DOI:10.13350/j.cjpb.221007

·论著·

冰冻对弓形虫GT1速殖子体外感染力的影响*

郭海婷,谭洁,何玉林,梁雪,李中原**
(桂林医学院基础医学院,广西脑与认知神经科学重点实验室,广西桂林 541199)

【摘要】 **目的** 探讨冰冻不同时间对弓形虫GT1速殖子体外感染力的影响。 **方法** 用HFF细胞传代GT1速殖子3次,刮净培养物,取2.0 mL充分破碎后计数。将剩余培养物中速殖子调至10 000个/mL,分装至1.5 mL离心管(100 μL/管),并随机分成3组。上述样品放置于4 ℃,分别在0、12、24、36、48、60 h时取出样品,5管/组,再置于4 ℃、-20 ℃或-80 ℃保存。待首批冰冻72 h时取出全部样品,于室温(约22 ℃)完全融化后全量加入预先铺有HFF细胞的6孔培养板内,继续培养。于10倍物镜下随机选取3个视野,观察并记录感染3、5、7 d时的噬斑形成数,若噬斑总数为0,则进行全视野扫描检测。盲传感染7 d仍未检测到噬斑形成的培养物,用全视野扫描盲传7 d时各瓶内的噬斑。 **结果** 随机观察3个视野,复苏前4 ℃放置的GT1速殖子仅在感染3 d时形成的噬斑清晰可辨,且各孔间无明显不同;-20 ℃和-80 ℃冰冻均能减弱GT1速殖子的体外感染力。盲传后观察,经-20 ℃和-80 ℃冰冻24 h的GT1速殖子仍能在感染HFF细胞7 d时形成噬斑,且-80 ℃较-20 ℃冰冻组形成的噬斑数更多;冰冻36 h及以上的GT1速殖子未形成噬斑。 **结论** 以1 000个速殖子/孔的剂量感染6孔培养板内的HFF细胞,4 ℃放置不超过72 h对GT1速殖子体外毒力无显著影响,-20 ℃或-80 ℃冰冻36 h及以上能减弱弓形虫GT1速殖子体外感染力。

【关键词】 弓形虫;GT1;冰冻;感染
【中图分类号】 R382.5 【文献标识码】 A 【文章编号】 1673-5234(2022)10-1145-05

[*Journal of Pathogen Biology*. 2022 Oct.;17(10):1145-1149.]

Effect of the freezing followed by melting at RT on the infection ability of *Toxoplasma gondii* GT1 tachyzoites *in vitro*

GUO Hai-ting, TAN Jie, HE Yu-lin, LIANG Xue, Li Zhong-yuan (*Guangxi Key Laboratory of Brain and Cognitive Neuroscience, Basic Medicine College, Guilin Medical University, Guilin, Guangxi* 541199, *China*)**

【Abstract】 **Objective** To explore the effect of the freezing followed by melting at room temperature (RT) on the infection ability of *Toxoplasma gondii* GT1 tachyzoites *in vitro*. **Methods** *T. gondii* GT1 tachyzoites were passaged for three times using HFF cells, and the cultures at the last time were scraped, 2.0 mL of which were broken and subsequently used for counting parasite. The residual cultures in which the number of parasites was switched to 10 000 per milliliter, were loaded into 1.5 mL tube, 100 μL each. After divided into three groups randomly, all the samples were simultaneously placed at 4 ℃ for freshness, and then the time was as 0 h. Five tubes each group were taken out at 0, 12, 24, 36, 48 and 60 h post placement, and followed to store at 4 ℃, -20 ℃, or -80 ℃. All the samples were fully melted at RT about 22 ℃ when the first ones were frozen for 72 h, and were completely added into 6-well plates to continuously co-culture with HFF cells. The plaques within three visual fields were totally recorded under 10 objective lens at 3, 5, and 7-day post infection (dpi), respectively. If the total number was zero, the full-vision scanning would be performed, which was also used to identify the plaques at 7 d post the blind passage of the cell cultures with no plaque formation detected at 7 dpi. **Results** The results from the three random visual fields showed that the plaques formed by the GT1 tachyzoites stored at 4 ℃ were distinguished only at 3 dpi, and no statistic difference was detected among all the wells containing these parasites. Moreover, the infection abilities of *T. gondii* GT1 tachyzoites were both significantly reduced by the freezing at -20 ℃ and -80 ℃ followed by melting at RT *in vitro*. The data from the blind passages showed that the plaques of the GT1 tachyzoites were still detected at 7 dpi, which were frozen at -20 ℃ or -80 ℃ for 24 h followed by melted at RT before, and the number of plaques formed at -80 ℃ was more than that of -20 ℃. Noteworthily, the plaque cannot be detected during passaging the cell cultures in which the parasites were frozen for not less than 36 h followed by melted at RT. **Conclusion** 1 000 tachyzoites of *T. gondii* GT1 strain each well is suitable for infecting host cells in 6-well plate, and

