JOURNAL OF CLINICAL MICROBIOLOGY, June 1992, p. 1432–1435 Vol. 30, No. 6
0095-1137/92/061432-04$02.00/0
Copyright © 1992, American Society for Microbiology

A Serotype 10 Human Rotavirus

G. BEARDS,[1] L. XU,[2] A. BALLARD,[1] U. DESSELBERGER,[1] AND M. A. McCRAE[2]*

Regional Virus Laboratory, East Birmingham Hospital, Birmingham BS9 5ST,[1] and Department of Biological Sciences, University of Warwick, Coventry CV4 7AL,[2] United Kingdom

Received 25 November 1991/Accepted 20 March 1992

Rotaviruses with genome rearrangements isolated from a chronically infected immunodeficient child (F. Hundley, M. McIntyre, B. Clark, G. Beards, D. Wood, I. Chrystie, and U. Desselberger, J. Virol 61:3365–3372, 1987) are the first recognized human isolates of serotype 10. This was shown by both a direct enzyme-linked immunosorbent assay and virus neutralization assays using serotype specific monoclonal antibodies. The serotype was confirmed by sequence analysis of the gene encoding VP7, which revealed a 96% amino acid homology to the bovine serotype 10 isolate B223.

Rotaviruses are the major cause of acute viral gastroenteritis in human infants and in the young of a wide variety of mammalian and avian species (13). Viruses in the genus *Rotavirus* have been classified into groups (A to E) (16), subgroups (I, II, I+II, and non-I non-II) (8, 9, 22), and serotypes (1 to 14) (4, 6, 24). The two proteins in the virion outer shell, VP4 and VP7, have been shown to elicit a neutralizing antibody response. Although the possibility of classifying virus serotypes on the basis of epitopes present on VP4 (P specific) is being actively investigated (7, 10, 15, 18, 23, 24), currently virus serotypes are defined on the basis of epitopes present on glycoprotein VP7 (G specific) (6). Virus isolates from a number of serotypes, e.g., 3, 4, and 5, have been obtained from both humans and animals, whereas others such as 1 and 10 have to date been found only in humans and cattle, respectively (3, 11, 21).

We have previously described a number of rotavirus isolates, obtained from chronically infected children, containing a variety of concatemeric rearrangements in their genome segments (12, 17). Serological analyses carried out at the time of their original isolation showed these isolates to be of subgroup II and to be unreactive with a panel of monoclonal antibodies covering serotypes 1 through 4 (12). During the course of defining the precise molecular nature of the genomic rearrangements of these viruses (1), their serological classification has been reanalyzed. This report presents the results of these studies, which have revealed that these viruses belong to a virus serotype previously found only in cattle.

MATERIALS AND METHODS

Viruses and antibodies. Human rotavirus isolates A28 and A64 (1), which carry rearrangements in RNA segments 8, 10, and 11, were propagated in MA104 cells as previously described (12). The following virus isolates were used as controls in enzyme-linked immunosorbent assays (ELISAs) and virus neutralization assays: human isolate Wa (serotype 1), human isolate DS-1 (serotype 2), simian isolate SA11 (serotype 3), human isolate Hochi (serotype 4), porcine isolate OSU (serotype 5), bovine isolate UKtc (serotype 6), human isolate 69M (serotype 8), and bovine isolate B223 (serotype 10).

The monoclonal antibodies used in this study and their specificities were as follows: A3M4 (VP6), group A specific; 631/9/104/56 (VP6), subgroup I specific; 255/60/125 (VP6), subgroup II specific; 60-F2D4 (VP7), cross-reactive; RV4:2 (VP7), serotype 1 specific; RV5:3 (VP7), serotype 2 specific; RV3:1 (VP7), serotype 3 specific; ST3:1 (VP7), serotype 4 specific; 5OSU (VP7), serotype 5 specific; 5632 UK7 (VP7), serotype 6 specific; B37:1 (VP7), serotype 8 specific; and 5725 B223 (VP7), serotype 10 specific (5, 19, 21, 25).

