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SYSTEMIC MICROSPORIDIOSIS IN INLAND BEARDED DRAGONS (*POGONA VITTICEPS*)

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Abstract: One laboratory-hatched and -reared inland bearded dragon (*Pogona vitticeps*) (No. 1) and two privately owned inland bearded dragons (Nos. 2 and 3) died, showing nonspecific signs of illness. Light microscopic examination of hematoxylin and eosin-stained tissue sections from lizard No. 1 revealed severe hepatic necrosis with clusters of light basophilic intracytoplasmic microorganisms packing and distending hepatocytes and free in areas of necrosis. Similar microorganisms were within cytoplasmic vacuoles in distended renal epithelial cells, pulmonary epithelial cells, gastric mucosal epithelial cells, enterocytes, and capillary endothelial cells and ventricular ependymal cells in the brain. In lizard Nos. 2 and 3, microorganisms of similar appearance were in macrophages in granulomatous inflammation in the colon, adrenal glands, and ovaries. The microorganism was gram positive and acid fast and had a small polar granule that stained using the periodic acid–Schiff reaction. Electron microscopic examination of deparaffinized liver of lizard No. 1 revealed merogonic and sporogonic stages of a protozoan compatible with members of the phylum Microspora. This report provides the first description of microsporidiosis in bearded dragons and is only the second report of this infection in a lizard.

Key words: Microsporidia, lizard, bearded dragon, *Pogona vitticeps.*

INTRODUCTION

The phylum Microspora consists of obligate intracellular protozoans, microsporidia, that have an unusual life cycle. Infection begins with injection of sporoplasm into the host cell followed by a proliferating merogonic phase.[25] Eventually a sporogonic phase begins in which meronts of simple structure transform into sporonts of relatively complex structure. The morphology, internal and external, of both stages is used to distinguish microsporidia. The first microsporidian described was *Nosema bombycis,* a parasite of the silkworm (*Bombyx mori*).[13] Over 100 genera and almost 1,000 species have been reported in a variety of invertebrates and in all classes of vertebrates.[27]

Several microsporidia may infect amphibians.[2,8,18,19,28] In reptiles, *Pleistophora* sp. was reported in the tuatara (*Sphenodon punctatus*) in a zoological collection,[15] *Encephalitozoon lacerate* was reported in the common wall lizard (*Podarcis muralis*) in France,[1] *Pleistophora* (*Glugea*) *danilewskyi* was found in the European grass snake (*Natrix natrix*) in Italy,[7,9] and *Pleistophora atretii* was found in the split keelback snake (*Atretium schistosum*) in India.[17] In mammals, microsporidiosis has been reported most commonly in rabbits, with *Encephalitozoon cuniculi* the causative agent.[21] At least five genera of microsporidia have been identified as possible opportunistic pathogens in humans infected with human immunodeficiency virus.[20]

Bearded dragons (*Pogona*) have become popular in the reptile pet trade. Bearded dragons are native to Australia, and of the five species, the inland bearded dragon (*P. vitticeps*) is the most commonly sold bearded dragon in the United States. Over the last few years several infectious diseases have been reported in captive bearded dragons, including adenoviral enteritis and hepatic necrosis[5,14] and coccidiosis of the intestinal tract caused by *Isospora amphiboluri.*[16] In this report, we describe systemic microsporidiosis as another health problem of the inland bearded dragon.

CASE REPORTS

Four fertile inland bearded dragon eggs received from a zoological collection/breeding colony were incubated at 30°C and allowed to hatch. Following hatching, the neonates were maintained in an aquarium, with heat provided by an overhead incandescent bulb and reflector directed to a basking site of rocks, and a sand substrate. Lizards were fed commercially purchased domestic crickets (*Acheta domestica*) that were coated with a mineral/vitamin supplement (Reptocal, Tetra, Terafuana, Morris Plains, New Jersey 07950, USA). Water was available at all times. At approximately 3 mo of age, one of the lizards became anorexic and listless and

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died several days later. At the time of death, the lizard (No. 1) was thin and weighed approximately 3 g. No gross lesions were noted at necropsy.

