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Astrovirus Infection in Hatchling Turkeys: Histologic, Morphometric, and Ultrastructural Findings

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SUMMARY. In three separate experiments, 2- or 5-day-old commercial turkey poults were inoculated orally with astrovirus and examined for clinical signs and for gross and microscopic lesions over a period of 14 days. By day 2 postinoculation (PI), inoculated poults had developed diarrhea, generalized loss of intestinal tone, and dilated ceca that contained light-yellow fluid feces and gas; these changes persisted through day 10 PI. Mild crypt hyperplasia was the only change discernible by light microscopy, and it was first noted in the proximal jejunum on day 1 PI, in the distal jejunum and ileum on day 3 PI, and in the duodenum on day 5 PI. A significant ($P < 0.05$) increase in crypt depth and area was documented by image analysis on day 3 PI. Ultrastructural evaluation revealed intracytoplasmic aggregates of astrovirus in enterocytes on the sides and base of villi in the ileum and distal jejunum on day 3 PI. Based on the findings, it was concluded that astrovirus caused lesions and replicated in both upper and lower segments of the small intestine in turkey poults.

RESUMEN. Infección por astrovirus en pavitos recién nacidos: Resultados histológicos, morfométricos y ultraestructurales.

En tres experimentos separados, se inocularon oralmente pavitos de dos o cinco días de edad con astrovirus y se examinaron para observar la presencia de signos clínicos y lesiones macro y microscópicas durante un periodo de 14 días. Dos días después de la inoculación, los pavitos inoculados mostraron diarrea, pérdida generalizada de tono intestinal y ciegos dilatados que contenían heces líquidas amarillentas con presencia de gas. Estas lesiones se observaron durante 10 días. La única lesión encontrada al microscopio fue una leve hiperplasia de las criptas, observada primero en el yeyuno proximal un día después de la inoculación, en el yeyuno distal e íleo al tercer día después de la inoculación, y en el duodeno al quinto día. Se observó un aumento significativo ($P < 0.05$) en la profundidad y en el área de las criptas al tercer día después de la inoculación. La evaluación ultraestructural reveló agregados de astrovirus intracitoplasmáticos en los enterocitos, a los lados y en la base de la vellosidad del íleo y en el yeyuno distal al tercer día después de la inoculación. Basados en estos resultados, los astrovirus causaron lesiones y se replicaron en la parte superior e inferior del intestino delgado de los pavitos.

Astroviruses are single-stranded RNA viruses that have been isolated from fecal specimens of a variety of species, usually in association with mild diarrhea (2,5,6,7,8,10,22,27,28). Astrovirus is one of several different enteric viruses identified in association with turkey viral enteritis (15,16,20). Turkey viral enteritis is a serious problem for turkey producers (1). The disease

occurs in poults 1 to 4 weeks of age and results in diarrhea, decreased growth, and flock unevenness (21). Mortality is usually low, but morbidity is high, resulting in significant economic losses.

Studies over the past 10 years have identified several different enteric viruses associated with turkey viral enteritis, including groups A and D rotaviruses (15,19,20), astrovirus (14,15,20), enterovirus (15,19,20), parvovirus (26), reovirus (15,20), and adenovirus (20). However, few

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studies have examined the role of an individual virus as an enteropathogen in turkey poults (14,19,21,24,30). Pathogenicity studies of individual viruses include astrovirus (14), group A rotavirus (30,31), rotavirus-like virus (group D rotavirus) (19), reovirus (12), and adenovirus (3). Surveys of enteric viruses found in turkey poults from placement to 7 weeks of age indicate that astrovirus is the most frequently detected virus (15,16). In addition, astrovirus was the earliest post-hatch virus detected (16). The most frequent viral combination in poults with clinical signs of enteritis is astrovirus and group D rotavirus (15).

The ability of astrovirus to produce enteric disease in specific-pathogen-free (SPF) turkeys has been documented and is characterized by diarrhea, nonspecific gross lesions, and significant decreases in both weight gain and intestinal D-xylose absorption (14). However, there are no reports of concomitant microscopic, morphometric, or ultrastructural changes in astrovirus-infected poults. The present report documents the disease caused by astrovirus in experimentally inoculated commercial poults, emphasizing the gross, microscopic, and ultrastructural lesions.

