DETECTION OF *TOXOLASMA GONDII* IN CAPTIVE WILD FELIDS

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Abstract. *Toxoplasma gondii* can infect all species of warm-blooded animals, including humans, and causes serious diseases in immunocompromized hosts. Live tachyzoites derived from serial passage in HeLa culture were used in the Sabin-Feldman dye test for detection of *Toxoplasma gondii* antibody in serum samples of 21 captive wild felids including one fishing cat (*Prion nailurus viverrina*), one leopard (*Panthera pardus*), two flat-headed cats (*Prion nailurus planiceps*), 6 tigers (*Panthera tigris*), two leopard cats (*Felis bengalensis*), two clouded leopards (*Felis nebulosa*), 3 pumas (*Puma concolor*), and 4 jungle cats (*Felis chaus*). Antibodies to *Toxoplasma gondii* were founded in 9 of 21 felids (42.8%). This study revealed that cell culture-derived tachyzoites can be used successfully as a source of live organisms in a gold standard Sabin-Feldman dye test, which is simpler, cheaper and less ethically sensitive than *in vivo* inoculation.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular coccidian parasite that infects virtually all species of warm-blooded animals, including humans, and causes serious disease in immunocompromized hosts. It causes mental retardation and loss of vision in children and abortion in livestock. Cats and other felids are the main reservoir host for this parasite. The diagnosis of primary infection during the life of the host is difficult. The Sabin-Feldman dye test is the gold standard serological test for the detection of *Toxoplasma* infection, but it remains the most difficult test to maintain because it requires fresh, viable tachyzoites as test antigen. The use of tachyzoites in conventional method is a major ethical consideration, and maintenance is laborious and expensive. In the present study, *in vitro* culture using HeLa cell culture system was used to produce fresh viable tachyzoites for the dye test to detect *Toxoplasma* infection in captive wild felids.

MATERIALS AND METHODS

Serum samples

Twenty-one serum samples were collected from captive wild felids in Khao Kheow Open Zoo (KKOZ) and Khao Pratab Chang Breeding Center, Wildlife Conservation Division of National Parks, Wildlife and Plants Department, Thailand. Animals were selected from groups positive to FeLV p27 antigen detecting by nested PCR and had present sign(s) of Feline Leukemia Virus (FeLV) infection. Serum samples included one fishing cat (*Prion nailurus viverrina*), one leopard (*Panthera pardus*), two flat-headed cats (*Prion nailurus planiceps*), 6 tigers (*Panthera tigris*), two leopard cats (*Felis bengalensis*), two clouded leopards (*Felis nebulosa*), 3 pumas (*Puma concolor*), and 4 jungle cats (*Felis chaus*).

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Stock cell culture

HeLa cells, ordered from CLS Germany, were grown in 25 cm^2 tissue culture flasks (Nunc™, Roskilde, Denmark) in 5 ml of culture medium: minimum essential medium with Earle's salt (Gibco, Invitrogen UK)/Hepes solution supplemented with L-glutamine, gentamicin 40,000 IU (Gentacol® M&H Manufacturing, Thailand), Penicillin 40,000 IU, fungizone (amphotericin B; Gibco, Invitrogen UK) 1 mg and fetal bovine serum (FBS; Gibco, Invitrogen UK) at a concentration of 10% for the growing medium and 2% for the maintenance medium. The cells were grown in MEM/Hepes with 10% FBS (growth medium) and incubated at 37°C with 5% CO_2 in an incubator (Thermo Electron Corporation, USA). When a confluent monolayer was obtained, the medium was changed to MEM/Hepes with 2% FBS (maintenance medium). Cells were routinely subcultured every 2-3 days by trypsinization.

In vitro culture of *Toxoplasma gondii*

Toxoplasma gondii RH strain taken from the peritoneal cavity of mice maintained in the Protozoology

Laboratory, Department of Protozoology, Faculty of Tropical Medicine, Mahidol University were used in this study. The peritoneal fluid obtained by aseptic technique contained ≥1×10^8 *Toxoplasma* tachyzoites/ml, 99% viable assessed by phase-contrast microscopy. HeLa cell monolayers in 25 cm^2 tissue culture flasks were infected at a multiplicity of infection of 1:1 in maintenance medium. After 24 hours, the media were replaced with serum-free MEM/Hepes. *Toxoplasma* tachyzoites were harvested 3-4 days post-infection, or ≥5 plaque-forming units per field under 400 magnification were obtained. The cultures harvested were considered to meet dye test quality when the yield was ≥1×10^6 *Toxoplasma* tachyzoites/ml, and viability was >90% assessed by trypan blue dye under 400 magnification phase-contrast microscopy.

