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Serotypic Differentiation of Rotaviruses in Field Samples from Diarrheic Pigs by Using Nucleic Acid Probes Specific for Porcine VP4 and Human and Porcine VP7 Genes†

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Of 216 fecal and intestinal samples collected from nursing or weaned diarrheic pigs in the United States and Canada, 57 were identified as group A rotavirus positive by RNA electrophoresis and silver staining. Fifty-seven and 52 rotavirus-positive samples were analyzed by hybridization with Gottfried and OSU PCR-derived gene 9 and 4 probes, respectively. Only 17 samples were identified with either homologous VP4 (P)- or VP7 (G)-coding genes or both. One rotavirus identified as G4 and P7 was similar to the previously characterized interserotype rotavirus, SB-1A. Additional hybridization analyses were performed with PCR-derived probes prepared from gene 9 cDNA of the human rotaviruses Wa (G1), DS-1 (G2), and P (G3) and of the porcine rotavirus YM (G11). Eleven of 52 samples collected and analyzed from swine in Ohio, California, and Nebraska were identified as G11. No samples with G1-, G2-, or G3-type specificities were detected among the 25 of 57 rotavirus-positive samples analyzed with human rotavirus-derived probes. Further investigations with a PCR-derived gene 4 probe prepared from porcine rotavirus YM revealed hybridization specificities similar to those of the OSU gene 4 probe.

Rotaviruses are double-stranded RNA viruses that are intestinal pathogens of human infants and the young of many animal species. In swine as in other animal species, rotaviruses cause intestinal malabsorption resulting in diarrhea and sometimes death (3). Rotaviral diarrhea occurs primarily in nursing and newly weaned pigs.

Serotypic characterization of group A rotaviruses is based on neutralization determinants located on two outer capsid proteins, VP4 (a protease-sensitive protein that determines the P-serotype specificity) and VP7 (a glycoprotein that determines the G-serotype specificity). Genes coding for the P and G neutralization determinants may segregate independently in dual infections. Evidence for this has been found in serological and hybridization studies of a porcine rotavirus field isolate (SB-1A) with mixed serotype specificities, which was originally isolated by our laboratory (23a) and characterized by Hoshino et al. (9) and Midthun et al. (15), and of a porcine isolate with a similar mixed serotype specificity described in a report from Australia (17).

Two G-serotype porcine rotaviruses have been characterized in the United States (3, 25). These viruses, the prototype Gottfried (G4, P5) and OSU (G5, P7) strains, were initially reported in 1984 and 1977, respectively. Rotaviruses with similar G-serotype specificities have been detected in swine in several other countries (10, 14). Recently, two other G serotypes of rotavirus were isolated from swine and characterized. These include the YM rotavirus (G11) isolated in Mexico and several strains of G3 rotaviruses isolated in Australia (1, 16). Serological evidence for G11 (reported in Venezuela) and G3 (reported in Thailand and Argentina) rotavirus infections in swine exists (2, 12, 19). Genetic and serological analyses of the YM rotavirus confirmed that its G-serotype specificity is distinct from the specificities of other known animal and human rotaviruses (23), while the deduced amino acid sequence encoded by its gene segment 4 (P type) is similar to that of the OSU rotavirus (13). Other serotypes of rotavirus have been detected in swine but have not been fully characterized.

Information related to the serotype diversity and prevalence of rotaviruses in swine has direct implications for the development of effective vaccines. The present porcine rotavirus vaccines in the United States contain serotype G4 and G5 porcine rotavirus strains (27). Investigators have reported variable efficacies for porcine rotavirus vaccines (8, 27). Although efforts are being made to design rotavirus vaccines that offer heterotypic protection (11), most current rotavirus vaccine strategies have been directed toward the induction of homotypic immunities to rotavirus serotypes prevalent in a particular area (18).

In this study, the diversity of P- and G-type specificities of rotaviruses in fecal or intestinal contents of diarrheic pigs within the United States and to a limited extent from Canada was investigated. The type specificities were determined with cDNA probes developed in this study and previous studies and directed against the genes coding for VP4 and VP7. Primary emphasis was placed on probes prepared from the Gottfried (G4, P6) and OSU (G5, P7) strains of porcine rotaviruses. Additional cDNA probes were prepared from

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the VP7-coding genes of the human rotaviruses Wa (G1), DS-1 (G2), and P (G3) and the porcine rotavirus YM (G11). These probes were included to determine whether rotaviruses with G-type specificities reported in swine outside the United States could be detected.

