Pathogenicity of Turkey Coronavirus in Turkeys and Chickens

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SUMMARY. We designed this study to compare the replication potential of turkey coronavirus (TCV) and its effect in chickens and turkeys and to study the effect of single and combined infection of turkey poults with TCV and astrovirus. We studied the pathogenicity of TCV in experimentally inoculated turkey poults and chickens by observing the clinical signs and gross lesions. Two trials were conducted with 1-day-old and 4-wk-old specific-pathogen-free turkey poults and chickens. One-day-old turkey poults developed diarrhea at 48 hr postinoculation. Poults euthanatized at 3, 5, and 7 days postinoculation had flaccid, pale, and thin-walled intestines with watery contents. The 4-wk-old turkeys had no clinical signs or gross lesions. One-day-old and 4-wk-old chicks developed no clinical signs or gross lesions although the TCV was detected in gut contents of the birds throughout the experimental period (14 days). In another experiment, mean plasma D-xylose concentrations in 3-day-old turkey poults inoculated with TCV, turkey astrovirus, or a combination of both viruses were significantly lower than in the uninoculated controls.

RESUMEN. Patogenicidad del coronavirus del pavo en pavos y en pollos

Se diseñó un estudio para comparar el potencial de replicación del coronavirus del pavo y su efecto en pollos y en pavos y estudiar el efecto en pavipollos de una infección sola o combinada con el coronavirus del pavo y astrovirus. Se estudió la patogenicidad del coronavirus del pavo en pavipollos y pollos inoculados experimentalmente mediante la observación de los signos clínicos y las lesiones macroscópicas. Se realizaron dos ensayos en pavipollos y pollos libres de patógenos específicos de 1 día y 4 semanas de edad. Se observó diarrea en los pavipollos de un día de edad a las 48 horas posteriores a la inoculación. Se observó flacidez, palidez y adelgazamiento de la pared intestinal con contenidos acuosos en los pavipollos sacrificados a los 3, 5 y 7 días posteriores a la inoculación. No se observaron signos clínicos ni lesiones macroscópicas en los pavos de 4 semanas de edad. No se observaron signos clínicos ni lesiones macroscópicas en los pollos de 1 día y 4 semanas de edad, sin embargo, se aisló el coronavirus del pavo a partir de los contenidos del tracto intestinal de las aves durante el tiempo de duración del experimento (14 días). En otro experimento se observaron niveles promedio significativamente menores de concentración de D-xilosa en plasma en pavipollos de 3 días de edad inoculados con el coronavirus del pavo, astrovirus del pavo o con ambos virus al ser comparados con los niveles de los controles no inoculados.

Key words: turkey coronavirus, astrovirus, bluecomb, poult enteritis–mortality syndrome, diarrhea, enteritis, D-xylose, turkeys, chickens

Abbreviations: DEPC = diethyl pyrocarbonate; dNTP = deoxynucleoside triphosphate; DPI = days postinoculation; EID_{50} = 50% embryo infectious dose; GI = gastrointestinal tract; IBV = infectious bronchitis virus; IEM = immune electron microscopy; M = membrane;

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N = nucleocapsid; PCR = polymerase chain reaction; PEMS = poult enteritis–mortality syndrome; PTA = phosphotungstic acid; RT =reverse transcriptase; SPF = specific-pathogen free; TCV = turkey coronavirus

Turkey coronavirus (TCV) is the cause of an economically important enteric disease of turkeys designated transmissible enteritis or bluecomb. The disease affects turkeys of all ages and results in increased morbidity and mortality and economic losses due to mortality and loss in body weight (17). The virus is also associated with astrovirus and probably other yet unconfirmed infectious agents in producing poult enteritis–mortality syndrome (PEMS) in turkeys, a disease that affects turkeys between 1 and 4 wk of age (23). This syndrome is characterized by diarrhea, growth depression, immune dysfunction, and significantly high mortality (3,23). In a previous study in our laboratory, we found that TCV is antigenically and genomically related to infectious bronchitis virus (IBV) of chickens (15). Results from other laboratories (4,5,8,13,14,22) indicated that TCV is closely related to IBV of chickens. TCV also was successfully propagated in turkey and chicken embryos as well when inoculated into the amniotic cavity (1,17). Brown *et al.* (6) reported on a coronavirus and a birnavirus administered to day-old broilers and adult cattle, and the coronavirus was shed in the feces in the absence of clinical signs. Whether these authors were dealing with a TCV or a bovine coronavirus is not clear. Because of this close relatedness of TCV and IBV and the ability of TCV to grow on chicken embryos, we speculated that TCV could probably replicate in chickens.

