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Identification of Potentially Human-Pathogenic *Enterocytozoon bieneusi* Genotypes in Various Birds[▽]

Maria Luísa Lobo,[1] Lihua Xiao,[2] Vitaliano Cama,[2] Nuno Magalhães,[1] Francisco Antunes,[3] and Olga Matos[1]*

Instituto de Higiene e Medicina Tropical/Unidade de Protozoários Oportunistas/VIH e outras Protozooses/UPMM, Rua da Junqueira 96, 1349-008 Lisboa,[1] and Clinica Universitária de Doenças Infecciosas, Faculdade de Medicina, Hospital de Santa Maria, Universidade de Lisboa, Ava Professor Egas Moniz, 1649-035 Lisboa,[3] Portugal, and Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia[2]

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***Enterocytozoon bieneusi* was detected in 24 of 83 samples from birds of the orders Columbiformes, Passeriformes, and Psittaciformes. It was identical to or closely related to the Peru6 genotype, which was previously found in humans in Peru. Thus, various birds can be a significant source of environmental contamination by potentially human-pathogenic *E. bieneusi*.**

The four most common human microsporidian species, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Encephalitozoon cuniculi*, have been reported in a wide range of domestic and wild animals (3, 4, 7, 9, 11, 13, 14, 18). Thus, it has been debated for some time now whether animals, namely birds, could be a source of microsporidiosis for humans (9, 15). This is especially true for *E. bieneusi*, the most common microsporidian parasite in humans.

Thus far, *E. bieneusi* has been reported in 2 of 8 symptomatic chickens examined in Germany and 17 of 124 healthy pigeons examined in Spain (5, 12). The zoonotic potential of *E. bieneusi* from birds is not clear. Only four *E. bieneusi* samples of bird origin have been genotyped. The two samples from chickens in Germany had *E. bieneusi* genotype J, one of the several host-adapted genotypes found in cattle (12). Two sequences from pigeons in Spain also produced two *E. bieneusi* genotypes different from any genotypes described so far (5). However, the knowledge of the broad host range of these species does not by itself present direct evidence that any of these hosts function as a reservoir for human infection. The finding by molecular tools of previously unrecognized intraspecific genetic differences has improved our understanding of the epidemiology and zoonotic transmission of these microorganisms (9).

This study intended to examine the occurrence of *E. bieneusi* in several bird species in close contact with humans (pet birds and pigeons from public parks) and to characterize the parasites found at the genotype level.

Bird specimens. A total of 83 birds in the orders Psittaciformes, Passeriformes, and Columbiformes, including 39 caged pet birds and 44 pigeons, were surveyed for possible infection with microsporidia. The Psittaciformes studied included an *Agapornis* sp. ($n = 1$), *Agapornis fischeri* ($n = 5$), *Agapornis personatus* ($n = 3$), *Agapornis roseicollis* ($n = 4$), *Amazona aestiva* ($n = 1$), *Forpus coelestis* ($n = 1$), *Forpus conspicillatus* ($n = 1$), *Melopsittacus undulatus* ($n = 4$), *Nymphicus hollandicus* ($n = 3$), *Platycercus eximius* ($n = 1$), and *Psittacus erithacus* ($n = 8$). The Passeriformes included *Bathilda ruficauda* ($n = 1$), *Erythrura gouldiae* ($n = 1$), *Leiothrix lutea* ($n = 1$), *Lonchura domestica* ($n = 2$), *Padda oryzivora* ($n = 1$), and *Serinus canaria* ($n = 1$). They were mostly from an avian breeder (31 birds), and several pet owners (8 birds) in Lisbon, Portugal. Fecal droppings were collected from these birds. The pigeons (*Columba livia*, order Columbiformes) studied were captured in two public parks in Lisbon by personnel from the Lisbon Health Department. These birds were submitted for necropsy, when the intestinal contents were collected for this study.

***E. bieneusi* genotyping.** DNA was extracted from feces or intestinal contents using the Mini-BeadBeater/silica method or the FastDNA SPIN kit for soil (6, 10, 11). A nested-PCR protocol was used to amplify a fragment consisting of the partial small subunit and large subunit and the entire internal transcribed spacer (ITS) region of the rRNA gene of *E. bieneusi* (18). For primary PCR, a fragment of 410 bp was amplified. For secondary PCR, a fragment of 392 bp was amplified from 2.5 μl of the primary PCR product. PCR products were analyzed by 2% agarose gel electrophoresis and ethidium bromide staining. The secondary PCR products of the expected size were sequenced in both directions on a CEQ 2000XL instrument (Beckman Coulter Inc., Fullerton, CA.) or an ABI3100 automated sequencer (Applied Biosystems, Foster City, CA.). The accuracy of the nucleotide sequence was confirmed by sequencing two separate PCR products from the same specimen. The sequences obtained were analyzed together with the reference sequences from the GenBank database using the BLASTN (www.ncbi.nlm.nih.gov) and ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/) programs.

