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Virus Research 74 (2001) 17–25

Virus Research

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Nucleotide sequence variation of the VP7 gene of two G3-type rotaviruses isolated from dogs

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Received 21 April 2000; received in revised form 22 September 2000; accepted 2 October 2000

Abstract

The sequence of the VP7 gene of two rotaviruses isolated from dogs in southern Italy was determined and the inferred amino acid sequence was compared with that of other rotavirus strains. There was very high nucleotide and amino acid identity between canine strain RV198/95 and other canine strains, and to the human strain HCR3A. Strain RV52/96, however, was found to have about 95% identity to the G3 serotype canine strains K9, A79-10 and CU-1 and 96% identity to strain RV198/95 and to the simian strain RRV. Therefore both of the canine strains belong to the G3 serotype. Nevertheless, detailed analysis of the VP7 variable regions revealed that RV52/96 possesses amino acid substitutions uncommon to the other canine isolates. In addition, strain RV52/96 exhibited a nucleotide divergence greater than 16% from all the other canine strains studied; however, it revealed the closest identity (90.4%) to the simian strain RRV. With only a few exceptions, phylogenetic analysis allowed clear differentiation of the G3 rotaviruses on the basis of the species of origin. The nucleotide and amino acid variations observed in strain RV52/96 could account for the existence of a canine rotavirus G3 sub-type.

Keywords: Canine rotavirus; VP7; G3 serotype

1. Introduction

Group A rotaviruses are a major cause of neonatal diarrhea in humans and animals. Rotaviruses are non-enveloped and possess a triple-layered capsid, enclosing 11 fragments of double-stranded RNA (dsRNA). The outer viral capsid proteins, VP7 and VP4, elicit neutralizing antibody responses and form the basis of the current dual classification system by G and P types. The VP7 expresses the major neutralizing antigen and is distinguishable by means of both serological and genomic techniques in 14 G types, with good correlation between the serological and genomic classifications. Also, since the VP4 ex-

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0168-1702/01/$ - see front matter
PII: S0168-1702(00)00230-6

presses the minor neutralizing antigen, the serological classification of the P types is much more difficult than the genomic classification. To date, 13 P serotypes (including subtypes) and 20 P genotypes have been defined, but no precise correlation has been made between the serological and the genomic classifications. Thus a different designation has been adopted for the P serotype (open numbers) and the P genotype (numbers in square brackets) (Estes and Cohen, 1989; Estes, 1996). As regards canine rotavirus, all the strains isolated display the G3 and P5A[3] specificities (Hoshino et al., 1984; Nakagomi et al., 1989; Gouvea et al., 1994a,b; Taniguchi et al., 1994).

Canine rotavirus is usually responsible for subclinical or mild forms of enteritis, associated with anorexia and vomiting, especially in pups younger than 2 weeks of age (Pollock and Carmichael, 1990). The canine infection is considered a minor disease problem in pups (Pollock and Carmichael, 1990); however, serological investigations have shown a high prevalence of antibodies to canine rotavirus in adult dogs (Mochizuki et al., 1986).

There are only a few reports on the isolation of canine rotavirus from dogs affected with gastroenteritis. To date, three strains have been isolated in the US, CU-1, A79-10 and LSU79C-36 (also referred to as K9); one isolate (RS15) has been reported from Japan (Fulton et al., 1981; Hoshino et al., 1982, 1983; Mochizuki and Hsüan, 1984). In addition, two strains have been isolated from humans in the USA, HCR3A (Li et al., 1993) and HCR3B (Santos et al., 1998), and one isolate has been reported in Israel, Ro1845 (Aboudy et al., 1988), all which have been shown to be genetically related to canine rotavirus and to share the same G3P5A[3] specificity (Gouvea et al., 1990; Nakagomi et al., 1990; Li et al., 1993; Nakagomi et al., 1993; Vonsover et al., 1993; Li et al., 1994; Taniguchi et al., 1994; Santos et al., 1998).

