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A rapid and simple single-step method for the purification of *Toxoplasma gondii* tachyzoites and bradyzoites

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Abstract

This study describes a simple method for the large-scale isolation of pure *Toxoplasma gondii* tachyzoites and bradyzoites. *T. gondii* tachyzoites were obtained from infected human foreskin fibroblasts (HFFs) and peritoneal exudates of mice, while tissue cysts containing bradyzoites were collected from chronically infected mice. Harvested cells and brain tissues were incubated in Hanks balanced salt solution (HBSS), containing 0.25% trypsin and 0.5% taurodeoxycholic acid (TDC) for 5 min. Subsequent washes in phosphate buffered saline (PBS) were conducted, and the cell viability of the preparations was good, as determined by flow cytometry and ability to reinfect HFF cells and propagate in mice. The purification procedure allowed for a rapid preparation of

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pure *T. gondii* tachyzoites and bradyzoites in sufficient quantity that can be used for downstream procedures. The advantage of the new method is that it is convenient and inexpensive.

KEYWORDS

flow cytometry, purification, *T. gondii* tachyzoites and bradyzoites, trypsin, taurodeoxycholic acid

1 | INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite. Felids are the definitive hosts and a wide range of warm-blooded animals, including humans, rodents, birds, livestock and marine mammals are considered intermediate hosts. Sexual reproduction only occurs in felids. Sporozoites attach and penetrate the felid enterocytes after oocyst excystation in the intestinal lumen. Sporozoites undergo multiplication by endodyogeny to form tachyzoites, while bradyzoites are able to invade the intestinal cells, entering into the enteric phase of the sexual cycle. Bradyzoites switch to tachyzoites and go through several rounds of asexual division. After the sporulated oocysts are being ingested by intermediate hosts, sporulated oocysts will be excysted into the intestinal lumen. Sporozoites will find their way into extraintestinal sites to develop into tissue cysts in visceral organs, muscular and neural tissues (El-Ashram et al., 2015). A substantial amount of research on separation of eimerian oocysts and nematode eggs from faecal materials has been published over the last decade. Techniques for the concentration and purification of oocysts and eggs from faecal samples include saturated salt solution floatation, sucrose density, zinc sulphate and Percoll discontinuous density gradient centrifugation (Kawazoe et al., 1992). For example, sucrose gradient ultracentrifugation has been exploited to separate subcellular fractions of *Eimeria tenella* sporozoites. Recently, attention has focused on the isolation of endocytic organelles from macrophages by sucrose gradient (Lamberti et al., 2015). Furthermore, discontinuous sucrose gradients and isopycnic Percoll gradients have been used to isolate *Cryptosporidium* spp. oocysts and sporozoites (Arrowood & Sterling, 1987). Discontinuous sucrose gradient has been reported to be a simple and rapid procedure to obtain viable *Cryptosporidium* spp. oocysts (Bautista et al., 1999). In addition, *T. gondii* tachyzoites, which were obtained by two consecutive discontinuous sucrose gradient separations maintain their biological activity (Garberi et al., 1990). However, the aforementioned procedures are cumbersome, and tachyzoite yield is low. Although, it is technically possible to obtain pure zoites from oocysts collected from cat faeces using the sucrose gradient method; however, the method remains complicated. Currently, in vivo research on *Toxoplasma* using cats is very expensive because cats are an expensive species that are difficult to breed as their yields are very low. Therefore, most research on *T. gondii* is carried out using tachyzoites, which can be harvested in significant quantities from experimentally infected human foreskin fibroblasts (HFFs), the peritoneal cavity/exudates of experimentally infected mice, brain tissues and HFF cells. However, to obtain *T. gondii* tachyzoites from purification of murine peritoneal exudates, brain tissues and HFF cells remains a serious challenge. In this short communication, we report a very simple approach for the isolation of highly purified tachyzoites and bradyzoites from infected human foreskin fibroblasts (HFFs) and peritoneal exudates of mice.

Highlights

- Purification of *Toxoplasma gondii* tachyzoites and bradyzoites
- Harvested cells and tissues were incubated in digestive solution
- Preparation viability was assessed by flow cytometry and re-infection
- The new method is convenient and inexpensive.