Serotyping by ELISA. ELISA serotyping was carried out by using serotype-specific monoclonal antibodies basically as previously described (2). Polystyrene microtiter plates were coated overnight at 4°C with 100 μl of a 1/10,000 dilution of hyperimmune serum obtained from a rabbit immunized with a mixture of virus isolates covering serotypes 1 to 4. Virus samples for testing were diluted 1/4 in TBS-T-BSA (0.1 M Tris-buffered saline [TBS], pH 7.5, containing 0.1% [vol/vol] Tween 20 and 3% [wt/vol] bovine serum albumin [BSA]) and added to each of 16 wells in pairs across each plate. The plates were again incubated overnight at 4°C, the virus samples were removed, and the wells were washed six times with TBS-T. Ascitic fluids containing monoclonal antibodies to serotypes 1 to 6, 8, and 10 (5, 19, 21, 25) were diluted 1/10,000 in TBS-T-BSA, and 100 μl of diluted antibody was added to two wells for each antibody. The plates were incubated at 37°C for 2 h and then washed six times with TBS-T. Goat anti-mouse alkaline phosphatase conjugate (Sigma) was diluted 1/1,000 in TBS-BSA, and 100 μl was added to each well. The plates were incubated at 37°C for 90 min and then washed six times with TBS-T. Alkaline phosphatase NADP substrate (100 μl; IQ Bio Ltd., Cambridge, United Kingdom) was added to each well, and the plates were incubated at room temperature for 15 min. Two hundred microliters Ethanol-INT violet amplifier (IQ Bio Ltd.) was then added, and incubation was continued at room temperature for another 15 min before the reaction was stopped by the addition of 100 μl of 3 M sulfuric acid. Optical densities at 492 nm were read with a Flow plate reader. A sample was considered to yield a positive result with a particular monoclonal antibody if the optical density was at least 2.5 times the average density obtained with the other antibodies when that average was less than 0.1.

Serotyping by virus neutralization. This serotyping procedure was done by a modification of the method described by Beards et al. (3). Briefly, 50 μl of serial fourfold dilutions (1/100 to 1/102,400 in phosphate-buffered saline [PBS]) of serotype-specific neutralizing monoclonal antibodies covering serotypes 1 to 6, 8, and 10 (5, 19, 21, 25) were mixed with

* Corresponding author.

TABLE 1. Reactivities of serotype-specific monoclonal antibodies with human rotavirus isolates A28 and A64 in an ELISA

Virus	Reactivity of monoclonal antibody[a] specific for serotype:							
	1	2	3	4	5	6	8	10
A28	0.04	0.12	0.12	0.07	0.03	0.32	0.19	0.40
A64	0.01	0.06	0.11	0.15	0.05	0.23	0.25	0.52

[a] The particular serotype-specific monoclonal antibodies used are listed in Materials and Methods.

an equal volume of virus suspension (in Eagle's minimal essential medium) containing 200 fluorescent focus-forming units of virus. After 1 h of incubation at 37°C, the virus-antibody mixtures were added to confluent monolayers of MA104 cells in tissue culture microtiter plates. After adsorption for 1 h at 37°C, the virus inocula were removed and replaced with Eagle's minimal essential medium containing 10% fetal calf serum. Incubation was continued at 37°C for a further 18 h. The monolayers were then washed three times with PBS and fixed with cold 75% methanol containing 0.75% hydrogen peroxide. The fixative was removed after 20 min, and the monolayers were allowed to air dry. One hundred microliters of a group A-specific peroxidase-labelled monoclonal antibody conjugate, diluted 1/1,000 in PBS containing 3% BSA, was added to each well, and the plates were incubated overnight at 4°C. The plates were then washed six times with PBS containing 0.01% Tween 20, and 100 μl of peroxidase substrate (0.1 mg of 3,3′,5,5′-tetramethylbenzidine in 0.1 M citrate-phosphate buffer, pH 6.0, containing 1.3 mM hydrogen peroxide) was added to each well. After 15 min of incubation at room temperature, the reaction was stopped by the addition of 50 μl of 3 M sulfuric acid, and the optical density at 450 nm was read with a Flow plate reader.

