G-typing of rotavirus strains enables the study of molecular epidemiology and gathering of information to promote disease prevention and control. Rotavirus strains in fecal specimens from neonatal calves in Swedish cattle herds were therefore characterized by using G1 to -4-, G6-, G8-, and G10-specific primers in reverse transcription (RT)-PCR. Fecal samples were collected from one dairy herd (herd A) for 4 consecutive years and from 41 beef and dairy herds (herd B) experiencing calf diarrhea outbreaks. Altogether, 1,700 samples were analyzed by group A rotavirus enzyme-linked immunosorbent assay, and 98 rotavirus-positive specimens were selected for G-typing by RT-PCR. The effect of herd type, time, geographic region, and clinical symptoms on the G-type distribution was evaluated. Altogether (herds A and B), G10 was found in 59 (60.2%) fecal specimens, G6 was found in 30 (30.6%) specimens, G3 was found in 1 (1.0%) specimen, and G8 was found in 1 (1.0%) specimen. Seventy-seven (7.1%) fecal specimens were not typeable. Herd type specificity in the G-type distribution was demonstrated in the herds in herd B. In the 6 beef suckler herds, only G6 was detected, while rotavirus strains from the 35 dairy herds were predominantly (54%) G10. The G-type distribution was restricted in herds A and B. Twenty-nine of 30 strains from herd A were characterized as G10. In the vast majority of herds in herd B, a single G-type was identified. The serotype G10 and the electropherotype persisted over time in herd A. No characteristic G-type variation in the geographic distribution of cattle herds in herd B was obvious. There was no difference in the G-type distributions between the strains from clinically and subclinically rotavirus-infected calves in dairy herd A. The results from this study strongly indicate a pronounced stability in the rotavirus G-type distribution in Swedish cattle herds, which emphasizes the importance of continuous preventive measures for control of neonatal calf diarrhea. A future bovine rotavirus vaccine in Sweden should contain G10 and G6 strains.

Neonatal enteritis caused by bovine group A rotavirus (BRV) is a common and costly disease in cattle populations worldwide. In Sweden, a clear association between infections with BRV and calf diarrhea (6a) and reduction of weight gain in diarrheic calves has been demonstrated (6b). Control of neonatal diarrhea caused by BRV is based on prophylaxis. Useful information for vaccine development and other prevention strategies may be gained by insight into the BRV molecular epidemiology (e.g., the serotype distribution). BRV strains are classified into G- and P-types according to the two type-specific, outer capsid proteins VP7 and VP4 (7). Serotype-specific classification is based on neutralization tests, but other assays are also available for typing, including monoclonal antibodies in enzyme-linked immunosorbent assays (ELISAs) nucleic acid hybridization, reverse transcription-PCR (RT-PCR), and nucleotide sequence analysis (for review, see reference 11).

Significant information about the distribution of rotavirus serotypes in different species has been obtained, especially about the G-types. Out of 14 identified rotavirus G-types, at least 4 epidemiologically important BRV G-types (G1, G6, G8, and G10) have been described in cattle populations (2, 22). The G6 and G10 types are regarded as the main types, while G1 and G8 are less common. The occurrence of G2, G3, G7, and G11 BRV strains has also been reported (4, 12, 13). The distribution of BRV serotypes has been suggested to be associated with herd type, region, management conditions (1, 17, 18), clinical symptoms (14), and calf age (6).

In Sweden, the cattle herd structure comprises approximately 31,000 herds, equally divided between dairy and suckled beef herds. Herds are comparatively small; the average size of dairy herds is 28 cows, and that of beef cattle herds is 10 cows. The cattle population in the northern regions of Sweden has a sparse density (100 cows/km² of grazed land), while the density in the south is three times as high (Statistics Sweden 1998).

The aim of this study was to evaluate for the first time the effects of time, herd type, geographic region, and clinical symptoms on the BRV G-type distribution in Swedish cattle herds, in order to gather epidemiological information to be used in disease prevention and control.

