A Survey on Complement Fixation Antibody against Bovine Rotavirus in Light Horses of Japan

Hiroshi Imagawa,* Yasumasa Ando* and Yutaka Akiyama*

A survey was conducted on complement fixation (CF) antibody against bovine rotavirus (BRV) in the sera of a total of 1,873 light horses in a breeding region of Hokkaido, the Tokyo and Nakayama Racecourses, and the Ritto Training Center of the Japan Racing Association during a period from 1976 to 1978. The following results were obtained. (1) CF antibody against BRV was detected in 60.9% of the 1,873 sera tested. (2) In the breeding region, 15.1% (853) of foals, 56.3% (99/176) of 2-year-old horses, and 43.3% (91/210) of horses 4 years or more of age possessed this antibody. (3) When 200 sera of 3-year-old horses were examined in each of the Tokyo and Nakayama Racecourses and the Ritto Training Center, the antibody was detected in 86 (43.0%), 114 (57.0%) and 73 (36.5%) horses, respectively. (4) In the Nakayama Racecourse, 46.3% (31/67) of 2-year-old, 75.2% (32/43) of 3-year-old, 84.6% (176/208) of 4-year-old, and 92.2% (118/128) of 5- to 7-year-old horses possessed the antibody. This result suggested that light horses might be severely infected with rotavirus in some wide district in Japan.

Introduction

Rotavirus is one of the viruses which have caused acute gastroenteritis mainly in infants of many animal and avian species. Since Mebus et al.1) isolated it from feces of diarrheal calves in 1969, it has come to attract attention of many researchers. Then rotavirus was isolated from children,2,3) mice,4,5) piglets,6) lambs,7) foals,8–10) deer,11) rabbits12) and avian species.13)

In 1975, 1976, and 1978, Flewett et al.,8) Kanitz9) and Tzipori et al.10) isolated a rotavirus from diarrheal foals. Kanitz9) succeeded in producing experimental infection of this virus in foals.

From their serological survey on rotavirus infection in 68 horses in Japan, Takahashi et al.14) demonstrated that there were positive horses at a high rate.

As mentioned above, equine rotavirus was isolated in England, U.S.A. and Australia, and then experimental infection of foals was successful. In Japan or any other country, however, there is no report of detailed examination on the shift of antibody against rotavirus in horses.

This report presents the state of propagation of rotavirus in each age group of horses in a breeding region, racecourses and a training center in Japan.

Materials and Methods

Virus. The bovine rotavirus used was the Lincoln strain of NCDV1) supplied by
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M. Kodama of the National Institute of Animal Health, Kodaira, Tokyo. After two passages in primary bovine embryonic kidney cells at this laboratory, it had been stored at −70°C until use as seed virus.

**Antigen for CF test.** The growth medium used was modified Eagle’s MEM (Flow Laboratories) containing 10% bovine fetal serum, 200 units/ml of penicillin, 200 µg/ml of streptomycin, 5 µg/ml of amphotericin B, and 100 µg/ml of non-essential amino acids. It was removed from confluent monolayers of MA-104 cells established from Macacus rhesus kidney in bottles (5 × 10 × 15 cm). After washing 3 times with cation-free phosphate-buffered saline (PBS), tenfold dilutions of seed virus were prepared in maintenance medium (the growth medium exclusive of bovine fetal serum) and inoculated in 5 ml amounts. After adsorption at 37°C for 60 min, 45 ml of maintenance medium was added to the culture. After incubated at 37°C for 5 days, each culture was harvested and centrifuged at 5 000 rpm for 20 min. A small quantity of PBS was added to the resulting sediment, which was then frozen and thawed 5 times. Cell debris were deposited by centrifugation at 5 000 rpm for 20 min. The resulting supernatant was collected and mixed with the previous supernatant. The mixed supernatant virus was concentrated with 8% polyethylene glycol No. 6 000. The concentrated virus was resuspended in PBS to make 1/25 original volume and treated with trichloro-trifluoroethane. CF antigen titer was determined by box titration with hyperimmune guinea pig serum against BRV.

