Case Report—

Microsporidian Infection in the Pied Peach-Faced Lovebird (Agapornis roseicollis)

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Received 10 August 1977

SUMMARY

A microsporidian infection was diagnosed in a pied peach-faced lovebird (*Agapornis roseicollis*) which had died after an illness of 2 days. The parasite was observed in the liver, kidney, and small intestine. Lesions were most severe in the liver, with numerous organisms adjacent to areas of hepatic necrosis. Although the organisms were seen within renal tubules and within the intestinal mucosa, inflammatory lesions in those sites were minimal. The microsporidian nature of the parasite was confirmed by morphologic studies. The genus of the organism was not determined.

INTRODUCTION

Reports of naturally occurring microsporidian infections in birds are rare. The first report of a confirmed microsporidian infection in an avian species was apparently in 1975 (2). The organisms were found in the renal tubular epithelial cells, hepatocytes, bile ducts, and intestinal epithelial cells. From morphology and staining reactions, the authors tentatively classified the organism as *Encephalitozoon* sp. Previous reports of infections in sparrows (3) and in parakeets and pigeons (8) have been questioned since the mice used as the experimental host are known to be frequently infected with the organism. The present report described

a spontaneous microsporidial infection in a pied peach-faced lovebird (*Agapornis roseicollis*). To our knowledge, this is the second confirmed case of a microsporidian infection in an avian species.

CASE HISTORY AND METHODS

A 6-month-old pied peach-faced lovebird was purchased from a commercial aviary. Six weeks later the bird had anorexia, weakness, and closed eyes. The bird was sick for 2 days before it died. Chlortetracycline in water was given to the bird for 24 hours before death. The carcass was frozen and submitted by the owner to the Maryland Department of Agriculture, Animal Health Laboratory, for postmortem examination. The bird had been frozen for about 36 hours before necropsy.

At necropsy, samples of lung, liver, spleen, and intestine were collected for routine microbiologic cultures. Tissues for histopathologic examination were fixed in 10% buffered neutral formalin, embedded in paraffin, cut at 6 μm and stained with hematoxylin and eosin (H and E). Used on selected sections were special staining methods, including McCallum-Goodpasture (MG), toluidine blue, and periodic acid-Schiff (PAS). Deparaffinized formalin-fixed tissue from the liver was postfixed in 3% glutaraldehyde and 3% osmium tetroxide and embedded in Epon 812 mixture. Thin sections were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined with a Hitachi HS-8F electron microscope.

LABORATORY FINDINGS

At necropsy, the bird was emaciated. Congestion of the liver, spleen, and kidneys were observed. Scattered white foci, 1–2 mm in diameter, were seen in the liver. The most significant lesions found by light microscope were in the liver. Many focal areas of coagulative necrosis were seen in midzonal and periportal regions (Fig. 1). Typically, these consisted of residual hepatic nuclei, amorphous eosinophilic material, and occasionally lightly stained granular bodies surrounded by mononuclear cells and erythrocytes. Hepatocytes bordering areas of necrosis also contained pale basophilic bodies in dense cystlike clusters. Seen in the MG-stained sections were gram-positive short rods (1.3×1.7 μm) (Figs. 2, 3). With the PAS reaction, a small curved area was deeply stained at one end of the organism. The organisms were also seen in renal tubules (Fig. 4) and small intestine with little or no inflammatory reaction. No lesions or organisms were seen in the brain, trachea, lung, esophagus, or pancreas.

