JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1986, p. 695–699
0095-1137/86/040695-05$02.00/0

Vol. 23, No. 4

Comparison of Immune Electron Microscopy and Genome Electropherotyping Techniques for Detection of Turkey Rotaviruses and Rotaviruslike Viruses in Intestinal Contents†

KENNETH W. THEIL,* DONALD L. REYNOLDS, AND YEHIA M. SAIF

Food Animal Health Research Program, Ohio Agricultural Research and Devlopment Center, The Ohio State University, Wooster, Ohio 44691

Received 1 November 1985/Accepted 17 December 1985

Seventy-nine intestinal contents specimens from 65 turkey flocks were examined for rotavirus and rotaviruslike virus (RVLV) by immune electron microscopy (IEM) and genome electropherotyping. The IEM procedure was slightly more sensitive in detecting these viruses; 7 of 48 specimens (14.6%) positive for virus by IEM were negative by the genome electropherotyping technique. The genome electropherotyping technique more readily differentiated the rotaviruses and RVLVs than did the IEM procedure; 15 of 48 specimens (31%) positive for virus by IEM could not be differentiated into rotavirus or RVLV, whereas only 4 of the 41 specimens (9.7%) positive by genome electropherotyping produced incomplete genome electropherotypes and could not be differentiated. Thirty-one specimens negative by IEM were also negative by genome electropherotyping. Specimens determined to contain only rotavirus by IEM produced only rotavirus genome electropherotypes. Likewise, specimens determined to contain RVLV alone by IEM produced only RVLV genome electropherotypes. Three specimens contained viruses morphologically resembling rotaviruses that were not aggregated by either the anti-turkey rotavirus serum or the anti-turkey RVLV serum and possessed genome electropherotypes distinct from those of the turkey rotavirus and RVLV. These rotaviruses may represent a third, previously unrecognized serogroup of turkey rotaviruses.

Rotaviral infections cause diarrhea in many mammalian species (6), and early studies demonstrated that rotaviruses recovered from different species were antigenically related (17, 18). More recent investigations have disclosed that certain rotaviruses recovered from diarrheic pigs were antigenically unrelated to other mammalian rotaviruses (2, 4). These findings have led to the proposal that rotaviruses be subdivided into antigenically distinct serogroups, with members of each group sharing their own distinctive group antigens (11). According to the proposed nomenclature, the originally recognized rotaviruses would comprise group A, whereas the rotaviruslike agent (4) and the pararotavirus (2) would be prototype members of groups B and C, respectively.

Turkey rotaviruses were first detected in the feces of young poults with diarrhea and enteritis in South Dakota (1) and then later were detected in the intestinal contents of diarrheic 2- to 5-week-old poults in Northern Ireland (8). Although the viruses detected in these two studies were morphologically indistinguishable from mammalian rotaviruses, their antigenic relationship to the mammalian rotaviruses was not established by any serologic assay. Subsequently, it was demonstrated that turkey rotaviruses isolated and propagated in cell cultures shared antigens with the mammalian group A rotaviruses (9, 19; K. W. Theil, D. L. Reynolds, and Y. M. Saif, Avian Dis., in press). However we have recently reported that intestinal contents specimens from 2- to 3-week-old poults often contain rotaviruses that are antigenically distinct from the group A and group C rotaviruses (13). Additionally, these viruses were antigenically unrelated to a bovine rotaviruslike virus (13) that we provisionally consider a group B rotavirus (14). We currently refer to these antigenically distinct turkey viruses as turkey rotaviruslike viruses (RVLVs). Attempts to serially propagate turkey RVLVs in cell culture with techniques suitable for the propagation of turkey rotaviruses were unsuccessful (Theil, et al., in press).

Turkey RVLVs can be readily differentiated from rotaviruses in intestinal contents specimens by immune electron microscopy (IEM), provided specific antisera are used (13; Theil et al., in press). Because the quantities of RVLV antigen available have been small, we have relied exclusively upon convalescent serum obtained from experimentally infected specific-pathogen-free poults as the source of the anti-turkey RVLV serum used in the IEM procedure. Unfortunately, this reagent can be produced only in limited quantities. Moreover, it may be difficult for other laboratories to produce anti-turkey RVLV serum that is devoid of rotavirus antibody.