* 【基金项目】 广西自然科学基金项目(No. 2019GXNSFBA185031);广西研究生教育创新计划项目(No. JGY2022198);广西高等学校千名中青年骨干教师培育计划第4期资助项目(No. 2020-58);桂林医学院新引进博士科研启动项目(No. 31304018021)。

** 【通讯作者】 李中原,E-mail:lzy210930@163.com
【作者简介】 郭海婷(1991-),女,黑龙江哈尔滨人,硕士研究生。主要研究方向:病原生物学。E-mail:byndguoht@163.com

their infection abilities in vitro, which were not disturbed by storing at 4 ℃ for not more than 72 h, are significantly weakened by the freezing at −20 ℃ and −80 ℃ for not less than 36 h followed by melting at RT about 22 ℃.

【Key words】 *Toxoplasma gondii*; GT1; freezing; infection

刚地弓形虫(*Toxoplasma gondii*)简称弓形虫,是一种重要的人兽共患的机会致病性原虫,呈世界性分布,能感染包括人在内的所有温血动物,影响人类健康和畜牧业生产[1-2]。弓形虫拥有速殖子、缓殖子和卵囊等多种感染形态,感染途径多样,误食被污染的水与食物和接触包括家畜在内的被感染动物均可引起弓形虫感染[3-5]。此外,弓形虫基因型丰富[6],不同基因型的毒力间有时差异很大[7-8]。加之速殖子能够突破血脑屏障进入脑组织,且易于体外培养和体内传代,不同感染类型间容易通过人工诱导实现,弓形虫已经成为深入探究人兽共患性病原微生物致病机制和脑内感染引起人或动物行为变化的重要研究对象[9-10]。

GT1 速殖子(Genotype I)是实验室常见毒株,常被用作研究弓形虫未知基因功能、发病机理和致病机制的重要实验模型[11-13],由其引起的实验室安全隐患亦倍受关注。目前,实验室多用消毒试剂、火灼或水煮的方式对所用物品和废弃物进行消杀,但具体效果并不明确,且消毒试剂处理废液时存在因稀释而达不到理想浓度,火灼或水煮非耐温物品如培养瓶、培养板和细胞爬片时极易引发火灾。有资料显示,冰冻能够影响组织内弓形虫包囊的毒力[14],实验室均具有冰冻设施,与消毒试剂、火灼、水煮相比,冰冻对实验室安全与环境保护更加有利。本研究将模拟实验室日常操作,旨在探究室冰冻对弓形虫 GT1 速殖子体外感染力的影响,为保障实验室安全提供参考。

材料与方法

1 材料

1.1 宿主细胞系和弓形虫毒株 HFF 细胞系和弓形虫 GT1 速殖子均由广西脑与认知神经科学重点实验室保存。

1.2 主要试剂和仪器 青、链霉素混合液和高糖型 DMEM 培养基购自美国 Gibco 公司;胰蛋白酶-EDTA 消化液购自中国 Solarbio 公司;胎牛血清为以色列 Biological Industries 公司产品;T25 细胞培养瓶和 6 孔细胞培养板为中国 NEST Biotechnology 公司产品;CKX53 型荧光倒置显微镜购自日本 Olympus 公司;−80 ℃医用低温冰箱购自日本 Panasonic 公司;BCD-315TNGS 型冰箱为海尔智家股份有限公司生产。

2 方法

2.1 细胞复苏与传代 参考文献[15]的方法用 37 ℃水浴复苏 HFF 细胞和 GT1 速殖子,用胰酶消化和以一传三的方式传代 HFF 细胞,5.0 mL/瓶。待 GT1 速殖子感染 HFF 细胞 80%以上时,刮取培养物,吹打混匀,移取 200 μL 进行传代。共重复传代 3 次。用 6 孔细胞培养板对 HFF 细胞进行培养,备用。

