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Evaluation of reverse transcription and polymerase chain reaction (RT/PCR) for the detection of rotaviruses: applications of the assay

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SUMMARY

Our aim was to evaluate the reverse transcription and polymerase chain reaction (RT/PCR) technique for the detection of rotavirus shedding by infected children as a routine diagnostic procedure, in comparison to the enzyme-linked immunosorbent assay (ELISA), electron microscopy (EM) and polyacrylamide gel electrophoresis (PAGE) of rotavirus double-stranded RNA.

Two-hundred and twenty stool specimens were collected from infants and young children with diarrhoea, and 10-20% faecal suspensions were made. Several methods of rotavirus dsRNA extraction were assayed. Electrophoretic analysis of viral RNA was carried out on 10% polyacrylamide gels followed by silver staining. RT/PCR was performed using oligonucleotide primers specific for both 3' and 5' ends of the rotavirus gene encoding VP7 which are highly conserved among group A rotaviruses.

Following RNA extraction with phenol-chloroform and ethanol precipitation, RT/PCR could detect rotaviral RNA in only 11 of 25 samples known to contain rotaviruses by conventional methods. The purification of RNA extracts by CF11 cellulose and the application of the RNAID method were equally effective in extracting RNA and/or removing inhibitory substances from the faecal samples. RT/PCR led to the detection of 66 positive samples from 220 specimens tested (30%), whilst 64 specimens were positive by ELISA (29%), 59 (26.8%) by PAGE and 56 (25.4%) by EM. In our study, RT/PCR was 100 times more sensitive than the ELISA test in detecting rotaviruses serially diluted in a faecal suspension. Although RT/PCR is theoretically much more sensitive than ELISA, PAGE and EM for detection of rotaviruses, great care must be taken to remove inhibitory substances from the enzymatic reactions. We do not consider that RT/PCR should replace immunoassays with high sensitivity and specificity for rotavirus testing in faecal samples, although this technique has other applications, like the search for rotavirus in different clinical specimens (sera, cerebrospinal fluid, respiratory secretions, etc.) and in environmental samples, as well as the typing of viral strains using serotype-specific primers.

Key-words: Rotavirus, RT/PCR; ELISA, PAGE, Electron microscopy, Viral analysis, Sensitivity.

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INTRODUCTION

Group A rotaviruses cause an estimated 140 million cases of gastroenteritis in infants and children and one million deaths worldwide each year (Kapikian *et al.*, 1986; Glass *et al.*, 1994). Rapid diagnosis of this infection is of great value in the formulation of the prognosis for children with acute diarrhoea and in decisions concerning management and control measures. Since the cultivation of rotaviruses from clinical specimens is difficult to achieve, the most widely used diagnostic techniques for rotavirus detection in faecal samples include immunoassays such as ELISA and latex agglutination, electron microscopy (EM) and immune electron microscopy, and polyacrylamide gel electrophoresis (PAGE) of viral double-stranded RNA (Kapikian and Chanock, 1996). Nucleic acid hybridization techniques in the format of "dot blots" have also been applied to the detection of rotaviral RNA (Flores *et al.*, 1983). However, RNA detection assays have generally not proven to be substantially more sensitive than optimized immunoassay procedures (Wilde *et al.*, 1990).

The reverse transcription/polymerase chain reaction (RT/PCR) should enable the detection of rotaviruses at concentrations substantially lower (1,000 times less) than those required for detection by means of immunoassays and electron microscopic studies (Wilde *et al.*, 1991). RT/PCR has been applied by several authors to the detection of rotaviral RNA (Gouvea *et al.*, 1990; Wilde *et al.*, 1990, 1991; Xu *et al.*, 1990; Ushijima *et al.*, 1992, Husain *et al.*, 1995) and for typing virus strains, characterizing both G and P serotypes (Gouvea *et al.*, 1990; Ushijima *et al.*, 1992; Gentsch *et al.*, 1992).

We have evaluated the application of RT/PCR in the detection of group A rotavirus RNA in faecal specimens as a routine diagnostic procedure compared to the enzyme-linked immunosorbent assay (ELISA) currently employed in our laboratory, and to EM and PAGE. Special efforts have been made in the search for an efficient, simple and fast procedure to extract viral nucleic acids and to avoid interference with the enzymatic reactions by inhibitory substances present in faecal samples.