D-xylose is a pentose sugar that is readily absorbed from the upper small intestine mainly by passive diffusion (7). Because it is poorly metabolized and readily excreted unchanged in the urine, we used D-xylose to assess the absorptive function of the intestines by measuring either its excretion in urine or its concentration in plasma (11,12).

MATERIALS AND METHODS

Birds. We obtained specific-pathogen-free (SPF) 1-day-old chicks, turkey poults, and eggs used in these studies from SPF flocks maintained by the Food Animal Health Research Program. The flocks are free of detectable chicken and turkey pathogens including enteric viruses as indicated by clinical signs and periodic testing. All the birds used in the different experimental groups were kept in wire cages inside high-security isolation rooms provided with HEPA-filtered intake and exhaust air and provided with feed

and water *ad libitum.* Different experimental groups were kept in separate rooms.

Viruses. TCV, ATCC V R-911, was propagated in our laboratory with 22-day-old embryonated SPF turkey eggs inoculated via the amniotic sac route. The intestines of inoculated embryos were collected 48 hr postinoculation, homogenized, and clarified by centrifugation at $3000 \times g$ for 30 min. The supernatants were filtered through 0.45-μm syringe filters (Nalgene, Nalge Co., Rochester, NY), frozen at −70 C, and then thawed out and used for inoculation. The turkey astrovirus was obtained from gut contents of diarrheic turkey poults and was passaged once in SPF turkey poults in our laboratory.

Immune electron microscopy. Samples from the gastrointestinal (GI) tract were collected at necropsy. The GI contents of birds from the same treatment at each necropsy period were pooled and diluted 1/10 (w/v) in 0.05 M Tris-HCl buffer, pH 7.5, and then clarified by low-speed centrifugation at $3000 \times g$ for 30 min at 4 C. The supernatants were filtered through 0.45-μm disposable syringe filters. Two hundred microliters of the filtrate was incubated overnight at 4 C with 200 μl of turkey hyperimmune TCV antiserum diluted 1/100 in 0.05 M Tris-HCl buffer, pH 7.5. After incubation, the mixtures were centrifuged for 15 min at $160,000 \times g$ through a 50-μl cushion of 40% sucrose with a Beckman tabletop Airfuge® (Beckman Coulter Inc., Porterville, CA). Pellets were suspended in 400 μl of filtered distilled water and centrifuged again without the sucrose cushion as previously described. The pellets obtained were resuspended in 25 μl of sterile distilled water. One drop of the resuspended solution was placed on carbon-coated 300 mesh Formvar® copper grids and stained with a drop of phosphotungstic acid (PTA) solution (3% PTA, 0.4% sucrose, pH 7.0). The grids were examined for the presence of virus at 80 kV with a transmission electron microscope (Philips 201; Philips Norelco, Eindhoven, The Netherlands).

Statistical analysis. Statistical comparison of body weights between challenged and control birds was performed with the use of one-way analysis of variance (ANOVA). (21).

Extraction of viral RNA. The pooled intestinal contents supernatants (1:10 dilutions) from birds killed at selected days postinoculation were concentrated by ultracentrifugation at $100,000 \times g$ for 3 hr and then used for RNA extraction by the guanidium isothiocyanate–phenol chloroform method with a commercial kit (Trizol LS reagent; Life Technologies, Grand Island, NY) according to the manufacturer's instructions. Briefly, 0.25 ml of concentrated TCV was mixed with

0.75 ml of Trizol LS reagent. After 5 min at room temperature, 0.2 ml of chloroform was added for phase separation. The mixture was centrifuged at $12,000 \times g$ for 15 min at 4 C and the aqueous phase was collected. Isopropyl alcohol was added to the aqueous phase to precipitate the RNA. After centrifugation at $12,000 \times g$ for 10 min the pelleted RNA was washed with 75% ethanol, dried for 10 min at room temperature, and dissolved in 20 µl of diethyl pyrocarbonate (DEPC)-treated water (24). Intestinal homogenates from control SPF birds were used as negative control.

