JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1989, p. 126–131
0095-1137/89/010126-06$02.00/0

Vol. 27, No. 1

# Molecular Epidemiology and Subgroup Determination of Bovine Group A Rotaviruses Associated with Diarrhea in Dairy and Beef Calves†

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Received 1 August 1988/Accepted 26 September 1988

**The genome electropherotyping technique was used to examine group A rotaviral infections of diarrheic calves ranging from 1 to 85 days of age in 2 beef and 27 dairy herds. Coelectrophoresis studies demonstrated 38 distinct bovine group A rotavirus genome electropherotypes; all were long genome electropherotypes, and none had extra segments or unusual segment rearrangements. Genome electropherotypes in fecal specimens from diarrheic calves previously inoculated orally with a commercial, modified-live group A rotavirus vaccine differed from the vaccine genome electropherotype. Generally, when fecal specimens for genome electropherotyping were collected from two or more different calves within the same herd over a relatively short time, only one genome electropherotype was detected within a given herd. Different genome electropherotypes were detected in the same herd, however, when fecal specimens were obtained from different diarrheic calves over longer intervals (6 months or more). Twenty-three group A rotavirus strains with distinct genome electropherotypes, from diarrheic calves in 22 herds, were isolated and plaque purified in cell culture, and all were subgroup 1 group A rotaviruses. Non-group A rotavirus genome electropherotypes were not detected in 131 fecal specimens, negative for group A rotavirus, collected from diarrheic calves in 17 dairy herds.**

The 11 double-stranded RNA (dsRNA) segments of the rotaviral genome separate upon polyacrylamide gel electrophoresis to produce characteristic patterns called genome electropherotypes (17, 18). Because rotavirus isolates are distinguishable by subtle genomic variations, the genome electropherotyping technique has been used globally during the past decade to reveal aspects of human group A rotaviral infections unobtainable by conventional serologic methods (1, 2, 5–7, 9, 12, 13, 21, 22, 24, 26–28). Although bovine group A rotaviruses frequently cause calf diarrhea, there are only a few, often limited, genome electropherotyping studies on these viruses (3, 8, 16, 19, 20, 22, 23, 32), and none have been reported in the United States. In this study the genome electropherotyping technique was used to investigate group A rotaviral infections associated with diarrhea in dairy and beef calves located within a limited geographic area. In addition, the subgroup specificities of 23 group A rotaviruses isolated from diarrheic calves in 22 herds were determined by a monoclonal antibody enzyme-linked immunosorbent assay.

## MATERIALS AND METHODS

**Specimens.** Each fecal specimen consisted of a single collection from an individual animal. Specimens were collected over a 4-year period from diarrheic calves, 1 to 85 days of age, in 1 beef (AT) and 26 dairy herds located in northeastern Ohio within 35 km of the Ohio Agricultural Research and Development Center. The dairy operations maintained closed herds (i.e., raised their own replacement cows) or did not add new animals as neonates. Most of the local dairy herds had been in operation for over 15 years, and each maintained at least 80 cows, which were usually Holsteins. However, one herd (BV) had Brown Swiss, one herd (NO) had Holstein and Guernsey, and one herd (DB) had Holstein and Jersey cows. Dairy calves were kept in individual housings or stalls in all but one herd (GS). Fecal specimens from diarrheic calves in one dairy herd (IN) and one beef herd (MO), located in Indiana and southwestern Montana, respectively, were also included.

In seven dairy herds (BG, CA, CS, DW, GS, RP, and SN) 1-day-old calves were inoculated orally with a commercially available, modified-live vaccine containing bovine group A rotavirus (Lincoln NCDV isolate; 14, 15). In two herds (CS and NO), cows were injected intramuscularly with this vaccine late in gestation.

**Virus detection, isolation in cell culture, and subgroup analysis.** Group A rotaviruses in fecal specimens were detected by a cell culture immunofluorescence assay (29). Twenty-three group A rotaviruses, from diarrheic calves in 2 beef and 20 dairy herds, were propagated in MA104 cell cultures and plaque purified at passage 11, as described previously (29). After plaque purification, these isolates were passaged twice more in cell culture before their genome electropherotypes were determined. By using coelectrophoresis described below, the genome electropherotype of each cell culture-passaged isolate was compared with that of the group A rotavirus in the fecal specimen from which it was obtained.