MATERIALS AND METHODS

Collection of specimens

During the period October 1994 to March 1996, two-hundred and twenty faecal specimens were obtained from infants and young children with signs of gastroenteritis who were followed at the Pediatric Departments of the Hospital Clinico Universitario of Valencia, Spain, and at the Hospital of Sagunto, Spain. Faecal suspensions (10-20%) were made in Tris-NaCl-$CaCl_2$ buffer (0.05 M Tris-HCl pH 7.5, 0.15 M sodium chloride, 0.01 M calcium chloride) and stored at –70°C. Samples were assayed by ELISA for rotavirus antigens, by EM for viral particles and by PAGE and RT/PCR for rotavirus RNA.

ELISA for the detection of rotavirus antigens

Solid phase enzyme immunoassays were carried out on all samples. The "Rotaclone" rotavirus diagnostic kit (Cambridge Biotech, Worcester, MA, USA) was applied to detect rotavirus antigens in faecal specimens following the procedure described by the manufacturer. Results were read spectrophotometrically at 450 nm. The Rotaclone test utilizes a monoclonal antibody directed against the rotavirus VP6 protein in a solid-phase sandwich-type ELISA. According to the manufacturer, the sensitivity limit of the test is 5×10^5 viral particles per ml.

EM (electron microscopy)

Previously prepared 10-20% faecal suspensions were centrifuged at 50,000 rpm for 90 min (L8-70M Ultracentrifuge, Beckman Instruments), absorbed onto carbon-formvar-coated 400-mesh grids and

CSF = cerebrospinal fluid.
ELISA = enzyme-linked immunosorbent assay.
EM = electron microscopy.
RT/PCR = reverse transcription/polymerase chain reaction.
PAGE = polyacrylamide gel electrophoresis.
PFU = plaque-forming unit.
SDS = sodium dodecyl sulphate.

negatively stained with 2% phosphotungstic acid, pH 4.5, as described by Nakata *et al.* (1987). The preparations were examined under an electron microscope (Zeiss EM10C/CR, voltage 60 KV) at a magnification of 31,500x. Samples were considered negative when no virus particles were found during 15 min of observation.

Viral RNA

The following methods to extract viral dsRNA from faecal suspensions were assayed: (i) phenol-chloroform extraction and ethanol precipitation; (ii) "RNAID" method (Bio 101, Inc., La Jolla, CA, USA); (iii) "GeneReleaser protocol (BioVentures, Inc., Murfreesboro, TN, USA); and (iv) phenol-chloroform extraction and purification through CF11 cellulose.

Rotavirus dsRNA was extracted from stool suspensions by a modification of the procedure originally described by Herring *et al.* (1982) and commonly used in our laboratory for subsequent RNA electrophoresis in polyacrylamide gels (PAGE). Briefly, 400-µl stool suspensions were adjusted to contain 1% SDS and 0.1 M sodium acetate (pH 5.0), and incubated for 15 min at 37°C. The suspension was extracted with phenol-chloroform (1:1). The RNA in the aqueous phase was ethanol-precipitated at –70°C and dissolved in 50 µl of water.

The RNAID kit (Bio 101, Inc., La Jolla, CA, USA) was also applied to extract viral dsRNA from stool suspensions following the instructions of the manufacturer with the modifications described by Gentsch *et al.* (1992). Briefly, 250 µl of 6 M guanidinium thiocyanate (Fluka) were mixed with an equal volume of stool suspension. A volume of 12 µl of the RNAID glass powder was added and the mixture was vortexed and rotated at room temperature for 10 min. After centrifugation for 30 s at 650 *g* in a Beckman microcentrifuge, the supernatant was removed, the pellet washed twice with 400 µl of the RNAID kit wash buffer and recentrifuged at 850 *g*. After a third wash in the same buffer, the sample was finally centrifuged at 10,000 *g* for 60 s, dried under vaccum, redissolved in 25 µl of deionized water and incubated for 10 min at 65°C. The samples were centrifuged at 10,000 *g* for 60 s, and the supernatants stored at –20°C until use for the reverse transcriptase reaction and PCR. Just before use, the samples were heated at 56°C for 5 min and centrifuged at 10 000 *g* for 10 s to pellet any residual RNAID matrix from the sample.

The Gene Releaser protocol (BioVentures Inc., Murfreesboro, TN, USA) was also tested. This commercial polymeric matrix was originally described to release DNA from heterogeneous biological samples and sequester cell lysis products which might inhibit polymerases. Levy *et al.* (1994) have described the use of this method for PCR and RT/PCR detection and identification of viruses and viroids in plant tissue extracts. Both thermocycler and microwave protocols for sample preparation described by the manufacturer were assayed. In the microwave procedure, 2-5 µl of faecal suspension was placed in a 0.5-ml thermocycling tube together with 16-19 µl of GeneReleaser matrix, yielding a total volume of 21 µl. The mixture was vortexed for 30 s and the tube was placed in a microwave oven and heated at the maximal power setting for 5 min. The tube was then heated in a pre-warmed thermocycler at 90°C, incubated on ice for 5 min and then microfuged at maximal speed at 4°C. An aliquot of the supernatant was used to perform RT/PCR reactions as described in this paper.