MATERIALS AND METHODS

Field samples. Fecal samples were collected from (i) a single dairy herd on a long-term basis and (ii) 41 different herds experiencing calf diarrhea outbreaks. Herd A. Fecal sampling was performed for 4 consecutive years (1993 to 1996) in a large, closed dairy herd, as previously described (6b). In brief, at 4, 14, and 28 days of age, calves were sampled on a regular basis by farm personnel. In addition, fecal samples were also collected on the day of onset from all diarrheic calves, up to 31 days of age. All 1,400 fecal samples were submitted to the National Veterinary Institute (SVA) by mail. From each sample, a 10% fecal slurry was prepared in 0.9% NaCl and centrifuged at 1,000 × g for 5 min, after which the supernatant was collected. The clarified fecal specimens were stored at −20°C before analysis or examined directly by a group A rotavirus ELISA (23, 24). A total of 104 fecal specimens had an absorbance value (optical density) equal to or more than 0.10 and were thus considered BRV positive. Out of these 104 fecal specimens, 30 were selected for G-typing. The selection of fecal specimens for G-typing was performed to comprise symptomatic as well as asymptomatic infections (15 specimens originating from diarrheic calves and 15 from nondiarrheic calves) and also to cover the entire sampling time period.
Herds B. Roughly 300 fecal samples from diarrheic calves were collected by veterinary practitioners in 1992 to 1997 and submitted to SVA for enteropathogenic agent analysis. The samples were delivered directly to the laboratory or submitted by mail. Preparation of fecal slurries and examination and storage of the fecal specimens for BRV analysis were performed as for herd A. A total of 68 fecal samples, originating from calves in 35 dairy herds and 6 suckled beef herds, were tested positive by group A rotavirus ELISA (23, 24). Each herd contributed either 1 (24 herds), 2 (10 herds), 3 (4 herds), or 4 (3 herds) BRV-positive fecal samples. All 68 BRV-positive fecal samples were analyzed by G-type RT-PCR.

Reference strains. The BRV reference strains UK (G6), G8, and B223 (G10) and the rotavirus reference strains Wa (G1), DS-1 (G2), and BRV (G3) were used as controls in the RT-PCR assays. The BRV reference strain NCDV (G6) was used in the RNA polyacrylamide gel electrophoresis (RNA-PAGE). All reference strains had been cultured in MA 104 cells.

Extraction of dsRNA. Extraction of double-stranded RNA (dsRNA) from the fecal specimens and the reference strains was performed by a modification of the previously published guanidinium thiocyanate and silica extraction method (3). Briefly, 50 μl of 10% clarified fecal suspension was mixed and incubated for 15 min at 20°C. The silica was pelleted by centrifugation and washed twice with a buffer consisting of a mixture of 120 g of guanidinium thiocyanate (GTC), 100 ml of 0.1 M Tris-HCl (pH 6.4), 22 ml of 0.2 M EDTA (pH 8.0), and 2.6 g of Triton X-100 (KEBO Lab, Stockholm, Sweden). The silica was pelleted by centrifugation and washed twice with a buffer consisting of 120 g of guanidinium thiocyanate (GTC), 100 ml of 0.1 M Tris-HCl (pH 6.4), 22 ml of 0.2 M EDTA (pH 8.0), and 2.6 g of Triton X-100 (KEBO Lab, Stockholm, Sweden). The silica was pelleted by centrifugation and washed twice with a buffer consisting of 120 g of guanidinium thiocyanate (GTC), 100 ml of 0.1 M Tris-HCl (pH 6.4), 22 ml of 0.2 M EDTA (pH 8.0), and 2.6 g of Triton X-100 (KEBO Lab, Stockholm, Sweden). The dsRNA was then eluted with 25 μl of 100 mM methanol and incubation of this mixture for 5 min at 56°C for 15 min. The dsRNA was used directly in the RT-PCR assay or stored at −20°C.

