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Age-Related Infections with Rotavirus, Rotaviruslike Virus, and Atypical Rotavirus in Turkey Flocks†

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The genome electropherotyping technique was used in longitudinal surveys to detect group A rotavirus, rotaviruslike virus (RVLV), atypical rotavirus (ATR), and reovirus in intestinal contents or fecal specimens collected from turkeys in 10 commercial and 2 research station flocks. These viruses were detected in turkeys from 8 of 10 commercial flocks surveyed. Of 278 specimens collected from turkeys less then 29 days old in commercial flocks, 79 (28.4%) contained one or more viruses, whereas only 1 of 120 specimens collected from turkeys older than 28 days had virus. Viruses were detected in commercial turkeys between 3 and 35 days old, and over a third of the specimens collected from birds during their first week of life were positive for group A rotavirus. Between 8 and 28 days of age, commercial turkeys were infected with group A rotavirus, RVLV, ATR, and reovirus. ATR was the only virus detected in birds older than 28 days. Overall, group A rotavirus and RVLV were each detected in 39 specimens, and ATR was detected in 7 specimens; reovirus was detected in 2 specimens. Eight of the positive specimens contained two viruses. All 102 specimens collected from turkeys 1 to 56 days old in the two research station flocks were negative for virus.

Virus particles resembling rotaviruses have been detected in the feces and intestinal contents of diarrheic turkey poults only recently (1, 4). Subsequent studies have demonstrated that some turkey rotaviruses share a common antigen with mammalian group A rotaviruses (5, 7, 9, 11). However, it has also been established that turkey poults in the United States are frequently infected with rotaviruses that are antigenically distinct from the group A turkey rotaviruses (7, 9, 10). We currently refer to these antigenically distinct turkey viruses as turkey rotaviruslike viruses (RVLVs). Turkey RVLVs contain 11 double-stranded RNA (dsRNA) segments that produce genome electropherotypes in polyacrylamide gels that are distinct from the genome electropherotypes produced by the 11 dsRNA segments of turkey group A rotaviruses (7, 9, 10). More recently, we have detected additional turkey rotaviruses that possess a novel genome electropherotype and appear to be antigenically distinct from the turkey group A rotaviruses and turkey RVLVs (10). These second antigenically distinct turkey rotaviruses probably represent a third serogroup of turkey rotaviruses and for the purposes of this report will be referred to as turkey atypical rotaviruses (ATRs). Although the etiologic significance of turkey rotaviruses, RVLVs, and ATRs in the enteric disease of poults remains to be delineated, their occurrence in specimens derived from diarrheic turkeys suggests a causative role.

Little is known regarding the epidemiology of turkey rotavirus, RVLV, and ATR infections. Although previous investigations have provided some data concerning the prevalence of these viruses, they have relied upon a single or only a few specimens collected from each flock under study (7, 10). This study was undertaken to obtain additional information on the epidemiology of rotavirus, RVLV, and ATR infections by using the genome electropherotyping technique in prospective, longitudinal surveys to monitor these infections in turkeys raised in commercial and research station flocks.

MATERIALS AND METHODS

Specimens. Specimens were obtained from turkeys at two different locations during a 16-month period. Ten of the flocks studied contained market turkeys raised under commercial brooder-growout conditions. The poults for the commercial operation were all obtained from the same hatchery but were placed in various houses located within this operation. These flocks ranged from 20,000 to 25,000 birds, with five flocks having 24,000 or more birds. The other two flocks surveyed each contained approximately 100 turkeys raised in a small closed operation at our research station. Most of the 398 specimens from turkeys in the commercial flocks were intestinal contents, but a few from the oldest birds were fecal specimens. In addition, three yolk specimens (each comprising seven yolks) and three intestinal tract specimens (each comprising seven intestinal tracts) were obtained from unhatched embryos of flock 37-3 at the time of placement. Composite yolk specimens were prepared by thorough mixing, whereas composite intestinal tract specimens from unhatched poults were prepared by emulsification with an equal volume of Earle minimum essential medium by using a mechanical mixing apparatus (Stomacher 400; Tekmar Co., Cincinnati, Ohio). All 102 specimens from the research station turkey flocks were intestinal contents collected from birds 1 to 56 days old.

