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Parvovirus-Like Enteropathy in Missouri Turkeys

D. W. Trampel, D. A. Kinden, R. F. Solorzano, and P. L. Stogsdill

Veterinary Medical Diagnostic Laboratory
University of Missouri
Columbia, Missouri 65211

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SUMMARY

A viral enteric disease of young turkeys characterized by stunting of affected birds, diarrhea, and increased mortality is described. Eosinophilic intranuclear inclusion bodies were found in the absorptive epithelial cells of the ileum. Electron microscopy of formalin-fixed tissue revealed that the intestinal inclusions contained numerous loosely packed 15-to20-nm hexagonal particles. The size, shape, and intranuclear location have been used to tentatively identify these particles as parvoviruses.

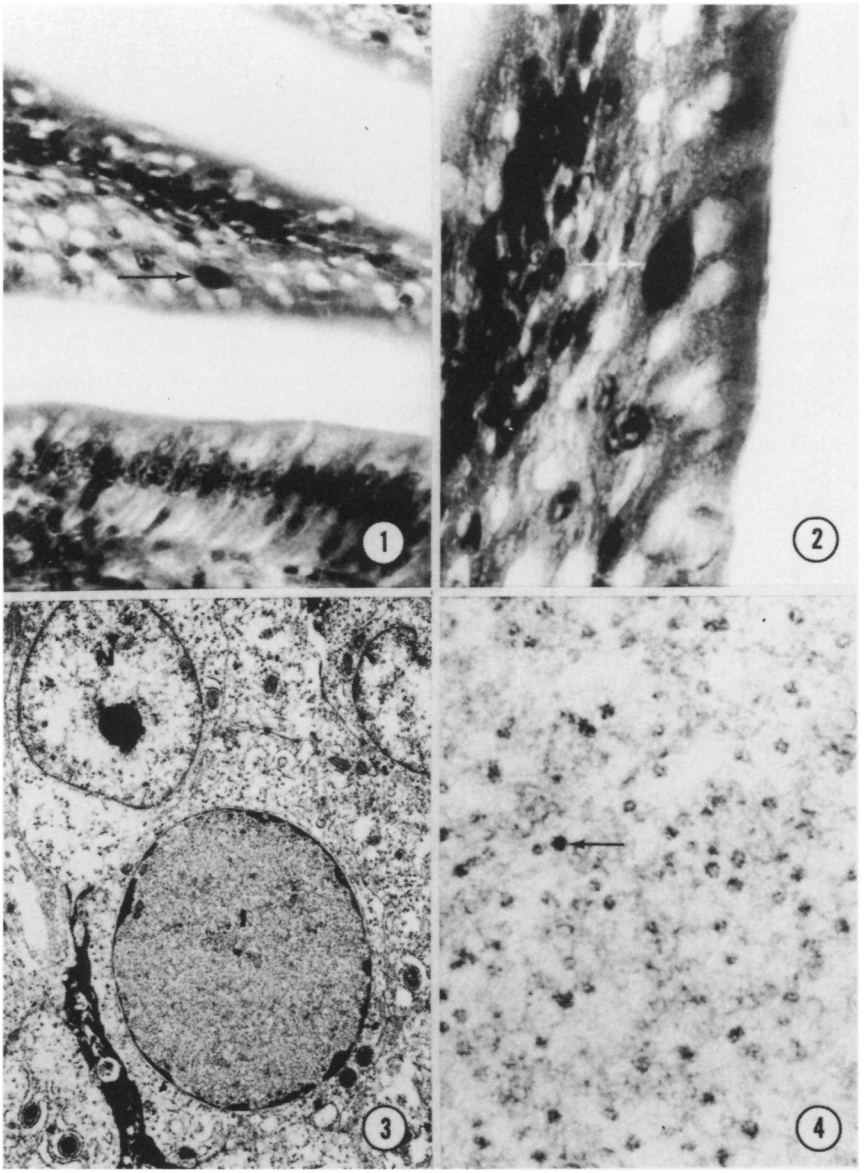
INTRODUCTION

Diarrhea of undetermined etiology is frequently encountered in young turkeys throughout the United States. Accompanying economic losses result from increased mortality, reduced feed efficiency, and stunting of poults in affected flocks. Because potential bacterial, nutritional, and toxic causes of diarrhea can usually be eliminated from a list of possible etiologies, viral infections are frequently suspected but seldom confirmed. This paper describes a viral enteric disease of turkeys that to our knowledge has not been reported in the literature.