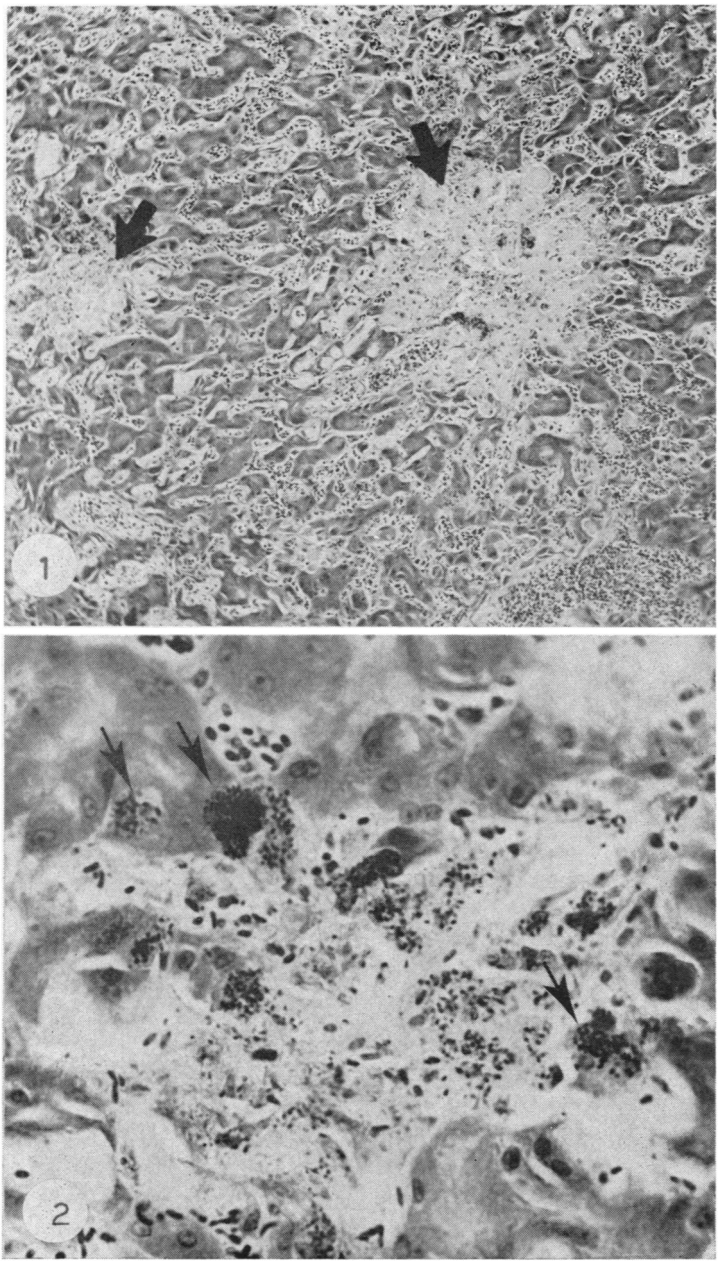
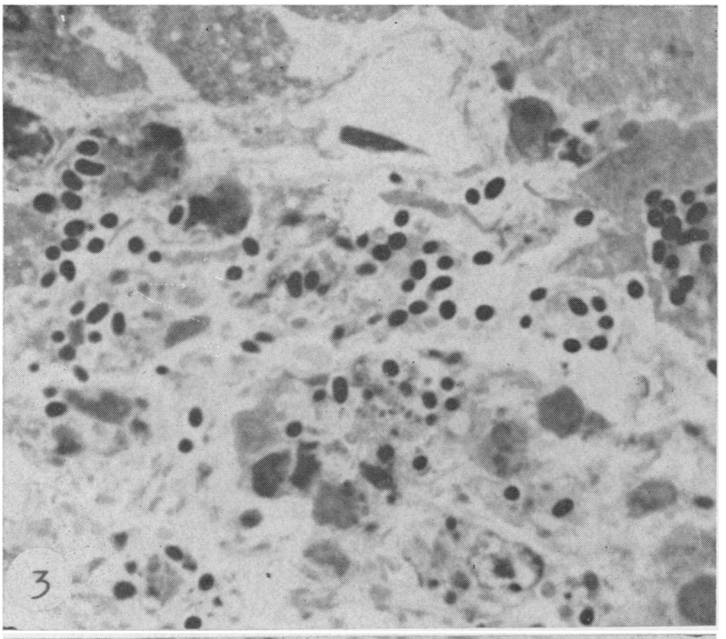


Fig. 1. Photomicrograph of liver from lovebird showing necrotic foci (arrows). H & E stain; ×100.

Fig. 2. Photomicrograph of liver from lovebird showing numerous gram-positive organisms. Note clusters of microsporidian organisms within some hepatocytes (arrows). McCallum-Goodpasture stain; ×485.