MATERIALS AND METHODS

Field samples. A total of 216 porcine samples consisting of feces or intestinal contents were submitted to our laboratory from several locations in the United States and Canada, including samples collected locally from herds in Ohio, and were analyzed for rotaviruses. Fifty-seven samples, collected between the years 1985 and 1992, were determined to be group A rotavirus positive by RNA electrophoresis. The 57 group A rotavirus-positive samples were obtained from pigs in California (n = 1), Illinois (n = 2), Indiana (n = 1), Kansas (n = 2), Nebraska (n = 10), Iowa (n = 1), South Dakota (n = 2), Ohio (n = 34), and Canada (n = 4).

Rotavirus double-stranded RNA (dsRNA) extracted from the 57 rotavirus-positive field samples was analyzed by hybridization with the porcine rotavirus-derived Gottfried and OSU gene 9 probes. Because of the limited quantities of some fecal and intestinal samples, not all of the samples could be analyzed with the probes. Thus, 52 samples were analyzed with the porcine rotavirus-derived Gottfried (P6) and OSU (P7) gene 4 probes and the YM (G11) gene 9 probe, and 25 samples were analyzed with the human rotavirus-derived Wa (G1), DS-1 (G2), and P (G3) gene 9 probes.

RNA extraction, electrophoresis, and quantitation. The extraction and deproteinization of rotavirus dsRNA from cell culture-passaged rotaviruses, rotavirus-positive and -negative fecal and intestinal samples, and mock-infected MA104 cells were performed as previously described (20, 21).

Nucleic acid extracted from cell culture-passaged rotaviruses and fecal or intestinal samples were examined by polyacrylamide gel electrophoresis (PAGE) in 12.5% polyacrylamide gels (20). The RNA electrophoretic patterns of rotavirus-positive samples were visualized by staining with silver nitrate.

The relative amounts of rotavirus dsRNA extracted from cell culture-passaged rotaviruses were estimated by comparisons with the intensities of silver-stained viral dsRNA following analysis by PAGE. Estimates of the amounts of rotavirus dsRNA were made to enable dotting of equivalent amounts of RNA for dot hybridizations in experiments to determine the specificities of the probes. Nucleic acid extracted from the intestinal contents of rotavirus-infected and noninfected gnotobiotic pigs and recombinant plasmids were applied to membranes as previously described (20).

Preparation of ^{32}P-labeled probes. Plasmid vectors containing full- or partial-length cDNA copies of gene segments 4 and 9 (coding for VP4 and VP7, respectively) of the porcine and human rotavirus strains were used for the preparation of cDNA probes. The recombinant plasmids were extracted from individual cultures of transformed *Escherichia coli* by the alkali lysis minipreparation procedure of Sambrook et al. (24).

Gene 4 cDNAs were produced from the Gottfried, OSU, and YM gene 4 recombinant plasmids by using specific oligonucleotide primers and PCR as previously described (20). The partial-length cDNAs encompassed nucleotides 211 to 607 of the Gottfried gene 4 segment and 212 to 612 of the OSU and YM segments. The variable nucleic acid sequences in this region were selected for amplification because of previous associations of this region with the neutralization determinants responsible for P-serotype differences among VP4 proteins (4).

The recombinant plasmids containing gene 9 cDNA segments of the Gottfried, OSU, and YM porcine rotaviruses and of the human rotaviruses Wa, DS-1, and P were used as templates for the production and amplification of gene 9 cDNA by PCR. The gene 9 cDNAs encompassed nucleotides 51 to 392 of the porcine and human rotavirus gene 9 segments. Previous nucleic acid sequence analyses have determined that this region contains three of six variable regions involved in determining G-serotype specificity (7). The specific oligonucleotide primers used in PCR were identical to the primers described for the production of human rotavirus gene 9 probes (5), except that the primer complementary to nucleotides 51 to 71 was 5′GTATGG TACTGAATATACCAC3′. PCR was performed as previously described, and PCR products of the expected size were semipurified by centrifugation through Centricon 30 microconcentrators (20). The gene 4 and 9 PCR-derived cDNA probes were prepared by nick translation (nick translation system; GIBCO BRL, Gaithersburg, Md.) with [^{32}P]dCTP (specific activity, 650 Ci/mmol; ICN Biomedicals Inc., Irvine, Calif.).

