Isolation of Feline Rotaviruses and Their Relationship to Human and Simian Isolates by Electropherotype and Serotype

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SUMMARY

Rotaviruses were detected by electron microscopy in the faecal specimens of six clinically well cats and virus was subsequently isolated from four of them. Analysis of the RNA of the isolates showed the existence of three electrophoretic types characteristic of the 'long' RNA electrophoretic pattern exhibited by rotaviruses. All feline isolates were neutralized only by antiserum to SA11 rotavirus, indicating that these isolates were serotype 3 rotaviruses. Antiserum prepared against a feline strain neutralized all the feline isolates as well as SA11 but showed no neutralizing activity against human isolates of serotype 1, 2 or 4.

Rotavirus is established as a common cause of gastroenteritis in the young of many species, and has been previously reported to cause both symptomatic and asymptomatic infections in cats (McNulty et al., 1978; Chrystie et al., 1979; Snodgrass et al., 1979; Hoshino et al., 1981). In two of these reports (Chrystie et al., 1979; Snodgrass et al., 1979) rotavirus was associated with symptoms of diarrhoea. In another (Hoshino et al., 1981) a feline rotavirus was isolated from a cat with chronic arthritis. In addition, antibody to rotavirus was detected in apparently healthy cats in Belfast, U.K. (McNulty et al., 1978). Two of these reports (Chrystie et al., 1979; Hoshino et al., 1981) concluded that feline rotaviruses do not share the antigenic characteristics of human serotype 1 rotavirus, although they possess the common antigen expressed by rotaviruses of all species. A number of animal rotaviruses have been reported to share serotype specificity with human rotavirus; two are of simian origin (MMU 18006 and simian agent 11, SA11), one is a canine rotavirus (CU-1) and a fourth is the recently isolated equine rotavirus, H-2 (Hoshino et al., 1983). A feline isolate has been shown to exhibit a one-way cross-reaction with the canine rotavirus (Hoshino et al., 1982) and has subsequently been classified as belonging to the third rotavirus serotype (Hoshino et al., 1984). The aims of the study reported here were (i) to establish whether rotaviruses could be detected in domestic cats in Melbourne, Australia, and to characterize the illness, if any, associated with infection with these agents, and (ii) to assess the relationship of human and animal viruses of known serotype to feline rotaviruses by comparing their electropherotypes and neutralization antigens.

Faecal specimens were collected from 100 cats housed at an animal shelter in a Melbourne suburb between March and October 1984. The cats were of domestic origin and were admitted to the shelter as strays, for sale, or as boarders. All were examined by a veterinary surgeon on entry to the shelter, were vaccinated against feline panleukopenia virus and treated for parasites. Specimens were collected only from cats caged singly in an enclosure. The cats ranged in age from 3 months to adult (more than 1 year of age). Because of the relatively short stay of the animals at the shelter, long-term observation of the cats was not possible and usually only one specimen could be collected from each animal.

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Attempts to isolate rotavirus from processed faecal extracts were as previously described (Birch et al., 1983). Faecal extracts were pretreated with trypsin (20 μg/ml), and trypsin (1.5 μg/ml) was incorporated into serum-free maintenance medium when passaging virus. RNA was extracted using a phenol/SDS procedure as previously described (Birch et al., 1983). RNA segments were separated by electrophoresis on 10% polyacrylamide slab gels, 1.0 mm thick, for 4 h at a current of 30 mA. Gels were stained using a silver staining method (Sammons et al., 1981) before photography.

Antisera against rotavirus strains previously adapted to growth in CV-1 cells and representative of serotypes 1, 2, 3 and 4 and against one of the cat isolates (cat 2) were prepared in guinea-pigs. Serotypes 1, 2 and 4 were of human origin (strains FH4232, Hu7 and FH4453, respectively); antibodies to serotype 3 rotavirus were prepared using SA11 as antigen. SA11 has previously been defined as a serotype 3 rotavirus (Hoshino et al., 1984). The animals were given four intramuscular injections, at 2- to 3-week intervals, of a concentrated, partially purified preparation containing at least 500 intact rotavirus particles per electron microscope grid square.