The neutralization titer was defined as the highest monoclonal antibody dilution giving a 60% or greater reduction in the optical density in comparison to that of the nonneutralized controls.

cDNA cloning and sequence analysis of the VP7-encoding gene. Cloning and sequence analysis were carried out by the strategy described by Xu et al. (27). In brief, a confluent monolayer of MA104 cells in one well of a six-well dish (approximately 3×10^6 cells) was infected with the A64 virus isolate at a multiplicity of infection of 1 to 5, and virus growth was allowed to continue at 37°C for 16 h. Cells were then harvested by scraping them into the medium and concentrated by centrifugation. The cell pellet was resuspended in lysis buffer (100 mM Tris-HCl buffer [pH 8.0], 50 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40) and incubated at 4°C for 10 min. Nuclei and unlysed cells were then removed by centrifugation (Beckman GPR centrifuge; 2,000 rpm, 10 min, 4°C). The cytoplasmic extract present in the supernatant was then incubated at 37°C with 200 μg of proteinase K per ml for 3 h before being extracted twice with phenol saturated with 50 mM Tris-HCl buffer (pH 8.0). After the residual phenol was removed by ether extraction (four times), the extracted nucleic acids were concentrated by ethanol precipitation. Viral mRNA and genomic double-stranded RNA for the gene encoding VP7 were then reverse transcribed and amplified by polymerase chain reaction with gene 8-specific terminal primers as previously described (27). The amplified cDNA was then cloned after G-C tailing as described by McCrae and McCorquodale (14), except that Bluescribe (Stratagene) was employed as the cloning vector. Screening for clones containing viral inserts was done on the basis of the blue or white color of colonies on L agar plates containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and IPTG (isopropyl-β-D-thiogalactopyranoside). Sequencing of a cDNA clone shown to contain a full-length insert of gene 8 was carried out by the dideoxy sequencing technique (20) with a series of gene 8-specific sequencing primers as previously described (28). Both strands of the clone were completely sequenced and all primer positions were sequenced through.

Nucleotide sequence accession number. The nucleotide sequence in Fig. 1 has been deposited in the EMBL data base under accession number X63156.

RESULTS AND DISCUSSION

The initial serotyping of the A28 and A64 virus isolates was carried out by an ELISA using monoclonal antibodies specific for serotypes 1 to 6, 8, and 10. The results (Table 1) revealed that the highest reactivity was obtained with the serotype 10-specific antibody, although in both cases there was some reactivity with the antibodies specific for serotypes 6 and 8. To further investigate this, serotyping assays were performed on the A28 isolate by a completely different assay procedure, namely, neutralization of virus infectivity. The results of these assays (Table 2) confirmed those of the ELISA in that the highest neutralization titer was obtained with the serotype 10-specific monoclonal antibody. A low level of neutralization was seen with the serotype 6-specific monoclonal antibody (Table 2); however, because it showed a titer 16-fold lower than that with the homologous virus, we tentatively concluded that A28 and A64 were serotype 10 viruses.

TABLE 2. Results of cross-neutralization studies of human rotavirus strain A28

Virus isolate	Serotype	Virus neutralization titers with monoclonal antibody[a] specific for serotype:							
		1	2	3	4	5	6	8	10
A28		200	<200	200	<200	200	800	<200	51,200
Wa	1	51,200							
DS-1	2		51,200						
SA11	3			51,200					
Hochi	4				51,200				
OSU	5					>102,400			
UKtc	6						12,800		<200
69M	8							12,800	
B223	10						<200		51,200

