Journal Pre-proof

Temperature is a key factor influencing the invasion and proliferation of *Toxoplasma gondii* in fish cells

Yun Yang, Shao-Meng Yu, Ke Chen, Geoff Hide, Zhao-Rong Lun, De-Hua Lai

PII: S0014-4894(20)30203-4

DOI: https://doi.org/10.1016/j.exppara.2020.107966

Reference: YEXPR 107966

To appear in: *Experimental Parasitology*

Received Date: 22 April 2020

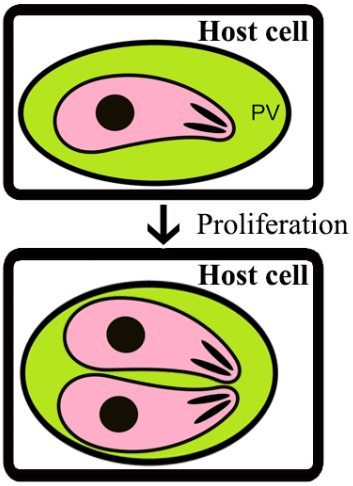
Revised Date: 15 July 2020

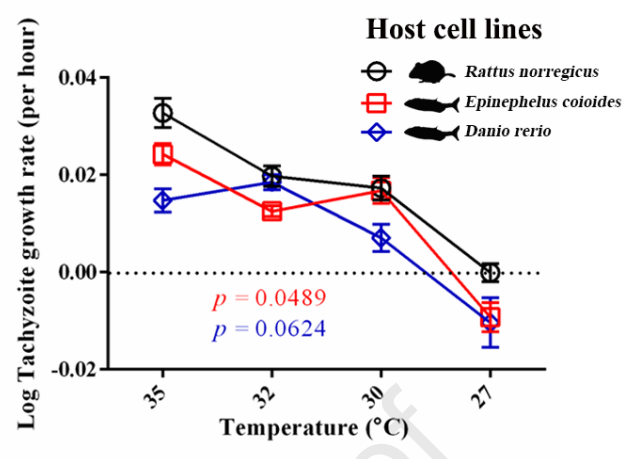
Accepted Date: 5 August 2020

Please cite this article as: Yang, Y., Yu, S.-M., Chen, K., Hide, G., Lun, Z.-R., Lai, D.-H., Temperature is a key factor influencing the invasion and proliferation of *Toxoplasma gondii* in fish cells, *Experimental Parasitology* (2020), doi: https://doi.org/10.1016/j.exppara.2020.107966.

CRediT authorship contribution statement

Yun Yang: Formal analysis, Investigation, Visualization, Writing - Original Draft. **Shao-Meng Yu:** Validation, Investigation. **Ke Chen:** Investigation, Resources. **Geoff Hide:** Writing - Review & Editing. **Zhao-Rong Lun:** Conceptualization, Methodology, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **De-Hua Lai:** Conceptualization, Writing - Original Draft, Writing - Review & Editing, Funding acquisition.





Temperature is a key factor influencing the invasion and proliferation of *Toxoplasma gondii* in fish cells

Yun Yang[a,#], Shao-Meng Yu[a], Ke Chen[a], Geoff Hide[b], Zhao-Rong Lun[a,b*], De-Hua Lai[a*]

[a] Center for Parasitic Organisms and Guangdong Provincial Key Laboratory of Aquatic Economic Animals, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, PR China

[b] Biomedical Research Centre and Ecosystems and Environment Research Centre, School of Science, Engineering and Environment, University of Salford, Salford, M5 4WT, UK

[#] Present address: Tsinghua-Peking Center for Life Sciences, School of Medicine, Tsinghua University, Beijing 100084, PR China

[*]Corresponding authors at: ZRL, lsslzr@mail.sysu.edu.cn; DHL, laidehua@mail.sysu.edu.cn

Abstract:

Toxoplasma gondii has long been considered a ubiquitous parasite possessing the capacity of infecting virtually all warm-blooded animals globally. Occasionally, this parasite can also infect cold-blooded animals such as fish if their body temperature reaches 37°C. However, we are currently lacking an understanding of key details such as the minimum temperature required for *T. gondii* invasion and proliferation in these cold-blooded animals and their cells. Here, we performed *in vitro T. gondii* infection experiments with rat embryo fibroblasts (REF cells), grouper (*Epinephelus coioides*) splenocytes (GS cells) and zebra fish (*Danio rerio*) hepatocytes (ZFL cells), at 27°C, 30°C, 32°C, 35°C and 37°C, respectively. We found that *T. gondii* tachyzoites could penetrate REF, GS nd ZFL cells at 27°C but clear inhibition of multiplication was observed. Intriguingly, the intracellular tachyzoites retained the ability to infect mice after 12 days of incubation in GS cells cultured at 27°C as demonstrated by bioassay. At 30°C, 32°C and 35°C, we observed that the mammalian cells (REF cells) and fish cells (GS and ZFL cells) could support *T. gondii* invasion and replication, which showed a temperature-dependent relationship in infection and proliferation rates. Our data demonstrated that the minimum temperature for *T. gondii* invasion and replication was 27°C and 30°C respectively, which indicated that temperature is a key factor for *T. gondii* invasion and proliferation in host cells. This suggests that temperature-dependent infection determines the differences in the capability of *T. gondii* to infect cold- and warm-blooded vertebrates.

