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Characterization of Full-Length and Polymerase Chain Reaction-Derived Partial-Length Gottfried and OSU Gene 4 Probes for Serotypic Differentiation of Porcine Rotaviruses†

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To determine the VP4 (P type) specificity of porcine rotaviruses, full- and partial-length gene 4 probes were produced from cloned Gottfried and OSU porcine rotavirus genomic segment 4 cDNAs. The gene 4 segments from the prototype Gottfried (VP7 serotype 4) and OSU (VP7 serotype 5) porcine rotavirus strains were selected for study because of their distinct P types and the occurrence of rotaviruses with similar serotypes among swine. Partial-length gene 4 cDNAs were produced and amplified by the polymerase chain reaction (PCR) and encompassed portions of the variable region (nucleotides 211 to 612) of VP8 encoded by genomic segment 4. The hybridization stringency conditions necessary for optimal probe specificity and sensitivity were determined by dot or Northern (RNA) blot hybridizations against a diverse group of human and animal rotaviruses of heterologous group A serotypes and against representative group B and C porcine rotaviruses. The PCR-derived gene 4 probes were more specific than the full-length gene 4 probes but demonstrated equivalent sensitivity. The Gottfried PCR-derived probe hybridized with Gottfried, SB2, SB3, and SB5 G serotype 4 porcine rotaviruses. The OSU PCR-derived probe hybridized with OSU, EE, A580, and SB-1A porcine rotaviruses and equine H1 rotavirus. Results of the hybridization reactions of the PCR-derived gene 4 probes with selected porcine rotavirus strains agreed with previous serological or genetic analyses, indicating their suitability as diagnostic reagents.

Group A rotaviruses are enteric pathogens of children, swine, and many other animal species (11, 21). The viral genome consists of 11 segments of double-stranded RNA which is surrounded by two capsid layers. Group A rotavirus serotypes were initially established by polyclonal antibody-based serum neutralization assays (13, 25). The serotypes defined by these assays were based on the antigenic specificities of VP7 (17, 18, 31, 54). Genetic and monoclonal antibody-based assays later determined that the neutralization specificities of rotaviruses were determined by two outer capsid proteins, VP4 and VP7, which are capable of eliciting neutralizing antibodies (40, 42, 51). Currently, at least 11 and potentially 14 serotypes of rotaviruses have been classified in human beings and animals on the basis of their VP7 or G type specificities (5, 6, 10, 53). Four G types infect swine, and other potential G types have been reported (1, 3, 36, 45).

The outer capsid protein VP4 is protease sensitive and is cleaved by trypsin into two polypeptides (VP5 and VP8) which play a major role in viral infectivity and virulence (9, 43). Serological analyses of rotavirus variants with monoclonal antibodies or bacterially expressed VP5 and VP8 subunit proteins with hyperimmune antisera have identified distinct neutralization regions located primarily on VP8 (30, 34). Studies on immunity and cross-protection have shown that VP4 may be as important as VP7 in eliciting rotavirus-neutralizing antibodies and heterotypic immunity (22, 24, 44). Genomic segment 4, coding for VP4, segregates independently of the genomic segment coding for VP7 (24). As a result, gene reassortment can occur, and interserotype bridging due to VP4 and VP7 has been reported for human and porcine isolates (23, 24, 39). Characterization of VP4 serotypes (P types) is as important as VP7 for the development of potential vaccines. Despite the importance of VP4, antigenic relationships among the VP4s of different porcine rotaviruses have only recently been investigated (16, 32, 33, 38).

Two assays have been developed for the direct detection and differentiation of porcine rotavirus G serotypes. One is based on the detection of G-type-specific epitopes with monoclonal antibodies in an enzyme-linked immunosorbent assay (37). The second assay involves hybridization of specific nucleic acid probes to differentiate rotavirus serotypes on the basis of differences in the nucleic acid sequences of the VP7-encoding gene (27, 46, 47). Variability of certain regions of the VP7-encoding gene has been associated with serotypic differences among rotavirus strains (8, 17). The advantage of nucleic acid hybridization is its potential for detecting variants which could be missed by monoclonal antibody-based assays (7, 32).

Molecular studies of genomic segment 4 have determined variable nucleic acid sequences located in the VP8-encoding region of VP4 (10, 34). The variable regions have been associated with serotypic differences among VP4 proteins (P types) (30, 34, 48). In this study, full- and partial-length VP4 probes were produced and investigated to determine their ability to differentiate genomic segment 4 among group A porcine rotaviruses. The specificities of the probes were tested against rotaviruses isolated from humans and animals.

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TABLE 1. Cell culture-adapted and gnotobiotic pig-passaged rotaviruses used for nucleic acid hybridization with Gottfried and OSU full- and partial-length gene 4 probes

Rotavirus strain	Origin of virus	Serotype (defined by G type) or group	Serotype defined by P type[a]
Wa[b]	Human	1	8 (1)
M37[c]	Human	1	6 (3)
DS-1[b]	Human	2	4 (2)
1076[c]	Human	2	6 (3)
M[b]	Human	3	—
McN13[c]	Human	3	— (3)
SA11	Simian	3	2
Rhesus	Simian	3	3
VA70[b]	Human	4	8 (1)
ST3[c]	Human	4	6 (3)
Gottfried	Porcine	4	6
SB-1A[d]	Porcine	4	7
SB2	Porcine	4	6[e]
SB3	Porcine	4	6[e]
SB5	Porcine	4	6[e]
OSU	Porcine	5	7
EE	Porcine	5	7[e]
A580	Porcine	5	7[e]
H1	Equine	5	7[e]
NCDV[f]	Bovine	6	1
B223	Bovine	10	—
—	Porcine	Group B	—
—	Porcine	Group C	—

[a] According to the tentative P serotyping scheme for animal and human rotaviruses proposed by Estes and Cohen (10). Serotypes in parentheses are according to the P serotyping scheme for human rotaviruses proposed by Gorziglia et al. (16). —, not determined.