[a] The particular serotype-specific monoclonal antibodies used are listed in Materials and Methods.

```
                                                   M  Y  G  I
   1 GGCTTTAAAAGCGAGAATTTCCGTTTGGCTAGCGGTTAGCTCCTTTTAATGTATGGTATT   60

     E  Y  T  T  F  L  I  Y  L  I  S  I  I  L  F  N  Y  I  L  K
  61 GAATATACCACATTTCTAATCTACTTAATATCAATTATATTGTTTAATTACATATTAAAA  120

     S  I  T  R  M  M  D  Y  I  I  Y  K  F  L  L  I  I  T  I  T
 121 AGCATAACTAGAATGATGGACTACATAATTTACAAGTTTTTACTTATAATTACGATCACC  180

     S  I  F  D  S  A  Q  N  Y  G  I  N  L  P  I  T  G  S  M  D
 181 TCGATTTTCGATAGTGCCCAGAATTATGGAATCAACTTGCCAATAACTGGATCAATGGAT  240

     V  S  Y  V  N  A  T  K  D  E  P  F  L  T  S  T  L  C  L  Y
 241 GTGTCATATGTGAACGCTACAAAGGACGAACCATTCTTAACGTCGACACTATGTCTATAC  300

     Y  P  T  E  A  R  T  E  I  N  D  N  E  W  T  S  T  L  S  Q
 301 TATCCGACTGAGGCTAGAACAGAAATAAATGATAATGAGTGGACGAGCACATTGTCACAG  360

     L  F  L  T  K  G  W  P  T  G  S  V  Y  F  K  E  Y  D  D  I
 361 TTGTTTCTGACAAAAGGGTGGCCAACTGGTTCAGTATACTTTAAAGAATACGATGATATA  420

     P  T  F  S  V  D  P  Q  L  Y  C  D  Y  N  I  V  L  M  R  Y
 421 CCTACCTTCTCAGTAGATCCGCAACTGTATTGTGATTATAATATAGTTTTAATGCGGTAC  480

     N  S  S  L  K  L  D  M  S  E  L  A  N  L  I  L  N  E  W  L
 481 AATTCAAGTTTAAAGCTAGACATGTCAGAATTAGCGAACTTAATACTAAATGAATGGCTA  540

     C  N  P  M  D  I  T  L  Y  Y  Y  Q  Q  T  D  E  A  N  K  W
 541 TGCAATCCAATGGATATCACACTATATTATTATCAACAAACGGATGAAGCAAATAAATGG  600

     I  A  M  G  Q  S  C  T  I  K  V  C  P  L  N  T  Q  T  L  G
 601 ATAGCAATGGGACAATCATGCACAATAAAAGTGTGTCCATTGAATACTCAAACACTGGGG  660

     I  G  C  Q  T  T  N  T  G  T  F  E  E  V  A  T  A  E  K  L
 661 ATAGGATGTCAGACCACAAATACTGGGACATTTGAAGAGGTAGCAACAGCTGAAAAACTG  720

     V  I  T  D  V  V  D  G  V  N  H  K  L  D  V  T  T  A  S  R
 721 GTAATTACTGACGTAGTTGACGGTGTAAATCATAAACTAGATGTCACTACCGCGTCTCGT  780

     T  I  R  N  C  K  K  L  G  P  R  E  N  V  A  V  I  Q  I  G
 781 ACTATTAGAAATTGTAAGAAATTGGGGCCGAGAGAGAACGTGGCAGTAATACAAATTGGC  840

     G  A  D  I  L  D  I  T  S  D  P  T  T  T  P  Q  T  E  R  M
 841 GGTGCTGACATTCTTGATATAACATCTGATCCAACGACTACTCCACAAACAGAGCGGATG  900

     M  R  I  N  W  K  K  W  W  Q  V  F  Y  T  I  V  D  Y  V  N
 901 ATGCGAATAAATTGGAAAAAATGGTGGCAAGTATTTTACACTATAGTAGATTACGTAAAC  960

     Q  I  V  Q  T  M  S  K  R  S  R  S  L  D  S  A  A  F  Y  Y
 961 CAAATTGTACAAACAATGTCCAAAAGGTCCAGATCACTAGATTCTGCAGCATTTTACTAT  1020

     R  V  *
1021 AGAGTGTAGATATTATGTAGATTAGAGTTGTATGATGTGACC  1062
```

FIG. 1. Nucleotide and derived amino acid sequences of the VP7 gene of human rotavirus isolate A64.

Unfortunately, the currently available panel of serotype-specific monoclonal antibodies (19) covers only a subset of the 14 rotavirus serotypes that have been defined to date. Therefore, direct sequencing of the VP7 gene was performed. This allowed the exclusion of two possibilities: first, that these viruses belonged to a serotype not covered by the serological assays carried out, and second, that they represented a completely new serotype. Gene sequencing has shown that genes encoding VP7 of virus isolates in the same serotype exhibit less than 7% sequence divergence at the amino acid level, whereas the VP7s from different serotypes diverge by between 20 and 30% (13). The sequence of the VP7 gene of isolate A64 is shown in Fig. 1. Like other VP7-encoding genes, the gene was 1,062 nucleotides long and contained a single long open reading frame extending from nucleotide 49 to 1026 (Fig. 1). A comparison of the sequence with those of the VP7 genes of other virus isolates is shown in Table 3. This revealed that A64 had a 94 to 96% amino acid homology and an 86 to 87% nucleotide homology to bovine isolates 61a and B223, the latter of which has been defined as the type isolate of serotype 10 (21, 26). By contrast, the homologies with virus isolates representing the 12 other serotypes for which sequence information is currently available were between 73 and 83% at the amino acid level and 73 and 77% at the nucleotide level. On the basis of these results, it is concluded that the A64 virus is the first recognized human isolate of serotype 10.