Key words: *Toxoplasma gondii*; invasion; proliferation; fish cells; *in vitro* cultivation;

cold-blooded animals

1. Introduction

Toxoplasma gondii is an important intracellular protozoan parasite, which is distributed globally and can infect almost all warm-blooded animals, including humans, but is thought to be incapable of infecting cold-blooded animals except as transport hosts (Dlugonska, 2017). Felids are the definitive host of *T. gondii*, in which sexual reproduction occurs in the epithelial cells of the small intestine, leading to the production of unsporulated oocysts in feces. Virtually, all warm-blooded vertebrates can be infected with oocysts and serve as intermediate hosts in which *T. gondii* undergoes asexual reproduction ultimately forming dormant tissue cysts (Halonen and Weiss, 2013). In general, warm-blooded animals and humans can also be infected via consumption of water or meat contaminated with *T. gondii* oocysts or cysts (Dubey and Beattie, 1988). In most warm-blooded animals, including humans, infection is asymptomatic but acute infection during pregnancy is detrimental to the developing fetus and can result in devastating neurological impairment or abortion (Hampton, 2015).

There is growing concern that toxoplasmosis is a waterborne zoonosis being transmitted to animals and humans through water contaminated with oocysts (Dubey, 2004). A large outbreak of human toxoplasmosis was shown to be linked with oocyst contamination of a municipal water reservoir in Canada by wild felids infected with *T. gondii* (Bowie et al., 1997), suggesting that infective oocysts are stable in aquatic environments. This is further supported by reports that sporulated *T. gondii* oocysts can survive in seawater for at least 24 months (Lindsay and Dubey, 2009). Meanwhile,

71 substantial evidence has shown that poikilothermic animals are capable of serving as
72 vectors of *T. gondii* oocysts, including oysters (Lindsay et al., 2001), mussels (Arkush
73 et al., 2003, Miller et al., 2008), filter feeding fish (Massie et al., 2010), abalones
74 (Schott et al., 2016) and gammarids (Bigot-Clivot et al., 2016). These findings above
75 suggest that *T. gondii* is likely to encounter cold-blooded animals in aquatic
76 environments, which raises an interesting question as to whether *T. gondii* can
77 penetrate cells of cold-blooded animals and replicate as tachyzoites.
78 As an obligate intracellular parasite, invasion of host cells is the most important
79 first step for *T. gondii* to establish a successful infection. Studies on cells from
80 cold-blooded animals indicated that they could be infected by *T. gondii* via
81 tachyzoites at a temperature of 37°C (Omata et al., 2005). Additionally, an *in vivo*
82 study on zebrafish at 37°C showed that intraperitoneal injection of *T. gondii* cysts led
83 to the multiplication of tachyzoites in different tissues including heart, blood vessel,
84 liver parenchyma, spleen and brain with corresponding histological and pathological
85 changes, resembling the situation found in acute infection in mammals (Sanders et al.,
86 2015). However, so far as we know, there is no evidence that *T. gondii* tachyzoites
87 invade and proliferate in the cells of cold-blooded animals at temperatures below
88 37°C. This suggests that the major limitation on *T. gondii* establishing an infection in
89 cold-blooded vertebrates may be highly dependent on temperature.
90 In order to find the minimum temperature for *T. gondii* invasion and replication,
91 we performed experiments at a range of temperatures with cells from rat (*Rattus*
92 *norvegicus*), grouper (*Epinephelus coioides*) and zebra fish (*Danio rerio*).

2. Materials and methods

2.1. Mice and rats

All mice (Swiss Webster, male) and rats (SD, female) were purchased from the Experimental Animal Center of Sun Yat-Sen University, at age of 6-8 weeks (weighing 20 - 25 g for mice and 150 - 200 g for rats). All animals were strictly maintained in a special pathogen free room with ad libitum access to food and water according to protocols approved by the Laboratory Animal Use and Care Committee of Sun Yat-Sen University under license NO. 2017YFD0500400.

2.2. Cell culture and staining

Rat embryo fibroblasts (REF cells) were derived from an embryo collected from the uterus of a healthy pregnant SD rat after euthanization. Grouper (*Epinephelus coioides*) splenocytes (GS cells) and zebrafish (*Danio rerio*) hepatocyte (ZFL cells) cell lines were gifted by Professor Qi-Wei Qin from the College of Marine Sciences, South China Agricultural University and Professor Wen-Shen Li from the School of Life Sciences, Sun Yat-Sen University. REF cells were maintained in RPMI 1640 medium supplemented with 10% FBS (fetal bovine serum) and 100 units/ml of penicillin and 100 μg/ml streptomycin at 37°C. GS cells were maintained in L-15 medium supplemented with FBS, penicillin and streptomycin as described at 27°C (Liang et al., 2018). While, ZFL cells were maintained in 50% L-15 medium and 40% RPMI 1640 medium supplemented with 10% FBS, penicillin and streptomycin at

115 27°C as described (Lopes et al., 2018). After digestion with 0.25% trypsin and 0.02%
116 EDTA, REF, GS and ZFL cells were harvested and they were seeded onto the wells of
117 a 24-well plate at an initial density of 10^5/well, and non-adherent cells were washed
118 away after 12 h incubation at the temperature described above.
119
120 2.3. *Toxoplasma gondii* strains and infection
121 Tachyzoites of the *T. gondii* RH strain, a type I strain initially isolated from a
122 toxoplasmic encephalitis case in 1939 which showed faster growth, enhanced
123 extracellular survival rate and inability to form tissue cysts after a long history of
124 passage in mice and *in vitro* cultivation (Yang et al., 2013). The RH strain used in this
125 study was genetically manipulated to express green fluorescence protein (Nishikawa
126 et al., 2003) and was cryopreserved in liquid nitrogen before use. After thawing,
127 tachyzoites were inoculated into two mice and passaged into another two mice. At the
128 late stage of infection (usually 4 days post infection), infected animals were
129 euthanized, ascites were collected after injection of 5 ml PBS (phosphate buffer saline,
130 pH 7.4) into the peritoneal cavity. Macrophages were removed from ascites by adding
131 0.25% trypsin and 0.02% EDTA as a final concentration (Derouin et al., 1987). After
132 centrifugation at 4°C, 2000 ×g for 10 min, pelleted tachyzoites were resuspended in
133 culture medium at 27°C (see below) and quantified using a haemocytometer.
134 The host cells (REF, GS and ZFL cells) were placed at the designed experimental
135 temperatures (27°C, 30°C, 32°C, 35°C and 37°C) for over one hour before an
136 infection assay. After 12 hours of incubation with *T. gondii* using a 5 to 10 multiplicity

