Comparison of Atypical Rotaviruses from Calves, Piglets, Lambs and Man

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(Accepted 8 February 1984)

SUMMARY

Some rotaviruses from calves, piglets, and lambs were detected by electron microscopic examination of faeces but not by an enzyme-linked immunosorbent assay which relies on detection of group antigen. On further examination by polyacrylamide gel electrophoresis, these viruses had 11 segments of dsRNA, as had typical rotaviruses, but arranged in atypical patterns. From humans, three rotaviruses with atypical electrophoretypes were also detected. Gnotobiotic animals were infected with atypical calf, piglet and lamb rotaviruses, and used to provide antigen and antiserum for an immunofluorescent comparison of these rotaviruses with conventional rotaviruses and other previously described atypical rotaviruses from piglets and chickens. Two atypical rotaviruses from humans failed to infect gnotobiotic piglets. The atypical rotaviruses could be tentatively categorized into two groups serologically distinct from each other and from conventional rotaviruses, and these distinctions were consistent with electrophoretypes. The atypical chicken rotavirus may form a fourth distinct group. These findings are consistent with the hypothesis that rotaviruses belong to at least four separate groups definable by serology and electrophoretype.

INTRODUCTION

Most rotaviruses from mammalian and avian species share a common antigen demonstrable by techniques such as immunofluorescence, immune electron microscopy, complement fixation, and enzyme-linked immunosorbent assay (Woode et al., 1976; Yolken et al., 1978; McNulty et al., 1979). Recently, however, viruses with characteristic rotavirus morphology which nevertheless lack this group antigen have been described from piglets (Bohl et al., 1982; Bridger et al., 1982), chickens (McNulty et al., 1981), and children (Rodger et al., 1982; Dimitrov et al., 1983; Nicolas et al., 1983). Although these antigenically distinct rotaviruses have 11 segments of dsRNA, their genome segment electrophoretic migration patterns are consistently different from those of conventional rotaviruses, and terminal fingerprint analysis also distinguishes the segments of the atypical rotaviruses (Pedley et al., 1983).

As comparatively few of these atypical rotaviruses have been described, serological and genomic comparisons are limited (Pedley et al., 1983). This paper describes such a study, using rotaviruses detected in the faeces of calves, piglets, lambs, and humans, and a chicken tissue culture isolate (McNulty et al., 1981).

METHODS

Rotavirus strains. Faeces samples from diarrhoeic animals submitted to the Moredun Research Institute for rotavirus diagnosis were examined by electron microscopy (EM) (Snodgrass et al., 1976) and enzyme-linked immunosorbent assay (ELISA) (Fahey et al., 1981). Any samples that were rotavirus-positive by EM but negative by ELISA (and thus potentially missing the group antigen) were examined for their rotavirus dsRNA segment pattern by polyacrylamide gel electrophoresis (PAGE) (Herring et al., 1982). Viruses showing atypical electrophoretypes were selected for further study. Stool samples containing atypical rotavirus from diarrhoeic humans were also included.
A plaque-purified strain of the U.K. calf rotavirus (Bridger & Woode, 1975) grown in MA104 cells was used as a representative of conventional rotaviruses for serological tests, and a lamb rotavirus (Snodgrass et al., 1976) as a conventional rotavirus for PAGE.

**Animals.** Gnotobiotic piglets, lambs, and calves were used. They were infected orally usually at 1 day of age with 2 ml of either 10 to 20% faecal suspension or faecal filtrate (0-45 µm). The animals were observed clinically, and faeces were collected daily. Some animals were killed after infection for collection of intestinal contents, and for preparation of intestinal sections from small and large intestine for histological and immunofluorescent examination (Snodgrass et al., 1979).

**Antisera.** Experimental animals were bled for convalescent antiserum from 21 to 25 days after inoculation. In addition, hyperimmune antisera to some viruses were prepared by inoculating convalescent animals with virus purified from their own faeces by the method described in Ojeh et al. (1984), although yields of virus were poor due to the tendency of the atypical rotavirus particles to aggregate. Purified virus was emulsified in oil adjuvant and inoculated intramuscularly to animals 3 to 4 weeks after primary infection. Blood was then collected 2 weeks after hyperimmunization.

