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

Saeed El-Ashram¹,² | Yu Zhang¹ | Yongsheng Ji³ | Dina Salama⁴,⁵ | Kun Mei¹ | Li Zhi³ | Huang Shujian¹ | Haoji Zhang¹ | Shawky M. Aboelhadi⁵ | Reem A. Alajmi⁶ | Dina M. Metwally⁶ | Manal F. El-Khadragy⁷ | Billy M. Hargis⁸ | Guillermo Tellez-Isaias⁸ | Beniamino T. Cenci-Goga⁹ | Musafiri Karama¹⁰ | Munyaradzi C. Marufu¹¹ | Fathi Abouhajer¹² | Gamal Ali Abdelhafez Hamady¹³ | Abeer El Wakil¹⁴ | Ibrahim Al Nasr¹⁵,¹⁶ | Xun Suo¹⁷

¹College of Life Science and Engineering, Foshan University, Foshan, Guangdong Province, China
²Faculty of Science, Kafrelsheikh University, Kafr el-Sheikh, Egypt
³School of Basic Medical Sciences, Division of Life Science and Medicine, University of Science and Technology of China, Hefei, China
⁴Department of Parasitology and Animal Disease, National Research Centre, Dokki, Giza, Egypt
⁵Parasitology Department, Faculty of Veterinary Medicine, Beni Suef University, Beni-Suef, Egypt
⁶Department of Zoology, Faculty of Science, King Saud University, Riyadh, Saudi Arabia
⁷Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt
⁸Department of Poultry Science, University of Arkansas, Fayetteville, AR, USA
⁹Dipartimento di Medicina Veterinaria, Laboratorio di Ispezione degli Alimenti di Origine Animale, University of Perugia, Perugia, Italy
¹⁰Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
¹¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
¹²Faculty of Education, Asmara University for Islamic Sciences, Zliten, Libya
¹³Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt
¹⁴Department of Biological & Geological Sciences, Alexandria University, Alexandria, Egypt
¹⁵College of Science and Arts in Unaizah, Qassim University, Unaizah, Saudi Arabia
¹⁶College of Applied Health Sciences in Ar Rass, Qassim University, Ar Rass, Saudi Arabia
¹⁷National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing, China

**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...
purity (Garberi et al., 1990). However, the aforementioned procedures of continuous sucrose gradient separations maintain their biological activity. 

Toxoplasma gondii has been reported to be a simple and rapid procedure to obtain tachyzoites (Arrowood & Sterling, 1987). Discontinuous sucrose gradient 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). 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.

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.

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 T. gondii RH strain tachyzoites (1x10⁷ 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...
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), centrifugation at 4,000 × g for 5 min, and collection of tachyzoites or bradyzoites

Toxoplasma-infected HFF cells in a T-75 flask

Trypsin-TDC medium (5 ml)

Collection and washing (three times in PBS)

HBSS containing 0.25 % trypsin and 0.5 % TDC (5 volumes of digestive medium /1 volume of cell- containing tachyzoites or tissue-containing bradyzoites)

Incubation at 40 °C for 5 min with intermittent shaking, and monitoring free zoites using the light microscopy

Dilution in HBSS solution at 1:10(v:v), centrifugation at 4,000 x g for 5 min, and collection of tachyzoites or bradyzoites

Tachyzoite or bradyzoite washing (three times with PBS) and centrifugation at 4,000 x g for 5 min

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

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.

3 | RESULTS AND DISCUSSION

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 purification 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...
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.

**ACKNOWLEDGEMENTS**

This work was supported by the National Natural Science Foundation of China (No. 31502071), Youth Innovative Talents Project of Guangdong province Education Department (No. 2017KQNCX212), Guangdong province (2017GDK07), Start-up Research Grant Program.
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. Aboelhaidid, Reem A. Alajmi, Dina M. Metwally, Manal F. El-Khadragy, Billy M. Hargis, Guillermo Tellez-Isais, Beniamino T. Cenci-Goga, Musafiri Karama, Munyaradzi C. Marufu, Fathi Abouhajer, Gamal Ali Abdelhafiez Hamady, Abeer El Wakil, Ibrahim Al Nasr and Xun Suo: Writing-original draft, Writing-review and editing.

AUTHOR CONTRIBUTIONS
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 Aboelhaidid: Writing-original draft; Writing-review & editing. Reem Reem Alajmi: Writing-original draft; Writing-review & editing. Manal F. 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 Abdelhafiez 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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How to cite this article: El-Ashram S, Zhang Y, Ji Y, et al. A rapid and simple single-step method for the purification of Toxoplasma gondii tachyzoites and bradyzoites. Vet. Med. Sci. 2021;7:357–361. https://doi.org/10.1002/vms3.364

ORCID
Saeed El-Ashram https://orcid.org/0000-0003-0389-1980