Characterization of Field Strains of Group A Bovine Rotaviruses by Using Polymerase Chain Reaction-Generated G and P Type-Specific cDNA Probes†  
ANIL V. PARWANI, HUSSEIN A. HUSSEIN, BLAIR I. ROSEN, ALEJANDRO LUCHELLI, LORENZO NAVARRO, AND LINDA J. SAIF†*  
Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio 44691  
Received 1 March 1993/Accepted 5 May 1993

Dot and Northern blot hybridization assays were used to analyze field strains of group A bovine rotaviruses (BRVs) by using nucleic acid probes representing P and G type specificities. The probes were prepared by polymerase chain reaction amplification of hyperdivergent regions of the cloned VP4 (nucleotides 211 to 686) and VP7 (nucleotides 51 to 392) genes from four serotypically distinct (in P or G types) strains of rotaviruses: NCDV (G6, P1), IND (G6, P5), 69M (G8, P10), and Cr (G10, P11). The P and G type cDNA probes were radiolabeled with [32P]dCTP and hybridized with RNA extracted from reference cell culture-passaged rotavirus strains or the field samples. The field samples were obtained from young diarrheic calves from Ohio, Nebraska, Washington State, and Canada. The cDNA probes were specific for their respective G or P types on the basis of analysis of known P and G type reference strains. The G typing analysis of 102 field samples revealed that 36.3% (37 of 102) were G6, 2.9% (3 of 102) were G8, 12.7% (13 of 102) were G10, and 23.5% (24 of 102) were untypeable. The P typing results for 93 samples indicated that 2.2% (2 of 93) were P1 (NCDV-like), 20.4% (19 of 93) were P5 (UK-like), 9.3% (10 of 93) were P11 (B223-like), and 40.8% (38 of 93) were untypeable. This is the first report of the identification among BRV strains in North America of a G type other than G6 or G10. Our report further confirms that G6, P5 rotaviruses are predominant among the BRV field strains that we examined, and the P types of these strains differ from that of the BRV vaccine strain used in the United States (G6, P1). The large number of untypeable G (23.5%) and P (40.8%) types suggests that other or new P and G types exist among BRV field strains.

Group A bovine rotaviruses (BRVs) are a major cause of enteric disease in young calves (1 to 3 weeks of age) (13, 20, 27). Affected calves may die as a result of severe dehydration or secondary bacterial infections (27). No highly effective vaccines for the prevention or control of BRV infections are available in the United States (20, 21). The serotypic classification of rotavirus is based on the reactivities of the outer capsid proteins, VP4 and VP7, which determine the P (for protease-susceptible) and G (for glycoprotein) serotypes, respectively (4, 10). Group A rotaviruses are classified into at least 14 G serotypes and 12 P serotypes (2-4, 10, 25). The information on the diversity of BRV P and G types in the field is important for the future development and monitoring of effective vaccines, because rotaviruses which possess distinct G and P types may not induce heterotypic cross-protection (9, 20, 27, 29).

Isolates of at least four G serotypes (G1, G6, G8, and G10) infect cattle (15, 24), of which serotypes G6 and G10 (represented by prototype strains NCDV and B223, respectively) have been identified in the United States (15, 29). Recently, a G1 BRV isolate, T449, was identified in the feces of a calf with diarrhea in Argentina (1). Others have reported G8 BRV isolates in the United Kingdom (24). At least four P types (P1 [NCDV], P5 [UK], P11 [B223], and P12 [678]) have been reported among BRV strains (16, 25). It has been suggested that the difference between P types may have contributed to the lack of homotypic (G type-specific) protection observed between the G6 strains NCDV (P1) and B641 (P5) (9, 29). To better understand the epidemiology of BRV infections and to elucidate the antigenic and molecular relationships that exist among BRV strains, sensitive and reliable methods for the characterization of P and G types of BRV are very important.

