Isolation and Characterization of an Equine Rotavirus

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A rotavirus, designated as the H-1 strain, was isolated from a diarrheic foal in primary African green monkey kidney cells and MA104 cells. This cell culture-adapted strain hemagglutinated erythrocytes of human group O, rhesus monkeys, guinea pigs, and sheep. It was found to be similar, if not identical, to porcine rotaviruses (strains OSU, EE, and A-580) by plaque reduction neutralization and hemagglutination inhibition tests, and, in addition, it was found to belong to subgroup 1. This equine rotavirus has an RNA electrophoretic migration pattern which was distinct from those of the three strains of porcine rotavirus. The serological relationship established by plaque reduction neutralization and hemagglutination inhibition tests between the equine (H-1) and porcine (OSU, EE, and A-580) rotaviruses is an example of a rotavirus of the same serotype being isolated from different species. The H-1 strain was distinct from four human rotavirus serotypes (Wa, DS-1, P, and St. Thomas 4) as well as from bovine rotavirus NCDV, simian rotavirus MMU18006, and canine rotavirus CU-1 by plaque reduction neutralization tests. This equine isolate (H-1) was found to be related antigenically to canine CU-1 and bovine NCDV rotaviruses in a one-way fashion by hemagglutination inhibition tests.

Rotaviruses are well established as important etiological agents of acute gastroenteritis in various mammalian and avian species, including newborn horses. In 1975, Flewett et al. (8) reported scouring in newborn foals in northern Humberside, England, and detected rotaviruses in feces from foals by electron microscopy. In 1976, Kanitz (C. L. Kanitz, Proc. Ann. Conv. Am. Assoc. Equine Practnr., Dallas, Tex., 1976, p. 155–165) successfully reproduced a neonatal foal diarrhea syndrome experimentally and identified a rotavirus as the causative agent. Woode et al. (43) reported that foal rotaviruses were indistinguishable from those from children, calves, piglets, and mice morphologically by electron microscopy and antigenically by complement fixation, fluorescent antibody tests, and immune electron microscopy. In 1977, Thouless et al. (40) reported that foal rotaviruses were neutralized by at least a fourfold greater dilution of homologous (foal) antisera than by heterologous (human, calf, piglet, lamb, mouse, and rabbit) serum, as determined by fluorescent focus neutralization tests. In 1978 from Australia (37, 41) and from the United States (7), in 1979 from New Zealand (6), and in 1982 from Ireland (36), visualization by electron microscopy of rotaviruses in feces from diarrheic foals was reported. In 1979, Smith and Tzipori (35) reported that foal rotaviruses have an RNA electrophoretotype that is distinct from those of calf, pig, mouse, deer, and human rotaviruses. Rodger et al. (33), in 1980, reported that different equine rotavirus isolates showed variations in the electrophoretic mobilities of some double-stranded (ds) RNA segments. Subsequently, the serological evidence by complement fixation and fluorescent antibody tests of widespread infection of horses with rotaviruses was reported from Japan (23, 38) and from the United States (5, 32). In 1981, Imagawa et al. (22) successfully propagated a foal rotavirus in MA104 cells and demonstrated that their isolate was distinct from bovine (NCDV) rotavirus by using tube neutralization tests. To develop effective rotavirus vaccines for both human and veterinary medicine, it is important to elucidate the serotypic diversity of these agents. It is of particular interest that simian, canine, and certain equine rotaviruses have recently been shown to be similar, if not identical, to the third human rotavirus serotype (19a–21, 44). This report describes the isolation, propagation, and partial characterization of an equine rotavirus, as well as a serological comparison of this agent with human, simian, canine, porcine, and bovine rotaviruses.
MATERIALS AND METHODS

Preparation of fecal inoculum. A 20% clarified fecal suspension of diarrhea from a foal at a racing stable at Newmarket, England, was kindly supplied by T. H. Flewett under U.S. Department of Agriculture permit 6328 (46).

