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Polymerase Chain Reaction Amplification and Typing of Rotavirus Nucleic Acid from Stool Specimens

VERA GOUVEA,[1,2]* ROGER I. GLASS,[1] PATRICIA WOODS,[1] KOKI TANIGUCHI,[3] H FRED CLARK,[4] BARBARA FORRESTER,[1] AND ZHAO-YIN FANG[1]

Viral Gastroenteritis Unit, Division of Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333[1]; Division of Microbiology, Food and Drug Administration, Washington, D.C. 20204[2]; Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo, Japan[3]; and The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104[4]

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The rotavirus gene segment coding for the major outer capsid glycoprotein vp7 was amplified directly from stool specimens by the polymerase chain reaction (PCR). Double-stranded RNA extracted from stool samples was used as the template for reverse transcription, which was followed immediately and in the same reaction mix with amplification, using the *Taq* polymerase. Various conditions were examined to optimize the yield of the amplified gene. The concentrations of $MgCl_2$, dimethyl sulfoxide, and template RNA were critical. The choice of primer pairs allowed amplification of the entire segment or specific portions. By using type-specific primers derived from distinct regions on the gene, we devised a PCR typing method in which each human serotype virus produced a characteristic segment size, readily identifiable in agarose gels. The PCR typing method was applied to 10 rotavirus reference strains, including all 6 known human serotypes (serotypes 1, 2, 3, 4, 8, and 9), and to 34 stool specimens previously serotyped by an enzyme immunoassay with monoclonal antibodies. An absolute correlation was found between the molecular and serologic methods. In addition, 14 stool specimens nonserotypable by an enzyme immunoassay with monoclonal antibodies could be typed by the PCR method. Besides the application for rotavirus detection and typing directly from stools, the PCR method provides a rapid and efficient means of obtaining large quantities of cDNA suitable for sequencing, cloning, and other genetic studies, precluding the need for cell culture and virus purification.

Worldwide, rotavirus is the major viral agent of severe diarrhea in children and the current focus of intense research to develop an effective vaccine (6, 15, 33). Although detection of rotavirus in clinical specimens has became a routine diagnostic procedure, characterization of these strains for serotype or specific gene products has remained difficult despite the need for such information to interpret the results of vaccine studies and epidemiologic surveillance. Currently, there are four well-established serotypes (serotypes 1 to 4) and two newly described serotypes (serotypes 8 and 9) of human rotavirus that have been defined by in vitro neutralization assays (2, 3, 16, 20, 34). Proper determination of serotype requires that each strain be adapted for cultivation and neutralized with reference sera. Shortcut methods such as the use of monoclonal antibodies (MAbs) in enzyme-linked immunoassays (EIA-MAbs) (4, 25, 27, 29–31) require serotype-specific neutralizing MAbs, which are not yet available for all serotypes and which still leave 20 to 30% of the strains untypable (14, 31, 32). In this study we used a novel, molecular approach to identify human rotavirus serotypes in stool specimens.

The rotavirus particle consists of 11 double-stranded RNA (dsRNA) genome enclosed in a double-shelled capsid. The outer shell is composed of a major glycoprotein with a molecular weight of 34,000 (vp7) and a minor, trypsin-sensitive protein with a molecular weight of 84,000 (vp4, previously designated vp3). Both proteins appear to be involved in virus neutralization (1, 12, 26), but serotype specificity is defined by vp7, which is encoded by gene segment 9 (or segment 8, depending on the strain) (6, 15). Comparative studies on the amino acid sequences of the vp7 proteins of the various serotypes have identified six discrete regions (A to F) with significant amino acid divergence (8). These regions, although very distinct among different serotypes, were highly conserved within each given serotype (10); furthermore, the serotype of a given rotavirus isolate could be predicted from the sequence of two of these divergent regions (11).

Using the sequence data of gene 9, we designed serotype-specific primers and devised a strategy for typing rotavirus by the polymerase chain reaction (PCR). This new and powerful technique of in vitro DNA amplification developed by Mullis and Faloona (22) had been extended for the detection of single-stranded RNA sequences after copy into cDNA by reverse transcriptase (7, 13, 18). However, to our knowledge, no amplification from a dsRNA template has yet been reported. We describe here the use of the PCR for the amplification of a dsRNA virus and a method for typing rotaviruses from clinical specimens by direct visualization of the amplified segment in agarose gels.