of infection (MOI), free tachyzoites were washed away with fresh medium and more than 300 infected cells were counted under a fluorescent microscope. The infection rate was represented by the ratio of infected cells/total cells. Meanwhile, at the time points of 12 h, 24 h and 36 h, numbers of *T. gondii* tachyzoites in 100 host cells were counted by fluorescence microscopy (Vert A1, Zeiss).

Otherwise, cells were seeded onto coverslips as above, and were washed with fresh medium and stained by Diff-Quik. Samples were then mounted with neutral resins and observed by microscopy (Imager A1, Zeiss).

2.4. Mouse bioassay

In order to detect the ability of *T. gondii* to infect mice when continually cultured in GS cells at 27°C, tachyzoites were collected from the infected GS cells after 12 days infection and were intraperitoneally inoculated into 10 mice, each with 3×10^4 tachyzoites (Gao et al., 2017). Tachyzoites kept in media at 27°C for 12 days were inoculated as an extracellular group (10 mice), and GS cells were used as negative control (10 mice). Inoculated mice were monitored daily.

2.5. Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 6.0 software. All data were showed as the means $\pm$ standard deviations of at least three independent experiments. Data were compared and analyzed by the Student's paired *t* test, and $P < 0.05$ was accepted as statistically significant.

3. Results

3.1 Growth of *T. gondii* in mammalian and fish cells at 27°C and 37°C

We firstly investigated the possible invasion and development of *T. gondii* tachyzoites in the mammalian REF cells at 27°C. *T. gondii* tachyzoites were collected from an infected mouse and resuspended in 27°C culture medium, added to the REF cells, and incubated at 27°C. To our surprise, a few tachyzoites could be found in parasitophorous vacuoles (PV), although proliferation was obviously limited and could only be observed at a later stage at 72 hours post inoculation (hpi). While the control group at 37°C, which was treated in the same way, showed normal multiplication of tachyzoites in PV at 48 hpi. We also applied the same test in cold-blooded fish cells. Similarly, in the GS cells cultured at 37°C, multiplication of tachyzoites was found at 24 hpi even though some of the GS cells shrunk and rounded up as 37°C is not a suitable temperature for them. While at 27°C, *T. gondii* tachyzoites were still able to penetrate into the GS cells but the proliferation was not observed at 72 hpi (Fig. 1A). A further observation, taking advantage of detection of fluorescence in the *T. gondii* strain, also revealed a decreasing number of intracellular tachyzoites from day 1 till day 9 (data not shown).

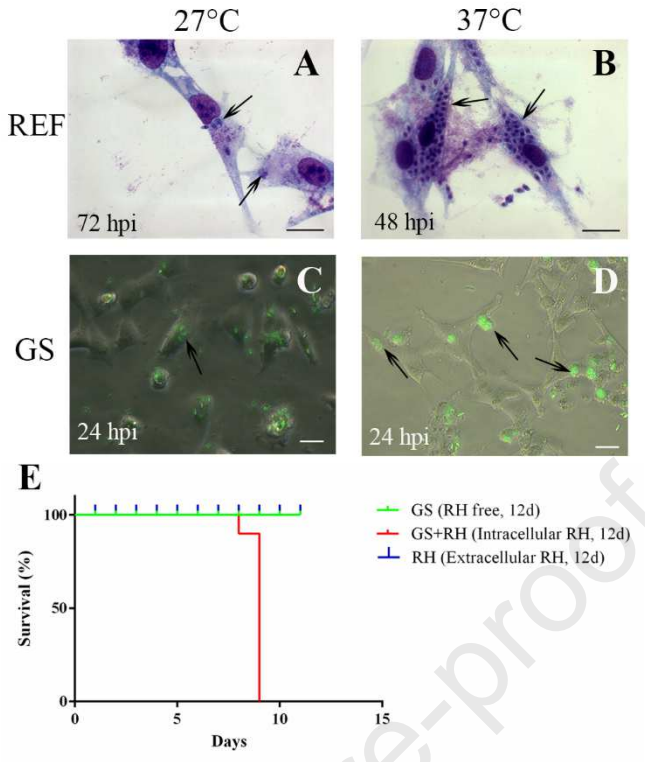


Fig. 1. REF and GS cells infected with *T. gondii* tachyzoites at 27°C and 37°C respectively and subsequent bioassays after 12 days cultured at 27°C.

A. A few tachyzoites (arrows) were observed within the REF cells cultured at 27°C, 72 hours post infection (hpi). B. Many tachyzoites (arrows) were found within the infected REF cells cultured at 37°C, 48 hpi (A, B. Cells were stained with Diff-Quik). C. Proliferation of tachyzoites was significantly inhibited in GS cells cultured at 27°C, 24 hpi (arrows showing several intracellular tachyzoites located in few parasitophorous vacuoles (PVs) in a one-to-one manner but multiplication was not found). D. Replication of tachyzoites was observed in GS cells at 37°C, 24 hpi (arrows showing multiplying intracellular tachyzoites). All infections in A-D were done with MOI = 10 unless specified and all scale bars are equal to 20 μm. E. The survival rate of mice inoculated with intracellular *T. gondii*, extracellular *T. gondii* and GS cells respectively. GS cells infected with *T. gondii* (MOI = 5, GS+RH group) and extracellular tachyzoites without host cells (RH group) were incubated at 27°C for 12 days, which were then harvested, quantified and inoculated into 10 mice intraperitoneally (3 x 10^4 tachyzoites per mouse) each group. GS cells (GS group) without *T. gondii* infection were inoculated to 10 mice as a control.

