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Unexpected oocyst shedding by cats fed *Toxoplasma gondii* tachyzoites: In vivo stage conversion and strain variation

J.P. Dubey *

Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Agricultural Research Service, United States Department of Agricultural, Building 1001, Beltsville, MD 20705, USA

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Abstract

Tachyzoites, bradyzoites (in tissue cysts), and sporozoites (in oocysts) are the three infectious stages of *Toxoplasma gondii*. The prepatent period (time to shedding of oocysts after primary infection) varies with the stage of *T. gondii* ingested by the cat. The prepatent period (pp) after ingesting bradyzoites is short (3–10 days) while it is long (18 days or longer) after ingesting oocysts or tachyzoites, irrespective of the dose. The conversion of bradyzoites to tachyzoites and tachyzoites to bradyzoites is biologically important in the life cycle of *T. gondii*. In the present paper, the pp was used to study in vivo conversion of tachyzoites to bradyzoites using two isolates, VEG and TgCkAr23. *T. gondii* organisms were obtained from the peritoneal exudates (pex) of mice inoculated intraperitoneally (i.p.) with these isolates and administered to cats orally by pouring in the mouth or by a stomach tube. In total, 94 of 151 cats shed oocysts after ingesting pex. The pp after ingesting pex was short (5–10 days) in 50 cats, intermediate (11–17) in 30 cats, and long (18 or higher) in 14 cats. The strain of *T. gondii* (VEG, TgCKAr23) or the stage (bradyzoite, tachyzoite, and sporozoite) used to initiate infection in mice did not affect the results. In addition, six of eight cats fed mice infected 1–4 days earlier shed oocysts with a short pp; the mice had been inoculated i.p. with bradyzoites of the VEG strain and their whole carcasses were fed to cats 1, 2, 3, or 4 days post-infection. Results indicate that bradyzoites may be formed in the peritoneal cavities of mice inoculated intraperitoneally with *T. gondii* and some bradyzoites might give rise directly to bradyzoites without converting to tachyzoites.
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Keywords: *Toxoplasma gondii*; Tachyzoites; Bradyzoites; Oocysts; Cats; In vivo stage conversion

1. Introduction

Tachyzoites and bradyzoites are structurally and biologically different stages of *Toxoplasma gondii* found in tissues of infected animals and humans (Dubey et al., 1998). Post-natally, humans or animals become infected with *T. gondii* by ingesting food or water contaminated with oocysts from infected cat feces or by ingesting tissue cysts from uncooked and undercooked infected meat (Dubey and Beattie, 1988). After ingestion, sporozoites or bradyzoites convert to tachyzoites inside the host tissues (Dubey, 1997; Dubey et al., 1997). Tissue cysts begin to form

* Tel.: +1 301 504 8128; fax: +1 301 504 9222.
E-mail address: jdubey@anri.barc.usda.gov.

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the first week p.i. and are thought to persist for life (Dubey and Frenkel, 1976). Reactivation of latent infection, for example, in patients with acquired immunodeficiency syndrome (AIDS) can lead to fatal toxoplasmosis. Reactivation is thought to be due to rupture of tissue cysts and formation of new tachyzoites and bradyzoites. It is uncertain whether bradyzoites from older tissue cysts can directly give rise to new tissue cysts or have to go though the tachyzoite stage first. Bradyzoites are less susceptible to chemotherapy that is effective against tachyzoites. Therefore, the fate of bradyzoites in host tissues is of clinical significance. Recent in vitro studies indicate that bradyzoites may give rise to bradyzoites without first converting into tachyzoites (Weiss et al., 1995; Dzierszinski et al., 2004).

There has been great interest in studying conditions needed for stage conversion of *T. gondii* (Weiss et al., 1995; Frenkel, 1996; Gross et al., 1996; Bohne et al., 1996; Jerome et al., 1998; Dubey et al., 1998; Dzierszinski et al., 2004). Structurally, tachyzoites have a centrally located nucleus, have a few or no PAS-positive granules, and are found during acute infection. Bradyzoites have a terminally located nucleus, have many PAS-positive granules, are enclosed in a resistant tissue cyst wall, and are more prevalent during the chronic stage (Dubey and Frenkel, 1976). Several monoclonal antibodies specific for tachyzoites and bradyzoites and genetic markers have been used to investigate the transition (Weiss et al., 1995; Bohne et al., 1996; Dzierszinski et al., 2004). However, the transition stage between tachyzoites and bradyzoites and vice versa is not well defined structurally or antigenically (Frenkel, 1996; Dubey et al., 1998).

Biologically, bradyzoites are resistant to gastric digestion and thus are infectious orally whereas tachyzoites are destroyed by gastric juice (Jacobs et al., 1960). However, recent studies indicate that susceptibility to acid-pepsin digestion is not a reliable criterion to distinguish bradyzoites from tachyzoites because occasionally tachyzoites survived pepsin digestion and tachyzoites were infective orally to mice and cats (Dubey, 1998a). Cell culture is not a reliable method to obtain pure culture of tachyzoites because bradyzoites develop in cell culture (Hoff et al., 1977); further, a proportion of bradyzoites are thought to divide directly into bradyzoites, without conversion to tachyzoites (Weiss et al., 1995). One method to distinguish tachyzoites from bradyzoites is by bioassay in cats using prepatent period (pp) to oocyst shedding as a criterion. Cats fed bradyzoites shed oocysts with a pp of 3–10 days, irrespective of the dose or the strain of *T. gondii* (Dubey and Frenkel, 1972, 1976; Dubey, 2001) whereas those fed tachyzoites of the M-7741 strain shed oocysts with a pp of 19 days or longer. These latter observations were based on feeding cats tissues of mice that had been infected up to 2 days after systemic inoculation with any stage of *T. gondii* (Dubey and Frenkel, 1976). Cats fed tachyzoites from the peritoneal exudate (pex) shed oocysts with a long pp (>19 days). These observations indicated that bradyzoites are not found in pex. In the present paper, I report shedding of oocysts by cats fed pex from mice infected with two other isolates (VEG and TgCKAr23) of *T. gondii* suggesting the presence of bradyzoites in pex.

2. Materials and methods

2.1. Strains of T. gondii

The VEG strain of *T. gondii* was isolated from the blood of an AIDS patient (Dubey et al., 1996). Its early maintenance history is unknown. Since 1995 it has been maintained in this laboratory by tissue cyst–oocyst passage. It has been used extensively to study the biology of toxoplasmosis in mice after oral infection with bradyzoites and oocysts (Dubey, 1997; Dubey et al., 1997). It is a type III strain using the classification of Howe and Sibley (1995) and is mildly pathogenic to mice.

The TgCkAr23 isolate was obtained from an asymptomatic chicken from Argentina in September 2004 (Dubey et al., 2005). It is a type I strain and is virulent for Swiss Webster (SW) mice. The original passage of the strain was used in this study. Five SW mice were inoculated subcutaneously (s.c.) with brain homogenate of this chicken. All five mice became sick and died or were euthanized on days 19, 19, 35, 35, and 35 post-inoculation (p.i.). Tachyzoites were seen in the lungs of mice at day 19 p.i. and tissue cysts were seen in the brains of mice at 35 day p.i.; these tissue cysts were used to obtain tachyzoites and oocysts in the present study.