Comparative diagnostic value of the gel diffusion test and virus isolation in cell culture for detecting equine herpesvirus type 1 (EHV-1)

S.C. TEWARI* and S. PRASAD**

Summary: The gel diffusion test was applied direct to field samples from cases of equine abortion, foal mortality and equine paralysis due to equine herpesvirus type 1 (EHV-1). Following polyethylene glycol concentration of viral antigen, one line was formed against EHV-1 antiserum after 24 hours incubation at 25°C. Tests carried out simultaneously on 41 field samples showed a high correlation between diagnosis based on this test and virus isolation in primary lamb kidney cell culture. Advantages of gel diffusion over isolation of virus in cell culture were its rapidity and simplicity. The precipitating antigen did not lose activity after heating at 56°C for 30 minutes. A positive reaction was observed with a contaminated sample, which failed to yield virus in the cell culture system.

INTRODUCTION

The usual procedures for diagnosis of equine abortion, foal mortality and equine paralysis cases are histopathological examination of tissues and isolation of virus from tissue suspension in cell culture. Direct immunofluorescence of tissue materials has also been found to be useful by some workers (16, 15, 2). However, these techniques are laborious and some are time-consuming. Immunofluorescence requires costly equipment and reagents.

The gel diffusion test has been used by several workers either for differentiating equine herpesvirus type 1 (EHV-1) from other types of equine herpesvirus or for detecting antibodies against EHV-1 in equine sera (13, 6, 7). The

* Scientist, Equine Rhinopneumonitis Project, Department of Veterinary Microbiology, Haryana Agricultural University, Hissar 125004, India.
** Professor and Head, Department of Veterinary Microbiology, Haryana Agricultural University, Hissar 125004, India.
gel diffusion test was used for detecting EHV-1 in liver and brain tissues of two mares which were destroyed following paralysis (5); the authors failed to get precipitin reaction in gel on direct field material.

The present paper describes the use of the gel diffusion test for direct examination of samples originating from field cases of abortion, stillbirth, foal mortality and paralysis. The results obtained were compared with isolation of virus in cell culture.

MATERIALS AND METHODS

Preparation of antigen.

For preparing antigen for the gel diffusion test, the technique described by Wilks and Coggins (1979) was used with suitable modifications. Lung, liver and spleen tissues from aborted fetuses or dead foals, and brain tissue from animals which had died of paralysis were ground in a mortar, and a 20% suspension was prepared in phosphate buffer saline (PBS) pH 7.2. After centrifugation at 5,000 rpm for 20 minutes, the supernatant fluid was collected. Cells in the sediment pellet were disrupted by three cycles of freezing and thawing, and then suspended in a small quantity of PBS and recentrifuged at 5,000 rpm for 20 minutes. The supernatant fluid was collected, mixed with that collected earlier and then concentrated with 8% w/v polyethylene glycol (PEG) 6000. The virus was eluted from PEG with tris buffer and used as antigen in the gel diffusion test without further treatment.

Known positive control antigen was prepared in the same way from the liver, lung and spleen tissues of dead baby hamsters infected with a standard strain of EHV-1. Known negative antigen was prepared from the tissues of healthy uninfected baby hamsters.

Hyperimmune serum.

Hyperimmune serum against EHV-1 was produced in rabbits by repeated inoculations of cell culture virus. The virus, supplied by Dr. N.C. Jain, had been identified as EHV-1 Ky-D strain by the Animal Virus Research Institute, Pirbright, England.

Gel diffusion test.

The gel medium contained 0.76% w/v agarose in gel buffer containing 3 g sodium chloride, 3 g potassium chloride, 2.5 g sodium barbital and 2 g magnesium sulphate per litre of distilled water. Two grams of sodium azide were added as a preservative.

The central well on a glass slide was surrounded by 6 wells, 4 mm diameter
and 4-5 mm apart. EHV-1 hyperimmune serum was placed in the central well and known positive antigen in two opposing outer wells. Test antigens were placed in the remaining wells. The tests were read after incubation for 24 h at 25°C. The reaction was considered positive if identical reactions were given by known positive and test antigens. In the absence of a line similar to that of the known positive antigen, the test was considered to be negative. Reactions were recorded and photographed after staining with 1% amido black.

Isolation of virus.

Primary lamb kidney monolayer cultures were used in a technique essentially the same as that described by Jain et al. (1978). The identity of the virus isolates was confirmed by the neutralization test with EHV-1 antiserum.

RESULTS

In the gel diffusion test, one precipitin line was formed when standard herpesvirus-infected hamster tissue antigen was allowed to react against EHV-1 hyperimmune serum, but it did not form against normal rabbit serum. Uninfected normal hamster tissue antigen did not react with EHV-1 hyperimmune serum. In positive reactions the precipitin lines formed by test antigen and EHV-1 infected hamster tissue antigen were identical (Fig. 1). Adsorption of hyperimmune serum with the EHV-1 infected hamster tissue antigen abolished the precipitin reaction. In negative reactions, no line formed with known positive antigen.

In all, 41 samples were processed simultaneously for virus isolation and the gel precipitin reaction (Table I). Of the 41 samples, 33 proved viable in cell culture and seven were not. The viable samples were neutralised by EHV-1

<table>
<thead>
<tr>
<th>History</th>
<th>Total number of samples processed</th>
<th>Virus isolation in cell culture</th>
<th>Gel diffusion test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Viable</td>
<td>Non-viable</td>
</tr>
<tr>
<td>Equine abortion</td>
<td>31</td>
<td>23</td>
<td>7 + 1*</td>
</tr>
<tr>
<td>Foal mortality</td>
<td>8</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Equine paralysis</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>33</td>
<td>7 + 1*</td>
</tr>
</tbody>
</table>

* Sample found to contaminate the cell culture.