MATERIALS AND METHODS

Poults and housing. Turkey poults were obtained at hatch from a commercial source. Poults were housed in separate pre-sterilized, positive-pressure plastic isolators equipped with intake and exhaust air filters. All poults were provided with identical feed and water *ad libitum*. The temperature was maintained at approximately 30 C. Cloacal swabs for bacterial culture were taken from all poults before placement in isolators to check for enteropathogenic bacterial infections. Swabs were incubated for 24 and 48 hr in selenite enrichment media and then streaked on brilliant green agar. Suspect colonies were placed on triple-sugar-iron agar and enterotubes (9).

Virus inoculation. Virus for the inoculum was derived from diarrhetic poults in Wisconsin (14). The original sample contained both rotavirus and astrovirus before it was purified by sonication and serial filtration to 0.05 μm with disposable filters (Millipore Corp., Bedford, Mass.). This preparation was evaluated by immune electron microscopy (IEM) for astrovirus and rotavirus to verify the presence of astrovirus only. SPF poults were inoculated with this preparation to increase the pool of astrovirus and ensure that no viral contaminants were present. Intestinal contents were collected from these SPF poults on day

6 postinoculation (PI), filtered, examined by IEM, and found to contain only astrovirus. This filtered pool of intestinal contents was used as the inoculum in all experiments.

Experimental design. Three separate experiments were performed, which varied slightly as described below. For all experiments, poults were randomly allotted into two equal groups and placed in separate sterile isolators. One group was inoculated orally with 0.2 ml of bacteria-free preparation containing only astrovirus, as previously determined by immune electron microscopy. The inoculum was administered with a sterile plastic tuberculin syringe and teat canula. The second group of poults served as a control and was not inoculated. Poults were observed twice daily for clinical signs of disease. At various intervals PI, several poults from each group were selected at random, euthanatized, and necropsied. In all experiments, intestine and intestinal contents were collected at necropsy for light microscopy, transmission electron microscopy, and immune electron microscopy, as described below. Poults were euthanatized by intraperitoneal or intravenous injection with 5% pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, Ill.). The intestinal tract was immediately exteriorized, removed, and divided into the following four segments: duodenum (from the curve of the duodenal loop to pancreo-biliary ducts), proximal jejunum (from pancreo-biliary ducts to yolk stalk), distal jejunum (from yolk stalk to 2 cm proximal to cecal tips), and ileum (remainder of the small intestine terminating at the ileo-cecal junction).

Expt. 1. Poults were 2 days old when inoculated; two control and two inoculated poults were necropsied on each of days 1, 3, 5, 7, 10, and 14 PI.

Expt. 2. Poults were 5 days old when inoculated; two control and three inoculated poults were necropsied on each of days 0.5, 1, 2, 7, and 14 PI. Morphometric evaluation of light microscopic sections was performed by computerized image analysis.

Expt. 3. Poults were 5 days old when inoculated; four control and four inoculated poults were necropsied on each of days 0.5, 1, 3, and 7 PI. As in Expt. 2, morphometric evaluation of light microscopic sections was performed by image analysis.

Light microscopy and morphometry. Each intestinal segment processed for light microscopy was fixed with 10% neutral buffered formalin by immediate intraluminal infusion and immersion. Segments were fixed for 3 hrs, transferred to 70% ethanol, processed routinely, embedded in paraffin, sectioned at 4 μm , and stained with hematoxylin and eosin.

Intestinal sections from all three experiments were evaluated for light microscopic changes by one pathologist and reviewed by a second pathologist. In addition, intestinal sections from Expts. 2 and 3 were evaluated at the Iowa State University image analysis facility using a Zeiss SEM-IPS image analysis system

(Zeiss-Kontron, IBAS version 1.31 and 2.00; Carl Zeiss Inc., Thornwood, N.Y.). Sections were viewed with a Zeiss Axiophot microscope (Carl Zeiss Inc.), and images were captured with a Sony 3 CCD color video camera (Sony Corp. of America, San Diego, Calif.). The intestinal images were normalized, interactively discriminated, and edited to isolate the villi, crypts, and submucosa. The following parameters were quantified for each section: area and height of villi, area and depth of crypts, villus-to-crypt ratio, and area and thickness of submucosa. Parameters were statistically analyzed using analysis of variance. Differences were determined to be significant at $P \leq 0.05$.