Nucleic acid sample preparation and hybridization conditions. The nucleic acid samples were analyzed by dot hybridization. Heat-denatured nucleic acid samples were dotted onto nylon membranes or applied with a filtration manifold (HybriDot Manifold; Bethesda Research Laboratories) (22). The nucleic acid samples were fixed to the membranes by baking for 0.5 h at 80°C in a vacuum oven (National Appliance Company, Tualatin, Oreg.).

The hybridization procedure, solutions, and final wash steps were as previously described (21), except that dextran sulfate was omitted from the hybridization solution. The hybridizations of the PCR-derived gene 9 probes were initially conducted at 42 or 52°C in 50% formamide and 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) to determine the stringency conditions necessary for optimal probe specificity and sensitivity. Visual inspection of the intensity and specificity of the autoradiograph signals resulted in later hybridizations being conducted at 42°C. The stringency conditions necessary for optimal probe specificity of the PCR-derived Gottfried and OSU gene 4 probes were previously established and determined to be 42°C and 50% formamide–5× SSC for the Gottfried probe and 42°C and 40% formamide–5× SSC for the OSU probe (20). The optimal stringency conditions for the YM gene 4 probe were similar to those for the OSU probe. Approximately 10^6 cpm of heat-denatured probe was added per ml of hybridization solution.

Virus strains. Human and animal cell culture-adapted rotaviruses of known serotype were used to confirm probe serotype specificity. Rotavirus strains used in the assays included the following: Wa (G1, P8), DS-1 (G2, P4), M (G3, P6), SA11 (G3, P2), rhesus rotavirus (G3, P3), VA70 (G4, P8), ST3 (G4, P6), Gottfried (G4, P6), OSU and equine H1 (G5, P7), Nebraska calf diarrhea virus (G6, P1), and B223 (G10, P11). Porcine rotavirus strains propagated by passage in gnotobiotic pigs were as follows: G4 strains Gottfried, SB2, SB3, SB5, and SB-1A (all P6 except SB-1A, which is P7); OSU (G5); porcine serogroup B rotavirus (Ohio strain); and porcine serogroup C rotavirus (Cowden strain).

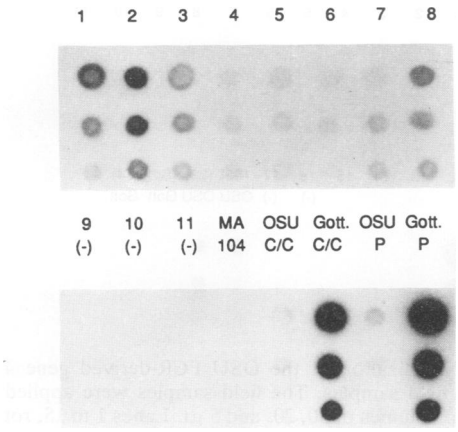

FIG. 1. Dot hybridization of the PCR-derived Gottfried gene 9 probe with serotype G4 gnotobiotic pig-passaged rotaviruses. The nucleic acid samples were diluted fivefold and applied to the membranes. Lanes: 1 to 3, Gottfried rotavirus; 4, SB2 rotavirus; 5 and 6, SB3 rotavirus; 7, SB5 rotavirus; 8, SB-1A rotavirus; 9 to 11, nucleic acid extracted from the intestinal contents of rotavirus-negative gnotobiotic pigs; MA 104, nucleic acid extracted from mock-infected MA104 cells; OSU C/C and Gott. C/C, OSU and Gottfried cell culture-passaged rotaviruses, respectively; and OSU P and Gott. P, OSU and Gottfried recombinant plasmids, respectively.

