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Rapid, Simple Method of Preparing Rotaviral Double-Stranded Ribonucleic Acid for Analysis by Polyacrylamide Gel Electrophoresis†

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A procedure for extracting rotaviral double-stranded ribonucleic acid (RNA) directly from fecal and intestinal specimens collected from calves and pigs is described. This procedure provides a rapid, simple, reproducible method of obtaining rotaviral double-stranded RNA preparations suitable for electrophoretic analysis in polyacrylamide-agarose composite gels. The rotaviral genome electrophoretic migration pattern produced by double-stranded RNA extracted directly from a specimen by this procedure was qualitatively identical to the electrophoretic migration pattern obtained with double-stranded RNA extracted from purified rotavirus derived from the same specimen. Direct extraction of specimens containing porcine rotavirus-like virus by this procedure gave preparations that had electrophoretic migration patterns similar, but not identical, to the characteristic electrophoretic migration pattern of the rotaviral genome. Sufficient rotaviral double-stranded RNA could be extracted from 6 ml of fecal or intestinal specimen by this procedure to permit 15 or more electrophoretic assays.

After the recognition of rotaviral infections as an important cause of diarrhea in calves (16), rotaviruses have been associated with neonatal diarrhea in many mammals, including humans (1, 9, 32), and, more recently, in avian species (15). Although rotaviral infections, occurring either alone or concomitantly with other enteropathogens, are frequently among the most common causes of neonatal diarrhea in many of these species, our knowledge of their epidemiology and epizootiology remains fragmentary. Among the first requisites for epidemiological and epizootiological studies of viral infections are the availability of suitable, practical procedures whereby the viruses can be detected and differentiated. The paucity of information concerning the epidemiology and epizootiology of rotaviral infections stems directly from the failure of these viruses to propagate readily in conventional cell culture systems, thereby hindering development of in vitro assays for detecting and quantifying these viruses and their antibodies. Rotaviruses recovered from different species are morphologically indistinguishable and possess a common antigen or antigens (9, 32); consequently, the need for convenient, definitive methods of differentiating rotavirus isolates exists. While noteworthy progress has been made in the development of serological methods to differentiate rotavirus isolates recovered from diverse species (7, 29, 33), such methods may lack sufficient sensitivity to discriminate between isolates recovered from the same species.

Rotaviruses possess a double-stranded ribonucleic acid (RNA) genome comprising 11 discrete segments of various molecular weights (13, 23, 27), and rotaviruses isolated from different host species can be distinguished by the electrophoretic migration patterns that their genomes produce in polyacrylamide gels (13, 14, 22, 27). This technique of electrophoretic analysis of rotaviral double-stranded RNA was also extended to differentiate isolates recovered from the same host species (14, 22, 27, 30). Although this technique provides little information at present regarding possible antigenic differences among rotaviruses, it does provide a powerful tool to use as a molecular probe into the epidemiology and epizootiology of rotaviral infections. A major impediment to the establishment of this technique as a routine laboratory procedure has been the need for purified or partially purified virus for extraction of nucleic acid. We thus undertook to develop a rapid, simple procedure

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by which rotaviral double-stranded RNA preparations suitable for electrophoretic analysis could be extracted directly from specimens, thereby obviating elaborate virus purification procedures.

MATERIALS AND METHODS

Virus detection and specimens. Rotavirus was detected in intestinal contents and fecal specimens by negative stain immune electron microscopy (24). Similarly, this technique was used for detection of bovine coronavirus in fecal specimens. Rotaviral and transmissible gastroenteritis (TGE) viral antigens were detected in small intestinal mucosal smears by immunofluorescent microscopy (28). In certain instances, bovine fecal smears were prepared according to Mebus et al. (16) and examined for exfoliated enterocytes containing rotaviral antigens by immunofluorescent microscopy as above (28) except that a K 530 barrier filter was used. Calicivirus-like virus and 23-nm virus-like particles were detected in porcine intestinal contents and small intestinal mucosal smears by electron and immunofluorescent microscopy, respectively, according to previously described procedures (25). Porcine rotavirus-like viral antigens were detected in small intestinal mucosal smears by immunofluorescent microscopy as described previously (25).