Table I
Comparison between virus isolation technique and gel diffusion test
The present report describes a simple and quick method for diagnosing abortion, stillbirth, foal mortality and equine paralysis cases due to infection with EHV-1. Diagnosis could be made within 24 hours by this test. Compared to this, virus isolation in cell culture and identification by serum neutralisation

When tested by gel diffusion, 30 of the 33 viable samples proved positive. Of the remaining seven non-viable samples, three proved positive with the gel test. The sample which contaminated the cell culture also proved positive with the gel test.

In order to study the storage life of PEG-concentrated antigen, it was stored at −20°C for up to two weeks. There was no appreciable loss in its antigenicity during this period.

The antigen was also heat-stable, as its activity remained intact when subjected to heat at 56°C for 30 minutes; on the other hand such treatment resulted in loss of viability when tested in the cell culture system.

DISCUSSION

The present report describes a simple and quick method for diagnosing abortion, stillbirth, foal mortality and equine paralysis cases due to infection with EHV-1. Diagnosis could be made within 24 hours by this test. Compared to this, virus isolation in cell culture and identification by serum neutralisation

![Fig. 1](image)

**Fig. 1**

Precipitin reaction with equine herpesvirus type 1 (central well—hyperimmune serum; 2 and 5—known standard antigens; 3 and 4—positive reaction; 1 and 6—negative reaction).
takes several days. Although pathognomonic histopathological lesions are present in cases of abortion and foal mortality caused by EHV-1, such lesions are not very characteristic in the paralytic syndrome (12, 1). Moreover, recovery of virus from the paralysis syndrome is also difficult (3, 8, 5, 9). It would therefore appear that the gel diffusion test would be particularly useful in cases of paralysis.

In the present study, 33 of 41 samples were positive for isolation of virus, and 30 proved positive with the gel diffusion test (a correlation of 84.4%).

It was interesting to note that 3 of the 7 non-viable samples in cell culture proved positive by gel diffusion. The virus present in these samples may have been killed or inactivated during dispatch of the samples to the laboratory. Burrows (1968) reported a half life for EHV-1 of seven hours at 37°C. Kawakami et al. (1962) reported EHV-1 to be inactivated within 10 minutes when exposed to 56°C. In the present investigation the antigen was found to react in the gel diffusion test even after heating at 56°C for 30 minutes, although the virus had lost its viability.

Three viable samples were missed by the gel diffusion test, perhaps because they did not contain enough viral antigen for detection by this test. One sample which was led to fungal contamination of the cell culture system, was positive by gel diffusion, so this test could prove useful when an aborted fetus has been trampled by the mare, or when a sample becomes contaminated during collection or dispatch to the laboratory. Considering the close correlation between the two tests, it may be concluded that a diagnosis can be made by gel diffusion. Samples which prove negative by this test should be further processed in the laboratory for detection and isolation of EHV-1.

ACKNOWLEDGEMENTS

Thanks are extended to the Indian Council of Agricultural Research, New Delhi, for providing financial help to carry out this investigation. The supply of cell culture-adapted equine herpesvirus type 1 by Dr. N.C. Jain, National Fellow, Department of Veterinary Microbiology is gratefully acknowledged. Sincere thanks are due to the officers of the Remount and Veterinary Corps for the supply of field samples.

* *

Résumé : L'immunodiffusion en gélose a été appliquée aux prélèvements provenant de chevaux atteints d'avortement, de mortalité néo-natale et de paralysie, provoqués par l'herpèsvirus équin type 1 (EHV-1).

Après concentration de l'antigène viral par le polyéthylène-glycol, il se forme une ligne de précipitation en face de l'antisérum EHV-1 après une incubation de 24 heures à 25°C. L'épreuve réalisée simultanément sur 41 échantillons du terrain s'est montrée en forte corrélation, pour le diagnostic, avec l'isolement du virus sur culture cellulaire de rein d'agneau. L'immunodiffusion en gélose a l'avantage sur l'autre méthode de la rapidité et de la simplicité.

L'antigène précipitant ne perd pas d'activité après chauffage à 56°C pendant 30 mn. La réaction de précipitation s'observe avec un prélèvement contaminé alors qu'il est difficile d'isoler le virus à partir d'une culture cellulaire.

* *


Resumen : Se aplicó la inmunodifusión en gelosa en las muestras procedentes de caballos atacados de aborto, mortalidad neonatal y parálisis, provocados por el herpesvirus equino tipo 1 (EHV-1).

Tras concentración del antígeno viral por el polietileno-glycol, se forma una línea de precipitación frente al antisero EHV-1, previa incubación de 24 horas a 25°C. La prueba realizada simultáneamente con 41 muestras de campo puso de manifiesto su alta correlación, para el diagnóstico, con el aislamiento del virus en cultivo celular de riñón de cordero. La ventaja de la inmunodifusión en gelosa, respecto al otro método, es la rapidez y sencillez.

El antígeno precipitante no pierde actividad después de calentamiento a 56°C durante 30 mn. Se observa la reacción de precipitación con una muestra contaminada, cuando resulta difícil aislar el virus a partir de un cultivo celular.

* *

* *
REFERENCES