Serotyping of rotavirus isolates was carried out using a combination of neutralization and immunofluorescence. Each virus was pre-titrated and used in the test at that dilution producing infection in 75 to 100% of cells (equivalent to 300 to 1000 TCID₅₀) after 16 h incubation at 37 °C. This dilution of virus (in a 50 μl volume) was added to an equal volume of each antiserum dilution and the mixture incubated at 37 °C for 1 h before being transferred to a 96-well microtitre plate (Disposable Products, Adelaide, Australia) seeded 7 days previously with 2 x 10⁴ CV-1 cells per well. An additional volume of 100 μl of maintenance medium was added per well and the plate incubated for 16 h at 37 °C. The cells were then fixed in ice-cold methanol for 15 min then stained for 1 h with a hyperimmune rabbit serum prepared against a human rotavirus strain purified from a faecal extract, followed by a fluorescein-conjugated antiserum to rabbit IgG (Kallestad, Austin, Tx., U.S.A.) for 1 h at 37 °C. Readings were obtained by inverting the plate and examining each well using the 10 x objective on a Zeiss incident light fluorescence microscope. Complete neutralization of the virus was considered to have occurred only in those wells where no specific fluorescence was visible and the endpoint of the titration was taken as the highest dilution of antiserum causing complete neutralization.

Rotaviruses of characteristic morphology, and with a mean diameter of 76 nm for virus with an outer shell and 67 nm for virus without an outer shell, were seen in six of 100 specimens examined (Fig. 1). All six cats in which rotavirus was identified were at least 1 year of age and were considered to be healthy, although two (cats 6 and 97) had faeces which were considered diarrhoeal. The number of rotaviruses shed in the faeces by each of the cats varied considerably, ranging from one to three particles per grid square in the concentrate of both diarrhoeal specimens to more than 800 per grid square in the normal specimen of cat 3.

Rotavirus was isolated from four of the specimens positive by electron microscopy (cats 2, 3, 22 and 97). The other specimens (from cats 6 to 19) contained non-viral agents, toxic to CV-1 cells within 2 to 3 h of inoculation, thereby preventing isolation attempts. The virus was relatively easily adapted to growth in cell culture, immunofluorescence specific for rotavirus being visible in up to 50% of cells at primary inoculation and after one passage a characteristic c.p.e. was readily observed, consisting of increasing granularity in most of the cells and a few (approx. 1%) attaching to the monolayer at a single point. The c.p.e. was a consistent observation from the first passage onwards. Supernatant fluids of cultures frozen and thawed before passage contained up to ten virus particles per grid square.

All isolates and faeces-derived virus in which RNA could be detected possessed a 'long' electrophoretic pattern characteristic of human rotavirus serotypes 1, 3 and 4 (subgroup II). However three distinct electropherotypes were represented among the four cat viruses. Electrophoresis of faeces-derived RNA from cats 2, 3 and 22 revealed identical genome profiles which, in the case of cats 2 and 3, remained constant during passage of the virus (results not shown). However, the original electropherotype of cat 22 virus was predominantly replaced by a rotavirus of a second distinctive electropherotype by the level of fifth passage (Fig. 2). At the third passage of cat 22 virus, there was a roughly equivalent amount of the two distinct
Fig. 1. Typical rotavirus particles from cat faeces. Bar marker represents 100 nm.

Fig. 2. RNA analysis of rotavirus isolates. Lanes (a) to (f): RNA extracted from cat 22 at the level of faecal extract (a) and first to fifth passages (lanes b to f, respectively). Lanes (g) to (i) contain RNA extracted from human isolates (FH4232 serotype 1, Hu7 serotype 2 and FH5972 serotype 3, respectively).

Electrophoretotypes. Re-isolation, re-passage and re-extraction of RNA at each level of passage showed this to be a repeatable phenomenon, indicating that the appearance of the new electropherotype at the third passage was not due to laboratory contamination. The RNA of the selected electropherotype differed from that of the original faecal extract in segments 1, 3, 4, 5, 6, 10 and 11, suggesting the presence of two distinct electropherotypes in the original extract (one of which was at a titre too low for detection, but which was selected out during passage) rather than a change in electropherotype of a single strain during passage. At third passage level, cat 97 possessed a genome profile not previously recognized in the other cats but which still conformed to a rotavirus 'long' electrophoretic pattern (there was insufficient virus in the faecal extract of this animal to allow detection of its RNA).

Table 1 shows that all four isolates of the cat rotavirus were neutralized only by antiserum to SA11, indicating that these isolates are serotype 3 rotaviruses. Antiserum raised against one of the isolates (cat 2) neutralized all four cat isolates and SA11 but did not neutralize human serotypes 1, 2 or 4. Virus present at each level of passage of the cat 22 strain was neutralized only by antiserum to SA11 and cat 2, suggesting that although two viruses with distinct electropherotypes were present in this animal, they were of the same serotype. The three most recent human isolates (FH5972, FH6136 and FH6139) were also serotype 3 (Table 1). These isolates were neutralized equally well by antiserum to SA11 and feline (cat 2) rotavirus.