MATERIALS AND METHODS

Viruses. The following cultivable human rotavirus strains were used in this study: Wa (serotype 1), DS1 (serotype 2), P (serotype 3), Hochi (serotype 4), 69M (serotype 8), WI61 (serotype 9), and F45 (serotype 9, supplied by Nobuco Ikegami, Osaka National Hospital, Osaka, Japan). Three animal rotavirus strains were included as controls: simian SA11 and RRV (serotype 3) and bovine UK (serotype 6).

A number of human stool specimens from different collections of the Viral Gastroenteritis Unit at the Centers for Disease Control were used in this study; 48 specimens were positive for group A rotavirus and 6 specimens were negative for group A rotavirus but known to contain other human

* Corresponding author.

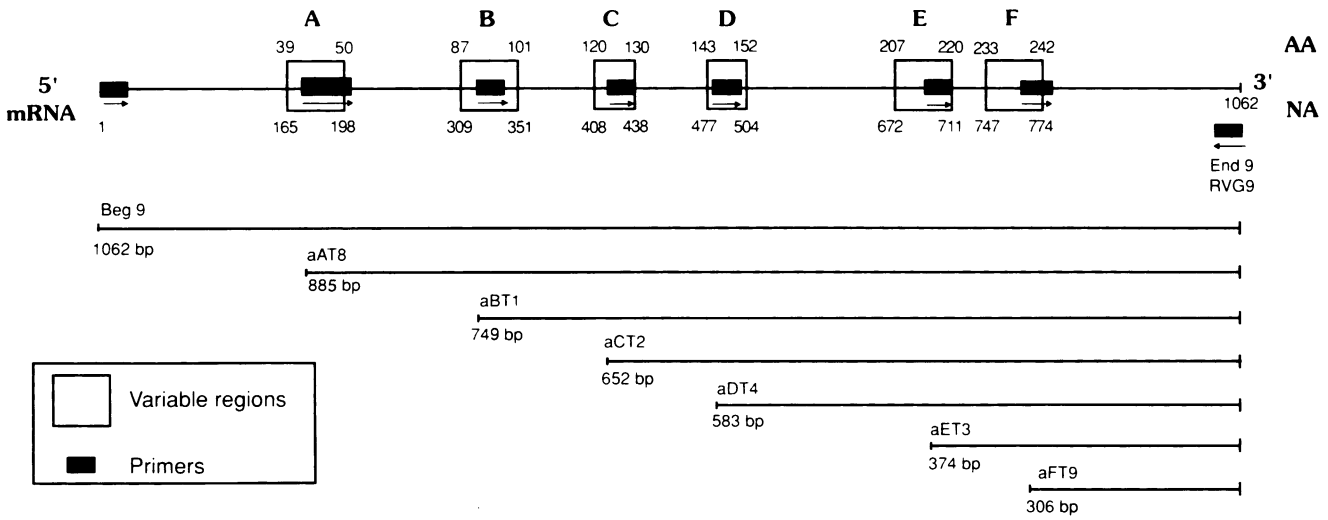

FIG. 1. Rotavirus gene 9 (or gene 8), which encodes vp7 glycoprotein. Shown are locations of variable regions and PCR primers and expected lengths of amplified segments.

enteric viruses: adenovirus, reovirus, calicivirus, astrovirus, small round particles, or group B rotavirus.

Viral RNA. Rotavirus dsRNA was extracted from stool suspensions or infected tissue culture in the manner commonly used for RNA electropherotyping in polyacrylamide gels (6). Briefly, a 400-μl stool suspension or tissue culture fluid was adjusted to contain 1% sodium dodecyl sulfate and 100 μg of proteinase K per ml and incubated for 1 h at 37°C. The suspension was extracted once with phenol-chloroform (1:1) and again with chloroform alone. The RNA in the aqueous phase was precipitated with 2 volumes of ethanol at −70°C overnight, collected by centrifugation, and suspended in 100 μl of water.

Purified SA11 virus. Simian rotavirus SA11 was grown in MA104 cells, harvested, and purified through a CsCl gradient by standard techniques (15). The concentration of viral dsRNA was calculated by determining the optical density at 260 nm and used as control viral RNA.

Polyacrylamide gel electrophoresis (PAGE) of rotavirus dsRNA. Electropherotyping of viral RNA on all extracted RNA samples was performed in 12% polyacrylamide gels with the Laemmli buffer system, and the gel was silver stained as previously described (24).