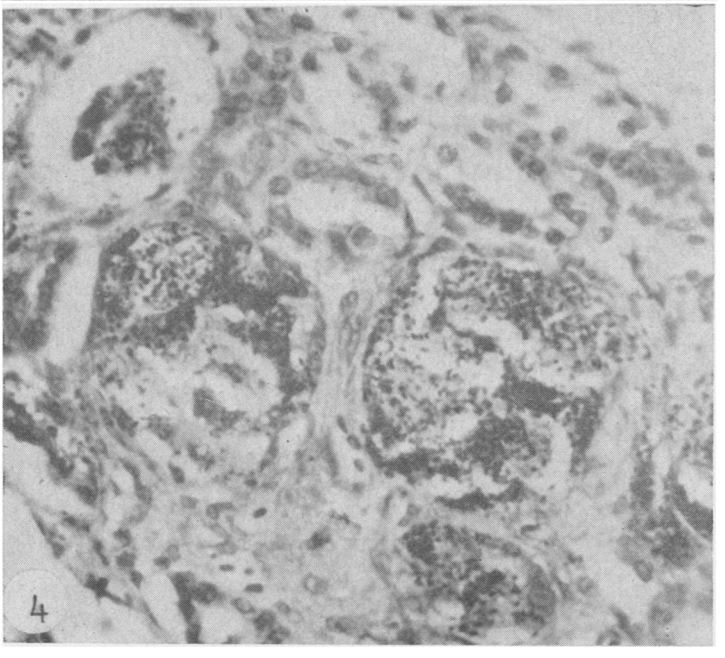


Fig. 3. Higher magnification of microsporidian organisms in area of necrosis. Epon-embedded section; toluidine blue; ×1600.

Fig. 4. Photomicrograph of renal medulla from lovebird showing collections of microsporidian organisms within tubules. McCallum-Goodpasture stain; ×440.

Electron-microscope evaluation of liver sections revealed alterations in cell structure attributed to freezing and formalin fixation. The organisms were usually found free within areas of necrosis; however, occasional host cells were seen with organisms in various stages of development. The organisms were well enough preserved to permit certain observations (Fig. 5). The mature spores contained an electron-dense outer membrane with a wider electron-lucent area internally. In some spores, the coiled polar filament could be seen, a feature which establishes the microsporidian nature of the parasite. Electron-dense lamellae were also seen, apparently representing the polaroplast.

Pasteurella sp. was cultured from aerobic bacterial cultures of liver and splenic specimens. *Escherichia coli* was cultured from the lung.

DISCUSSION

The large numbers of microsporidian organisms seen in the affected organs and their association with areas of necrosis and inflammation, especially in the liver, suggest an active infection with the organism. The significance of isolating *Pasteurella* sp. from the liver and spleen is not clear. A careful search of liver sections stained with the MG stain did not reveal these organisms in association with the lesions. Inadvertently, the *Pasteurella* sp. isolate was not identified as to species. Also, a section of spleen was not available for histopathologic evaluation and for correlation with microbiologic findings. The isolation of *E. coli* from the lung was probably due to postmortem invasion. It is not possible to unequivocally rule out intercurrent infection in this bird.

Interestingly, the first confirmed microsporidian infection reported in the avian species also involved a lovebird (2). However, that occurred in the blue-masked lovebird (*Agapornis personata*), in contrast to the pied peach-faced lovebird reported here. The finding of organisms in the liver, kidney, and intestine was similar to that described previously, including the minimal inflammatory response in some tissues (2).

Morphologic features and staining reactions were similar to those reported for microsporidia in various mammalian host tissues (4, 7). The organisms stained poorly in H-and-E-stained sections. Although many organisms were gram-positive, this staining reaction was variable, apparently a characteristic related to the developmental stage of the organism. In Epon-embedded sections stained with toluidine blue, the organisms usually stained darkly. A PAS-

positive area at one end of the organism is related to the polar cap (6). The ultrastructural features of the spores resembled those described for mammalian microsporidia. The presence of a polar filament conclusively identifies the parasite as a member of the order Microsporida.