Dye test

The dye test was performed as previously described. A suspension of ≥2×10^6 tachyzoites in 50% accessory factor was added to doubling dilutions of serum samples and control sera in sterile normal saline in flat-bottomed microtiter plates. Accessory factor was composed of Alserver's solution, negative serum, and *Toxoplasma* antigen. After incubation at 36°C for one hour, titers were read at the end-point dilution for 50% tachyzoites killing, assessed by 400 magnification phase-contrast microscopy and the addition of methylene blue.

RESULTS

The overall prevalence of *T. gondii* antibodies was 45.5% in 9 of 21 felids. The titer varied from 1:4 (3 samples) to 1:16 (one sample) (Table 1). *Toxoplasma gondii* tachyzoites derived from cell culture can successfully be used as an antigen in the Sabin-Feldman dye test to detect *Toxoplasma* infection in captive wild felids. The first and second passages were used for testing. The cell culture harvest was considered to be of dye test quality when the yield was ≥1×10^6/ml and viability was >90%. The harvests met the quality requirement and could be used to perform the dye test.

DISCUSSION

Cell culture-derived tachyzoites can be used successfully as organisms in the Sabin-Feldman dye test to detect *Toxoplasma gondii* antibodies in serum samples from human patients by using tachyzoites derived from the HeLa culture system, which met the dye test criteria for yield at ≥1×10^6 *Toxoplasma* tachyzoites/ml with viability >90% (Evan *et al*, 1999; Ashburn *et al*, 2000; Chatterton *et al*, 2002; Mavin *et al*, 2003). Some difference had been shown between low and high passes harvested, but it was not significant (Chatterton *et al*, 2002). Some reported that different lineages of *Toxoplasma gondii* RH strain could be performed could be performed differently in cell culture and consistently produced tachyzoites successfully used in the dye test, while some were not (Mavin *et al*, 2004). Nine of 21 serum samples were positive in the high risk group. There were some reports about the seroprevalence of *Toxoplasma gondii* infection in domestic cats and captive felids around the world. About 7.3% of domestic cats in Thailand were found seropositive to *Toxoplasma* infection by dye test (Sukthana *et al*, 2004). Seropositivity (64.9%) was found by modified agglutination test in exotic wild felids in 12 zoos from 6 Brazilian states; also, in captive neotropical felids from Brazil, 54.6% antibodies were found to *Toxoplasma gondii* in various zoos (Silva *et al*, 2002). Another report from Brazil showed 50.5% in

Table 1
Dye test results in 21 captive wild felids.

Species	Number	Results		Titer
		Positive	Negative	
Fishing cat	1	1	-	1:8
Flat-headed cat	2	1	1	1:8
Leopard	1	1	-	1:8
Leopard cat	2	1	1	1:8
Jungle cat	4	-	4	-
Puma	3	-	3	-
Clouded leopard	2	2	-	1:4
Tiger	6	3	3	1:4(2), 1:16(1)
Total	21	9	12	

stray dogs and 40% in feral cats using ELISA (Meireles *et al,* 2004); in the USA, found seropositivity in feral cats of 63%, and 34.2% in pet cats in North Carolina (Cassity *et al*, 2002). In Barcelona, Spain, 45% of domestic cats were seropositive to *Toxoplasma gondii* (Gauss *et al*, 2003). Another report show that, cats that were seropositive to virus infection, such as feline immunodeficiency virus (FIV) or feline leukemia virus (FeLV) were strongly positive for *Toxoplasma gondii* (Lickey et al, 2005). This was related to this study, where co-infection can lead to high seropositive results for *Toxoplasma gondii* infection in captive wild felids in Thailand. These results will be of interest to public health authorities, zoo workers, veterinarians, and wildlife biologists. Further passages of cell culture will be performed to replace animal culture.

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