RNAs extracted from faecal samples with phenol-chloroform were purified using CF11 cellulose fibre powder as described by Wilde *et al.* (1990). After phenol/chloroform/isoamyl alcohol extraction (25:24:1), the aqueous phase was mixed with 95% ethanol to bring the final solution to 15% ethanol (vol/vol). To this mixture, 30 mg of CF11 cellulose (fibrous medium cellulose, Sigma) was added, vortexed and rotated for 90 min at 4°C. After centrifugation at 6 000 *g* for 60 s, the supernatant was removed and the pellet washed three times with STE buffer (0.1M NaCl, 0.001M EDTA, 0.5M Tris-HCl pH 7.0) containing 15% ethanol (vol/vol). After a final wash in STE without ethanol to elute the RNA from the cellulose, the sample was centrifuged for 5 min to pellet the CF11 cellulose. RNA in the supernatant was precipitated with ethanol in the presence of 0.3 M sodium acetate pH 5.2. The RNA pellet was redissolved in 50 µl of deionized water and stored at –20°C.

In the preliminary phase of our study, viral RNA was extracted by the methods noted above from 25 faecal samples which were previously found to be positive for rotaviruses by ELISA, PAGE and EM.

PAGE of rotavirus dsRNA

The PAGE technique for rotavirus RNA detection and analysis was performed on 10% polyacrylamide gel slabs lacking SDS and silver staining as previously described (Herring *et al.*, 1982; Buesa *et al.*, 1987).

Primers

The oligonucleotide primers used, Beg9 and End9, were described previously (Gouvea *et al.*, 1990). These primers are specific for the gene cod-

ing for VP7 which may be either RNA segment 7, 8 or 9, depending on the viral strain. They produce full-length copies of the gene from any group A rotavirus strain.

RT/PCR amplification

The following RT/PCR method was used: 1 µl of dimethyl sulphoxide was added to 9 µl of the extracted RNA and heated at 97°C for 5 min to denature nucleic acids, cooled on ice, and the reaction buffer (6 µl 5 × AMV buffer [Promega]), 0.25 µl RNasin (Promega), 3 µl of 10 mM solutions of each of the deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP), 2.5 µM of each primer, Beg9 and End9, and 10 units AMV reverse transcriptase (Promega) were added; reactions were incubated at room temperature for 10 min and subsequently at 42°C for 35 min, ending with 92°C for 2 min to inactivate the reverse transcriptase.

The cDNA amplification reactions were carried out in 50 µl 1 × PCR buffer (10 mM Tris-HCl pH 8.8) containing 1 µl of the RT reaction, 1.5mM $MgCl_2$, 50mM KCl, 0.1% Triton X-100, 2 mM of each dNTP, 1 µM each primer and 2 units of "Dynazyme" DNA polymerase (Finnzymes Oy, Finland). Reaction mixtures were overlaid with 50 µl of light mineral oil (Sigma) and PCR was conducted in a thermal cycler (GenE, Techne) for 40 cycles (94°C for 1 min, 47°C for 2 min and 72°C for 2 min) with a final 7-min incubation at 72°C. Negative and positive controls were included in every RT/PCR assay. The positive control was viral RNA from SA11 rotavirus cultivated in MA-104 cells and extracted by the method of Herring *et al.* (1982).

PCR products were analysed by electrophoresis on 1.2% agarose gels with ethidium bromide at 100 V for 30 min and photographed under UV light.

The sensitivity of the RT/PCR in detecting rotaviruses was determined by using ten-fold serial dilutions in a negative faecal suspension of a SA11 rotavirus preparation containing 5×10^7 PFU/ml, as titrated by plaque assay. RNA was extracted by the RNAID method and RT/PCR was done as previously described.