Subgroup antigen specificity of each plaque-purified isolate was determined at passage 13 by a monoclonal antibody enzyme-linked immunosorbent assay (29) with some modifications. The modifications were the use of biotinylated affinity-purified goat anti-mouse immunoglobulin G antibody (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.), peroxidase-conjugated streptavidin (Kirkegaard and Perry, Inc.), and the chromogenic substrate 2,2′-azino-di-(3-ethylbenzthiazolinesulfonic acid) to detect bound monoclonal antibody. Cell culture-passaged bovine (ID isolate) and

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† Journal article no. 193-88, Ohio Agricultural Research and Development Center, The Ohio State University.

porcine (Gottfried isolate) group A rotaviruses served as subgroup 1 and 2 control antigens, respectively. Criteria for subgroup determination were those used previously (29).

**Extraction and electrophoresis of viral dsRNA.** Viral dsRNA was extracted from fecal specimens and cell culture lysates containing group A rotavirus by CF11 cellulose chromatography (30), and electrophoresis was performed with Laemmli 7.5% polyacrylamide slab gels (4). Gels were then stained with silver as described previously (4) and examined for rotaviral genome electropherotypes. Sixty-five fecal specimens, negative for group A rotavirus by cell culture immunofluorescence assay, collected from diarrheic calves in one dairy herd (DB) also were extracted in an attempt to detect non-group A rotaviruses. Likewise, another 66 fecal specimens, negative for group A rotavirus by cell culture immunofluorescence assay, from diarrheic calves in 16 of the local dairy herds were similarly extracted.

Differences among bovine group A rotavirus genome electropherotypes were determined by coelectrophoresis of viral dsRNA with a standard reference dsRNA preparation derived from the Lincoln NCDV isolate of bovine group A rotavirus or, sometimes, with dsRNA preparations derived from different fecal specimens. Occasionally, dsRNA extracted from the ID isolate of bovine group A rotavirus was included in the electrophoresis run as a short genome electropherotype control (29).

## RESULTS

Although the overall incidence of group A rotavirus in fecal specimens collected throughout this study was not determined, 76 of 350 (22%) representative specimens from diarrheic calves in 20 local herds were positive for the virus by the cell culture immunofluorescence assay; these positive specimens were from animals 1 to 28 days old. Routinely, dsRNA obtained from 60 μl of fecal specimen was sufficient to produce a complete genome electropherotype in silver-stained gels. Genome electropherotypes produced by group A rotavirus-positive fecal specimens, collected from diarrheic calves in 27 dairy and 2 beef herds, had segment distributions among the four size classes characteristic of group A rotavirus; all were long genome electropherotypes, none possessed extra segments, and none had unusual segment rearrangements. Seven representative bovine group A rotavirus genome electropherotypes are shown in Fig. 1. No preparation derived from the 131 fecal specimens negative for group A rotavirus produced a genome electropherotype.

Coelectrophoresis of the Lincoln NCDV isolate dsRNA with dsRNA extracted from group A rotavirus-positive fecal specimens revealed 38 distinct genome electropherotypes (Table 1). These genome electropherotypes varied from the Lincoln NCDV isolate genome electropherotype by differences in mobility of one (genome electropherotype 32) to seven (genome electropherotypes 12, 13, 26, and 27) segments, and none was identical to it. Some variation was detected in each of the 11 genome segments. If several genome electropherotypes differed from the Lincoln NCDV isolate genome electropherotype by the same segments (i.e., genome electropherotypes 1, 2, and 3, or genome electropherotypes 22, 23, and 24), appropriate coelectrophoresis experiments established that each of these genome electropherotypes differed from the others in the mobility of at least one segment. Genome electropherotypes detected in each herd were unique to that herd except for genome electropherotype 25, which was first detected in a diarrheic calf in the RU herd and then, a year later, in a diarrheic calf in the WE herd about 15 km away.

