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Case Report—

Microsporidiosis in a Young Ostrich (Struthio camelus)

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SUMMARY. Microsporidia are obligate, intracellular, protozoan parasites of a wide variety of vertebrates and invertebrates. Confirmed reports of microsporidial infection of avian species are few (lovebirds, a parrot, and a group of budgerigar chicks). At slaughter, a 14-mo-old ostrich was found to have small intestinal serosal hemorrhages during postmortem inspection. Histologic examination of the small intestine revealed a chronic lymphoplasmacytic to purulent enteritis with mucosal hyperplasia, muscular hypertrophy, and numerous microsporidia that were located within the superficial enterocytes and the lamina propria. Microsporidia have a ubiquitous distribution in nature and are suspected as possible zoonotic agents.

RESUMEN. *Reporte de Caso*—Microsporidiosis en una avestruz joven (Struthio camelus). Los microsporidios son protozoarios intracelulares y parásitos obligados de una amplia variedad de vertebrados e invertebrados. Los reportes de infecciones microsporidiales confirmadas en especies aviares son muy pocos (aves mascota, un loro y un grupo de pericos recién nacidos). Se sacrificó un avestruz de 14 meses de edad y durante el examen a la necropsia se observó una hemorragia en la capa serosa del intestino delgado. El examen histopatológico de este órgano mostró enteritis desde linfoplasmacítica crónica hasta purulenta con hiperplasia de la mucosa. Se observó también hipertrofia muscular y numerosos microsporidios que se encontraron localizados entre los enterocitos superficiales y la lámina propia. Los microsporidios están ampliamente distribuídos en la naturaleza y se sospecha que pueden actuar como agentes zoonóticos.

Key words: ratite, ostrich, microsporidiosis, enteritis

Abbreviations: AIDS = acquired immunodeficiency syndrome

Microsporidia are obligate intracellular protozoan parasites that belong to the phylum Microspora and infect most invertebrates and all classes of vertebrates (17). Microsporidiosis is an emerging human infectious disease that has been described in patients with acquired immunodeficiency syndrome (AIDS) (5) as well as in an immunocompetent person not infected with AIDS (16). In addition, these parasites cause economically significant disease in laboratory animals and fish (3). Few descriptions of infection and disease in avian species have been described (lovebirds (2,6,9,10,11,13,15), a parrot (12), and a group of budgerigars (1)). Commercial production of ratites as a meat source has resulted in the recognition of a wide range of clinical and postmortem abnormalities that could affect the wholesomeness and safety of the carcass as a food source. This report describes microsporidial infection of the intestine of an ostrich in which serosal hemorrhage and a chronic enteritis with mucosal hyperplasia and muscular hypertrophy were present.

CASE REPORT

A clinically normal, 14-mo-old male ostrich was presented for slaughter in a United States

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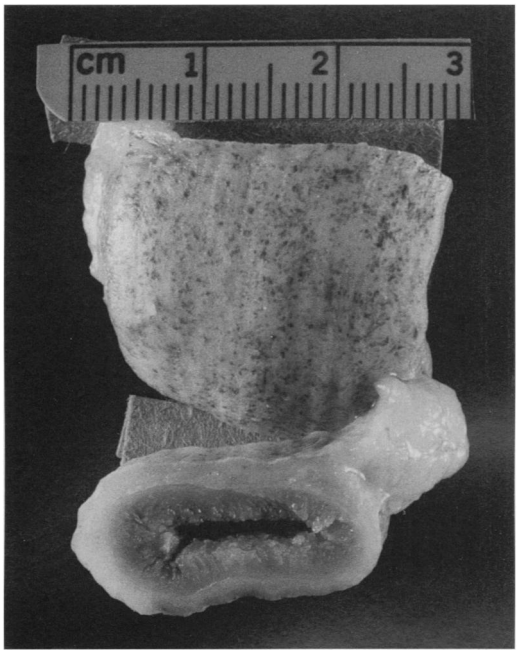

Fig. 1. Cross and longitudinal sections of ostrich jejunum demonstrating the thickened wall and serosal congestion and hemorrhage.