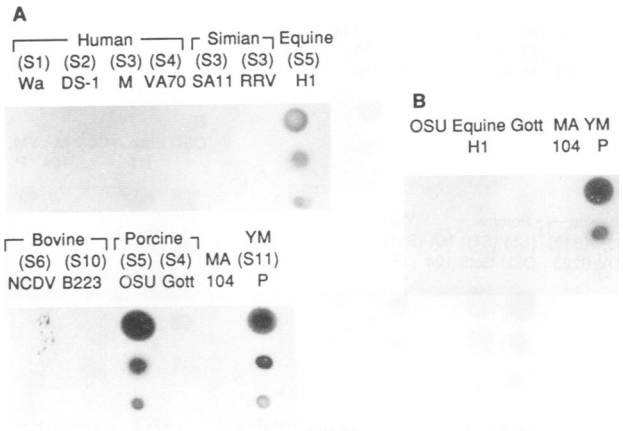

FIG. 2. Dot hybridization of the PCR-derived YM and OSU gene 9 probes with human and animal rotaviruses. (A) Hybridization of equivalent amounts (in terms of counts per minute) of the YM and OSU gene 9 probes with cell culture-passaged group A rotaviruses, nucleic acid extracted from mock-infected MA104 cells (MA 104), and the homologous YM recombinant plasmid (YM P). The G-serotype specificities (S1 to S6 and S10) of the group A rotaviruses are shown in parentheses above the strain designations. (B) Hybridization reactions of the YM gene 9 probe with group A animal rotaviruses. The amount of probe used (in terms of counts per minute) was equivalent to the amount of YM gene 9 probe used for panel A. The hybridization signals in panels A and B were produced simultaneously by exposure of the membranes to a single X-ray film. RRV, rhesus rotavirus; NCDV, Nebraska calf diarrhea virus; Gott, Gottfried.

RESULTS

Determination of the gene 9 cDNA probe specificities. The specificities of the gene 9 cDNA probes were determined by hybridization reactions with dsRNA extracted from rotaviruses of human and animal origins. Rotavirus strains of several known serotype specificities were included in the assay to ensure the specificities of the probes for subsequent use as diagnostic reagents for analyses of field samples. The hybridizations of the human (Wa, DS-1, and P) and porcine (Gottfried and OSU) PCR-derived gene 9 cDNA probes were similar to those of probes previously prepared from excised full-length gene 9 cDNA segments (22). The probes were specific and produced hybridization signals with the homologous cell culture-passaged rotaviruses and recombinant plasmids containing the respective gene 9 cDNA inserts (data not shown). Hybridization signals were not observed with heterologous serotypes of group A human and animal rotaviruses and the porcine group B and C rotaviruses.

The Gottfried PCR-derived gene 9 cDNA probe, unlike our full-length probe (22), did not produce hybridization signals with the serotype G4 human rotaviruses, VA70 and ST3. The overall objectives of this study were to develop diagnostic probes capable of characterizing rotaviruses of similar G-serotype specificities in swine. In order to confirm this capability, the Gottfried gene 9 cDNA probe was hybridized with several animal-passaged porcine rotaviruses previously demonstrated to be serologically related to Gottfried (Fig. 1). The Gottfried probe produced hybridization signals with the homologous Gottfried rotavirus and the SB2, SB3, SB5, and SB-1A strains of serotype G4 porcine rotaviruses. The OSU gene 9 cDNA probe was hybridized to RNA extracted from porcine rotaviruses serologically related to the OSU rotavirus (data not shown). The OSU probe hybridized with the A580 (G5) and EE (G5) strains of porcine rotaviruses; however, the hybridization signals with the A580 strain were weak compared with the hybridization signals with the OSU and EE rotaviruses.

Investigations of the specificity of the YM gene 9 cDNA probe (G11) were conducted in a similar manner (Fig. 2). In this case, however, we did not have access to the YM porcine rotavirus strain (because of importation restrictions) or another serologically confirmed G11 rotavirus strain to use as a positive control. The recombinant plasmid containing the YM gene 9 cDNA insert was used in this assay as the positive control. In order to correlate studies of the specificity of the YM gene 9 cDNA probe with those of the other gene 9 probes investigated, hybridizations were conducted with a mixture of the YM and OSU gene 9 cDNA probes (Fig. 2A). The exposure time of the autoradiograph was adjusted to allow sufficient time for the detection of hybridization signals with the OSU cell culture-passaged rotavirus. Hybridization reactions of the YM and OSU porcine rotavirus gene 9 cDNA probes with rotaviruses of human and animal origins produced strong signals with the OSU (G5) and equine H1 (G5) rotaviruses and the YM recombinant plasmid (containing a YM gene 9 cDNA insert). The hybridization reactions of the YM gene 9 probe alone did not produce hybridization signals with the OSU, equine H1, or Gottfried rotavirus strains (Fig. 2B).