[b] Symptomatic strains of human rotaviruses.

[c] Asymptomatic strains of human rotaviruses.

[d] Naturally occurring reassortant rotavirus: VP7 is similar to that of Gottfried porcine rotavirus; VP4 is similar to that of OSU porcine rotavirus.

[e] P serotype as determined using the Gottfried and OSU gene 4 PCR-derived probes in this study and classified according to the serotyping scheme of Estes and Cohen (10).

[f] NCDV, Nebraska calf diarrhea virus.

The objective of the study was to develop reagents suitable for the routine P typing of porcine rotavirus field samples.

MATERIALS AND METHODS

Viruses. Human and animal cell culture-adapted group A rotaviruses were propagated in MA104 cells as previously described (47). The rotavirus strains of known serotype (as defined by their VP7 specificities) used in the assays included the following: Wa and M37 (serotype 1); DS-1 and 1076 (serotype 2); rhesus rotavirus, SA11, McN13, and M (serotype 3); VA70, ST3, SB-1A, and Gottfried (serotype 4); OSU, EE, A580, and equine H1 (serotype 5); Nebraska calf diarrhea virus (serotype 6); and B223 (serotype 10) (Table 1). The human rotavirus strains selected for this study possessed three of the four VP4 gene alleles currently documented among human rotaviruses (15). They included (i) the symptomatic human rotaviruses Wa, M, and VA70, which represent genetic group 1; (ii) the symptomatic human rotavirus DS-1, which represents genetic group 2; and (iii) the asymptomatic human rotaviruses M37, 1076, McN13, and ST3, which represent genetic group 3. The naturally occurring porcine rotavirus reassortant SB-1A was selected for study because of its dual serotype specificities (23, 24): its VP7 protein is similar to that of Gottfried rotavirus (G serotype 4), while its VP4 is similar to that of OSU rotavirus (G serotype 5).

Several porcine rotaviruses were propagated by passage through gnotobiotic pigs (49). The animal-passaged rotaviruses used were as follows: Gottfried, SB2, SB3, SB5, and SB-1A rotaviruses (G serotype 4); OSU rotavirus (G serotype 5); porcine serogroup B rotavirus; and porcine serogroup C rotavirus (Table 1).

Nucleic acid extraction and quantitation. Rotavirus double-stranded RNA (dsRNA) was extracted from virus-infected MA104 cells and intestinal samples by modifications of procedures previously described (46). Briefly, virus-infected cell culture supernatants and diluted intestinal samples were clarified by centrifugation for 10 to 20 min at 430 × *g* or 1,200 × *g*, respectively. Rotaviruses in cell culture supernatants were semipurified by centrifugation at 122,000 × *g* for 3 h through a 40% sucrose cushion. Sodium dodecyl sulfate and sodium acetate were added to the clarified or semipurified virus preparations to concentrations of 1.0% and 0.1 M, respectively. The virus suspensions were deproteinized with phenol-chloroform, and rotavirus dsRNA was precipitated with ethanol overnight at −20°C. The precipitated dsRNA was suspended in sterile water treated with diethyl pyrocarbonate (Sigma Chemical Co., St. Louis, Mo.) and stored at 4°C.

Nucleic acid was extracted from negative-control samples, which included mock-infected MA104 cells and intestinal samples from rotavirus-negative gnotobiotic pigs. The procedures described above for rotavirus dsRNA extraction were used for extraction of negative controls. Positive controls, which consisted of recombinant plasmids containing Gottfried or OSU genomic segment 4 cDNA inserts, were used. Positive-control samples were extracted by the procedures described below for preparation of ^{32}P-labeled probes.

The concentration of nucleic acid in rotavirus-positive and -negative samples was determined in a spectrophotometer at 260 nm. The absorbance readings of rotavirus-positive samples were used as estimates of rotavirus dsRNA concentrations for dot hybridization experiments. The spectrophotometer measurements did not discriminate between rotavirus dsRNA and nucleic acid from cells or other sources. In Northern (RNA) blot experiments, the amount of rotavirus dsRNA used was adjusted on the basis of A_{260} readings and the staining intensity of rotavirus RNA segments when visualized in polyacrylamide gels.

RNA electrophoresis and Northern blot. The presence or absence of rotavirus RNA in cell culture preparations, intestinal samples, and the negative-control samples (nucleic acid extracted from mock-infected MA104 cells and the intestinal contents of rotavirus-negative pigs) was confirmed by polyacrylamide gel electrophoresis. The dsRNA was resolved in 12.5% polyacrylamide separation gels (1.5 mm thick) by the discontinuous buffer system of Laemmli (29). The silver staining method of Herring et al. (20) was used for detection of rotavirus dsRNA in extracted samples. Rotavirus dsRNA for Northern blot studies was electrophoresed in 10% polyacrylamide gels (0.75 mm thick) and stained with ethidium bromide. The dsRNA segments were electrophoretically transferred to nylon membranes (Nytran; Schleicher & Schuell, Keene, N.H.) as previously described (52).