RESULTS

Efficiency of RNA extraction methods

In a preliminary set of assays, 25 faecal samples previously found to be rotavirus-positive by ELISA, PAGE and EM were tested using different methods of RNA extraction in order to determine the most reliable and efficient procedure. The results of these assays are shown in table I. Phenol-chloroform extraction followed by binding of RNA to CF11 cellulose in STE-ethanol and elution with STE, as described by Wilde *et al.* (1990), was an efficient method to recover rotaviral RNA from stool samples free of inhibitory activity. All 25 samples analysed by RT/PCR were positive when phenol-extracted and purified by CF11 cellulose, whereas only 11 (44%) were positive when purification with CF11 was omitted. Equally effective appeared to be the RNAID method, which also allowed the detection of viral RNA in all 25 samples tested. However, the GeneReleaser method was, in our experience, inefficient in purifying rotaviral RNA or removing inhibitors from the faecal samples.

Having established the efficacy of both CF11 cellulose purification and the RNAID method in extracting viral RNA and/or removing inhibitory substances from faecal samples, we used the RNAID method with all the specimens tested.

Sensitivity of the RT/PCR assay

The limit of detection of the RT/PCR using an SA11 rotavirus suspension serially diluted in a 10% faecal extract was 5×10^3 PFU/ml (fig. 1). In comparison, the ELISA test used in this study could detect 5×10^5 PFU/ml.

Table I. Results of VP7-specific RT/PCR after using different RNA extraction procedures on 25 human faecal samples previously found to be positive for group A rotaviruses by ELISA, PAGE and EM.

Rotavirus dsRNA extracted from human faecal samples by:	No. of positive samples by RT/PCR
phenol-chloroform extraction and ethanol precipitation	11/25
phenol-chloroform extraction and CF11 cellulose purification	25/25
RNAID method	25/25
GeneReleaser method	0/25

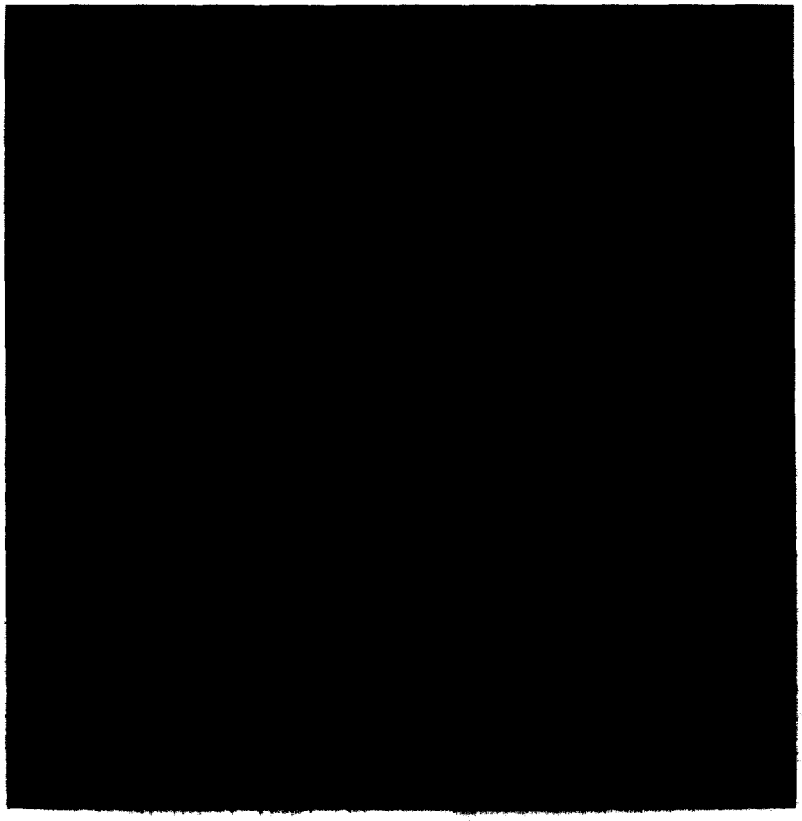

Fig. 1. Sensitivity of RT/PCR assay in detecting rotaviruses.

Rotavirus strain SA11 was serially diluted in a 10% faecal suspension and RNA was extracted by the RNAID method (Bio 101, Inc.) as described in "Materials and Methods". M=1 kb DNA ladder (Gibco BRL). Lanes 1 to 6=RT/PCR products obtained from viral suspensions containing 5×10^7, 5×10^6, 5×10^5, 5×10^4, 5×10^3 and 5×10^2 PFU/ml; a faint band is still visible in lane 5 (5×10^3 PFU/ml); lanes 7 and 8 are negative and positive controls, respectively.

Comparison between ELISA, EM, PAGE and RT/PCR for detecting rotavirus

The results obtained from analyses by electron microscopy, ELISA, PAGE and RT/PCR are presented in table II. Sixty-eight samples (30.9%) were found to be positive by one or more of the techniques applied. In two specimens, the result of RT/PCR was negative, whereas the ELISA test showed positive results. These were considered to be false-negative PCR results. Conversely, four samples were positive by RT/PCR but negative by ELISA.