Nucleic acid probes and the polymerase chain reaction (PCR) have been useful for the detection and serotypic classification of human and animal rotaviruses in clinical specimens (5, 7, 12, 15–18, 20). Full-length cDNA probes prepared from rotavirus gene segments coding for VP7 or partial-length PCR probes amplified from hyperdivergent regions of the VP7 gene have been used for the G typing of human (7), bovine (15), and porcine (18) rotaviruses. Similar methods have been reported for the P typing of human (12), bovine (16), and porcine (17) group A rotaviruses.

The objective of the present study was to clone the full-length VP4 and VP7 genes of the BRV strains NCDV, IND (UK-like), and Cr (B223-like) and to generate partial-length PCR probes for the P and G type characterization of 93 and 102 BRV field strains, respectively, from Ohio, Nebraska, Washington State, and Ontario, Canada.

MATERIALS AND METHODS

Viruses and cells. The reference viruses were grown in rhesus monkey kidney (MA-104) cells in roller bottles and titrated by a plaque assay as described previously (22). The strains and serotypes (P and G) of the reference human and animal rotaviruses used in the present study are listed in Table 1, and their sources have been described previously.

* Corresponding author.
† Journal article 36-93 of The Ohio Agricultural Research and Development Center.
TABLE 1. Cell culture-adapted and gnotobiotic calf-passaged rotaviruses used for nucleic acid hybridization with BRV G and P type PCR-derived cDNA probes

<table>
<thead>
<tr>
<th>Rotavirus strain</th>
<th>Group</th>
<th>Species</th>
<th>G type</th>
<th>P type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonbovine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wa</td>
<td>A</td>
<td>Human</td>
<td>1 (a)</td>
<td>8 (a)</td>
</tr>
<tr>
<td>DS-1</td>
<td>A</td>
<td>Human</td>
<td>2 (a)</td>
<td>4 (a)</td>
</tr>
<tr>
<td>ST3</td>
<td>A</td>
<td>Human</td>
<td>4 (a)</td>
<td>6 (a)</td>
</tr>
<tr>
<td>VA70</td>
<td>A</td>
<td>Human</td>
<td>8 (a)</td>
<td>10 (a)</td>
</tr>
<tr>
<td>69M</td>
<td>A</td>
<td>Human</td>
<td>8 (a)</td>
<td>10 (a)</td>
</tr>
<tr>
<td>SA11</td>
<td>A</td>
<td>Simian</td>
<td>3 (b)</td>
<td>2 (b)</td>
</tr>
<tr>
<td>Gottfried</td>
<td>A</td>
<td>Porcine</td>
<td>4 (b)</td>
<td>6 (b)</td>
</tr>
<tr>
<td>OSU</td>
<td>A</td>
<td>Porcine</td>
<td>5 (b)</td>
<td>7 (b)</td>
</tr>
<tr>
<td>Bovine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K124 (a)</td>
<td>A</td>
<td>Bovine</td>
<td>6 (c)</td>
<td>1 (c)</td>
</tr>
<tr>
<td>T1309 (a)</td>
<td>A</td>
<td>Bovine</td>
<td>6 (c)</td>
<td>1 (c)</td>
</tr>
<tr>
<td>C486</td>
<td>A</td>
<td>Bovine</td>
<td>5 (d)</td>
<td>5 (d)</td>
</tr>
<tr>
<td>IND</td>
<td>A</td>
<td>Bovine</td>
<td>4 (d)</td>
<td>5 (d)</td>
</tr>
<tr>
<td>OK</td>
<td>A</td>
<td>Bovine</td>
<td>6 (e)</td>
<td>5 (e)</td>
</tr>
<tr>
<td>ID</td>
<td>A</td>
<td>Bovine</td>
<td>6 (e)</td>
<td>5 (e)</td>
</tr>
<tr>
<td>AT11</td>
<td>A</td>
<td>Bovine</td>
<td>6 (e)</td>
<td>5 (e)</td>
</tr>
<tr>
<td>RV584</td>
<td>A</td>
<td>Bovine</td>
<td>6 (e)</td>
<td>5 (e)</td>
</tr>
<tr>
<td>B223</td>
<td>A</td>
<td>Bovine</td>
<td>10 (e)</td>
<td>11 (e)</td>
</tr>
<tr>
<td>Cr</td>
<td>A</td>
<td>Bovine</td>
<td>10 (e)</td>
<td>11 (e)</td>
</tr>
<tr>
<td>ATIB</td>
<td>B</td>
<td>Bovine</td>
<td>ND (f)</td>
<td>ND (f)</td>
</tr>
<tr>
<td>Shintoku</td>
<td>C</td>
<td>Bovine</td>
<td>ND (f)</td>
<td>ND (f)</td>
</tr>
</tbody>
</table>