Preparation of oligonucleotide primers. The genome of TCV was amplified with primers described earlier that amplified a 1082-base pair region spanning portions of the region encoding membrane (M) and nucleoprotein (N) proteins of IBV (2).

Reverse transcriptase (RT)–polymerase chain reaction (PCR) procedure. The RT-PCR procedure was done as described previously (2) but with some modifications. In addition, a reverse transcription step was added as follows. The tube containing 5 µl of RNA sample and 2 µl of downstream IBV primer (25 pmol) and 4 µl of DEPC-treated water was incubated in a boiling water bath for 2 min, then quenched on ice for 5 min. Subsequently, 9 µl of the RT-PCR mixture was added. The RT-PCR mixture consisted of 4 µl of 5× RT buffer, 2 µl of 0.1 dithiothreitol, 2 µl of 10 mM deoxynucleoside triphosphates (dNTPs) (Promega Corporation, Madison, WI), 0.5 µl RNAsin (Promega), and 0.5 µl avian myeloblastosis virus RT (Promega). The mixture was incubated at 42 C for 90 min. After incubation, 10 µl of the resulting template was mixed with 50 µl of PCR mixture. The PCR mixture consisted of 5 µl of 10× buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), 5 µl of MgCl Ch (25 mM) (Promega), 4 µl 10 mM dNTP (Promega), 1 µl of upstream primer (25 pmol), 1 µl of downstream primer (25 pmol), 0.5 µl of Taq polymerase (Promega), and 26.5 µl DEPC-treated water. The mixture was preheated at 94 C for 5 min, then subjected to 35 cycles of 1 min at 94 C, 2 min at 37 C, 5 min at 72 C, and a final 15 min of incubation at 72 C. The PCR products were visualized on 1.2% agarose gels stained with ethidium bromide.

D-xylose estimation in plasma. Plasma D-xylose concentrations were determined by the micromethod as described previously (10,12). Two milliliters of phloroglucinol reagent (Sigma Chemical Co., St. Louis, MO) was mixed with 20 µl of plasma sample and heated to 100 C for 4 min with multi-block heater (Lab-Line Instruments Inc., Melrose Park, IL) for color development. The mixture was then cooled to room temperature with a water bath. Sample absorption was then determined with a spectrophotometer (Spectronic 1001 Split-Beam Spectrophotometer; Milton Roy Co., Rochester, NY).

Experimental inoculation of turkey poults.

Trial 1. Forty 1-day-old SPF turkey poults were each orally inoculated with 100 50% embryo infective dose (EID_{50}) of TCV. Another group of the same number of poults was kept in a separate room and served as unexposed controls.

Trial 2. Seventy 1-day-old SPF turkey poults were allotted into two groups of 35 birds each. Each of the poults from the first group was orally inoculated with 100 EID_{50} of TCV. The other group of 35 birds was kept separately to serve as unexposed controls.

Trial 3. Fifty 4-wk-old SPF turkey poults were each orally inoculated with 100 EID_{50} of TCV. Another group of the same number of poults was kept in a separate room and served as unexposed controls.

Trial 4. Forty 4-wk-old SPF turkey poults were each orally inoculated with 100 EID_{50} of TCV. Another group of the same number of poults was kept in a separate room and served as unexposed controls.

In all the trials, the poults were observed twice daily for clinical signs. Equal numbers of poults from each treatment were randomly selected, weighed, and euthanized at 3, 5, 7, and 14 days postinoculation (DPI). Birds were examined for lesions, and intestinal contents were collected for immune electron microscopy (IEM) and RT-PCR.

Experimental inoculation of chickens. *Trial 1.* Thirty-two 1-day-old SPF chicks were orally inoculated with 100 EID_{50} of TCV. Another group of the same number was kept in a separate room to serve as controls.