Rotavirus particles were detected by EM in 56 specimens (25.4%). Other viruses were also detected among the 220 faecal samples studied, mainly adenovirus (8.1%), astrovirus (2.2%), calicivirus (1.8%) and coronavirus-like particles (1.3%).

PAGE analysis was positive with 59 faecal samples (26.8%). Most rotavirus strains showed "long" electrophoresis pattern, with the exception of 5 strains (8.4%) which were found to have "short" electrophoretypes. Ten different electrophoretypes were detected during the course of this study (results not shown).

PCR amplification products were easily detected by agarose gel electrophoresis (figs. 2 and 3). A clear and distinctive fragment of about

Table II. Comparison of ELISA, PAGE and EM with RT/PCR in detecting rotavirus in 220 faecal samples of children with gastroenteritis.

	ELISA		PAGE		EM	
	+	−	+	−	+	−
RT/PCR$^+$	62	4	59	7	56	10
RT/PCR$^-$	2	152	0	154	0	154

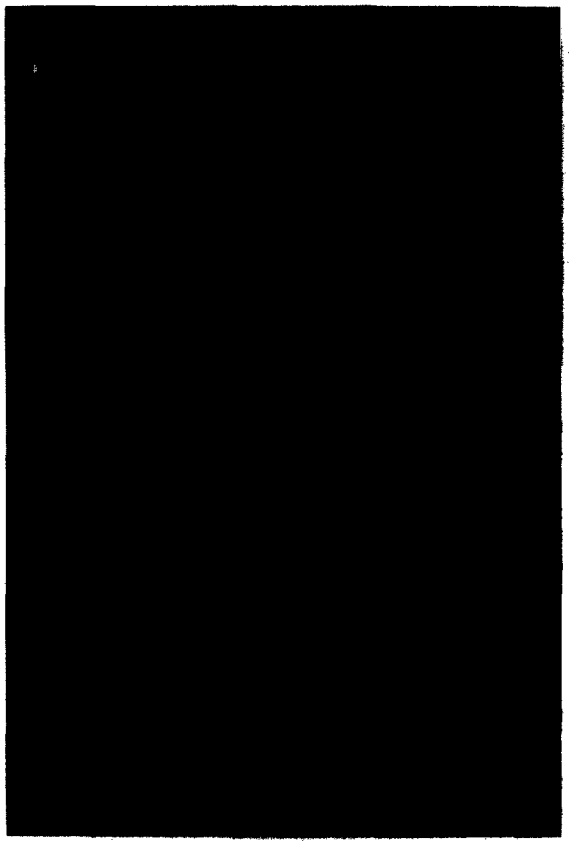

Fig. 2. Rotavirus RT/PCR products analysed by agarose gel electrophoresis.

M = "mass ladder" (Gibco BRL). Lanes 1 and 2 are RT/PCR products from faecal samples extracted with phenol-chloroform and purified using CF11 cellulose. Lanes 3 and 4 are from faecal samples extracted with the RNAID method as described in "Materials and Methods". Lanes 5 and 6 are positive (RNA from simian rotavirus SA11) and negative controls, respectively. K = 1-kb DNA ladder. The oligonucleotide primers used are specific for the viral gene encoding VP7 from any group A rotavirus strain.

1 kb was considered to be the product of a specific amplification of the rotavirus gene 9 (or gene 8).

DISCUSSION

Many authors have applied RT/PCR to the detection of rotaviruses in clinical specimens (Gouvea *et al.*, 1990; Wilde *et al.*, 1990; Xu *et al.*, 1990; Ushijima *et al.*, 1992; Husain *et al.*, 1995). Moreover, direct "serotyping" of rotavirus strains (VP7, G types; VP4, P types) is feasible by means of RT/PCR using type-specific primers derived from distinct regions of the genes (Gouvea *et al.*, 1990; Ushijima *et al.*, 1992; Gentsch *et al.*, 1992). However, not many surveys have been performed with the purpose of making a comparison between RT/PCR and other methods currently in use in diagnostic laboratories for the detection of rotaviruses in stool specimens.