Cell culture. An established cell line of fetal rhesus monkey kidney (MA104) was used for rotavirus isolation and for plaque reduction neutralization (PRN) tests. Primary African green monkey kidney (AGMK) cells were used for reisolation of rotavirus. Growth medium was Eagle minimal essential medium, with 8 to 9% fetal calf serum or calf serum and antibiotics. Maintenance medium was the same but did not contain serum.

Isolation and propagation of equine rotavirus in cultured cells. Confluent monolayer cultures (culture tubes, 16 by 125 mm) were washed three times with Leibovitz L-15 medium or Eagle minimal essential medium and were inoculated with 0.1 ml of fecal suspension (pretreated for 60 min at 37°C with trypsin at a final concentration of 5 μg/ml). After a 1-h adsorption at 37°C, cell cultures were washed once with maintenance medium, fed with 1.0 ml of maintenance medium supplemented with 0.25 μg of trypsin per ml, and incubated on a roller apparatus at 37°C. At 1 week after infection if cytopathic effects (CPE) were not observed or at the time the cells showed 50 to 80% CPE, the culture tubes were frozen and thawed quickly three times, and cell lysates were inoculated into fresh cell cultures as described above.

Subgroup antigen assay. The immune adherence hemagglutination assay (IAHA) (27), enzyme-linked immunosorbent assay (ELISA) (10), or both were employed to subgroup equine rotavirus. Human rotavirus strains DS-1 (subgroup 1) and Wa (subgroup 2) were used as positive controls. Solid-phase radioimmunoassays (RIA) were performed as described previously (10).

Viruses. The following rotaviruses were used as reference strains: porcine rotavirus OSU, EE, A-580 (furnished by E. H. Bohl), and P-1 (furnished by J. G. Leecce); bovine rotavirus NCDV (furnished by C. A. Mebus); rhesus monkey rotavirus MMU18006 (furnished by N. J. Schmidt); canine rotavirus CU-1; and human rotavirus Wa, DS-1, P, and St. Thomas 4. The St. Thomas 4 strain belongs to a newly defined fourth serotype (45) and was used in the form of a reassortant virus between a temperature-sensitive mutant of a cultivatable bovine rotavirus (UK strain) and a human rotavirus (St. Thomas 4) (13). All rotaviruses were triply plaque purified before use.

Antisera. All hyperimmune antisera used in PRN and hemagglutination inhibition (HI) tests were prepared in guinea pigs as described previously (11, 44).

Preparation of ds RNA and polyacrylamide gel electrophoresis. ds RNAs of equine and porcine rotavirus- infected MA104 cell lysates were extracted from the infected MA104 cell lysates by a previously described method (26). Segmented ds RNA was run overnight in a 12% polyacrylamide slab gel with 3.5% stacking gel at 15 to 20 mA of constant current by the method of Laemmli (30), except that sodium dodecyl sulfate was omitted from all solutions. Ethidium bromide staining and photography of RNA under UV light were performed as reported previously (26).

RESULTS

Isolation and propagation of rotavirus in cultured cells. CPE were not observed during the first two passages of strain H-1 in either the MA104 cells or the reisolation attempts in primary AGMK cells. The first sign of CPE was detected 3 days postinfection at passage 3 in both cell types. CPE consisted of increased cell granularity, obscure cell boundaries, cell rounding, and eventual cell detachment from the glass walls; CPE were consistently observed thereafter.

Subgroup antigen assay. The rotavirus in the original fecal material from a diarrheic foal was found to belong to subgroup 2 by using RIA (10). However, the same fecal material was not subgrouped using ELISA (Table 1), and the tissue culture-adapted equine rotavirus (H-1) grown in either AGMK or MA104 cells was shown to belong to subgroup 1 by using ELISA (Table 1) and IAHA (Table 2). In attempting to reconcile these conflicting data, subgroup testing of the original feces was reattempted by using RIA. Common monoclonal antibodies detected rotavirus common antigen(s); however, both subgroup 1 and 2 monoclonals failed to detect subgroup-specific antigen(s) on repeated RIA tests (Table 3). Strains EE, A-580, and P-1 of porcine rotavirus were found to belong to subgroup 1 (Table 1).