2.2 速殖子的处理与感染 待 GT1 速殖子感染 HFF 细胞 80%时,刮净培养物并吹打混匀,移取 2.0 mL,用 5 号针头充分破碎,用血细胞计数板重复计数 5 次,取平均值。用 DMEM 培养基将剩余培养物中 GT1 速殖子调至 10 000 个/mL,并分装至 1.5 mL 离心管,100 μL/管,共 90 管,随机分成 3 组,30 管/组,放置于 4 ℃冰箱,分别在 0、12、24、36、48、60 h 时依次随机取出 5 管/组,并分别置于 4 ℃、−20 ℃或−80 ℃条件保存。待首批样品冰冻 72 h 即最后一批冰冻 12 h 时取出全部样品,于室温(约 22 ℃)下至完全融化。

将管内液体充分混匀,全量加入上述 6 孔细胞培养板,培养板放入湿盒内,置于 37 ℃、5% CO_2 培养箱培养。分别在感染后的第 3、5、7 d 用 10 倍物镜随机选取 3 个视野进行观察,分别记录各孔内的噬斑形成情况。若 3 个视野内形成的噬斑总数为 0,则对该孔内的细胞进行全视野扫描检测。

2.3 细胞培养物的盲传 用胰酶对感染 7 d 仍未检测到有噬斑形成孔内的培养物进行消化,补加 DMEM 培养基(含 10%胎牛血清)至 5.0 mL,吹打混匀后全量加入 T25 细胞培养瓶,于 37 ℃、5% CO_2 培养箱内静置培养 6 h 后更换培养基,继续培养至 7 d。用荧光倒置显微镜全视野扫描拍照,记录各瓶内 HFF 细胞生长情况与噬斑形成总数。

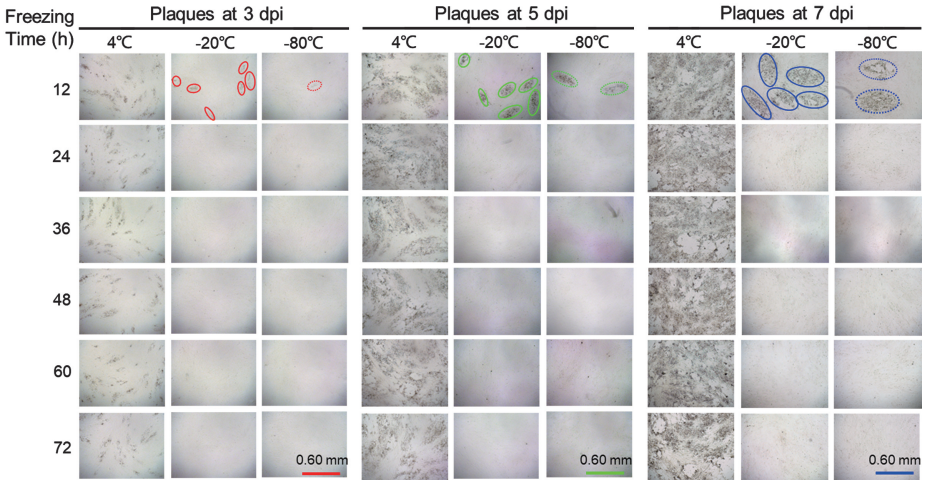
2.4 统计学分析 运用 SPSS PASW Statistics v18.0 系统进行统计学分析,各组所得噬斑数比较采用 Student's t 检验,$P<0.05$ 为差异有统计学意义。用 GraphPad Prism 8 软件绘制所得数据图形。

结 果

1 GT1 速殖子感染 HFF 细胞噬斑形成情况

取 4 ℃、−20 ℃和−80 ℃条件下保存 12、24、36、48、60、72 h 的弓形虫 GT1 速殖子,置室温(约 22 ℃)完全融化后分别感染 6 孔培养板内的 HFF 细胞,记录感染 3、5、7 d 时的噬斑形成情况,结果如图 1。−20 ℃和−80 ℃条件下冰冻的弓形虫 GT1 速殖子噬斑形成能力减弱,除−20 ℃和−80 ℃冰冻 12 h 形成的噬斑外,置于 4 ℃保存的 GT1 速殖子仅在感染第 3 d 时

的噬斑清晰可辨，表明以1 000个速殖子/孔的剂量感染6孔培养板内的HFF细胞较为适合。此外，在用荧光倒置显微镜进行全视野扫描时，未检测到－20 ℃和－80 ℃冰冻24 h及以上的细胞孔内有噬斑形成。



