Sero-epizootiological Studies on Viral Diseases in Horses on a Breeding Farm of Japan during a Period from 1981 to 1985

Tomio MATSUMURA,* Michio KOMANO,** Takeo SUGIURA,*
Masanobu KAMADA* and Yoshio FUKUNAGA*

Sero-epizootiological studies were conducted on viral diseases among horses on a breeding farm in the Kanto district for 5 years. Their purpose was to make a plan to control equine herpesvirus type 1 (EHV-1) infection and other viral diseases in horses in breeding areas and at training centers to which horses for racing were transported from these areas. EHV-1 infection was prevalent three times among foals under 2 years of age in 1983 and 1985. Besides, serological examination revealed that primary infection and reinfection with EHV-1 occurred frequently among foals in various seasons in 5 years, except in 1984. These results suggest the possibility that EHV-1 may be disseminated among foals in every season of the year and reinf ect them readily in a relatively short time after primary infection. Therefore, broodmares must be separated completely from foals after the 6th month of gestation, and the time and frequency of inoculation with inactivated vaccines must be reconsidered for the prevention of abortion caused by EHV-1 in breeding areas. At training centers, it will be necessary to perform a strict quarantine on racehorses transported from breeding farms in every season, as these horses may possibly be a source of EHV-1 infection. The present studies also showed that equine adeno-and rhinoviruses were disseminated among foals in a particular season, from January to June, and that equine rotavirus was disseminated sporadically among horses in various seasons.

Key words: sero-epizootiological study, equine herpesvirus type 1, equine adenovirus, equine rotavirus, equine rhinovirus type 1

Introduction

Some of the viruses which cause infection and disease to horses inflict heavy economic damage upon the owners of breeding farms or racehorses. In Japan, many reports have been published on the prevalence of viruses in horses. These viruses include equine herpesviruses,1,2) equine influenza virus,3) equine infectious anemia virus (EIAV),4) Japanese encephalitis virus (JEV), equine adenovirus (EAdV),5) equine rhinovirus type 1 (ERhV-1),6) equine rotavirus (ERoV),7) and Getah virus (GV).8)

Under close surveillance by law in Japan, there have been few outbreaks of EIA among horses in the whole country for the past several years. JE has been controlled very well by inoculation with a commercial inactivated vaccine. In addition, there are inactivated vaccines availa-
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ble for the control of spread of equine herpesvirus type 1 (EHV-1), equine influenza viruses and GV among horses. Up to date, there has been no occurrence of equine influenza or GV infection among horses inoculated with these vaccines.

On the other hand, the abortion caused by EHV-1 infection is still one of the important problems for breeders, although the vaccination against EHV-1 is widely carried out among breeding mares. For the past 2 years, the abortion caused by EHV-1 has been observed even among vaccinated mares. Furthermore, infections caused by EHV-1, EAdV, ERoV and ERhV-1 have been observed seasonally or sporadically every year among racehorses at the two training centers of the Japan Racing Association. At present, no vaccines are available for the prevention of horses from infections with these viruses. Few reports have been made on the sero-epizootiology of these viruses in broodmares and foals in a breeding area. These viruses cause infections among racehorses which are frequently transferred from a breeding farm to a training center in every season of the year. To control them, it is absolutely necessary to know the epizootiological situation of such viruses among horses in a breeding area.

The present paper deals with sero-epizootiological research conducted on EHV-1, EAdV, ERoV and ERhV-1 among horses on a breeding farm in the Kanto district for 5 years. The purpose of the research was to get basic data for making a plan to control infections with these viruses among horses on breeding farms and at training centers.

Materials and Methods

Sera. Serum samples were collected at monthly intervals over a period from January, 1981 to May, 1985 from Thoroughbred broodmares and foals under 2 years old on a breeding farm located in Tochigi Prefecture of the Kanto district, which is in the eastern part of Japan. On this farm, about 50 horses have been raised constantly every year. Horses have been moved less frequently from this farm than any breeding farm in Hokkaido, on the northernmost part of Japan, where more than 70% of the breeding farms of Japan are located.

The horses of the farm were divided into two groups. One group consisted of 11 to 16 broodmares and the other of 22 to 34 foals each year. No serum samples were collected from foals 2 years of age in 1981 and foals born in 1985. Rectal temperature was taken in both groups once a day. All the serum samples obtained were stored at \(-20°C\) until use.

Antigens. The viruses used for the complement fixation (CF) tests were the HH-1 strain of EHV-1,\(^1\) the T-1 strain of EAdV\(^9\) and the Lincoln strain of bovine rotavirus,\(^9\) which was used for the detection of CF antibody against ERoV. Viral antigens were prepared by the methods mentioned in the previous reports.\(^1,9,10\) The NM-11 strain of ERhV-1\(^10\) was used for the serum neutralization (SN) test.

