Application of a Complement Fixation Test Using Inactivated Antigen to the Safe Diagnosis of Equine Viral Arteritis

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An attempt was made to develop a complement fixation (CF) test for equine viral arteritis (EVA) which can be safely used in the countries free from EVA. The antigen was inactivated by 0.1% formalin, 50% ether, ultraviolet (UV) irradiation and heat at 70°C respectively. It was efficiently affected in viral infectivities by ether and formalin, but not at all in CF titers by formalin and UV irradiation. Ether degraded CF titers readily and UV irradiation caused random inactivation of virus. Heat showed incomplete effect on viral infectivities and declined CF titers. Therefore, the antigen inactivated by formalin was selected in the subsequent CF studies.

To evaluate the CF test using the inactivated viral antigen, it was conducted with 6 sero-positive horse sera against EAV in serum neutralization (SN) test. Three sera possessing SN titers of 1:128 to 1:256 revealed titers of 1:4 to 1:8 in CF test. The rest which had SN titers of less than 1:64 were negative.

Key words, Equine viral arteritis, complement fixation test, inactivated viral antigen.

Several serological tests have been reported for detection of antibodies to equine arteritis virus (EAV) by serum neutralization1-3) (SN), complement fixation4-6) (CF), immunodiffusion4), immunofluorescence4) and enzyme-linked immunosorbent assay7,8). Although SN test is currently and widely carried out by the method of micro-neutralization3) in the presence of complement for diagnosis and sero-survey of equine viral arteritis (EVA), a set of live virus, susceptible cell culture and fresh guinea pig serum should be kept all the time and maintained to be best suited. The CF test, however, can be simply and readily used in order to meet an unexpected requirement for diagnosis of EVA. It has been reported that antigen of CF test for EVA was prepared by virus grown in cell cultures4-6). Such antigen may contain live viruses and is not always allowed to use in all of the diagnostic laboratories in the countries free from EVA. The purpose of the present study is to develop and evaluate a safe CF test for EVA using inactivated virus antigen.

CF antigen was prepared by roller bottle culture of E. Derm (Equine dermal) cells inoculated with modified Bucyrus strain of equine arteritis virus as described previously8), and the finally obtained culture fluid was partially purified and concentrated to 1/25 volume by polyethylene glycol precipitation as described in the previous paper9). The

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The procedure of CF test has been described previously\(^6\), but modified from the previous method to microtiter system. The titration of virus was performed according to previously described plaque assay\(^2\).

The standard antisera to EAV were prepared by inoculating intranasally with Bucyrus strain of EAV to experimental horses and collected 4 weeks after inoculation as described previously\(^2\). Six imported horse sera positive to EAV by SN test were used for evaluation of CF test.

The effects of inactivation of the antigen by 0.1% formalin, 50% ether, ultraviolet irradiation and heat at 70°C on viral infectivities and CF titers are shown in Fig. 1 A, B, C and D respectively. Inactivation of virus was made efficiently by ether and formalin, and CF titers were little affected by UV irradiation and formalin treatments. Heat inactivation resulted in not only incomplete effect on viral infectivities, but decrease of CF titers. UV irradiation seemed to inactivate the virus and had no effects on CF titers. However, the inactivation of this treatment was not complete nor reproducible. CF titers of ether-treated antigen degraded promptly as well as viral infectivities. It must be due to physicochemical property of EAV which is sensitive to lipid solvents\(^10\). In our previous study, EAV was inactivated by treatment of 0.1% formalin at 37°C for at least 6 hrs\(^9\). Therefore, the treatment of formalin was completed for 24 hrs in the present examination. Antigenicities of the formalin-inactivated CF antigen were stable and then no significant effect on CF reactions was observed (data not shown) when residual formalin was eliminated by dialysis as described\(^9\). In the subsequent experiments, the formalin treatment without dialysis was selected for preparation of inactivated CF antigen and it was stored at -40°C until use. Crawford and Henson\(^4\) described that antibody which was detected by CF test was reacting with an antigen on the surface of the intact viral particle. Inactivation of CF antigen by formalin would fix steadily the surface antigen of virus and thus antigenicities could be preserved. Viral antigen used by previous workers\(^4-6\) in CF test has been prepared by growing EAV in cell culture after cytopathic effect became apparent and then centrifuged to remove cellular debris. Gaskin et al.\(^11\) and Cook et al.\(^8\) indicated that induced antibodies to bovine serum which contained in tissue culture vaccine were inevitable to react as false-positive in some serological tests using horse sera, because horses were exposed to widespread usage of several
C. F. Test of Equine Viral Arteritis