Small and large intestinal mucosa and contents were harvested from rotavirus-infected pigs by scraping into 25 to 40 ml of 0.05 M tris(hydroxymethyl)-aminomethane buffer, pH 7.5, containing 0.1 M NaCl and 0.001 M $CaCl_2$ (TSC buffer). Small and large intestinal mucosa and contents of rotavirus-infected calves were harvested similarly into 100 ml of TSC buffer. Bovine fecal specimens containing rotavirus were used as collected. Small and large intestinal mucosal scrapings and contents derived in a similar manner from normal pigs and from pigs infected with enterotoxigenic *Escherichia coli*, TGE virus, porcine rotavirus-like virus, or porcine calicivirus-like virus and 23-nm virus-like particles served as control specimens. Likewise, small and large intestinal mucosal scrapings and contents from normal calves and from calves infected with enterotoxigenic *E. coli* or other microbial pathogens (i.e., cryptosporidia) were harvested as described above and served as control specimens. Fecal specimens collected from diarrheic calves with coronaviral infections or from calves with undifferentiated diarrhea (but negative for rotaviral infection) served as additional control specimens.

Bacteriological and histological examinations. Enterotoxigenic *E. coli* strains were isolated and identified according to previously described procedures (1). Cryptosporidia associated with diarrhea in calves were detected by histological examination of Formalin-fixed thin sections of the small intestine stained with azure and eosin (4).

Virus purification and nucleic acid extraction. Virus purification was performed at 4°C. Intestinal scrapings and contents or fecal specimens containing rotavirus were diluted twofold with TSC buffer, homogenized, and centrifuged at 12,000 × *g* for 20 min. The supernatant fluid was then homogenized with an equal volume of trichlorotrifluoroethane (Fisher Scientific Co.) and centrifuged at 800 × *g* for 20 min, and the resultant aqueous phase was collected and extracted again with trichlorotrifluoroethane. The fluorocarbon phase derived from the initial extraction of the specimen was homogenized with an equal volume of TSC buffer, and the resultant aqueous phase was combined with the aqueous phase obtained with the fluorocarbon-extracted supernatant fluid. Virus was pelleted from the combined aqueous phases by centrifugation at 69,000 × *g* for 1 h and resuspended in TSC buffer by sonication. The virus suspension was then clarified by centrifugation at 7,700 × *g* for 10 min, and the virus was pelleted through a 40% (wt/vol) sucrose cushion by centrifugation at 100,000 × *g* for 2 h in an SW41 rotor. Virus was resuspended in TSC buffer by sonication, layered onto preformed linear cesium chloride density gradients, and centrifuged overnight at 100,000 × *g* in an SW41 rotor. Virus banding at 1.36 to 1.38 g/cm^3 was collected, diluted in TSC buffer, and pelleted by centrifugation at 69,000 × *g* for 1 h. Purified virus was then resuspended in 2.5 ml of TSC buffer by sonication.

Double-stranded RNA was extracted from purified rotavirus by a modification of the phenol-chloroform method described by Perry et al (20). Sterile buffer solutions and sterile glassware coated with silicone (Sigmacote, Sigma Chemical Co., St. Louis, Mo.) were used throughout; water-saturated phenol was prepared according to Diener and Schneider (5). To 2.5 ml of purified virus suspension was added an equal volume of 0.02 M tris(hydroxymethyl)aminomethane buffer, pH 7.4, containing 0.2 M NaCl, 0.004 M disodium ethylenediaminetetraacetate, and 1% (wt/vol) sodium dodecyl sulfate (SDS), and the mixture was allowed to stand at room temperature for 30 min. Then 5 ml of water-saturated phenol-chloroform (1:1, vol/vol) was added with moderate agitation, and the resultant mixture was centrifuged at 800 × *g* for 20 min at 4°C. The lower phenol-chloroform phase was removed, and the upper aqueous phase was extracted twice more with phenol-chloroform. The extracted aqueous phase was mixed with 0.1 volume of 4 M sodium acetate, and ethanol was then added to a final concentration of 67% (vol/vol). Rotaviral double-stranded RNA was precipitated overnight at −20°C and pelleted by centrifugation at 12,000 × *g* for 30 min at 0°C. Pelleted RNA was dried under a stream of nitrogen, resuspended in approximately 1 ml of 0.01 M tris(hydroxymethyl)aminomethane buffer, pH 8.0, containing 0.1 M NaCl and 0.005 M disodium ethylenediaminetetraacetate, and dialyzed overnight at 4°C against the same buffer. Before electrophoresis, rotaviral double-stranded RNA preparations were heated for 2 min at 45°C and then diluted to an appropriate concentration in tris(hydroxymethyl)aminomethane-borate buffer, pH 8.3, containing disodium ethylenediaminetetraacetate (Peacock buffer; 19). In some instances, portions of rotaviral double-stranded RNA preparations were mixed and subjected to coelectrophoresis.