Bearded dragon Nos. 2 and 3 were privately owned and fed a diet consisting of 50% domestic crickets and giant mealworms (*Zoophobus morio*) and 50% assorted vegetables. Lizard No. 2 was a 3-mo-old female that collapsed unexpectedly and died within 24 hr. The lizard was captive hatched and reared by the owner. The lizard behaved normally, grew, and was the largest of clutch cohorts at the time of death. Other lizards in the household included green iguanas (*Iguana iguana*) and New Guinea frilled dragons (*Chlamydosaurus kingii*). No gross lesions were noted at necropsy.

Lizard No. 3. was a 19-mo-old, 362-g adult male that had been thin and unthrifty in appearance for many months. The lizard was purchased as a hatchling and was from the same household as lizard No. 2. The lizard had a positive fecal culture for *Salmonella arizonae* 48:R-Z, and coccidial oocysts also were detected in the feces. The lizard was administered enrofloxacin (Baytril, 10 mg/kg p.o. s.i.d. for 14 days; Bayer B.V., Mijdrecht, Belgium), amoxicillin (5 mg/kg p.o. s.i.d. for 14 days; SmithKline Beecham Animal Health, West Chester, Pennsylvania 19380, USA), sulfadimethoxine (Albon, 75 mg/kg p.o. s.i.d. for 14 days; SmithKline Beecham), and metronidazole (Flagyl, 100 mg/kg p.o. s.i.d. for 14 days; Sidmak Labs, East Hanover, New Jersey 07936, USA). However, the lizard failed to improve, and euthanasia was elected when it developed hemorrhagic diarrhea. No gross lesions were noted when the attending veterinarian performed a necrospy.

Multiple tissues were collected from all three lizards, fixed in neutral buffered 10% formalin, and processed routinely for light microscopy. Paraffin-embedded tissue was sectioned at either 6 or 7 μm and treated with hematoxylin and eosin (H&E), modified Twort Gram stain[4] or Brown and Brenn Gram stain, and Ziehl–Neelsen acid-fast stain and by the periodic acid–Schiff (PAS) reaction. The heart, lung, trachea, liver, gall bladder, spleen, esophagus, stomach, duodenum, pancreas, mid and distal small intestine, colon, adrenal glands, ovaries, testes, and skeletal muscle were examined. Brain and kidneys were evaluated only in lizard No. 1.

For transmission electron microscopy, the liver of lizard No. 1 was removed from the paraffin block, deparaffinized in xylene, hydrated, postfixed in 1% (w/v) osmium tetroxide, and washed in 0.1 M cacodylate buffer (pH 7.2). Next, the liver was washed in distilled water, dehydrated in graded solutions of ethanol through 100% acetone, and infiltrated and embedded in Embed 812 (EMS, Fort Washington, Pennsylvania 19034, USA). One-micrometer sections were cut on an ultramicrotome and stained with 0.1% toluidine blue. Ultrathin sections (70–80 nm) were cut, placed on a copper grid, stained with uranyl acetate and Reynold's lead citrate, and viewed on a Hitachi H-7000 electron microscope.

Approximately 50 domestic crickets were purchased from the supplier that provided crickets used to feed lizard No. 1. Representative samples of feces/urine from the bearded dragon colony from which lizard No. 1 was derived were collected for four sequential days on two separate occasions, placed in a glass container to which an equal volume of water was added or in plastic bags, refrigerated or frozen, and screened for micosporidian spores. Crickets and feces/urine were homogenized separately in water using a glass tissue grinder. Samples were then filtered through cotton packed to a depth of 2–5 mm in the bottom of a glass syringe. The homogenate was poured into the syringe and the plunger was used to force as much of the homogenate as possible through the cotton. A density gradient mixer and a previously described methodology were used in an attempt to extract spores from the filtrate.[22] The colloidal silica Ludox® HS40 (Aldrich Chemicals, Milwaukee, Wisconsisn 53233, USA) was used in the gradient extraction process, and ammonium chloride (0.01–0.05 M) was added to each chamber of the gradient mixer to reduce the risk of spores germinating in the gradient. A continuous gradient, with a density of 1.303 g/ml at the bottom grading to 1.000 at the top, was produced. The filtrate was layered on top of the gradient, and the gradient was then centrifuged at about 16,000 × *g* for 30 minutes. The centrifuge was allowed to decelerate slowly, preventing mixing of the upper region of the gradient. A Pasteur pipette was passed into the gradient, about ¾ of the way from the original top of the gradient to the bottom. All of the liquid in the density region expected to contain microsporidian spores[23] was withdrawn. Ludox was removed from the gradient, diluted with water so that potential spores would not remain suspended in it, and then centrifuged. The water–Ludox mixture was decanted, resuspending the residue in a minimal volume of water. The resuspended residue was examined with a phase contrast microscope.