Sera were collected from patients JM and HM 5 and 6 weeks respectively after onset of illness diagnosed as an atypical rotavirus infection.

Other antisera used were: convalescent gnotobiotic calf and hyperimmune rabbit to conventional calf rotavirus; gnotobiotic piglet convalescent to piglet rotavirus-like agent (Bridget et al., 1982) supplied by Dr J. C. Bridger (Compton); gnotobiotic piglet convalescent to piglet pararotavirus (Bohl et al., 1982) supplied by Dr L. J. Saif (Ohio); and chicken antiserum to chicken rotavirus 132 (McNulty et al., 1981) supplied by Dr M. S. McNulty (Stormont).

**Immunofluorescence (IF) tests.** Test antigens used for our atypical rotaviruses were cryostat sections of small intestine. In addition, chicken rotavirus 132 grown in MA104 cells on coverslips was supplied by Dr M. S. McNulty, and cryostat sections of piglet intestine infected with piglet rotavirus-like agent were supplied by Dr J. C. Bridger. Calf rotavirus grown in MA104 cells on microtitre plates was used as an antigen representative of conventional rotaviruses.

Fourfold dilutions of each antiserum were tested with each antigen, with appropriate anti-species IgG conjugated with fluorescein isothiocyanate (FITC), except for antiserum to chicken rotavirus 132 which was conjugated with FITC for a direct IF test. Positive and conjugate controls were included in each test.

**PAGE.** Double-stranded RNA was extracted from faeces by the methods of Herring et al. (1982), and further purified by one cycle of CF 11 phosphocellulose chromatography (Franklin, 1966) performed as described by Bevan et al. (1973). Porcine pararotavirus dsRNA was kindly provided by Dr E. H. Bohl. PAGE was then performed using a 7.5% discontinuous gel; this was stained with silver (Ojeh et al., 1984).

**RESULTS**

**Detection of atypical rotaviruses by PAGE**

Of 598 samples of calf faeces examined for rotavirus by EM and ELISA over 2 years, 179 were positive by both techniques, and 14 came into the category EM + ELISA –. Only two of these 14 had atypical electrophoretypes when examined by PAGE (D522 and D531). In addition, faeces samples from one piglet (D238) and multiple faeces samples from an outbreak of diarrhoea in lambs (E1101) also had atypical electrophoretypes. Stool samples from three humans (HM, JM and LK) from different sources also had atypical electrophoretypes. No viruses other than rotaviruses were detected by EM examination.

The genome profiles of the atypical rotaviruses were compared with conventional rotavirus (group A of Pedley et al., 1983) and with porcine pararotavirus (group C) by PAGE (Fig. 1). The atypical rotaviruses had two distinctive electrophoretypes as described by Pedley et al. (1983). Both calf viruses (D522 and D531) and lamb virus E1101 were of the pattern described as group B, typified by close migration of segments 5 and 6 and the migration of segment 9 near 10 and 11. Piglet rotavirus D238 and the three human rotavirus strains were of the pattern described as group C, typified by segment 7 migrating near segments 5 and 6. However, as with conventional rotaviruses, minor variations within this overall pattern were apparent for each rotavirus strain.

**Passage in experimental animals**

Rotavirus D238 from a diarrhoeic piglet was given orally in faecal suspension to two piglets, and faecal filtrate from one of these piglets was used to infect four further piglets. All piglets
Atypical rotaviruses

Fig. 1. Genome profile of rotaviruses by PAGE. Lane 1, human LK rotavirus; lane 2, piglet D238 rotavirus; lane 3, piglet pararotavirus (Ohio); lane 4, conventional lamb rotavirus; lane 5, calf D522 rotavirus; lane 6, calf D531 rotavirus; lane 7, lamb E1101 rotavirus. Lanes 1 to 3 contain rotaviruses exhibiting group C electrophoretype, lane 4 a typical group A electrophoretype, and lanes 5 to 7 group B electrophoretypes.

excreted D238 rotavirus in faeces within 24 h of infection. Severe diarrhoea, vomiting, anorexia, and dehydration occurred in piglets infected at 1 day of age, but milder diarrhoea only occurred in two piglets infected at 5 days old.