Primers. Each of the 11 rotavirus genomic segments has been shown to possess unique sequences both at 3′ and at 5′ ends, which are highly conserved among all strains (8). Oligonucleotide primers specific for gene segment 9 (or segment 8), which encodes vp7, were synthesized, complementary to the 3′ ends of both viral RNA strands. These primers—Beg9 and End9, which were 28 and 27 nucleotides long, respectively—were selected to produce full-length copies of gene 9 (or gene 8) from any group A rotavirus strain.

Six serotype-specific primers ranging from 19 to 25 nucleotides long were synthesized, complementary to the negative RNA strand by using each of the six variable regions on gene 9 as the blueprint for a distinct serotype (Fig. 1). Thus, the sequence of primer aAT8 maps to variable region A of strain 69M (serotype 8), the sequence of primer aBT1 maps to region B of strain Wa (serotype 1), and so forth. The sequence of each primer, its position in the genomic segment, and the prototype viruses from which the sequences were copied are displayed in Table 1. These primers were selected to be pooled as a primer mix and used in combination with the common primer RVG9, 19 nucleotides at the 5′ end of primer End9. The serotype-specific primers form the basis of the PCR typing method, since they were chosen to produce a characteristic segment length for each serotype (Fig. 1).

PCR amplification from dsRNA (first amplification). Por-

TABLE 1. Oligonucleotide primers for PCR amplification

Primer	Sequence[a] (5′–3′)	Position	Region	Strain (serotype)
Beg9[b]	GGCTTTAAAAGAGAGAATTTCCGTCTGG	1–28		Wa (1)
End9[b]	GGTCACATCATACAATTCTAATCTAAG	1062–1036		SA11 (3)
RVG9	GGTCACATCATACAATTCT	1062–1044		SA11 (3)
aAT8	GTCACACCATTTGTAAATTCG	178–198	A	69M (8)
aBT1	CAAGTACTCAAATCAATGATGG	314–335	B	Wa (1)
aCT2	CAATGATATTAACACATTTTCTGTG	411–435	C	DS1 (2)
aDT4	CGTTTCTGGTGAGGAGTTG	480–498	D	ST3 (4)
aET3	CGTTTGAAGAAGTTGCAACAG	689–709	E	P (3)
aFT9	CTAGATGTAACTACAACTAC	757–776	F	WI61 (9)

[a] The nucleic acid sequences were obtained from GenBank, Computer System Division, Bolt Beranek and Newman, Inc., Cambridge, Mass.; European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; and National Biomedical Research Foundation, Georgetown University Medical Center, Washington, D.C.

[b] Primers used to amplify full-length sequence.

tions of rotavirus dsRNA were used as templates for reverse transcriptase to synthesize cDNA copies from both viral strands that were then amplified by the PCR. Both reactions were performed in a single, siliconized 600-μl Eppendorf tube containing 100 μl of reaction mixture. Various conditions regarding the concentrations of $MgCl_2$, dimethyl sulfoxide (DMSO), primers, and template were tested. Optimization of these parameters resulted in the following standard procedure. Extracted RNA (1 or 10 μl) was added to the reaction mixture consisting of 10 mM Tris (pH 8.3); 40 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM each dATP, dCTP, dTTP, and dGTP; 7% DMSO; and 1 μM each the desired set of oligonucleotide primers. The pair Beg9-End9 was used for the amplification of full-length gene 9, or the primer mix containing all six serotype-specific primers aBT1, aCT2, aET3, aDT4, aAT8, and aFT9 and the common primer RVG9 were used for direct PCR typing. Before the addition of the enzymes, dsRNA was denatured by heating at 97°C for 5 min and quickly quenched in a dry ice-ethanol bath. The solution was allowed to thaw at room temperature. Avian myeloblastosis virus super reverse transcriptase (4 U) (Molecular Genetic Resources, Tampa, Fla.) and 2.5 U of *Taq* polymerase (or AmpliTaq; Perkin Elmer Cetus, Norwalk, Conn.) were added, and the mixture was homogenized and overlaid with mineral oil. The tubes were placed in a thermocycler (Ericomp Inc., San Diego, Calif.) for an initial 30-min incubation at 42°C, followed by 25 cycles of PCR (94°C for 1 min, 42°C for 2 min, and 72°C for 1 min) and a final 7-min incubation at 72°C.