The Abney isolate of reovirus type 3 (ATCC VR-232) propagated in L929 cell cultures (ATCC CCL-1) was liberated from infected cells by sonication and clarified by centrifugation at 6,000 × *g* for 20 min at

4°C. Virus was then pelleted from clarified supernatant fluids by centrifugation at 69,000 × *g* for 90 min at 4°C, suspended in 2 ml of TSC buffer by sonication, and purified by ultracentrifugation in linear cesium chloride density gradients as described above. Double-stranded RNA was extracted from purified reovirus according to the procedures described for rotaviral double-stranded RNA extraction.

Direct extraction of rotaviral double-stranded RNA from specimens. The direct extraction procedure was essentially an adaptation of the method of Franklin (10) as modified by Morris and Dodds (18). Unless noted, all steps were performed at 4°C; silicone-coated glassware was used throughout. This procedure was initially performed by using 25 ml of specimen, but later extractions were conducted with 6 ml of specimen and using proportionately fewer volumes of the other reagents. The procedure performed with 6 ml of specimen is given. Approximately 6 ml of specimen was homogenized with an equal volume of extraction buffer (0.2 M glycine, 0.1 M Na_2HPO_4, 0.6 M NaCl, and 1% SDS, pH 9.5) at room temperature and clarified by centrifugation at 12,000 × *g* for 20 min. The supernatant fluid was then homogenized with 6 ml of water-saturated phenol and 6 ml of chloroform-pentanol (25:1, vol/vol), and the resultant emulsion was broken by centrifugation at 800 × *g* for 20 min. An occasional specimen yielded a homogenate so viscous that the emulsion failed to separate; in these instances, the homogenate was rehomogenized with sufficient additional extraction buffer to reduce the viscosity, and the emulsion was recentrifuged. The upper aqueous phase containing the nucleic acids was drawn off, and ethanol was added to it to a final concentration of 15% (vol/vol). To this solution was added 0.5 g of chromatographic cellulose fiber powder (CF11 powder; Whatman, Inc., Clifton, N.J.), and the mixture was stirred for 1 h. The mixture was then centrifuged at 800 × *g* for 20 min, and the cellulose pellet was suspended in 0.05 M tris(hydroxymethyl)-aminomethane buffer, pH 7.0, containing 0.1 M NaCl and 0.001 M disodium ethylenediaminetetraacetate (STE buffer), to which ethanol was added to a final concentration of 15% (vol/vol). This suspension was centrifuged as above, and the cellulose was resuspended in fresh STE buffer-ethanol (85:15, vol/vol) and centrifuged as before. The pelleted cellulose was resuspended in STE buffer-ethanol (85:15, vol/vol) and poured into a glass chromatographic column; the packed cellulose column was then washed at room temperature with approximately 70 ml of STE buffer-ethanol (85:15, vol/vol). Rotaviral double-stranded RNA was eluted from the cellulose column with STE buffer without ethanol; the eluate containing the rotaviral double-stranded RNA was detected by the Schlieren effect produced by the STE buffer without ethanol as it emerged from the column (visualization of this effect was facilitated by oblique illumination provided by a 100-W incandescent bulb mounted in a gooseneck lamp). Rotaviral double-stranded RNA was precipitated overnight from the collected eluate (approximately 10 ml) with sodium acetate and 67% ethanol as described for double-stranded RNA extracted from purified rotavirus. Precipitated double-stranded RNA was pelleted by centrifugation at 12,000 × *g* for 30 min at 0°C, dried under a stream of nitrogen, and suspended in 150 μl of Peacock buffer (rotaviral double-stranded RNA extracted from 25 ml of specimen was resuspended in 750 μl of Peacock buffer). Before electrophoresis, these preparations were heated and diluted in Peacock buffer according to the procedure described for double-stranded RNA preparations obtained from purified rotavirus.

