Frequent Occurrence of Human-Associated Microsporidia in Fecal Droppings of Urban Pigeons in Amsterdam, The Netherlands

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Human-associated microsporidia were frequently observed in fecal samples of 331 feral pigeons in Amsterdam, The Netherlands, obtained during high- and low-breeding periods. Thirty-six of 331 samples (11%) contained the human pathogens Enterocytozoon bieneusi (n = 18), Encephalitozoon hellem (n = 11), Encephalitozoon cuniculi (n = 6), and Encephalitozoon intestinalis (n = 1); 5 samples contained other microsporidia. Pigeon feces can be an important source of human microsporidian infection.

The microsporidia form a diverse group of organisms consisting of more than 1,200 species in various vertebrate and invertebrate hosts. Four species emerged as frequent and important opportunistic pathogens when AIDS became pandemic: Enterocytozoon bieneusi, Encephalitozoon intestinalis, Encephalitozoon hellem, and Encephalitozoon cuniculi. The high seroprevalence against Encephalitozoon species in Dutch blood donors and French women suggested that contact with Encephalitozoon spp. is also common in immunocompetent subjects. Animal reservoirs (10), surface water (12), and humans (1) are thought to be sources of human infection. However, which animal species are relevant reservoirs or sources is still unclear.

In many European cities, the feral pigeon (Columba livia [forma domestica]) is an abundant bird species that often lives in close contact with humans. Recently, E. bieneusi and other microsporidia were detected in fecal droppings of these pigeons (5, 9). The objective of the present study was to investigate the presence of microsporidia in urban pigeons in Amsterdam.

DNA was isolated from 331 pigeon fecal samples from 331 pigeons obtained during a study of Chlamyphila psittaci, as described by Heddema et al. (7, 12). Briefly, at nine locations, pigeons were attracted with food, and their fresh fecal droppings were collected from pavements by using sterile cotton swabs (MW&E, United Kingdom) during a low-breeding period (between 3 February and 8 March 2005) and when breeding was frequent (2 May 2005). The cotton swabs were placed in 1.5-ml tubes in 300 µl of Baker water (Boom B.V., Meppel, The Netherlands) and vortexed thoroughly. Fifty microliters of each fecal suspension was used as input for the DNA extraction procedure using a modified Boom extraction (6) or a High Pure DNA purification kit (Roche). PCR inhibition was excluded by amplification of a control target, as previously described (7). PCR was performed using the primers and conditions described by Notermans et al. (11), directed to a conserved region of the small-subunit rRNA gene of microsporidia. PCR products were size separated by agarose gel electrophoresis and visualized by UV illumination after ethidium bromide staining. A unidirectional workflow was maintained with separation of the PCR mixture preparation and the DNA extraction from all (post) amplification activities, and negative controls were included in all steps of the detection process. Amplicons were sequenced using BigDye Terminator chemistry (Applied Biosystems) and analyzed on an ABI 3900 sequencer. Resulting sequences were analyzed using CodonCode Aligner (CodonCode Corp.) and MEGA (13) software.

Sequence-confirmed microsporidium-positive PCR products were obtained at all sampling locations for 41/331 samples (12%) (Table 1). In the low-breeding period, 6% (10/160; 95% confidence interval [CI], 2 to 10) of the samples were positive, whereas during the breeding period, this number increased significantly to 18% (31/171; 95% CI, 12 to 24; Fisher’s exact test, P = 0.001). This indicates that either infection with mi-

TABLE 1. Number of microsporidium-positive feral pigeon fecal samples by location in Amsterdam, The Netherlands

<table>
<thead>
<tr>
<th>Town council</th>
<th>Low-breeding period</th>
<th>Breeding period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oost Watergraafsmeer</td>
<td>2/15</td>
<td>2/15</td>
</tr>
<tr>
<td>Oud Zuid</td>
<td>0/15</td>
<td>6/20</td>
</tr>
<tr>
<td>Binnenstad (Dam)</td>
<td>1/20</td>
<td>4/27</td>
</tr>
<tr>
<td>Binnenstad (Leidseplein)</td>
<td>4/25</td>
<td>2/27</td>
</tr>
<tr>
<td>Zeeburg</td>
<td>0/15</td>
<td>4/15</td>
</tr>
<tr>
<td>Zuider Amstel</td>
<td>1/15</td>
<td>5/15</td>
</tr>
<tr>
<td>Geuzenveld</td>
<td>0/15</td>
<td>4/15</td>
</tr>
<tr>
<td>Bos en Lommer</td>
<td>0/20</td>
<td>1/15</td>
</tr>
<tr>
<td>Oud West</td>
<td>2/20</td>
<td>3/22</td>
</tr>
<tr>
<td>Total</td>
<td>10/160</td>
<td>31/171</td>
</tr>
</tbody>
</table>

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Since microsporidia are important opportunistic pathogens, the risk of such exposure can be of significance for the immunocompromised human host. Frequent exposure to bird droppings might partly explain the observed high seroprevalence against *Encephalitozoon* species in Dutch blood donors (14).

In conclusion, this study supports the finding that microsporidiosis is a zoonotic disease (10) and suggests that excreta of urban feral pigeons can be an important source of human infection with *Enterocytozoon bieneusi*, *Encephalitozoon cuniculi*, and *E. hellem* DNA in multiple samples of different pigeons suggests true infection.

The minimal infectious dose for microsporidia is unknown but is thought to be comparable to those of other intestinal parasites, i.e., 10 to 100 spores (3). In a quantitative study of pigeon guano, Graczyk et al. found 3,500 *E. bieneusi* spores/g (4). It was calculated that a person with a 30-min exposure to pigeon guano through nearby sweeping with a broom could inhale >1,000 viable *E. bieneusi* spores. Close interaction of feral pigeons with humans (especially children and elderly persons and tourists) is common in Amsterdam and is higher during the breeding period than the low-breeding period. Also, pigeon nests that are built near ventilation inlets could result in the inhalation of spores.

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