PCR amplification from dsDNA (second amplification). PCR typing could also be performed from dsDNA that was obtained from the first amplification of the entire gene 9. In this case, 1 to 10 μl of the dsDNA product served as the template for this second typing amplification. The same reaction buffer was used, but with reduced concentrations of primer mix and RVG9 (200 nM each primer) and *Taq* polymerase (1 U). Reverse transcriptase was omitted. The same PCR program was used with only 10 to 15 cycles, followed by a final 7-min extension at 72°C.

Agarose gels. PCR products (10 μl) were loaded on 1.2% SeaKem agarose (FMC Bio Products, Rockland, Maine) in Tris-borate buffer (0.089 M Tris–0.089 M boric acid–0.002 M EDTA, pH 8) containing 0.5 μg of ethidium bromide per ml. After electrophoresis at 120 V for 1 h, the gels were photographed under UV light on Polaroid type 57 film.

EIA-MAb. Rotaviruses in stool specimens were serotyped by an EIA-MAb method previously described (31), using the MAbs KU-4, S2-2G10, YO-IE2, and ST-2G7 specific for serotypes 1, 2, 3, and 4, respectively. MAb YO-2C2, reactive against the vp4 protein of rotaviruses (30), was used to indicate the presence of double-shelled particles in the stool suspensions. A serotype was assigned when the reaction with one MAb was at least twice the mean optical density reading of the other three MAbs to the same sample.

RESULTS

Optimization of reaction conditions. Our efforts to reverse transcribe dsRNA and amplify the cDNA products were dependent on the concentrations of DMSO and Mg^{2+} ions. The presence of 5 to 7% DMSO in the reaction buffer was critical for the amplification of nucleic acid from a dsRNA template (Fig. 2). Addition of 7% DMSO decreased the quantities of dsRNA needed for the amplification of gene 9 from micrograms in the absence of DMSO to nanograms when DMSO was present. The recommended 6 mM Mg^{2+} concentration for maximal reverse transcriptase activity was tested for the reverse transcription reaction that was performed as a separate step before the polymerase amplification, as well as under conditions in which the reactions were performed together. The best results were obtained when the 1.5 mM Mg^{2+} concentration recommended for the *Taq* polymerase was used for both reactions (Fig. 2). This simplified our method, since both enzymes could be added to the same buffer, sealed in reaction tubes, and incubated sequentially for both reverse transcription and polymerase reactions without any additional tube manipulation.

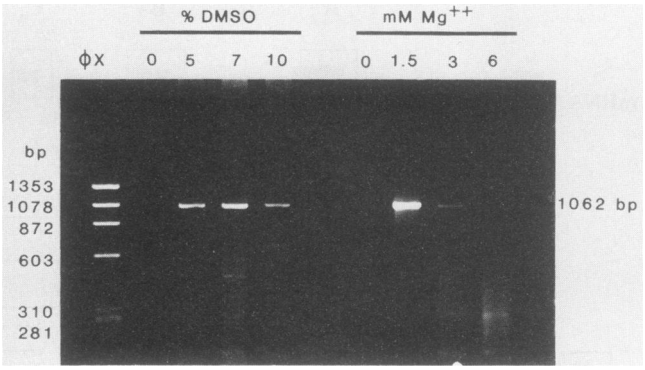

FIG. 2. Optimization of DMSO and $MgCl_2$ concentrations in the reaction buffer for combined reverse transcription and PCR amplification of approximately 1 ng of rotavirus dsRNA gene 9 segment. φX, *Hae*III-digested φX 174 DNA size markers.

PCR amplification from dsRNA. We recognized how important the amount of viral dsRNA could be to the amplification when PCR was applied to stool specimens containing a broad range of rotavirus concentrations. To determine the best amount of viral dsRNA for PCR amplification, we titrated the dsRNA extracted from two stool samples both by PAGE and by PCR. Both samples gave similar results (the titration of one of the samples is shown in Fig. 3). Portions of the extracted dsRNA suspension in volumes ranging from 0.01 to 10 μl were used as templates for the amplification reaction and were loaded into the wells of a polyacrylamide gel. Known amounts of purified rotavirus SA11 RNA were loaded in adjacent wells of the same gel to estimate the amount of viral RNA in the stool specimen. Quantities of total viral RNA ranging from approximately 4 to 40 ng produced good amplification. This represents approximately 200 pg to 2 ng of dsRNA template, as gene 9 accounts for approximately 5% of the total rotavirus RNA. The sensitivity of the test was between 20 and 100 pg of template dsRNA, as 100 pg still produced a visible 1,062-base-pair (bp) band. Excess dsRNA template was detrimental to the reaction, particularly in the presence of contaminating substances, e.g., RNA extracted from stools. Thus, 10 μl of this particular RNA preparation containing an estimated 400 ng of total RNA or 20 ng of template produced no visible amplification. This upper limit of template varied for different samples and was also detected in RNA suspensions extracted from infected tissue culture. However, corresponding amounts of RNA obtained from purified virus gave quantitative results with up to 1 μg of RNA template (results not shown). It is possible that coprecipitated substances other than viral dsRNA, when present in high quantities, inhibited one of the early steps in the PCR amplification method such as dsRNA denaturing or primer annealing. The amount of RNA suspension appropriate for