Rotavirus D522 from a diarrhoeic calf was given orally by faecal filtrate to one calf and two lambs, and faecal suspension from the calf was used to infect two further lambs. All animals excreted D522 rotavirus in faeces. The calf passed a large volume of loose yellow faeces on the day following infection, but was otherwise clinically normal. Only the lambs infected with the second passage level virus developed diarrhoea.

A suspension of lamb faeces containing rotavirus E1101 was given orally to one piglet. Intestinal contents from this piglet were given orally as suspension and filtrate to two and four further piglets respectively. Piglets excreted E1101 rotavirus in faeces within 17 h of inoculation, approximately coincident with the onset of a severe watery diarrhoea. Piglets infected at less than 3 days of age died within 48 h.
Table 1. Serological relationships (IF) between typical and atypical rotaviruses

<table>
<thead>
<tr>
<th>Antiserum to rotavirus</th>
<th>Group A*</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>Group A*</td>
<td>Group B</td>
<td>Group C</td>
<td>Group D</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>A*</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Calf U.K.</td>
<td>2560</td>
<td>40</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>B calf D522</td>
<td>&lt;10</td>
<td>160</td>
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<td>&lt;10</td>
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<tr>
<td>Piglet rotavirus-like</td>
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<td>&lt;10</td>
<td>160</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Lamb E1101</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>640</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Piglet D238</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>320</td>
</tr>
<tr>
<td>Chicken 132</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>1280</td>
</tr>
</tbody>
</table>

* Groups A, B, C, D: see text.
† Hyperimmune rabbit antiserum to calf rotavirus (U.K.).
‡ Convalescent gnotobiotic calf antiserum to calf rotavirus (U.K.).
§ Hyperimmune gnotobiotic calf antiserum to D522 rotavirus.
‖ Hyperimmune gnotobiotic piglet antiserum to D238 rotavirus.
¶ Not tested.

Suspensions of human stools containing rotaviruses JM and LK were given orally to one and two piglets respectively. These piglets continued to pass formed faeces, and no rotavirus excretion was detected by EM or PAGE during the 7 days following infection.

Rotavirus D531 from a diarrhoeic calf was given orally as faecal filtrate to one lamb. The lamb excreted D531 rotavirus in faeces but remained clinically normal.

For each virus studied, the electrophoretic pattern detected by PAGE remained constant from the initial faecal sample through sequential passage.

Histopathological examination

Histopathological examination of intestine taken 2 days after infection from piglets infected with D238 and E1101 rotaviruses and from a lamb infected with D522 rotavirus revealed lesions of the small intestine typical of rotavirus infection (Snodgrass et al., 1977). There were no abnormalities detected in the small intestine of a lamb killed 4 days after infection with D531 rotavirus.

IF examination

Immunofluorescence in small intestinal enterocytes with homologous antiserum was detected in piglets infected with D238 rotavirus, the most extensive staining being present in the duodenum of a piglet killed 15 h after infection. Immunofluorescence with homologous antiserum was detected in ileal enterocytes of a lamb killed 12 h after infection with D522 rotavirus. Immunofluorescence with antiserum to calf D522 rotavirus was detected in piglets infected with lamb E1101 rotavirus. Infected enterocytes were present throughout the small intestine in piglets killed at 11 h and 14 h, but not at 18 h after infection.

Serological comparisons

The serological comparisons using as antigen gut sections from the animals described in the previous paragraph are summarized in Table 1. Calf and rabbit antisera to conventional (group A) calf rotavirus reacted with U.K. calf rotavirus antigen, but with none of the atypical rotavirus antigens used.

Within the group B rotaviruses, antiserum to D522 calf rotavirus reacted with homologous antigen and with E1101 and piglet group B antigens. However, antiserum to piglet group B virus reacted with homologous antigen but not with either D522 or E1101 antigens. Neither of these antisera reacted with any other of the rotavirus antigens used.

Within the group C rotaviruses, antisera to D238 piglet rotavirus and to piglet group C rotavirus both reacted with D238 antigen, but not with any other of the rotavirus antigens tested.

Antiserum to chicken 132 rotavirus reacted only with homologous antigen.
Sera from humans HM and JM both had antibody titres to group A rotaviruses of 80 and to group B rotaviruses of < 10. Titres to group C rotaviruses were 40 and 160 respectively.