196

197 To reveal the viability of the tachyzoites, a bioassay was carried out by inoculating
198 10 mice with a fixed amount of fluorescent tachyzoites (3×10^4) from the infected GS
199 cells at 27°C harvested on day 12. A survival curve revealed that the intracellular
200 tachyzoites remained infective, while the extracellular tachyzoites and
201 tachyzoites-free GS cells from parallel experiments (12 days in medium) were not
202 (Fig. 1B).

203 Together, the tachyzoites of *T. gondii* could invade both mammalian and fish cells
204 at 37°C and 27°C, and retain viability for at least 12 days post infection at 27°C,
205 although the proliferation was clearly inhibited.

206

207 **3.2 Growth of *T. gondii* tachyzoites at 35°C, 32°C, 30°C and 27°C**

208 To identify how the temperature affects the growth of tachyzoites, we performed
209 experiments with a temperature series at 35°C, 32°C, 30°C and 27°C, respectively,
210 using mammalian (REF) and fish (GS, ZFL) cells. It is intriguing that GS and ZFL
211 cells grew well at 35°C, as did the invaded *T. gondii* parasites, both in a similar
212 manner to growth in the REF cells (Fig. 2 A). Quantitative analysis revealed that
213 tachyzoites could grow well in REF, GS and ZFL cells from 30°C to 35°C, but not at
214 27°C (Fig. 2 A-D). Infection rates in these groups were also determined at 12 hpi, and
215 mild host preferences were observed at the tested temperatures (Fig. 2E). Clearly, the
216 growth of *T. gondii* in REF, GS and ZFL cells at the tested temperature series
217 indicated a temperature-dependent manner (Fig. 2F). However, to our surprise, a

significant difference in growth rates was found between mammalian REF and fish GS cells (p = 0.049), while the difference between REF and fish ZFL cells approached significance (p = 0.062).

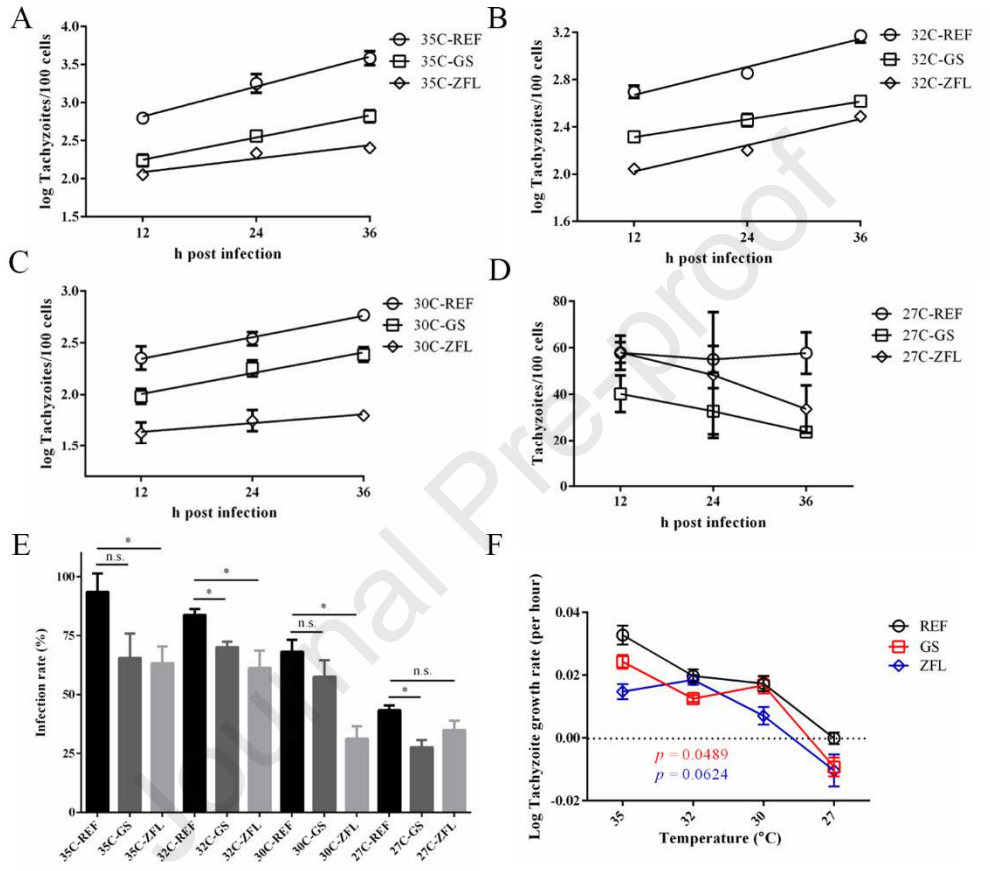


Fig. 2. The status of growth and infection rates of *T. gondii* in REF, GS and ZFL cells cultured at 35°C, 32°C, 30°C and 27°C.