Preparation of ^{32}P-labeled probes. Plasmid vectors pTZ18R containing full-length cDNA copies of Gottfried and OSU porcine rotavirus gene 4 segments were used (16, 41). The DH5α strain of *Escherichia coli* containing the recombinant plasmids was cultivated in medium containing ampicillin. Extraction of the recombinant plasmids from cleared lysates of *E. coli* was performed by the procedure of

Birnboim and Doly (2). Purification of the plasmids and excision of the genomic segment 4 cDNA inserts were performed as previously described (47), except *Bam*HI was used for excision of the cloned inserts. Probes were prepared and labeled by using nick translation and ^{32}P-dCTP (47).

Two partial-length gene 4 cDNAs were produced and amplified by using specific oligonucleotide primers and the polymerase chain reaction (PCR) in a DNA thermal cycler (Perkin-Elmer Cetus Corp., Norwalk, Conn.). The partial-length cDNAs encompassed portions of the variable region (nucleotides 213 to 612) in genomic segment 4, which encodes VP8 (10, 14). Purified recombinant plasmids containing Gottfried and OSU genomic segment 4 cDNA inserts were used as templates. The primers used for production of the partial-length Gottfried gene 4 segment were 5′CCATAT CAGCCAACGAGT3′, complementary to nucleotides 211 to 228, and 5′TTACTACTTCTACATCAGGT3′, complementary to nucleotides 607 to 588. The partial-length OSU gene 4 segment was produced with primers 5′CATACCAACCAA CCACTTTC3′ and 5′TGATGTCATATTTACTGTGT3′, complementary to nucleotides 212 to 231 and 612 to 593, respectively. The PCR was terminated after 30 cycles of denaturation (94°C for 1 min), primer annealing (42°C for 1.5 min), and primer extension (72°C for 3 min). To confirm the production of cDNA of the appropriate length, the PCR products were analyzed by agarose gel electrophoresis and compared with molecular size markers following ethidium bromide staining. Fragments of the expected size were purified by excision from 1% low-melting-point agarose (Bethesda Research Laboratories, Gaithersburg, Md.) gels and extracted with phenol (50). Partial-length ^{32}P-dCTP-labeled PCR-derived probes were prepared from purified PCR products as described for full-length genomic segment 4 cDNA inserts.

Nucleic acid sample preparation and hybridization conditions. Nucleic acid samples for dot hybridizations were heat denatured and dotted onto nylon membranes (Zeta Probe; Bio-Rad Laboratories, Richmond, Calif.) (46). The samples were applied to the membranes in volumes of 5 μl or less, and sample application was repeated when necessary until the desired quantity was applied. Samples for dot and Northern blot hybridizations were fixed to the nylon membranes by baking for 0.5 and 1 h, respectively, at 80°C in a vacuum oven (National Appliance Company, Tualatin, Oreg.) and stored at 4°C.

The hybridization procedure, solutions, and final wash steps were performed as previously described (46), with minor modifications. Hybridization temperatures (37 to 52°C) and formamide concentrations (25 to 50%) were varied to determine the optimum hybridization conditions for full- and partial-length gene 4 probes. The hybridization stringency conditions necessary for optimal probe specificity and sensitivity were determined by visual inspection of the intensity and specificity of the reactions. Optimal stringency conditions were determined to be 42°C and 50% formamide concentration for the full-length Gottfried and OSU gene 4 probes and the Gottfried PCR-derived probe. The optimal stringency conditions for the OSU PCR-derived probe were 42°C and 40% formamide concentration. Approximately 4×10^6 cpm of denatured probe was added per 10 ml of hybridization solution.

Following hybridizations and autoradiography, disassociation of nucleic acid probes from target sequences on selected nylon membranes was performed according to the manufacturer's instructions (Bio-Rad Laboratories). The nucleic acid samples fixed on nylon membranes were then rehybridized with a second probe by the hybridization procedures described above.

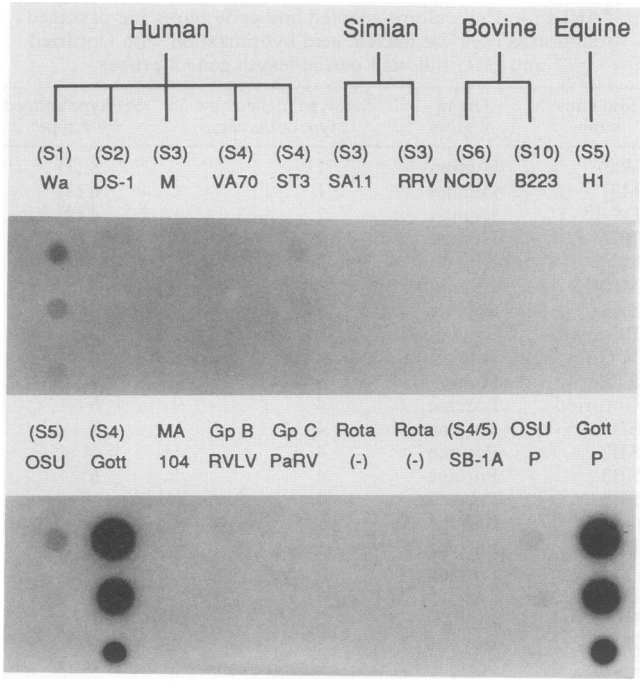

FIG. 1. Dot hybridization of the full-length Gottfried gene 4 probe with human and animal rotaviruses. The G and G/P serotype designations of the group A rotaviruses are shown in parentheses above the strain designations. Nucleic acid samples were diluted fivefold and applied to the membranes as follows: (i) cell culture-adapted group A rotaviruses (human, simian, bovine, equine, and porcine OSU and Gottfried [Gott]) and mock-infected MA104 cells, applied in amounts from 500 to 20 ng; (ii) OSU and Gottfried recombinant plasmids (OSU P and Gott P, respectively) applied in amounts from 250 to 10 ng; and (iii) nucleic acid extracted from the intestinal contents of rotavirus-infected gnotobiotic pigs, group B rotavirus (Gp B RVLV), group C rotavirus (Gp C PaRV), the SB-1A group A rotavirus, and rotavirus negative [Rota (−)] gnotobiotic pigs, applied in amounts from 1,000 to 40 ng.