CF and SN tests. The CF and SN tests were conducted by the microtiter methods described by Wasserman and Levine\(^11\) and by Imagawa et al.,\(^8\) respectively. Initial 1: 4 dilution of a serum sample was heated at 56°C for 30 min. Serial two-fold dilutions of the serum sample were prepared in a range from 1: 4 to 1: 256 for each test.
The CF test was performed with 0.025 ml of diluted serum, 0.025 ml of antigen prepared as 4 units, 0.05 ml of complement prepared as 2 units, and 0.05 ml of 1.25% sensitized sheep erythrocytes. The SN test was performed with 0.05 ml of diluted serum, 0.05 ml of virulent virus prepared as 100 TCID<sub>50</sub>/0.05 ml, and 0.075 ml of Vero cell suspension containing 2 x 10<sup>5</sup> cells/ml in a CO<sub>2</sub> incubator at 37°C for 5 days. Serum from a horse experimentally infected with each virus was used as reference serum for each test. Antibody titer was expressed as the reciprocal of the highest dilution of serum that had reduced hemolysis to 50% or less in the CF test, or that had neutralized the virus completely in the SN test. Each test was assumed to be positive when the titer was 1:4 or greater. It was regarded that the serological conversion was caused by the infection when the difference in titer was 8 times or more, or the titer increased from <1:4 to 1:8 or 1:16, between two serial sera from an individual horse.

Vaccination. Vaccination against EHV-1 was performed in most broodmares with 3 doses of inactivated vaccine (Nisseiken Co. Ltd., Ohme, Japan) in 1982. In 1982 and 1984, it was also performed with 2 doses in 6 foals at 6 to 7 months of age in each year. No vaccination was performed against any other test virus.

Results

Clinical signs. The number of animals which showed apparent pyrexia during the experimental period was as follows: 3 foals at 3 to 5 months of age in July, 1981; 11 and 4 foals at 1 year of age in May and August, 1983, respectively; 6 foals at 6 to 7 months of age in November, 1983; 11 foals at 10 to 12 months of age in March, 1985. Of the foals, only those affected in May and November, 1983 and in March, 1985 were revealed to be infected with EHV-1 when examined by the serological tests. On the other hand, abortion occurred sporadically in 5 broodmares every year, except in 1981, in which abortion took place in 4 animals in the middle stage of gestation and in one animal in the terminal stage. The cause of abortion was unrevealed in any broodmare by the serological tests.

Antibody reactions. A significant increase was seen in CF titer against EHV-1 from <1:4 to 1:8 to 1:64 in 11 and 16 foals in June and December, 1983, respectively, and in 11 foals in April, 1985, as shown in Tables 1 and 2. It occurred simultaneously with the prevalence of a febrile disease in May and November, 1983 and in March, 1985. Therefore, it was judged that the disease had been caused by EHV-1 infection. Furthermore, 6 of 11 foals born in 1982 which were all involved in the first prevalence of EHV-1 infection in May, 1983, showed a second increase in CF titer against EHV-1 from <1:4 to 1:8 to 1:16 after the second prevalence in November, 1983. In this second prevalence, 6 of 11 foals born in this year showed pyrexia and a serological conversion against EHV-1 was seen in 10 of the 11 foals in the next month, December, 1983 (Tables 1 and 2).

All the foals vaccinated with inactivated vaccines in 1982 and 1984 presented a rise in antibody titer from <1:4 to 1:4 to 1:16 after the first or the second vaccination. They lost, however, their antibody titers detectable within 3 months after the second vaccination and were involved in the first or the third prevalence of EHV-1 infec-
Table 1. Number of horses showing a significant increase in antibody titers against viruses over a period from 1981 to 1985

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>EHV-1</td>
<td>Broodmare</td>
<td>0/11</td>
<td>0/16</td>
<td>0/16</td>
<td>0/13</td>
<td>0/13</td>
</tr>
<tr>
<td></td>
<td>Foal</td>
<td>7/23</td>
<td>5(2)/34</td>
<td>21(6)/28</td>
<td>0/33</td>
<td>11/22</td>
</tr>
<tr>
<td>EAdV</td>
<td>Broodmare</td>
<td>0/11</td>
<td>1/16</td>
<td>0/16</td>
<td>0/13</td>
<td>0/13</td>
</tr>
<tr>
<td></td>
<td>Foal</td>
<td>1/23</td>
<td>9/34</td>
<td>3(2)/28</td>
<td>5(1)/33</td>
<td>2/22</td>
</tr>
<tr>
<td>ERoV</td>
<td>Broodmare</td>
<td>0/11</td>
<td>0/16</td>
<td>0/16</td>
<td>0/13</td>
<td>0/13</td>
</tr>
<tr>
<td></td>
<td>Foal</td>
<td>5/23</td>
<td>6(2)/34</td>
<td>4/28</td>
<td>9(2)/33</td>
<td>1/22</td>
</tr>
<tr>
<td>ERhV-1</td>
<td>Broodmare</td>
<td>0/11</td>
<td>0/16</td>
<td>0/16</td>
<td>0/13</td>
<td>0/13</td>
</tr>
<tr>
<td></td>
<td>Foal</td>
<td>9/23</td>
<td>1/34</td>
<td>1/28</td>
<td>2/33</td>
<td>6/22</td>
</tr>
</tbody>
</table>