A, B, C and D, logarithmic scale (or linear scale for D) growth curves of tachyzoites in REF, GS and ZF-L cells cultured at 35°C (A), 30°C (B), 32°C (C) and 27°C (D), respectively. Tachyzoites were assessed in around 100 cells per well using fluorescent microscopy. E, infection rates of REF, GS and ZFL cells cultured at 35°C, 32°C, 30°C and 27°C, respectively. More than 200 cells per well were counted under an inverted fluorescence microscope at 12h post infection with tachyzoites and the infection rate was expressed as the ratio of infected cells to total cells. F, Logarithmic scale curves of tachyzoite growth rates (per hour) in REF, GS and ZFL cells cultured at 35°C,

32°C, 30°C and 27°C, respectively. All infections were done with a MOI = 6. Each measurement was performed in triplicate and repeated three times (n = 3) and all presented values are meaning ± SD. Statistical significance was indicated by using a paired Student's *t* test between different groups. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; N.S., not significant.

Furthermore, we selected temperatures of 32°C and 27°C to get a clearer visual observation of tachyzoite infection in REF, GS and ZFL cells at 24 hpi. Consistent with the experiments above, *T. gondii* showed obvious multiplication in REF, GS and ZFL cells at 32°C, while no signs of proliferation were observed at 27°C (Fig. 3).

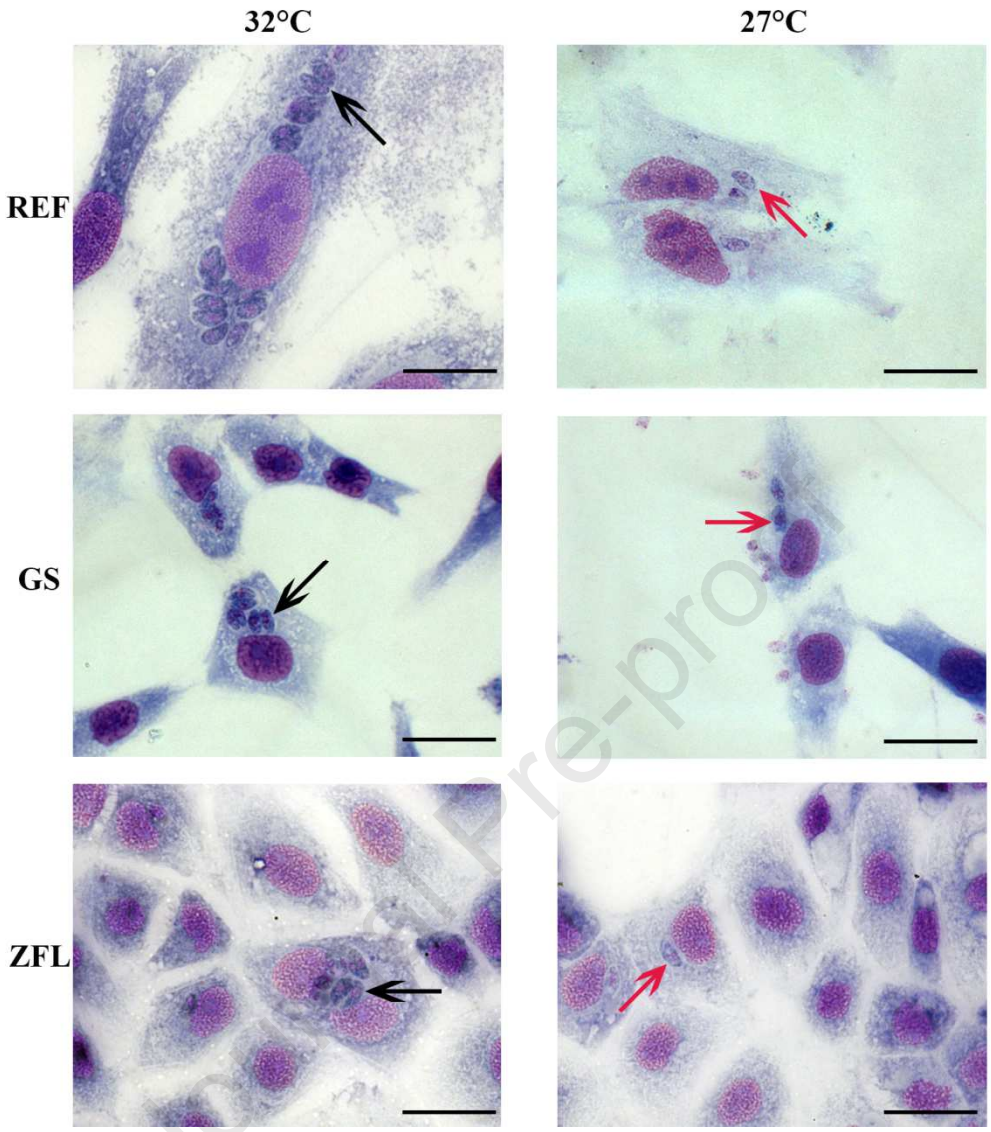


Fig. 3. The growth of *T. gondii* in REF, GS and ZFL cells cultured at 32°C and 27°C respectively and stained with Diff-Quik.

Black arrows indicate the tachyzoites within the PVs, while red arrows indicate the tachyzoites without PVs. Images were taken at 24 hpi, MOI = 6, scale bar for all photos = 20 µm.

Taken together, these results showed that at 30 - 35°C, *T. gondii* tachyzoites could invade and multiply in both marine and fresh water fish cells, although this was not as efficient as in mammalian cells.