G-typing by RT-PCR. G-typing was performed by a modification of the previously published RT-PCR method (8–10). The nucleotide sequences of the primers are shown in Table 1. Typing with G1 to -4 primers and G6, G8, and G10 primers were performed in two separate assays. Briefly, 0.8 μl of 100 mM methylmercuric hydroxide (LabKemi, Stockholm, Sweden) was mixed in a PCR tube with either 3 μl of End 9 and End 9 UK (33 μM each), (G1 to -4) or 9 μl of 10 μM primer sBeg9 (G6-G8-G10), followed by addition of 10 μl of dsRNA and subsequent denaturation for 5 min. Denaturation was stopped by addition of 0.8 μl of 700 mM β-mercaptoethanol and incubation of this mixture for 5 min at room temperature. The denaturated dsRNA was mixed to a final volume of 30 μl with an RT reaction mixture consisting of 3 μl of 10% PCR buffer (Perkin-Elmer), 1.8 μl of 25 mM MgCl2 (Perkin-Elmer), 3 μl of 2 mM deoxynucleoside triphosphates (dNTPs) (Pharmacia Biotech), 0.5 μl of RNasin (40 U/μl) (Promega), and dH2O. The dsRNA was reverse transcribed by incubation in a Perkin-Elmer MJ2000 PCR machine for 60 min at 42°C. The cDNA produced was amplified in the presence of 70 μl of PCR mixture, which consisted of 10 μl of 10% PCR buffer, 6 μl of 25 mM MgCl2, 10 μl of 2 mM dNTPs, a serotype-specific primer mix (either 4 μl of 33 μM sBeg1-aCT2-aET3-aDT4 or 12 μl of 10 μM sBeg9-aBT1-aCT2-aET3-aDT4, or 12 μl of 10 μM sBeg9-aBT1-aCT2-aET3-aDT4) and 12 μl of 10 μM Taq polymerase (5 U/μl) (Perkin-Elmer), and dH2O. The PCR was conducted for 30 cycles at 94°C for 2 min, 55°C for 1 min, and 72°C for 1 min, followed by 72°C for 5 min. The G-type-specific PCR product was visualized by electrophoresis for 30 min at 100 V on a 2% agarose–ethidium bromide gel in Tris borate buffer and photographed under UV light on Polaroid film.

RT-PCR of full-length VP7. For the BRV-positive fecal specimens that were not typeable by G-type RT-PCR, amplification of the full-length VP7 gene was performed. The VP7 RT-PCR procedure was identical to the G6-G8-G10-typing procedure, with the exception that a VP7 primer mix (33 μM sBeg 9-End 9-End 9 UK) was used instead of the type-specific primer mix in the PCR mixture.

Demonstration of RNA electrophoretic migration pattern and detection of BRV by RNA-PAGE. The RNA electrophoretic patterns of BRV strains from dairy herd A were examined by RNA-PAGE (24). The RNA segment bands were visualized by silver staining with the Silver Stain Plus kit (Bio-Rad), according to the manufacturer’s instructions.

RESULTS

A total of 91 of 98 BRV strains from dairy herd A and the herds in herd B were successfully G-typed. Types G10 and G6 predominated; G10 was detected in 59 (60.2%) and G6 was detected in 30 (30.6%) of the BRV-positive fecal specimens. Both G3 and G8 were each demonstrated in 1 (1.0%) of the BRV-positive fecal specimens. Figure 1 illustrates PCR results. No fecal specimen with double G-types (i.e., more than one amplified G-type-specific PCR product visualized) was identified.