In the present note, the VP7 nucleotide and the corresponding inferred amino acid sequences of two canine rotaviruses isolated in Italy are reported and analyzed. A phylogenetic tree based on G3 human and animal rotaviruses was also elaborated.

2. Materials and methods

2.1. Viruses

Two crossbred pups of 2.5 and 6 months of age (# 52/96 and # 198/95, respectively) with acute gastroenteritis were presented, on separate occasions, to the Small Animal Clinic of the Veterinary Faculty, University of Bari, for clinical examination and treatment. Both pups recovered 1 week after the onset of clinical signs. Electron microscopy (EM) examination of fecal specimens from both pups revealed the presence of viral particles with rotaviral morphology. In addition, parvovirus- and coronavirus-like particles were observed in the feces of pup # 198/95.

Two rotavirus isolates (RV52/96 and RV198/95) were made on MA-104 (foetal monkey kidney) cells in the presence of trypsin (5 μg/ml in maintenance medium). Viral growth was monitored by an indirect immunofluorescence assay. Viral dsRNA was recovered from the infected cells by standard phenol-chloroform extraction procedures and subjected to electrophoresis. Both the isolates had virtually identical mobilities of the genomic segments with an electrophoretical pattern 4:2:3:2, typical of group A rotaviruses (data not shown).

2.2. Polymerase chain reaction (PCR) amplification

For PCR amplification, rotaviral dsRNA was extracted from infected MA-104 cells, using the RNeasy Kit (Qiagen Gmbh, Hilden, Germany). Reverse transcription (RT) PCR was carried out as previously described (Gouvea et al., 1990; Isegawa et al., 1993). The pair of generic primers Beg9 and End9, which amplifies the entire VP7 gene, was used both for reverse transcription of genomic RNA and for PCR.

2.3. Sequencing and sequence analysis

The RT-PCR amplicons were purified on Ultrafree-DA Columns (Amicon, Millipore) and then sequenced directly with ABI-PRISM 377 (Perkin Elmer, Applied Biosystem Instruments) using an

overlapping strategy; i.e. starting with the generic primers Beg9 and End9 and choosing additional primers on the basis of the sequence obtained, in order to completely sequence the VP7 gene in both directions. The sequence was determined twice for each virus. The primers used for sequencing are listed in Table 1.

Sequence analysis was performed with NCBI's and EMBL's analysis tools. The alignment of sequences was performed with CLUSTAL W (Thompson et al., 1994). Phylogenetic analysis was carried out with TREECOON package (Van De Peer and De Wachter, 1993). A neighbor-joining tree (Saitou and Nei, 1987) was generated using the gamma distribution model (Ota and Nei, 1993), with bootstrapping over 1000 replicates. For comparative analysis, selected VP7 sequences of G3 human and animal rotaviruses were used (Table 2). The nucleotide sequences of strains CU-1, A79-10, CAT2 and CAT97 were kindly supplied by Dr Y. Hoshino. The sequences of strains RV198/95 and RV52/96 are available in GenBank under accession numbers AF271089 and AF271090, respectively.

Table 1
Primers used for sequencing strains RV198/95 and RV52/96[a]

Primer	Sequence 5′–3′	Sense
Beg9[b] (1–28)	GGC TTT AAA AGA GAG AAT TTC CGT CTG G	+
End9[b] (1062–1036)	GGT CAC ATC ATA CAA TTC TAA TCT AAG	−
198R1 (245–226)	GCA GTA TCC ATT GAA CCA GT	−
198R2 (600–583)	CCA CTT ATT AGC TTC ATC	−
198F1 (361–377)	CTA TTT CTA ACT AAA GG	+
198F2 (723–742)	GAT CAC TGA TGT CGT TGA TG	+
198F3 (900–918)	GAT GCG TAT TAA TTG GAA G	+
52R1 (278–259)	GGA AAG TCT CTT CCT GTG TG	−
52R2 (672–653)	TAG ACA CCC AAT TCC AAG AG	−
52F1 (357–374)	CAC AGT TGT TTT TGA CC	+
52F2 (620–637)	GTA CAA TAA AAG TGT GTC	+

[a] The position and the sense of the primers is also reported.
[b] Gouvea et al. (1990).