RESULTS

Specificities of the full-length Gottfried and OSU gene 4 probes. The specificity of the Gottfried probe with rotaviruses of human and animal origin was tested by dot hybridization (Fig. 1). The Gottfried probe produced strong hybridization signals with the homologous Gottfried cell culture-propagated rotavirus and recombinant plasmid and a weak hybridization signal with the ST3 human rotavirus. Moderate to weak hybridization signals were also observed, however, with the Wa strain of human rotavirus, the OSU strain of porcine rotavirus, and the recombinant plasmid containing an OSU genomic segment 4 cDNA insert. Hybridization was not observed with nucleic acid extracted from other human and animal group A or porcine group B and C rotaviruses or with the negative controls (mock-infected MA104 cells and rotavirus-negative intestinal samples).

Hybridization reactions of the OSU gene 4 probe with human and animal rotavirus strains are shown in Fig. 2. The OSU probe produced strong hybridization signals with OSU porcine rotavirus and recombinant plasmid and with equine H1 rotavirus. Weak hybridization signals were observed

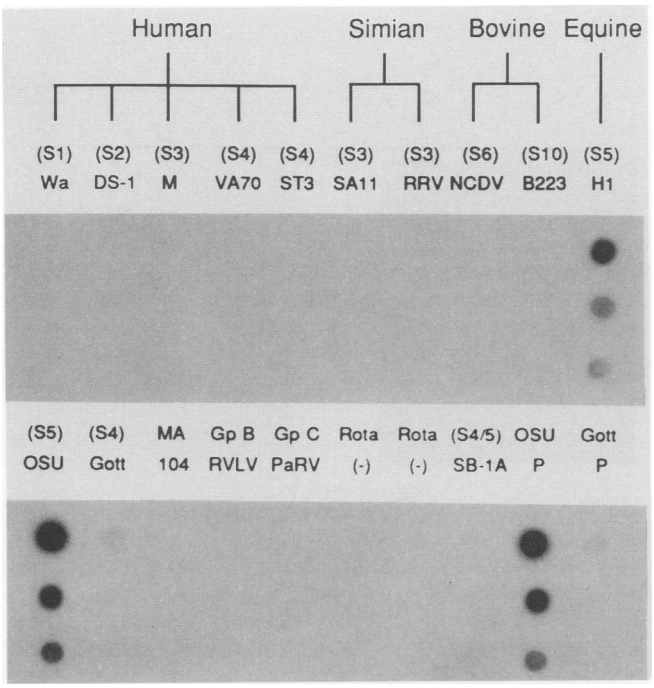

FIG. 2. Dot hybridization of the full-length OSU gene 4 probe with human and animal rotaviruses. The samples probed were the same as those in Fig. 1.

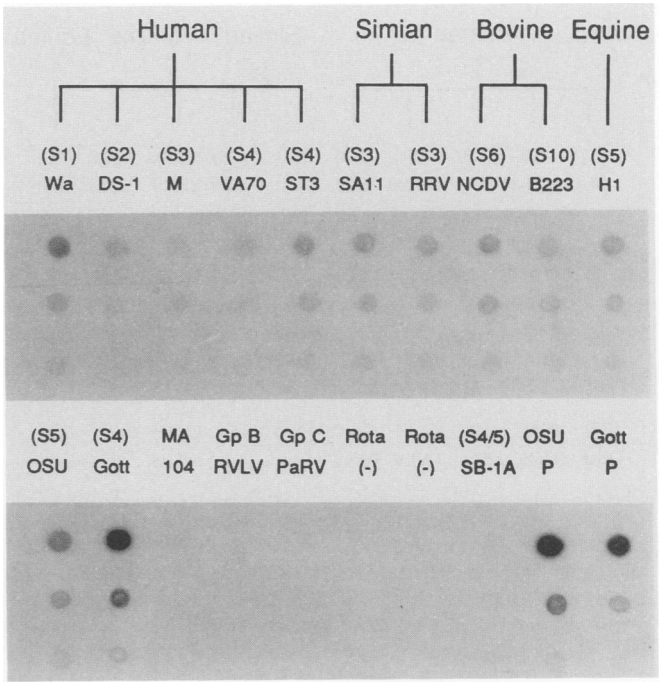

FIG. 3. Rehybridization of the membrane shown in Fig. 1 with a full-length Gottfried gene 9 probe. The full-length Gottfried gene 4 probe was stripped from the nylon membrane as described in the text. The membrane was reprobed with a Gottfried gene 9 probe under low-stringency conditions (42°C, 0% formamide, and 5× SSC) to confirm the fixation of rotavirus dsRNA samples on the membrane.

with SB-1A animal-passaged rotavirus, Gottfried cell culture-propagated rotavirus, and the Gottfried recombinant plasmid. Positive results were not observed with other rotavirus specimens and the negative controls.