Trial 2. Forty 1-day-old SPF chicks were orally inoculated with 100 EID_{50} of TCV. Another group of the same number was kept in a separate room as unexposed controls.

Trial 3. Forty 4-wk-old SPF chicks were orally inoculated with 100 EID_{50} of TCV. Another group of the same number was kept in a separate room to serve as controls.

Trial 4. Thirty-two 4-wk-old SPF chicks were orally inoculated with 100 EID_{50} of TCV. Another group of the same number was kept in a separate room as unexposed controls.

In all the trials, the chickens were observed twice daily for clinical signs. Equal numbers of chickens from each treatment were weighed and euthanized at 3, 5, 7, and 14 DPI. Birds were examined for lesions, and intestinal contents were collected for IEM and RT-PCR.

Combined infections. Sixty 1-day-old SPF turkey poults were allotted into four groups of 15 each. Each group was kept separately in an isolation unit. When the birds were 3 days old, the first group received 100 EID_{50} of the TCV, the second group received turkey astrovirus, and the third group received a combination of TCV and turkey astrovirus. The fourth group was kept as negative control. At 24, 72, and 120 hr after oral inoculation of the viruses, five poults from each group were randomly caught and

Table 1. Mean body weights of 1-day-old turkey poults inoculated with TCV in two trials.

Treatment	Mean body weight (g) ± SD at days postinoculation (DPI)							
	3 DPI		5 DPI		7 DPI		14 DPI	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Control	60.29 ± 2.72	59.59 ± 2.73	98.48 ± 1.50	98.42 ± 2.04	118.67 ± 3.64	121.30 ± 6.23	169.31 ± 4.76	171.13 ± 5.30
Inoculated	56.78* ± 2.35	50.90* ± 5.35	89.14* ± 1.64	89.19* ± 1.97	103.87* ± 4.25	105.17* ± 4.59	167.92 ± 4.48	169.50 ± 6.21

* = Significantly different from controls ($P < 0.05$).

placed in another isolation unit for a 12-hr fasting period prior to D-xylose administration. Individual poults were weighed to calculate the D-xylose dosage. A solution of D-xylose (Sigma Chemical Co.) was prepared in deionized water to contain 5% (w/v) concentration of the sugar. The dose of D-xylose was 0.5 g/kg body weight. The solution was delivered to the crop of each bird with a tomcat urethral catheter (Cooke Veterinary Products, Bloomington, IN). Heparinized blood samples were collected from the superficial medial wing vein of each bird with 25-gauge needles as lancettes at 30, 90, and 120 min after D-xylose administration with hematocrit capillary tubes (Drummond Scientific Co., Broomall, PA). The hematocrit tubes were then centrifuged to collect the plasma. At the end of the experimental period and after collection of blood samples from the last five birds from each group, the birds were killed and intestinal contents were collected for virus detection by IEM.

RESULTS

Experimental inoculation of turkey poults. In both trials in I-day-old poults, inoculated poults were depressed and stunted. Diarrhea with frothy droppings started at 2 days PI and lasted for about 4 days. The morbidity was 100% with average 6% mortality in the two trials. On postmortem examination, the intestines were markedly enlarged and filled with frothy yellowish loose contents. The intestines were flaccid with pale thin walls. In both trials, inoculated poults had significantly lower body weight compared with unexposed control poults at 3, 5, and 7 DPI (Table 1). The TCV was detected from pooled intestinal contents of challenged poults by IEM and RT-PCR at 3, 5, 7, and 14 DPI (Fig. 1). In both trials with 4-wk-old poults, no clinical sign or postmortem lesion was observed throughout the experimental period. The body weight of inoculated poults was not significantly different from that of unexposed control poults at any point during the experiment (Table 2). Nevertheless, the TCV could be detected from the pooled intestinal contents of TCV-inoculated poults by IEM and RT-PCR at 3, 5, 7, and 14 DPI in the first trial. In the second trial, the TCV was detected by IEM until 7 DPI and by RT-PCR until 14 DPI. The control poults remained negative throughout the trial.

