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Serial Monoxenous Transmission of *Toxoplasma gondii* in Cats

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**Abstract:** Oral administration of *Toxoplasma gondii* oocysts to cats (i.e., monoxenous transmission) typically induces patent infections in fewer than half of test subjects. In the present study, oral administration of *T. gondii* oocysts to 5 kittens induced a patent infection in 2 of them, but only 1 kitten shed enough oocysts to enable further study. Those monoxenously-produced oocysts were administered to another kitten, which produced a second generation of monoxenous oocysts, and then those were used to induce a third generation of monoxenous oocysts. These results provide a rationale to develop a strain of *T. gondii* that has efficient direct transmission. The isolate of *T. gondii* that was able to be passaged in this manner has been designated the Dubey strain and cultured tachyzoites have been donated to a repository.

The protozoan *Toxoplasma gondii* has a 2-host transmission cycle (dixenous: prey–predator). Definitive hosts are felids and intermediate hosts include most investigated species of mammals and birds. Alternative transmission methods exist such as transgenerational "endogenous transplacental transmission" (Trees and Williams, 2005) in mice (Beverley, 1959; Owen and Trees, 1998), transmission to intermediate hosts by carnivorism (Choi et al., 1997), and direct transmission in cats by ingestion of sporulated oocysts (monoxenous: fecal–oral).

Compared with ingestion of infected mice, oral administration of *T. gondii* oocysts to cats is relatively inefficient at inducing patent infections. Dubey et al. (1970) reported that 23 of 24 (96%) cats shed oocysts following consumption of infected mice while only 8 of 17 (47%) cats shed oocysts after oral administration of oocysts. Dubey (1996) tabulated results of 15 previously reported direct transmission studies in which only 34 of 155 cats (22%) developed patent infections following oral administration of oocysts; the shortest prepatent period recorded in those studies was 22 days.

Although monoxenous transmission of *T. gondii* in cats is inefficient, we hypothesize that a strain with efficient monoxenous transmission could be developed by serial passage through cats. Here we report serial passage of *T. gondii* in kittens by direct oral transmission of sporulated oocysts.

All cats used in this study were 8- to 12-wk-old, purchased from Harlan Laboratories (Indianapolis, Indiana). To begin, dixenously-produced oocysts were obtained for 3 different strains of *T. gondii*.

**ME 49 strain:** A mouse latently infected with the ME 49 strain of *T. gondii* was donated by the laboratory of David Sibley (Washington University, St. Louis, Missouri); the mouse was euthanized, and the skin and gastrointestinal tract were removed and discarded, and the carcass was placed on ice, transported to the University of Illinois, and fed to a kitten within a period of 6 hr. This kitten developed a light patent infection with a peak daily production of approximately 8 × 10⁴ oocysts.

**Beverley strain:** Cultured tachyzoites of the Beverley strain of *T. gondii* were also donated by the Sibley lab. Approximately 600 tachyzoites were inoculated subcutaneously into 3 mice. Two of these were euthanized after 2 wk because of illness, at which time the third clinically healthy mouse was treated with sulfamethoxazole and trimethoprim in drinking water. This mouse appeared healthy 83 days after inoculation, when it was euthanized and fed to a kitten (as above). This kitten developed a patent infection with a peak daily production of approximately 10⁸ oocysts.

**Dubey strain:** Oocysts of a previously un-named isolate of *T. gondii* were donated by the laboratory of J. P. Dubey (U.S.D.A. Agriculture Research Service, Bethesda, Maryland). This isolate was originally obtained by feeding tissues from a slaughtered pig to a cat, which shed 5.25 × 10⁶ oocysts (Dubey et al., 2002). Oocysts were stored refrigerated until 2006, at which time fresh oocysts were produced by passage through a mouse and a cat (J. P. Dubey, pers. comm.). Approximately 6 × 10⁷ sporulated oocysts in 50 ml solution were received at the University of Illinois in July 2006.

In addition to performing the direct transmission studies described below, the following procedures were used to isolate the Dubey strain into cell culture. Between 50 and 200 oocysts were orally administered to 4 male outbred white ICR mice (Harlan Laboratories). The mice did not develop signs of illness. One of these mice was euthanized 15 wk after inoculation, the brain was removed aseptically and divided in half, and one-half was used for microscopic examination of tissue squashes. Well-developed tissue cysts were observed (Fig. 1A). The remaining brain tissue was macerated in a sterile tissue grinder, suspended in culture medium, inoculated onto a monolayer of MA104 cells (African Green Monkey Kidney), and the medium was replaced after 30 min. The culture flask was maintained at 37 C, 5% CO₂, in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% horse serum and examined frequently for growth of organisms. Tachyzoites were first confidently observed in the primary culture flask 34 days after inoculation. The rate of growth of the organisms gradually increased in subsequent passages and by 80 days (relative to euthanasia) the fifth passage was burgeoning with tachyzoites. This isolate of *T. gondii* is designated the “Dubey” strain and a specimen has been deposited with the American Type Culture Collection (Manassas, Virginia), accession number ATCC PRA-394.