2 | MATERIALS AND METHODS

Six- to eight- week-old specific pathogen free (SPF) female BALB/c mice were purchased from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences (Beijing, China). Two genotypes of *T. gondii* were used in this study: the avirulent type II *T. gondii* Prugniaud (Pru) strain and the virulent *T. gondii* RH strain. The avirulent type II *T. gondii* Prugniaud (Pru) strain maintained by passage every 4–5 weeks in mice by oral infection with five cysts where tachyzoites of *T. gondii* RH strain were kept stored in liquid nitrogen. The experimental infection was carried out by inoculating tachyzoites of the *T. gondii* RH strain to mice intraperitoneally. Successful *T. gondii* infection was achieved within 4–5 days after intraperitoneal infection, followed by recovery of the parasites from the peritoneal exudate. Three days after infection, mice were sacrificed by cervical dislocation. Furthermore, HFF cells were incubated in DMEM medium (HyClone) supplemented with 8% fetal bovine serum (HyClone) and infected with the *T. gondii* RH strain tachyzoites ($1x10^7$ parasites) for 72 hr. Tachyzoites and tachyzoites were collected from the peritoneal cavity and brain tissues, respectively in 5 ml phosphate buffered saline (PBS). Peritoneal cells and brain tissues

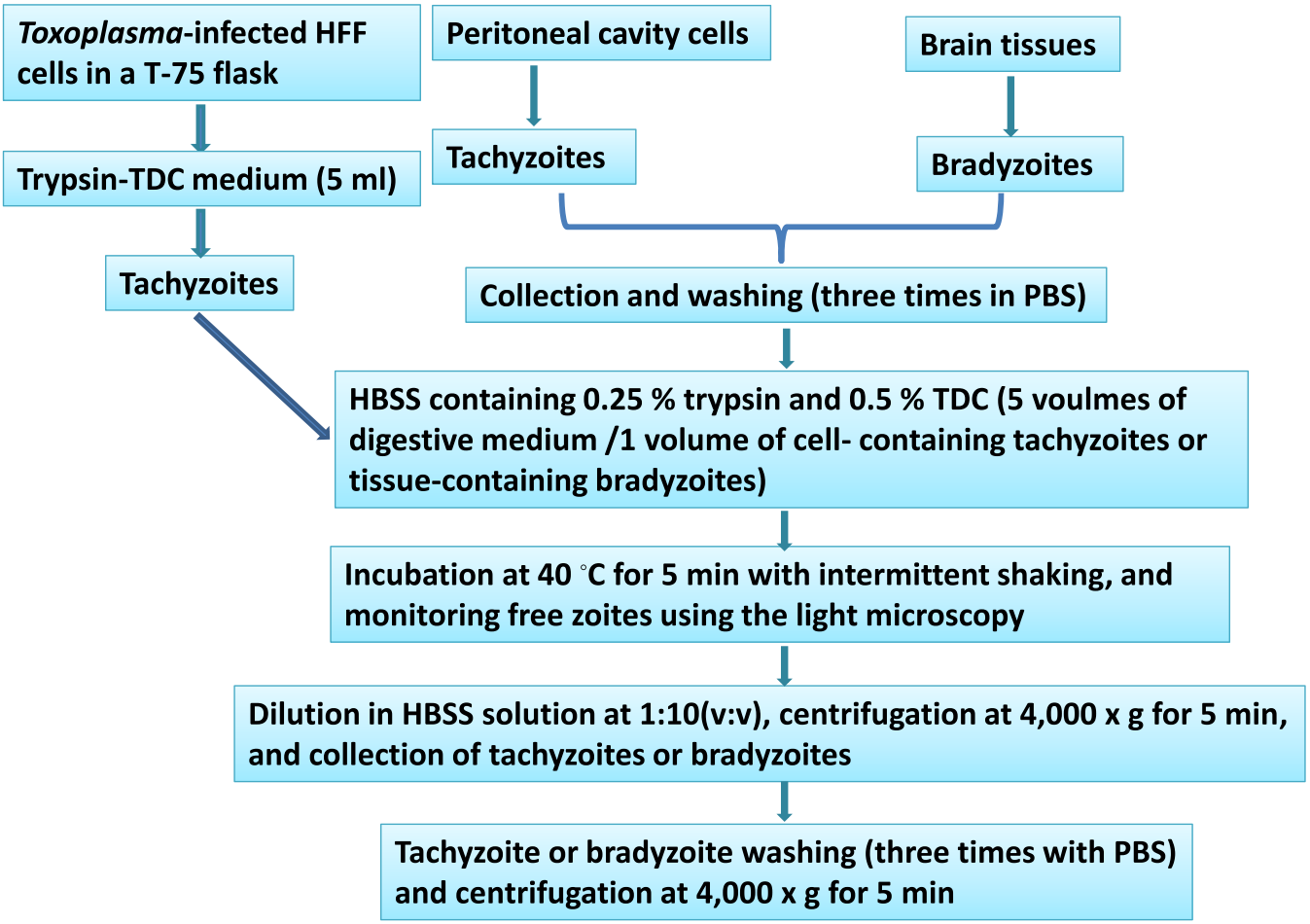

FIGURE 1 Flow chart summarizing the purification steps of *T. gondii* tachyzoites and bradyzoites

were washed three times in PBS, placed in 5 volumes of digestive medium (i.e. 5 volumes of digestive medium/ 1 volume of cell- or tissue-containing zoites)—Hanks balanced salt solution (HBSS) containing 0.25% trypsin and 0.5% taurodeoxycholic acid (TDC). After their washing thrice with PBS, 5 ml of the trypsin-TDC medium was added to a T-75 flask containing *Toxoplasma*-infected HFF cells. The mixture was incubated at 40°C for 5 min with intermittent shaking and free zoites were monitored by the light microscopy. The zoite-containing solution was diluted 10 times in HBSS solution at 1:10 (v:v). Tachyzoites or bradyzoites were collected by centrifugation at 4,000 × g for 5 