注：红色、绿色、蓝色的实线和虚线椭圆分别指示－20 ℃和－80 ℃冰冻12 h的GT1速殖子室温完全融化后感染HFF细胞3、5、7 d时的噬斑，其他时间未见噬斑形成。红色、绿色和蓝色Bar＝0.60 mm。

图1 弓形虫GT1速殖子感染HFF细胞的噬斑形成情况

Notes: Plaques on HFF cells at 3, 5 and 7 dpi formed by GT1 parasites that were placed at －20 ℃ or －80 ℃ for freezing 12 h followed by melting at RT, were marked with red, green and blue ellipses with the full and dotted lines, respectively. No plaque formation was detected at other time points. Red, green and blue bars equal 0.60 mm.

Fig. 1 Plaques on HFF cells formed by *T. gondii* GT1 tachyzoites at 3, 5, 7 days post infection (dpi)

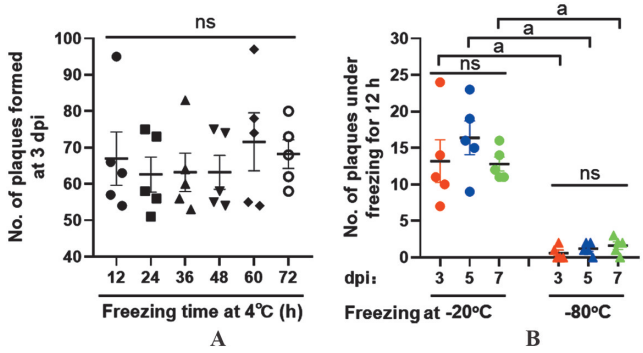
2 冰冻对GT1速殖子体外感染力的影响

用4 ℃条件作用12、24、36、48、60、72 h的GT1速殖子经室温放置后感染HFF细胞3 d，于10倍物镜下随机选取3个视野，统计噬斑形成总数，并进行差异性分析。结果显示，各细胞孔内噬斑形成数目间均差异无统计学意义（t = 0.503 for 12 h *vs* 24 h, 0.421 for 12 h *vs* 36 h, 0.438 for 12 h *vs* 48 h, 0.425 for 12 h *vs* 60 h, 0.145 for 12 h *vs* 72 h, 0.084 for 24 h *vs* 36 h, 0.090 for 24 h *vs* 48 h, 0.965 for 24 h *vs* 60 h, 0.905 for 24 h *vs* 72 h, 0.000 for 36 h *vs* 48 h, 0.877 for 36 h *vs* 60 h, 0.761 for 36 h *vs* 72 h, 0.908 for 48 h *vs* 60 h, 0.822 for 48 h *vs* 72 h 和 0.382 for 60 h *vs* 72 h, P＞0.05）（图2 A）。即4 ℃条件放置不超过72 h对GT1速殖子的体外毒力无显著影响。

检测冰冻的GT1速殖子再感染HFF细胞噬斑形成情况，结果如图2B。－20 ℃和－80 ℃冰冻12 h的弓形虫GT1速殖子体外噬斑形成能力减弱，以－80 ℃条件下冰冻对噬斑形成的抑制作用更强（t＝4.272 for 3 d, 6.481 for 5 d 和 10.224 for 7 d, P＜0.01）。

3 无噬斑形成细胞培养物的盲传鉴定

针对弓形虫GT1速殖子经－20 ℃、－80 ℃冰冻和室温融化后再感染HFF细胞7 d时未检测到噬斑形成孔内的细胞培养物，用胰酶消化后全部加入T25培养瓶，继续培养7 d，用全视野扫描对各培养瓶内的噬斑形成情况进行盲传鉴定。结果显示，－20 ℃和－80 ℃冰冻24 h的弓形虫GT1速殖子复苏后仍能在感染HFF细胞7 d时形成噬斑，且－80 ℃冰冻24 h较－20 ℃冰冻24 h的速殖子形成的噬斑数更多（t＝2.772, P＜0.05）；检查盲传－20 ℃和－80 ℃冰冻36 h及以上的速殖子感染的细胞培养物，所有培养瓶内均未见噬斑形成（图3）。