At scheduled intervals, randomly selected turkeys were removed from the commercial flocks and sacrificed by farm personnel. The abdominal viscera were frozen and later shipped frozen to our laboratory. Fecal specimens were collected by us. A similar procedure was used to obtain specimens from the research station flocks, except live birds were delivered to our laboratory for sacrifice. Before testing, intestinal tracts were thawed, and the contents were collected. The volume of intestinal contents collected from individual birds less than 11 days old was often small. In these cases, unless noted, a single composite specimen was

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TABLE 1. Detection of dsRNA viral infections in turkeys by genome electropherotyping

Operation	Hatch	Date placed	House	Age of poults/days[a]										
				0	1	2	3	4	5	6	7	8	9	10–11
Commercial	37-5	9/84	NR											
Commercial	40-3	10/84	ER					Neg (1)						RV + RL (1)
Commercial	49-2	12/84	NR				Neg (1)			RV (1)		RV (1)		
Commercial	7-4	2/85	B					Neg (1)			Neg (1)			Neg (6)
Commercial	1-3	1/85	ER					Neg (1)		Neg (1)				Neg (1)
Commercial	27-4	6/85	B	Neg (1)			RV (1)			Neg (1)				AR (1)
										RV (4)				RL (1)
														Neg (3)
Commercial	33-2	8/85	NR	Neg (1)			Neg (1)	Neg (1)	Neg (1)	RV (5)	Neg (1)			Neg (1)
											RV (4)			RV (2)
														RL (1)
														RV + AR (1)
Commercial	37-3	9/85	ER	Neg (1)	Neg (1)	Neg (1)	Neg (5)	Neg (5)		Neg (1)				Neg (4)
										RV (4)				RL (1)
Commercial	45-2	11/85	NR	Neg (1)			RV (1)	RV (1)	RV (1)	Neg (1)	Neg (4)			Neg (5)
											RV (1)			
Commercial	51207	12/85	ER	Neg (1)		Neg (1)	Neg (1)		Neg (1)	Neg (1)			Neg (4)	RL (1)
Research	1	7/85	A		Neg (1)			Neg (1)			Neg (1)			Neg (6)
Research	2	12/85	B		Neg (1)			Neg (1)	Neg (1)			Neg (1)		Neg (5)

prepared from all the birds (usually five) sacrificed on the same day. Intestinal content specimens from older turkeys were maintained individually.

Genome electropherotyping technique. Viral dsRNA was extracted from 2-ml samples of specimens by CF11 cellulose chromatography (8) and suspended in 150 μl of sample buffer (10). A 20-μl sample was then subjected to electrophoresis in Laemmli 7.5% polyacrylamide gel slabs and stained with silver, and the genome electropherotype was determined as described previously (10).

Cell culture. Monolayers of MA104 cells were grown and maintained in screw-cap tubes or 96-well plates as described previously (9). Before inoculation, monolayers were washed three times and fed serum-free maintenance medium containing 2 μg of trypsin (type IX; Sigma Chemical Co., St. Louis, Mo.) per ml.

CCIF assay for detection of turkey group A rotaviruses. Specimens were diluted 25-fold in serum-free medium and treated with garamycin as described previously (9). Trypsin was then added to a final concentration of 20 μg/ml, and the specimen was incubated at 37°C for 1 h. After incubation, 0.2 ml of treated specimen was inoculated into each of two refed MA104 monolayers in screw-cap tubes. Inoculated monolayers were incubated on a roller-drum apparatus at 37°C for 48 to 96 h. Culture medium from the two inoculated monolayers was pooled, and 0.1-ml samples were then used to inoculate refed MA104 monolayers in two or three wells of a 96-well plate. Inoculated monolayers were incubated overnight and then fixed in 80% (vol/vol) acetone. Fixed monolayers were stained with fluorescein-conjugated bovine anti-bovine rotavirus globulin (9) and examined by immunofluorescence microscopy as described previously (2). Specimens were considered positive for group A rotavirus if specifically immunofluorescing infected cells were detected in any well inoculated with the specimen. Previous studies have shown that some turkey specimens negative for group A rotavirus by the genome electropherotyping technique do contain some infective group A rotavirus demonstrable by this cell culture immunofluorescence (CCIF) assay (K. W. Theil, unpublished observation).

RESULTS

Viruses were detected by the genome electropherotyping technique in 8 of 10 commercial turkey flocks surveyed (Table 1). The genome electropherotypes of the turkey group A rotavirus, RVLV, and ATR were distinctive and readily differentiated (Fig. 1). In addition, these genome electropherotypes were also distinct from the characteristic genome electropherotype produced by the 10 dsRNA segments of reovirus (Fig. 1).