Determination of the gene 4 cDNA probe specificities. The specificities of the OSU and Gottfried gene 4 cDNA probes with heterologous serotypes of rotaviruses have previously been demonstrated (20). Hybridization reactions of the YM gene 4 probe with rotaviruses of human and animal origins are shown in Fig. 3. The strategy used for the determination of the specificity of the YM PCR-derived gene 4 probe was similar to that used for the YM gene 9 probe. In this study, however, cohybridizations were conducted with the YM and

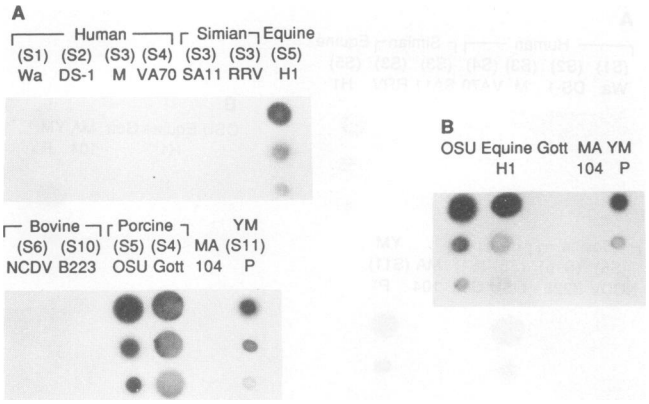

FIG. 3. Dot hybridization of the PCR-derived YM and Gottfried gene 4 probes with human and animal rotaviruses. (A) Hybridization of equivalent amounts (in terms of counts per minute) of the YM and Gottfried gene 4 probes with cell culture-adapted group A rotaviruses, nucleic acid extracted from mock-infected MA104 cells (MA 104), and the homologous YM recombinant plasmid (YM P). The serotype specificities of the group A rotaviruses (S1 to S6 and S10), as determined by their VP7 or G specificities, are shown in parentheses above the strain designations. (B) Hybridization reactions of the YM gene 4 probe with group A animal rotaviruses. The amount of probe used (in terms of counts per minute) was equivalent to the amount of YM gene 4 probe used for panel A. The hybridization signals in panels A and B were produced as described for Fig. 2. RRV, rhesus rotavirus; NCDV, Nebraska calf diarrhea virus; Gott, Gottfried.

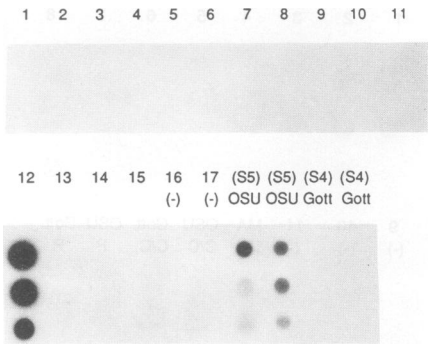

FIG. 4. Hybridization of the OSU PCR-derived gene 4 probe with porcine field samples. The field samples were applied to the membranes in volumes of 50, 20, and 5 μl. Lanes 1 to 15, rotavirus-positive field samples; lanes 16 and 17, rotavirus-negative field samples. Positive controls included two individual samples of both the OSU and Gottfried (Gott) rotavirus strains extracted from the intestinal contents of rotavirus-infected gnotobiotic pigs. S4 and S5, serotype specificities.

Gottfried gene 4 probes (Fig. 3A). The Gottfried and YM gene 4 probes produced hybridization signals with the equine H1, OSU, and Gottfried rotavirus strains and the YM recombinant plasmid (containing a YM gene 4 cDNA insert). Hybridizations of the YM gene 4 probe alone produced signals with the OSU and equine H1 rotaviruses and the YM recombinant plasmid (Fig. 3B).