<table>
<thead>
<tr>
<th>Primer (reference)</th>
<th>Nucleotide sequence (5'→3')</th>
<th>Position</th>
<th>G-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>sBeg9 (10)</td>
<td>GCC TTT AAA AGA GAG AAT TTC</td>
<td>1–21</td>
<td>1</td>
</tr>
<tr>
<td>End 9 (8)</td>
<td>GGT CAC ATC ATA CAA TTC TAA TCT AAG</td>
<td>1062–1036</td>
<td>2</td>
</tr>
<tr>
<td>End 9 UK (9)</td>
<td>GGT CAC ATC ATA CAA CTC TAA TCT</td>
<td>1062–1039</td>
<td>3</td>
</tr>
<tr>
<td>aBT1 (8)</td>
<td>CAA GTA CTC AAA TCA ATG ATG G</td>
<td>314–335</td>
<td>1</td>
</tr>
<tr>
<td>aCT2 (8)</td>
<td>CAA TGA TAT TAA CAC ATT TTC TGT G</td>
<td>411–435</td>
<td>2</td>
</tr>
<tr>
<td>aET3 (8)</td>
<td>CGT TTT AAG TTG CAA CAG</td>
<td>689–709</td>
<td>3</td>
</tr>
<tr>
<td>aDT4 (8)</td>
<td>CGT TTT AAG TTG CAA CAG</td>
<td>480–498</td>
<td>4</td>
</tr>
<tr>
<td>DT6 (10)</td>
<td>CTA GGT CCT GTG TAG AAT C</td>
<td>499–481</td>
<td>6</td>
</tr>
<tr>
<td>HT8 (10)</td>
<td>CCG TCC CGG ATT AGA CAC</td>
<td>273–256</td>
<td>8</td>
</tr>
<tr>
<td>ET10 (10)</td>
<td>TTC AGC CGT TGC GAC TCT</td>
<td>714–697</td>
<td>10</td>
</tr>
</tbody>
</table>

FIG. 1. G-typing of bovine field strains by RT-PCR. Lanes: 6 to 10, field strains; 2 to 5, rotavirus reference strains G6 (UK), G8 (678), G10 (B223), and G3 (RRV), respectively; 1 and 11, molecular weight marker (100-bp DNA ladder).
The G-type distribution in dairy herd A from 1993 to 1996 is summarized in Table 2 and shows that, with one exception, G10 strains were the only ones circulated. Furthermore, it was interesting to observe that all identified RNA patterns in the herd showed great similarity (four RNA patterns are shown in Fig. 2), suggesting that a single G-type strain circulated in herd A. No effect of time or clinical symptoms on the G-type distribution was apparent.

TABLE 2. Rotavirus G-types in 30 fecal specimens from diarrheic and nondiarrheic calves in dairy herd A

<table>
<thead>
<tr>
<th>Yr/quarter</th>
<th>Distribution in G-type*:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1–4</td>
</tr>
<tr>
<td>1993/1</td>
<td>NN</td>
</tr>
<tr>
<td>1994/4</td>
<td>D</td>
</tr>
<tr>
<td>1995/1</td>
<td>D</td>
</tr>
<tr>
<td>1995/2</td>
<td>D</td>
</tr>
<tr>
<td>1995/3</td>
<td>D</td>
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<tr>
<td>1996/1</td>
<td>D</td>
</tr>
<tr>
<td>1996/3</td>
<td>D</td>
</tr>
</tbody>
</table>

* D, diarrheic; N, nondiarrheic.

The typing results from the B herds are shown in Table 3. Most interesting was to find that in 15 out of 17 herds where multiple samples were collected, a single G-type was identified. In the remaining two herds, two fecal specimens from one herd were not typeable, and two G6 plus two untyped strains were identified in the other herd. All strains from the beef suckler herds in herd B were characterized as G6, whereas in the dairy herds, G10 was the predominating G-type (54%) and G6 was less frequently detected (32%). The geographic distribution of the herds in study B is illustrated in Fig. 3.