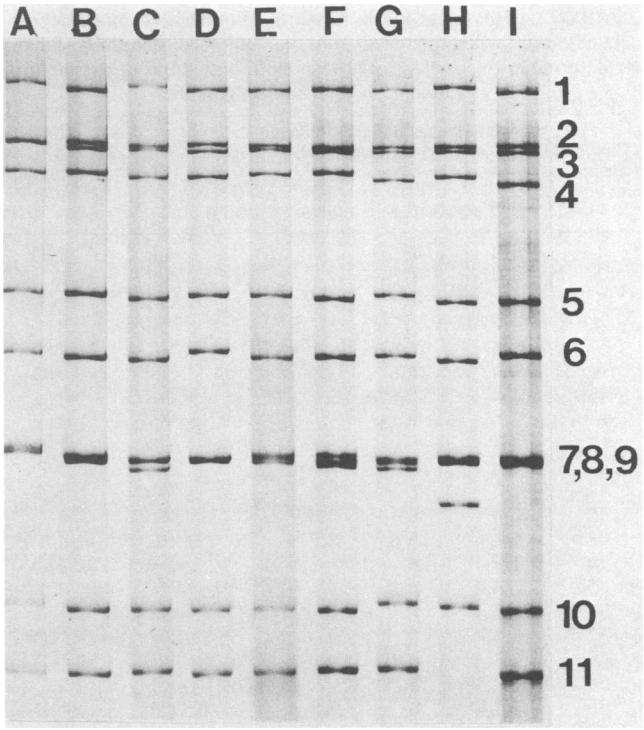


FIG. 1. Some group A rotaviral genome electropherotypes detected in fecal specimens collected from diarrheic dairy calves. Electrophoresis was conducted in the same polyacrylamide gel slab, and migration is from top to bottom. Numbers to the right designate segments of the Lincoln NCDV isolate genome. Lanes: A, genome electropherotype from the GS herd; B, genome electropherotype 5 from the SF herd; C, genome electropherotype 12 from the DE herd; D, genome electropherotype 32 from the MI herd; E, genome electropherotype 26 from the OR herd; F, genome electropherotype 27 from the CA herd; G, genome electropherotype 37 from the RO herd; H, short genome electropherotype of the ID isolate; I, genome electropherotype of the Lincoln NCDV isolate.

Although numerous genome electropherotypes were recognized, generally only one genome electropherotype was detected in a herd if fecal specimens were taken from diarrheic calves within a several-month span (Fig. 2). Nonetheless, if fecal specimens were collected from calves in a herd over a longer period (6 months or more), other genome electropherotypes were detected. For example, group A rotaviruses in fecal specimens collected over a 6-month interval from 11 different diarrheic calves in the DB herd had genome electropherotype 24. During the following 6 months, however, group A rotaviruses shed by seven different diarrheic calves in this herd had genome electropherotype 11. Then, during the next 2 months, five different calves shed group A rotaviruses with genome electropherotype 17. Two years later, group A rotaviruses in fecal specimens collected within 1 week from three different diarrheic calves had genome electropherotype 7 (Fig. 2).

Only one genome electropherotype was detected in each of 11 other dairy herds (CA, EM, FI, GS, IN, NO, OR, RO, SF, SN, and WE) from which fecal specimens were obtained from two or more different diarrheic calves during an interval of <6 months. A similar situation was noted in the two beef herds. The group A rotaviruses in fecal specimens collected from three different calves on the same day in the

TABLE 1. Bovine group A rotavirus genome electropherotypes detected in fecal specimens from diarrheic beef and dairy calves as determined by coelectrophoresis with bovine group A rotavirus (Lincoln NCDV isolate) genome