MATERIALS AND METHODS

In 1979 in Missouri, idiopathic diarrhea occurred in poults from numerous flocks that contained a total of more than one million birds. On November 9, 18 two-week-old tom turkeys were submitted to the Veterinary Medical Diagnostic Laboratory in Columbia, Missouri. Turkeys in the flock of origin were experiencing depression and high mortality. Microscopic examination of the small intestine revealed intranuclear inclusion bodies in absorptive epithelial cells. Electron microscopy of formalin-fixed tissues showed that the inclusions were composed of numerous 15-to-20-nm viral particles. Six additional cases characterized by diarrhea and intestinal inclusion bodies were identified in the following months.

Diagnostic accessions were the source of all turkeys. The turkeys were necropsied, and appropriate specimens were cultured for bacteria. Multiple tissues were fixed in 10% phosphate-buffered neutral formalin and processed routinely in preparation for viewing with the light microscope.



An ileum from a poult in the initial accession was selected for examination by electron microscopy. The ileum was deparaffinized, minced into 1-mm cubes, and rinsed with several changes of phosphate buffer. The specimens were then fixed with phosphate-buffered 1% OsO_4, dehydrated in a graded ethanol series, and embedded in Spurr's low-viscosity resin (13). Ultrathin sections were cut with a diamond knife on an LKB Ultratome III ultramicrotome and were post-stained with uranyl acetate and lead citrate (8). Micrographs were taken on an RCA EMU-3G electron microscope operating at an accelerating voltage of 100 kV.

RESULTS

Case histories consistently included listlessness, depression, and stunting of 5–10% of the birds in a flock. Male and female turkeys from both commercial and breeder candidate flocks were represented in the seven cases. The age of affected poults ranged from 1 to 5 weeks. Turkeys in two flocks were reported to suffer from "splayed legs." Poults in one flock were known to have originated from eggs characterized by poor hatchability.

Five of the seven cases appeared to be uncomplicated viral infections, since no bacterial pathogens were detected. In the two other cases, *Escherichia coli* was isolated from the livers and air sacs.

Lesions observed at necropsy were usually restricted to the gastrointestinal tract when no other pathogens were found. Gizzards contained large quantities of litter and grit but very little feed. The small intestine, and occasionally the ceca, were distended by mucus, gas, and fluid feces. In two flocks, the bones of numerous turkeys were extremely soft and pliable and would bend before breaking. Many poults in these flocks could no longer walk and tended to congregate in small groups on the floors of the houses. The quantity and ratio of calcium, phosphorus, and vitamin D in the ration was checked and found to be within recommended limits.

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Figs. 1–4. (*1*) Villi from the ileum of a 2-week-old poult. An intranuclear inclusion body is present within the layer of epithelial cells covering a villus (arrow). Note the normal tall columnar morphology of epithelial cells on the adjacent villus. 975×. (*2*) An oval intranuclear inclusion body within the epithelial cell layer of a villus (arrow). Adjacent unaffected nuclei are much smaller than the inclusion body. 1,575×. (*3*) Electron micrograph of formalin-fixed ileal epithelium. Margination of chromatin can be seen around the periphery of an intranuclear inclusion body (I). Empty space and a prominent nucleolus can be seen within an unaffected nucleus (N). 6,075×. (*4*) Electron micrograph of the center of an inclusion body. Numerous particles with an electron-dense outer shell and an electron-lucent center can be seen. An occasional particle is completely electron-dense (arrow). 108,000×.

Histopathological examination of tissues from poults in each of the seven flocks disclosed the presence of intranuclear inclusion bodies within absorptive epithelial cells of the ileum (Figs. 1, 2). The inclusions occurred at various points along the length of a villus but were not observed in the crypts. The length and thickness of villi and the length of crypts were essentially normal. Inclusion bodies were round to oval, eosinophilic, and completely filled the nuclei. Most inclusions were larger than adjacent normal nuclei, and margination of chromatin was frequently observed around the periphery of the inclusion.

Electron micrographs of ileal mucosa showed that intranuclear inclusions were well preserved but that ultrastructural detail of cytoplasmic structures and unaffected nuclei was poor (Fig. 3). Nuclei without inclusions were somewhat irregular in shape and contained leached-out areas in the nucleoplasm. In contrast, nuclei containing inclusions were round to oval and completely filled by loosely packed, finely granular particles (Fig. 4). Individual particles had hexagonal profiles and were approximately 15–20 nm in diameter. The majority of particles had an electron-dense outer shell surrounding an electron-lucent center. However, a few particles were completely electron-dense.

Spleens in some birds had marked depletion of lymphocytes from periarteriolar lymphoid sheaths. The liver of one bird had a single small focal area of hepatocellular necrosis. Poorly preserved round bodies were observed within the necrotic hepatic tissue and probably represent intranuclear inclusion bodies (Figs. 5, 6).