The taxonomic classification of various miscrosporidian genera (*Nosema, Encephalitozoon, Glugea,* and *Perezia*) is currently under revision. Earlier workers had considered *Encephalitozoon* to be a junior synonym of *Nosema*; however, recent investigators using electron microscopy, have shown important morphologic differences which apparently justify the taxonomic separation of these genera (1, 7). *Nosema* are characterized by nuclei which are paired in

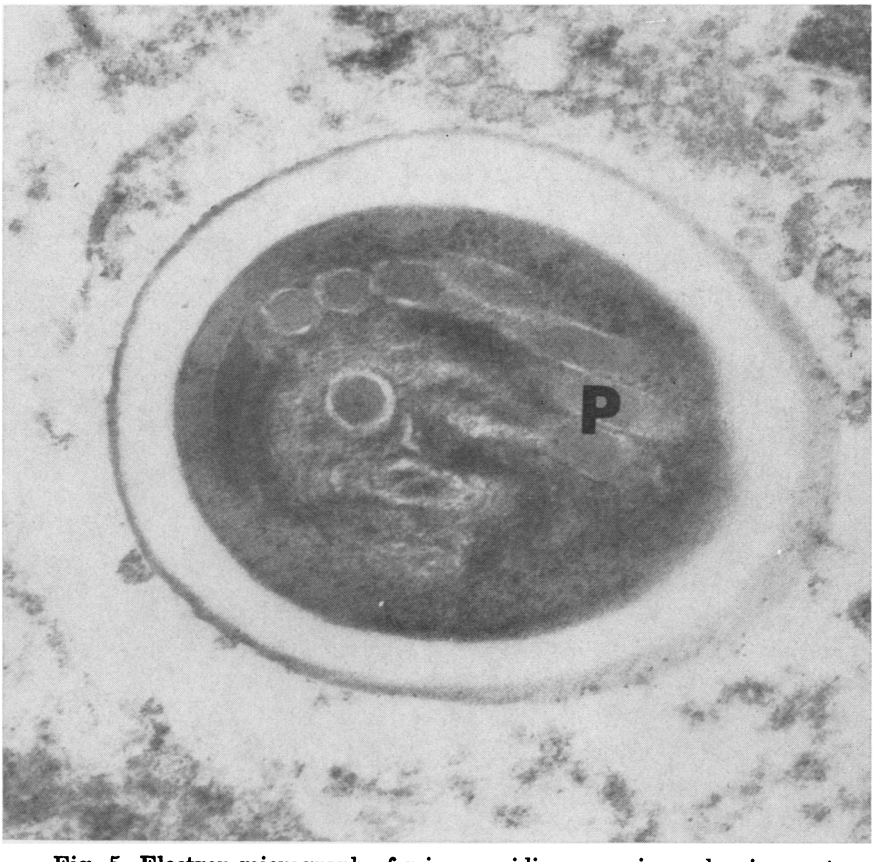


Fig. 5. Electron micrograph of microsporidian organisms showing mature spore with electron-dense outer layer of spore wall. Note coils of polar filament (P) in sporoplasm; ×60,000.

diplokaryon arrangement throughout most stages of the life cycle. They develop diffusely in the host cytoplasm. In contrast, *Encephalitozoon* has nuclei which are unpaired throughout most stages of development and develop free within a vacuole of the host cell. Our electron-microscope studies did not permit detailed evaluation of these characteristics and subsequent generic determination. In many cases the host-cell membranes were disrupted, artifacts of freezing or fixation.

Some taxonomists (7) recognize the genus *Glugea* in the Nosematidae. *Glugea,* a parasite of fish, can be excluded from consideration in this case since it produces a massive cellular hypertrophy resembling a tumor. *Perezia,* another organism formerly treated as a valid genus, is considered by some to be a junior synonym for *Nosema* (7).

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ACKNOWLEDGMENTS

The authors thank Drs. Ann Cali and Victor Sprague for consultations, and Ms. Vivian Shinski, Mr. Joe Phelan, Mr. Michael Tinsley, Ms. Karen Bunting, and Ms. Ellen Lauer for technical assistance.