The presence of rotavirus nucleic acid on the membranes was confirmed by disassociation of the Gottfried gene 4 probe followed by hybridization of the membrane with a full-length Gottfried gene 9 probe under low-stringency conditions (42°C, 0% formamide, and 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]) (Fig. 3). Previous studies in our laboratory have shown this probe to be cross-reactive with heterologous serotypes of rotaviruses under low-stringency conditions (47) as documented in Fig. 3.

Serological and deduced amino acid sequence analyses of the VP4 polypeptide of Gottfried porcine rotavirus and the asymptomatic human rotaviruses have indicated a high degree of homology (16). We investigated the homology and the rotavirus gene segment specificity of the Gottfried gene 4 probe with the asymptomatic human rotaviruses of G serotypes 1 to 4 by Northern blot hybridization (Fig. 4). The Gottfried probe produced moderate to strong hybridization signals with the genomic segment 4 of Gottfried porcine rotavirus and the asymptomatic human rotaviruses M37 (G serotype 1), 1076 (G serotype 2), McN13 (G serotype 3), and ST3 (G serotype 4). Comparatively weak hybridization signals were observed with the symptomatic human rotaviruses Wa and VA70, while no hybridization signal was observed with OSU porcine rotavirus.

Specificities of the partial-length Gottfried and OSU gene 4 PCR-derived probes. The specificity of the Gottfried probe with rotaviruses of human and animal origin is shown in Fig. 5. The Gottfried PCR-derived probe was more specific than the full-length Gottfried probe and hybridized only with the homologous Gottfried porcine rotavirus and recombinant plasmid. Hybridization was not observed with other human and animal rotavirus samples, the OSU recombinant plasmid, and the negative controls.

Hybridization reactions of the OSU PCR-derived probe

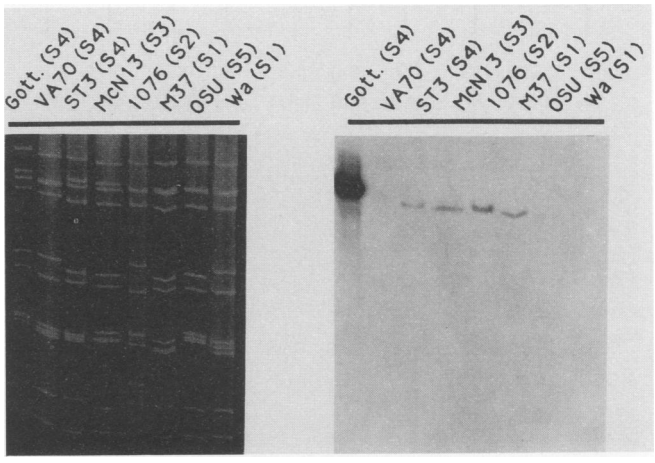

FIG. 4. Northern blot hybridization of the full-length Gottfried gene 4 probe. The G serotype designations of the group A rotaviruses are shown in parentheses following the strain designations. (Left) dsRNA electrophoretic patterns of eight group A human and animal rotaviruses resolved in a 10% polyacrylamide gel and stained with ethidium bromide; (right) autoradiogram depicting the hybridization of the Gottfried gene 4 probe with the eight group A rotaviruses following electrophoretic transfer of the rotavirus RNA to a nylon membrane.

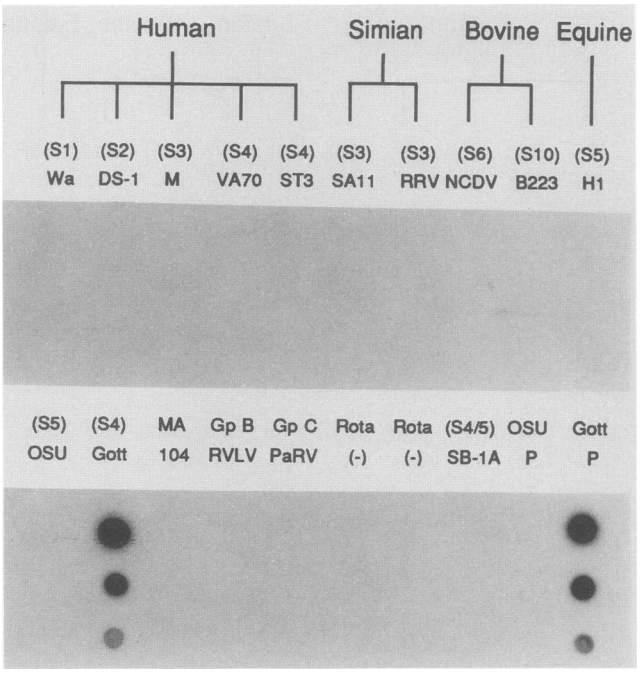

FIG. 5. Dot hybridization of the Gottfried PCR-derived gene 4 probe with human and animal rotaviruses. The samples probed were the same as those in Fig. 1.

with human and animal rotavirus strains also showed a higher degree of specificity in comparison with the full-length OSU probe (Fig. 6). The OSU PCR-derived probe produced strong hybridization signals with OSU cell culture-propagated rotavirus, OSU recombinant plasmid, and