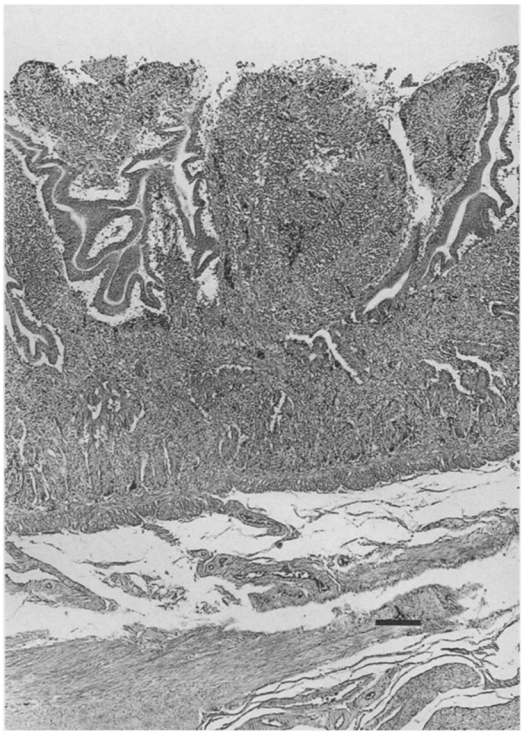

Fig. 2. Elongate, broadened villi of the ostrich jejunum are congested, with necrosis and erosion of the villous tips. Hematoxylin and eosin stain. Bar = 250 μm.

Department of Agriculture–inspected slaughter establishment in Texas. The inspection veterinarian noted that the carcass was in good condition, with the only change consisting of serosal congestion and petechial hemorrhage limited to a 2-ft section of the jejunum (Fig. 1). Portions of the affected jejunum were preserved in 10% neutral buffered formalin and submitted for histopathologic examination. The tissue was processed routinely, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin, Gram's, acid-fast, Giemsa, and Goodpasture's stains. In addition, the jejunal mucosa was sectioned into 2-mm cubes, rinsed in distilled deionized water for 30 min, placed in picric acid fixative (2% glutaraldehyde, 2% paraformaldehyde, 0.2% picric acid in 0.1 M cacodylate buffer), and processed for electron microscopy.

The jejunal tunica muscularis was thickened, and the mucosal and serosal vessels were congested. Within the serosa, there were mild, multifocal, perivascular hemorrhages and edema with a very mild infiltrate of heterophils, lymphocytes, plasma cells, and macrophages that extended into the adjacent serosa and superficial muscularis. The mucosa was thickened and characterized by elongate, broad villi in which frequent mitotic figures were observed among the crypt enterocytes (Fig. 2). Within the lamina propria, there were mild multifocal hemorrhages. Inflammation consisted of a mild-to-moderate, diffuse infiltrate of lymphocytes, plasma cells, and macrophages and mild, multifocal aggregates of heterophils. Multifocally, there were enterocytes along the superficial tips and sides of the villi with cytoplasmic vacuoles that contained numerous 1–3-μm, thin-walled, gram-positive, oval, protozoal organisms (Fig. 3). Occasionally, the protozoa were observed in the lamina propria. The protozoal-infected enterocytes were associated with necrosis and erosion of the villus tips and deposition of a thin adherent layer of fibrin, cellular debris, hemorrhage, mixed mononuclear cells, and small numbers of a mixed population of gram-negative bacterial rods.

Electron microscopy revealed enterocytes with a cytoplasmic parasitophorous vacuole