Of the 278 specimens collected from turkeys less than 29 days old in commercial flocks, 79 (28.4%) were positive for virus by the genome electropherotyping technique, whereas only 1 of 120 specimens collected from the turkeys older than 28 days was positive (Tables 1 and 2). Group A rotavirus was detected in 39 specimens, RVLV was detected in 39 specimens, ATR was detected in 7 specimens, and reovirus was detected in 2 specimens. Eight specimens contained two viruses (Table 1). All specimens determined to contain group A rotavirus by the genome electropherotyping technique were also positive for group A rotavirus by the CCIF assay.

Viruses were detected in specimens obtained from commercial turkeys between the ages of 3 and 35 days (Tables 1 and 2). Over a third of the specimens collected from commercial turkeys during their first week of life were positive for virus; all viruses detected in these young birds were group A rotavirus (Table 2). In those flocks infected with group A rotavirus at an early age, many individual birds were affected. For example, in flocks 27-4, 33-2, and 37-3, 17 of 20 (85%) individual birds were positive for virus. In flocks 40-3 and 7-4, group A rotavirus was first detected in specimens obtained from older birds. In flock 49-2, two episodes of group A rotavirus infection were detected, the first at 6 to 8 days of age and the second at 21 days of age. This latter

TABLE 1—*Continued*

Age of poults/days[a]														
12–13	14–15	16–17	18–19	20–21	22–23	24–28	29–34	35–41	42–48	49–55	56–62	63–69	70–76	84–90	91–97
						Neg (1)		Neg (1)	Neg (1)		Neg (2)	Neg (5)	Neg (3)	Neg (1)	
			RV + RL (1)			Neg (6)	Neg (6)	Neg (2)	Neg (3)	Neg (6)	Neg (3)	Neg (3)	Neg (3)		Neg (1)
			RV (1)												
Neg (6)	Neg (6) REO (1)			Neg (1) RL (3) RV + RL (4)				Neg (5)		Neg (3)				Neg (1)	
Neg (6)		Neg (6)	Neg (6)	Neg (6)	Neg (1) RV (5)	Neg (4) RV (2)		Neg (5)		Neg (1)	Neg (1)		Neg (1)		
	Neg (1)		Neg (1)					Neg (3)							
		Neg (3) RL (2)	Neg (5)		Neg (5)	Neg (5)	Neg (5)	Neg (5)	Neg (4)						
	Neg (3) RL (2)		Neg (2) RL (2) AR (1)	Neg (5)		Neg (5)	Neg (5)	Neg (4) AR (1)							
	RL (5)		Neg (5)							Neg (3)					
Neg (3)	Neg (3) AR (2)	Neg (3) AR (1)	RL (5)		Neg (3)	Neg (7)		Neg (5)	Neg (4)						
RL (4) RL + REO (1)		Neg (5)	Neg (5)		Neg (5)	Neg (5)	Neg (9)	Neg (10)	Neg (5)						
		Neg (6)	Neg (6)	Neg (6)		Neg (12)		Neg (6)	Neg (6)	Neg (6)	Neg (6)				
	Neg (5)		Neg (5)		Neg (5)	Neg (5)	Neg (5)	Neg (5)							

[a] RV, Rotavirus genome electropherotype detected; RL, RVLV genome electropherotype detected; AR, ATR genome electropherotype detected; REO, reovirus genome electropherotype detected; NEG, no genome electropherotype detected. Single specimens tested from poults less than 11 days old usually represented pooled intestinal contents from two to six birds. Number in parentheses indicates number of specimens with the genome electropherotype.

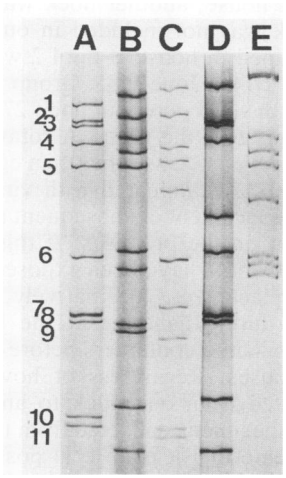

FIG. 1. Comparison of turkey group A rotavirus, RVLV, ATR, and reovirus genome electropherotypes in the same polyacrylamide gel slab. A group A mammalian rotavirus genome electropherotype is included for reference purposes. Numbers to the left designate segments of the group A turkey rotavirus genome. Migration is from top to bottom. Lanes: A, group A turkey rotavirus genome detected in flock 33-2; B, turkey RVLV genome electropherotype detected in flock 37-3; C, turkey ATR genome electropherotype detected in flock 27-4; D, group A bovine rotavirus genome electropherotype; E, turkey reovirus genome electropherotype. The turkey reovirus genome electropherotype was derived from a cell culture-passaged virus since the genome electropherotype detected in flock 49-2 was too faint to produce sufficient photographic contrast. Segments 7 and 8 comigrate in the group A turkey rotavirus genome and in the group A bovine rotavirus genome. Segments 6 and 7 of the turkey ATR genome comigrate.

episode occurred simultaneously with RVLV infections, and both viruses were detected in specimens derived from four individual 21-day-old birds.