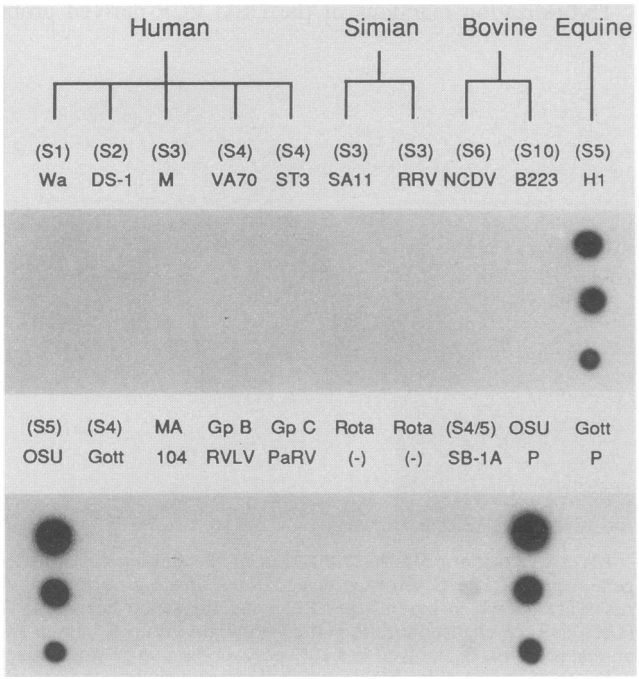

FIG. 6. Dot hybridization of the OSU PCR-derived gene 4 probe with human and animal rotaviruses. The samples probed were the same as those in Fig. 1.

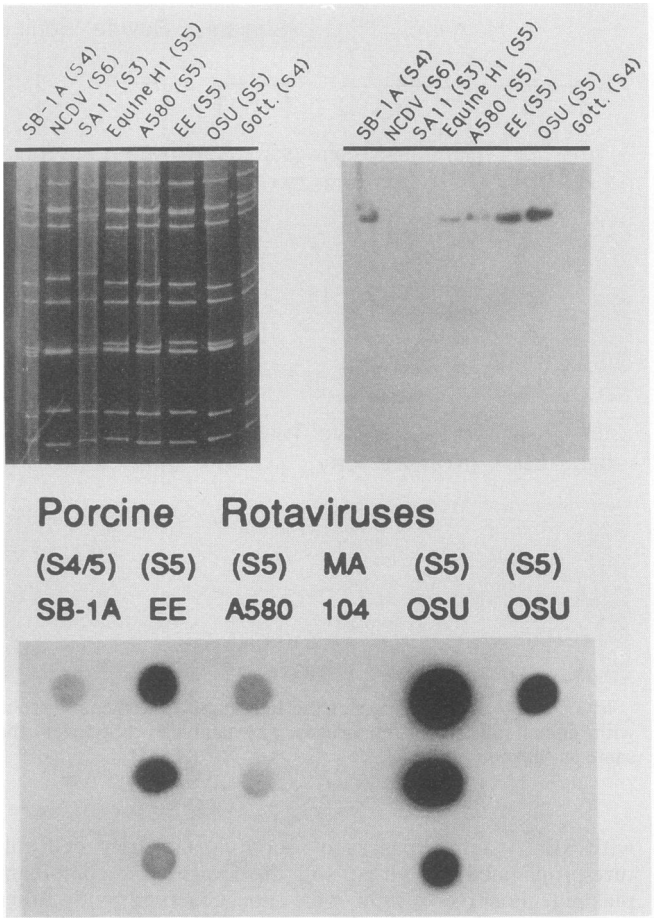

FIG. 7. Northern and dot hybridizations of the OSU PCR-derived gene 4 probe. The G and G/P serotype designations of the group A rotaviruses are shown in parentheses following or above the strain designations. (Top left) dsRNA electrophoretic patterns of eight group A animal rotaviruses resolved in a 10% polyacrylamide gel and stained with ethidium bromide; (top right) autoradiogram depicting the hybridization of the OSU probe with the eight group A rotaviruses following electrophoretic transfer of the rotavirus RNA to a nylon membrane; (bottom) dot hybridization of the OSU probe with cell culture-adapted porcine rotaviruses and nucleic acid extracted from mock-infected MA104 cells. The amount of sample applied was the same as in Fig. 1, except for the OSU sample on the far right, which was applied at a single undetermined amount to aid in blot orientation.

equine H1 rotavirus. A weak hybridization signal was also observed with the homologous SB-1A animal-passaged rotavirus. Hybridization signals were not observed with other human and animal rotavirus samples (including Gottfried cell culture-propagated rotavirus and recombinant plasmid) and the negative controls.

The sensitivity of the OSU PCR-derived probe for the detection of other strains of animal rotaviruses with OSU-like VP4 segments and the specificity of the probe for genomic segment 4 were examined by dot and Northern blot hybridization (Fig. 7). The OSU probe hybridized with the genomic segment 4 of the cell culture-adapted OSU, EE, A580, and SB-1A strains of porcine rotaviruses and the equine H1 rotavirus by Northern blot hybridization. Similar hybridization reactivities were observed with these porcine rotavirus strains by dot hybridization. As in the dot hybrid-