Light microscopy of H&E-stained liver sections of lizard No. 1 revealed severe, multifocal to diffuse hepatic necrosis, with minimal infiltrates of macrophages and small mononuclear cells. Clusters

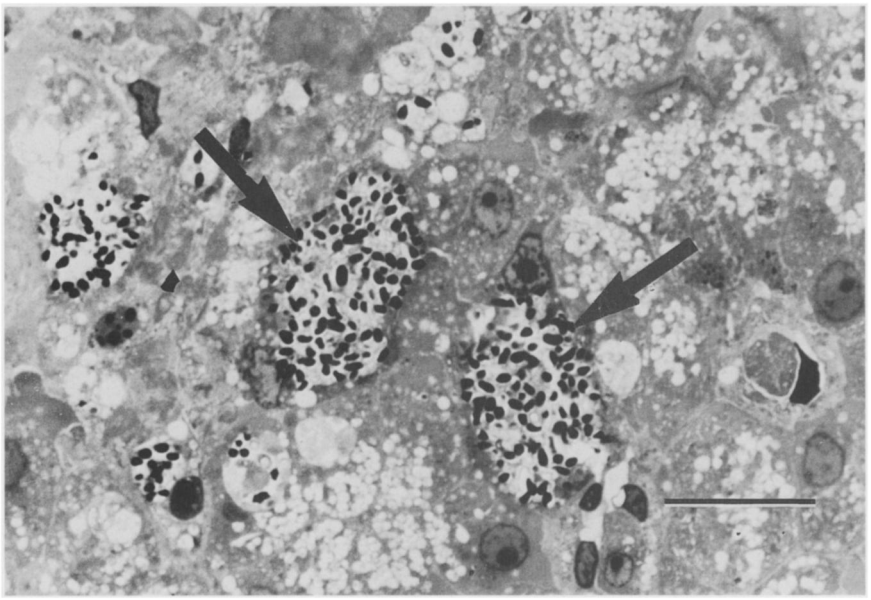

Figure 1. Liver of bearded dragon No. 1. Intracytoplasmic microorganisms (arrows) pack and distend the cytoplasm of hepatocytes. Toluidine blue. Bar = 5 μm.

of small, lightly basophilic rod-shaped microorganisms filled and distended the cytoplasm of hepatocytes. Microorganisms were also seen scattered throughout areas of necrosis. In toluidine blue-stained sections, the organisms were oval and stained dark blue (Fig. 1). Twelve spores were 1.8–2.2 μm in length and 1.3–1.8 μm in diameter. In the kidney, numerous renal epithelial cells had a vacuolated cytoplasm that was filled and distended by similar microorganisms. Using a modified Twort Gram stain,[4] organisms were gram positive, making them easy to localize (Fig. 2). Organisms contained a small PAS-positive granule and were acid fast. Organisms similar in appearance and staining char-

