TRIALS TO ATTENUATE EHV-1 ON CELL CULTURE
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A B S T R A C T

The present study was designated to obtain an attenuated strain from local isolate of virulent equine herpes virus type 1 (EHV-1) by successive serial passages on Vero cell line at 37°C (13 passages) and 26°C (51 passages). The obtained results showed that the attenuated EHV-1 was found to be avirulent, but immunogenic in horses if injected intramuscularly. Also, the attenuated EHV-1 was safe, immunogenic and potent when inoculated intranasal in pregnant mice as an animal model instead of horses.

KEY WORDS: EHV-1, Equine, Mice, Vero cell line.

1. INTRODUCTION

Equine herpes viruses comprise a group of antigenic distinct biological agents which cause a variety of infection in horses ranging from subclinical to fatal disease. The most important one of them is the equine herpes virus-1 (EHV-1) which belongs to family Herpesviridae subfamily alpha-herpes viruses, genus varicellovirus [4].

EHV-1 can be contracted through inhalation causing one of four syndromes (abortion, respiratory disorders, neonatal foal disease or neurological syndrome) [9]. So, it considered a sever threat to horses which have in close groups, such as studs. Therefore, prevention of infection is of major economic importance.

Since controlling of this disease was achieved by vaccination [11], current vaccines against this virus include chemically inactivated EHV-1 or live attenuated virus [6]. Inactivated vaccines are generally known to be weak inducers of cell-mediated immunity that is responsible for controlling EHV-1 infection. In contrast, modified live vaccines usually induce adequate cellular and humeral long-lasting immune response in inoculated animals [10].

The present study was undertaken to obtain an attenuated strain from the locally strains of virulent EHV-1 by serial passages of it on Vero cell line [8] as a primary step for production of attenuated EHV-1 vaccine

2. MATERIAL AND METHODS

2.1. Virus:
Freeze dried local strain of EHV-1 egg passage 3 (EP3) was isolated and characterized by Equine Vaccine Research Dept., Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

2.2. Tissue culture:
African green monkey kidney cell line (Vero) was grown, maintained and used for virus passage and titration.
2.3. Animals:
2.3.1. Mice:
Groups of pregnant BALB/C mice were used as a model of EHV-1 in horses [1, 2] for determination of the stage of EHV-1 attenuation and its immunogenicity and potency.

2.3.2. Horses:
Three pregnant mares at last third of gestation period were used to prove the attenuated EHV-1 was immunogenically and avirulent.

2.4. EHV-1 attenuation assay:
A process for the production of an immunogenically active attenuated live EHV-1 was carried out according to Purdy et al. [7], briefly: EHV-1 (egg passage 3) was inoculated into monolayer sheet of Vero cell, after adsorption time 1 hour at 37°C, maintenance media were added. Infected cell cultures were incubated at 37°C for 4 to 5 days until cytopathic effect (CPE) becomes evident. Serial passages were carried out for 13 times at incubation temperature of 37°C. At passage No. 14, virus adsorption time was at 37°C while the infected cell cultures were incubated at 26°C until CPE was completed. Further serial passages were done following the same process (C) until the immunogenicity active live avirulent EHV-1 was produced.

2.5. Determination of the passage of EHV-1 at which it becomes avirulent through several passages of EHV-1 (passage No. 30, 40, 50, 55, 60, 62, 64 on Vero cell at 26°C) were inoculated intra-nasally (45 µl/mouse) at late stage of pregnancy.

To ensure that the EHV-1 was avirulent and immunogenically, pregnant mares and mice were inoculated as follows: Passage No. 64 of EHV-1 (titer 6.5 log10 TCID50/ml) was inoculated I.N. into three groups of mice at 16th or 17th days of pregnancy (45µl/mouse) and after 10 days post inoculation, mice of group (A) were bled for serological test (ELISA), and mice of group (B) were inoculated I.N. by virulent EHV-1 of titre 7 log10 TCID50/ml (45 µl/mouse). Mice of group (C) were kept as control. The same passage EHV-1 (No. 64) was inoculated intramuscularly into two pregnant mares (2ml/dose) [7], and the 3rd mare was kept as control. Pre- and post-inoculation (after 21 days) serum samples were collected from all horses for screening of EHV-1 antibodies by using ELISA and serum neutralization test. All horses were kept under observation till parturition.

2.6. Passage No. 62, 64 of EHV-1 incubated at 26°C and passage No. 13 at 37°C were titrated in tissue culture plate.

3. RESULTS AND DISCUSSION

In this paper we reported a process for preparation of an immunogenically active attenuated EHV-1 as a preliminary step for production of attenuated EHV-1 vaccine which usually induce adequate humeral and cellular immune response inoculated animals [10].

EHV-1 locally isolated strain (egg passage 3) was propagated on Vero cell line via 13 passages at 37°C then at 26°C until the passage 64. Different passages were inoculated I.N. into groups of pregnant mice to assay its attenuation.

The results presented (table 1, fig. 2) showed that the abortion in pregnant mice was decreased from the passage 55 and disappeared by the passage 62. This result agree with Purdy et al. [7] who reported that the EHV-1 which passed for 50 passages through Vero cell line at 26°C were found to be avirulent.