3. Results

Similar to most of the group A rotaviruses (Estes and Cohen, 1989; Bellamy and Both, 1990), the VP7 nucleotide sequence of strains RV198/95 and RV52/96 is 1062 nucleotides long. The ORF starts at nucleotide 49 with an AUG (ATG) start codon and ends at nucleotide 1029 with a UAG (TAG) termination codon, comprising 981 nucleotides. The inferred VP7 amino acid sequence of strains RV198/95 and RV52/96, aligned with the sequences of other G3 animal and human rotaviruses, is reported in Fig. 1. As expected, the sequence of both the strains is 326 amino acids long and conserves the potential glycosylation site NST at residues 69–71 (Bellamy and Both, 1990).

On the basis of sequence analysis, both strains RV198/95 and RV52/96 clearly belong to the G3 serotype. Rotaviruses belonging to the same G serotype generally share more than 91% VP7 amino acid similarity (Kapikian and Chanock, 1996; Palombo et al., 1997), even if strains displaying an overall identity of only 88.7% are included into the G3 serotype (Li et al., 1994). As shown in Table 3, strain RV198/95 had the highest nucleotide identity (about 96–97%) to the canine strains K9, CU-1, A79-10 and to the human strain HCR3A. Sequence similarity of strain RV52/96 was highest (90%) to the rhesus rotavirus RRV, followed by the equine strain ERV316 (85.8%), rather than to the canine strains (83–84%). In regard to the amino acid composition, strain RV198/95 revealed the highest identity to the G3 human strain HCR3A (100%), followed by the canine strains K9, CU-1, A79-10 (97.5–99%) and the feline strain CAT97 (99.4%). As regards strain RV52/96, a high amino acid similarity was found to the simian strains RRV (96.1%) and SA11 (95.1%), whereas identity to the canine strains was about 94–95%. The two Italian isolates showed about 84.0% nucleotide and 96.2% amino acid similarity to each other.

In Fig. 2 the neighbor-joining tree based on the VP7 amino acid sequence of human and animal

Table 2
List of the G3 animal and human rotaviruses used in this study[a]