\(a\) According to the unified serotyping scheme on the basis of virus neutralization assays originally proposed by Hoshiba et al. (10).
\(b\) According to the tentative P serotyping scheme originally proposed by Estes and Cohen (4) and extended by Snodgrass et al. (25).
\(c\) Nebraska calf diarrhea virus (NCDV), Covy strain.
\(d\) G serotype as determined in the present study on the basis of hybridization analysis with G-type (G6, IND; G10, Cr) cDNA probes and numbered according to the uniform serotyping scheme (10). The G type cDNA probes did not cross-react with heterologous G types.
\(e\) P serotype as determined in the present study on the basis of hybridization data obtained with P-type (P1, NCDV; P5, IND; P11, Cr) cDNA probes and numbered according to the P typing system proposed by Estes and Cohen (4) and extended by Snodgrass et al. (25) for group A bovine rotaviruses. The P type cDNA probes did not cross-react with heterologous P types.
\(f\) Nebraska calf diarrhea virus, Lincoln strain.

(15–18, 20). Ten reference group A BRV laboratory strains were also used as controls and are listed in Table 1. In addition to the BRV strains described previously (15, 16), strain C486 was obtained from D. Benfield, South Dakota State University, strain AT11 was obtained from the Agricultural Technical Institute (ATI) herd in Wooster, Ohio (20a), and the cell culture-adapted strain RV584 was obtained from Salah Hammani, University of California at Davis. These G serotypes of these BRV strains were previously characterized by using G6- and G10-neutralizing serotype-specific monoclonal antibodies (MAbs) or by two-way cross-neutralization tests (20a). A group C BRV, Shintoku strain, previously adapted to growth in cell culture was used as a negative control (28). The group A BRV strains NCDV (Cody), Cr, OK, ID, B223, and IND (15) and a bovine group B rotavirus (AT1 B strain) (19) were also passaged in gnotobiotic or colostrum-deprived calves as described previously (21) (Table 1).

**Field strains of BRV.** Two rotavirus-negative and 102 BRV-positive samples for the hybridization assays were obtained from Ohio \((n = 57)\), Nebraska \((n = 27)\), Washington \((n = 3)\), and Ontario, Canada \((n = 15)\). The majority of the samples were provided as calf diarrhea survey samples (Ohio) (13) or as diagnostic samples from calves with diarrhea (1 to 30 days of age) and were collected between 1988 and 1992. Before testing with nucleic acid probes, all the samples were tested for group A rotaviruses by polyacrylamide gel electrophoresis (PAGE) (18) and at least one or more of the following assays: enzyme-linked immunosorbent assay (ELISA) (13) and immune electron microscopy (13).

**Extraction and electrophoresis of RNA.** Rotavirus double-stranded RNA (dsRNA) was extracted from cell culture-propagated viruses or fecal samples from the field by previously described procedures (18). The dsRNA in extracted samples was analyzed by PAGE to confirm the presence of dsRNA and to examine the genomic electropherotypes as described previously (15). Electrophoresis was conducted at 12 mA for 14 to 16 h. The dsRNA bands were visualized by silver staining or staining with ethidium bromide (0.5 μg/ml; Sigma Chemical Co., St. Louis, Mo.).