Analysis of porcine field samples. Hybridization analyses were performed on porcine field samples determined to be group A rotavirus positive by PAGE (data not shown). Of 216 porcine field samples analyzed, 57 had dsRNA patterns characteristic of group A rotaviruses. The group A-positive samples were selected for further study with the human and porcine nucleic acid probes. Seven group A-positive field samples were characterized with more than 11 dsRNA segments, indicating multiple rotavirus strains. The 159 group A rotavirus-negative field samples contained nucleic acid patterns characteristic of group B rotavirus (n = 1) or group C rotavirus (n = 3) or contained no recognizable or visible nucleic acid (n = 151). Each of the four remaining samples contained two segments of nucleic acid with electrophoretic patterns similar to those previously observed in fecal preparations from diarrheic cattle or swine (6, 26).

The results of hybridization of the OSU gene 4 cDNA probe with 15 representative porcine field samples are shown in Fig. 4. Hybridization signals were observed with the homologous gnotobiotic pig-passaged OSU rotavirus and field sample 12. The Gottfried gene 9 cDNA probe was hybridized with a replicate membrane that contained the samples used for Fig. 4 (data not shown). The Gottfried gene 9 cDNA probe produced hybridization signals with the gnotobiotic pig-passaged Gottfried rotavirus and field samples 1, 12, and 13. As noted above, the OSU gene 4 cDNA probe and the Gottfried gene 9 cDNA probe produced hybridization signals with field sample 12 (isolated from a pig in Ohio). These results indicated the detection of another porcine rotavirus isolate with VP4- and VP7-coding genes similar to those of the SB-1A rotavirus. The hybridization reactions of the following Gottfried and OSU probes with the rotavirus-positive porcine field samples identified the indicated numbers of serologically related viruses: Gottfried gene 9 probe (G4), 15.8% (9 of 57); Gottfried gene 4 probe (P6), 3.8% (2 of 52); OSU gene 9 probe (G5), 7.0% (4 of 57); and OSU gene 4 probe (P7), 13.5% (7 of 52).

Because of the large number of samples which were unreactive with the Gottfried and OSU gene 4 and 9 cDNA probes, investigations were conducted to determine whether other serotypes of rotaviruses were present in swine. Hybridization analyses were conducted with gene 9 cDNA probes prepared from the human rotaviruses Wa (G1), DS-1 (G2), and P (G3) and the porcine rotavirus YM (G11). Hybridization reactions with the human rotavirus P and the YM gene 9 cDNA probes were of particular interest because of previous studies reporting either serological evidence or the isolation of G3 and G11 rotaviruses from swine (12, 16, 19). Although G1 and G2 rotaviruses have not previously been isolated from swine, one research group reported serological evidence for these serotypes on the basis of serotyping enzyme-linked immunosorbent assays with monoclonal antibodies specific for human G1 and G2 rotaviruses (2).

No rotaviruses with homologous serotype specificities to the Wa (G1), DS-1 (G2), and P (G3) rotaviruses were detected in hybridizations with the respective human rotavirus PCR-derived gene 9 probes in 25 porcine field samples analyzed. In contrast, hybridization signals were detected with 21.1% (11 of 52) of the field isolates analyzed with the YM gene 9 cDNA probe. The results of hybridization of the YM gene 9 cDNA probe with 12 representative porcine field samples that contained G11-positive field specimens are shown in Fig. 5. The G11-positive samples were collected (between the years 1989 and 1992) from swine from three farms in Ohio and from swine maintained at the Ohio Agricultural Research and Development Center. The G11-positive samples collected from two different locations were obtained during outbreaks of diarrhea in nursing pigs. The G-