We have taken advantage of the previous work performed by Gouvea *et al.* (1990), and Ushijima *et al.* (1992) to establish the optimal conditions ($MgCl_2$, dimethyl sulphoxide and template RNA concentrations) for the RT/PCR procedure. Our first attempts to amplify rotavirus RNA from faecal extracts were sometimes hampered by an apparent inhibition of the reactions. This faecal inhibition of the RT/PCR has been reported by others (Wilde *et al.*, 1990) and may constitute a significant limitation on the detection of RNA viruses in faecal specimens. A variety of extraction procedures have been proposed to remove inhibitory substances from faecal extracts as well

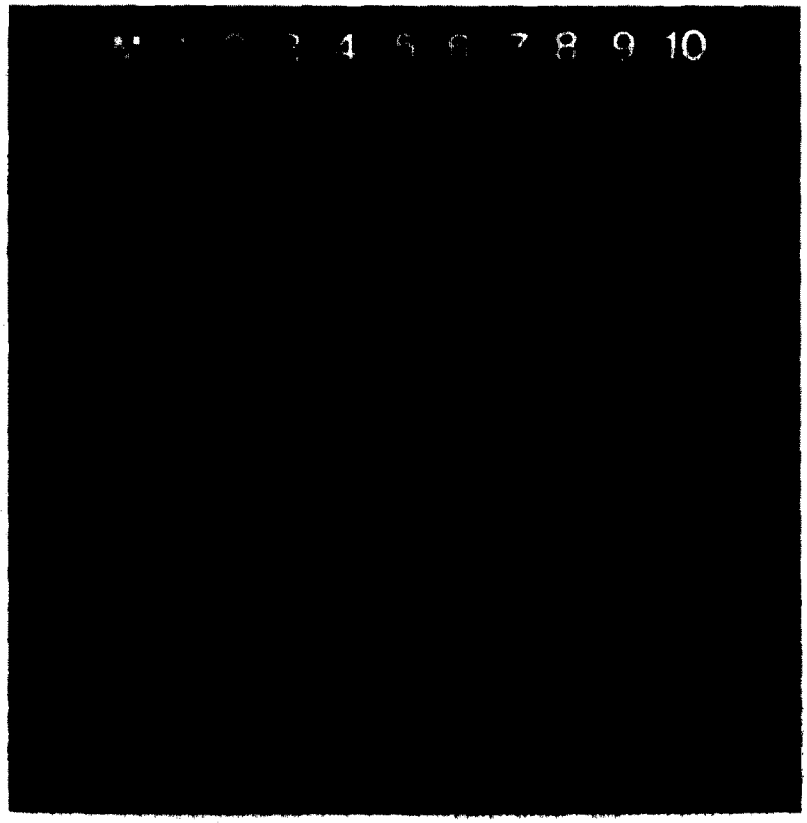

Fig. 3. Agarose gel electrophoresis of rotavirus RT/PCR products from stool specimens.

Viral RNAs were extracted by the RNAID method. M=1-kb DNA ladder; lane 1=positive control (RNA from simian rotavirus SA11); lanes 2 to 9=RT/PCR products obtained from faecal samples; lane 10=negative control. A clear and distinctive band of about 1 kb was considered to be the product of a specific amplification of rotavirus dsRNA.

as from environmental samples (Le Guyader *et al.*, 1994). Our results suggest that both CF11 purification and the RNAID procedure for extracting viral RNA may be used prior to performing RT/PCR.

According to our data, RT/PCR is not substantially more sensitive for detecting rotavirus than other techniques such as the ELISA test used in our study, in agreement with the results obtained by Gouvea *et al.* (1990) and Ushijima *et al.* (1992). The use of a double amplification system, nested or semi-nested PCR, has been reported by some authors to improve the sensitivity of rotavirus detection (Gouvea *et al.*, 1990; Gentsch *et al.*, 1992; Le Guyader *et al.*, 1994), though others did not observe an increase in sensitivity using these procedures (Ushijima *et al.*, 1992).

It has been reported that the RT/PCR assay can be approximately 100 times (Husain *et al.*, 1995) and even 1,000 times (Wilde *et al.*, 1990) more sensitive than ELISA tests, as determined by serial dilutions of faecal specimens. Although this increase in sensitivity may be feasible, in practice it may be very difficult to achieve. In our study, four samples were positive by RT/PCR but negative by ELISA, probably due to the higher sensitivity of the first technique. Moreover, great care must be taken to avoid inhibitory substances in the enzymatic reactions. PAGE can detect as little as 10 ng rotavirus RNA (Herring *et al.*, 1982) and in our hands, appears to be a very

reproducible technique. Gouvea *et al.* (1990) reported the requirement of at least 2 ng (10^8 copies) of viral RNA in the faecal specimen in order to be detected as rotavirus-positive by RT/PCR. Xu *et al.* (1990) were able to detect 8×10^3 virus particles by RT/PCR under experimental conditions. However, we do not consider that RT/PCR should replace the more conventional immunoassays in the diagnosis of rotavirus infections performed in clinical virology laboratories. Obviously, RT/PCR is a very sensitive and specific assay, but it is more expensive due to the costs of enzymes and more time-consuming, and the results are obtained later than with other techniques such as immunoassays and/or EM. Both G and P typing of rotavirus strains with specific primers is a main application of RT/PCR (Gouvea *et al.*, 1990; Ushijima *et al.*, 1992; Gentsch *et al.*, 1992) as are other studies on the genomic variability of these viruses (Vonsover *et al.*, 1993).