Table 1. Comparative results of antibody titers between SN and CF tests conducted with serum samples of six imported horses.

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>SN antibody titer</th>
<th>CF antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 : 32</td>
<td>&lt;1 : 4*</td>
</tr>
<tr>
<td>2</td>
<td>1 : 32</td>
<td>&lt;1 : 4</td>
</tr>
<tr>
<td>3</td>
<td>1 : 64</td>
<td>&lt;1 : 4</td>
</tr>
<tr>
<td>4</td>
<td>1 : 128</td>
<td>1 : 4</td>
</tr>
<tr>
<td>5</td>
<td>1 : 128</td>
<td>1 : 8</td>
</tr>
<tr>
<td>6</td>
<td>1 : 256</td>
<td>1 : 4</td>
</tr>
</tbody>
</table>

*CF antibody titer at <1 : 4 was regarded as negative.

Six horse sera positive to EAV by SN test were conducted by CF test using formalin inactivated CF antigen and titers of CF antibodies were compared with those of SN antibodies (Table 1). The CF titers were much lower and CF antibodies were no longer detectable from 3 horse sera (Serum Nos. 1–3) possessing SN antibody titers less than 1 : 64. The other three (Serum Nos. 4–6) had titers of 1 : 128 to 1 : 256, but CF titers were 1 : 4 to 1 : 8. Huntington et al. described that SN titers of 1 : 128 was relatively high in the field. Developmental features of CF and SN antibodies in sera of horses experimentally exposed to EAV indicated that CF titers peaked after 2 to 3 weeks and then decreased while high-titered SN antibodies lasted during the period of examination. Accordingly, these 6 sero-positive horses might have been infected with virus some times ago and thereafter introduced in Japan.

CF test using formalin inactivated antigen would seem to be most useful detecting horses shortly after infection which may be a source of EAV infection in the countries free from EVA. However, it remains to be elucidated the fate of CF antibody of carrier stallions of EVA which must be the most epidemiological significance as a source of EVA.

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Literature Cited

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要 約

不活化抗原を用いた安全な補体結合試験の馬ウイルス性動脈炎診断への応用：福永昌夫, 今川 浩, 和田隆一, 兼丸卓美, 藤田正信（日本中央競馬会競走馬総合研究所栃木支所）—馬ウイルス性動脈炎（EVA）の清浄国で安全に検査できる血清反応として, 不活化抗原を用いた補体結合（CF）試験の開発を試みた。抗原の不活化はそれぞれ紫外線照射, 50％エーテル, 70° C 加熱, 0.1％ホルマリンで実施した。抗原はウイルス感染陰においてエーテルとホルマリンで効率良く不活化されたが, 紫外線照射とホルマリン処理は CF 価へ何等影響を及ぼさなかった。エーテル処理は CF 価を容易に低減させ, 紫外線照射のウイルス感染価への作用は不確実であった。加熱処理はウイルスを充分に不活化しないばかりか, CF 価も低下させた。従って, 以降の CF 試験にはホルマリン不活化抗原を用いて実験した。

不活化抗原を用いた CF 試験の評価を行うため, 中和試験において EVA 陽性の 6 頭の馬血清について試験した。中和抗体価 1:128-1:256 を有する 3 頭の血清は CF 抗体価 1:4-1:8 を示したが, 中和抗体価 1:64 以下 3 頭の血清は CF 反応で陰性であった。