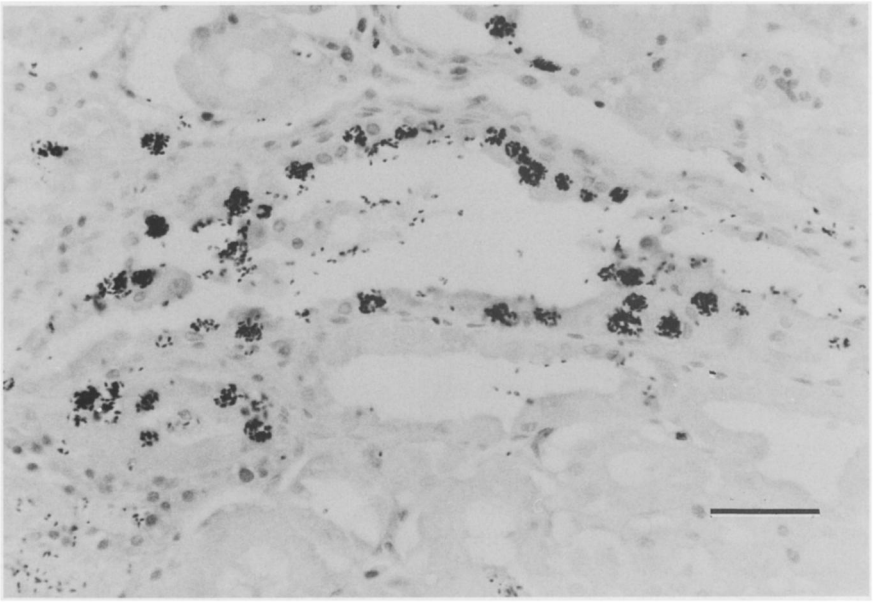

Figure 2. Kidney of bearded dragon No. 1. Numerous microorganisms can be seen within the cytoplasm of renal epithelial cells. Twort Gram stain. Bar = 50 μm.

Figure 3. Colon of bearded dragon No. 3. The submucosa (SU) is diffusely infiltrated by macrophages. Fewer macrophages are present in the lamina propria, tunica muscularis, and serosa. Brown and Brenn Gram stain. Bar = 500 μm.

acteristics were seen within pulmonary epithelial cells in the lung, gastric mucosal epithelial cells, enterocytes, capillary endothelial cells, and ventricular ependymal cells in the brain. In the stomach, clusters of microorganisms were scattered in the lumen among partially digested crickets.

Light microscopic evaluation of tissue from lizard Nos. 2 and 3 revealed granulomatous inflammation in the adrenal glands, ovaries (lizard No. 2), and wall of the colon. Inflammation in the adrenals and ovaries was so extensive that few normal cortical cells or follicles remained. In the colon, the inflammation was multifocal, occasionally nodular, and transmural (Fig. 3). Macrophages, epithelioid macrophages, fibroblasts, lymphocytes, plasmacytes, granulocytes, and small multinucleated giant cells were evident in decreasing frequency. Gram-positive, acid-fast microorganisms occasionally were seen within the distended cytoplasm of macrophages. Twelve spores were 1.8–2.9 μm in length and 0.8–1.5 μm in width. Many had a minute PAS-positive granule. Microorganisms also were found within colonic mucosal epithelial cells. No hypertrophied host cells were found and microorganisms were not detected in the lungs, liver, spleen, heart, and tests (lizard No. 3).

Several concurrent infectious diseases were evident histologically in lizard Nos. 2 and 3. Lizard No. 2 had enterocolic coccidiosis, and lizard Nos. 2 and 3 had intranuclear inclusion body enterocolitis. Lizard No. 3 also had multifocal pyogranulomatous and caseous pneumonia, intranuclear inclusion body pneumonitis, and chronic hepatobiliary disease of undetermined etiology.

Electron microscopy revealed clusters of microorganisms in liver sections of lizard No. 1 (Fig. 4).