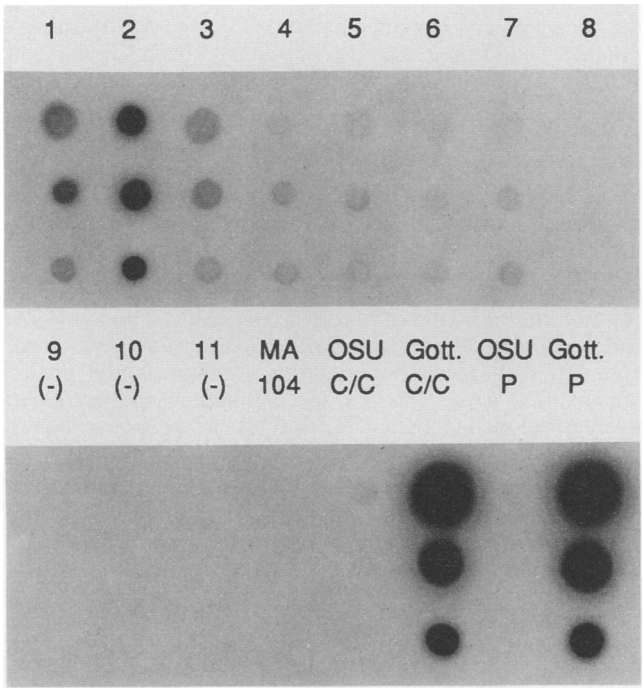

FIG. 8. Dot hybridization of the Gottfried PCR-derived gene 4 probe with gnotobiotic pig-passaged G serotype 4 porcine rotaviruses. Samples 1 to 3, Gottfried rotavirus; sample 4, SB2 rotavirus; samples 5 and 6, SB3 rotavirus; sample 7, SB5 rotavirus; sample 8, SB-1A rotavirus; samples 9 to 11, nucleic acid extracted from the intestinal contents of rotavirus-negative gnotobiotic pigs; MA104, nucleic acid extracted from mock-infected MA104 cells; OSU C/C and Gott. C/C, OSU and Gottfried cell culture-adapted rotaviruses, respectively; OSU P and Gott. P, OSU and Gottfried recombinant plasmids, respectively. Nucleic acid samples were diluted fivefold and applied to the membranes as follows: samples 1 to 11, applied in amounts from 1,000 to 40 ng; all other samples, applied in amounts from 250 to 10 ng.

izations in Fig. 6, positive signals were not observed by Northern blot hybridization with the NCDV, SA11, and Gottfried strains of cell culture-propagated rotaviruses (Fig. 7).

Dot hybridization reactions of the Gottfried PCR-derived probe with animal-passaged G serotype 4 porcine rotaviruses possessing Gottfried-like VP4 segments are shown in Fig. 8. The Gottfried probe hybridized with the animal-passaged Gottfried (samples 1 to 3), SB2 (sample 4), SB3 (samples 5 and 6), and SB5 (sample 7) strains of G serotype 4 porcine rotaviruses and with the positive controls (Gottfried cell culture-propagated rotavirus and recombinant plasmid). Weak hybridization signals were observed with the OSU control samples (OSU cell culture-propagated rotavirus and recombinant plasmid); however, the signals were not as strong as those observed for the animal-passaged G serotype 4 rotaviruses and Gottfried positive controls. Hybridization was not observed with the animal-passaged G serotype 4 reassortment rotavirus SB-1A (sample 8) or with the negative controls (nucleic acid extracted from mock-infected MA104 cells and the intestinal contents from three rotavirus-negative gnotobiotic pigs, samples 9 to 11).

Hybridization reactions of the OSU PCR-derived probe with animal-passaged OSU rotaviruses were similar to the homologous reactions observed with the Gottfried PCR-derived probe. However, cross-reactivity was not observed with the Gottfried cell culture-propagated rotavirus and recombinant plasmid (data not shown).

DISCUSSION

In this study, full- and partial-length gene 4 probes were produced from Gottfried and OSU genomic segment 4 cDNA. The probes were prepared for use as potential diagnostic reagents for the differentiation of the genomic segment 4 of porcine rotaviruses. To determine the hybridization stringency conditions necessary for optimal probe specificity and sensitivity, the probes were tested against a diverse group of human and animal rotaviruses of heterologous group A serotypes and against representative group B and C porcine rotaviruses.

The full-length Gottfried and OSU gene 4 probes produced strong hybridization signals with the homologous porcine rotaviruses and recombinant plasmids containing Gottfried or OSU gene 4 cDNA inserts. Moderate to weak hybridization signals were observed, however, with some heterologous rotavirus strains. In general, rotaviruses of homologous P serotypes could be differentiated from heterologous P serotypes of rotaviruses by using cell culture-adapted rotavirus strains in the dot hybridization assays. The heterologous hybridization signals, however, are a potential source of problems for differentiating low-titer rotaviruses which give weak homologous reactions from high-titer rotaviruses which give strong heterologous reactions in field samples. Consequently, the partial-length PCR-derived gene 4 probes, which were more specific and equally sensitive, were better candidates for use as diagnostic reagents. Hybridization signals were not observed with either full- or partial-length gene 4 probes with group B or C rotaviruses.