Besides being antigenically unrelated to turkey rotaviruses, turkey RVLVs contain 11 double-stranded RNA (dsRNA) segments that produce electrophoretic migration patterns (genome electropherotypes) in polyacrylamide gels that differ from the genome electropherotypes produced by the 11 dsRNA segments of turkey rotaviruses (13; Theil et al., in press). Herring et al. (7) reported that the genome electropherotyping technique with silver-stained polyacrylamide gels was a sensitive method, equivalent to electron microscopy, for detecting rotaviruses in bovine and human stool specimens. Because the turkey rotaviruses and RVLVs have distinctive genome electropherotypes, we conducted a study to determine whether the genome electropherotyping technique could be used as an alternative to IEM for detecting and differentiating these viruses in intestinal contents specimens.

* Corresponding author.
† The Ohio State University journal article no. 193-85.

TABLE 1. Comparison of the genome electropherotyping technique with IEM for the detection of turkey rotavirus and RVLV in intestinal contents specimens from poults

Diagnosis by IEM[a]	Diagnosis by genome electropherotyping					
	Negative	Rotavirus	RVLV	Rotavirus and RVLV	Undifferentiated[b]	Other
Negative (31)	31	0	0	0	0	0
Rotavirus (3)	0	2	0	0	1	0
RVLV (27)	2	0	23	0	2	0
Rotavirus and RVLV (3)	0	0	0	3	0	0
Undifferentiated[c] (15)	5	4	1	1	1	3

[a] Numbers in parentheses indicate numbers of specimens.
[b] Specimen produced an incomplete genome electropherotype.
[c] Specimen contained too few rotavirus particles to permit an accurate determination of their reaction with antisera.

MATERIALS AND METHODS

Specimens. Seventy-nine intestinal contents specimens were obtained from turkeys, ranging in age from 1 day to 12 weeks old, in 65 flocks located in North Carolina, Ohio, and Wisconsin. Many of these specimens were composite intestinal contents collected from several birds of the same age. The vast majority were from turkeys less than 4 weeks of age.

IEM. The IEM procedures used in this study were similar to those described previously (12; Theil et al., in press). Convalescent antisera were prepared in specific-pathogen-free turkey poults (14) maintained in isolation units. The anti-turkey rotavirus serum was collected 42 days after exposure to the A-4 isolate of turkey rotavirus and was used at a 1:50 dilution. The anti-turkey RVLV serum was collected 25 days after exposure to the AE-49B isolate of turkey rotavirus and was used at a 1:50 dilution.

Approximately 1-ml samples of specimens were diluted sixfold in phosphate-buffered saline (pH 7.4) and sonicated at room temperature for six 15-s intervals. Sonicated specimens were clarified by centrifugation at 500 × *g* for 20 min followed by filtration of the supernatant fluid through 0.8- and 0.45-μm (average pore diameter) filters (Millipore Corp., Bedford, Mass.). Samples (0.2 ml) of each filtrate were reacted overnight at 4°C with 0.8 ml of each diluted antiserum and then pelleted by ultracentrifugation at 57,000 × *g* for 45 min in a fixed-angle rotor. Pellets were suspended in 1 ml of sterile distilled water and pelleted again by ultracentrifugation as described above. These pellets were suspended in a drop of distilled water, transferred to Formvar-coated, carbonized copper grids (300 mesh; Ted Pella, Inc., Tustin, Calif.), and negatively stained with 1.5% phosphotungstic acid (pH 7.3). Specimens were then examined for virus aggregates with a Philips 201 electron microscope (Philips Norelco, Eindhoven, The Netherlands) at 80 kV. For each antiserum treatment five grid squares were examined (for approximately 15 min). Specimens with one or more aggregates containing five or more virions were considered positive for virus and were designated as rotavirus or RVLV depending upon which antiserum induced the aggregation. Specimens containing individual rotavirus particles not aggregated by either antiserum were also considered positive for virus, but could not be differentiated as rotavirus or RVLV. Specimens containing no rotavirus particles were considered negative.