**Preparation of guinea pig antiserum against BRV.** The supernatant of the culture of infected primary bovine embryonic kidney cells harvested at 5 days of incubation was centrifuged at 36 000 rpm for 3 h in Hitachi RP 45 Roter. The virus pellet was suspended in 1/50 of the original volume of PBS and treated with trichloro-trifluoroethane. Then 0.2 ml of concentrated virus mixed with Freund complete adjuvant of the same quantity was injected into the foot pads of 5 guinea pigs. One week later, 0.2 ml of concentrated virus without adjuvant was injected into the abdominal cavity 4 times at 1-week intervals. The guinea pigs were bled 1 week after the last injection. Each pre-immunization serum of the 5 guinea pigs had a titer of <1:4 in CF test against NCDV. On the other hand, each post-immunization serum had a CF antibody titer of 1:1 600 to 1:3 200.

**CF test.** The CF test was done by the microtiter method with 0.025 ml of test serum, 0.05 ml of complement of 2 units, 0.025 ml of antigen of 4 units, and 0.05 ml of 1.25% sensitized erythrocytes. Three units of hemolysin and 2.5% sheep erythrocytes were mixed to prepare the sensitized erythrocytes. Each test serum was diluted 1:4 in veronal-buffered saline with 0.05% gelatine and heated at 60°C for 20 min to inactivate complement. CF antibody titer was expressed as the highest dilution of serum that reduced hemolysis to 50% at least. A serum with a CF antibody titer of ≥1:4 was taken as positive for antibody.

**Test sera.** A total of 1 873 sera were obtained from light horses in a breeding region of Hokkaido, the Tokyo and Naka-yama Racecourses, and the Ritto Training Center for the CF test. Details of the test sera are shown in Table 1 with distinction
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to three groups. Group A consisted of 439 sera harvested from foals and horses 2, 4, or more years of age in the breeding region of Hokkaido in 1977 and 1978. Group B consisted of 600 sera harvested from horses 3 years old of the Tokyo and Nakayama Racecourses and the Ritto Training Center in 1977. Group C consisted of 834 sera harvested from horses 2 to 7 years old of the Nakayama Racecourse in 1978.

Results

CF antibody in the breeding region of Hokkaido. In the sera harvested from the breeding region of Hokkaido and classified into group A of Table 1, CF antibody was detected as shown in Table 2. In 53 foal sera, 8 (15.1%) were positive for CF test and 45 (80.4%) negative. Of the 8 positive sera, 1, 6 and 1 had a titer of 1:4, 1:8 and 1:16, respectively. Of 176 sera of 2-year-old horses, 99 (56.3%) were positive and 77 (43.7%) negative. Of the 99 positive sera, 35, 55 and 9 had a titer of 1:4, 1:8 and 1:16, respectively. Of 210 sera of horses 4 years or more of age, 91 (43.3%) were positive and 119 (56.7%) negative. Of the 91 positive sera, 34, 51 and 6 had a titer of 1:4, 1:8 and 1:16, respectively.

Table 1. List of test sera used for serological survey

<table>
<thead>
<tr>
<th>Group</th>
<th>Place harvested</th>
<th>Age (year)</th>
<th>Month harvested</th>
<th>No. of sera tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Hokkaido</td>
<td>&lt; 1</td>
<td>1977 June</td>
<td>53</td>
</tr>
<tr>
<td>A</td>
<td>Hokkaido</td>
<td>≥ 4</td>
<td>1977 Feb.—May</td>
<td>210</td>
</tr>
<tr>
<td>B</td>
<td>Tokyo</td>
<td>3</td>
<td>1977 Mar.</td>
<td>200</td>
</tr>
<tr>
<td>B</td>
<td>Nakayama</td>
<td>3</td>
<td>1977 Mar.</td>
<td>200</td>
</tr>
<tr>
<td>B</td>
<td>Ritto</td>
<td>3</td>
<td>1977 Mar.</td>
<td>200</td>
</tr>
<tr>
<td>C</td>
<td>Nakayama</td>
<td>2</td>
<td>1978 Feb.</td>
<td>67</td>
</tr>
<tr>
<td>C</td>
<td>Nakayama</td>
<td>3</td>
<td>1978 Feb.</td>
<td>431</td>
</tr>
<tr>
<td>C</td>
<td>Nakayama</td>
<td>4</td>
<td>1978 Feb.</td>
<td>208</td>
</tr>
<tr>
<td>C</td>
<td>Nakayama</td>
<td>5-7</td>
<td>1978 Feb.</td>
<td>128</td>
</tr>
</tbody>
</table>