TABLE 3. Levels of sequence homology between human rotavirus strain A64 and other rotavirus strains

Virus isolate	Serotype	Species of origin	% Homology to A64[a]	
			Nucleotide	Amino acid
61a	10	Bovine	86.7	94.2
B223	10	Bovine	86.5	95.7
Wa	1	Human	75	77.3
Hu-5	2	Human	72.9	72.7
SA11	3	Simian	77.3	82.8
ST-3	4	Human	74.7	74.5
OSU	5	Porcine	74	81
UKtc	6	Bovine	75.6	80.1
69M	8	Human	75.1	79.8
WI-61	9	Human	76.7	81.3
YM-1	11	Porcine	74.1	80.7
L-26	12	Human	73.6	77.9
L338	13	Equine	73.6	74.8
F-123	14	Equine	74.7	77.9

[a] The sequence data necessary to construct this table were taken from the relevant entries in the EMBL data base.

It was of great interest to find that the viruses isolated from a chronically infected immunodeficient child were of a serotype which had previously been isolated only from cattle (21). It could be speculated that immunodeficient hosts are more tolerant for the replication of viruses originating from a different host species than for replication of virus from the same species. Future virus isolations from immunocompromised hosts will confirm or deny this hypothesis.

REFERENCES

1. **Ballard, A., L. Xu, M. A. McCrae, and U. Desselberger.** 1991. Nucleotide sequences of normal and rearranged RNA segments 10 of human rotaviruses. J. Gen. Virol. **73:**633–638.
2. **Beards, G.** 1987. Serotyping of rotavirus by NADP-enhanced enzyme immunoassay. J. Virol. Methods **18:**77–85.
3. **Beards, G. M., M. E. Thouless, and T. H. Flewett.** 1980. Rotavirus serotypes by serum neutralisation. J. Med. Virol. **5:**231–237.
4. **Browning, G. F., T. A. Fitzgerald, R. M. Chalmers, and D. R. Snodgrass.** 1991. A novel group A rotavirus G serotype: serological and genomic characterization of equine isolate FI23. J. Clin. Microbiol. **29:**2043–2046.
5. **Coulson, B. S., L. E. Unicomb, G. A. Pitson, and R. F. Bishop.** 1987. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotaviruses. J. Clin. Microbiol. **25:**509–515.
6. **Estes, M. K., and J. Cohen.** 1989. Rotavirus gene structure and function. Microbiol. Rev. **53:**410–449.
7. **Gorziglia, M., G. Larralde, A. Z. Kapikian, and R. M. Chanock.** 1990. Antigenic relationships among human rotaviruses as determined by outer capsid protein VP4. Proc. Natl. Acad. Sci. USA **87:**7155–7159.
8. **Greenberg, H. B., J. Flores, A. R. Kalica, R. G. Wyatt, and R. Jones.** 1983. Gene cloning assignments for growth restriction, neutralisation and subgroup specificities in the W and DS-1 strains of human rotavirus. J. Gen. Virol. **64:**313–320.
9. **Hoshino, Y., M. Gorziglia, J. Valdesuso, J. Askaa, R. I. Glass, and A. Z. Kapikian.** 1987. An equine rotavirus (FI-14 strain) which bears both subgroup I and subgroup II specificities on its VP6. Virology **157:**488–496.
10. **Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralisation of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82:**8701–8704.
11. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian.** 1984. Serotypic similarity and diversity of rotaviruses

of mammalian and avian origin as studied by plaque-reduction neutralisation. J. Infect. Dis. **49**:694–702.