**Cloning of BRV VP4 and VP7 genes.** The oligonucleotide primers used in the present study are listed in Table 2. The single-stranded RNA (ssRNA) was produced by in vitro transcription of purified viral cores by previously described procedures (6). Amplification of ssRNA templates required an initial reverse transcription step. First-strand cDNA was synthesized by incubating heat-denatured ssRNA (0.2 to 1 μg) for 30 min to 1 h at 42°C in a reaction mixture containing 25 mM Tris (pH 8.3), 25 mM KCl, 5 mM MgCl₂, 1 mM dNTP (each), dATP, dCTP, dGTP, and dTTP, 200 ng of primers 7B (VP7 gene) or 4B (VP4 gene), and 10 μl of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) (Fig. 1).

TABLE 2. Sequences of oligonucleotides used for PCR amplification of full- or partial-length VP4 and VP7 genes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Gene Location</th>
<th>Strand</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>4A</td>
<td>VP4 (a) 5' end</td>
<td>Sense</td>
<td>5'-CCG GGG ATG GCA ATT GGG GAT TAA AAT GGC TGG GCT-3'</td>
</tr>
<tr>
<td>4B</td>
<td>VP4 (a) 3' end</td>
<td>Antisense</td>
<td>5'-TGG GCA ATT CTA GAG GTC ACG TGG TGG GCA GAC-3'</td>
</tr>
<tr>
<td>4C</td>
<td>VP4 Nucleotides 211 to 230</td>
<td>Sense</td>
<td>5'-CCG TAT GAG GCC GGG GGA TT-3'</td>
</tr>
<tr>
<td>4D</td>
<td>VP4 Nucleotides 677 to 686</td>
<td>Antisense</td>
<td>5'-GGG GCC GCG AGG GGG TTG TGT AT-3'</td>
</tr>
<tr>
<td>7A</td>
<td>VP7 (a) 5' end</td>
<td>Sense</td>
<td>5'-CCG GATG GTC GAA GCG CCG TGG TTA AAAG GAA TAT TTT-3'</td>
</tr>
<tr>
<td>7B</td>
<td>VP7 (a) 3' end</td>
<td>Antisense</td>
<td>5'-GTA TGG TAT GAG ATG ACA GAA TAC AAG GAC-3'</td>
</tr>
<tr>
<td>7C</td>
<td>VP7 Nucleotides 51 to 71</td>
<td>Sense</td>
<td>5'-GAC TGA GAA TGA AGG AAC TAA GCC A-3'</td>
</tr>
<tr>
<td>7D</td>
<td>VP7 Nucleotides 376 to 392</td>
<td>Antisense</td>
<td>5'-GAT CAT GTT GGC CTT GAT-3'</td>
</tr>
</tbody>
</table>

\(a\) Strand denotes the sense or antisense of the primer, which indicates the sequence of the primer is either identical or complementary to the bovine rotavirus mRNA (VP4 and/or VP7 genes), respectively.
\(b\) The sequences of the oligonucleotides used for partial- and full-length amplification of the VP4 genes were based on the published sequence for the VP4 gene of the UK strain of group A bovine rotaviruses (11).
\(c\) The sequences of the oligonucleotides used for partial- and full-length amplification of the VP7 genes were based on the published sequence for the VP7 gene of the NCDV strain of group A bovine rotaviruses (8).
The PCR mixture contained 200 μM (each) dATP, dCTP, dGTP, and dTTP, 20 mM Tris-HCl (pH 8.3), 2.5 mM MgCl₂, 0.05% gelatin, 200 ng of each primer (VP4 gene, primers 4A and 4B; VP7 gene, primers 7A and 7B), 1 to 10 ng of DNA template, and 2.5 U of Taq polymerase (Boehringer Mannheim Biochemicals). Thirty amplification cycles were used, with each cycle consisting of 94°C for 1 min (denaturation), 42°C for 1.5 min (annealing), and 72°C for 3.5 min (extension). The full-length PCR products were purified by electrophoresis and analyzed on 1% agarose gels by standard procedures (23).