Extraction and electrophoresis of viral dsRNA. Viral dsRNA was extracted from 2-ml samples of specimens by CF11 cellulose chromatography (15) and subjected to electrophoresis as described previously (3). Briefly, extracted dsRNA was resuspended in 150 μl of Laemmli sample buffer (prepared without sodium dodecyl sulfate and 2-mercaptoethanol) containing 20% (vol/vol) glycerol, and a 20-μl sample was then subjected to electrophoresis at 40 mA for 4 to 5 h in Laemmli 7.5% polyacrylamide gel slabs. Polyacrylamide gels were then stained with silver and examined for genome electropherotypes by using transilluminated white light provided by an X-ray film viewer. Specimens that produced a complete genome electropherotype (all 11 segments detected) were considered positive for virus and were designated as RVLV or rotavirus depending upon the genome electropherotype produced. Specimens that produced complete genome electropherotypes distinct from those of the turkey rotaviruses and RVLVs were also considered positive for virus and were designated as "other" rotaviruses. Those specimens that produced incomplete genome electropherotypes (usually containing only the five largest segments) were considered positive but could not be differentiated as rotavirus or RVLV. Specimens that did not produce genome electropherotypes were considered negative for virus.

RESULTS

Virus particles with rotavirus morphology were detected in 48 specimens (60.7%) by IEM (Table 1); of these positive specimens, 27 (34.2%) contained particles that aggregated with anti-turkey RVLV serum, 3 (3.8%) contained particles that aggregated with anti-turkey rotavirus serum, and 3 (3.8%) contained partilces that aggregated with both the anti-turkey and RVLV serum and the anti-turkey rotavirus serum. The remaining 15 positive specimens (19.0%) contained too few virus particles to permit an accurate determination of their reactions with the antisera.

Forty-one of the 79 specimens (51.9%) produced complete or incomplete genome electropherotypes and were considered positive for virus (Table 1). Twenty-four specimens (30.4%) produced genome electropherotypes characteristic of turkey RVLVs, whereas six specimens (7.6%) produced genome electropherotypes characteristic of turkey rotavirus. The genome electropherotypes of turkey RVLVs and rotaviruses were easily distinguishable (Fig. 1). The turkey RVLV genome electropherotype had two segments (segments 6 and 7) in the second size class and two segments (segments 8 and 9) in the third size class. Moreover, segments 10 and 11 in the fourth size class of the turkey RVLV genome electropherotype migrated as a widely spaced couplet. The segments of the turkey RVLV genome electropherotypes always migrated sufficiently apart so that all

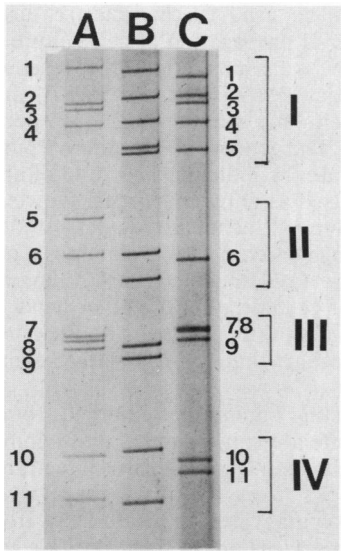

FIG. 1. Comparison of the turkey rotavirus and turkey RVLV genome electropherotypes in the same polyacrylamide gel slab. Migration is from top to bottom. Roman numerals on the right indicate dsRNA size classes. Numbers to the left designate segments of a reference group A mammalian rotavirus genome, and numbers to the right designate segments of the turkey rotavirus genome. Lanes: A, bovine rotavirus genome electropherotype; B, turkey RVLV genome electropherotype; C, turkey rotavirus genome electropherotype. Segments 7 and 8 in the turkey rotavirus genome electropherotype comigrated.