Transmission electron microscopy. Intestinal tracts processed for transmission electron microscopic examination were immersed in and flushed with chilled 3% glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.2–7.4) with 0.2 M sucrose. Intestinal samples were taken from the same locations as samples taken for light microscopy. Intestinal segments were cut open with a razor blade, rinsed vigorously with fixative to remove adherent ingesta and mucus, chopped into 1-mm³ tissue segments, and left in fixative for 3 hr. Specimens were then rinsed two times for 15 minutes each time in 0.1 M Sorensen's phosphate buffer (pH 7.2–7.4) with 0.2 M sucrose, postfixed for 1 hour in 1% OsO₄ in 0.1 M Sorensen's phosphate buffer (pH 7.2–7.4) with 0.2 M sucrose, rinsed in distilled water, and dehydrated through a graded series of ethanol ending in absolute ethanol. Specimens were then cleared with propylene oxide, embedded in Embed 812 (Electron Microscopy Sciences, Fort Warrington, Pa.), sectioned with a diamond knife, mounted on copper grids, stained for 9 minutes in 2% methanolic uranyl acetate and 9 minutes in Reynold's lead citrate, and examined at 75 V on a Hitachi H500 transmission electron microscope.

Immune electron microscopy. Intestinal samples were evaluated for the presence of astrovirus by negative-stain IEM. The method used was a modified version of that described by Saif *et al.* (18). In Expt. 1, the entire small intestine and ceca with intestinal and cecal contents were examined; in Expts. 2 and 3, the ceca and small intestinal and cecal contents were examined. The samples were pooled by treatment group and day PI. Samples were weighed, diluted 1:2 with sterile phosphate-buffered saline solution, and frozen and thawed three times. Samples were then homogenized in a Stomacher Blender (Tekmar Corp., Cincinnati, Ohio), sonicated, and centrifuged at 1500 rpm (1000 × *g*) for 20 minutes. The supernatant was serially filtered through 0.8- μ m and 0.45- μ m disposable filters (Millipore), incubated overnight at 4 C with convalescent antisera prepared against astrovirus, and allowed to form immune complexes. Following the incubation, samples were pelleted through 33% sucrose by ultracentrifugation (55,000 × *g*) for 45 minutes at 10 C. The pellet was resuspended in 1

ml double-distilled water and ultracentrifuged a second time. The resulting pellet was resuspended in 20 μ l of 2% phosphotungstic acid in double-distilled water (pH 7.1).

Material from the pellet was applied to #200-mesh carbon-coated copper grids and evaluated at 75 kV in a Hitachi H500 transmission electron microscope (Nissei Sanjyo Co., Ltd., Hitachi division, Tokyo, Japan). For each sample evaluated, five grid squares were scanned. Samples having at least one aggregate of astrovirus were identified as positive.

Convalescent antisera that were used in IEM identification of astroviruses were prepared by inoculating 1-day-old commercial poults with astrovirus inoculum as described above. Serum samples were collected 3 to 4 weeks after the initial inoculation, mixed with astrovirus, and evaluated by IEM for the presence of astrovirus aggregates.

RESULTS

Clinical signs, gross and microscopic lesions, and results from virologic and bacteriologic examinations were similar for comparable times PI in all three experiments.

Clinical disease and gross lesions. Clinical signs and gross lesions were first noted on day 2 PI and persisted through day 10 PI. The predominant clinical sign was diarrhea. Gross lesions included dilated ceca containing light-yellow fluid feces and gas, variable hyperemia of the intestinal tract, and thinning or loss of intestinal tone of the jejunal and ileal intestinal walls.

Virology and bacteriology. Cultures from all poults were negative for *Salmonella* species.

Light microscopy and morphometry. Mild crypt hyperplasia resulting in increased crypt depth was the most consistent histopathological finding. Hyperplastic crypt epithelial cells were slightly basophilic and had large, oval nuclei and multiple prominent nucleoli. Changes appeared first in the proximal jejunum day 1 PI and persisted through day 7 PI. Most of the small intestine was involved by day 3 PI, when changes were also noted in the distal jejunum (Fig. 1A,B) and ileum. The duodenum was inconsistently affected before day 5 PI, but changes were noted in the duodenum from day 5 through day 7 PI. Elongation of crypts persisted in the proximal and distal jejunum through day 7 PI (Fig. 1C,D). No significant lesions were found at days 10 or 14 PI.