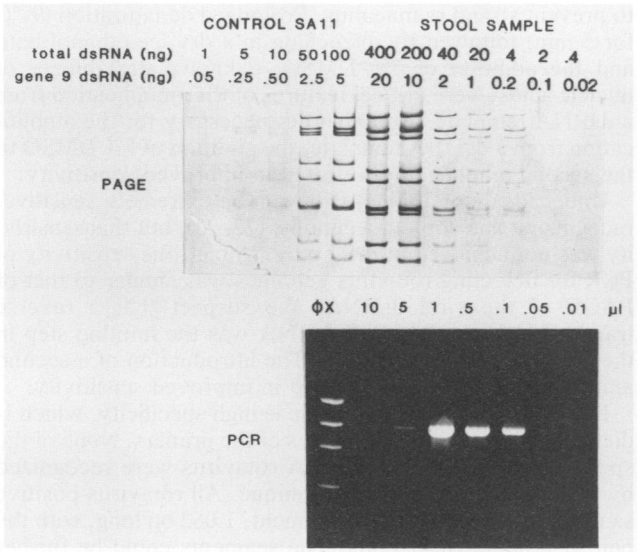

FIG. 3. Titration of dsRNA by PAGE and PCR. Portions containing 10 to 0.01 μl of dsRNA extracted from a stool were used for both PAGE analysis and PCR amplification as indicated between the two gels. Known amounts of purified SA11 rotavirus (as indicated) were loaded in adjacent wells of a 12% polyacrylamide gel. After electrophoresis at 150 V for 2 h, the gel was silver stained, and the amount of RNA in the stool sample was estimated by comparison with control SA11 (e.g., a 10-μl portion was estimated to contain 400 ng of dsRNA or 20 ng of template). The PCR products (10 μl) were loaded on ethidium bromide-agarose gel, run at 120 V for 1 h, and photographed under UV light. Different amounts of a 1,062-bp segment were produced. The best amplification was obtained with 2 to 0.2 ng (1 to 0.1 μl) of dsRNA template. ϕX, *Hae*III-digested ϕX 174 DNA size markers.

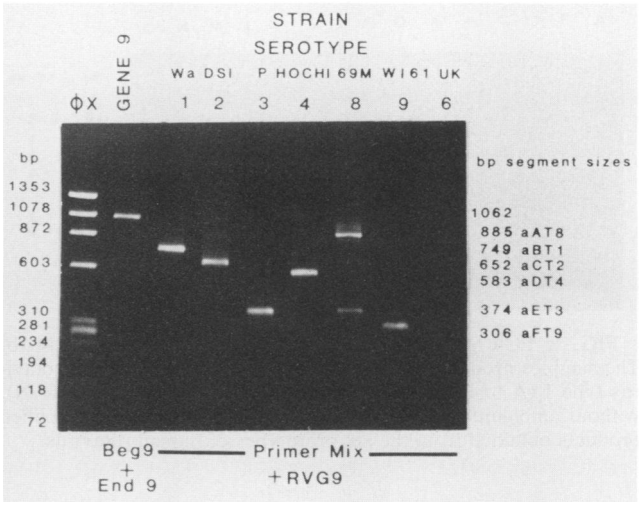

FIG. 4. PCR typing of human rotavirus reference strains. Lanes show DNA-amplified segments corresponding to whole gene 9 and to each of the human rotavirus serotypes. Lane UK, Bovine UK rotavirus; lane ϕX, *Hae*III-digested ϕX 174 DNA size markers.

good amplification could be estimated by PAGE of the viral RNA extracted from each stool specimen.

The reaction was specific, as none of the six stools that contained other viruses showed any reactivity with the primers specific for gene 9 of group A rotavirus.