RT/PCR has been applied to the detection of rotaviral RNA not only in faecal samples but also in other types of clinical specimens (throat swabs, sera and cerebrospinal fluids) with some intriguing results. Ushijima *et al.* (1994) reported the detection by RT/PCR of human rotavirus RNA in throat swabs from seven children with rotavirus gastroenteritis and in CSF from seven other patients with gastoenteritis and convulsions, as well as in two sera samples. Such studies will lead to a better understanding of the pathogenesis of rotavirus infections and may confirm the detection of rotavirus antigens in respiratory secretions reported by others (Fragosa *et al.*, 1986; Zheng *et al.*, 1991). In addition, RT/PCR is also a powerful technique to further investigate the mechanisms of transmission of these viruses and their presence in the environment (Wilde *et al.*, 1992; Le Guyader *et al.*, 1994).

EM continues to be a mainstay in the diagnosis of rotaviral diseases and has been used in the past as the final arbiter when discrepancies occurred with other techniques (Kapikian and Chanock, 1996). It remains a very useful tool, especially in the detection of unexpected viral agents for which suitable probes may not be available with immunological or nucleic acid-based techniques.

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Évaluation de la RT/PCR pour la détection des rotavirus: applications de cette technique

La RT/PCR a été évaluée pour la détection des rotavirus dans les selles des enfants ayant une infection due à ces virus. Les résultats ont été comparés à ceux de la technique ELISA, de la microscopie électronique (ME) et de l'électrophorèse de l'ARN viral bicaténaire en gel de polyacrylamide (PAGE).

Nous avons préparé des suspensions fécales à 10-20 % en Tris-NaCl-$CaCl_2$ à partir de 220 échantillons provenant d'enfants souffrant de gastro-entérite aiguë et utilisé différentes méthodes d'extraction de l'ARN viral. Dans la RT/PCR, des oligonucléotides spécifiques des extrémités 3' et 5' du gène codant la glycoprotéine VP7 ont été utilisés. La purification des extraits d'ARN avec la CF11 cellulose et le méthode "RNAID" ont été également efficaces pour l'obtention de l'ARN viral.

L'amplification par la RT/PCR a permis de détecter 66 échantillons positifs (30 %), pendant que 64 ont été positifs par l'ELISA (29 %), 59 (26.8 %) par l'électrophorèse de l'ARN viral et 56 (25.4 %) par la ME.

La RT/PCR est plus sensible que l'ELISA, la PAGE et la ME pour la détection des rotavirus. Cependant il faut veiller à éliminer les possibles inhibiteurs des réactions enzymatiques. Malgré cette efficacité, nous considérons que la RT/PCR ne doit pas remplacer les techniques immunoenzymatiques ayant une grande sensibilité et une haute spécificité pour la détection des rotavirus dans les selles. Mais cette technique est utilisable pour la recherche des rotavirus dans d'autres prélèvements (sérum, LCR, sécrétions respiratoires etc.) ou dans des échantillons provenant de l'environnement; elle peut, de plus, permettre de caractériser les sérotypes viraux.

Mots-clés : Rotavirus, RT/PCR ; ELISA, PAGE, Microscopie électronique, Analyse virale, Sensibilité.

References

Buesa, F.J., Duato, M., Gimeno, C. & Garcia de Lomas, J. (1987), Sequential variation in genomic RNA patterns of human rotaviruses isolated from infantile gastroenteritis. *Ann. Virol. (Inst. Pasteur)*, 138, 307-314.

Flores, J., Purcell, R.H., Perez, I., Wyatt, R.G., Boeggeman, E., Sereno, M., White, L., Chanock, R.M. & Kapikian, A.Z. (1983), A dot hybridization assay for detection of rotavirus. *Lancet* I, 555-559.

Fragosa, M., Kumar, A. & Murray, D.L. (1986), Rotavirus in nasopharingeal secretions of children with upper respiratory tract infections. *Diagn. Microbiol. Infec. Dis.*, 4, 87-88.