Polyacrylamide-agarose gel electrophoresis. Composite 0.5% agarose (Seakem ME grade; Marine Colloids, Inc., Rockland, Maine)–2.5% acrylamide (Bio-Rad Laboratories, Richmond, Calif.) slab gels were prepared in a vertical electrophoresis cell (model 470; E. C. Apparatus Corporation, St. Petersburg, Fla.) similar to that of Raymond (21), maintained at constant temperature by coolant circulated through the cooling plates with an external constant-temperature circulator (Lauda K-2/RD; Brinkmann Instruments, Inc., Westbury, N.J.). Gels were prepared and electrophoresis was conducted by a modification of the method described by Peacock and Dingman (19). Agarose and acrylamide (19:1, acrylamide/bisacrylamide) were mixed at 37°C in Peacock buffer containing 0.03% (vol/vol) *N,N,N',N'*-tetramethylethylenediamine, and ammonium persulfate was then added to a final concentration of 0.05% (wt/vol) to effect polymerization. This solution was quickly cast into an electrophoresis cell precooled to 20°C and allowed to polymerize overnight at 20°C. Polymerized gels were then pre-electrophoresed at 200 V while the temperature of the cell was lowered to 0°C; after this, samples containing 20 μl of diluted rotaviral double-stranded RNA preparations mixed with 5 μl of 75% (wt/vol) sucrose–0.05% (wt/vol) bromphenol blue solution were introduced into the gel slots, and electrophoresis was continued at 200 V (approximately 35 mA) at 0°C for 5 h. Gels were stained at room temperature for 30 min with ethidium bromide (0.5 μg/ml) in 0.05 M tris(hydroxymethyl)aminomethane buffer, pH 7.8, containing 0.14 M NaCl, and the fluorescent double-stranded RNA bands manifested by transillumination with short-wave ultraviolet light (model C-61 transilluminator; Ultraviolet Products, San Gabriel, Calif.) were photographed with Polaroid type 665 film, using a red filter (Wratten 23A, Eastman Kodak Co., Rochester, N.Y.).

RESULTS

The electrophoretic migration pattern of a rotaviral double-stranded RNA preparation derived by the direct extraction procedure was qualitatively identical to the electrophoretic migration pattern obtained with double-stranded RNA extracted from rotavirus purified from the same specimen (Fig. 1). Consistent, reproducible electrophoretic migration patterns could be obtained with rotaviral double-stranded RNA preparations derived by the direct extraction procedure, even if the specimens varied considerably in their extraneous constituents. The reproducibility of the electrophoretic migration patterns produced with rotaviral double-

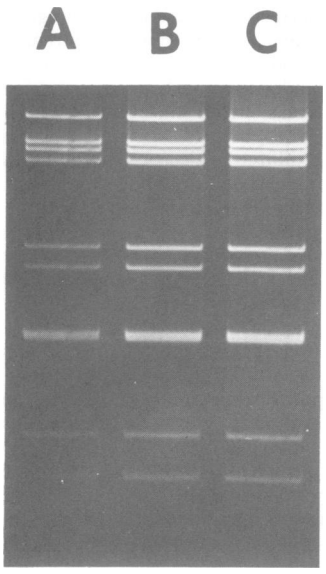

FIG. 1. *Comparison of the electrophoretic migration patterns of porcine rotaviral double-stranded RNA preparations derived by two methods. Lanes (A) Double-stranded RNA extracted from purified virus obtained from the small and large intestinal mucosa and contents pooled from four gnotobiotic pigs infected with the OSU isolate of porcine rotavirus; (B) coelectrophoresis of a mixture containing equal amounts of the double-stranded RNA preparations used in lanes A and C; (C) rotaviral double-stranded RNA extracted directly from the same specimen used in lane A.*

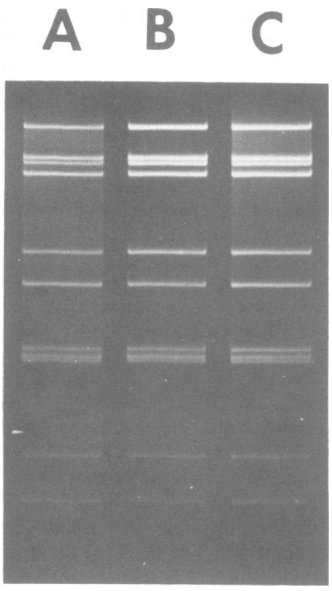

FIG. 2. *Comparison of the electrophoretic migration patterns of rotaviral double-stranded RNA preparations derived by direct extraction from specimens collected sequentially from a colostrum-deprived calf experimentally infected with bovine rotavirus (EM isolate). (A and B) Preparations derived from fecal specimens collected at 44 and 69 h post-inoculation, respectively; (C) preparation derived from the small and large intestinal mucosa and contents of the calf found dead at 81 h post-inoculation.*