PCR typing method. The six sets of primers used for typing yielded bands of distinct lengths—749, 652, 374, 583, 885, and 306 bp for the reference strains Wa, DS1, P, Hochi, 69M, and WI61 belonging to serotypes 1, 2, 3, 4, 8, and 9, respectively (Fig. 4). The two serotype 3 simian rotaviruses SA11 and RRV and the other known serotype 9 strain F45 were assigned the same types (3 and 9, respectively) by the PCR typing method (data not shown). No cross-reaction was observed for any serotype except strain 69M, which was recognized by primer aET3 producing an additional but weak 374-bp band characteristic of type 3 strains, along with a strong 885-bp band specified by the type 8 primer aAT8. The six type-specific primers—aBT1, aCT2, aET3, aDT4, aAT8, and aFT9—were fully compatible among themselves and with the common primer RVG9, demonstrated no spurious primer annealing, and could be used together in a single reaction tube. The reactions were specific for serotype, and none of the human type-specific primers recognized the serotype 6 bovine strain UK used as a negative control.

PCR typing from stool specimens. From a large collection of stool specimens serotyped by an EIA-MAb, 48 were selected for PCR typing. For 34 samples, a distinct serotype could be detected by EIA: 12 specimens were serotype 1, 6 specimens were serotype 2, 9 specimens were serotype 3, and 7 specimens were serotype 4. In all cases, PCR typing gave the same result, demonstrating 100% agreement between the two typing methods. The remaining 14 samples could not be serotyped by the EIA-MAb in spite of strong reaction with the MAb that binds to the outer capsid protein vp4 (Table 2). Those specimens could be divided into three groups according to their reactivity with the serotype-specific MAbs: group 1 (samples A to E), which had high optical density values with all four MAbs; group 2 (samples F and G), in which no reaction with any of the MAbs was detected; and group 3 (samples H to N), in which a positive reaction was observed with both serotype 1 and 2 MAbs. By PCR, all five samples in group 1 were assigned to type 3, group 2 specimens were assigned to type 1 (F) and type 3 (G), and six of the seven samples from group 3 were assigned to type 1; the remaining sample was of type 2 (Fig. 5). Each of these 14 samples reacted exclusively with one type-specific primer. Nonspecific reactions were not detected by the PCR typing method.

PCR typing (second amplification). PCR typing was also

TABLE 2. PCR typing of 14 samples nonserotypable by EIA-MAb

Sample	Reactivity with EIA-MAb:					PCR type
	YO-2C2 (vp4)	KU-4 (1[a])	2S-2G10 (2[a])	YO-IE2 (3[a])	ST-2G7 (4[a])	
A	+	+	+	+	+	3
B	+	+	+	+	+	3
C	+	+	+	+	+	3
D	+	+	+	+	+	3
E	+	+	+	+	+	3
F	+	−	−	−	−	1
G	+	−	−	−	−	3
H[b]	+	+	+	−	−	2
I	+	+	+	−	−	1
J	+	+	+	−	−	1
K	+	+	+	−	−	1
L	+	+	+	−	−	1
M	+	+	+	−	−	1
N	+	+	+	−	−	1

[a] vp7 Serotype.
[b] Short-pattern electropherotype; all others were long patterns.

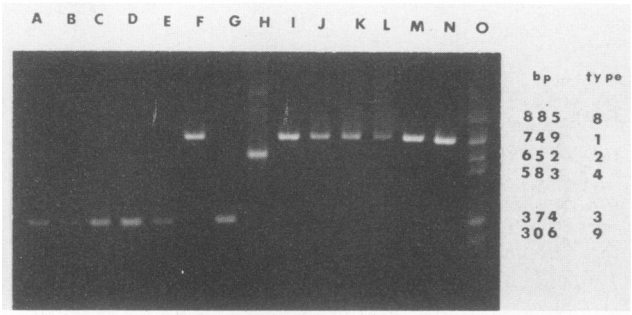

FIG. 5. PCR typing of 14 samples nonserotypable by EIA-MAb. The samples produced amplified segments corresponding to rotavirus type 1 (A to E and G), type 2 (H), and type 3 (F and I to N), without ambiguity. Markers (O) represent a pool of the amplified products obtained from the six prototypes of human rotavirus.

conducted with amplified material obtained after a first PCR amplification of the full-length gene 9 segment. Use of this second amplification increased the sensitivity of the test. A good second amplification was produced by 100 times less than the limit of detectability of ethidium bromide-stained gels (Fig. 6). Indeed, 3 of the 48 samples (5%) in this study did not produce a visible 1,062-bp band in the first amplification but gave strong typing bands in the second.