HA and HI tests. The partially purified H-1 strain of equine rotavirus hemagglutinated erythrocytes of human group O (titer, 1:32), rhesus monkeys (1:32), guinea pigs (1:4), and sheep (1:4). Since the hemagglutinating pattern of human group O erythrocytes was firmer and...
better than that of the rhesus monkeys, human group O erythrocytes were used for the HI test. The H-1 strain of equine rotavirus and the OSU strain of porcine rotavirus were found to be similar, if not identical, by the HI test (Table 4). The CU-1 strain of canine rotavirus and the NCDV strain of bovine rotavirus were related to the H-1 strain in a one-way fashion, and rhesus rotavirus was distinct from equine H-1. The canine and simian viruses were similar, if not identical, by HI test.

**Antigenic relationships between equine and other mammalian rotaviruses.** Table 3 summarizes the results of the antigenic relationships between equine and other mammalian rotaviruses studied by PRN tests. The equine (H-1) and porcine (OSU) rotaviruses were found to be similar, if not identical, by this reciprocal cross-neutralization test. It is striking that the equine (H-1) rotavirus was distinct from the four serotypes of human rotavirus (Wa, DS-1, P, and St. Thomas 4), as well as from rhesus (MMU18006), canine (CU-1), and bovine (NCDV) rotaviruses by the PRN test.

**Antigenic relationship of equine and three strains of porcine rotaviruses.** To further study the close antigenic relationship between this isolate of equine rotavirus and porcine rotaviruses, two additional porcine rotavirus strains (EE and A-580) were examined by using PRN tests. Table 6 shows a two-way antigenic relationship between the H-1 strain of equine rotavirus and OSU, EE, and A-580, which together represent a single serotype of porcine rotavirus.

**Polyacrylamide gel electrophoresis of segment-ed viral ds RNAs.** Since equine rotavirus was found to be closely related antigenically to porcine rotaviruses and since there is always a possibility of cross-contamination in the laboratory, electrophoretic migration patterns of ds RNAs of equine and porcine rotaviruses were compared. The H-1 strain of equine rotavirus has an electropherotype of ds RNA distinct from those of OSU, EE, and A-580 of porcine rotaviruses (Fig. 1). The H-1 strain was also shown to be distinct from porcine rotavirus P-1 in ds RNA electrophoretic migration pattern.

### Table 1. Subgroup specificity of equine (H-1), and porcine (OSU, EE, A-580, and P-1) rotaviruses determined by using ELISA with monoclonal antibodies

<table>
<thead>
<tr>
<th>Rotavirus (strain)</th>
<th>Optical density with a single dilution of indicated monoclonal</th>
<th>Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subgroup 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Subgroup 2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Human (DS-1)</td>
<td>280</td>
<td>13</td>
</tr>
<tr>
<td>Human (Wa)</td>
<td>13</td>
<td>293</td>
</tr>
<tr>
<td>Original foal feces (H-1)</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>MA104-grown equine (H-1)</td>
<td>299</td>
<td>14</td>
</tr>
<tr>
<td>Porcine (OSU)</td>
<td>298</td>
<td>12</td>
</tr>
<tr>
<td>Porcine (EE)</td>
<td>294</td>
<td>13</td>
</tr>
<tr>
<td>Porcine (A-580)</td>
<td>296</td>
<td>14</td>
</tr>
<tr>
<td>Porcine (P-1)</td>
<td>293</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sum of optical density of two wells × 100 at 400 nm.
<sup>b</sup> Monoclonal 255/60 with subgroup 1 specificity.
<sup>c</sup> Monoclonal 631/9 with subgroup 2 specificity.