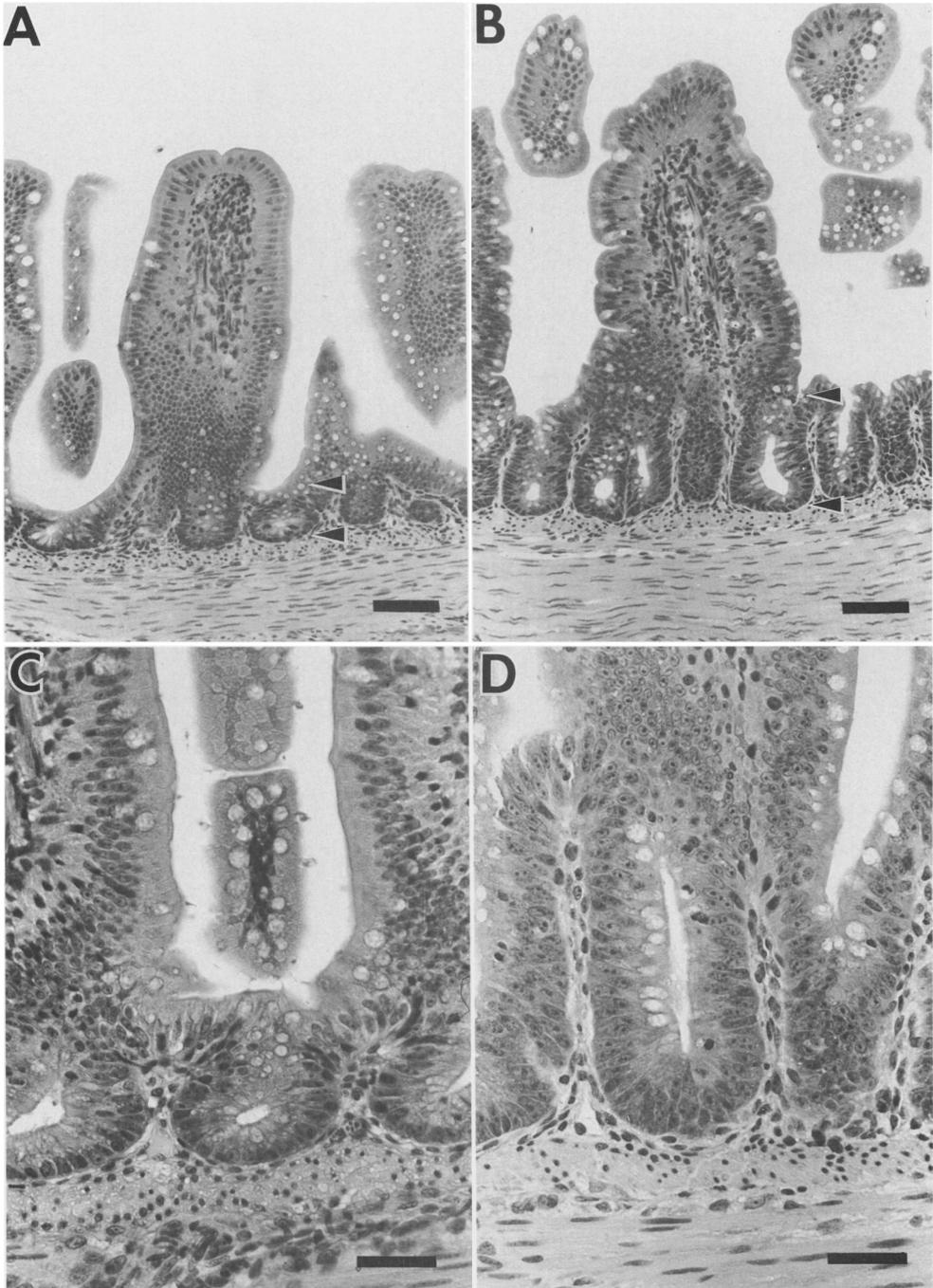


Fig. 1. Distal jejunum. **A)** 3 days PI. Intestine from a control poult demonstrating normal crypt depth (between arrowheads). Bar = 50 μm . **B)** 3 days PI. Intestine from an inoculated poult demonstrating significantly increased crypt depth (between arrowheads). Bar = 50 μm . **C)** 7 days PI. Intestine from a control poult demonstrating normal crypt depth. Bar = 30 μm . **D)** 7 days PI. Intestine from an inoculated poult with crypt epithelial hyperplasia resulting in significantly increased crypt depth. Most crypt epithelial cells contain multiple, prominent nucleoli. Bar = 30 μm .