Experimental inoculation of chickens. In all trials with 1-day-old and older chickens, we found no clinical sign or gross lesion in chickens necropsied throughout the experimental period. The body weight of inoculated chickens was not signifi-
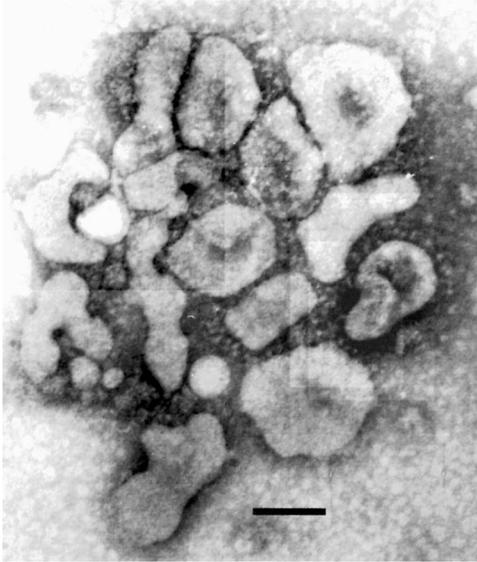
Fig. 1. Immune electron micrograph of the TCV particles detected from pooled intestinal contents of inoculated turkey poults at 3 DPI. Bar = 100 nm.

cantly different from control chickens (Tables 3, 4). The TCV was detected by IEM and RT-PCR from guts of chickens up to 14 DPI in all TCV-inoculated groups (Fig. 2). The control chickens remained negative throughout the trial.

Combined infection. Results of D-xylose absorption by intestines of control and virus-inoculated turkey poults at 24, 72, and 120 hr postinoculation are presented in Table 5. All inoculated groups had significantly lower plasma D-xylose than unexposed control groups at 30 and 90 min after D-xylose administration. In all groups, we found no difference between control and inoculated birds at 120 min after xylose administration. The effect was greater in the group inoculated with a combination of the two viruses. The mean plasma peak of xylose was achieved earlier in control birds (90 min) and later in inoculated birds (120 min). The intestinal contents collected from the five birds killed at the last interval in each group were positive for the corresponding virus used for challenge (Fig. 3). No virus was detected from the control birds.

DISCUSSION

Studies on antigenic relationships between TCV and IBV indicated that the TCV was closely related to the serogroup 3 of avian coronaviruses that includes IBV (13,15). Subsequent sequence analysis of

Table 2. Mean body weights of 4-wk-old turkey poults inoculated with TCV in two trials.

| | Mean body weight (g) ± SD at days postinoculation (DPI) | | | | | | | |
| | 3 DPI | | 5 DPI | | 7 DPI | | 14 DPI | |
Treatment	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Control	677.3 ± 154.9	655.8 ± 135.4	733.8 ± 168.9	707.4 ± 129.6	995.9 ± 103.8	987.9 ± 100.3	1120.7 ± 174.4	1151.2 ± 159.4
Inoculated	737.4 ± 112	739.6 ± 108.2	776.0 ± 140.9	768.4 ± 147.3	970.6 ± 121.0	970.8 ± 112.3	1230.6 ± 193.5	1215.3 ± 175.2

Table 3. Mean body weights of 1-day-old chickens inoculated with TCV in two trials.

| Treatment | Mean body weight (g) ± SD at days postinoculation (DPI) | | | | | | | |
| | 3 DPI | | 5 DPI | | 7 DPI | | 14 DPI | |
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Control	39.68 ± 2.36	39.91 ± 2.66	54.89 ± 3.19	56.45 ± 4.08	64.33 ± 3.67	64.61 ± 5.41	104.01 ± 3.61	105.12 ± 4.20
Inoculated	38.94 ± 1.73	40.02 ± 1.71	53.86 ± 3.43	55.79 ± 4.31	64.02 ± 4.64	65.11 ± 3.99	102.88 ± 3.05	106.59 ± 3.66

Table 4. Mean body weights of 4-wk-old chickens inoculated with TCV in two trials.