The PCR-amplified full-length VP4 and VP7 genes of BRV strains IND, NCDV, and Cr were cloned into the plasmid pGEM 3zf (Promega, Madison, Wis.) or pBS (Stratagene, La Jolla, Calif.) by standard recombinant DNA procedures (23). Recombinant plasmids were identified by colony blot hybridization with 32P-labeled PCR probes. The specificities of the clones were confirmed by Northern blot hybridization by previously described procedures (15).

The full-length VP7 gene of human rotavirus strain 69M (G8) cloned into the plasmid pUC 13 was obtained from Jorge Flores, National Institutes of Health. The recombinant plasmids were used as templates for the production by PCR of partial-length cDNA segments for use as probes in serotyping assays.

**PCR amplification and labeling.** Partial-length VP7 cDNA (nucleotides 51 to 392) and VP4 cDNA (nucleotides 211 to 686), encompassing areas of major sequence diversity, were prepared by PCR amplification with specific primers (Fig. 1). For amplification of VP7 cDNA segments (341 bp) from rotavirus strains IND (G6), 69M (G8), and Cr (G10), primer 7C (homologous to nucleotides 51 to 71 of the NCDV VP7 gene) and primer 7D (complementary to nucleotides 376 to 392 of the NCDV VP7 gene) were used (Table 2). For PCR amplification of VP4 partial-length cDNA segments (475 bp) from strains NCDV (P1), IND (P5), and Cr (P11), primer 4C (sequence homologous to nucleotides 211 to 630 of the UK VP4 gene) and primer 4D (sequence complementary to nucleotides 677 to 686 of the UK VP4 gene) were used (Table 2). These primers corresponded to highly conserved regions of the VP4 and VP7 genes and flanked the target “sequence-divergent” regions (Fig. 1).

The purified PCR products were radiolabeled by using a nick-translation kit (Bethesda Research Laboratories, Gaithersburg, Md.) and [32P]dCTP (specific activity, 650 Ci/mmol; ICN Biomedicals Inc., Irvine, Calif.) as described previously (16).

**Northern and dot blot hybridization assays.** The Northern and dot blot hybridization assays were performed by using modifications of previously described procedures (16). Hybridization was performed under high-stringency conditions (52°C, 5 × SSC [1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate] 50% formamide) in 3 to 5 ml of hybridization buffer containing 4.5% dextran sulfate and 3 × 10⁶ to 5 × 10⁷ cpm of heat-denatured PCR probe (~1.2 × 10⁷ cpm/μg of DNA template). The hybridizations were performed for 16 to 24 h at 52°C. The membranes were washed four times at room temperature in 2 × SSC-0.1% sodium dodecyl sulfate (SDS) and two times at the hybridization temperature in 0.4 × SSC-0.1% SDS. The washed membranes were rinsed once with water, blotted, and exposed to Kodak XAR films with intensifying screens at ~70°C for 1 to 7 days.

**RESULTS**

**Cloning of BRV VP4 and VP7 genes.** Full-length BRV VP4 genes were amplified from the BRV strains NCDV (P1), IND (P5), and Cr (P11). Figure 2A shows the full-length PCR product (2,362 bp) amplified from ssRNA obtained from the
BRV strain NCDV. The PCR products were purified and cloned into recombinant plasmids for use as templates in subsequent experiments and for further studies. The specificities of the clones were confirmed by dot and Northern blot hybridization assays. Figure 2B shows the ethidium bromide-stained electrophoretotypes of BRV strains NCDV, B223, and ID (short genome pattern). The dsRNA was electrotransferred onto nylon membranes which were hybridized with the radiolabeled NCDV VP4 clone. The clone was specific and hybridized only with the VP4 gene from the BRV strain NCDV and not with the heterologous P types (B223 [P11] and ID [P5]) (Fig. 2C). Similarly, full-length BRV VP7 genes from strains IND (G6), NCDV (G6), and Cr (G10) were generated by PCR and cloned into the recombinant plasmid pGEM. Under high-stringency conditions, the G6 clones hybridized only with the dsRNA samples obtained from G6 rotaviruses (IND and NCDV) (data not shown). Similarly, the G10 clone (Cr strain) hybridized only with the RNA extracted from G10 samples (B223 and Cr) (data not shown).