stranded RNA preparations derived by direct extraction of specimens is exemplified by the identical electrophoretic migration patterns produced with rotaviral double-stranded RNA preparations derived by direct extraction of specimens collected sequentially from the same animal (Fig. 2) or with rotaviral double-stranded RNA preparations derived by direct extraction of specimens collected from different animals infected with the same rotavirus isolate (Fig. 3).

The electrophoretic migration patterns of rotaviral genomes could easily be distinguished from the electrophoretic migration pattern of the reoviral genome (Fig. 4 and 5b); under conditions used in this study, all 10 segments of the reoviral genome were not resolved. Preparations derived by the direct extraction of specimens containing porcine rotavirus-like virus produced electrophoretic migration patterns with 11 resolved segments (Fig. 5a). This electrophoretic migration pattern, however, differs from the typical rotaviral genome electrophoretic migration pattern in several aspects; the most conspicuous differences were that the loosely migrating couplet of segments 5 and 6 characteristic of the rotaviral genome electrophoretic migration pattern was replaced by three segments of slightly greater mobility, whereas the closely migrating rotaviral genome segments 7, 8, and 9 were replaced by two segments. Furthermore, segments 10 and 11 in the electrophoretic migration patterns of porcine rotavirus-like virus preparations had a greater electrophoretic mobility than the corresponding rotaviral genome segments 10 and 11.

Preparations derived by direct extraction of small and large intestinal contents and mucosal scrapings obtained from normal pigs and from diarrheic pigs infected with TGE virus, a mixture of calicivirus-like virus and 23-nm virus-like particles, or enterotoxigenic *E. coli* were negative for fluorescent bands after electrophoresis in polyacrylamide-agarose gels. Preparations derived by direct extraction of small and large intestinal contents and mucosal scrapings obtained from normal calves and from calves infected with enterotoxigenic *E. coli* or other microbial enteropathogens were similarly negative. Furthermore, fecal specimens collected from diarrheic calves infected with bovine coronavi-

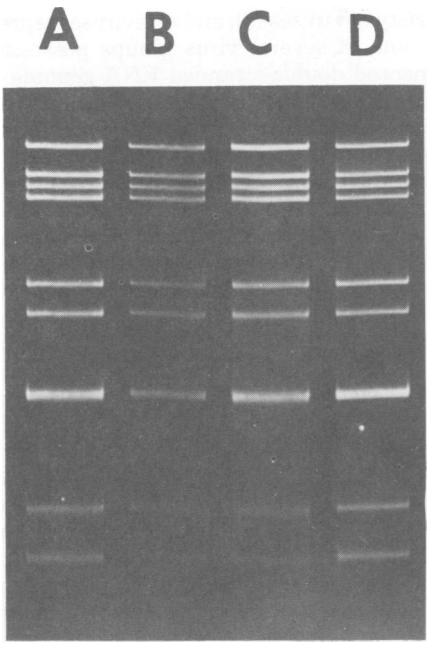

FIG. 3. *Comparison of the electrophoretic migration patterns of rotaviral double-stranded RNA preparations derived by direct extraction from specimens collected from four different calves experimentally infected with the same isolate of bovine rotavirus (NCDV). (A, C, and D) Preparations derived from fecal specimens collected from diarrheic colostrum-deprived calves; (B) preparation derived from small and large intestinal mucosa and contents of a diarrheic gnotobiotic calf.*