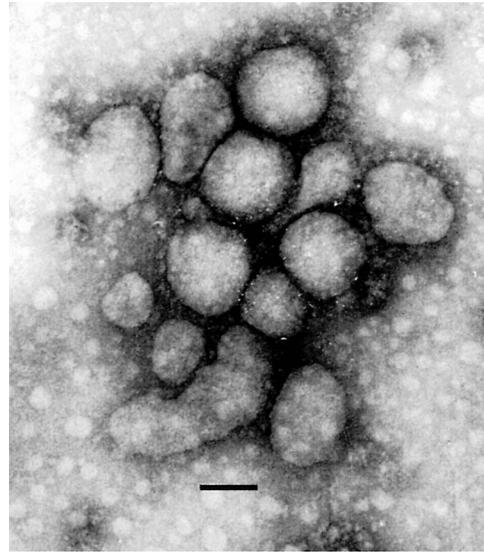| Treatment | Mean body weight (g) ± SD at days postinoculation (DPI) | | | | | | | |
| | 3 DPI | | 5 DPI | | 7 DPI | | 14 DPI | |
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Control	677.3 ± 154.9	655.8 ± 135.4	733.8 ± 168.9	707.4 ± 129.6	995.9 ± 103.8	987.9 ± 100.3	1120.7 ± 174.4	1151.2 ± 159.4
Inoculated	737.4 ± 112	739.6 ± 108.2	776.0 ± 140.9	768.4 ± 147.3	970.6 ± 121.0	970.8 ± 112.3	1230.6 ± 193.5	1215.3 ± 175.2

Fig. 2. Immune electron micrograph of the TCV particles detected from pooled intestinal contents of inoculated chickens at 3 DPI. Bar = 100 nm.

genes encoding the M and N proteins revealed a high identity between TCV and IBV gene sequences (4). In other studies, Guy et al. (14), on the basis of immunofluorescence and immunoperoxidase studies, found that TCV isolated from turkey flocks with either bluecomb or PEMS was closely related to IBV of chickens. Adams et al. (1) reported the propagation of TCV on turkey and chicken embryos via amniotic cavity route. These findings lead us to speculate that because of this close relatedness, and the growth of TCV on chicken embryos, TCV might replicate in the guts of chickens.

In the present study, 1-day-old turkey poults challenged with TCV had diarrhea and growth depression in the two trials conducted. The growth depression started as early as 3 DPI and continued to 7 DPI. Intestines of inoculated birds were markedly enlarged, with loose contents, and were thin walled. The TCV was detected by IEM and RT-PCR from guts of inoculated birds up to 14 DPI.

In the two trials with 4-wk-old turkey poults, we found no clinical sign or gross or microscopic gut lesion but detected the virus by IEM in intestinal contents. We found no significant difference in growth rate between control and inoculated groups. We concluded that the age factor is important in determining the pathogenicity of TCV. The lack of pathogenicity in 4-wk-old turkey poults in our

Table 5. Virus isolation and mean plasma D-xylose concentrations in control and virus-inoculated groups.

Hours postinoculation	Group	Virus detection[A]	Plasma D-xylose concentration (mg/dl)		
			30 min[B]	90 min[B]	120 min[B]
24	Control	None	26.60	42.82	30.23
	TCV	TCV	13.34[C]	29.75[C]	30.55
	Astrovirus	Astrovirus	13.28[C]	25.23[C]	31.68
	Combination	TCV + astrovirus	12.23[C]	20.72[C]	32.2
72	Control	None	26.63	43.8	29.83
	TCV	TCV	13.32[C]	29.86[C]	31.24
	Astrovirus	Astrovirus	13.14[C]	26.52[C]	30.11
	Combination	TCV + astrovirus	11.21[C]	24.01[C]	30.34
120	Control	None	28.04	41.97	32.05
	TCV	TCV	13.71[C]	27.08[C]	23.73
	Astrovirus	Astrovirus	13.07[C]	26.02[C]	21.77
	Combination	TCV + astrovirus	11.02[C]	23.08[C]	23.22

[A]From gut contents collected postmortem.
[B]Time after oral D-xylose administration.
[C]Significantly different from controls ($P < 0.05$).

study may be due to use of SPF poults, which differ from commercial poults in which other organisms may contribute to the appearance and severity of signs of the disease initiated primarily by TCV. In all trials in 1-day-old and 4-wk-old chickens, we found no clinical sign or gross lesion although the TCV was detected from gut contents of inoculated birds. These findings suggest that TCV is not pathogenic to chickens, but replication of the virus in the guts of chickens raises a question about the potential role of chickens as carriers of the virus passing the infection to susceptible turkey flocks. Further studies are needed to investigate the possibility of TCV presence in the guts of chickens under natural field conditions, especially in chickens reared in confinement in the vicinity of TCV-infected turkey flocks.