### Table 2. Subgroup specificity of equine (H-1) and porcine (OSU, EE, and A-580) rotaviruses determined by IAHA

<table>
<thead>
<tr>
<th>Rotavirus (strain)</th>
<th>Reciprocal of IAHA antigen titer of gnotobiotic calf postinfection antiserum to indicated rotavirus (strain)</th>
<th>Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human subgroup 1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Human subgroup 2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Human (DS-1)</td>
<td>16</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Human (Wa)</td>
<td>&lt;2</td>
<td>32</td>
</tr>
<tr>
<td>Original foal feces (H-1)</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>MA104-grown equine (H-1)</td>
<td>32</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Porcine (OSU)</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Porcine (EE)</td>
<td>64</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Porcine (A-580)</td>
<td>32</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

### Table 3. Subgroup specificity of equine (H-1) rotavirus as determined by RIA

<table>
<thead>
<tr>
<th>Rotavirus (strain)</th>
<th>Reciprocal of RIA antigen titer with a single dilution of indicated monoclones</th>
<th>Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subgroup 1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Subgroup 2&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Human (DS-1)</td>
<td>1,385</td>
<td>93</td>
</tr>
<tr>
<td>Human (Wa)</td>
<td>35</td>
<td>658</td>
</tr>
<tr>
<td>Original foal feces (H-1)</td>
<td>36</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> Monoclonal 255/60 with subgroup 1 specificity.
<sup>b</sup> Monoclonal 631/9 with subgroup 2 specificity.
<sup>c</sup> Mixture of monoclones 952/224, 260/9, 260/12, 631/16, 631/24, and 255/18, designed as a broadly reactive reagent.
**TABLE 4.** Comparison of equine (H-1), porcine (OSU), simian (MMU18006), canine (CU-1), and bovine (NCDV) rotaviruses by HI tests

<table>
<thead>
<tr>
<th>Rotavirus (strain)</th>
<th>Equine (H-1)</th>
<th>Porcine (OSU)</th>
<th>Simian (MMU18006)</th>
<th>Canine (CU-1)</th>
<th>Bovine (NCDV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equine (H-1)</td>
<td>5,120 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>10,240</td>
<td>40</td>
<td>80</td>
<td>5,120</td>
</tr>
<tr>
<td>Porcine (OSU)</td>
<td>5,120</td>
<td>40,960</td>
<td>40</td>
<td>40</td>
<td>2,560</td>
</tr>
<tr>
<td>Simian (MMU18006)</td>
<td>160</td>
<td>320</td>
<td>20,480</td>
<td>2,560</td>
<td>640</td>
</tr>
<tr>
<td>Canine (CU-1)</td>
<td>1,280</td>
<td>2,560</td>
<td>20,480</td>
<td>5,120</td>
<td>320</td>
</tr>
<tr>
<td>Bovine (NCDV)</td>
<td>80</td>
<td>20</td>
<td>10</td>
<td>80</td>
<td>40,960</td>
</tr>
</tbody>
</table>

<sup>a</sup> Homologous values are shown in boldface type.

**DISCUSSION**

The results of the present study demonstrated by PRN tests that equine rotavirus strain H-1 is distinct from four human rotavirus serotypes (Wa, DS-1, P, and St. Thomas 4) as well as being distinct from bovine (NCDV), rhesus monkey (MMU18006), and canine (CU-1) rotaviruses, based on the criterion of 20-fold or greater differences in antibody titer. This isolate (H-1) of equine rotavirus was found to be indistinguishable from one serotype of porcine rotavirus (OSU, EE, and A-380) by PRN and HI tests. However, it differs in the electrophoretic mobility of certain ds RNA segments with each of these porcine rotavirus strains (Fig. 1). It is not surprising that the rotaviruses from two different animal species belong to the same serotype, since it has been well established that simian (MMU18006 and SA11), canine (CU-1, A79-10, and LSU75C-36), some equine (H-2), and some human (M, P, B, 14, and 15) rotaviruses belong to the third human rotavirus serotype (19a–21, 44, 45); HI tests have revealed broader cross-reactivity among rotaviruses examined in this study than PRN tests. For example, although the H-1 strain of equine rotavirus was shown to be serotypically distinct from canine (CU-1) and bovine (NCDV) rotaviruses by PRN tests (Table 5), the former was found to be related to the latter in a one-way fashion by HI tests (Table 4). Kalica et al. (24) have recently reported by analyzing 16 simian (MMU18006) × bovine (UK) reassortants that rotaviral HA and neutralization are functions encoded by two different gene products: gene 4 encodes viral hemagglutinin, and gene 8 or 9 encodes a major neutralizing protein. More recently, Greenberg et al. (12) have demonstrated by analyzing a series of monoclonal antibodies directed against two surface proteins of simian rotavirus (MMU18006) that both viral HA and neutralization properties can be mediated by antibodies to gene 4 as well as to gene 8 or 9 products. They have also shown that both viral hemagglutinin and a major neu-