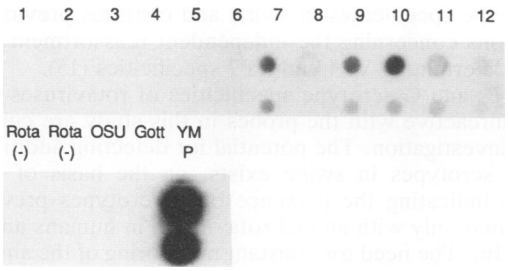

FIG. 5. Hybridization of the PCR-derived YM gene 9 probe with porcine field samples. The field samples were applied to the membranes in volumes of 50 and 15 μl. The samples shown in this autoradiogram differed from the field samples shown in Fig. 4. Lanes 1 to 12, rotavirus-positive field samples. The bottom row of the membrane shows two rotavirus-negative [Rota (−)] field samples, the OSU and Gottfried (Gott) rotavirus strains extracted from the intestinal contents of rotavirus-infected gnotobiotic pigs, and the homologous YM recombinant plasmid (YM P) containing a full-length YM gene 9 cDNA insert.

and P-serotype specificities of the typeable field isolates identified in this study are summarized in Table 1.

DISCUSSION

In this study, fecal and intestinal samples collected from group A rotavirus-infected swine from several states in the United States and to a limited extent from Canada were investigated to determine the diversity and prevalence of rotavirus P and G serotypes. The analyses were performed by nucleic acid hybridization, with initial efforts focused on the use of gene 4 and gene 9 cDNA probes prepared from the Gottfried (G4, P6) and OSU (G5, P7) porcine rotavirus strains. Because a relatively large number of samples (n = 48, gene 9 probes; n = 42, gene 4 probes) were unreactive with these probes, further studies were conducted with gene 9 cDNA probes prepared from the porcine rotavirus YM (G11) and the human rotaviruses Wa (G1), DS-1 (G2), and P (G3).

The gene 9 cDNA probes used in this study were similar to probes previously described in investigations of the G-serotype diversity of human rotaviruses in clinical specimens (5). The rotavirus probes used in the human study were specific, and their efficacy in determining the serotype specificities of rotaviruses was equal to that of monoclonal antibody-based

TABLE 1. Hybridization reactivities of porcine rotavirus PCR-derived gene 9 (coding for the VP7 or G-serotype specificity) and gene 4 (coding for the VP4 or P-serotype specificity) probes with group A rotavirus-positive field samples[a]

Rotavirus sample	Source of sample	Specificity of rotavirus probe[b]				
		Gottfried		OSU		YM (VP7 [G11])
		VP4 (P6)	VP7 (G4)	VP4 (P7)	VP7 (G5)	
A	Ohio	ND	+	ND	−	ND
B	Ohio	ND	+	ND	−	ND
C	Ohio	ND	−	ND	+	ND
D	South Dakota	ND	+	ND	−	ND
E	South Dakota	ND	+	ND	−	ND
F	Ohio	−	−	−	−	+
G	Ohio	−	−	−	−	+
H	Ohio	−	+	+	−	−
I	Ohio	+	−	−	−	−
J	Ohio	−	−	+	+	−
K[c]	Ohio	−	+	−	+	−
L	Illinois	−	+	−	−	−
M	California	−	−	−	−	+
N	Kansas	−	−	+	−	−
O	Ohio	−	−	−	−	+
P	Iowa	−	−	+	−	−
Q	Nebraska	−	−	−	−	+
R[d]	Kansas	−	+	−	−	+
S	Nebraska	−	−	+	+	−
T[c]	Nebraska	−	−	+	−	−
U	Ohio	+	+	−	−	−
V	Ohio	−	−	+	−	+
W	Ohio	−	−	−	−	+
X	Ohio	−	−	−	−	+
Y	Ohio	−	−	−	−	+
Z	Ohio	−	−	−	−	+
Total positive[e]		2	9	7	4	11

[a] Fifty-seven group A rotavirus-positive samples were analyzed with the Gottfried (G4) and OSU (G5) gene 9 probes, 52 samples were analyzed with the Gottfried (P6) and OSU (P7) gene 4 and YM (G11) gene 9 probes, and 25 samples were analyzed with the Wa (G1), DS-1 (G2), and P (G3) PCR-derived gene 9 probes. No hybridization signals were detected with the human rotavirus Wa, DS-1, and P gene 9 probes. Results for the probe-reactive samples are summarized in the table.

[b] The hybridization reactivity of the OSU gene 4 probe was equivalent to that of the YM gene 4 probe. The YM gene 4 probe results are not shown. ND, not determined because of insufficient quantity of field sample.