Hybridization reactions of the full-length Gottfried gene 4 probe with human and animal rotaviruses produced moderate to weak hybridization signals with the asymptomatic human rotaviruses (M37, 1076, McN13, and ST3) in dot or Northern blot hybridization assays. These results agreed with previous deduced amino acid sequence comparisons indicating a relatively high degree of homology (87.1 to 88%) between the VP4s of these rotavirus strains (16). Unexpected hybridization signals were observed with the heterologous symptomatic human rotavirus Wa and the porcine rotavirus OSU in dot hybridizations. Comparisons of the deduced amino acid sequences of VP4 between Gottfried rotavirus and the symptomatic human rotaviruses (e.g., Wa) have revealed 77.5 to 77.8% amino acid sequence homology (16). Similar comparisons between the Gottfried and OSU porcine rotaviruses have determined 72.1% VP4 amino acid sequence homology (16). A possible explanation for the cross-reactivity observed with the Wa and OSU rotavirus strains was revealed by hybridization of one membrane a second time with a full-length Gottfried gene 9 probe at low stringency (Fig. 3). Although attempts were made to dot equivalent amounts of rotavirus dsRNA based on A_{260} readings, differences in rotavirus titers in cell culture preparations resulted in the dotting of a larger quantity of Wa and OSU rotavirus RNA. The heterologous hybridization signals observed with these two rotavirus strains may have been due to the larger quantity of rotavirus RNA dotted and interactions of the conserved regions of the full-length Gottfried gene 4 probe with the OSU and Wa rotavirus RNA samples. This conclusion is supported by results from the Northern blot hybridization studies. In this assay, the amount of rotavirus RNA used was based on A_{260} readings and the intensity of ethidium bromide-stained rotavirus RNA visu-

alized in polyacrylamide gels. Hybridization reactions of the full-length Gottfried gene 4 probe with rotavirus RNA preparations of approximately equivalent staining intensity resulted in moderate to strong hybridization signals with the genomic segment 4 of Gottfried porcine rotavirus and the asymptomatic human rotaviruses, comparatively weak hybridization signals with the symptomatic human rotaviruses Wa and VA70, and no hybridization signals with OSU porcine rotavirus.

The hybridization of both full-length and PCR-derived OSU gene 4 probes with the cell culture-propagated G serotype 5 equine H1 rotavirus was not expected, since previous dot hybridization studies by our laboratory demonstrated hybridization of an OSU gene 9 probe with equine H1 rotavirus (47). These results indicate shared P and G serotype specificities, which further corroborates previous serological studies indicating shared neutralization specificities between G serotype 5 porcine rotaviruses and equine H1 rotavirus (25, 26). The genetic homology shared between other RNA segments of these two rotaviruses has not been determined. Equine rotaviruses with G serotype 5 specificities have rarely been detected in serological surveys of horses in the United States and England (4, 19). Equine H1 rotavirus, originally isolated from a foal in England, may represent a unique laboratory strain.

The Gottfried and OSU PCR-derived gene 4 probes were designed by using specific oligonucleotide primers to encompass areas of major sequence diversity among rotavirus serotypes in the VP8-coding region and to eliminate large areas of conserved nucleic acid sequences. Although hybridization results with the PCR-derived probes were more specific than those with the full-length gene 4 probes, equivalent hybridization signals were observed with the homologous porcine rotaviruses. Dot hybridizations of the Gottfried PCR-derived gene 4 probe with the pig-passaged G serotype 4 porcine rotaviruses produced hybridization signals with the SB2, SB3, and SB5 strains of rotavirus. These results paralleled previous dot hybridization studies in our laboratory indicating sequence homology between the VP7 coding genes of Gottfried rotavirus and the SB2, SB3, and SB5 rotavirus strains (46). Although the VP4 and VP7 coding genes of the SB2, SB3, and SB5 strains of rotavirus are similar to those of Gottfried rotavirus and indicate shared P and G serotype specificities, serological analyses have indicated that these porcine rotavirus strains are not identical (3, 25, 28).

The dot hybridization of the PCR-derived Gottfried gene 4 probe, in contrast to that of the full-length gene 4 probe, did not produce a hybridization signal with the asymptomatic human rotavirus ST3. Comparisons of the deduced amino acid sequence encoded by the PCR-derived Gottfried gene 4 cDNA with the corresponding region of the related asymptomatic human rotavirus 1076 indicated an amino acid homology of only 70%. The lower percentage of amino acid homology suggests a lower nucleic acid homology between the PCR-derived gene 4 probe and the asymptomatic human rotaviruses, which could account for this change in probe reactivity.

The ability of the OSU PCR-derived gene 4 probe to detect porcine rotaviruses with similar gene 4 segments was demonstrated by dot and Northern blot hybridizations with SB-1A, EE, A580, and OSU cell culture-adapted porcine rotaviruses. These results confirmed previous RNA-RNA hybridization studies indicating a high degree of homology between the gene 4 segments of OSU, EE, and SB-1A porcine rotaviruses (12, 35). Although genetic relationships between the gene 4 segments of the OSU and A580 strains of porcine rotavirus have not previously been established, serological studies in our laboratory have indicated cross-neutralization (unpublished data). The OSU PCR-derived probe was effective in the detection of OSU rotavirus extracted from intestinal contents of infected gnotobiotic pigs in addition to the cell culture-propagated rotavirus strains.

Extensive characterization of the partial-length PCR-derived OSU and Gottfried gene 4 probes developed in this study has shown them to be specific and sensitive reagents for the differentiation of porcine rotaviruses. The hybridization reactivity of the probes with porcine rotaviruses corresponded with results of previous serological and genetic studies. The gene 4 probes, in conjunction with our previously developed Gottfried and OSU gene 9 probes, currently provide a full complement of reagents for determining the prevalence of the Gottfried and OSU P and G serotypes among field specimens. The analysis of rotaviruses from swine herds with both gene 4 and gene 9 probes should provide information for determining which vaccine strains in relation to P and G neutralization specificities should be developed and administered for controlling rotavirus diarrheal disease. An additional benefit will be the capability to detect new or previously unrecognized P or G rotavirus serotypes in swine herds. Investigations are currently under way in our laboratory to develop nonradioactive detection procedures for the potential adaptation of these reagents for use in diagnostic laboratories.

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