Specificities of the partial-length G and P type PCR probes. Dot and Northern blot hybridization assays with the cell culture-passaged rotavirus samples and the BRV VP4 and VP7 cDNA probes showed that the three VP4 (P1, P5, and P11) and the three VP7 (G6, G8, and G10) cDNA probes did not hybridize with other group A rotaviruses, including the human, porcine, and simian strains, with different G and P specificities. Furthermore, none of the probes showed any reaction with other rotavirus serogroups (groups B and C) (data not shown).

Characterization of the G and P types of the BRV field strains. Rotavirus dsRNA was extracted from the samples obtained from the field. The dsRNA was heat denatured and tested by dot blot hybridization assays. All of the 102 rotavirus-positive and 2 rotavirus-negative samples were tested by dot blot hybridization by using the three G type-specific partial-length cDNA probes. For P typing, 93 rotavirus-positive and 2 rotavirus-negative samples were tested. The results of the G typing of the 102 BRV field samples and the 10 reference BRV strains by using the partial-length PCR-generated cDNA probes are presented in Table 3. The 24 PAGE-positive field samples were untypeable because they failed to hybridize with any of the three G type-specific probes. For P typing, a total of 93 BRV field samples and the 10 reference BRV strains were examined, and the results of the P typing assays are presented in Table 4. The 38 PAGE-positive field samples were considered untypeable because they tested negative with all three P type probes.

The G and P typing results were compared to investigate the various combinations of G and P types that occurred in the field specimens tested. The most frequently detected combination of G and P types in the samples that we examined was G6, P5 (n = 14). Other P type combinations with G6 were G6, P1 (n = 2) and G6, P11 (n = 4). There were no P1, P5, or P11 types detected among the three type G8 field samples. For the G10 strains, the combinations were G10, P5 (n = 2) and G10, P11 (n = 4), but there were no G10, P1 strains.

**DISCUSSION**

In the present study, we cloned full-length VP4 and VP7 genes from three serotypically distinct (in P or G types) strains of rotaviruses: NCDV (G6, P1), IND (G6, P5), and Cr (G10, P11). The cloned BRV VP4 and VP7 genes were used as templates in PCR to generate partial-length cDNA segments representing serotype-specific regions of the genes. Similarly, a human rotavirus (69M) G8 cDNA partial-length probe was also prepared from the recombinant plasmid made available for the present study. These partial-length segments were radiolabeled and used to characterize field strains of group A bovine rotaviruses.

The serotype classification of BRV has also been defined on the basis of VP7 specificities by ELISA with VP7-specific MAbs (20, 24). The neutralization specificities of rotavirus, however, are also dependent on VP4, and the VP4 gene segregates independently from the VP7 gene (4). It has therefore been proposed that serotypic classification of rotaviruses should account for both VP4 (P) and VP7 (G) specificities (4, 10, 25). Previous investigators have reported that it is difficult to prepare VP4-specific MAbs (26). Therefore, at least for VP typing, cDNA probes offer great promise. In addition, the cDNA probes have the potential to detect genomic variants or monotypes not detectable by MAbs (5). We demonstrated in the present study that cDNA probes can be used as a reliable means of characterizing the G and P type specificities of BRV field specimens.