Strain	Origin	Accession	Reference
A79-10	Dog	n.r.	Nishikawa et al., 1989
CU-1	Dog	n.r.	Nishikawa et al., 1989
K9 or LSU79C-36	Dog	U97199	Nishikawa et al., 1989Kobayashi, unpublished
RV198/95	Dog	AF271089	This paper
RV52/96	Dog	AF271090	This paper
CAT97	Cat	n.r.	Nishikawa et al., 1989
CAT2	Cat	n.r.	Nishikawa et al., 1989
EL	Mouse	U08427	Dunn et al., 1994
EC	Mouse	U08422	Dunn et al., 1994
EDIM	Mouse	U08430	Choi et al., 1998
EB	Mouse	U08420	Nishikawa et al., 1989; Dunn et al., 1994
YR-1	Mouse	D45216	Ushijima et al., 1995
R-2	Rabbit	n.r.	Nishikawa et al., 1989
ALA	Rabbit	n.r.	Nishikawa et al., 1989
Bap2	Rabbit	U62153	Ciarlet et al., 1997a
C11	Rabbit	n.r.	Nishikawa et al., 1989
FI-14	Horse	n.r.	Nishikawa et al., 1989
H-2	Horse	n.r.	Nishikawa et al., 1989
ERV-316	Horse	L49043	Browning and Begg, 1996
SA11	Simian	V01190	Both et al., 1983
RRV	Simian	M21650	Mackow et al., 1988
AT-76	Pig	VGXRT6	Huang et al., 1989
Ben-307	Pig	AAB24409	Nagesha et al., 1992
CRW-8	Pig	VGXRW8	Huang et al., 1989
LCA843	Pig	L35057	Ciarlet et al., 1995
A131	Pig	L35055	Ciarlet et al., 1995
A138	Pig	L35079	Ciarlet et al., 1995
HCR3A	Man	L21666	Li et al., 1994
YO	Man	D86284	Wen et al., 1997; Nishikawa et al., 1989
MO	Man	D86280	Wen et al., 1997; Nishikawa et al., 1989
TK28	Man	D86283	Wen et al., 1997
TK15	Man	D86282	Wen et al., 1997
TK08	Man	D86281	Wen et al., 1997
J-12	Man	D86279	Wen et al., 1997
ST8	Man	n.r.	Nishikawa et al., 1989
W178	Man	n.r.	Nishikawa et al., 1989
M	Man	n.r.	Nishikawa et al., 1989
McN	Man	n.r.	Nishikawa et al., 1989
Nemoto	Man	n.r.	Nishikawa et al., 1989
Ito	Man	D86278	Nishikawa et al., 1989; Wen et al., 1997
AK-35	Man	n.r.	Nishikawa et al., 1989
Au-17	Man	D86272	Wen et al., 1997
P	Man	n.r.	Nishikawa et al., 1989
95-91	Man	D86266	Wen et al., 1997
107e1b	Man	U04350	Gentsch and Das, unpublished
No.14	Man	n.r.	Nishikawa et al., 1989
No.15	Man	n.r.	Nishikawa et al., 1989
Ai-53	Man	D86269	Wen et al., 1997
Ai-75	Man	D86270	Wen et al., 1997
Ai-36	Man	D86267	Wen et al., 1997
Ai-39	Man	D86268	Wen et al., 1997
02-92	Man	D86264	Wen et al., 1997
Au-1	Man	D86271	Nishikawa et al., 1989; Wen et al., 1997

[a] Multiple references are reported for viruses sequenced more times. n.r. not registered in the databases.

Table 3
VP7 nucleotide and amino acid identity among the G3 animal and human rotaviruses[a]