min, and supernatant was discarded (Figure 1). Tachyzoites were washed three times with PBS, centrifuged at 4,000 × g for 5 min and propagated in mice by intraperitoneal inoculation. HFFs with 80%–90% confluence were infected with tachyzoites. The viability of the purified zoites was detected by a flow cytometer (FCM) as follows: the isolated tachyzoites and bradyzoites were added to Eppendorf tubes containing 5 μl of Annexin V-FITC (fluorescein isothiocyanate conjugated), 5 μl propidium iodide (PI) or 1.5 ml PBS. Furthermore, Annexin$^+$/PI$^-$(early apoptosis) and Annexin$^+$/PI$^+$(late apoptosis or already dead) parasites were sorted by fluoresence-activated cell sorter (FACS)(Demchenko, 2013; Yin, et al., 2015; Yin et al., 2015).

3 | RESULTS AND DISCUSSION

HFF and murine peritoneal exudates of *T. gondii* RH strain-infected HFF cells (Figure 2a,b) and mice (Figure 2c,d) are shown below before and after purification, respectively. Additionally, the purified bradyzoites are displayed in Figure 3. The viability of tachyzoites and bradyzoites was established by flow cytometry and re-infectivity. As shown in Figure 4, more than 99% of collected parasites were Annexin$^-$/PI$^-$, indicating the high viability of parasites.

There are several published reports on purification of *Toxoplasma* tachyzoites by passing the parasite through a 25-gauge needle, filtration through a 3-μm pore size, sucrose gradient density centrifugation (Garberi et al., 1990; Khan & Grigg, 2017). *Toxoplasma* tachyzoite prurification can also be achieved by sonic vibration and trypsin digestion (Tsunematsu, 1960) or by a sintered glass filter. These procedures are often complex and pose scale-up challenges. Additionally, recovery rates can be low, and some methods have failed to produce pure tachyzoite preparations. In this study, we developed a rapid and simple method for purifying tachyzoites and bradyzoites with optimal yield and purity. The new proposed method has the following advantages: (a) zoites can be obtained quickly and easily; (b) the technique can be performed in low resource settings and capable of handling