Gentsch, J.R., Glass, R.I., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B.K. & Bhan, M.K. (1992), Identification of group A rotavirus gene 4 types by polymerase chain reaction. *J. Clin. Microbiol.*, 30, 1365-1373.

Glass, R.I., Gentsch, J. & Smith, J.C. (1994), Rotavirus vaccines: success by reassortment? *Science*, 265, 1389-1391.

Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B. & Fang, Z.Y. (1990), Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *J. Clin. Microbiol.*, 28, 276-282.

Herring, A.J., Inglis, N.F., Ojeh, C.K., Snodgrass, D.R. & Menzies, J.D. (1982), Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver stained polyacrylamide gels. *J. Clin. Microbiol.*, 16, 173-477.

Husain, M., Seth, P. & Broor, S. (1995), Detection of group A rotavirus by reverse transcription and polymerase chain reaction in feces from children with acute gastroenteritis. *Arch. Virol.*, 140, 1225-1233.

Kapikian, A.Z. & Chanock, R.M. (1996), Rotaviruses, *in* "Fields virology" (B.N. Fields, D.M. Knipe and P.M. Howley) (pp. 1657-1708). Lippincott-Raven Publishers, Philadelphia.

Kapikian, A.Z., Flores, J., Hoshino, Y., Glass, R.I., Midthun, K., Gorziglia, M. & Chanock, R.M. (1986), Rotavirus: the major etiologic agent of severe infantile diarrhea may be controllable by a "Jennerian" approach to vaccination. *J. Infect. Dis.*, 153, 815-822.

Le Guyader, F., Dubois, E., Ménard, D. & Pommepuy, M. (1994), Detection of hepatitis A virus, rotavirus, and enterovirus in naturally contaminated shellfish and sediment by reverse transcription-seminested PCR. *Appl. Environ. Microbiol.*, 60, 3665-3671.

Levy, L., Lee, I.M. & Hadidi, A. (1994), Simple and rapid preparation of infected plant tissue extracts for PCR amplification of virus, viroids and MLO nucleic acids. *J. Virol. Methods*, 49, 295-304.

Nakata, S., Petrie, B., Calomeni, E.P. & Estes, M. (1987), Electron microscopy procedure influences detection of rotaviruses. *J. Clin. Microbiol.*, 25, 1902-1906.

Vonsover, A., Shif, I., Silberstein, I., Rudich, H., Aboudy, Y., Mendelsonn, E., Shulman, L., Nakagomi, T. & Nakagomi, O. (1993), Identification of feline- and canine-like rotaviruses isolated from humans by restriction fragment length polymorphism assay. *J. Clin. Microbiol.*, 31, 1783-1787.

Wilde, J., Eiden, J. & Yolken, R. (1990), Removal of inhibitory substances from human fecal specimens for detection of group A rotaviruses by reverse transcriptase and polymerase chain reaction. *J. Clin. Microbiol.*, 28, 1300-1307.

Wilde, J., Van, R., Pickering, L., Eiden, J. & Yolken, R. (1992), Detection of rotaviruses in the day care environment by reverse transcriptase polymerase chain reaction. *J. Infect. Dis.*, 166, 507-511.

Wilde, J., Yolken, R., Willoughby, R. & Eiden, J. (1991), Improved detection of rotavirus shedding by polymerase chain reaction. *Lancet*, 337, 323-326.

Ushijima, H., Koike, H., Mukoyama, A., Hasegawa, A., Nishimura, S. & Gentsch, J. (1992), Detection and serotyping of rotaviruses in stool specimens by using reverse transcription and polymerase chain reaction amplification. *J. Med. Virol.*, 38, 292-297.

Ushijima, H., Xin, K.Q., Nishimura, S., Morikawa, S. & Abe, T. (1994), Detection and sequencing of rotavirus VP7 gene from human materials (stools, sera, cerebrospinal fluids, and throat swabs) by reverse transcription and polymerase chain reaction amplification. *J. Clin. Microbiol.*, 32, 2893-2897.

Xu, L., Harbour, D., & McCrae, M.A. (1990), The application of polymerase chain reaction to the detection of rotaviruses in faeces. *J. Virol. Methods*, 27, 29-38.

Zheng, B.J., Chang, R.X., Ma, G.Z., Xie, J.M., Liu, Q., Liang, X.R. & Ng, M.H. (1991), Rotavirus infection of the oropharynx and respiratory tract in young children. *J. Med. Virol.*, 34, 29-37.