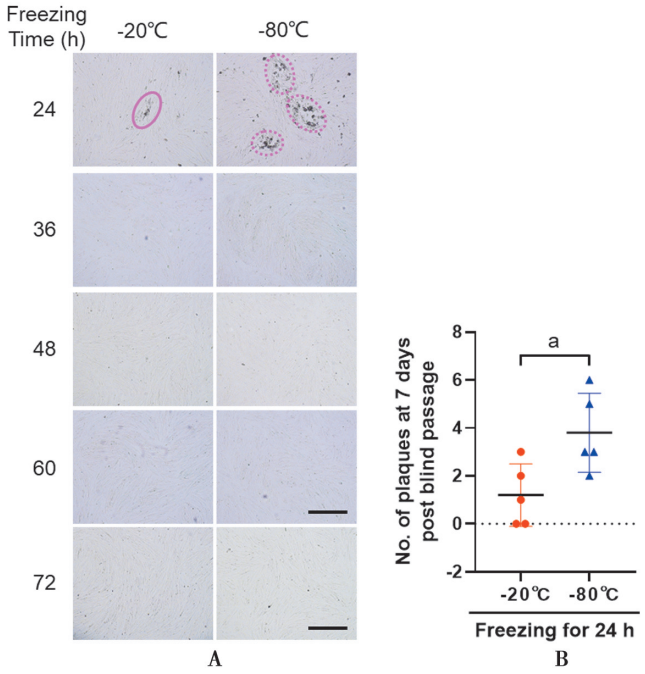


A GT1速殖子于4 ℃条件作用12、24、36、48、60、72 h，经室温放置后再感染HFF细胞3 d时形成的噬斑数 B 于－20 ℃或－80 ℃冰冻12 h和室温融化后再感染HFF细胞3、5、7 d时的噬斑数。组间比较，ns P＞0.05；a P＜0.01。

图2 冰冻对弓形虫GT1速殖子感染HFF细胞噬斑形成的影响

A Plaques formed by the GT1 tachyzoites on HFF cells at 3 dpi, before which the parasites were placed at 4 ℃ for 12 h, 24 h, 36 h, 48 h, 60 h, or 72 h followed by arranged at RT B Statistic analysis for the plaques on HFF cells formed at 3, 5, and 7 dpi by the GT1 parasites that were placed at －20 ℃ or －80 ℃ for freezing 12 h followed by melting at RT. ns P＞0.05; a P＜0.01.

Fig. 2 Effect of the freezing followed by melting at RT onthe plaque formation of *T. gondii* GT1 tachyzoites



A 全视野扫描无噬斑形成细胞孔内培养物盲传 7 d 时的生长情况(粉色实线、虚线椭圆分别示－20 ℃和－80 ℃冰冻 24 h 后于室温完全融化的 GT1 速殖子感染 HFF 细胞 7 d 时的培养物盲传 7 d 所形成的噬斑。盲传冰冻 36、48 、60 、72 h 的细胞培养物均未发现噬斑。黑色 Bar＝0.30 mm B 盲传－20 ℃与－80 ℃冰冻 24 h 所得细胞培养物噬斑形成比较，a $P<0.05$。

图 3 无噬斑形成细胞培养物的盲传鉴定

A The cell growth of cultures in which no plaque was determined using full-vision scanning before blind passage. The plaques formed by the GT1 tachyzoites in the cultures, which were produced at 7 dpi through co-incubating with the parasites that were placed at -20 ℃ or -80 ℃ for freezing 24 h followed by melting at RT, were marked at 7 days post blind passage using the pink ellipses with full and dotted lines. For freezing 36 h, 48 h, 60 h and 72 h, no plaque was detected based on the full-vision scanning. Black bar equals 0.30 mm B Statistic analysis for the plaques formed during the blind passage of cultures containing the parasites that were frozen for 24 h before. a $P<0.05$.

Fig. 3 Identification of the cells produced by blind passaging the cultures in which no plaque was detected before based on the full-vision scanning

讨 论

实验室安全非常重要，凡进入实验室工作和学习的人员，都必须严格遵守相关规定[16－17]。保障实验室安全，是每一位科研工作者和学习者应尽的义务，更是每一位科研工作者和学习者不可推卸的责任。作为实验室安全的重要组成部分，生物安全尤其是人兽共患性病原微生物的公共卫生安全举足轻重，理当受到重点关注[18－19]。

