Detection of *Encephalitozoon hellem* in Feces of Experimentally Infected Chickens

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*Encephalitozoon hellem*, initially isolated from an AIDS patient, was found infectious for athymic mice [5] and has been reported in natural infections of psittacine birds, an ostrich and hummingbirds [4,6]. The specific routes of transmission have not been well documented. However, like other feline borne pathogens, the infectious spore stage is probably transmitted by direct contact or by ingestion of contaminated food and water. Because the range of avian hosts has been found to be so great the likelihood of there being additional avian species susceptible to infection with *E. hellem* seems a reasonable expectation. Therefore, the present study was conducted to determine if chickens and turkeys, easily accessible laboratory species, species of economic importance, and species in close contact with humans, were also susceptible to infection with *E. hellem*.

**MATERIALS AND METHODS**

Spores of *Encephalitozoon hellem* (ATCC no. 50451) were obtained from the American Type Culture Collection (Manassas, VA) and propagated in human lung fibroblast cells (WI-38, CCL-75) in T-75 flasks in MEM medium with 10% fetal calf serum, 1% non-essential amino acids, 1% L-glutamine, 1% sodium pyruvate, 1% penicillin/streptomycin, and 2% HEPES. Spores were harvested from cell cultures and counted directly in well slides.

Sexual chicks and L-71 White turkey poults, obtained 3 to 5 days of age from a commercial source, were housed in groups of 3 to 6 birds in wire cages in an isolated building. Experimental chicks and poults were inoculated with an aqueous suspension of 1 × 10⁵ spores via gastric gavage. In each experiment, an equal number of birds served as uninoculated controls. Whenever spores were given to birds to test for infectivity, a portion was retained to test for infectivity in vitro.

For the duration of Experiments 1–3, each bird was held in a disposable plastic cup daily until ~5 g of feces were collected (Table 1). Feces passed through a no. 325-mesh sieve were further cleaned of debris by centrifuging over a cesium chloride gradient as described [1]. Three ml of supernatant from each gradient was resuspended in 12 ml of water and centrifuged at 1,000 g for 15 min. Pellets were aspirated, dried on a microscope slide, stained with Calcofluor white and examined by fluorescence microscopy at a wave length of 470 nm. Comparison slides were prepared using spores from in vitro cultures. At necropsy, tissues were collected from cecum, cloaca, and intestine, and fixed in 10% neutral buffered formalin. Paraffin embedded sections cut at 4 μm, were stained by the quick-hot gram-chromotrope method [2]. Stained slides were examined by bright field microscopy for the presence of microsporidia.

In Experiments 4–6, feces from chicks were collected daily beginning on the day of exposure, cleaned as described above, and tested for the presence of *E. hellem* by polymerase chain reaction (PCR) instead of microscopy. Total DNA was extracted from 50 ml of processed feces using QIAamp Tissue Kit (Qiagen, Valencia, CA). Microsporidial SSU-rRNA coding regions were amplified using the following primers Micro F (5′-CACCAGTGTATTCTGCTGA-3′) and Micro R (5′-CTCTCAGGGAAAACCCCTG-3′) [7]. PCR products were sequenced with forward and reverse primers using an ABI3100 automated sequencer. SSU-rRNA coding sequences were compared to SSU-rRNA sequences from GenBank. PCR positive by sequencing were further tested for the presence of microsporidia.

**RESULTS AND DISCUSSION**

In Experiments 1–6, microscopic examination of fecal smears, histologic sections, and impression smears failed to detect spores or other stages of microsporidia although all spores used as inoculum were found infective for cultured cells. All negative control birds were negative by either microscopy or PCR.

The present study has demonstrated the difficulty in detecting infections of *E. hellem* in chickens and turkeys by microscopic methods as compared with molecular methods. In titration studies (not shown), at a concentration of 10,000 *E. hellem* spores/g in 5 g of chicken feces, 10 of 10 and 5 of 10 specimens were positive by the PCR and microscopy methods used in the present study, respectively. In contrast, at 1,000 spores/g, 3 of 10 and none of 10 specimens were positive by PCR and microscopy, respectively. At 100 spores/g, detection by PCR was infrequent and sporadic.

In Experiments 4–6, the presence of *E. hellem* in feces was detected by PCR (Table 1). In pooled feces in Experiments 4 and 6 PCR-positive feces were found daily beginning one day after spores were given by gastric gavage. In Experiment 5, PCR-positive species for the 3 chicks lasted 5, 6, and 7 days. Although these findings suggest that some spores passed through the digestive tract within a day of exposure, daily positive PCR findings for as long as 19, 7, and 7 days after exposure strongly suggest that active infections were present to provide *E. hellem* DNA for such long periods of time. All PCR specimens positive for *E. hellem* were confirmed by sequence data.

Present findings suggest that in addition to *Enterocytozoon bieneusi*, detected in feces from 5-week-old broiler chickens in a poultry abattoir [3], chickens also serve as hosts to *E. hellem*.

**LITERATURE CITED**


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**Table 1. Oral inoculation of chicks (C) and turkey poults (P) with spores of *E. hellem***

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>No. of Birds</th>
<th>Age when inocul. (Days)</th>
<th>Days of Exposure until Necropsy</th>
<th>Histology</th>
<th>No. of Days PCR Found Positive</th>
<th>PCR Confirmed by Sequence Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (C)</td>
<td>6</td>
<td>3–5</td>
<td>21</td>
<td>Negative</td>
<td>ND 1</td>
<td>ND 1</td>
</tr>
<tr>
<td>2 (P)</td>
<td>6</td>
<td>7</td>
<td>21</td>
<td>Negative</td>
<td>ND 1</td>
<td>ND 1</td>
</tr>
<tr>
<td>3 (P)</td>
<td>5</td>
<td>14</td>
<td>11</td>
<td>Negative</td>
<td>ND 1</td>
<td>ND 1</td>
</tr>
<tr>
<td>4 (C)</td>
<td>4</td>
<td>3–5</td>
<td>17 &amp; 28</td>
<td>Negative</td>
<td>19</td>
<td>Y</td>
</tr>
<tr>
<td>5 (C)</td>
<td>3</td>
<td>3–5</td>
<td>7</td>
<td>ND</td>
<td>5,6, 7</td>
<td>Y</td>
</tr>
<tr>
<td>6 (C)</td>
<td>3</td>
<td>3–5</td>
<td>7</td>
<td>Negative</td>
<td>7</td>
<td>Y</td>
</tr>
</tbody>
</table>

* ND = Not done.