Between 8 and 28 days of age commercial turkeys were infected with all four viruses detected by the genome electropherotyping technique (Tables 1 and 2). However, RVLV was the most frequently detected virus in birds of this age group, and birds infected with this virus were detected in 7 of the 10 commercial flocks surveyed. ATRs were detected infrequently and in only 3 of the 10 commercial flocks surveyed. ATR was the only virus detected in birds older than 28 days and was present in just one specimen collected from birds of this age group. Reovirus was detected in two specimens, both derived from turkeys approximately 2 weeks of age. One reovirus-infected turkey was in flock 49-2 and the other was in flock 51207. The turkey from flock 51207 was also infected with RVLV.

All yolk and intestinal tract specimens derived from

TABLE 2. Age distribution of rotavirus, RVLV, ATR, and reovirus infections of turkeys in 10 commercial flocks

Age (days)	Virus infection[a]				No. of specimens positive for virus/no. of specimens tested
	Rotavirus	RVLV	ATR	Reovirus	
0–7	22	0	0	0	22/61 (36.1)[b]
8–21	10	39	6	2	49/157 (31.2)
22–28	7	0	0	0	7/60 (11.7)
29–97	0	0	1	0	1/120 (0.8)

[a] For specimens containing two viruses, each virus was included separately in the appropriate column.

[b] Number in parentheses represents percentage of specimens positive for virus.

unhatched turkey embryos of flock 37-3 were negative for virus by the genome electropherotyping technique. In addition, these specimens were also negative for group A rotavirus in the CCIF assay. Specimens obtained from birds in flocks 27-4, 33-2, and 37-3 before those specimens positive for group A rotavirus by the genome electropherotyping technique were negative for group A rotavirus by the CCIF assay. All specimens from flock 51207, which were negative for group A rotavirus by the genome electropherotyping technique, were also negative for group A rotavirus by the CCIF assay.

All specimens collected from turkeys raised in the two research station flocks were negative for virus by the genome electropherotyping technique and negative for group A rotavirus by the CCIF assay.

DISCUSSION

Viruses with segmented dsRNA genomes were detected by the genome electropherotyping technique in turkeys from 8 of 10 commercial flocks monitored. The failure to detect viruses in the two other commerical flocks was probably due to the sampling schedule used for these flocks. Specimen collection from flock 37-5 did not start until the turkeys were 28 days old, and specimen collection from flock 1-3 was less frequent than for the other flocks.

The genome electropherotyping technique was selected to monitor dsRNA virus infections of turkeys for several reasons. Although the genome electropherotyping technique is slightly less sensitive than immune electron microscopy for detecting turkey group A rotaviruses and RVLVs, it more readily permits the differentiation of these two viruses (10). Additionally, genome electropherotyping is the only method available at present for recognizing turkey ATRs, as specific antiserum is unavailable. Furthermore, the genome electropherotyping technique has the potential for detecting other dsRNA viruses possessing novel genome electropherotypes for which specific antiserum is also unavailable.

Aside from the reovirus genome electropherotypes detected in two specimens, all genome electropherotypes detected in the turkey specimens of this study were similar to one of three genome electropherotypes previously described for turkey viruses morphologically resembling rotaviruses (10). The turkey group A rotavirus and RVLV genome electropherotypes were the most frequently detected and were present in an equal number of specimens. In contrast, the ATR genome electropherotype was detected with considerably less frequency. This finding agrees with our previous study in which turkey ATRs were the least frequently detected virus in field specimens submitted from flocks in three states(10). In the previous study, however, turkey group A rotavirus was less frequently detected in specimens than in the present investigation. This difference could be explained by the differences in the specimens examined in the two studies. Over half of the group A rotaviruses detected in the present study were in specimens from turkeys 3 to 7 days old. In the earlier study, few specimens were from turkeys of this age group.