| Genome electropherotype | Differences detected in given size class (segments) | | | | Herd | Subgroup[a] |
|---|---|---|---|---|---|---|
| | I (1,2,3,4) | II (5,6) | III (7,8,9) | IV (10,11) | | |
| 1 | 1,4 | 6 | | | LR | ND[b] |
| 2 | 1,4 | 6 | | | RP[c] | 1 |
| 3 | 1,4 | 6 | | | GS[c] | ND |
| 4 | 1,4 | | 9 | | PV | 1 |
| 5 | 1,4 | 5,6 | | | SF | ND |
| 6 | 1,4 | 5,6 | | | SN[c] | ND |
| 7 | 1,4 | 5,6 | 9 | | DB | 1 |
| 8 | 1,4 | 6 | 9 | | WE | 1 |
| 9 | 1,4 | 6 | | 10 | EM | ND |
| 10 | 1,4 | 5 | 8,9 | | BG[c] | ND |
| 11 | 1,4 | 6 | 8,9 | | DB | ND |
| 12 | 1,4 | 6 | 7,8,9 | 11 | DE | 1 |
| 13 | 1,4 | 5,6 | 8,9 | 10 | FI | 1 |
| 14 | 1,3,4 | 6 | | | DW[c] | ND |
| 15 | 1,3,4 | 6 | | | IN | 1 |
| 16 | 1,3,4 | 6 | | | GS[c] | 1 |
| 17 | 1,3,4 | 6 | 9 | | DB | ND |
| 18 | 1,3,4 | 6 | 9 | 10 | SC | ND |
| 19 | 1,3,4 | 6 | | 10,11 | AT | 1 |
| 20 | 1,3,4 | 6 | 7,8 | | LR | ND |
| 21 | 1,2,4 | 6 | | | AY | 1 |
| 22 | 1,2,4 | 6 | 9 | | GW | 1 |
| 23 | 1,2,4 | 6 | 9 | | LR | ND |
| 24 | 1,2,4 | 6 | 9 | | DB | 1 |
| 25 | 1,2,4 | 5,6 | 9 | | RU | 1 |
| | | | | | WE | ND |
| 26 | 1,2,4 | 5,6 | 7,9 | | OR | 1 |
| 27 | 1,2,4 | 5 | 7,9 | 11 | CA[c] | 1 |
| 28 | 1,2,3,4 | | 9 | | EM | 1 |
| 29 | 1,2,3,4 | 6 | | 10 | CS[c,d] | ND |
| 30 | 2,3,4 | 5,6 | | | SN[c] | ND |
| 31 | 3,4 | 5,6 | | 10,11 | BO | 1 |
| 32 | 4 | | | | MI | ND |
| 33 | 4 | 5 | 7 | | MY | 1 |
| 34 | 4 | 5,6 | 9 | | RM | 1 |
| 35 | 4 | 5,6 | | 11 | BV | 1 |
| 36 | 4 | | 7,8,9 | 11 | NO[d] | 1 |
| 37 | 4 | 5 | 9 | 10,11 | RO | 1 |
| 38 | 4 | 6 | | 10 | MO | 1 |

[a] Subgroup determination conducted with cell culture isolate.
[b] ND, Not determined.
[c] Neonatal calves orally inoculated with commercial, modified-live bovine group A rotavirus vaccine.
[d] Cows intramuscularly inoculated with commercial, modified-live bovine group A rotavirus vaccine.

AT herd had genome electropherotype 19, whereas those in fecal specimens collected from five different calves during an outbreak of calf scours in the MO herd had genome electropherotype 38. A single exception was the LR dairy herd, in which three different genome electropherotypes (genome electropherotypes 1, 20, and 23) were detected in fecal specimens derived from three different diarrheic calves in 1 month.

As with group A rotavirus genome electropherotypes detected in the DB herd, the genome electropherotypes detected within some other herds changed if fecal specimens were collected from different diarrheic calves within a herd at intervals of 6 months or more. For instance, within 2 weeks, three calves in the SN herd shed group A rotaviruses with genome electropherotype 6 but, 1 year later, a fecal

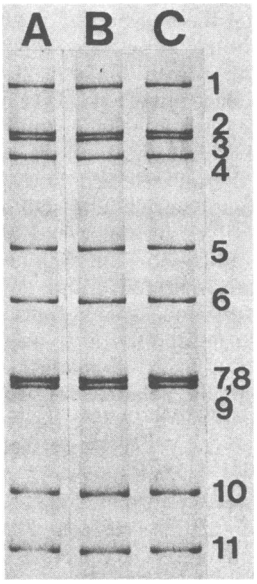


FIG. 2. Bovine group A rotavirus genome electropherotype in fecal specimens collected from three different diarrheic dairy calves in the DB herd during 1 week. Electrophoresis was in the same polyacrylamide gel slab, and migration was from top to bottom. Numbers to the right designate segments. Lanes A to C contain genome electropherotypes detected in each of the three fecal specimens. Coelectrophoresis studies (not shown) established that each specimen contained genome electropherotype 7.

specimen obtained from a calf in this herd contained a group A rotavirus with genome electropherotype 30. Likewise, during 3 months, three calves in the WE herd shed group A rotaviruses with genome electropherotype 8 but, 1 year later, a calf in this herd shed group A rotavirus with genome electropherotype 25. Moreover, a calf in the GS herd shed a group A rotavirus with genome electropherotype 3 but, 2 years later, within 3 weeks two different calves shed group A rotaviruses with genome electropherotype 16. Finally, a fecal specimen collected from a calf in the EM herd contained a group A rotavirus with genome electropherotype 28, whereas fecal specimens collected 6 months later on the same day from two other calves contained group A rotaviruses with genome electropherotype 9.