弓形虫是一种机会致病人兽共患性病原微生物，实验室在研究人兽共患性病原致病机制和脑内感染引起人或动物行为变化作用机制中的作用不可忽视[6－7]。需要注意的是，弓形虫具有多种感染途径，拥有 300 多种基因型[20]，且不同基因型之间的感染力有时差异很大[7－8]。据报道，全球已有约三分之一人群被弓形虫感染[21]，接触被感染动物和误食被弓形虫污染的水与食物已经成为弓形虫感染人的重要途径和方式[3－5]。如何排除因实验室消杀不彻底可能造成的生物安全隐患，比如如何避免弓形虫速殖子污染水源可能性事件的发生，值得认真思考。

目前，可能引起实验室对弓形虫速殖子消杀不彻底的因素主要有：①处理废液时所用的消毒试剂达不到理想消杀浓度；②火灼所用物品不完全；③水煮物品浮于水面和(或)游离锅外，导致受热不均。更为重要的是，含有消毒试剂的废液和火灼、水煮过程中产生的废气，均不利于实验室人员的身体健康与环境保护，且火灼和水煮时极易引发火灾。鉴于实验室具备低温冰冻条件，且已有文献报道显示冰冻可减弱组织内弓形虫包囊的感染力[14]。可见，运用低温冰冻对弓形虫实验过程中所用物品进行处理更为科学、环保、易行。

本研究以弓形虫常用毒株 GT1 速殖子为对象，经充分模拟实验室日常操作，着重探讨低温冰冻不同时间后对弓形虫速殖子体外感染 HFF 宿主细胞能力的影响。结果显示，用 1 000 个速殖子/孔的剂量对 6 孔细胞培养板内的 HFF 细胞进行感染，适合用于评价冰冻对弓形虫 GT1 速殖子体外感染力的影响，并且 4 ℃条件放置不超过 72 h 时的 GT1 速殖子体外感染力无明显变化。更为重要的是，－20 ℃和－80 ℃低温冰冻均可减弱弓形虫 GT1 速殖子体外感染 HFF 宿主细胞的能力，并且随着冰冻时间的延长，速殖子的感染力减弱越明显。当冰冻 36 h 及以上时，所有盲传培养瓶内均未发现噬斑形成。

综上所述，本研究通过探究室温融化前冰冻对弓形虫 GT1 速殖子体外感染能力的影响，明确了－20 ℃和－80 ℃冰冻不低于 36 h 均能减弱弓形虫 GT1 速殖子的体外感染能力，以－20 ℃冰冻的效果更好，可为实验室生物安全保障和实验室开展弓形虫 GT1 速殖子致病机制研究提供参考。关于冰冻对弓形虫 RH、ME49、PRU 和 VEG 等其他毒株速殖子体外感染能力的影响有待进一步研究。

【参考文献】

[1] Milne G, Webster JP, Walker M. *Toxoplasma gondii*: an underestimated threat? [J]. Trends Parasitol, 2020, 36(12): 959-969.

[2] Piao LX, Cheng JH, Aosai F, et al. Cellular immunopathogenesis in primary *Toxoplasma gondii* infection during pregnancy [J]. Parasite Immunol, 2018, 40(9): e12570.

[3] Almeria S, Dubey JP. Foodborne transmission of *Toxoplasma gondii* infection in the last decade. An overview [J]. Res Vet Sci, 2021(135): 371-385.

[4] Robert-Gangneux F, Dard ML. Epidemiology of and diagnostic strategies for toxoplasmosis [J]. Clin Microbiol Rev, 2012, 25(2): 264-296.