Table 2. Positive rate of CF antibody against BRV in age groups of light horses in a breeding region

<table>
<thead>
<tr>
<th>Calendar year harvested</th>
<th>Age (year)</th>
<th>No. of sera tested</th>
<th>No. of positive sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977</td>
<td>&lt; 1</td>
<td>53</td>
<td>8 15.1</td>
</tr>
<tr>
<td>1978</td>
<td>≥ 4</td>
<td>210</td>
<td>91 43.3</td>
</tr>
</tbody>
</table>

CF antibody of 3-year-olds in the Tokyo and Nakayama Racecourses and the Ritto Training Center. Sera were harvested from 200 horses 3 years old of each of the Tokyo and Nakayama Racecourses and the Ritto Training Center, as shown in group B of Table 1. CF antibody was detected from them, as shown in Table 3. Of the 200 sera of the Tokyo Racecourse, 86 (43.0%) were positive and 114 (57.0%) negative. Of the 86 positive sera, 23, 55 and 8 had a titer of 1:4, 1:8 and 1:16, respectively. Of the 200 sera of the Nakayama Racecourse, 134 (67.0%) were positive and 66 (33.0%) negative.

Table 3. Positive rate of CF antibody against BRV in 3-year-old horses of the Tokyo and Nakayama Racecourses and the Ritto Training Center in 1977

<table>
<thead>
<tr>
<th>Place harvested*</th>
<th>No. of sera tested</th>
<th>No. of positive sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tokyo</td>
<td>200</td>
<td>86 43.0</td>
</tr>
<tr>
<td>Nakayama</td>
<td>200</td>
<td>134 67.0</td>
</tr>
<tr>
<td>Ritto</td>
<td>200</td>
<td>73 36.5</td>
</tr>
</tbody>
</table>

Remarks. *: See the footnote of Table 1.
negative. Of the 134 positive sera, 50, 72, 10 and 2 had a titer of 1:4, 1:8, 1:16 and 1:32, respectively. Of the 200 sera of the Ritto Training Center, 73 (36.5%) were positive and 127 (63.5%) negative. Of the 73 positive sera, 22, 45 and 6 had a titer of 1:4, 1:8 and 1:16, respectively.

**CF antibody distinguished with age in the Nakayama Racecourse.** As shown in group C of Table 1 834 sera were harvested from horses 2 to 7 years old in the Nakayama Racecourse. CF antibody was detected from them, as shown in Table 4. Of 67 sera of the 2-year-old, 31 (46.3%) were positive and 36 (53.7%) negative. Of the 31 positive sera, 11, 15, 4 and 1 had a titer of 1:4, 1:8, 1:16 and 1:32, respectively. Of 431 sera of the 3-year-old, 324 (75.2%) were positive and 107 (24.8%) negative. Of the 324 positive sera, 55, 167, 8 7 and 15 had a titer of 1:4, 1:8, 1:16 and 1:32, respectively. Of 208 sera of the 4-year-old, 176 (84.6%) were positive and 32 (15.4%) negative. Of the 176 positive sera, 29, 80, 57 and 10 had a titer of 1:4, 1:8, 1:16 and 1:32 respectively. Of 128 sera of the 5 to 7-year-old, 118 (92.2%) were positive and 10 (7.8%) negative. Of the 118 positive sera, 17, 56, 32 and 13 had a titer of 1:4, 1:8, 1:16 and 1:32, respectively. The positive rate showed a tendency to increase with the advance in age.

**Discussion**

It is possible to detect rotavirus easily from the diarrheal feces of children and animal and avian species by using an electron microscope. It was confirmed that this virus possessed a group-reactive antigen detectable from the host by CF,15–17) fluorescent antibody,16,17) neutralization16,17) and other serological tests independently. Rotavirus has been detected from many kinds of hosts, but only calf rotavirus,18–21) simian rotavirus,22) and O agent22) multiplied in cultured cells with cytopathogenic effect. For this reason, viruses which multiply readily in cultured cells have been used for a serum reaction to human and other animal rotavirus infection.