[c] The sample exhibited multiple group A rotavirus RNA electrophoretic patterns in silver nitrate-stained PAGE gels.

[d] The VP7 specificity could not be determined because of equivalent hybridization signals with two different probes.

[e] Number of field samples positive with the indicated probe.

typing assays. The gene 9 human and porcine cDNA probes used in this study were also extensively investigated and were effective in differentiating previously characterized serologically defined rotaviruses. The hybridization stringency conditions for the gene 9 cDNA probes and our previously reported gene 4 cDNA probes (20) were adjusted to allow maximum sensitivity in detecting homologous serotypes of rotavirus while maintaining serotype specificity.

Fifty-seven and 52 group A rotavirus-positive porcine field samples were analyzed by hybridization with Gottfried and OSU gene 9 and 4 cDNA probes, respectively. Seventeen samples were identified with either combined homologous VP4- or VP7-coding genes or both. The large number of rotavirus-positive samples that were unreactive with the Gottfried and OSU probes suggested that rotaviruses with other P- and G-serotype specificities may be present in swine. Hybridization reactions with gene 9 cDNA probes prepared from the human rotaviruses Wa (G1), DS-1 (G2), and P (G3) did not detect rotaviruses with respective homologous serotype specificities in the 25 rotavirus-positive field samples for which sufficient quantities were available for analysis. Hybridization reactions with the porcine rotavirus YM (G11) gene 9 cDNA probe, however, produced hybridization signals with 11 of 52 porcine field samples analyzed. Ten of the G11-positive field samples identified were obtained from swine in Ohio ($n = 8$), California ($n = 1$), and Nebraska ($n = 1$). Another field sample from Kansas produced hybridization signals with the YM and Gottfried gene 9 probes and could not be serologically distinguished. This study represents the first report of G11 rotaviruses in the United States. The isolation of G11 rotaviruses from diverse regions of the United States indicates that G11 rotaviruses may be widespread in many swine herds in the United States. The discovery of G11 rotaviruses as the most frequently detected G-type among the swine samples we studied is of great importance to the swine industry, veterinary practitioners, and rotavirus vaccine producers in the United States. Porcine rotavirus vaccines currently in use are specifically directed only against rotavirus serotypes G4 and G5 (8, 27). The efficacy of these vaccines in stimulating immunological protection against G11 rotavirus strains is unknown.

The nucleic acid sequence of the YM gene 4 segment was previously reported in a study by López et al. (13). In this study, comparisons of the deduced amino acid sequence of the YM VP4 protein with the reported VP4 sequences of rotaviruses of different serotype specificities indicated a high degree of homology with the OSU VP4 protein (97% overall identity). Similar comparisons with the VP4 of other animal rotaviruses identified 82.6 and 81.9% identities with the SA11 and Nebraska calf diarrhea rotaviruses, respectively, while the lowest degree of homology was observed with human rotavirus strains (68.5 to 70.7%). Hybridization reactions of the YM gene 4 cDNA probe with the OSU and equine H1 rotaviruses in our study confirmed previous amino acid homologies and indicated that the serotype specificity of the YM VP4 is P7. Furthermore, the hybridization stringency conditions used in our study were sufficient to prevent hybridization signals with other rotavirus serotypes of human and animal origins. An important observation pertaining to the hybridization reactions of the OSU gene 4 probe (essentially equivalent to the YM gene 4 probe) with the G11-positive field samples was that unlike the original YM porcine isolate, only one field sample had a P-serotype specificity similar to the YM VP4 (Table 1, sample V). This observation suggests the presence of other P-serotype specificities in swine and confirms previous observations concerning the independent reassortment of the genes determining VP4 and VP7 specificities (15).

The P- and G-serotype specificities of rotaviruses which were unreactive with the probes in this study are currently under investigation. The potential for detecting additional P and G serotypes in swine exists, on the basis of recent studies indicating the presence of G serotypes previously associated only with animal rotaviruses in humans and vice versa (16). The need for constant monitoring of the antigenic diversity of rotaviruses in swine is important for the development of effective porcine rotavirus vaccines and for identification of new rotavirus serotypes which may have the potential to cause zoonotic infections in humans.

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