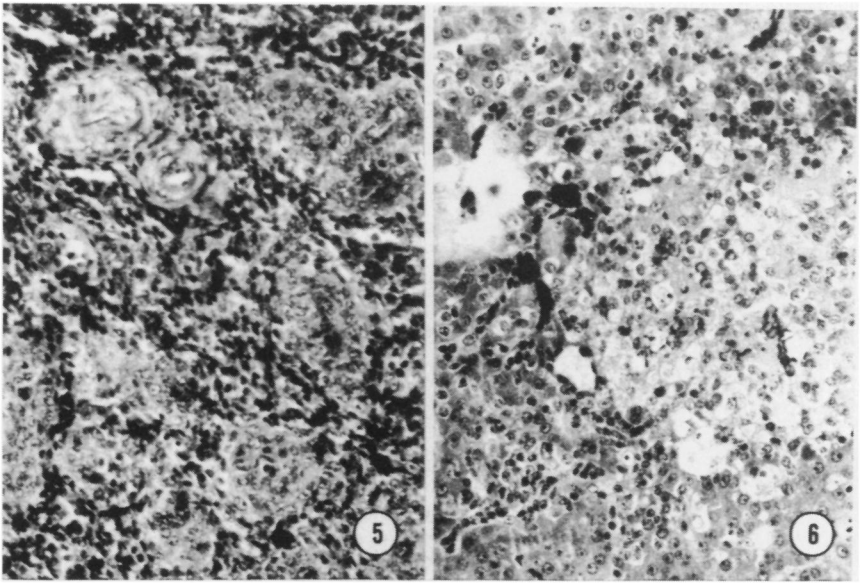
DISCUSSION

The size, morphology, and intranuclear location of particles within the inclusion bodies are consistent with parvovirus (12). Electron-lucent and electron-dense hexagonal particles have been interpreted to represent complete and incomplete virions, respectively (14). However, confirmation of the identity of this virus must await isolation and application of appropriate virological techniques. Although parvoviruses have been reported in laboratory chickens in the United States and in European geese, this paper represents the first report of a parvovirus-like infection in turkeys (2,3,6,7,9,10,11).

Histopathological change in the intestines was limited to the development of intranuclear inclusions bodies in the epithelial cells of the ileum. The absence of inclusions in crypt epithelium probably reflects the stage of infection at the time of tissue collection. Unlike feline and canine parvoviruses, this virus apparently does not cause

lysis of enterocytes. The notable lack of vascular and cellular changes associated with inflammation suggests that the intestinal lesions should be classified as an enteropathy and not as an enteritis.

Normal morphology of villi and crypts was unexpected after fluid feces were observed in the intestinal tracts at necropsy. This finding may indicate that the intestine was injured at the biochemical level. If the metabolism of mature intestinal epithelial cells was diverted from the normal goals of absorption and digestion and redirected toward the goal of virion production, then a malabsorption syndrome could develop in spite of normal histology. Increased osmotic pressure caused by unabsorbed nutrient particles would hold water within the intestinal lumen and thereby create the fluid feces recognized clinically as diarrhea (5). Malabsorption of nutrients from the intestinal tract would account for the stunting of large numbers of birds in affected flocks. Malabsorption of calcium and phosphorus could explain the bone changes noted grossly in these turkeys and associated with some cases of parvovirus enteropathy.



Figs. 5-6. (*5*) Spleen from a 2-week-old turkey. Splenic arterioles are in close proximity to one another due to reduced numbers of lymphocytes in the parenchyma. 975×. (*6*) Liver from a 2-week-old turkey. A pale-staining focal area of hepato-cellular necrosis is present within the parenchyma. Aggregates of spherical bodies within the necrotic hepatic tissue may represent poorly preserved intranuclear inclusions. 975×.

The absence of diarrhea in some cases most likely results from a combination of inadequate observation of the birds before death and extreme fluidity of the feces. When feces approach the consistency of water, the fecal material does not adhere to feathers around the vent and is rapidly absorbed by the litter. Consequently, pasting around the vent and loose droppings in the litter cannot be seen. If affected birds are not observed while defecating, diarrhea may not be detected.

Immunosuppression is potentially a more important manifestation of turkey parvoviral infection than is its effect upon the intestinal tract. Parvovirus replication and lysis of host cells occur in tissue characterized by high mitotic activity, such as that occurring in lymphoid tissue. Thymic atrophy is a consistent lesion in cats infected by the feline parvovirus (1). In turkeys, depletion of lymphocytes from splenic periarteriolar lymphoid sheaths, believed to be of thymic origin, could be an indication of thymic damage similar to that reported in cats (4). Unfortunately, this hypothesis could not be confirmed because neither thymus nor bursa from affected birds was saved for microscopic examination.

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