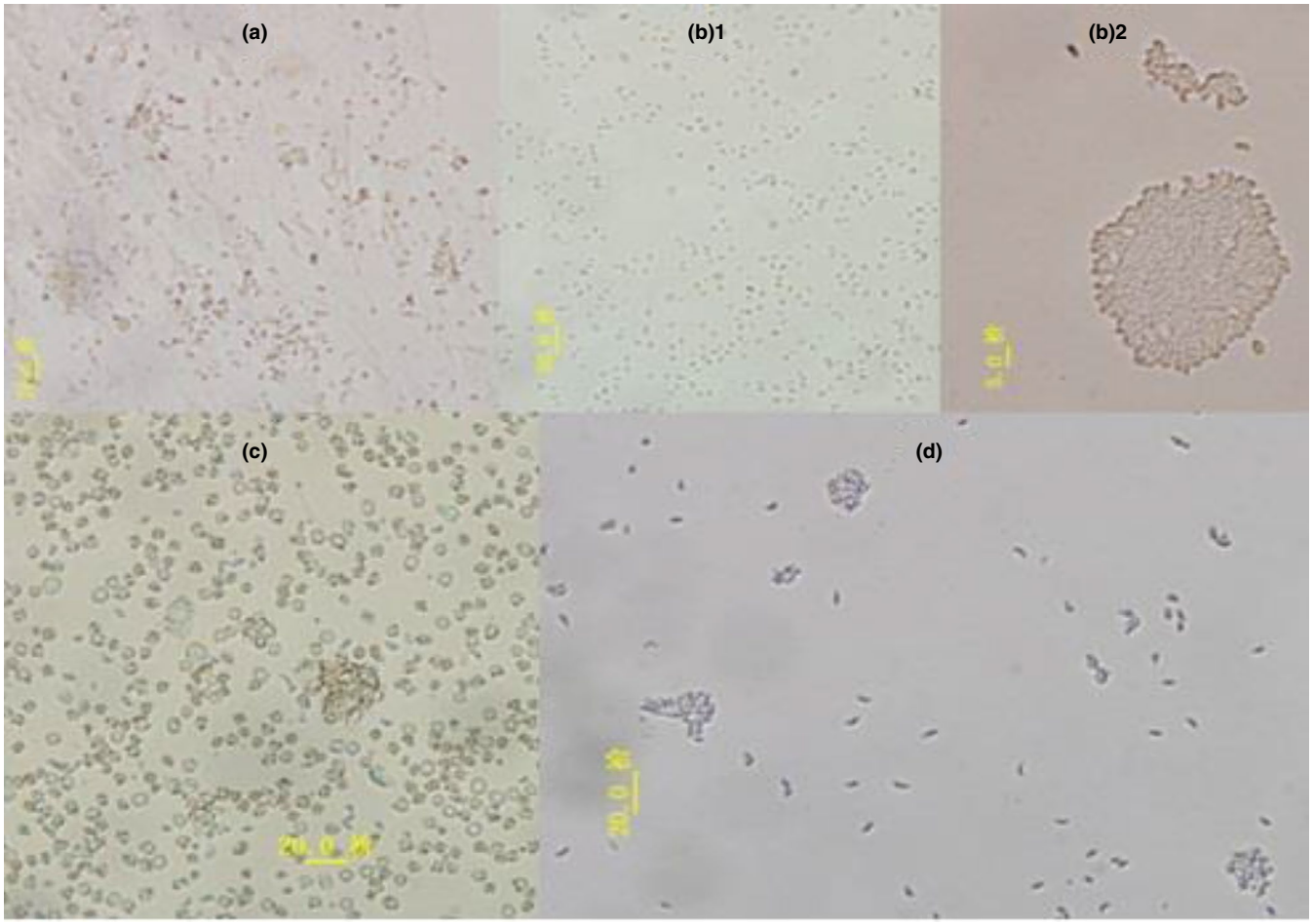

FIGURE 2 Tachyzoite-infected human foreskin fibroblast (HFF) cell line (a) and purified tachyzoites from HFF cell line (b1 and b2); and tachyzoite-infected peritoneal cells (c) and purified tachyzoites from HFF cell line (d)