Table 1. Expt. 2. Morphometric data comparing mean crypt death and mean crypt area between astrovirus-inoculated and control poult.

Days PI	Organ	Mean crypt depth (mm) ^A		Crypt area (mm ²) ^A	
		Control ^B	Astrovirus ^C	Control ^B	Astrovirus ^C
0.5	Duodenum	0.043 ± 0.006	0.043 ± 0.007	0.065 ± 0.012	0.069 ± 0.016
	Proximal jejunum	0.040 ± 0.001	0.036 ± 0.005	0.060 ± 0.004	0.055 ± 0.007
	Distal jejunum	0.040 ± 0.004	0.039 ± 0.001	0.060 ± 0.008	0.061 ± 0.003
	Ileum	0.039 ± 0.004	0.045 ± 0.008	0.058 ± 0.007	0.070 ± 0.013
1	Duodenum	0.043 ± 0.005	0.039 ± 0.008	0.067 ± 0.012	0.060 ± 0.014
	Proximal jejunum	0.042 ± 0.002	0.041 ± 0.006	0.065 ± 0.004	0.060 ± 0.009
	Distal jejunum	0.046 ± 0.007	0.045 ± 0.003	0.065 ± 0.015	0.069 ± 0.003
	Ileum	0.045 ± 0.003	0.049 ± 0.009	0.066 ± 0.006	0.072 ± 0.012
2	Duodenum	0.045 ± 0.013	0.062 ± 0.010	0.069 ± 0.020	0.085 ± 0.013
	Proximal jejunum	0.05 ± 0.007	0.058 ± 0.007	0.068 ± 0.007	0.082 ± 0.011
	Distal jejunum	0.046 ± 0.003	0.077 ± 0.030	0.064 ± 0.001	0.101 ± 0.033
	Ileum	0.051 ± 0.004	0.065 ± 0.009	0.074 ± 0.002	0.093 ± 0.008
7	Duodenum	0.049 ± 0.003	0.110 ± 0.039	0.111 ± 0.005	0.229 ± 0.072
	Proximal jejunum	0.055 ± 0.006	0.130* ± 0.018	0.115 ± 0.021	0.300* ± 0.044
	Distal jejunum	0.096 ± 0.011	0.195* ± 0.022	0.185 ± 0.015	0.335 ± 0.044
	Ileum	0.056 ± 0.006	0.108* ± 0.013	0.114 ± 0.011	0.260* ± 0.027
14	Duodenum	0.058 ± 0.010	0.066 ± 0.002	0.131 ± 0.024	0.153 ± 0.001
	Proximal jejunum	0.059 ± 0.007	0.05 ± 0.011	0.134 ± 0.028	0.112 ± 0.018
	Distal jejunum	0.069 ± 0.005	0.055 ± 0.004	0.152 ± 0.013	0.112 ± 0.116
	Ileum	0.070 ± 0.013	0.087 ± 0.048	0.147 ± 0.029	0.159 ± 0.071

^AMean ± S.D. Values marked by asterisks are significantly ($P \leq 0.05$) different from control values.

^B $n = 2$.

^C $n = 3$.

Morphometric changes were inconsistent and variable before day 3 PI in Expts. 2 and 3; however, consistent morphometric changes were detected from day 3 through day 7 PI. The only morphometric changes that consistently differed significantly ($P < 0.05$) between inoculated and control poult were increases in crypt depth and area (Tables 1 and 2). No differences in crypt depth or area were detected from day 0.5 through day 2 PI in any small intestinal section from either experiment. However, crypt depth and area were increased in the distal jejunum and ileum beginning at day 3 PI; the majority of the small intestine was affected by day 7 PI. An exception occurred in Expt. 2, at day 7 PI, when no significant changes were detected in the duodenum. By day 14 PI, there were no significant differences in crypt depth or area.