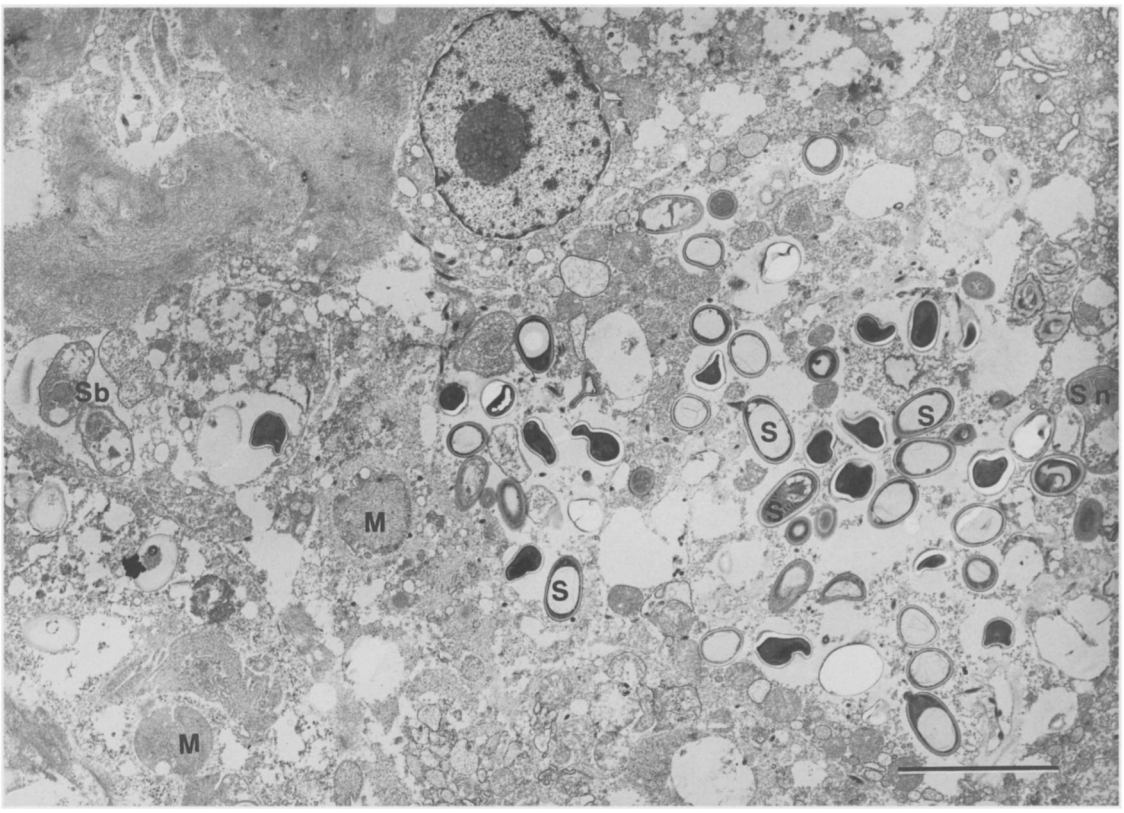

Figure 4. Transmission electron micrograph. Liver of a bearded dragon. Presporogonic and sporogonic stages of development of a microsporidium can be seen. M = meront; Sb = sporoblast; Sn = sporont; S = spore. Lead citrate and uranyl acetate. Bar = 5 μm.

Merogonic and sporogonic developmental stages were recognized. Developmental stages were free in the cytoplasm and were not surrounded by a membrane. The morphology of these developmental stages was consistent with that of the phylum Microspora.[25]

Presporulation stages (meronts), approximately 3 μm in diameter, had a diffusely granular, lightly radiodense cytoplasm with a more dense single nucleus (Fig. 4). Some presporulation stages were binucleate during division into uninucleate forms. The outer cytoplasmic membrane was smooth.

The sporulation stage consisted of sporonts, sporoblasts, primary spores, and secondary spores (Fig. 4). Sporoblasts had a granular deposit on their surface, which condensed during spore formation into a thin electron-dense layer forming the exospore. Primary spores were oval, had broadly oval poles, and had a thin exospore and a thicker endospore (Fig. 5). The anchoring disc of the filament was constricted, elongated, and bulbous and covered the end of the filament. A polar sac did not cover the polaroplast. The filament was isofilar, short, approximately 0.1 μm in diameter, and coiled in six turns (Fig. 5). A single nucleus was located in the center of the spore. In the posterior end of the spore, tubular coils of the Golgi system were seen. Many primary spores had germinated, as seen by an absence of contents (Fig. 6). In germinated spores, the everted polar tube was anchored subapically, at an angle to the longitudinal axis of the spore (Fig. 6). Twelve spores were 2.0–2.5 μm × 1.0–1.1 μm.

Secondary spores had a thin exospore and an endospore that was thicker than that of primary spores (Fig. 7). The polar filament was coiled in six turns. Because of a radiodense interior, no further details were discernible.

Microsporidian spores were not identified in crickets or feces/urine of the bearded dragon colony from which lizard No. 1 was derived.