12. **Hundley, F., M. McIntyre, B. Clark, G. Beards, D. Wood, I. Chrystie, and U. Desselberger.** 1987. Heterogeneity of genome rearrangements in rotaviruses isolated from a chronically infected immunodeficient child. J. Virol. **61**:3365–3372.
13. **Kapikian, A. Z., and R. M. Chanock.** 1990. Rotaviruses, p. 1353–1404. *In* B. N. Fields, D. M. Knipe, et al. (ed.), Virology, 2nd ed. Raven Press, New York.
14. **McCrae, M. A., and J. G. McCorquodale.** 1982. Molecular biology of rotaviruses. IV. Molecular cloning of the bovine rotavirus genome. Virology **126**:204–212.
15. **Offit, P. A., H. F. Clark, G. Blavat, and H. B. Greenberg.** 1986. Reassortant rotaviruses containing structural proteins vp3 and vp7 from different parents induce antibodies protective against each parental serotype. J. Virol. **60**:491–496.
16. **Pedley, S., J. C. Bridger, D. Chasey, and M. A. McCrae.** 1986. Definition of two new groups of atypical rotaviruses. J. Gen. Virol. **67**:131–137.
17. **Pedley, S., F. Hundley, I. Chrystie, M. A. McCrae, and U. Desselberger.** 1984. The genome of rotaviruses isolated from chronically infected immunodeficient children. J. Gen. Virol. **65**:1141–1150.
18. **Qian, Y., and K. Y. Green.** 1991. Human rotavirus strain 69M has a unique VP4 as determined by amino acid sequence analysis. Virology **182**:407–412.
19. **Raj, P., D. O. Matson, B. S. Coulson, R. F. Bishop, K. Taniguchi, S. Urasawa, H. B. Greenberg, and M. K. Estes.** 1992. Comparisons of rotavirus VP7-typing monoclonal antibodies by competition binding assay. J. Clin. Microbiol. **30**:704–711.
20. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
21. **Snodgrass, D. R., T. Fitzgerald, I. Campbell, F. M. M. Scott, G. F. Browning, D. L. Miller, A. J. Herring, and H. B. Greenberg.** 1990. Rotavirus serotypes 6 and 10 predominate in cattle. J. Clin. Microbiol. **28**:504–507.
22. **Svensson, L., L. Grahnquist, C.-A. Pettersson, M. Grandien, G. Stintzing, and H. B. Greenberg.** 1988. Detection of human rotaviruses which do not react with subgroup I- and II-specific monoclonal antibodies. J. Clin. Microbiol. **26**:1238–1240.
23. **Taniguchi, K., K. Nishikawa, T. Urasawa, S. Urasawa, K. Midthun, A. Z. Kapikian, and M. Gorziglia.** 1989. Complete nucleotide sequence of the gene encoding VP4 of a human rotavirus (strain K8) which has unique VP4 neutralization epitopes. J. Virol. **63**:4101–4106.
24. **Taniguchi, K., T. Urasawa, N. Kobayashi, M. Gorziglia, and S. Urasawa.** 1990. Nucleotide sequence of VP4 and VP7 genes of human rotaviruses with subgroup I specificity and long RNA pattern: implication for new G serotype specificity. J. Virol. **64**:5640–5644.
25. **Tursi, J. M., M. J. Albert, and R. F. Bishop.** 1987. Production and characterization of neutralizing monoclonal antibodies to a human rotavirus strain with a "super-short" RNA pattern. J. Clin. Microbiol. **25**:2426–2427.
26. **Woode, G. N., N. E. Kelso, T. F. Simpson, S. K. Gaul, L. E. Evans, and L. Babiuk.** 1983. Antigenic relationships among some bovine rotaviruses: serum neutralization and cross-protection in gnotobiotic calves. J. Clin. Microbiol. **18**:358–364.
27. **Xu, L., D. Harbour, and M. A. McCrae.** 1990. The application of the polymerase chain reaction to the detection of rotaviruses in faeces. J. Virol. Methods **27**:29–38.
28. **Xu, L., D. Harbour, and M. A. McCrae.** 1991. Sequence of the gene encoding the major neutralisation antigen (VP7) of serotype 10 rotavirus. J. Gen. Virol. **72**:177–180.