In general, the best results of PCR amplification are obtained with a low quantity of template. Excess DNA can produce high background, spurious bands, or even a streak of nucleic acids instead of or in addition to the correct amplified segment, as can be seen in Fig. 6 for a type 1 rotavirus.

DISCUSSION

We have adapted the PCR technique for the amplification of dsRNA of rotavirus gene 9 (or gene 8) directly from stools and applied it to viral diagnosis and type identification. The PCR technique, originally described for amplification of DNA segments, has been extended for the detection of single-stranded RNA viruses such as the human immunodeficiency virus (12) and rhinovirus (7) following reverse transcription into cDNA copies. Using a similar approach, we were able to amplify cDNA from a dsRNA template. Because of the stronger binding forces between RNA-RNA strands as compared with DNA-DNA or DNA-RNA strands, more drastic denaturing conditions were necessary to prevent strand reannealing. Prolonged denaturation (97°C for 5 min) followed by quenching in a dry ice-ethanol bath and the addition of 7% DMSO, a known destabilizer of nucleic acids, were critical features of the amplification from a dsRNA template. Although not necessary for the amplification from a dsDNA template, the addition of 7% DMSO to the second amplification resulted in improved sensitivity.

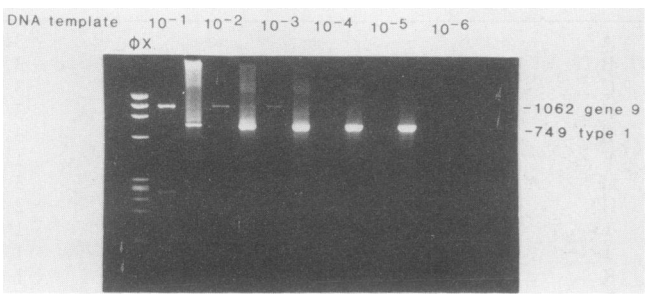

FIG. 6. Titration of DNA template for PCR typing. Ten microliters of 10-fold serial dilutions of the first PCR product (DNA template) was used for the second PCR amplification (PCR typing). Lanes show the pairs of DNA template and its corresponding PCR product. ϕX, *Hae*III-digested ϕX 174 DNA size markers.

Under ideal conditions, PCR can be extremely sensitive, recognizing one copy of a genome (22, 23), but this sensitivity was not achieved in this study. In fact, the sensitivity of PCR for detecting rotavirus genomes was similar to that of PAGE of the viral dsRNA. We suspect that a reverse transcriptase reaction with dsRNA was the limiting step in the sensitivity of this method. The introduction of a second amplification, however, resulted in improved sensitivity.

Intrinsic to the PCR technique is high specificity, which is dictated by the unique sequences of the primers. None of the specimens negative for group A rotavirus were recognized by the primers used in our technique. All rotavirus-positive samples produced only one segment, 1,062 bp long, with the pair of primers Beg9-End9. The segments could be further recognized by a distinct gene 9-specific typing primer in a second PCR amplification.

Serotype is, by definition, a classification based on neutralization of viral infectivity. Rotavirus serotyping has been performed by plaque reduction, tube neutralization, or fluorescent focus reduction test and recently by EIAs, using neutralizing MAbs. Presently, however, enough information on the gene 9 sequences of rotavirus strains allows for the prediction of the serotype of a given strain by the limited sequence of the variable regions. The strategy used in the present study was to design each type-specific primer to anneal to one of the variable regions A to F, which are at different distances from the 3′ end of the gene. The characteristic size of the segment amplified is, then, in itself an identification of the viral type. Thus, the amplified segment does not require further confirmation by hybridization to a specific, labeled probe (18, 23). Yet the same high specificity obtained with a confirmatory hybridization to a third probe is obtained with the PCR typing technique performed as a second amplification with a third rotavirus gene 9-specific primer.