**DISCUSSION**

The three criteria available for grouping atypical rotaviruses are serological distinction, distinct electrophoretype, and distinct RNA segment terminal fingerprint. Our results provide evidence that the atypical rotaviruses included in this study can be grouped on the basis of the first two of these criteria.

We detected two consistent atypical electrophoretypes, in both of which the typical group A rotavirus pattern of the 7th, 8th, and 9th segments migrating as a tight triplet was altered. Our D522 and D531 calf rotaviruses, and E1101 lamb rotavirus had electrophoretypes similar to the piglet rotavirus-like virus (Bridger *et al.*, 1982) proposed as group B by Pedley *et al.* (1983), with one of the triplet segments migrating more rapidly. Similarly our D238 piglet rotavirus and HM, JM, and LK human rotaviruses had electrophoretypes similar to piglet pararotavirus (Bohl *et al.*, 1982) proposed as group C (Pedley *et al.*, 1983), with one of the tight triplet segments migrating more slowly.

The serological results support this division of these atypical rotaviruses into two groups. Our D238 piglet rotavirus cross-reacted with piglet pararotavirus (group C), and our D522 calf rotavirus and E1101 lamb rotavirus showed a one-way cross-reaction with rotavirus-like virus (group B).

Thus there is good evidence provided by electrophoretype and serogroup that these atypical rotaviruses can be classified under the scheme of Pedley *et al.* (1983) as group B (D522 and E1101) and group C (D238). Others for which there is so far only electrophoretypic evidence can be more tentatively assigned to group B (D531) and group C (HM, JM, and LK). It will be interesting to compare the RNA segments by terminal fingerprint analysis (McCrae & McCorquodale, 1983) to ascertain if this method also can be consistently useful in atypical rotavirus classification, as so far only very limited data are available from one group B and one group C virus (Pedley *et al.*, 1983).

The presence of antibody in the human convalescent antisera to groups A and C but not to group B rotaviruses does not in itself indicate that these are group C rotaviruses, but is consistent with that hypothesis. So far all atypical human rotavirus strains appear from published electrophoretypes to fall within the group C rotaviruses (Rodger *et al.*, 1982; Dimitrov *et al.*, 1983; Nicolas *et al.*, 1983).

The failure of antiserum to piglet group B rotavirus to react with D522 and E1101 antigens is puzzling. It may reflect a greater specificity of the porcine serum for type-specific rather than group-specific antigens, with the existence of subgroup or serotypes within the group B rotaviruses as well as within the group A rotaviruses (Kapikian *et al.*, 1981).

Other variations within these groups of atypical rotaviruses are shown by the failure of human group C rotaviruses to multiply in gnotobiotic piglets. This is in contrast to the marked pathogenicity for piglets of group C rotaviruses isolated from pigs. Biological variations of this type may be as extensive among atypical viruses as in group A rotaviruses.

These studies confirmed that chicken 132 rotavirus does not cross-react serologically with rotaviruses of groups A, B, or C, and so is a potential group D rotavirus. The existence of at least four serologically distinct rotavirus groups poses an obvious diagnostic problem when techniques based on immunological specificity, such as ELISA, are used. This problem can be overcome with no loss of sensitivity by the use of diagnostic PAGE techniques (Herring *et al.*, 1982).

There are reasons to suppose that the atypical rotaviruses are comparatively uncommon: they remained undetected for many years in spite of widespread rotavirus research; they have been detected at levels varying from 0.25 to 4% of human rotavirus cases (Rodger *et al.*, 1982; Nicolas *et al.*, 1983; Dimitrov *et al.*, 1983); and we have detected only two atypical rotaviruses from 222 rotavirus-containing calf faeces (< 1%). On the other hand, antibody to groups B and C occurs commonly in cattle and pigs (Bridger *et al.*, 1983). This discrepancy could be accounted for if atypical rotaviruses were less virulent than group A rotaviruses, but evidence from this study and others suggests that group C rotaviruses are pathogenic for piglets (Bohl *et al.*, 1982; Bridger...
et al., 1982), and one of our group B rotaviruses induced typical rotavirus lesions. Their overall importance remains to be established.

We gratefully thank those identified in the text for supplying us with viruses and antisera. Neil Inglis supplied able technical assistance, and K. W. Angus examined the histological sections.

REFERENCES


(Received 6 December 1983)