Two previous reports of domestic cats having diarrhoea with concurrent rotavirus shedding (Chrystie et al., 1979; Snodgrass et al., 1979) and this report, where two of the six cats shedding the virus had diarrhoea, suggests that in this species infection may be either symptomatic or asymptomatic. Rotavirus present in a faecal extract obtained from a kitten with transient diarrhoea produced gastroenteritis in a colostrum-deprived and a colostrum-fed kitten (Snodgrass et al., 1979). However, oral inoculation of cats with a rotavirus isolated from a cat with chronic arthritis (Hoshino et al., 1981) did not produce clinical symptoms but resulted in a rise in antibody titre and shedding of virus in the faeces. There appears to be no relationship...
### Table 1. Results of serotyping experiments as determined by neutralization

<table>
<thead>
<tr>
<th>Virus†</th>
<th>Serotype 1 (FH4232)</th>
<th>Serotype 2 (Hu7)</th>
<th>Serotype 3 (SA11)</th>
<th>Serotype 4 (FH4453)</th>
<th>Cat 2</th>
<th>Serotype</th>
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<tr>
<td>FH4232</td>
<td>2000</td>
<td>&lt;250</td>
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<td>&lt;250</td>
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<tr>
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<td>&lt;250</td>
<td>2000</td>
<td>&lt;250</td>
<td>8000</td>
<td>3</td>
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<tr>
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<td>&lt;250</td>
<td>&lt;250</td>
<td>2000</td>
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<td>&lt;250</td>
<td>4000</td>
<td>&lt;250</td>
<td>4000</td>
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</tr>
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<td>&lt;250</td>
<td>&lt;250</td>
<td>4000</td>
<td>&lt;250</td>
<td>8000</td>
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<td>&lt;250</td>
<td>4000</td>
<td>3</td>
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<tr>
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<td>&lt;250</td>
<td>&lt;250</td>
<td>2000</td>
<td>&lt;250</td>
<td>4000</td>
<td>3</td>
</tr>
<tr>
<td>Cat 97</td>
<td>&lt;250</td>
<td>&lt;250</td>
<td>2000</td>
<td>&lt;250</td>
<td>2000</td>
<td>3</td>
</tr>
</tbody>
</table>

* Reciprocal of highest dilution of antiserum producing complete neutralization.
† Viruses other than SA11 and the cat isolates were of human origin.
‡ Results obtained at fifth level of passage.

between the amount of virus shed in the faeces and the presence or absence of symptoms, although this may depend on the stage of infection at which the specimen is obtained. Cats 6 and 97, with counts of only one to three rotavirus particles per grid square had diarrhoea, while cats 2, 3 and 22 with counts of 300 to 800 particles per grid square were asymptomatic. These differences are too great to be accounted for by possible counting error.

A previous study (Hoshino et al., 1982) has demonstrated a one-way cross-reaction between a feline rotavirus isolate (Taka) and a canine rotavirus (CU-1), and a two-way relationship between CU-1 and SA11 using a plaque-reduction neutralization assay. The feline isolate has been subsequently classified as serotype 3 (Hoshino et al., 1984). However, neither of these surveys directly examined the relationship between the feline isolate and SA11. Our results have confirmed the classification of the feline isolate as serotype 3 and have demonstrated a two-way relationship between SA11 and the feline isolates, suggesting they are either very similar or identical.

The feline rotaviruses isolated in this study possessed the RNA genome profile characteristic of all rotaviruses (in particular that of human serotypes 1, 3 and 4, all of which have a 'long' profile). These profiles also showed inter-species variation in a manner similar to that of the human rotaviruses. However the comparison of feline and human electropherotypes did not provide any measure of the degree of relatedness or otherwise of feline to human strains.

The most recent human infections from which isolation has been successful (March to May 1985) have been due to serotype 3. Hence it cannot be ruled out that cats might act as a source of rotavirus infection in man. Without human volunteer studies, proof that cat rotaviruses can cross the species barrier can only be obtained indirectly. However, some evidence that cats could act as a source of infection in humans might come from closer epidemiological investigations of rotavirus excretion in cats in conjunction with seroepidemiological surveillance of human rotavirus within the community. Production of monoclonal antibodies to human serotype 3 strains, SA11 and feline rotaviruses should also provide further evidence as to the extent of the relatedness of these rotavirus strains.

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