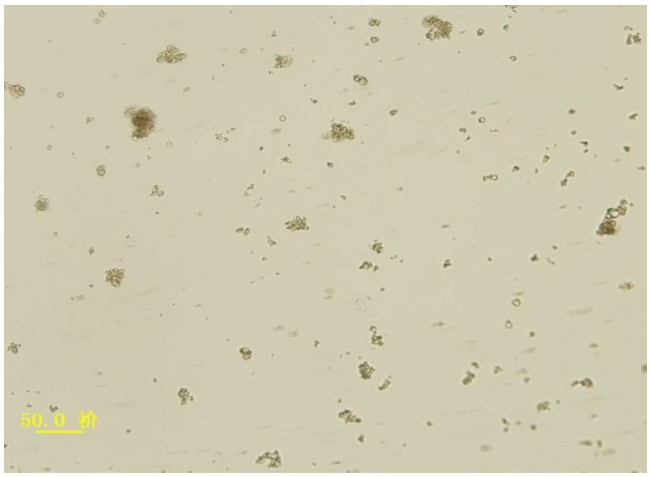

FIGURE 3 Purified bradyzoites from infected murine brain

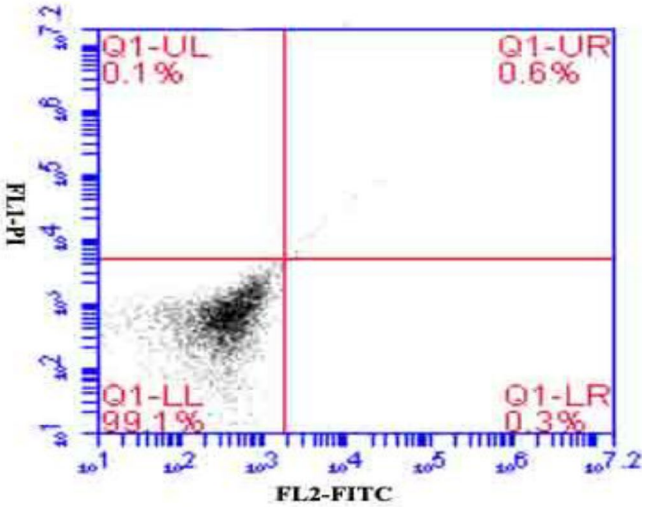

FIGURE 4 Viability of *T. gondii* tachyzoites by flow cytometry

larger numbers of cells and tissues; (c) zoites obtained in this way are free of cellular debris; and (d) the viability and infectivity of tachyzoites and bradyzoites are maintained. A more detailed study is required to explore the effect of trypsin on surface antigens of parasites.

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CONFLICTS OF INTEREST

The author declares no conflicts of interest.

AUTHOR CONTRIBUTIONS

Saeed El-Ashram: Conceptualization, Investigation and Methodology.

Saeed El-Ashram, Yu Zhang, Yongsheng Ji, Dina Salama, Kun Mei, Li Zhili, Huang Shujian, Haoji Zhang, Shawky M. Aboelhadid, Reem A. Alajmi, Dina M. Metwally, Manal F. El-Khadragy, Billy M. Hargis, Guillermo Tellez-Isaias, Beniamino T. Cenci-Goga, Musafiri Karama, Munyaradzi C. Marufu, Fathi Abouhajer, Gamal Ali Abdelhafez Hamady, Abeer El Wakil, Ibrahim Al Nasr and Xun Suo: Writing-original draft, Writing-review and editing.

AUTHOR CONTRIBUTION

Saeed El-Ashram: Conceptualization; Investigation; Methodology; Writing-original draft; Writing-review & editing. **Yu Zhang:** Writing-original draft; Writing-review & editing. **Yongsheng Ji:** Writing-original draft; Writing-review & editing. **Dina Salama:** Writing-original draft; Writing-review & editing. **Kun Mei:** Writing-original draft; Writing-review & editing. **Li Zhili:** Writing-original draft; Writing-review & editing. **Huang Shujian:** Writing-original draft; Writing-review & editing. **Haoji Zhang:** Writing-original draft; Writing-review & editing. **Shawky M Aboelhadid:** Writing-original draft; Writing-review & editing. **Reem Reem Alajmi:** Writing-original draft; Writing-review & editing. **dina metwally:** Writing-original draft; Writing-review & editing. **Manal El-Khadragy:** Writing-original draft; Writing-review & editing. **Billy Hargis:** Writing-original draft; Writing-review & editing. **Guillermo Tellez:** Writing-original draft; Writing-review & editing. **Beniamino Cenci-Goga:** Writing-original draft; Writing-review & editing. **Musafiri Karama:** Writing-original draft; Writing-review & editing. **Munyaradzi Marufu:** Writing-original draft; Writing-review & editing. **Fathi Abouhajer:** Writing-original draft; Writing-review & editing. **gamal ali abdelhafez hamady:** Writing-original draft; Writing-review & editing. **Abeer El Wakil:** Writing-original draft; Writing-review & editing. **Xun Suo:** Writing-original draft; Writing-review & editing.

ETHICAL STATEMENT

All experiments were conducted according to the ethical standards and protocols approved by the Committee of Animal Experimentation of College of Life Science and Engineering, Foshan University, China (permission number 2019- FOSU- 04).

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