Strain	198/95	52/96	HCR3	CU-1	A79-10	CAT2	CAT97	SA11	RRV	Erv316	A-138	YR-1	BAP-2	TK28	Y0	Au-1
K9 Dog	96.9/97.5	82.9/94.2	95.6/98.5	96.4/98.8	96.6/98.8	79.8/90.5	96.0/99.1	82.5/93.3	84.3/93.9	87.8/93.6	80.1/89.6	74.9/87.2	84.4/92.1	80.0/90.2	78.9/89.0	78.7/90.2
RV198/95 Dog	■	84.0/96.2	96.1/99.8	96.2/99.1	96.6/99.1	80.4/91.5	96.8/99.4	83.3/94.2	84.7/94.8	89.1/94.5	80.5/90.2	76.0/87.5	86.6/93.0	81.4/91.2	80.3/90.8	80.1/91.2
RV52/96 Dog		■	83.8/95.1	84.1/95.1	83.9/95.1	82.8/92.1	83.9/94.5	83.6/95.1	90.4/96.1	85.8/94.5	80.0/90.8	76.6/89.4	82.7/92.7	82.1/91.5	82.9/90.5	82.4/90.8
HCR3 Man			■	97.0/99.1	97.2/99.1	80.9/91.5	95.1/99.4	83.9/94.2	85.0/94.8	88.5/94.5	80.9/90.2	76.3/87.5	85.0/92.3	81.1/91.2	80.5/90.8	80.0/91.1
CU-1 Dog				■	98.7/99.4	80.8/91.5	95.8/99.1	83.6/93.9	85.7/94.5	89.1/94.2	81.3/89.9	76.2/87.2	85.9/92.7	81.3/90.8	80.7/90.5	80.3/90.8
A79-10 Dog					■	80.8/91.8	96.2/99.1	83.9/94.5	85.3/94.5	89.1/94.2	80.7/90.5	76.2/87.8	85.6/92.7	81.3/91.5	80.5/91.2	80.3/91.5
CAT2 Cat						■	80.6/90.8	81.1/91.8	82.4/91.8	82.3/90.5	87.1/95.8	75.3/84.1	78.9/89.7	91.2/95.4	91.6/96.1	91.1/96.7
CAT97 Cat							■	83.2/93.6	85.0/94.2	89.9/93.9	80.9/89.9	76.4/86.9	86.3/82.4	81.6/90.5	81.1/90.2	80.7/90.5
SA11 Monkey								■	84.7/95.4	84.3/93.9	80.3/91.8	78.1/88.1	81.1/92.4	81.9/91.4	81.9/92.2	81.6/92.4
RRV Monkey									■	85.8/95.1	79.6/90.8	77.2/87.8	82.1/93.6	82.4/91.8	82.6/90.8	82.1/91.8
Erv316 Horse										■	80.9/90.2	77.1/87.8	85.2/93.6	82.5/91.5	82.6/90.2	82.3/90.5
A138 Pig											■	76.3/83.2	78.9/89.0	86.4/94.2	87.1/94.8	87.3/95.4
YR-1 Mouse												■	75.4/86.9	74.9/84.7	75.2/83.5	74.8/83.8
BAP-2 Rabbit													■	80.1/89.9	80.0/88.4	79.8/88.7
TK-28 Man														■	96.5/96.3	96.4/97.0
Y0 Man															■	97.8/98.2

[a] The strains are referenced in Table 2. The nucleotide percent values are indicated on the left, the amino acid percent value on the right.

G3 rotaviruses is presented. Human G3 rotaviruses differ from the animal G3 isolates, with exception of the human strain HCR3A, which is clustered together with the canine strains, and the feline strain CAT2, which is grouped with the human isolates. Strain RV52/96 seems to be genetically more similar to the simian strain RRV.

4. Discussion

G3 serotype rotaviruses have been found in a broad range of host species, including humans, monkeys, dogs, cats, horses, rabbits, mice, sheep and pigs (Hoshino et al., 1984; Paul et al., 1988; Fitzgerald et al., 1995).

Studies of escape mutants made with neutralizing monoclonal antibodies and sequence analysis of viruses with different serotypes have identified six variable regions in the rotaviral VP7 protein. However, only region A (residues 87–101), B (residues 141–150), C (residues 208–224) and F (residues 235–242) have been confirmed to have a major role in neutralization (Dyall-Smith et al., 1986; Green et al., 1987; Coulson and Kirkwood, 1991; Kirkwood et al., 1993; Ciarlet et al., 1997b). Comparative analysis of the VP7 protein of G3 human and animal rotaviruses showed, at the amino acid level, that sequence divergence among G3 strains from different host species occurs primarily in regions A, B and C. In contrast, those regions exhibit a high degree of conservation among G3 strains belonging to the same host species (Nishikawa et al., 1989).

The high degree of conservation of region A is believed to be very important for G3 serotype strains to maintain serotype specificity (Nishikawa et al., 1989; Ciarlet et al., 1997b). Strain RV52/96 has a substitution at amino acid 87 (asn for thr), which is considered a key contact residue of the immunodominant epitopes defining serotype G3 rotavirus strains (Ciarlet et al., 1997b). In region B, the sequence LMKYDAALQL is considered spe-

