

# Avian Microsporidiosis

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A variety of parasites from the phylum Microspora have been increasingly recognized as opportunistic infections in immunocompromised humans. One such species, *Encephalitozoon hellem* was first described in 1991 based on immunologic and biochemical characteristics of 3 isolates from AIDS patients.<sup>1</sup> Subsequent molecular analysis of the small subunit ribosomal RNA gene verified that *Enc. hellem* was a new species that was closely related to *Enc. cuniculi*, commonly found in rabbit and rodent hosts, and *Enc. (Septata) intestinalis* found in humans.<sup>2</sup> *Enc. hellem* has been identified as a cause of ocular, respiratory and systemic disease in AIDS patients.<sup>3-6</sup>

In contrast to human data, only a few cases of natural microsporidial infections in birds are reported in scientific literature. These reports describe single animal infections or isolated flock outbreaks, and all reported cases so far are in psittacine birds. Peach-faced lovebirds (*Agapornis roseicollis*) are the most frequently reported hosts<sup>7-10</sup> followed by masked lovebirds (*Agapornis personata*).<sup>11-13</sup> Microsporidiosis has also been reported in a mixed flock of black-masked and Fischer's lovebirds (*Agapornis fischeri*),<sup>13</sup> a flock of budgerigars (*Melopsittacus undulatus*),<sup>14</sup> a single case in a double yellow-headed Amazon parrot (*Amazona ochrocephala*)<sup>15</sup> and in 2 unrelated eclectus parrots (*Eclectus roratus*).<sup>16</sup>

In the following report, we describe our ongoing efforts to determine, to the species level, the microsporidia that infect birds, the host range of avian microsporidia, and the prevalence of infection of microsporidia in pet birds.

## PREVALENCE AND HOST RANGE

Using convenience sampling, cloacal swabs and/or samples of avian droppings were collected from a total of 208 birds of 8 species from 7 sources. Avian species surveyed focused on peach-faced, masked and Fischer's lovebirds, but also included several African Grey parrots and 4 Cockatoo species. Smears from cloacal swabs or droppings were screened for the presence of spores using a Calcofluor staining method commonly used in clinical screening for human microsporidial infections in AIDS patients.

The overall prevalence of spores in these birds was approximately 25% with peach-faced and masked lovebirds showing the highest prevalence in species with large sample sizes. Prevalence varied depending on the source of bird and the species of bird, but parasites were identified in at least 1 or more birds from each source. To date, all microsporidial infections reported in the scientific literature have been in psittacine birds. However, recently at the California Veterinary Diagnostic Laboratory in San Bernadino, CA., a veterinary pathologist (Dr. Barbara Daft) diagnosed intestinal microsporidiosis in multiple hummingbirds from a wildlife rehabilitation center (unpublished data). Samples from these birds have been obtained. From these samples an isolate has been established in culture and a partial sequence of this organism has been obtained. Additionally, in a limited survey of wild-caught hummingbirds, screening droppings for spores, a near 30% prevalence infection was found (personnal communication with B. Daft).

## SPECIES IDENTIFICATION

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Using PCR amplification and automated sequencing, the partial sequence of the 5' end of the *ssrRNA* gene has been determined for microsporidia infecting two eclectus parrots, a lovebird, and a hummingbird. The avian isolates were shown to have > 99% homology with published sequence from human isolates of *Encephalitozoon hellem*.

### PBFDV AND MICROSPORIDIA

Viral immunosuppression may play a role in the prevalence and quantity of *Enc. hellem* shedding and the onset of clinical disease in parrots. The psittacine beak and feather disease virus (PBFDV) frequently infects African grey and eclectus parrots, cockatoos, and budgerigars.<sup>17</sup> It is an especially common viral infection of lovebirds.<sup>18</sup> Circumstantial evidence suggests that this virus is immunosuppressive, as birds with PBFDV infections commonly die from opportunistic infectious agents including *Candida albicans*,<sup>17</sup> members of the *Enterobacteriaceae*,<sup>17</sup> the avian polyomavirus,<sup>19</sup> and *Cryptosporidia* sp.<sup>17</sup> Using a commercial polymerase chain reaction based assay for PBFDV (Research Associates Laboratory Inc. Milford, OH) two eclectus parrots with *Enc. hellem* infection and disease were both found to be co-infected with PBFDV.<sup>16</sup> Using the same assay PBFDV-positive lovebirds were found to have a 3 times greater prevalence of spore shedding as compared to PBFDV-negative lovebirds. The number of spores found per fecal smear was also significantly higher in PBFDV-positive birds (Snowden et al, unpublished data). These data suggest that PBFDV in birds, like HIV in humans, alters the immune system and either makes infection more likely or interferes with the bird's ability to control the parasites replication or both.

### CONCLUSION

While this investigation is ongoing, our preliminary data suggests that microsporidia have a broad avian host range that may include many species of cage birds as well as wild birds. In the past, microsporidia have only been recognized when they caused disease and have thus been thought of primarily as a pathogen. Our data, however, suggests that they may be normal flora in many species of birds and only be opportunistic pathogens. Our data also suggests that the psittacine beak and feather virus may influence the prevalence of microsporidial infection and shedding and may allow microsporidia to cause disease in a host that would otherwise would be resistant to disease.

Prior to our discoveries, the definitive hosts of *Enc. hellem* were not known. Our data, while still preliminary, suggests that spores shed from one or more bird species may be the source *Enc. hellem* infections for humans.

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