253

254 **4. Discussion**

255 *Toxoplasma gondii* has been widely known to be capable of invading virtually all
256 nucleated cells of warm-blooded animals including humans since the discovery of this
257 marvelous parasite (Dubey et al., 1998). However, whether animals apart from
258 homothermic ones can serve as potential host for *T. gondii* remains a controversy.
259 Interestingly, *T. gondii* tachyzoites could survive in three *Aedes* cell lines at 35°C for
260 at least 15 days, as verified using intraperitoneally inoculated mice with washed cell
261 suspensions, nevertheless, no data on *T. gondii* tachyzoite proliferation was mentioned
262 in the study (Buckley, 1973). Attempts to infect insects with *T. gondii* indicated a
263 maximum of three days survival of the parasite in an *in vivo* study of four species of
264 mosquito, judged by the retained infectivity of either cysts or tachyzoites orally fed to
265 mosquitoes, whether the temperature was 26°C or 37°C (Chapman and Ormerod,
266 1966). However, the combination of the above results, suggested *T. gondii* failed to
267 penetrate mosquito cells during the *in vivo* test, whereas *T. gondii* survived for a much
268 shorter time than in the *in vitro* test. In other words, *T. gondii* may not be capable of
269 infecting insects.

270 In addition, results from some previous studies indicated that *T. gondii* could
271 survive in cold-blooded animals from fish to reptiles both under natural (Nasiri et al.,
272 2016) and experimental conditions (Sanders et al., 2015). However, cold-blooded
273 animals including filter-feeding fish and other aquatic invertebrates were only
274 considered as carriers of *T. gondii* oocysts in the wild (Arkush et al., 2003, Miller et

al., 2008, Massie et al., 2010, Bigot-Clivot et al., 2016). To our knowledge, there is no direct evidence in the literature shows that *T. gondii* can naturally multiply in the cells of wild cold-blooded animals to expand its population. Therefore, it still remains an attractive question as to what is the key factor that limits the invasion and proliferation of *T. gondii* in cold-blooded animals or their cells.

By definition, one of the fundamental distinctive differences between cold-blooded and warm-blooded animals is their body temperature; therefore, we assumed that temperature could be the prerequisite for *T. gondii* to establish invasion and proliferation in various host cells. As expected, using marine and fresh water fish cells in the present study, we demonstrated that *T. gondii* could invade and proliferate in these cells at temperatures of 30°C, 32°C and 35°C. At 27°C, a temperature lower than 30°C, invasion and proliferation of *T. gondii* tachyzoites was significantly inhibited, although parasites remained infective to mice for at least 12 days. These findings are supported by previous observations at an experimental temperature of 37°C in which *T. gondii* could establish an infection in goldfish (*Carassius auratus*) cells *in vitro* (Omata et al., 2005) and zebra fish *in vivo* (Sanders et al., 2015).

More importantly, our study also revealed a temperature dependent manner for *T. gondii* invasion and proliferation at the range of 27°C to 35°C, with higher temperature associated with greater invasion efficiency and faster growth of the parasite. Indeed, Omata and his colleagues found a similar tendency using two temperature conditions of 33°C and 37°C with goldfish cells (Omata et al., 2005). Interestingly, this temperature dependent manner is not restricted to *T. gondii* infecting

297 cold-blooded hosts, as a similar behavior was observed in the rat cells. We found that
298 tachyzoites had a slightly better growth and invasion efficiency in the rat cells than
299 the two types of fish cells, which may indicate an adaption by *T. gondii* to its natural
300 warm-blooded host. On the other hand, the viability of *T. gondii* in the fish cells for
301 12 days at 27°C also indicated a tolerance to ambient temperature (27°C) of this
302 parasite, retaining its infectivity to new host for at least 12 days.

303 It is well known that *T. gondii* is a parasite which can infect most warm-blooded
304 vertebrates, while many other related species in the Apicomplexa, such as *Eimeria*
305 *tenella* only naturally infects the cecum of chickens (Clark et al., 2017), and
306 *Plasmodium falciparum* specifically infects humans and chimpanzees (Miller et al.,
307 2002). Our findings suggest a mechanism limiting the broad host range of *T. gondii*
308 that is determined by a low-temperature-barrier preventing the infection of *T. gondii*
309 in poikilothermal animals. Therefore, although results from the earlier investigations
310 demonstrated that *T. gondii* was found in amphibians and reptiles (Nasiri et al., 2016),
311 it is unlikely that *T. gondii* can establish a persistent infection in these cold-blooded
312 animals. In other words, cold-blooded vertebrates inhabiting regions that have
313 temperatures at 30°C to 37°C (Sun, 1996, Engeszer et al., 2007, Zhang et al., 2010,
314 Woolway et al., 2016, Chaidez et al., 2017, North Temperate Lakes, 2010), may be at
315 risk of *T. gondii* infection. Therefore, an epidemiological survey for *T. gondii*
316 infections in fish and other cold-blooded animals, especially in tropical areas, could
317 obtain some very interesting results pertinent to understanding the endemic status of *T.*
318 *gondii* in these animals. Based on our current results and related published data, we

consider that temperature, rather than cell type or host, is a key contributor to the successful development of *T. gondii* in cells (and hosts) at least in vertebrates. This raises an interesting question as to the nature of host-parasite interactions that determine this temperature dependence. It can be inferred that *T. gondii* is capable of infecting nucleated cells of virtually all vertebrates, which reflects the advantages of employing *T. gondii* as a perfect model to explore the evolutionary relationships between intracellular parasites and host cells. Furthermore, the high plasticity of host-parasite interactions in *T. gondii* may be determined by such studies and provides insights into the potential of this fascinating parasite to evolve the capability of infection in cold-blooded vertebrates.

Data Availability Statement

The datasets generated during the study are available from the corresponding author on reasonable request.

Declaration of Interests

The authors declare no competing financial interests.

Acknowledgements

The GS and ZFL cell lines were kindly provided by Prof. Q.W. Qin from South China Agricultural University and Prof. Wen-Sheng Li from Sun Yat-Sen University. We also appreciate help from Prof. R. Iestyn Woolway (Centre for Ecology & Hydrology,

Lancaster, UK) for access to water temperature data. This work was supported by grants from The National Key R&D Program of China (2017YFD0500400) and The National Natural Science Foundation of China (31772445).