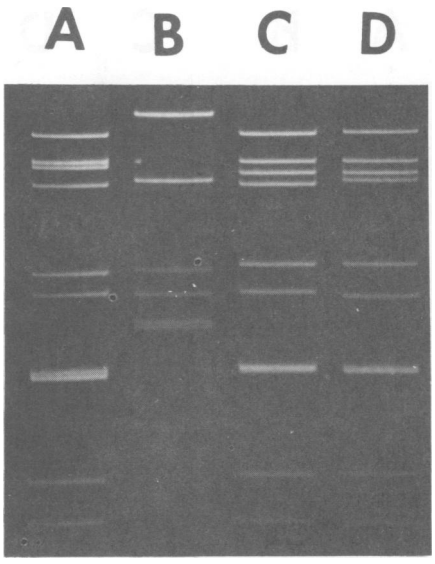

FIG. 4. *Comparison of the electrophoretic migration patterns of reoviral and rotaviral genomes. (A) Porcine rotavirus (SB 9/3/80 isolate); (B) reovirus type 3, Abney isolate; (C) bovine rotavirus (NCDV isolate); (D) bovine rotavirus (DB isolate). All double-stranded RNA preparations were obtained by extraction from purified virus.*

rus or from calves with undifferentiated diarrhea (but negative for rotavirus) gave preparations that were negative for fluorescent bands after polyacrylamide-agarose gel electrophoresis.

DISCUSSION

The procedure of extracting rotaviral double-stranded RNA directly from specimens provides a rapid, simple, reproducible method of obtaining rotaviral double-stranded RNA preparations suitable for electrophoretic analysis in polyacrylamide-agarose gels. Rotaviral double-stranded RNA derived from a specimen by this procedure is qualitatively identical to that extracted from purified rotavirus obtained from the same specimen.

The direct extraction procedure used in this study represents an adaptation of a cellulose chromatographic procedure (10) as modified by Morris and Dodds (18). Franklin (10) found that only double-stranded RNA binds to cellulose powder in significant quantities in the presence of 15% ethanol. Thus, the remaining unbound nucleic acids (single-stranded RNA, double-stranded deoxyribonucleic acid) can be readily removed from the preparation by thorough washing of the cellulose powder with buffer containing 15% ethanol. Double-stranded RNA relatively free of other nucleic acids can then be eluted from the cellulose powder simply by washing with buffer containing no ethanol. Variations of this method have previously been used to recover replicative intermediates of RNA-containing bacteriophage and plant viruses (6, 10, 11, 12) and viral double-stranded RNA from infected fungi (2, 18). To our knowledge, however, this method has not been previously applied to investigations of rotaviral double-stranded RNA.

Although intestinal and fecal specimens are often grossly contaminated with extraneous matter (undigested milk, digestive secretions, mucus, cellular debris, etc.), our experience during the past 2 years with the direct extraction of over 300 of these specimens has demonstrated that this procedure produces very few artifacts. Preparations derived by direct extraction of mucosal scrapings from small intestines with scant fluid contents, however, occasionally contained material that migrated as a diffuse band near the largest rotaviral double-stranded RNA segment (segment 1) and stained with ethidium bromide. A few other preparations contained

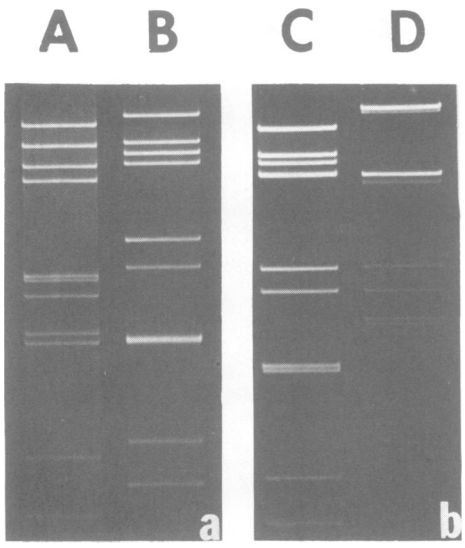

FIG. 5. *Comparison of the electrophoretic migration patterns produced by preparations derived by direct extraction of specimens containing rotavirus, reovirus, and rotavirus-like virus. Panel a: (A) preparation (presumably double-stranded RNA) derived from the small and large intestinal mucosa and contents of a gnotobiotic pig infected with porcine rotavirus-like virus; (B) preparation derived from a fecal specimen collected from a diarrheic colostrum-deprived calf infected with bovine rotavirus (NCDV isolate). Bovine rotaviral genome segments in lane B are numbered 1 through 11 according to decreasing molecular weight (from top to bottom); segments 7, 8, and 9 comigrate and appear as one fluorescent band. Panel b: (C) preparation derived from the small and large intestinal mucosa and contents from a gnotobiotic pig infected with porcine rotavirus (OSU isolate); (D) preparation derived from culture fluids harvested from L929 cell cultures infected with reovirus type 3 (Abney isolate).*

