CULTIVATION OF *GIARDIA DUODENALIS* IN MONGOLIAN GERBILS

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Abstract. The Mongolian gerbil (*Meriones unguiculatus*) is susceptible to infection with *Giardia duodenalis* trophozoites. Each animal was orally infected with 0.5 ml Diamond's TYIS-33 culture medium containing 10^6 trophozoites. Cysts were then collected and concentrated by sucrose gradient centrifugation. *G. duodenalis* cysts were first observed in feces on day 5 post-infection. The characteristic of *G. duodenalis* infection in gerbils was intermittent cyst release. The range in the number of cysts released per gerbil for a 4-hour collection period was $0\text{-}1.5 \times 10^3$.

INTRODUCTION

Giardia duodenalis (syn. *Giardia intestinalis*, *Giardia lamblia*) is a flagellated unicellular eukaryotic microorganism that commonly causes diarrheal disease worldwide. Experimental infections of *Giardia* have been studied in a variety of animals. Mice (Vinayak *et al,* 1979; Hill *et al,* 1983), rats (Anand *et al,* 1980; Craft, 1982; Kanwar *et al,* 1986) and gerbils (Belosevic *et al,* 1983) have been most commonly used in the development of laboratory models; sometimes, cats (Kirkpatrick and Green, 1985), dogs (Hewlett *et al,* 1982), rabbits (Schleinitz *et al,* 1983), and sheep (Olson *et al,* 1995) have also been utilized. The Mongolian gerbil (*Meriones unguiculatus*) is widely regarded as the best experimental host of *G. duodenalis* infections and offers a much better alternative to mice as a laboratory model. In this study, we aimed to determine whether *in vitro*-grown trophozoites were infective to gerbils, and to describe the *G. duodenalis* cyst collection technique for viability, inactivation, or elimination study.

MATERIALS AND METHODS

Experimental animals

Male Mongolian gerbils (*Meriones unguiculatus*) 6-10 weeks old, weighing 40-50 g, were used in this experiment. The animals were purchased from the Laboratory Animal Unit, Faculty of Medicine, Khon Kaen University, Thailand. On arrival at the facility, the gerbils were placed in filter-top cages (one gerbil per cage) with food and water provided *ad libitum*. Gerbils were treated for 3 consecutive days with a solution (20 mg per gerbil per day) of metronidazole (Flagyl®; Rhone-Poulenc, Montreal, Quebec, Canada), administered by gavage before experimental infection. This treatment ensured that the gerbils were free from all previous infections of the small intestine, as demonstrated by 3 consecutive examinations of feces of gerbils chosen at random.

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Preparation of *G. duodenalis* trophozoites

Trophozoites of *G. duodenalis* were routinely maintained *in vitro* in Diamond's TYIS-33 culture medium at the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Thailand. Actively growing *G. duodenalis* trophozoites (48-to-96 hour-old culture) were incubated on ice for 20 minutes to detach the parasite from the walls of the screw-cap 16 by 125-mm culture tube. The organisms were then sedimented by centrifugation at 2,500 rpm for 10 minutes at 4°C, counted, and resuspended in 0.5 ml culture medium to contain 10^6 trophozoites.

Inoculation of *G. duodenalis* trophozoites

Gerbils were inoculated orally through a feeding tube with 0.5 ml of culture medium containing 10^6 *G. duodenalis* trophozoites.

G. duodenalis cyst collection

Cysts released in 4 hours' fecal collection from the gerbils were collected in 12 by 75-mm plastic tubes during days 1-14 post-infection. The cysts were separated from the feces by filtration through stainless-steel sieves and concentrated by sucrose gradient centrifugation technique, as described by Roberts-Thomson *et al* (1976). Briefly, fresh stools from the infected animals were broken up in distilled water, and 3 ml of fecal suspension were layered on 2.5 ml of 1 M sucrose (specific gravity, 1.11) in a 75 by 12-mm plastic tube and centrifuged at 400*g* for 15 minutes at 20°C. Cysts concentrated at the

water-sucrose interface were carefully removed with a Pasteur pipette, washed by resuspension in 4 ml of normal saline, and sedimented by centrifugation at 600*g* for 10 minutes. The supernatant was removed, the cysts resuspended in phosphate-buffered saline and counted in a hemacytometer chamber. Finally, the cysts were stored at 4°C in phosphate-buffered saline and 0.01% Tween 20 with antibiotic cocktail (100 U penicillin and 0.1 mg/ml streptomycin) to inhibit bacteria growth until used.