346 **References**

347

348 Arkush, K.D., Miller, M.A., Leutenegger, C.M., Gardner, I.A., Packham, A.E.,
349 Heckeroth, A.R., Tenter, A.M., Barr, B.C., Conrad, P.A., 2003. Molecular and
350 bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels
351 (*Mytilus galloprovincialis*). Int J Parasitol. 33(10), 1087-1097.
352 https://doi.org/10.1016/s0020-7519(03)00181-4

353 Bigot-Clivot, A., Palos Ladeiro, M., Lepoutre, A., Bastien, F., Bonnard, I., Dubey, J.P.,
354 Villena, I., Aubert, D., Geffard, O., Francois, A., Geffard, A., 2016.
355 Bioaccumulation of *Toxoplasma* and *Cryptosporidium* by the freshwater
356 crustacean *Gammarus fossarum*: Involvement in biomonitoring surveys and
357 trophic transfer. Ecotoxicol Environ Saf. 133, 188-194.
358 https://doi.org/10.1016/j.ecoenv.2016.07.006

359 Bowie, W.R., King, A.S., Werker, D.H., Isaac-Renton, J.L., Bell, A., Eng, S.B.,
360 Marion, S.A., 1997. Outbreak of toxoplasmosis associated with municipal
361 drinking water. Lancet. 350(9072), 173-177.
362 https://doi.org/10.1016/s0140-6736(96)11105-3

363 Buckley, S.M., 1973. Survival of *Toxoplasma gondii* in mosquito cell lines and
364 establishment of continuous infection in Vero cell cultures. Exp Parasitol. 33(1),
365 23-26.
366 https://doi.org/10.1016/0014-4894(73)90005-2

367 Chaidez, V., Dreano, D., Agusti, S., Duarte, C.M., Hoteit, I., 2017. Decadal trends in

368 Red Sea maximum surface temperature. Sci Rep. 7(1), 8144.
369 https://doi.org/10.1038/s41598-017-08146-z
370 Chapman, J.M., Ormerod, W.E., 1966. The survival of *Toxoplasma* in infected
371 mosquitoes. J Hyg (Lond). 64(3), 347-355.
372 https://doi.org/10.1017/s0022172400040626
373 Clark, E.L., Tomley, F.M., Blake, D.P., 2017. Are *Eimeria* Genetically Diverse, and
374 Does It Matter? Trends Parasitol. 33(3), 231-241.
375 https://doi.org/10.1016/j.pt.2016.08.007
376 Derouin, F., Mazeron, M.C., Garin, Y.J., 1987. Comparative study of tissue culture
377 and mouse inoculation methods for demonstration of *Toxoplasma gondii*. J Clin
378 Microbiol. 25(9), 1597-1600.
379 Dlugonska, H., 2017. Are poikilothermic animals real hosts for *Toxoplasma gondii*?
380 Ann Parasitol. 63(1), 3-5.
381 https://doi.org/10.17420/ap6301.77
382 Dubey, J.P., 2004. Toxoplasmosis - a waterborne zoonosis. Vet Parasitol. 126(1-2),
383 57-72.
384 https://doi.org/10.1016/j.vetpar.2004.09.005
385 Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of Animals and Man. In: Beattie, C.P.
386 (Ed.), CRC Press, Boca Raton, Florida (pp. 220)
387 Dubey, J.P., Lindsay, D.S., Speer, C.A., 1998. Structures of *Toxoplasma gondii*
388 tachyzoites, bradyzoites, and sporozoites and biology and development of tissue
389 cysts. Clin Microbiol Rev. 11(2), 267-299.

390 Engeszer, R.E., Patterson, L.B., Rao, A.A., Parichy, D.M., 2007. Zebrafish in the wild:
391 a review of natural history and new notes from the field. Zebrafish. 4(1), 21-40.
392 https://doi.org/10.1089/zeb.2006.9997
393 Gao, J.M., Xie, Y.T., Xu, Z.S., Chen, H., Hide, G., Yang, T.B., Shen, J.L., Lai, D.H.,
394 Lun, Z.R., 2017. Genetic analyses of Chinese isolates of *Toxoplasma gondii*
395 reveal a new genotype with high virulence to murine hosts. Vet Parasitol. 241,
396 52-60.
397 https://doi.org/10.1016/j.vetpar.2017.05.007
398 Halonen, S.K., Weiss, L.M., 2013. Toxoplasmosis. Handb Clin Neurol. 114, 125-145.
399 https://doi.org/10.1016/B978-0-444-53490-3.00008-X
400 Hampton, M.M., 2015. Congenital Toxoplasmosis: A Review. Neonatal Netw. 34(5),
401 274-278.
402 https://doi.org/10.1891/0730-0832.34.5.274
403 Liang, Y., Ding, X., Yu, X., Wang, Y., Zhou, Y., He, J., Shi, Y., Zhang, Y., Lin, H., Lu,
404 D., 2018. Identification and functional characterization of Toll-like receptor 13
405 from orange-spotted grouper (*Epinephelus coioides*). Fish Shellfish Immunol. 74,
406 309-317.
407 https://doi.org/10.1016/j.fsi.2017.12.054
408 Lindsay, D.S., Dubey, J.P., 2009. Long-term survival of *Toxoplasma gondii* sporulated
409 oocysts in seawater. J Parasitol. 95(4), 1019-1020.
410 https://doi.org/10.1645/GE-1919.1
411 Lindsay, D.S., Phelps, K.K., Smith, S.A., Flick, G., Sumner, S.S., Dubey, J.P., 2001.