The choice of the variable region to serve as a serotype-specific primer was arbitrary; other combinations would probably give similar results. Nevertheless, the PCR typing results correlated with the serotype assigned by EIA-MAb in all samples tested. The extent to which the amino acid sites defined by these six variable regions are directly involved in neutralization is still unclear. Most studies using escape mutants have identified epitopes corresponding to regions B, D, and E (also designated A, B, and C, respectively, by some authors) as the immunodominant neutralization sites (5, 19, 28, 29). The other variable regions (A, C, and F) have not yet been directly implicated in viral neutralization. Morita et al. (21) have demonstrated the existence of at least five neutralizing epitopes in vp7 which overlap and collectively constitute a single large neutralization domain. It is possible that a close interrelationship (or restriction) exists among the six variable regions of gene 9 in such a way that each given serotype would possess all the characteristic regions for that serotype, and the identification of any individual variable region could reveal the strain serotype. Sequencing data on a variety of human strains (8, 10, 11) appear to confirm this hypothesis, as do our results. The only exception to date has been observed with the human serotype 8 strains 69M and B37. These strains share with the

serotype 3 viruses the epitope encoded by region E (9, 17). Therefore, it was not surprising that strain 69M was recognized by primer aET3 specific for region E of type 3 viruses, which presents only one mismatch with the 69M strain. It is of interest that although serotype 3 MAb YO-IE2 did not recognize strain 69M, serotype 8 MAb B37:1 (kindly supplied by Ruth Bishop, Royal Children's Hospital, Melbourne, Australia) strongly reacted with the three serotype 3 samples tested (strain YO and two stool specimens) in a limited EIA (our unpublished results). Since serotypes 3 and 8 share an epitope, it is suggested that MAb B37:1 is probably directed to this epitope, whereas MAb YO-IE2 is not. The PCR typing procedure may prove to be a good method for detecting this kind of intertypic sharing of neutralization epitopes in field strains. New type-specific primers designed for variable regions other than the ones described here, forming a panel of primers for PCR typing, might detect other unsuspected links among strains of distinct serotypes, including strains from different animal species. An additional advantage of this method is the ready availability of nucleic acid material for sequence analysis to confirm any unforeseen result. Conversely, a comparison of typing results obtained with a panel of MAbs and with a panel of PCR primers might help identify nucleic acid variations in the divergent regions within serotypes and monitor antigenic drifts.

Of unquestionable value for the identification of neutralizing epitopes and determination of their spatial allocation and distribution in the virion, MAbs often give no definitive indication of the virus serotype. Taniguchi et al. (31), using the same type-specific MAbs used in this study, found that 19 of 57 stool specimens (30%) could not be assigned a definitive serotype: 10 samples reacted with two or more MAbs, and 9 samples reacted with none. In another epidemiologic survey of rotavirus serotypes in Japan, Urasawa et al. (32) could not serotype 22% of rotavirus-positive stool samples. Using a different set of MAbs in a similar EIA, Heath et al. (14) could not serotype 30% of viruses grown in culture and more than 60% of rotavirus-positive fecal extracts. Part of the MAb inability to type certain rotavirus specimens has been blamed on a lack of complete virus particles; however, in our laboratory, we cannot serotype approximately 20% of stool specimens containing double-shelled particles, as shown by their positive reaction to MAb YO-2C2, which reacts with viral outer capsid protein vp4. The applicability of the PCR technique to type 14 of the stool samples was well demonstrated here, and the assigned PCR type was further supported by dsRNA electrophoretic analysis. Thus, sample H presented a short electropherotype characteristic of human serotype 2 strains; samples A to E and G presented the same electropherotype, indistinguishable from other serotype 3 human strains; and the remaining samples had electropherotypes identical to one or the other of the two electropherotypes found in known serotype 1 strains. These specimens, which were typed by PCR and not by EIA-MAb, are currently being sequenced and adapted to culture for further investigation.

The correlation demonstrated between the PCR typing and EIA-MAb methods supports previous findings that genotyping can be a reliable means of identifying rotavirus serotype. Molecular methods might present some advantages over serologic methods for large epidemiologic studies on gastroenteritis, particularly in regard to reagents and the handling of samples. The reagents, oligonucleotide primers, or probes are synthetic, chemically defined substances that allow the easy comparison of results obtained in different studies. The samples, nucleic acids extracted from stool specimens, can be stored easily for long periods and used as templates not only for rotavirus detection or amplification but also for a number of infectious agents.

As an amplification technique, PCR produces the desired segment of nucleic acid in amounts and purity adequate for genetic studies such as cloning and sequencing, completely bypassing the need for cell cultivation. Although we have so far studied only rotavirus gene 9, this procedure should be equally appropriate to amplify any rotavirus gene as well as genes of other segmented dsRNA viruses, provided the proper set of primers is selected. Similar methods may also prove useful to identify specific viral sequences or markers associated with virulence, species of origin, and ability to grow, provided enough nucleic acid sequence information regarding these traits is available.

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LITERATURE CITED

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