Occurrence of *E. bieneusi* in birds. ITS PCR products of the expected size (~392 bp) were obtained from 24 (19 pigeons, 2 African gray parrots, 1 cockatiel, 1 Fischer's lovebird, and 1 Star finch) of the 83 birds examined (Table 1). Thus, *E. bieneusi* was identified in specimens from all three orders of birds studied. However, pigeons had a significantly higher frequency (43.2% versus 12.8%) of microsporidian infection than pet

* Corresponding author. Mailing address: Unidade de Protozoários Oportunistas/VIH e outras Protozooses, Instituto de Higiene e Medicina Tropical, Rua da Junqueira 96, 1349-008 Lisboa, Portugal. Phone: (351) 21-363-2600. Fax: (351) 21-363-2105. E-mail: omatos@ihmt.unl.pt.

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TABLE 1. *Enterocytozoon bieneusi* genotypes identified in specimens from different birds

Order	Common name/scientific name	Specimen	*E. bieneusi* prevalence	*E. bieneusi* genotype[a]
Psittaciformes	Lovebird/*Agapornis* sp.	Droppings	1/13	Peru6[b]
	Cockatiel/*Nymphicus hollandicus*	Droppings	1/3	ND
	African gray parrot/*Psittacus erithacus*	Droppings	1/7	Peru6-var[c]
			1/7	ND
	Others	Droppings	0/8	
Passeriformes	Star finch/*Bathilda ruficauda*	Droppings	1/1	ND
	Others	Droppings	0/6	
Columbiformes	Pigeon/*Columba livia*	Intestinal contents	16/44	Peru6
			1/44	Peru6-var
			2/44	Peru6 and Peru6-var mixed infections

[a] ND, *E. bieneusi* genotype not determined.
[b] *E. bieneusi* genotype previously reported as Peru6 (AY371281).
[c] Peru6-var, *E. bieneusi* Peru6 genotype variant with one nucleotide change (G to A) near the 3′ end of the PCR fragment.

birds ($\chi^2 = 9.27$; $P = 0.0023$). None of the infected birds displayed clinical signs, except for one parrot that had decreased appetite and weight loss 1 week before death.

Genotypes of *E. bieneusi* in birds. DNA sequencing of PCR products was successful for specimens from 21 birds. All sequences obtained belonged to *E. bieneusi*. Sequence analysis revealed that the 16 pigeons and 1 lovebird were infected with an *E. bieneusi* genotype previously reported for AIDS patients as the Peru6 genotype (17). One pigeon and one parrot had an *E. bieneusi* genotype very similar to Peru6, but with one nucleotide change (G to A) near the 3′ end of the PCR fragment. Two pigeons had a mixed genotype consisting of Peru6 and the Peru6 variant, as judged by the electropherograms of DNA sequencing and repeated PCR analyses.

Public health significance. In this study, *E. bieneusi* was identified by PCR in the specimens of 28.9% of the birds sampled. Most infected birds were apparently healthy and might serve as asymptomatic carriers of microsporidian species, as already suggested by others for *Encephalitozoon hellem* (15). Previous reports of clinical microsporidiosis in birds involved mostly young animals and those coinfected with other pathogens (viruses and bacteria) (2). Thus, microsporidia in avian hosts could be largely opportunistic pathogens, as seen in humans.

Previously, the majority of cases of microsporidia in birds were documented for species in the order Psittaciformes and involved almost exclusively *Encephalitozoon hellem* (15). *Enterocytozoon bieneusi* was observed only in two chickens (order Galliformes) and 17 pigeons (order Columbiformes) (5, 12).

Airborne transmission, previously proposed for *E. hellem* (2), also might occur for *E. bieneusi*, since this species has been found in the lungs of AIDS patients (8, 16). As shown in this study, microsporidian spores are commonly shed in bird excrement. Because bird droppings dry quickly and produce dust, inhalation of dust containing viable spores into the respiratory tract, as happens for *Histoplasma capsulatum* and *Chlamydia psittaci* (2), might initiate an infection, especially for immunocompromised persons.

A high intraspecific variability in *E. bieneusi* has been described previously based on sequence differences of the ITS of the rRNA gene. More than 50 genotypes of *Enterocytozoon* spp. have been reported, with many distinct and probably host-adapted genotypes, which probably have no significant public health importance, associated with specific groups of animals (20). However, a large group of closely related *E. bieneusi* genotypes have no strict host specificity and are frequently found in both humans and animals (18). In the present study, the genotype found in bird specimens had ITS sequences identical or similar to those of an *E. bieneusi* genotype previously reported for Peruvian AIDS patients, the Peru6 genotype (17). To our knowledge, this is the first time that an *E. bieneusi* ITS genotype identical to one found in humans has been found in a variety of birds (18 pigeons and 1 lovebird). Unlike previous studies conducted in mammals, which had shown that humans and animals in a geographic area are usually infected with multiple *E. bieneusi* genotypes (1, 17, 18, 19), birds in this study were infected with only two closely related genotypes. This could be the result of the highly mobile nature of birds, which may select more-transmissible genotypes.

The finding in birds of an ITS genotype identical to one found in humans suggests a high zoonotic potential of avian *E. bieneusi*. This finding provides further support for the suggestion of a lack of transmission barriers in *E. bieneusi* between different animal species or even between different taxonomic classes of hosts. Moreover, the high prevalence of infection in pigeons and the large number of birds of this species in Portugal and other European countries (5) indicate that pigeons might be a potential source of human infection and a significant source of environmental contamination.

Nucleotide sequence accession numbers. The unique *E. bieneusi* ITS sequences obtained in this study have been deposited in GenBank under accession numbers DQ425107 and DQ425108.

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