Titre of different passages of EHV-1 were presented in Table (2) where the infectivity titre of the fully attenuated EHV-1 passage 62th and 64th was 6.5 Log10 TCID50/ml, that is in agreement with the result of Purdy et al. [7] in which the titre of attenuated EHV-1 passage 50th was 6.7 Log10 TCID50/ml. To evaluate the immunogenicity and potency of the attenuated EHV-1, pregnant mice were
used as a model instead of horses according to Awan et al. [3], Tsujimura et al. [10] and Walker et al. [12].

**Table 1** Determination of EHV-1 virulence in pregnant mice

<table>
<thead>
<tr>
<th>Passage No. of EHV-1</th>
<th>No. of inoculated mice</th>
<th>Aborted mice</th>
<th>Non-aborted mice</th>
<th>Incidence of abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100 %</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100 %</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>80 %</td>
</tr>
<tr>
<td>55</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>60 %</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>20 %</td>
</tr>
<tr>
<td>62</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0 %</td>
</tr>
<tr>
<td>64</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0 %</td>
</tr>
</tbody>
</table>

Pregnant mice of group (B) do not show any untoward reaction (roughness, abortion, loss of weight, nervous signs, deaths, hypersensitivity) while pregnant mice of group (C) which was kept as control were aborted after inoculation with virulent EHV-1. These results revealed that the attenuated EHV-1 was potent in mice. The attenuated EHV-1 passage 64 was inoculated into two pregnant mares to determine its safety and immunogenicity. The results in Table (4) showed that there was significant increase in neutralizing and ELISA antibody titre in serum samples of pregnant mares, 21 days after inoculation of attenuated EHV-1 while in control mare there is no any increase in antibody titre which revealed that the attenuated EHV-1 was immunogenic in horses as recorded by Vallat and Edwards [11], also all mares were get birth of healthy foal.

**Table 3** Antibody titres against EHV-1 in inoculated mice determined by ELISA

<table>
<thead>
<tr>
<th>Mice No.</th>
<th>Antibody titres by ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1580</td>
</tr>
<tr>
<td>2</td>
<td>1600</td>
</tr>
<tr>
<td>3</td>
<td>1700</td>
</tr>
<tr>
<td>4</td>
<td>1650</td>
</tr>
<tr>
<td>5</td>
<td>1800</td>
</tr>
<tr>
<td>Mean</td>
<td>1620</td>
</tr>
</tbody>
</table>

Pregnant mice of group (B) do not show any untoward reaction (roughness, abortion, loss of weight, nervous signs, deaths, hypersensitivity) while pregnant mice of group (C) which was kept as control were aborted after inoculation with virulent EHV-1. These results revealed that the attenuated EHV-1 was potent in mice. The attenuated EHV-1 passage 64 was inoculated into two pregnant mares to determine its safety and immunogenicity. The results in Table (4) showed that there was significant increase in neutralizing and ELISA antibody titre in serum samples of pregnant mares, 21 days after inoculation of attenuated EHV-1 while in control mare there is no any increase in antibody titre which revealed that the attenuated EHV-1 was immunogenic in horses as recorded by Vallat and Edwards [11], also all mares were get birth of healthy foal.

**Table 4** Antibodies titre against EHV-1 in horses before and after inoculation determined by ELISA and SNT

<table>
<thead>
<tr>
<th>Horse No.</th>
<th>ELISA Before</th>
<th>ELISA After</th>
<th>SNT Before</th>
<th>SNT After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>157</td>
<td>400</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>314</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>151</td>
<td>152</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

*Horse No. 3 was kept as control*
4. CONCLUSIONS

In conclusion, the obtained data clarify that:
1. Serial passages of EHV-1 on Vero cell line at incubation temperature of 37°C for 13 passages and 51 passages at 26°C led to evolution of fully attenuated strain.
2. The attenuated EHV-1 strain was immunogenic and potent in mice as model animal (instead of horses).
3. The attenuated EHV-1 was immunogenic and safe in pregnant mares.
4. The produced EHV-1 attenuated strain needs further studies for vaccine production.

5. REFERENCES

محاولات لإستضعاف فيروس الإجهاض المعدى في الخيول على الخلايا النسيجية الحية

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الملخص العربي

أجريت هذه الدراسة بهدف استضعاف العترة المعزولة محلياً لفيروس الأجهاض المعدى في الخيول بتمريرها على خلايا كلية الفرد الأخضر الأفريقي (Vero cell line) عند درجة حرارة 37 (13 تمريرة) و 26 درجة مئوية (51 تمريرة).

أظهرت نتائج هذه الدراسة أن العترة المستضعفة من فيروس الأجهاض المعدى في الخيل فاقت ضراوبها ولكن احتفظت بقدرها على إنتاج الأجسام المناعية عند حقنها في كل من الخيل والفئران الحوامل. ولم يحدث أي إجهاضات في الفئران المحقونة بالعترة المستضعفة عند إجراء اختبار التحدي بالعترة الضارية. نستخلص من هذه النتائج نجاح محاولات استضعاف فيروس الإجهاض المعدى في الخيول على الخلايا النسيجية الحية.