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

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Received 16 June 2006/Accepted 21 August 2006

*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 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 sequences from chicken samples 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), *Nymphaicus hollandicus* (n = 3), *Platycercus eximius* (n = 1), and *Psittacus erithacus* (n = 8). The Passeriformes included *Bathilda ruficauda* (n = 1), *Erythronia 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...
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 Perub 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 Columbiformes (17). The unique *E. bieneusi* genotype found in bird specimens had ITS sequences identical or similar to those of a *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 of 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.

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

<table>
<thead>
<tr>
<th>Order</th>
<th>Common name/scientific name</th>
<th>Specimen</th>
<th><em>E. bieneusi</em> prevalence</th>
<th><em>E. bieneusi</em> genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psittaciformes</td>
<td>Lovebird/Aquaporus spp.</td>
<td>Droppings</td>
<td>1/13</td>
<td>Peru6</td>
</tr>
<tr>
<td></td>
<td>Cockatiel/Nymphicus hollandicus</td>
<td>Droppings</td>
<td>1/3</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>African gray parrot/Psittacus erithacus</td>
<td>Droppings</td>
<td>1/7</td>
<td>Peru6-var</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>Droppings</td>
<td>1/7</td>
<td>ND</td>
</tr>
<tr>
<td>Passeriformes</td>
<td>Star finch/Bathilda ruficauda</td>
<td>Droppings</td>
<td>0/8</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>Droppings</td>
<td>0/6</td>
<td>ND</td>
</tr>
<tr>
<td>Columbiformes</td>
<td>Pigeon/Columba livia</td>
<td>Intestinal contents</td>
<td>16/44</td>
<td>Peru6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/44</td>
<td>Peru6-var</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2/44</td>
<td>Peru6 and Peru6-var mixed infections</td>
</tr>
</tbody>
</table>

* a ND, *E. bieneusi* genotype not determined.

* b *E. bieneusi* genotype previously reported as Peru6 (AY371281).

* c *E. bieneusi* genotype not determined.

* d *E. bieneusi* genotype identical to one found in humans has been 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.

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**REFERENCES**