Coelectrophoresis of dsRNA from each of the plaque-purified bovine group A rotavirus isolates with the dsRNA in the fecal specimens from which they were derived confirmed in all cases that the genome electropherotype of the cell culture-passaged isolate was identical to the genome electropherotype detected in the fecal specimen of origin. Typical results are shown in Fig. 3. All plaque-purified, cell culture-passaged isolates were subgroup 1 group A rotaviruses (Table 1).

## DISCUSSION

Our findings document the substantial genomic diversity of bovine group A rotaviruses recovered from diarrheic calves within a small defined region of the United States. Thirty-six genome electropherotypes were detected in 27 herds located within the 3,850 $km^2$ (about 3.6% of Ohio) surrounding the research center; two more genome electropherotypes were detected in specimens from diarrheic calves in 2 herds outside Ohio. Undoubtedly, the recognized genomic diversity represents only a small fraction of the

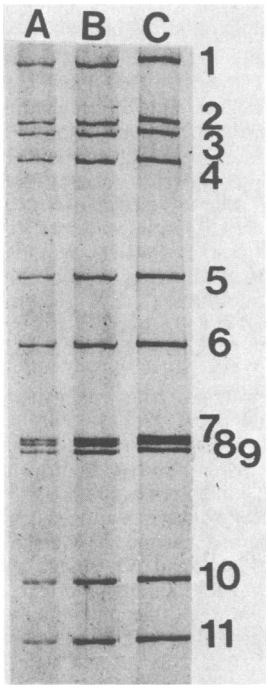


FIG. 3. Comparative coelectrophoresis of the group A rotavirus genome electropherotype detected in a fecal specimen (from a diarrheic dairy calf in the RU herd) with the genome electropherotype of its cell culture isolate derivative. Migration is from top to bottom and numbers to the right designate genome segments. Lanes: A, genome electropherotype of the cell culture-passaged, plaque-purified virus; C, genome electropherotype of the fecal specimen virus; B, coelectrophoresis of dsRNA preparations used in lanes A and C.

total variation, considering that specimens were not collected from calves in most of the more than 800 dairy herds located within this surrounding region. Nonetheless, these results confirm and extend similar, but often more limited, observations made in other countries on group A rotavirus strains shed by calves in a few or widely separated herds (3, 8, 16, 19, 20, 22, 23, 32).

Interestingly, whereas extensive genomic variation existed among the bovine group A rotaviruses, just one genome electropherotype was usually detected in fecal specimens obtained from diarrheic calves within a herd over several months. This agrees with previous observations made in France and Argentina associating one genome electropherotype with outbreaks of calf scours within a herd (3, 8, 32). In five instances, however, different genome electropherotypes were detected within the same dairy herd, provided specimens were obtained from diarrheic calves at intervals of 6 months or more. Changes in the genome electropherotype detected within a herd have been noted previously in only a few isolated instances (3, 8, 22). Our data indicate that such changes are not uncommon. Only once in our study was the simultaneous cocirculation of several genome electropherotypes within a herd (LR) detected, supporting findings by others (3, 8, 22) that simultaneous cocirculation of several genome electropherotypes within a herd does occur, although infrequently. The reason for the simultaneous presence of several genome electropherotypes within the LR herd is unknown. Mixed group A rotavirus infections, indicated by extra segments within a genome electropherotype, have been observed by others (3, 8, 16, 20, 22, 23, 32) but were not detected in our study.

Why new group A rotavirus genome electropherotypes associated with calf scours appear within a dairy herd remains unknown, but they may indicate the introduction of another virus strain into the herd or the emergence of a variant strain with some selective advantage. Moreover, a new genome electropherotype might be detected within a herd if a group A rotavirus strain, previously associated only with asymptomatic infections, became more virulent. Young calves were not added to these herds, so new strains would have to be introduced either by older animals or by some other means. On one occasion, older calves within a herd were the first to be identified as shedding a group A rotavirus with a new genome electropherotype. In this case, diarrheic calves, <28 days old, in the DB herd nursery had shed group A rotaviruses with genome electropherotype 24 until, during a break in calving, the empty nursery was cleaned and disinfected. Afterwards, two diarrheic calves, 56 and 61 days old, outside the nursery shed group A rotaviruses with genome electropherotype 11. Calves subsequently introduced into the clean nursery shed group A rotaviruses with this new genome electropherotype. Most likely, farm personnel tending to the older calves spread the virus to the nursery while performing their daily routines. It must be emphasized, however, that the older calves in this instance had been calved and raised in the DB herd. Group A rotaviruses with genome electropherotype 25 were detected in two geographically separated herds, but the temporal differences in detections suggests a coincidental sharing of a genome electropherotype by two strains rather than the herd-to-herd spread of one strain.