412 Removal of *Toxoplasma gondii* Oocysts from Sea Water by Eastern Oysters
413 (*Crassostrea virginica*). J Eukaryot Microbiol. 48, 197-198.
414 https://doi.org/10.1111/j.1550-7408.2001.tb00517.x
415 Lopes, F.M., Sandrini, J.Z., Souza, M.M., 2018. Toxicity induced by glyphosate and
416 glyphosate-based herbicides in the zebrafish hepatocyte cell line (ZF-L).
417 Ecotoxicol Environ Saf. 162, 201-207.
418 https://doi.org/10.1016/j.ecoenv.2018.07.005
419 Massie, G.N., Ware, M.W., Villegas, E.N., Black, M.W., 2010. Uptake and
420 transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish. Vet
421 Parasitol. 169(3-4), 296-303.
422 https://doi.org/10.1016/j.vetpar.2010.01.002
423 Miller, L.H., Baruch, D.I., Marsh, K., Doumbo, O.K., 2002. The pathogenic basis of
424 malaria. Nature. 415(6872), 673-679.
425 https://doi.org/10.1038/415673a
426 Miller, M.A., Miller, W.A., Conrad, P.A., James, E.R., Melli, A.C., Leutenegger, C.M.,
427 Dabritz, H.A., Packham, A.E., Paradies, D., Harris, M., Ames, J., Jessup, D.A.,
428 Worcester, K., Grigg, M.E., 2008. Type X *Toxoplasma gondii* in a wild mussel
429 and terrestrial carnivores from coastal California: new linkages between
430 terrestrial mammals, runoff and toxoplasmosis of sea otters. Int J Parasitol.
431 38(11), 1319-1328.
432 https://doi.org/10.1016/j.ijpara.2008.02.005
433 Nasiri, V., Teymurzadeh, S., Karimi, G., Nasiri, M., 2016. Molecular detection of

Toxoplasma gondii in snakes. Exp Parasitol. 169, 102-106. https://doi.org/10.1016/j.exppara.2016.08.002

Nishikawa, Y., Xuenan, X., Makala, L., Vielemeyer, O., Joiner, K.A., Nagasawa, H., 2003. Characterisation of *Toxoplasma gondii* engineered to express mouse interferon-gamma. Int J Parasitol. 33(13), 1525-1535. https://doi.org/10.1016/s0020-7519(03)00204-2

North Temperate Lakes. 2010. North Temperate Lakes LTER: High Frequency Water Temperature Data - Lake Mendota Buoy 2006 – current. https://doi.org/10.6073/pasta/8ceff296ad68fa8da6787076e0a5d992, (Accessed 18 September 2019).

Omata, Y., Umeshita, Y., Murao, T., Kano, R., Kamiya, H., Kudo, A., Masukata, Y., Kobayashi, Y., Maeda, R., Saito, A., Murata, K., 2005. *Toxoplasma gondii* does not persist in goldfish (*Carassius auratus*). J Parasitol. 91(6), 1496-1499. https://doi.org/10.1645/GE-3503RN.1

Sanders, J.L., Zhou, Y., Moulton, H.M., Moulton, Z.X., McLeod, R., Dubey, J.P., Weiss, L., M.Kent, M.L., 2015. The zebrafish, *Danio rerio*, as a model for *Toxoplasma gondii*: an initial description of infection in fish. J Fish Dis. 38(7), 675-679. https://doi.org/10.1111/jfd.12393

Schott, K.C., Krusor, C., Tinker, M.T., Moore, J., Conrad, P.A., Shapiro, K., 2016. Concentration and retention of *Toxoplasma gondii* surrogates from seawater by red abalone (*Haliotis rufescens*). Parasitology. 143(13), 1703-1712.

https://doi.org/10.1017/S0031182016001359

Sun, Z., 1996. Analysis of water temperature different depths of fish ponds. Journal of Lake Sciences 8(4), 325-329. (in Chinese)

Woolway, R.I., Jones, I.D., Maberly, S.C., French, J.R., Livingstone, D.M., Monteith, D.T., Simpson, G.L., Thackeray, S.J., Andersen, M.R., Battarbee, R.W., DeGasperi, C.L., Evans, C.D., de Eyto, E., Feuchtmayr, H., Hamilton, D.P., Kernan, M., Krokowski, J., Rimmer, A., Rose, K.C., Rusak, J.A., Ryves, D.B., Scott, D.R., Shilland, E.M., Smyth, R.L., Staehr, P.A., Thomas, R., Waldron, S., Weyhenmeyer, G.A., 2016. Diel Surface Temperature Range Scales with Lake Size. PLoS One. 11(3), e0152466. https://doi.org/10.1371/journal.pone.0152466

Yang, N., Farrell, A., Niedelman, W., Melo, M., Lu, D., Julien, L., Marth, G.T., Gubbels, M.J., Saeij, J.P., 2013. Genetic basis for phenotypic differences between different *Toxoplasma gondii* type I strains. BMC Genomics. 14, 467. https://doi.org/10.1186/1471-2164-14-467

Zhang, Z.Y., Zhang, Z.W., Zhang, C.J., Wu, G.J., Chen, Y.S., Liu, H.L., 2010. Analysis of water temperature of fishing pond in south Jiangsu coast area. Marine Science Bulletin 29(6), 674-677. (in Chinese)

Highlights

- *Toxoplasma gondii* RH strain tachyzoites are capable of invading fish cells and maintaining infectivity for at least 12 days post infection at 27°C although proliferation was clearly inhibited.
- Multiple temperatures (27°C, 30°C, 32°C and 35°C) were tested for *T. gondii* infection, and the minimum temperature for *T. gondii* invasion is 27°C and for replication is 30°C.
- *T. gondii* infection is highly dependent on temperature which determines the differences in the capability to infect cold- and warm- blooded vertebrates, rather than cell type or host.