Mean plasma D-xylose concentrations determined at 24, 72, and 120 hr after virus inoculations were lower in virus-inoculated birds than in control birds in samples collected at 30 and 90 min after xylose administration. The low levels of plasma xylose were evident in both TCV- and astrovirus-inoculated groups. Their effect was greater in the group that received the combined inoculum. This lower level of plasma xylose indicates an effect on absorptive functions of the small intestines by these viruses. The reduction in D-xylose absorption has been used by many investigators as a parameter of intestinal absorptive dysfunction in turkey poults with many disease conditions like reovirus (11), rotavirus (20), astrovirus (19), and PEMS (9). Although the avian intestinal tract is anatomically complete early in embryonic development, at hatch, the poult digestive system of the poult is still immature (16), which renders it vulnerable to various infectious and noninfectious agents that commonly bombard the digestive tract early during brooding. Such an immature system also has limited capacity of absorbing carbohydrates, lipids, and proteins (18). These findings indicate that both TCV and astrovirus had a significant effect on absorptive functions
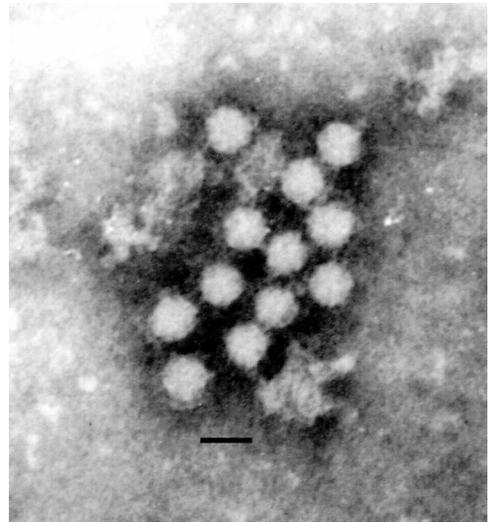

Fig. 3. Immune electron micrograph of the astrovirus particles detected from pooled intestinal contents of inoculated turkey poults at 120 hr postinoculation. Bar = 30 nm.

of the intestines of turkey poults, which may explain the retardation in growth observed in birds infected with these viruses. This effect was more marked in poults that had combined infection.

REFERENCES

1. Adams, N. R., and M. S. Hofstad. Isolation of transmissible enteritis agent of turkeys in avian embryos. Avian Dis. 15:426–433. 1971.

2. Andreasen, J. R., M. W. Jackwood, and D. A. Hilt. Polymerase chain reaction amplification of the genome of infectious bronchitis virus. Avian Dis. 35:216–220. 1991.

3. Barnes, H. J., and J. S. Guy. Poult enteritis–mortality syndrome ("spiking mortality") of turkeys. In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, IA. pp. 1025–1031. 1997.

4. Breslin, J. J., L. G. Smith, F. G. Fuller, and J. S. Guy. Sequence analysis of the turkey coronavirus nucleocapsid gene region of turkey coronavirus. Intervirology 4:22–29. 1999.

5. Breslin, J. J., L. G. Smith, F. J. Fuller, and J. S. Guy. Sequence analysis of the turkey coronavirus nucleocapsid gene and 3′ untranslated region identifies the virus as a close relative of infectious bronchitis virus. Virus Res. 65:187–193. 1999.

6. Brown, T. P., W. H. Emory, and D. R. Howell, Jr. Acute enteritis in turkey poults, chickens and cattle as subclinical carriers. In: Proc. 132nd Annual Meeting of the American Veterinary Medical Association. p. 26, 115. 1995.