Transmission electron microscopy. Virus particles were found in a few mature villous enterocytes on days 2 and 3 PI in the ileum, but only on day 3 PI in the distal jejunum. The

scattered infected cells were located on the sides or near the base of villi. Electron-dense viral aggregates found on day 2 PI were located in and around dilated cytocavitary spaces as crystalline arrays or free in the cytoplasm of ileal enterocytes (Fig. 2A). Individual viral particles were circular and either hollow or solid-cored and had an average diameter of approximately 30 nm (Fig. 2B). Virus was also identified in the form of large, crystalline perinuclear arrays on day 2 PI. Most infected villous enterocytes on day 3 PI had viral particles arranged in large, crystalline arrays in the cytoplasm (Fig. 2B). Some enterocytes contained intracytoplasmic electron-dense ovoid aggregates consisting of crystalline arrays of virus particles embedded in an amorphous matrix. Occasionally, viral aggregates were present in the intestinal lumen in the distal jejunum on day 3 PI. Intraluminal viral aggregates and free virus particles were adjacent to the luminal margin of degenerate villous enterocytes or scattered among cellular debris (Fig. 2C).

Table 2. Expt. 3. Morphometric data comparing mean crypt depth and mean crypt area between inoculated and control poults.

Days PI	Organ	Mean crypt depth (mm) ^A		Crypt area (mm ²)	
		Control	Astrovirus	Control	Astrovirus
0.5	Duodenum	0.045 ± 0.015	0.048 ± 0.006	0.072 ± 0.019	0.068 ± 0.008
	Proximal jejunum	0.042 ± 0.005	0.044 ± 0.005	0.065 ± 0.007	0.058 ± 0.004
	Distal jejunum	0.041 ± 0.004	0.047 ± 0.006	0.063 ± 0.004	0.061 ± 0.011
	Ileum	0.045 ± 0.003	0.043 ± 0.008	0.069 ± 0.007	0.056 ± 0.012
1	Duodenum	0.049 ± 0.006	0.045 ± 0.005	0.079 ± 0.013	0.073 ± 0.011
	Proximal jejunum	0.045 ± 0.005	0.041 ± 0.007	0.071 ± 0.009	0.060 ± 0.011
	Distal jejunum	0.040 ± 0.006	0.040 ± 0.004	0.066 ± 0.009	0.058 ± 0.006
	Ileum	0.040 ± 0.004	0.042 ± 0.004	0.061 ± 0.008	0.065 ± 0.002
3	Duodenum	0.045 ± 0.006	0.050 ± 0.011	0.076 ± 0.010	0.083 ± 0.018
	Proximal jejunum	0.042 ± 0.005	0.052 ± 0.010	0.069 ± 0.008	0.085 ± 0.018
	Distal jejunum	0.039 ± 0.005	0.051* ± 0.007	0.064 ± 0.008	0.082* ± 0.010
	Ileum	0.039 ± 0.004	0.047* ± 0.006	0.065 ± 0.008	0.076 ± 0.011
7	Duodenum	0.060 ± 0.005	0.107* ± 0.013	0.134 ± 0.026	0.229 ± 0.031
	Proximal jejunum	0.053 ± 0.009	0.113* ± 0.033	0.116 ± 0.024	0.241* ± 0.070
	Distal jejunum	0.058 ± 0.014	0.079* ± 0.018	0.112 ± 0.033	0.170* ± 0.039
	Ileum	0.110 ± 0.030	0.157 ± 0.060	0.172 ± 0.072	0.281 ± 0.079

^A Mean ± S.D. Values marked by asterisks are significantly ($P \leq 0.05$) different from control values. $n = 4$ at each sampling interval.

Immune electron microscopy. Astrovirus was found by IEM in pooled intestinal samples from inoculated poults but not from control poults. No other viruses or recognized enteropathogens were detected by IEM or direct examination of tissues from inoculated or control birds by transmission electron microscopy.