Direct transmission studies were performed in kittens as detailed below. No kitten had diarrhea, constipation, or any other sign of illness during these trials. Feces were examined daily by flotation in Sheather’s (sucrose) solution (Zajac and Conboy, 2012) between the 15th and 45th day after inoculation. Oocysts were purified by flotation in Sheather’s solution, washed in water, and suspended in 2% H₂SO₄. Sporulation was induced at room temperature by placing capped, half-filled 50-ml centrifuge tubes horizontally upon a rotary platform shaker at low speed for several days—tubes were opened daily to permit a fresh exchange of air. A 20-μl sample of oocyst suspension was placed under a coverslip, examined microscopically to count oocysts, and the number of oocysts produced was extrapolated by comparison with the total volume of solution.

One kitten was orally administered 10⁴ sporulated oocysts of the ME 49 strain. This did not induce a patent infection.

Two kittens were each administered 10⁵ oocysts of the Beverley strain. A patent infection was detected in 1 kitten, but the number of oocysts was too low to enable a reliable count for further use.

Next, direct transmission was attempted using the Dubey strain. Three monoxenous generations of oocysts were produced (a fourth generation was not attempted). Oocysts are depicted in Figures 1B, C. Detailed

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results are provided in Table I. These results provide support of our rationale to develop a *T. gondii* strain with efficient direct transmission in cats. To our knowledge, direct transmission of *T. gondii* has not previously been reported beyond a single generation.

Patent *T. gondii* infections occurred in only 2 of 5 kittens to which dixenously-produced oocysts had been orally administered, but patent infections occurred in all 3 kittens that were administered monoxenously-produced oocysts. Future experiments are needed to determine if these results are repeatable, to establish the minimum infectious dose, and to investigate the relationship between infectious dose and numbers of oocysts produced.

If a strain of *T. gondii* could be maintained indefinitely by monoxenous transmission, we speculate that its ability to infect intermediate hosts could be lost. Alterations of the *T. gondii* life cycle by other types of artificial passage have been demonstrated previously; for example, rapid passage of *T. gondii* tachyzoites in mice (with insufficient time for encystment of bradyzoites) has created strains that are incapable of encysting in tissues or producing oocysts (Frenkel et al., 1976; Buxton, 1993). The development of a monoxenous strain that could only infect cats would have potential use for vaccination of cats, and a vaccine containing oocysts would have advantages regarding storage and administration.

The poultry industry provides ample precedence for the manufacture of coccidial vaccines in vivo (Shirley et al., 2005). Artificial selection of precocious strains (i.e., with shortened prepatent periods) has been used to create attenuated strains of *Eimeria* spp. (Shirley et al., 2005). However, selection of precocious strains of *Eimeria* spp. does not change the efficiency of infection or the host range of the parasites. A disadvantage of precocious vaccines is that they produce fewer oocysts per animal and thus are more expensive to manufacture. In contrast, the strategy reported here for production of *T. gondii* oocysts in cats does not select for shortened prepatent periods; possible effects upon virulence or fecundity remain to be determined.

We thank L. David Sibley and J. P. Dubey for donation of organisms. We thank personnel of the University of Illinois, Division of Animal Resources for husbandry of *T. gondii*-infected cats. The use of animals in these studies was approved by the University of Illinois’ Institutional Animal Care and Use Committee. This study was supported in part by a U.S.D.A. formula grant administered by the University of Illinois (section 1433, Animal Health and Disease, ILLU-888-947).

**LITERATURE CITED**


**Table I.** Serial oral administration of *Toxoplasma gondii* Dubey strain oocysts to kittens and subsequent development of patent infections.

<table>
<thead>
<tr>
<th>Kitten ID</th>
<th>Source of oocysts</th>
<th>Number of oocysts administered</th>
<th>Days oocysts shed (postinoculation)</th>
<th>Total oocysts produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Wild type (dixenous)</td>
<td>$10^5$</td>
<td>—*</td>
<td>0</td>
</tr>
<tr>
<td>B2</td>
<td>Wild type (dixenous)</td>
<td>$10^5$</td>
<td>29–36</td>
<td>$4 \times 10^6$</td>
</tr>
<tr>
<td>C</td>
<td>Kitten B2 (monoxenous)</td>
<td>$10^5$</td>
<td>24–26</td>
<td>$2 \times 10^7$</td>
</tr>
<tr>
<td>D1</td>
<td>Kitten C (monoxenous)</td>
<td>$10^5$</td>
<td>29–34</td>
<td>$4 \times 10^6$</td>
</tr>
<tr>
<td>D2</td>
<td>Kitten C (monoxenous)</td>
<td>$10^5$</td>
<td>26–31</td>
<td>$2 \times 10^6$</td>
</tr>
</tbody>
</table>

* (—) = No oocysts were shed by this cat.

**Figure 1.** Photomicrographs of the Dubey strain of *Toxoplasma gondii*. Each bar = 10 μm. (A) Tissue cyst in a brain squash from a latently infected mouse. (B) Unsporulated oocysts. (C) Sporulated oocyst after incubation in the presence of air. Two sporocysts are clearly visible within the oocyst.