In the previous study (10), a few specimens contained both turkey group A rotavirus and RVLV. However, as these specimens represented composite specimens prepared from the intestinal contents of several turkeys, it was impossible to ascertain whether these results indicated mixed infections of individual birds. In the present investigation mixed infections of individual turkeys with two viruses were detected in six birds, 10 to 21 days old, in flocks 49-2, 33-2, and 51207.

Only group A rotavirus was detected in commercial turkeys less than 7 days old, and the observation that 3-day-old birds were infected raises questions concerning the transmission of this virus. For 3-day-old turkeys to be infected, the birds must have been exposed to group A rotavirus by no later than 2 days of age. In these cases, such an early exposure age suggests transmission via the egg. We were unable to detect group A rotavirus in yolk or intestinal tract specimens derived from unhatched turkey embryos collected at the placement of flock 37-3 (which was infected with group A rotavirus within 6 days). These results, however, do not preclude mechanical transmission of the virus to the poults via fecal contamination of egg surfaces. On the other hand, we were unable to detect group A rotavirus either by genome electropherotyping or CCIF assay in specimens derived from poults sacrificed on the day of placement in four flocks (27-4, 33-2, 37-5, and 45-2) that subsequently became infected within 3 to 6 days. This suggests that if group A rotavirus is transmitted by the egg surface, only a few birds are infected initially and that the infection must subsequently spread very rapidly throughout the flock. Additional studies are required to determine whether group A rotavirus is transmitted to poults via the egg.

Another possibility is that poults become infected with group A rotavirus (or RVLV, ATR, etc.) that has persisted in the brooder house between flocks. The findings with flocks placed in brooder house B suggest that virus from previous flocks might remain in the house and be transmitted to subsequent flocks. This house was empty for 6 months preceding the placement of flock 7-4 in February 1985. Group A rotavirus was not detected in this flock until the birds were over 3 weeks of age. Shortly after flock 7-4 was removed from this house, another flock was placed in this building (this flock was not included in our survey). This second flock remained in house B until 2 weeks before the placement of flock 27-4 in June 1985. Group A rotavirus was then detected in 3-day-old poults in flock 27-4. This pattern of infection suggests that the group A rotavirus was introduced into flock 7-4 by a means other than egg transmission. Once the house was contaminated with virus shed by this flock, the frequent placement of subsequent flocks permitted the virus to persist on the premises. If this was the case, poults in flock 27-4 would have been exposed to virus in the house soon after placement. Alternatively, poults may be exposed to virus during handling at the hatchery or by shipment in contaminated containers before their placement in the brooder houses. Regardless of how these viruses might be transmitted from one flock to another, the data obtained with the specimens collected from the two research station flocks indicate that it is at least possible to prevent these infections within small closed turkey flocks.

The lack of viruses in the research station flocks most likely stems from the fact that the small numbers of poults placed in these flocks are derived in a closed operation and flocks are infrequently placed. In contrast, the large numbers of poults for the commercial flocks are derived in an open system and obtained from many different flocks within the hatchery. Further, in a commercial operation, new flocks are placed on a continuous basis. Thus, the more intensive management conditions in the commercial operation are more conducive to the introduction and perpetuation of these viruses in the flocks.

The RVLV and ATR infections were never detected in commercial turkeys less than 10 days old. The reason for this is unclear, but it suggests that these viruses differ from the

group A rotaviruses in their mode of transmission or that the poults possess some age-related resistance factors.

Although it has been established that rotaviruses are important causes of diarrhea in many mammalian species (3), the roles of turkey group A rotavirus, RVLV, and ATR in enteric diseases of poults remain uncertain. Controlled experimental infections of turkey poults with these viruses are required to provide information concerning the pathogenicities of these viruses. The results of the present investigation provide useful insights into how such studies might be conducted to reflect more accurately some field circumstances. For example, pathogenicity studies with group A rotaviruses should be performed with turkey poults 3 to 4 days of age, as this is the age group commonly infected in commercial flocks. Second, since mixed infections were detected, pathogenicity studies with two viruses combined might also provide data relevant to the field situation.

The pattern of group A rotavirus, RVLV, and ATR infections in commercial turkeys differs from the pattern of infections in broiler chickens caused by four serogroups of rotavirus (6). Turkeys were often infected during the first week of life, whereas broiler chickens were not infected until later. However, broiler chickens were frequently infected after 4 weeks of age. In contrast, group A rotavirus, RVLV, and ATR infections of commercially reared turkeys appear to be limited almost exclusively to the first month of life.

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