DISCUSSION

When first seen, the microorganisms in the bearded dragons were thought to be bacteria. They were small (1.8–2.9 μm × 0.8–1.8 μm), lightly ba-

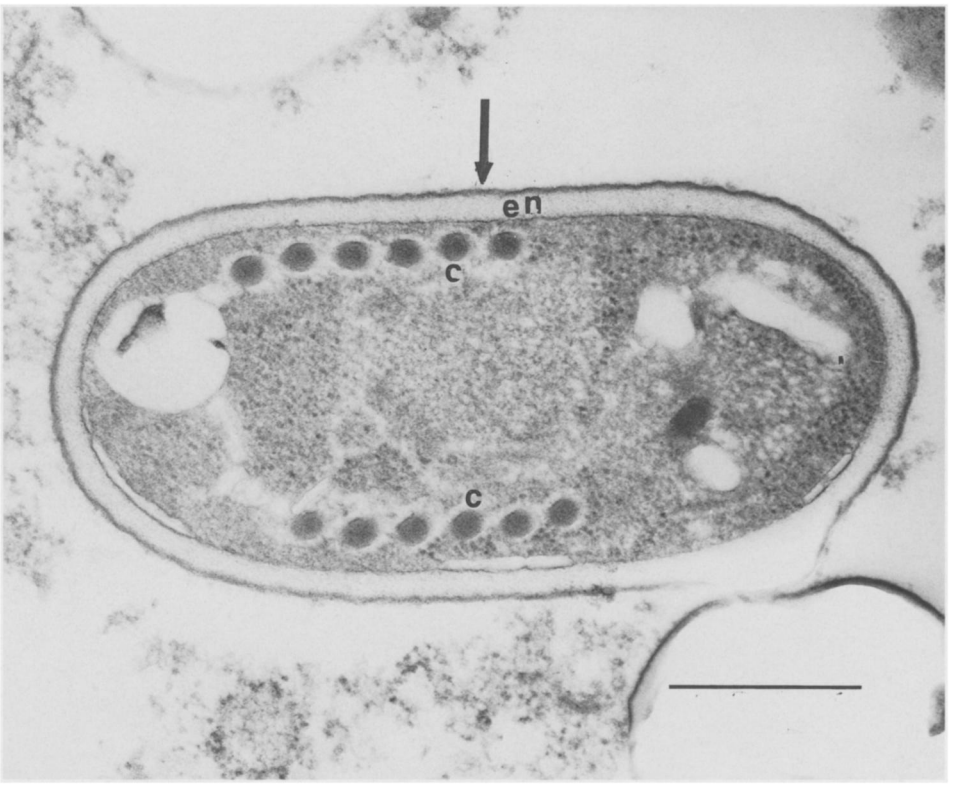

Figure 5. Transmission electron micrograph. Primary microsporidian spore. The wall is thin, consisting of an exospore (arrow) and endospore (en). Cross sections of the polar filament are seen as six pairs of coils (c). Lead citrate and uranyl acetate. Bar = 0.5 μm.

sophilic in H&E–stained tissue sections, commonly intracytoplasmic, and arranged in clusters. In the liver of one 3-mo-old lizard, the organisms were associated with areas of necrosis, where they appeared to be both intracellular and extracellular. The organisms also were seen within vacuolated, distended renal epithelial cells. In the second juvenile lizard and the adult lizard they were within centers of granulomatous inflammation in the colon, adrenal glands, and ovaries. A PAS-positive polar granule was identified, which is characteristic of micropsoridia.[6] Additional microsporidial staining features, such as gram positivity and acid-fast positivity also were demonstrated. The Gram and acid-fast staining allowed rapid localization of microorganisms throughout infected tissues. Many more organisms could be seen using these staining methods than by H&E staining alone.

By electron microscopy, the presence of spores with coils of the polar filament and a polar tube supported the identification as a microsporidian.[24] In the liver of lizard No. 1, primary and secondary spores were seen. The primary spores probably were responsible for autoinfection, as indicated by germination of many spores in the liver; the interior of many spores was electron lucent, indicating release of sporoplasm. The thick-walled secondary spores would be adapted for survival outside the host.

In previous reports of microsporidiosis in reptiles, there were limited lesions such as skeletal muscle necrosis or formation of parasitophorous vacuoles in infected enterocytes.[1,15] In the bearded dragons, as with microsporidiosis in mammals,[20] lesions varied between lizards. Tissues of lizard Nos. 2 and 3 were not examined by electron microscopy; these lizards may have been infected with a different microsporidian. Thus, differences in lesions between the cases may have resulted from infection with different organisms.