Two previous studies have investigated the frequencies of
infection of calves with serotypes G6 and G10. Woode et al. (29) used two-way neutralization assays and demonstrated that 89% of the isolates were G6 but that only 7.4% were G10. Snodgrass et al. (24) used a MAb-based ELISA for G typing and reported that 66% of the isolates that they tested were G6 but that only 7.4% were typed as G10. Those results are in accordance with the results of our study (although our overall percentage was lower), in which the predominant G type was G6 (37 of 102 samples tested; 36.3%), whereas 13 of 102 (12.7%) were G10. The reason for these discrepancies may reflect the more diverse (in geographic location) field samples used in our study. Furthermore, we have established in our laboratory that this discrepancy is not due to differences in antigenic versus genetic typing with MAbs versus cDNA probes, respectively, because the percent observed agreement between MAbs and probes for the samples described in the present study for G6 and G10 typing was 90.6% (29).

There is very limited information on the frequency and distribution of BRV P types. Such information is very useful from an epidemiological standpoint. Furthermore, there has been no systematic study of the P types of BRV field cases. In our studies, the predominant P type was P5 (19 of 93 samples tested; 20.4%). The P type found in the vaccine strain (NCDV, P1) was detected in only 2.2% of the samples. Comparison of the G and P typing results further confirms that the field strains with the G and P combination G6, P5 were detected most frequently (n = 14) among the BRV field strains examined. Therefore, a BRV strain with the G6, P5 combination may be a better vaccine candidate strain than the BRV vaccine strain used in the United States (G6, P1). Investigators have observed a lack of cross-protection between the two BRV G6 strains B641 (G6, P5) and NCDV (G6, P1) (29). Because the P types of these strains are distinct, it has been suggested that differences in P types may be a contributing factor to this lack of cross-protection (9).

In the present study, we identified at least three isolates that tested positive with the G8 cDNA probe. Snodgrass et al. (24) demonstrated that the two bovine strains J2538 and 678 from the United Kingdom belong to G8. Interestingly, Ohshima et al. (14) reported a moderate level of homology between the human G8 strain 69M and some bovine rotavirus strains. To our knowledge, ours is the first report of G8 rotaviruses identified among cattle in North America. There are at least three possibilities that may explain the occurrence of human G types (G1 and G8) (1, 24) in the bovine population. First, they may be the result of direct transmission between animals and humans. Second, the human strains may have evolved ancestrally from animal strains, and lastly, there may be natural reassortants between human and animal strains.

The large number of untypeable BRV field samples (G untypeables, 23.5%; P untypeables, 40.8%) remains a major area of concern (Tables 3 and 4). These samples previously tested positive by ELISA and PAGE. Since it is possible that any combination of P and G types may occur in nature, the untypeable samples may represent other existing or new G or P types. These G or P types may escape classification if there are no suitable diagnostic reagents available. These samples may also be classified as untypeable because they were tested only with cDNA probes representing the G and/or P types traditionally associated with the bovine population. In future studies, the samples that tested negative with the BRV G type (G6, G8, and G10) and P type (P1, P5, and P11) cDNA probes will be retested with human and other animal G and P type cDNA probes that are not commonly associated with the bovine population.

ACKNOWLEDGMENTS

The synthetic primers used in the present study were kindly provided by Mario Gorziglia, and the human rotavirus strain 69M VP7 clone was provided by J. Flores, both of the National Institutes of Health. Various rotavirus strains were kindly provided by Susan Lance and Paul Bartlett (13), The Ohio State University; Ram Mohan, Ohio Department of Agriculture; Fernando Osorio, University of Nebraska; Thomas Besier, Washington State University; and Susan Carman, Ontario Ministry of Agriculture and Food, Ottawa, Ontario, Canada.

Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, Ohio State University. This study was supported in part by Animal Health Competitive Research grant 86-CSRS-2-2910 from the Science and Education Administration, CSRS, U.S. Department of Agriculture.

REFERENCES