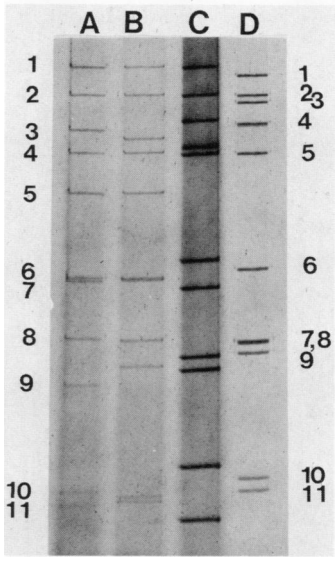

FIG. 2. Comparison of the turkey rotavirus and turkey RVLV genome electropherotypes with the genome electropherotypes of two rotavirus isolates with distinct genome electropherotypes in the same polyacrylamide gel slab. Migration is from top to bottom. Numbers to the left designate segments of the distinct genome electropherotypes, and numbers to the right designate segments of the turkey rotavirus genome electropherotype. Lanes: A, turkey rotavirus 202N, distinct genome electropherotype; B, turkey rotavirus 202J, distinct genome electropherotype; C, turkey RVLV genome electropherotype; D, turkey rotavirus genome electropherotype.

11 segments could be resolved. The turkey rotavirus genome electropherotype had only one segment (segment 6) in the second size class but had three segments (segments 7, 8, and 9) in the third size class. Further, the two smallest segments (segments 10 and 11) of the fourth size class migrated close together. Segments 7, 8, and 9 frequently migrated very close together, and it was not uncommon for two of these segments to comigrate. Four specimens (5.1%) produced genome electropherotypes of both turkey RVLVs and rotaviruses. Because each of these four specimens was composite intestinal contents collected from five or six poults, it was not possible to determine whether these results indicated mixed infections of individual birds. Four specimens (5.1%) produced incomplete genome electropherotypes and were considered positive for virus but it could not be determined whether these were turkey RVLV or rotavirus electropherotypes.

Three specimens (3.8%) produced genome electropherotypes that were distinct from the turkey rotavirus and RVLV genome electropherotypes (Fig. 2 and 3). These positive specimens were designated as other rotaviruses. These distinct electropherotypes possessed many similarities. Segment 5 of the distinct genome electropherotypes migrated further than the fifth segments of the turkey RVLV and rotavirus genome electropherotypes. Segments 6 and 7 in the distinct genome electropherotypes either migrated very close together or comigrated. Finally, segments 10 and 11 in the distinct genome electropherotypes migrated close together, but further than segments 10 and 11 in the turkey rotavirus genome electropherotype.

DISCUSSION

Overall, the IEM procedure was slightly more sensitive than the genome electropherotyping technique for detecting turkey rotaviruses and RVLVs in intestinal contents specimens. Seven of 48 specimens (14.6%) positive for virus by IEM were negative by the genome electropherotyping technique. It should be noted, however, that five of these genome electropherotype-negative specimens also contained insufficient virus particles to permit differentiation by IEM into turkey rotavirus or RVLV. Although less sensitive than the IEM procedure, the genome electropherotyping technique more frequently differentiated the rotavirus and RVLVs than did the IEM procedure. Fifteen of the 48 specimens (31%) positive for virus by IEM could not be differentiated by IEM into turkey rotavirus or RVLV, or possible other rotaviruses, whereas only 4 of 41 specimens (9.7%) positive by genome electropherotyping produced incomplete genome electropherotypes and could not be differentiated.

The inability of the IEM procedure to differentiate many positive specimens could possibly be explained, in part, by the more elaborate specimen preparation required for this test. Undoubtedly virus aggregates and debris-associated virus are removed during centrifugation and filtration of specimens before the addition of antisera. This virus would not be removed from the specimens used for extraction of the dsRNA and therefore could contribute additional dsRNA for the genome electropherotyping technique. In three instances, however, the rotaviruses not aggregated by either serum also had genome electropherotypes distinctly different from the turkey rotavirus and RVLV genome electropherotypes. Because of their distinct genome electropherotypes, it is possible that these rotaviruses represent a third, previously unrecognized, serogroup of turkey rotaviurses. Very recently, McNulty et al. (10) described several rotavirus serogroups that infected broiler chickens, and each of these new serogroups had a distinctive genome