7. Butterworth, C. E., Jr., E. Perez-Santiago, J. M. deJesus, and R. Santini. Studies on the oral and parental administration of D(+)xylose. N. Engl. J. Med. 261:157–163. 1959.

8. Cavanagh, D., K. Mawditt, M. Shanna, S. E. Drury, H. L. Ainsworth, P. Britton, and R. E. Gough. Detection of a coronavirus from turkey poults in Europe genetically related to infectious bronchitis virus of chickens. Avian Pathol. 30:355–368. 2001.

9. Doerfler, R. E., L. D. Cain, F. W. Edens, C. R. Parkhurst, M. A. Qureshi, and G. B. Havenstein. D-xylose absorption as a measurement of malabsorption in poult enteritis and mortality syndrome. Poult. Sci. 79:656–660. 2000.

10. Eberts, T. J., R. H. B. Sample, M. R. Glick, and G. H. Ellis. A simplified, colorimetric micromethod for xylose in serum or urine, with phloroglucinol. Clin. Chem. 25:1440–1443. 1979.

11. Goodwin, M. A., K. S. Latimer, B. N. Nersessian, and O. J. Fletcher. Quantitation of intestinal D-xylose absorption in normal and reovirus-inoculated turkeys. Avian Dis. 28:959–967. 1984.

12. Goodwin, M. A., K. S. Latimer, B. N. Nersessian, and O. J. Fletcher. Quantitation of intestinal D-xylose absorption in normal turkeys. Poult. Sci. 63:1742–1747. 1984.

13. Guy, J. S. Turkey coronavirus is more closely related to avian infectious bronchitis virus than to mammalian coronavirus: a review. Avian Pathol. 29:207–212. 2000.

14. Guy, J., H. J. Barnes, L. J. Smith, and J. Breslin. Antigenic characterization of a turkey coronavirus identified in poult enteritis and mortality syndrome–affected turkeys. Avian Dis. 41:583–590. 1997.

15. Ismail, M. M., K. O. Cho, M. Hasoksuz, L. J. Saif, and Y. M. Saif. Antigenic and genomic relatedness of turkey-origin coronaviruses, bovine coronaviruses, and infectious bronchitis virus of chickens. Avian Dis. 45:978–984. 2001.

16. Moran, E. T., Jr. Digestion and absorption of carbohydrates in fowl and events through prenatal development. J. Nutr. 115:665–674. 1985.

17. Nagaraja, K. V., and B. S. Pomeroy. Coronaviral enteritis of turkeys (bluecomb disease). In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, IA. pp. 686–692. 1997.

18. Phelps, P. V., F. W. Edens, and R. P. Gildersleeve. The post hatch physiology of the turkey poult. III. Yolk depletion and serum metabolites. Comp. Biochem. Physiol. 87A:409–415. 1987.

19. Reynolds, D. L., and Y. M. Saif. Astrovirus: a cause of an enteric disease in turkey poults. Avian Dis. 30:728–735. 1986.

20. Shawky, S. A., Y. M. Saif, and D. E. Swayne. Role of circulating maternal anti-rotavirus IgG in protection of intestinal mucosal surface in turkey poults. Avian Dis. 37:1041–1050. 1993.

21. Snedecor, G. W., and W. G. Cochran. One way classifications; analysis of variance. In: Statistical methods, 7th ed. The Iowa State University Press, Ames, IA. pp. 215–237. 1980.

22. Stephensen, C. B., D. B. Casebolt, and N. N. Gangopadhyay. Phylogenetic analysis of a highly conserved region of the polymerase gene from eleven coronaviruses and development of a consensus polymerase chain reaction assay. Virus Res. 60:181–189. 1999.

23. Yu, M., M. M. Ismail, M. A. Qureshi, R. N. Dearth, H. J. Barnes, and Y. M. Saif. Viral agents associated with poult enteritis and mortality syndrome: the role of a small round virus and a turkey coronavirus. Avian Dis. 44:297–304. 2000.

24. Yu, M., Y. Tang, M. Guo, Q. Zhang, and Y. M. Saif. Characterization of a small round virus associated with the poult enteritis and mortality syndrome. Avian Dis. 44:600–610. 2000.