produced good serological responses. Other formulation containing vegetable oil emulsion and Tween-80 responses. In earlier experimental vaccines also formulation containing non-ionic block co-polymers, in Vaccine design: The Subunit and adjuvant approach, edited by M F Powell and J Mark (Newman Pharmaceutical Biotech, USA) 1995, 297-311.

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References