anomalous materials that appeared as numerous faintly fluorescing discrete bands scattered throughout the rotaviral double-stranded RNA electrophoretic migration pattern after staining. The nature of these contaminants is unknown, but their occurrence within a rotaviral double-stranded RNA preparation had little, if any, effect upon the formation of the normal genome electrophoretic migration pattern. Undoubtedly the successful application of this procedure to the direct extraction of rotaviral double-stranded RNA from intestinal or fecal specimens depends greatly upon the relative resistance of double-stranded RNA to hydrolysis by ribonucleases, since these specimens are likely to contain pancreatic and possibly other ribonucleases (3).

Fecal and intestinal specimens often contain a variety of viruses (8), and rotaviruses represent only one of several virus groups possessing a segmented double-stranded RNA genome (31). If the direct extraction procedure is used to derive rotaviral double-stranded RNA from these specimens, the possibility of other double-stranded RNA viruses contributing segments to the rotaviral genome electrophoretic migration pattern must always be considered. Reoviruses, for example, have a segmented double-stranded RNA genome and are frequently recovered from fecal specimens, but under the conditions used in this study, the electrophoretic migration pattern of the reoviral genome can be readily distinguished from the rotaviral genome electrophoretic migration pattern. The only bacteriophage known to possess a segmented double-stranded RNA genome is bacteriophage $\phi 6$ of the phytopathogenic bacterium *Pseudomonas phaseolicola* (17). This bacteriophage, however, cannot replicate at temperatures above 31°C (26) and should not be encountered within the mammalian intestinal tract. Direct extraction of specimens containing the newly recognized porcine rotavirus-like virus (25), however, yielded preparations (presumably double-stranded RNA, since the extraction procedure selects for this type of nucleic acid) that produce electrophoretic migration patterns similar, though not identical, to the characteristic rotaviral genome electrophoretic migration pattern. Unfortunately, these rotavirus-like virus specimens contained insufficient quantities of virus to permit virus purification and further, more definitive biochemical characterization of the viral genome. Consequently, these observations on the rotavirus-like viral genome electrophoretic migration pattern using preparations derived by the direct extraction procedure must be regarded as preliminary and await confirmation by electrophoretic analysis of nucleic acid extracted from purified virus. It is likely that the electrophoretic migration pattern of the rotavirus-like viral genome obtained from purified virus will be qualitatively identical to that observed with preparations derived by the direct extraction procedure because we have found that rotaviral genome electrophoretic migration patterns obtained by this procedure are qualitatively identical to those produced with double-stranded RNA extracted from the corresponding purified virus. The electrophoretic migration patterns produced by the rotavirus-like virus preparations had some similarities to the rotaviral genome electrophoretic migration pattern, but were sufficiently distinctive to be differentiated.

The procedure of extracting rotaviral double-

stranded RNA directly from intestinal and fecal specimens affords several advantages over those extraction procedures requiring purified rotavirus. First, it is considerably more rapid; since four to five specimens may be processed in 1 day and analyzed by electrophoresis the next, this procedure extends the application of rotaviral RNA analysis to a greater number of specimens. Second, steps involving high-speed centrifugation are eliminated, thereby obviating the need for an ultracentrifuge. Most important, however, was that this procedure frequently made it possible to derive sufficient rotaviral double-stranded RNA for electrophoretic analysis from specimens from which we were unable to obtain purified virus. Thus, this procedure permitted the electrophoretic characterization of the genomes of rotaviruses in specimens that previously would have been regarded as unsuitable for this purpose. Finally, sufficient rotaviral double-stranded RNA for numerous electrophoretic analyses could be extracted directly from a small volume of specimen. For example, most rotaviral double-stranded RNA preparations derived by direct extraction produced suitable electrophoretic migration patterns when diluted twofold or more; therefore, the 150 μl of preparation obtained from 6 ml of specimen could be used for a minimum of 15 electrophoretic assays.

The results of the present study demonstrate that rotaviral double-stranded RNA suitable for electrophoretic analysis can be extracted directly from fecal or intestinal specimens collected from diarrheic calves or pigs. This simplified procedure of obtaining rotaviral double-stranded RNA preparations should facilitate those studies requiring informative electrophoretic analysis of the rotaviral genome.

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