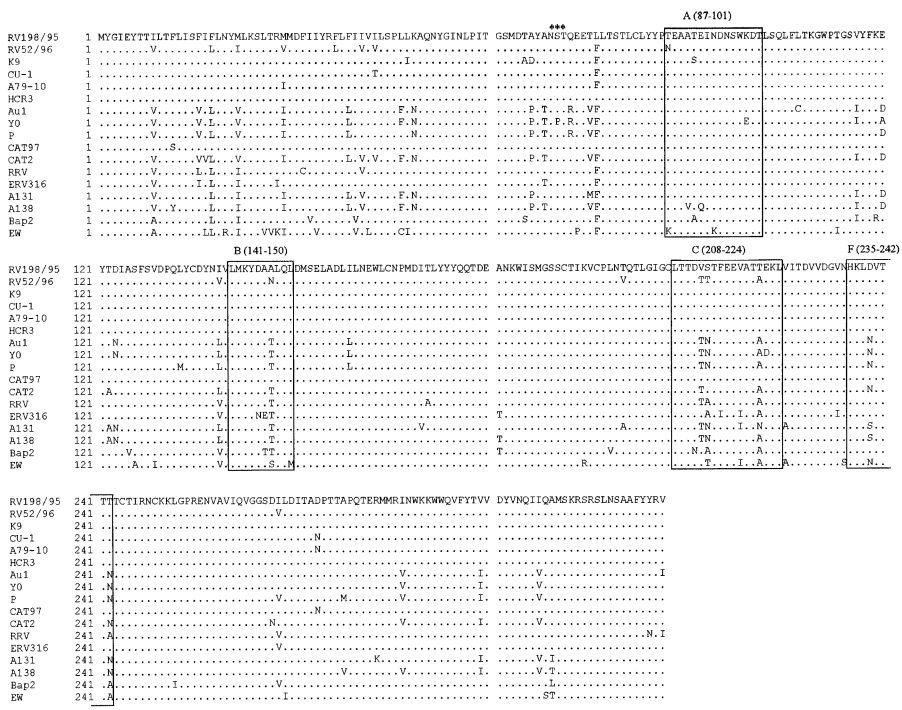

Fig. 1. Amino acid sequence of the VP7 protein of strains RV198/95 and RV52/96 aligned with that of other G3 animal and human rotaviruses. The strains are referenced in Table 2. The variable regions A (aa 87–101), B (aa 141–150), C (aa 208–224) and F (aa 235–242) are shown. The NST glycosylation site is indicated by asterisks.