In lizard No. 1, the major changes included severe hepatic necrosis and renal epithelial cells vacuolated and distended with organisms. In *Encephalitozoon cuniculi* infection in rabbits, similar renal changes have been seen.[21] With lysis of renal epithelial cells, spores are released into the lumen and exit from the host through the urine. Spores also were seen in the mucosa of gastrointestinal tract

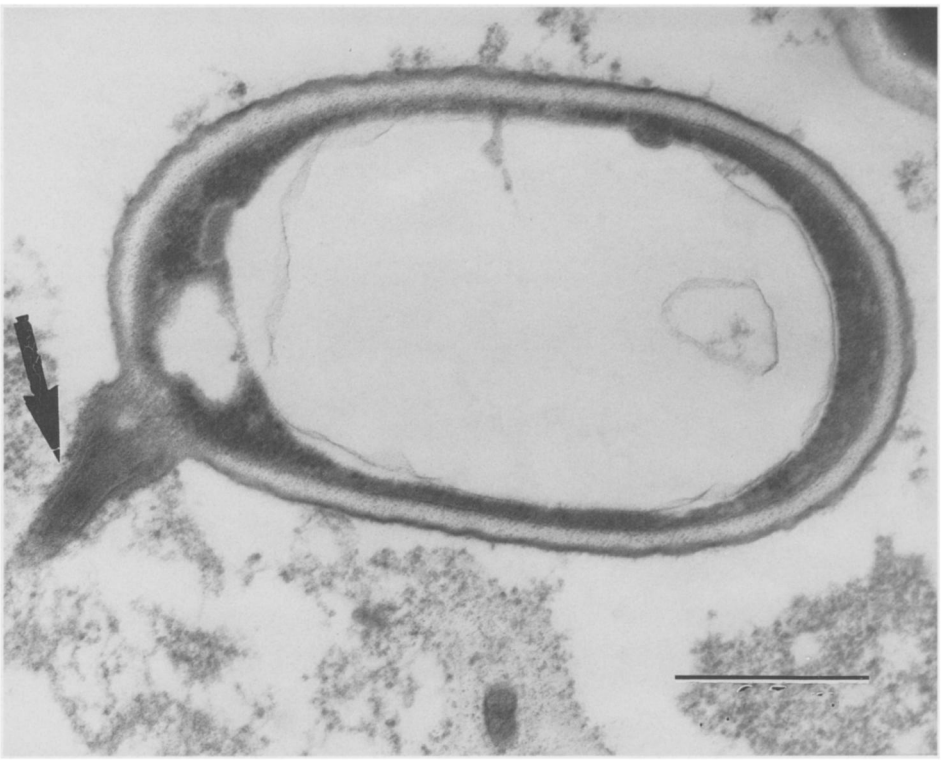

Figure 6. Transmission electron micrograph. Germinated primary microsporidian spore. There is an absence of contents, and the polar tube (arrow) is everted, subapical, and at an angle to the longitudinal axis of the spore. Lead citrate and uranyl acetate. Bar = 0.5 μm.

and free within the lumen, where they were mixed with digested crickets. Consequently, feces are probably another route of elimination from the host and contamination of the environment. Thus, feces and urine could serve as a mechanism for horizontal transmission. Microsporidian spores may persist up to 1 yr outside the host, especially in an aquatic environment.[29]

In the other juvenile and adult bearded dragons, spores were seen within regions of granulomatous inflammation in the colon, adrenal glands, and ovaries. Xenomas, representing hypertrophied cells associated with some microsporidian infections, as with *Alloglugea bufonis* in the marine toad[18] and *Glugea anomala* in fish,[30] were not seen.