Remarks. 1) Antibody titers were determined by the CF test, except the titer against ERhV-1 which was determined by the SN test.
2) EHV-1: equine herpesvirus type 1, EAdV: equine adenovirus, ERoV: equine rotavirus, and ERhV-1: equine rhinovirus type 1.
3) Numerator: the number of horses showing serological conversion. Denominator: the number of horses tested.
4) In parentheses is shown the number of horses which have experienced infection and which show a second increase in antibody titer.

Table 2. Seasonal occurrence of significant increase in antibody titers against viruses over a period from 1981 to 1985

<table>
<thead>
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<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>EHV-1</td>
<td>44(8)</td>
<td>1</td>
<td>2</td>
<td>11</td>
<td>2</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>10(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAdV</td>
<td>21(3)</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>13(2)</td>
<td>1(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERoV</td>
<td>25(4)</td>
<td></td>
<td></td>
<td></td>
<td>9(2)</td>
<td>5</td>
<td>8(2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERhV-1</td>
<td>19</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Remarks. For 1), 2), and 4) see the footnote of Table 1.
3) A total number of horses showing a serological conversion during the period of investigation.

Table 3. Distribution and positive rates of antibody titers against viruses over a period from 1981 to 1985

<table>
<thead>
<tr>
<th>Virus</th>
<th>Group</th>
<th>No. of horses tested</th>
<th>&lt;4</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>≥64</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHV-1</td>
<td>Broodmare</td>
<td>69</td>
<td>0</td>
<td>4</td>
<td>31</td>
<td>17</td>
<td>3</td>
<td>00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Foal</td>
<td>140</td>
<td>26</td>
<td>20</td>
<td>43</td>
<td>33</td>
<td>16</td>
<td>2</td>
<td>81.4</td>
</tr>
<tr>
<td>EAdV</td>
<td>Broodmare</td>
<td>69</td>
<td>12</td>
<td>34</td>
<td>19</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>82.6</td>
</tr>
<tr>
<td></td>
<td>Foal</td>
<td>140</td>
<td>93</td>
<td>17</td>
<td>27</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>33.6</td>
</tr>
<tr>
<td>ERoV</td>
<td>Broodmare</td>
<td>69</td>
<td>37</td>
<td>25</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>46.4</td>
</tr>
<tr>
<td></td>
<td>Foal</td>
<td>140</td>
<td>97</td>
<td>15</td>
<td>12</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>30.7</td>
</tr>
<tr>
<td>ERhV-1</td>
<td>Broodmare</td>
<td>69</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>14</td>
<td>24</td>
<td>21</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>Foal</td>
<td>140</td>
<td>109</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>21</td>
<td>22.1</td>
</tr>
</tbody>
</table>

Remarks. For 1) and 2) see the footnote of Table 1.
3) A total number of horses tested during the period of investigation.
4) Reciprocal.
5) Positive at serum dilution of ≥1:4.
6) The number of horses showing the titer indicated. This titer is the highest of the serial sera collected from an individual horse during the period of investigation in each year.
tion. There was no difference in antibody reaction between vaccinated and unvaccinated foals after EHV-1 infection.

In the other years, except 1984, some foals showed a serological conversion against EHV-1 in their sera. Infection with this virus was observed sporadically on this farm in various seasons of the year (Table 2). Furthermore, the positive rate of CF antibody against EHV-1 in foals throughout the years investigated was as high as 81.4%. The positive rates of CF antibodies against EAdV and ERoV and of SN antibody against ERhV-1 were comparatively low, or 33.6, 30.7 and 22.1%, respectively, as shown in Table 3.

All the broodmares maintained positive antibodies against EHV-1, the titers of which ranged from 1:4 to 1:64 constantly during the period of investigation. No broodmares showed a serological conversion against EHV-1 in their sera, as shown in Tables 1 and 3.