Altogether, seven BRV-positive fecal specimens (7.1%), originating from five distinct dairy herds in different parts of Sweden, were not typeable with the G1- to -4-, G6-, G8-, and G10-specific primers (Table 3). Amplification of full-length copies of the VP7 gene obtained from five of the seven untyped fecal specimens indicated that they are of group A rotavirus. The remaining two fecal specimens, originating from two diarrheic calves in the same herd, were examined by RNA-PAGE without detection of dsRNA bands. Their optical densities in ELISA were close to the 0.10 cutoff level (0.12 and 0.24).

Several different BRV G-types (G3, G6, G8, and G10) were detected in Swedish cattle herds A and B. G10 clearly dominated in dairy herd A and was also an important G-type in herd B. This differs from previously reported studies, in which G6 was shown to be the predominating G-type (1, 14, 17, 20, 22). The results from this study strongly suggest G-type restriction in the herds. From a vast majority (15 of 18) of the herds from which multiple samples were collected, only a single G-type was detected. Most interestingly, all strains from the suckled beef herds were G6, while G10 predominated or was the only G-type in the dairy herds. A large proportion of the BRV-positive fecal specimens originated from dairy herds, a circumstance that naturally reflects the G-type prevalence. The strong association between herd type and G-type distribution found in this study is supported by others (1, 17, 18). In the Swedish cattle population, exchange of animals between dairy herds and suckled beef herds is limited, which probably enhances the connection between herd type and BRV G-type. If cattle trading is restricted and herds are closed, BRV can still persist in a herd from one year to the next and serve as a source of infection to neonates. Long-term persistence of one G-type was apparent in the closed dairy herd A, which is consistent with a previous report (16). The RNA electrophoretic pattern of the BRV strains from herd A showed remarkable congruity. This suggests that the same BRV strain persisted in the herd for several years, possibly due to heavy contamination of BRV in the calf barn. No characteristic G-type variation was obvious in the geographic distribution. From the northern part of Sweden, two BRV strains were identified as G3 and G6, and two were untyped, which might indicate a different G-type entity compared with the rest of the BRV strains. However, the number of fecal specimens from the north, where the cattle population is sparse, was very low. In southern Sweden, G10 and G6 predominated. The untyped fecal specimens originated from different parts of the country. The results from this study support the opinion (22) that BRV forms a distinct epidemi-

DISCUSSION

Several different BRV G-types (G3, G6, G8, and G10) were detected in Swedish cattle herds A and B. G10 clearly dominated in dairy herd A and was also an important G-type in herd B. This differs from previously reported studies, in which G6 was shown to be the predominating G-type (1, 14, 17, 20, 22). The results from this study strongly suggest G-type restriction in the herds.
the occurrence of G-types other than G1- to -4, G6, G8, and G10 or antigenic variation within the G1- to -4, G6, G8, and G10 serotypes. Further studies, including nucleotide sequence determination, might reveal the G-type and origin of these strains. Two untyped fecal specimens proved BRV positive by ELISA, but no full-length copies of the VP7 gene were amplified and no dsRNA bands were detected by RNA-PAGE, suggesting that these were false positive by ELISA. The fact that these specimens had optical density values close to the cutoff supports this conclusion.

In conclusion, the results from this study strongly indicate a pronounced stability in the BRV G-type distribution in Swedish cattle herds, which probably reflects the resistant character of rotavirus. The resistance and persistence of BRV in a cattle herd are highly significant epidemiological factors and stress the importance of continuous preventive measures in control of the disease. Preventive measures for a herd should aim at reducing BRV contamination in the calf barn and elevating the calves’ resistance to disease through hygiene, management, and vaccination programs. In Sweden, no BRV vaccines are currently registered. An efficient vaccine would clearly be a useful agent for cattle herds experiencing problems related to calf diarrhea. A future vaccine in Sweden should contain both the G6 and G10 BRV strains.

ACKNOWLEDGMENTS

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REFERENCES


FIG. 3. Geographic distribution of rotavirus G-types in 41 Swedish cattle herds with calf diarrhea outbreaks.