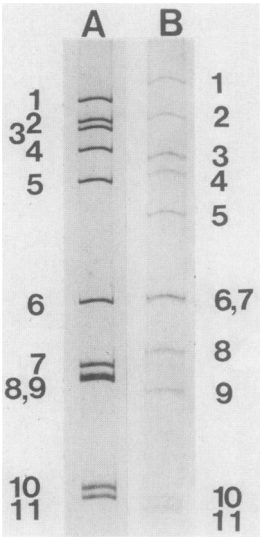

FIG. 3. Comparison of the turkey rotavirus genome electropherotype with the turkey rotavirus distinct genome electropherotype in the same polyacrylamide gel slab. Migration is from top to bottom. Numbers to the left designate segments of the turkey rotavirus genome electropherotype, and numbers to the right designate segments of the distinct genome electropherotype. Lanes: A, turkey rotavirus genome electropherotype (this isolate is different from the turkey rotavirus isolate used in Fig. 1 and 2; segments 8 and 9 in this genome electropherotype comigrate); B, turkey rotavirus 189U, distinct genome electropherotype.

electropherotype. The distinct genome electropherotypes of the turkey rotaviruses in the present study are very similar to the electropherotype 3 of the A4 isolate of chicken rotavirus reported by McNulty et al. (10). Whether or not these turkey rotaviruses with the distinct genome electropherotypes represent a new serogroup of turkey rotavirus awaits confirmation by tests with specific antiserum.

No specimen determined by IEM to contain turkey rotavirus alone produced a turkey RVLV genome electropherotype; similarly, no specimen determined by IEM to contain turkey RVLV alone produced a turkey rotavirus genome electropherotype. The 31 specimens negative for virus by IEM were also negative for virus by genome electropherotyping.

The results of our study indicate that the genome electropherotyping technique is a procedure, slightly less sensitive than IEM, suitable for the detection of turkey rotaviruses and RVLVs in intestinal contents specimens. Although this procedure lacks serologic specificity, our data demonstrate a correlation between genome electropherotype and antigenic relatedness. Genome electropherotyping does not require elaborate equipment and thus may be advantageous to those laboratories lacking electron microscope facilities. Further, IEM requires specific antiserum against each virus to be tested for, and monospecific sera may not be readily available to every laboratory. At present, genome electropherotyping is the only method available other than IEM for the detection of turkey RVLVs. In addition to the detection of rotaviruses and RVLVs, the genome electropherotyping technique also permits the detection of other rotaviruses possessing distinct genome electropherotypes. Three of the 79 specimens examined in this study contained rotaviruses with distinct genome electropherotypes, suggesting that infections with such rotaviruses are not uncommon in young turkeys. Moreover, the genome electropherotyping technique can be used to characterize turkey rotaviruses adapted to serial propagation in cell culture (Theil et al., in press). Finally, we realized that most researchers in laboratories investigating rotavirus and RVLV infections of turkeys will eventually want to prepare specific antisera to these viruses. The genome electropherotyping technique can greatly facilitate the judicious selection of appropriate specimens to be used as antigen sources. However, the genome electropherotyping technique detects only those viruses possessing segmented dsRNA genomes, and other procedures must be used to detect turkey enteric viruses with other types of genomes. On the other hand, electron microscopic examination of specimens does permit the detection of viruses other than those with segmented dsRNA genomes.

One interesting finding that emerged from this study was that RVLV infections were much more commonly detected in turkey poults than were rotavirus infections; 24 specimens from 24 flocks were positive for RVLV by genome electropherotyping, whereas 6 specimens from 6 flocks were positive for rotavirus and 4 were positive for both viruses. This observation confirms and extends our previous findings (13). The finding that infections with RVLV occur frequently in turkeys resembles the situation recently found with lambs (5) and broiler chickens (10), in which infections with rotaviruses other than group A rotaviruses are common.

ACKNOWLEDGMENTS

This research was supported in part by Special Grants Program No. 84-CRSR-2-2436, U.S. Department of Agriculture Science and Education Administration, Cooperative State Research Service. 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 M. Nelms, D. Karunakaran, and E. Polari for providing specimens. We thank Robert Dearth, Diane Miller, Christine McCloskey, and Ken Chamberlain for technical assistance and Robert Whitmoyer of the electron microscopy laboratory for his cooperation in these studies.