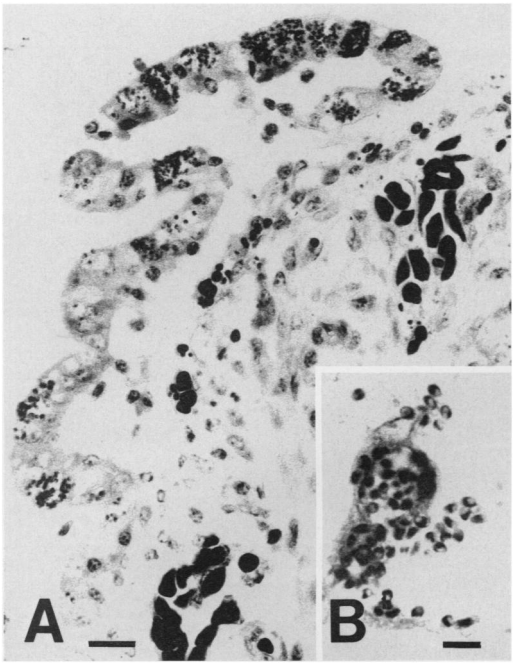

Fig. 3. Section of ostrich jejunum. (A) Numerous microsporidia are present within cytoplasmic vacuoles of the superficial enterocytes. Goodpasture's stain. Bar = 25 μm. (B) Higher magnification demonstrating the polar cap. Goodpasture's stain. Bar = 5 μm.

that contained numerous microsporidia. Uninucleate sporonts and mature spores with three to six turns of the polar filament were apparent (Fig. 4). The mature spores had a characteristic wall composed of the outer electron-dense exospore and the inner electron-lucent endospore as well as a posterior vacuole. Developing spores measured 0.8–1.0 × 1.0–2.0 μm.

DISCUSSION

Microsporidia are the only class of protozoal cysts and spores that stain gram positive (3). This, combined with the presence of unicellular spores without mitochondria that contained an extrusion apparatus with a filamentous polar tube and polar cap, confirmed the organism in this ostrich as a member of the phylum Microspora (8). Confirmed reports of microsporidiosis in birds have been limited to psittacine birds, with mention of "encephalitozoonosis" in pheasants and a duck in one report (9) and questionable cases in sparrows (7), parakeets, and pigeons (18). The cases in psittacine birds included descriptions of microsporidia in the intestine, liver, kidney, and lung, both with and without necrosis, (1,2,6,9,11,12,13,15), and bile duct infection and biliary hyperplasia (6,9,13,15). Other infectious conditions associated with microsporidiosis in these psittacines included megabacteriosis, giardiasis, and candidiasis (1,9,10).

Superficial enterocyte degeneration and necrosis were apparently directly related to the large number of microsporidia present in the jejunum of this ostrich. The mucosal hyperplasia and inflammation may have also been the direct result of this infection. However, the significance of the small number of gram-negative bacteria present among the superficial enterocytes and exudate as a contributing factor in the changes is unknown. Additionally, one of us has observed mucosal and smooth muscle hyperplasia of the tunica muscularis in the small intestine of slaughter-age ostriches. In these cases, there was minimal inflammation, consisting of lymphoplasmacytic infiltrates in the mucosa, and no infectious agent was detected by special stains (Puette, unpubl. obs.). Smooth muscle hyperplasia of unknown etiology has also been described in the small intestine of a rhea. However, in the affected rhea, the smooth muscle hyperplasia was without associated inflammation and involved both the tunica mucosa and muscularis (14). The lack of a clinical history and the submission of only intestine for histologic examination limit any conclusions as to the extent and clinical significance of the microsporidial infection in this bird. Possibly, another disease process could have contributed to the histologic changes and/or predisposed the bird to microsporidial infection.

Specific identification of the microsporidia by immunologic or genetic analysis was not attempted. However, membrane-bound parasitophorous vacuoles containing uninucleate, 1- × 2-μm microsporidia with an average of six turns of the polar filament are characteristic of *Encephalitozoon* species (4). Recognition that ratites may be infected by microsporidia may lead to increased surveillance for their presence, resulting in a more complete picture of the clinical symptoms and histologic lesions associated with this infection in these birds. Because microsporidiosis is an emerging opportunistic disease of persons with AIDS, the recognition and

Fig. 4. Ultrastructure of microsporidia within ostrich jejunal enterocytes. (A) Numerous microsporidia in various stages of sporogony, including uninucleate sporonts (open arrow) and mature spores (solid arrow), are present in parasitophorous vacuoles. L = intestinal lumen. Bar = 1 μm. (B) Higher magnification of an immature spore (arrow) with three to four turns of the coiled polar filament and degenerating spores with distinct exospore and endospore wall layers. Bar = 0.5 μm.

identification of microsporidia and their potential host range are of increasing importance.

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