DISCUSSION

The current research documented for the first time the microscopic, morphometric, and ultrastructural findings associated with experimental astrovirus infection in hatchling commercial turkey poults. Experimental astrovirus inoculation of commercial poults produced clinical signs and gross lesions similar to those described in a previous study (14), which used SPF poults. The duration of the disease in the present study was 10 days, which is comparable to the previously reported range of 10 to 14 days (14). Mild crypt hyperplasia, which resulted in increased crypt depth and area, was the most prominent microscopic change observed. Morphometric evaluation by computerized image analysis supported subjective observations made by light microscopy, detecting a significant increase in both crypt depth and area in the je-

junum and ileum from days 3 to 7 PI. There was progressive involvement of the small intestine beginning in the distal jejunum and spreading to most of the small intestine by day 7 PI. In the present study, turkey astrovirus produced microscopic and morphometric changes in the small intestine of turkeys that were similar to changes reported previously (23) in comparable regions of the small intestine in gnotobiotic lambs experimentally infected with ovine astrovirus. In the previous study, crypt hyperplasia progressively extended through the small intestine from 38 to 120 hours PI in astrovirus-infected lambs, but unlike the case with infected poults in the present study, the duodenum was not affected. Additionally, partial villous atrophy occurred in the "mid-gut" (distal jejunum) and ileum of astrovirus-infected lambs (23). Villous atrophy was neither observed directly nor detected with morphometric evaluation in astrovirus-inoculated poults. Lack of detectable villous atrophy is unusual, since villous atrophy typically precedes crypt hyperplasia in other intestinal viral diseases, such as rotavirus infections (11). Infection of gnotobiotic calves with bovine astrovirus in another study (29) failed to produce clinical signs but resulted in necrosis of M cells in the dome epithelium as-