A significant increase in antibody titer against EAdV, ERoV or ERhV-1 was observed sporadically in animals every year, as shown in Table 1. A serological conversion was observed in CF titer against EAdV only in a particular period between January and May and in SN titer against ERhV-1 between February and June every year. A serological conversion in CF titer against ERoV was observed sporadically over a period from March to December, as shown in Table 2. There was a serological evidence of reinfection with EAdV or ERoV in some foals during the period of investigation.

Discussion

Burrows et al. observed a serological conversion against EHV-1 among horses in Great Britain in every quarter of the year. Doll and Bryans mentioned that horses could be reinfected within 3 to 5 months. The results coincided with the findings of the present research that primary infection and reinfection with EHV-1 frequently occurred among foals on a breeding farm of Japan in various seasons. They suggest the possibility that EHV-1 may be disseminated among foals in any season of the year and that its reinfection may be induced readily in a relatively short time after its primary infection. In another way, it is highly possible for foals on breeding farms to be a source of EHV-1 prevalence in some season of the year.

In the recent years, there has been an increase in incidence of abortion caused by EHV-1 infection even among broodmares vaccinated with 2 doses of inactivated vaccines during a period from the 7th to 9th month of gestation. Doll and Bryans described that abortion caused by EHV-1 infection occurred among broodmares mostly in the late stage, or after the 8th month, of gestation. Furthermore, Bryans suggested that EHV-1 viremia might persist for as long as 3 weeks. Therefore, to prevent abortion caused by EHV-1 infection, broodmares should be separated completely from foals after the 6th month of gestation at the latest. Moreover, the time and frequency of inoculation of inactivated vaccines should be reconsidered. It is also desirable to develop a new effective vaccine.

On the other hand, a respiratory disease caused by EHV-1 has been observed every year mostly in the winter season, from December to March. It has attacked approximately 1 to 4% of the racehorses at the two training centers of the Japan
Racing Association where more than 4000 racehorses are kept for training (unpublished data). There is a frequent transportation of horses between training centers and breeding farms. Therefore, at training centers, it would be needed to perform a strict quarantine for horses transported from breeding farms in every season of the year for the prevention of EHV-1 prevalence.

Powell et al.15 reported that the rate of horses possessing precipitating antibody against EAdV was approximately twice as high in summer as in winter in England. They suggested that reinfection with EAdV might be common. Imagawa et al.16 observed that a serological conversion in CF titer against ERoV occurred among foals in February, June and August. Sugiura et al.17 pointed out that a serological conversion in SN titer against ERhV-1 took place among rearing horses mostly in winter, from November to February. In the present research, a particular seasonal occurrence was observed in EAdV and ERhV-1 infections in foals. EAdV infection was prevalent over a period from January to May, especially in March, and ERhV-1 infection over a period from February to June. Whereas, horses showing serological evidence of ERoV infection were observed sporadically in various seasons. There were also serological evidences of reinfection with EAdV or ERoV.

Acknowledgments

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Literature Cited

T. Matsumura et al.

Med. Assoc. 155, 294-300.


要 約

一軽種馬生産牧場における馬のウイルス感染症の血清学的調査—1981年〜1985年：松村富夫*・駒野道夫**・杉浦健夫*・鎌田正信*・福永昌夫*（*日本中央競馬会競走馬総合研究所畜木支所 **日本軽種馬協会町田種馬場）—生産地の馬群における馬のウイルス感染症の防制対策ならびにトレーニングセンター（トレセン）への入厩馬に対する検疫プログラムを検討するために、5年間にわたって関東地方の一産一牧場を監視下におき、トレセンで発生の認められる馬鼻肺炎、馬アデノ、馬ロタおよび馬ライノ1型の各ウイルスに対する血清学的調査を実施した。その結果、1983年と1985年に当該馬群と明け2歳馬群内に馬鼻肺炎の比較的大きな流行が3回認められ、その他の年度においても散発的に抗体上昇馬が認められた。また、馬鼻肺炎ウイルスに対する抗体上昇馬は2か月から12か月までのほとんど各月に認められた。これらのことから、生産場においては季節に関係なく高頻度に馬鼻肺炎ウイルスが若齢馬群間で伝播していることが明らかとなった。近年現行ワクチン接種馬においても流産の発生が認められる事実と今回得られた成績から、馬鼻肺炎ウイルスによる流産予防のためには、若齢馬群と妊娠6カ月以降の繁殖馬群の完全隔離を徹底するとともに、接種時期、接種回数などのワクチンプログラムの再検討が必要であると思われた。また、トレセンへの入厩馬の防制においては、いずれの時期における入厩馬も馬鼻肺炎の感染源となりうることを十分に考慮して検疫を実施する必要があると思われた。一方、馬アデノあるいは馬ライノ1型ウイルス感染若齢馬は春の一定の時期に集中して、また馬ロタウイルス感染若齢馬は季節に関係なく散発的に行、毎年認められた。