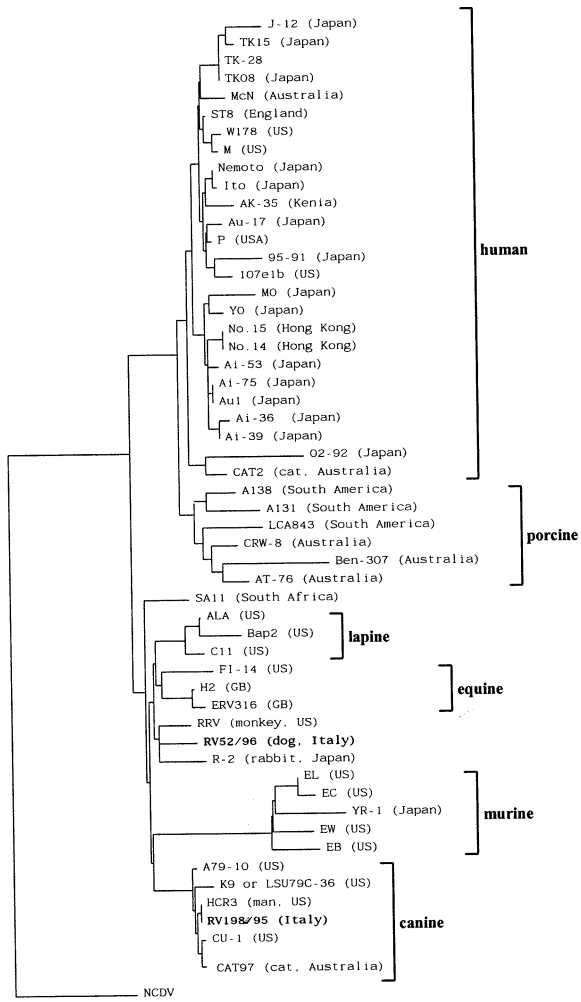

Fig. 2. Neighbour-joining tree based on the VP7 amino acid sequences of G3 human and animal rotaviruses. Horizontal branches are drawn to scale and the tree is rooted with the G6 serotype bovine strain NCDV (accession M63266). Bootstrap values are not shown. The strains are referenced in Table 2. The geographical origin of the strains is reported in brackets. The host species of isolation of the viruses is reported in brackets only when necessary.

cies-specific for canine and feline strains (Nishikawa et al., 1989) and residue 147 is considered to be critical for both serotype specificity (Dyall-Smith et al., 1986) and monotype specificity (Coulson and Kirkwood, 1991). In this region, strain RV52/96 shows a substitution at the critical residue 147 (asn for ala). In region C, which has been suggested to be an immunodominant antigenic site (Kirkwood et al., 1993; Ciarlet et al., 1997b), RV52/96 possesses three changes with respect to the other canine strains.

In summary, strain RV52/96 possesses amino acid variations in regions A, B and C and about 16–17% nucleotide divergence from to the other described canine isolates K9, CU-1, A79-10 and RV198/95. The differences observed demonstrate that variability exist among the canine rotaviruses. Similarly, nucleotide and amino acidic variation in the VP7 of human G3 rotaviruses has been recently described and serotype 3 viruses are currently considered to be intraserotypically more heterologous than serotype 1, 2 and 4 viruses (Wen et al., 1997; Suzuki et al., 1998). Furthermore, by cross-neutralization tests, monoclonal antibody analysis and RNA hybridization, two G3 subtypes of equine rotavirus have been established (Browning et al., 1992). Therefore, we hypothesize that the differences observed in strain RV52/96 could account for the existence of a G3-subtype of canine rotavirus. However, the analysis of additional canine isolates and serological evaluations are required to confirm this hypothesis.

The neighbor-joining tree based on the VP7 gene shows the presence of different clusters among the G3 serotype rotaviruses. With few exceptions, clustering seems to follow a species-specific pattern. The animal isolates are phylogenetically distinct from the human isolates. The porcine, murine, lapine, equine and canine strains, moreover, are clearly distinguishable from each other. In agreement with previous observations (Ciarlet et al., 1995), the porcine isolates seem to be more related to human than to animal rotaviruses. The human strain HCR3 shows a high homology with the canine strains, whereas the feline strain CAT2 is grouped into the human cluster. This is not surprising since strain HCR3A has been shown to be genetically highly related to the canine strains (Gouvea et al., 1990; Li et al., 1993, 1994; Taniguchi et al., 1994; Santos et al., 1998). On the other hand, strain CAT2 shares the VP4 specificity, P3A[9], with human strains such as Au1 and K8 (Gouvea et al., 1994a; Taniguchi et al., 1994). As regard strain RV52/96, the homology with strain RRV is consistent with previ-

ous findings (Taniguchi et al., 1994) describing the close genetic relationship between the VP4 of canine rotaviruses and RRV. These results point out the usefulness of phylogenetic analysis for obtaining a more comprehensive understanding of the complex ecology of rotaviruses.

In conclusion, this is the first report describing the isolation of canine rotaviruses outside the USA and Japan, and confirms that the G3 is the unique serotype within the canine rotaviruses. Finally, the data provided by sequence comparison and phylogenetic analysis revealed nucleotide and amino acid variability which has not been reported previously among the canine rotavirus strains.

Acknowledgements

We thank Mr Donato Narcisi for his technical collaboration and Dr Yasutaka Hoshino for supplying the VP7 nucleotide sequences of the canine and feline strains. We thank Professor Leland E. Carmichael for revising the English and for his suggestions.

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