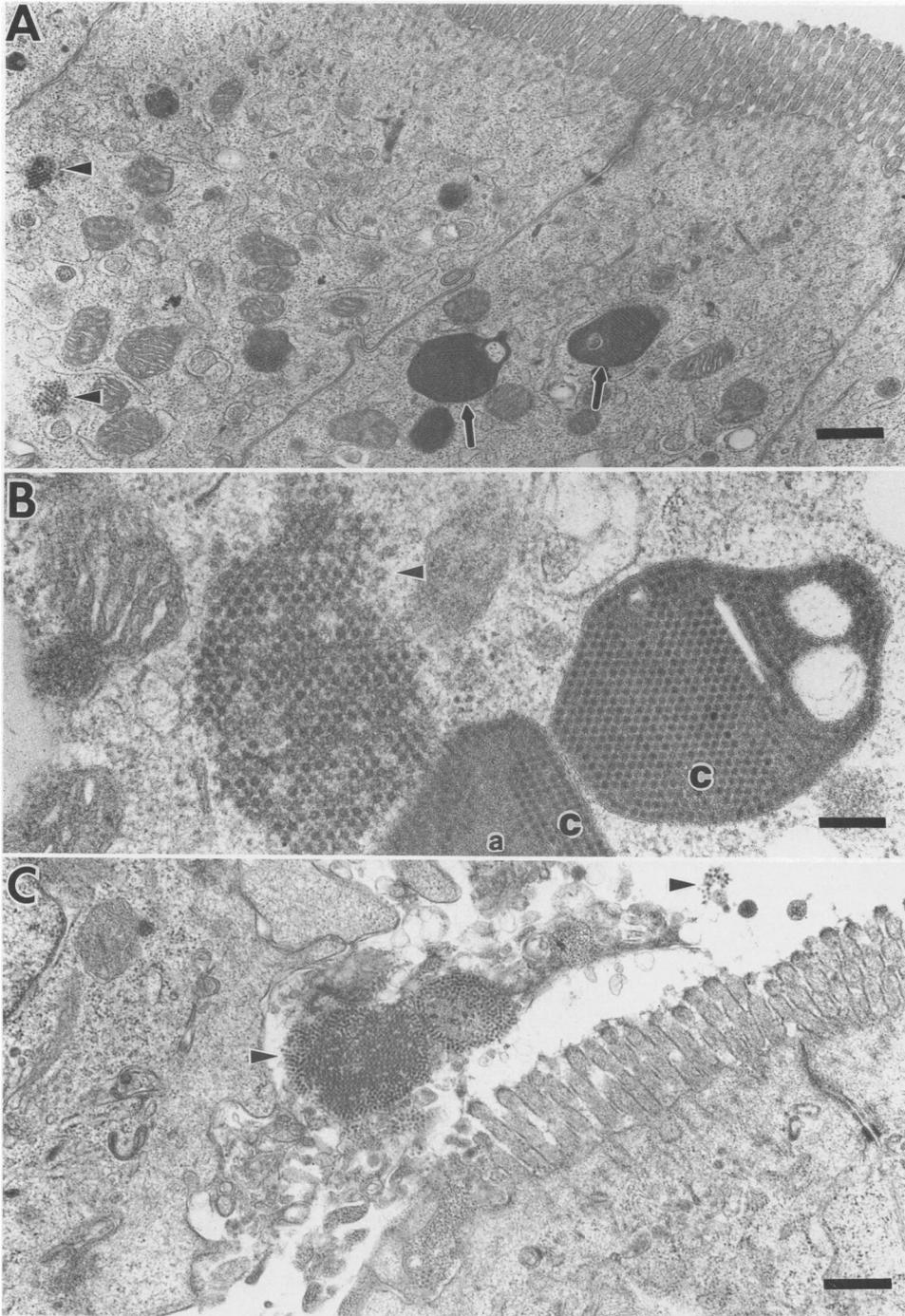


Fig. 2. A) Ileum. 2 days PI. Ileal enterocytes from an inoculated poult containing intracytoplasmic aggregates of astrovirus (arrowheads) and electron-dense crystalline arrays of astrovirus particles (arrows). Bar = 606 nm. B) Ileum. 2 days PI. Intracytoplasmic aggregate of astrovirus; a few virions are free in the cytoplasm. Virions are approximately 30 nm in diameter and have an electron-lucent center (arrowhead). Astrovirus is also organized into membrane-bound, crystalline arrays (c), which contain virions embedded within an electron-dense matrix (a). Bar = 174 nm. C) Distal jejunum. 3 days PI. Luminal aggregates of astrovirus (arrowheads) within debris adjacent to enterocytes. Bar = 441 nm.

sociated with Peyer's patches in the jejunum and ileum.

Ultrastructural evaluation of enterocytes in turkeys in the present study revealed intracytoplasmic crystalline arrays of astrovirus particles resembling those found in lambs (4). However, location of the infected enterocytes on villi differed between turkeys and lambs; enterocytes along the sides and near the base of villi were infected in turkeys, in contrast to enterocytes on the tips of villi in lambs (4). Similar to turkey astrovirus, human astrovirus is also found in the epithelium on the lower part of villi (13). The ultrastructural appearance and location of turkey astrovirus differs slightly from that of bovine astrovirus (29). Bovine astrovirus is confined to the dome epithelium of the ileum, and virions appear as large, perinuclear, electron-dense aggregates or small aggregates packed between apical tubules and vesicles. The mechanism of astrovirus entry into turkey enterocytes was not investigated in the present study, but apparent release of astrovirus from degenerate enterocytes was observed in the distal jejunum at day 3 PI. Entry of astrovirus into ovine enterocytes occurs via apical pits and tubules, and although the release of astrovirus has not been directly observed, it is presumed to result from disintegration of desquamated enterocytes (4).

Although astrovirus has not been formally classified into a viral taxon, evidence in the present study indicates that turkey astrovirus may be characterized as a type I enteropathogenic virus. Type I enteropathogenic viruses are transmitted by the fecal-oral route, have an intestinal cell tropism for villous enterocytes, and affect the distal small intestine most extensively (17). The mechanism for preferential infection by astrovirus of enterocytes in the distal small intestine has not been determined.

Diarrhea is the most common and significant clinical problem caused by enteropathogenic viral infections. The defined mechanisms of diarrhea that are operative in intestinal viral infections are malabsorption and maldigestion secondary to loss of mature enterocytes and villous atrophy (11). However, as mentioned above, significant villous atrophy is not a feature of intestinal disease of poult infected with astrovirus; therefore, the operative mechanism causing diarrhea is not obvious. It may be that astrovirus infection causes an increased turn-

over rate of enterocytes that results in a less mature population of villous enterocytes but is subtle enough so that significant villous atrophy does not develop. This hypothesis is consistent with our finding of crypt hyperplasia, which is usually a reparative response to loss of enterocytes. In addition, astrovirus infection significantly decreases the level of intestinal disaccharidase activity, which results in maldigestion and subsequent malabsorption of disaccharides (25). This could result from enterocyte immaturity secondary to increased turnover or direct interference with disaccharidase activity.

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