Diarrheic calves in dairy herds in which the commercially available, modified-live group A rotavirus vaccine was administered orally to calves, or intramuscularly to pregnant cows, shed group A rotaviruses with genome electropherotypes distinct from the vaccine genome electropherotype. Fecal specimens were collected from orally vaccinated diarrheic calves of appropriate ages to ensure a reasonable probability of detecting the vaccine genome electropherotype if the diarrhea was vaccine induced. For examples, genome electropherotypes were determined for group A rotaviruses in fecal specimens obtained from three different diarrheic vaccinates, 1 to 8 days old, in the CA herd and from three different diarrheic vaccinates, 8 to 16 days old, in the SN herd. Whereas our results demonstrate that group A rotavirus infections associated with diarrhea in orally vaccinated calves were not of vaccine origin, they also provide evidence that the vaccine is frequently inefficacious in preventing these infections under conditions prevailing in the local dairy herds.

Group A rotaviruses are antigenically characterized in part by their subgroup antigens encoded for by the sixth genome segment (10, 11). At present there is very little information regarding the subgroup specificities of bovine group A rotaviruses, and of the eight isolates characterized, all were subgroup 1 (10, 29). All 23 bovine group A rotavirus isolates characterized in this study also had the subgroup 1 antigen, even though each had a distinct genome electropherotype. In this regard, bovine group A rotavirus genomic diversity does not reflect antigenic diversity.

Human group A rotaviruses with short genome electropherotypes have been recognized for quite some time (1, 2, 5–7, 9, 13, 21, 24, 26–28), but a bovine group A rotavirus possessing a short genome electropherotype (ID isolate) has been reported only recently (29). This isolate was recovered from a diarrheic beef calf in southwestern Montana. Many genome electropherotypes were recognized in this study but

all were long, including those detected in specimens from five diarrheic beef calves in another southwestern Montana herd (MO). Thus, unlike human group A rotaviruses, bovine group A rotaviruses with short genome electropherotypes appear to be uncommon.

Rotaviruses lacking the group A antigen are at times recovered from diarrheic calves (25, 31, 33; L. J. Saif, K. W. Theil, and D. R. Redman, 63rd Annu. Meet. Conf. Res. Work. Anim. Dis., abstr. no. 98, 1982), and these viruses can be detected and differentiated from group A rotaviruses by the genome electropherotyping technique (17, 18, 25, 31, 33). In this study, however, non-group A rotavirus genome electropherotypes were not detected in any fecal specimen, either with group A rotavirus genome electropherotypes or in group A rotavirus-negative specimens. Consequently, if these viral infections occurred in these herds, they were not associated with calf diarrhea. Our findings support other studies demonstrating that non-group A rotaviruses are seldom, if ever, detected in fecal specimens from diarrheic calves (3, 25).

In conclusion, the genome electropherotyping technique can be useful for gaining insights into the epidemiology of rotaviral infections of calves, and our data show that the genomic diversity of bovine group A rotaviruses is comparable to that of human group A rotaviruses. The origin of this genomic diversity is among the interesting questions concerning group A rotaviruses that remain to be answered. As cattle within a dairy herd often represent a closed population, additional studies on group A rotaviruses with different genome electropherotypes recovered from calves within the same herd, using more sophisticated molecular techniques, could enhance our understanding of the mechanisms involved.

## ACKNOWLEDGMENTS

This research was supported in part by Special Grants Program 84-CRSR-2-2436, Cooperative State Research Service, U.S. Department of Agriculture Science and Education Administration. Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

We thank Arden Agnes, Ken Chamberlain, Cynthia Hardies, Mary Beth Kaps, Margaret Latta, Paula Lundin, and Lisa Thompson for technical help and E. Bohl, D. Hancock, D. Redman, the local dairy producers, and L. Myers (Bozeman, Mont.) for assistance in providing specimens.

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