Equine rotavirus was detected initially from the diarrheal feces of a foal by Flewett et al.8) in 1975. Subsequently, Kanitz9) tried to produce experimental infection of foals with the intestinal contents obtained from a foal affected with diarrhea. He succeeded in causing severe diarrhea in experimental animals. Recently, Tzipori et al.10) made an attempt to induce experimental infection of gnotobiotic piglets with rotavirus derived from a foal suffering from diarrhea. In their experiment, though foal rotavirus produced no diarrhea in the piglets, the virus was detected from their feces.

In Japan, Takahashi et al.14) demonstrated that of 44 yearlings studied, 50% possessed CF antibody and 72.2%, serum neutralizing (SN) antibody, and that of 28 5- to 6-year-old horses, 78% possessed CF antibody and 82.1%, SN antibody.
In the present study, CF antibody against BRV was detected in 60.9% of a total of 1,873 sera tested. This result indicated that light horses in Japan were highly infected.

Diarrhea has been frequently seen in foals of a breeding region in Japan. It is one of the causes of death in foals. In a survey conducted in a breeding region in 1977 and 1978, 15.1% (8/53) of foals, 56.3% (99/176) of horses 2 years old, and 43.3% (91/210) of horses 4 years or older possessed CF antibody against BRV. As stated above, it was already confirmed by some workers that rotavirus had caused diarrhea in foals. From these findings, it is assumed that rotavirus may act as a causative agent of diarrheal disease among foals in Japan.

In 200 racehorses 3 years old each of the Tokyo and Nakayama Racecourses and the Ritto Training Center in 1977, the rate of horses positive for rotavirus was 43.0, 67.0 and 36.5%, respectively. From this result, it was made clear that the Nakayama Racecourse was highly contaminated with this virus, and that the Tokyo Racecourse and the Ritto Training Center were also contaminated comparatively highly with the same virus. The herd of the Nakayama Racecourse which showed the highest positive rate of the three institutions was selected for estimation of the positive rate of each age group of racehorses. In a serological survey on nearly all the horses of the Nakayama Racecourse in 1978, a positive rate showed a tendency to rise with the advance in age and reached 92.8% (118/128) in horses 5 to 7 years old. This result suggests that horses may be involved in very mild or inapparent infection of rotavirus in the racecourse.

It is a problem to be settled in future to know the state of detailed propagation of rotavirus by annual and seasonal serological surveys. Furthermore, it is also important to make clear the relationship between rotavirus and the disease in the herd by performing a virological survey on diarrheal horses.

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日本の軽種馬のウシロタウイルスに対する抗体保有状況

今川 浩*・安藤泰正*・秋山 綾*

1976年から1978年にかけて採集された北海道の生産地、東京、中山両競馬場ならびに栗東トレーニングセンター所属の軽種馬の血清計1873例について、ウシロタウイルスのCF抗体保有状況を調べた。その結果、以下の成績を得た。

1. 全検査例（1873例）の60.9％にウシロタウイルスに対するCF抗体が検出された。

2. 北海道の生産地において、当歳馬の15.1％（8/53）、2歳馬の56.3％（99/176）ならびに4歳以上の馬の43.3％（91/210）がそれぞれウシロタウイルスに対するCF抗体を保持していた。

3. 東京、中山両競馬場および栗東トレーニングセンターの3歳馬から採集されたそれぞれの200例についてのウシロタウイルスに対するCF抗体の保有率は、東京競馬場では43.0％、中山競馬場では57.0％および栗東トレーニングセンターでは36.5％であった。

4. 中山競馬場の2歳馬の46.3％（31/67）、3歳馬の75.2％（324/431）、4歳馬の84.6％（176/208）ならびに5歳から7歳馬の92.2％（118/128）にウシロタウイルスに対するCF抗体が検出された。

以上の結果、ロタウイルスは日本の軽種馬において、広範間にわたって高率に感染していることが明らかになった。