**TABLE 5.** Antigenic relationship between equine rotavirus and porcine, bovine, simian, canine, and human rotaviruses by PRN tests with hyperimmune antisera

<table>
<thead>
<tr>
<th>Rotavirus (strain)</th>
<th>Equine (H-1)</th>
<th>Porcine (OSU)</th>
<th>Bovine (NCDV)</th>
<th>Rhesus (MMU18006)</th>
<th>Canine (CU-1)</th>
<th>Human (DS-1)</th>
<th>Human (Wa)</th>
<th>Human (P)</th>
<th>Human (St. Thomas 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equine (H-1)</td>
<td>46,816 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>82,687 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,524 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>689 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Equine (H-1)</td>
<td>68,343 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>500 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;80 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;80 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>982 &lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Equine (H-1)</td>
<td>65,785 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Porcine (OSU)</td>
<td>21,777 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>90,442 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>982 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;80 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bovine (NCDV)</td>
<td>840 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>32,482 &lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rhesus (MMU18006)</td>
<td>230 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>77,607 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Canine (CU-1)</td>
<td>1,666 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>30,465 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Human (DS-1)</td>
<td>&lt;80 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Human (Wa)</td>
<td>982 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>65,478 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>Human (P)</td>
<td>&lt;80 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>24,827 &lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Human (St. Thomas 4)</td>
<td>&lt;80 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> This is a composite table of values derived from three different PRN tests, a to c. Homologous values are shown in boldface type.

<sup>d</sup> Human-bovine reassortant rotavirus.

<sup>e</sup> Not tested.
TABLE 6. Comparison of equine rotavirus (H-1) and porcine rotaviruses (strains OSU, EE, and A-580) by PRN tests* with hyperimmune antiserum

<table>
<thead>
<tr>
<th>Rotavirus (strain)</th>
<th>Reciprocal of 60% plaque reduction neutralizing titer of hyperimmune antiserum to indicated rotavirus (strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equine (H-1)</td>
</tr>
<tr>
<td>Equine (H-1)</td>
<td>53,484</td>
</tr>
<tr>
<td>Porcine (OSU)</td>
<td>20,345</td>
</tr>
<tr>
<td>Porcine (EE)</td>
<td>11,890</td>
</tr>
<tr>
<td>Porcine (A-580)</td>
<td>13,932</td>
</tr>
</tbody>
</table>

* All values are from the same test.

Homologous values are shown in boldface type.

Neutralizing protein exhibit distinct type specificity. For example, simian (MMU18006) and canine (CU-1) rotaviruses have been shown to be indistinguishable from each other by PRN and HI tests using monoclonal antibodies directed against gene 8 or 9 product; however, a cross-reactivity between the two has not been detected by PRN and HI tests using monoclonal antibodies directed against gene 4 product (12). Therefore, although the qualitative nature and quantitative distribution of two biologically distinct antibodies (those directed against gene 4 products and gene 8 or 9 products as demonstrated in a monoclonal system) in polyclonal hyperimmune antiserum employed in this study are not known, it is conceivable that the antigenic relationships among rotaviruses studied by PRN tests would be different from those examined by HI tests as shown in the present study. A variety of human, animal, and avian rotaviruses are currently being studied for serotypic diversity in our laboratory.

In 1981, Kapikian et al. (27) proposed the first unified antigenic classification of human and other mammalian rotaviruses by demonstrating by IAHA and ELISA the existence of at least two distinct antigenic specificities designated subgroups 1 and 2. Moreover, Kalica et al. (25) demonstrated that subgroup antigens were encoded by the rotavirus gene 6 product (the major inner capsid protein). Subsequently, Greenberg et al. (12) confirmed and extended the previous findings by using 10 separate monoclonal antibodies directed against the protein product of gene 6. Bastardo et al. (1), using simian rotavirus SA11, and Killen and Dimmock (29), using bovine rotavirus UK, reported independently that the monospecific hyperimmune antiserum prepared to gene 6 products of each rotavirus had a low level of neutralizing activity against homologous viruses. In addition to its practical applications described previously (10, 27), subgroup specificity plays an important role in the studies of genetics and natural history of rotaviruses.