LITERATURE CITED

1. **Bergeland, M. E., J. P. McAdaragh, and I. Stotz.** 1977. Rotaviral enteritis in turkey poults, p. 129–130. *In* Proceedings of the 26th Western Poultry Disease Conference. University of California, Davis.
2. **Bohl, E. H., L. J. Saif, K. W. Theil, A. G. Agnes, and R. F. Cross.** 1982. Porcine pararotavirus: detection, differentiation from rotavirus, and pathogenesis in gnotobiotic pigs. J. Clin. Microbiol. **15**:312–319.
3. **Bohl, E. H., K. W. Theil, and L. J. Saif.** 1984. Isolation and serotyping of porcine rotaviruses and antigenic comparison with other rotaviruses. J. Clin. Microbiol. **19**:105–111.
4. **Bridger, J. C., I. N. Clarke, and M. A. McCrae.** 1982. Characterization of an antigenically distinct porcine rotavirus. Infect. Immun. **35**:1058–1062.
5. **Chasey, D., and J. Banks.** 1984. The commonest rotaviruses from neonatal lamb diarrhoea in England and Wales have atypical electropherotypes. Vet. Rec. **115**:326–327.
6. **Flewett, T. H., and G. N. Woode.** 1978. The rotaviruses. Brief review. Arch. Virol. **57**:1–23.
7. **Herring, A. J., N. F. Inglis, C. K. Ojeh, D. R. Snodgrass, and J. D. Menzies.** 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J. Clin. Microbiol. **16**:473–477.
8. **McNulty, M. S., G. M. Allan, and J. C. Stuart.** 1978. Rotavirus infection in avian species. Vet. Rec. **103**:319–320.
9. **McNulty, M. S., G. M. Allan, D. Todd, and J. B. McFerran.** 1979. Isolation and cell culture propagation of rotaviruses from turkeys and chickens. Arch. Virol. **61**:13–21.

10. **McNulty, M. S., D. Todd, G. M. Allan, J. B. McFerran, and J. A. Greene.** 1984. Epidemiology of rotavirus infection in broiler chickens: recognition of four serogroups. Arch. Virol. **81**:113–121.
11. **Pedley, S., J. C. Bridger, J. F. Brown, and M. A. McCrae.** 1983. Molecular characterization of rotaviruses with distinct group antigens. J. Gen. Virol. **64**:2093–2101.
12. **Saif, L. J., E. H. Bohl, E. M. Kohler, and J. H. Hughes.** 1977. Immune electron microscopy of transmissible gastroenteritis virus and rotavirus (reovirus-like agent) of swine. Am. J. Vet. Res. **38**:13–20.
13. **Saif, L. J., Y. M. Saif, and K. W. Theil.** 1985. Enteric viruses in diarrheic turkey poults. Avian Dis. **29**:798–811.
14. **Saif, Y. M.** 1974. Maintenance of a specific-pathogen-free turkey flock. Poult. Sci. **53**:544–546.
15. **Theil, K. W., C. M. McCloskey, L. J. Saif, D. R. Redman, E. H. Bohl, D. D. Hancock, E. M. Kohler, and P. D. Moorhead.** 1981. Rapid, simple method of preparing rotaviral double-stranded ribonucleic acid for analysis by polyacrylamide gel electrophoresis. J. Clin. Microbiol. **14**:273–280.
16. **Theil, K. W., L. J. Saif, P. D. Moorhead, and R. E. Whitmoyer.** 1985. Porcine rotavirus-like virus (group B rotavirus): characterization and pathogenicity for gnotobiotic pigs. J. Clin. Microbiol. **21**:340–345.
17. **Thouless, M. E., A. S. Bryden, T. H. Flewett, G. N. Woode, J. C. Bridger, D. R. Snodgrass, and J. A. Herring.** 1977. Serological relationships between rotaviruses from different species as studied by compelement fixation and neutralization. Arch. Virol. **53**:287–294.
18. **Woode, G. N., J. C. Bridger, J. M. Jones, T. H. Flewett, A. S. Bryden, H. A. Davies, and G. B. B. White.** 1976. Morphological and antigenic relationships between viruses (rotaviruses) from acute gastroenteritis of children, calves, piglets, mice, and foals. Infect. Immun. **14**:804–810.
19. **Yason, C. V., and K. A. Schat.** 1985. Isolation and characterization of avian rotaviruses. Avian Dis. **29**:499–508.