In two lizards, basophilic intranuclear inclusions in enterocytes were seen with H&E staining. The inclusions were consistent with adenoviral inclusions previously described in the bearded dragon.[14] Combined adenovirus and microsporidial infection of duodenal enterocytes has been reported in a human patient with AIDS.[26]

The infection in the laboratory-hatched and -reared bearded dragon could have resulted from infection of the lizard through its only dietary item (crickets), transovarian and/or transuterine infection of the egg prior to shell formation, or contamination of the surface of the egg at oviposition from spores in the cloaca or contamination from spores in the environment where the eggs were deposited. Neonates hatching from contaminated eggs could ingest spores at the time of hatching. Transovarial transmission has been reported for *Thelohania,* a microsporidium of the mosquitoes *Anopheles crucians* and *A. quadrimaculatus,*[10] and there are reports of transplacental transmission for *E. cuniculi* in caesarian-delivered rabbits[11] and mice.[12]

An invertebrate–vertebrate life cycle has not been reported for any microsporidia.[3] Crickets were examined for spores and were negative. However, only one batch of crickets was examined, and an insect/lizard life cycle cannot be completely ruled out.

Feces/urine collected from the lizard colony producing the eggs that gave rise to the laboratory-reared lizard in this report also were negative for spores. If only small numbers of spores were shed by the bearded dragon colony at the two times of collection, they could have been missed. Demonstrating transovarian/transuterine transmission will

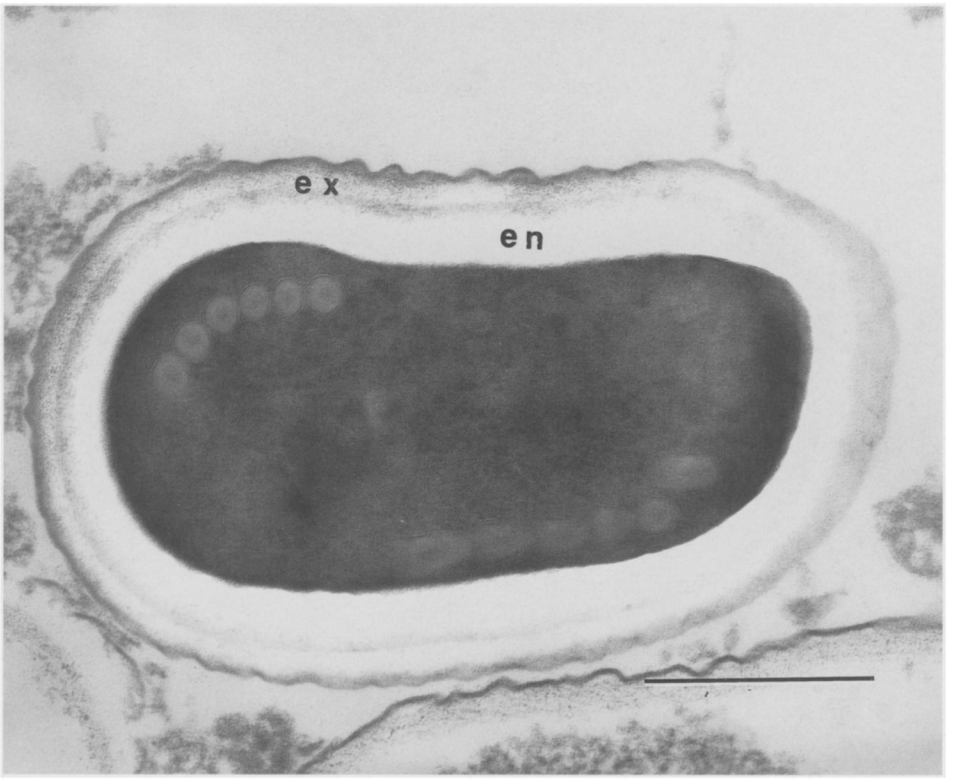

Figure 7. Transmission electron micrograph. Secondary microsporidian spore. The wall is thick, consisting of a exospore (ex) and endospore (en). Cross sections of the polar filament are seen as six pairs of coils. Lead citrate and uranyl acetate. Bar = 0.5 μm.

require collecting additional fertile eggs from an infected colony and examining embryos for spores. Elimination of this parasite from a colony will be difficult because currently there is no simple method for identifying carriers.

With the available material, the microsporidium could not be specifically identified. Presporulation (merogonic) stages observed by electron microscopy were poorly preserved, and the morphology of spores, such as the subapical extruded polar tube, was not consistent with characteristics of any other known microsporidia.

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