FIG. 1. Comparison of RNA electrophoretic migration patterns of H-1 strain of equine and three strains (OSU, EE, and A-580) of porcine rotaviruses. RNAs from H-1 were coelectrophoresed with OSU, EE, or A-580 as indicated. Migration was from top to bottom, and genome segments are numbered in descending order of molecular weight.
For example, we have recently demonstrated for
the first time the existence of two rotaviruses
from one animal species which belong to the
same serotype but differ in subgroup specificity
(Hoshino et al. manuscript in preparation). Fur- 
ther studies on its biological nature and genetic
significance need to be done.

The equine rotavirus (H-1) in the original fecal
material has been found on two separate occa-
sions to be a subgroup 2 virus by using RIA with
monoclonal antibodies (10); however, the culti-
vatable virus was found to be a subgroup 1 virus.
As noted earlier, however, on repeated RIA
testing of the original fecal material by using
RIA with the same monoclonal antibodies, a
subgroup determination could not be made (Ta-
ble 3). A rotavirus reisolated from the original
foal feces in primary AGMK cells was also a
subgroup 1 virus. It is possible that the virus in
the original feces was a mixture of subgroup 2
(predominantly) and subgroup 1 subpopulations,
and susceptible cell substrates (MA104 and
AGMK cells) selected the subgroup 1 subpop-
ulation preferentially over the subgroup 2 sub-
population during virus multiplication. Anti-
genic heterogeneity of the various constituent virus
particles comprising a virus strain has been well
established for certain enteroviruses (2), myxo-
viruses (3, 4), alphaviruses (14, 15), and rabdo-
viruses (42). Also, host-induced antigenic varia-
tion has been reported previously for animal
viruses such as variola (19), influenza A (18),
ECHO-6 (28), western equine encephalomyelitis
(31), eastern equine encephalomyelitis (17),
Semliki Forest, chikungunya, O'nyoung-nyoung
(T. B. Stim and J. R. Henderson, Bacteriol.
Proc., p. 182, 1968), and Getah (16). Very re-
cently Sabara et al. (34) reported the coexis-
tence of electrophoretically distinct bovine rotavi-
rus subpopulations within one animal by the use of
high-resolution gels. The existence of a sub-
group 1 (39) as well as subgroup 2 (J. H. Gilles-
pie, Cornell University, Ithaca, N.Y., personal
communication) equine rotaviruses obtained from
a different source than the H-1 strain has been
reported recently.

In cross-protection studies performed by Tzi-
pori and Walker (41) in 1978, apparent cross-
protection between pig and foal rotaviruses in
pigs was observed. This cross-protection be-
tween pig and foal rotaviruses can now possibly
be explained since certain porcine and equine
rotaviruses belong to the same serotype. How-
ever, it has been reported that there is a serotype
of porcine rotavirus distinct from the OSU strain
(E. H. Bohl, Ohio Agricultural and Develop-
ment Center, Wooster, personal communica-
tion), and this strain does not cross-react with
the H-1 strain of equine rotavirus (Hoshino et
al., manuscript in preparation). Moreover, we
have recently reported the existence of a second
equine rotavirus serotype which was found to be
different from the H-1 strain not only in serotypic
specificity but also in subgroup specificity (19a).

Gaul et al. (9) have recently concluded that
the cross-protection between rotaviruses is type
specific, and potential vaccines should include
serotypes which cause disease in the homolo-
gous animal species. If the H-1 strain of equine
rotavirus is found to be of low virulence or can
be further attenuated, and if the above findings
by Gaul et al. are confirmed further, the H-1
strain may become a potential vaccine for use
not only in foals but also in piglets.

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