RESULTS

Cysts were first observed in feces on day 5 post-infection (Fig 1). The characteristic of *G. duodenalis* infection in gerbils was intermittent cyst release, and the number of cysts released per gerbil for a 4-hour collection period ranged from 0 to 1.5×10^3.

DISCUSSION

This study demonstrated that Mongolian gerbils are susceptible to infection with *G. duodenalis* trophozoites. Intermittent cyst release was characteristic of *G. duodenalis* infection in gerbils. Our result is similar to previous studies with Mongolian gerbils (Belosevic *et al,* 1983; Bouza *et al,* 2000) and mongrel dogs (Hewlett *et al,* 1982). However, the number of cysts excreted by these gerbils was lower than recorded by Belosevic *et al* (1983). The difference is probably due to the fact that Belosevic *et al* (1983), infected orally via a stomach tube (intragastric gavage), while we infected orally via a feeding tube (intraesophagus gavage). Experimental infections of mice by a single trophozoite of *G. muris* (de Carneri *et al,* 1977), dogs (Hewlett *et al,* 1982), domestic cats (Kirkpatrick and Green, 1985) and Mongolian gerbils (Belosevic *et al,* 1983), by cultured trophozoites from humans, have been reported. Although the trophozoite is not thought to be the usual form of transmission, it is important to recognize that infection by this form of the parasite is possible and that gastric acidity does not seem to affect trophozoite infectivity. Mongolian gerbils appear to be an excellent model for the study of *G. duodenalis* because (1) they can be infected with isolates from a variety of hosts; (2) adult animals can be infected with either cysts or trophozoites cultured *in vitro*; (3) their prepatent period and pathogenesis are similar to those of the original hosts; (4) they are capable of maintaining strains of *G. duodenalis* in the laboratory by serial passage; (5) they are prolific and easy to breed; and (6) they can be infected with a low infectious dose (ID_{50} 5-15 cysts) (Stachan and Kunst, 1983; Hibler *et al,* 1987; Thompson, 1999). In this study, we also describe the collection technique for *G. duodenalis* cysts using sucrose gradient centrifugation (Roberts-Thomson *et al,* 1976). We found the sucrose gradient centrifugation technique suitable for viability, inactivation, or elimination studies.

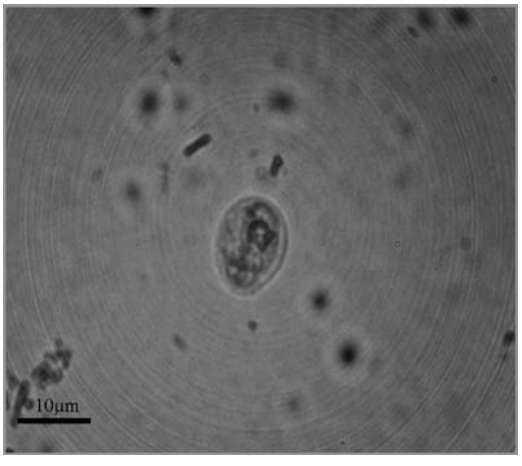

Fig 1- *Giardia duodenalis* cyst is ovoid and measures 10 ×14 µm, and contains a nucleus and axoneme (×1,000).

ACKNOWLEDGEMENTS

We are very grateful to the late Assist Prof Chutatip Siripanth and Ms Niramol Thima for providing *in vitro G. duodenalis* trophozoites, and Assoc Prof Vichit Pipitgool for providing the animals used in the experiments.

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