[5] Smith JR, Ashander LM, Arruda SL, et al. Pathogenesis of ocular toxoplasmosis [J]. Prog Retin Eye Res, 2021(81): 100882.
[6] Dubey JP, Cerqueira-Cezar CK, Murata FHA, et al. All about toxoplasmosis in cats: the last decade [J]. Vet Parasitol, 2020(283): 109145.
[7] Saraf P, Shwab EK, Dubey JP, et al. On the determination of *Toxoplasma gondii* virulence in mice [J]. Exp Parasitol, 2017(174): 25-30.
[8] Yang Y, Ren H, Xin S, et al. Comparative immunological response and pathobiology of mice in oculated with *Toxoplasma gondii* isolated from different hosts [J]. J Parasitol, 2021, 107(2): 179-181.
[9] Matta SK, Rinkenberger N, Dunay IR, et al. *Toxoplasma gondii* infection and its implications within the central nervous system [J]. Nat Rev Microbiol, 2021, 19(7): 467-480.
[10] Mendez OA, Koshy AA. *Toxoplasma gondii*: entry, association, and physiological influence on the central nervous system [J]. PLoS Pathog, 2017, 13(7): e1006351.
[11] Li ZY, Liang X, Guo HT, et al. *Toxoplasma* invasion delayed by TgERK7 eradication [J]. Parasitol Res, 2020, 119(11): 3771-3776.
[12] Antil N, Kumar M, Behera SK, et al. Unraveling *Toxoplasma gondii* GT1 strain virulence and new protein-coding genes with proteogenomic analyses [J]. OMICS, 2021, 25(9): 591-604.
[13] Olias P, Sibley LD. Functional analysis of the role of *Toxoplasma gondii* nucleoside triphosphate hydrolases I and II in acute mouse virulence and immune suppression [J]. Infect Immun, 2016, 84(7): 1994-2001.
[14] Mirza Alizadeh A, Jazaeri S, Shemshadi B, et al. A review on inactivation methods of *Toxoplasma gondii* in foods [J]. Pathog Glob Health, 2018, 112(6): 306-319.
[15] Khan A, Grigg ME. *Toxoplasma gondii*: laboratory maintenance and growth [J]. Curr Protoc Microbiol, 2017(44): 11-17.
[16] Wurtz N, Papa A, Hukic M, et al. Survey of laboratory-acquired infections around the world in biosafety level 3 and 4 laboratories [J]. Eur J Clin Microbiol Infect Dis, 2016, 35(8): 1247-1258.
[17] M nard AD, Trant JF. A review and critique of academic lab safety research [J]. Nat Chem, 2020, 12(1): 17-25.
[18] Gassiep I, Bauer MJ, Harris PNA, et al. Laboratory safety: handling *Burkholderia pseudomallei* isolates without a biosafety cabinet [J]. J Clin Microbiol, 2021, 59(7): e0042421.
[19] Yucesan B, Ozkan O. Laboratory safety in parasitology laboratory [J]. Turkiye Parazitol Derg, 2018, 42(2): 144-153.
[20] Valenzuela-Moreno LF, Mendez-Cruz ST, Rico-Torres CP, et al. SAG3 *Toxoplasma gondii* cloning reveals unexpected fivefold infection in the blood of feral cats in the Mexican Caribbean [J]. BMC Vet Res, 2022, 18(1): 33.
[21] Galvan-Ramirez Mde L, Gutierrez-Maldonado AF, Verduzco-Grijalva F, et al. The role of hormones on *Toxoplasma gondii* infection: a systematic review [J]. Front Microbiol, 2014(5): 503.

【收稿日期】 2020-04-20 【修回日期】 2020-07-15

(上接1144页)

[7] Rivaya B, Jordana-Lluch E, Fernandez-Rivas G, et al. Macrolide resistance and molecular typing of *Mycoplasma pneumoniae* infections during a 4 year period in Spain[J]. J Antimicrob Chemother, 2020, 75(10): 2752-2759.
[8] Zhou Y, Wang J, Chen W, et al. Impact of viral coinfection and macrolide-resistant mycoplasma infection in children with refractory *Mycoplasma pneumoniae* pneumonia[J]. BMC Infect Dis, 2020, 20(1): 633.
[9] Beeton ML, Zhang XS, Uldum SA, et al. *Mycoplasma pneumoniae* infections, 11 countries in Europe and Israel, 2011 to 2016[J]. Euro Surveill, 2020, 25(2): 1900112.
[10] Nakamura A, Takumi K, Miki K. Crystal structure of a thermophilic GrpE protein: insight into thermosensing function for the DnaK chaperone system[J]. J Mol Biol, 2010, 396(4): 1000-1011.
[11] Ziemienowicz A, Skowyra D, Zeilstra-Ryalls J, et al. Both the *Escherichia coli* chaperone systems, GroEL/GroES and DnaK/DnaJ/GrpE, can reactivate heat-treated RNA polymerase. Different mechanisms for the same activity[J]. J Biol Chem, 1993, 268(34): 25425-25431.
[12] Bracher A, Verghese J. GrpE, Hsp110/Grp170, HspBP1/Sil1 and BAG domain proteins: nucleotide exchange factors for Hsp70 molecular chaperones[J]. Subcell Biochem, 2015(78): 1-33.
[13] Murakami J, Terao Y, Morisaki I, et al. Group A streptococcus adheres to pharyngeal epithelial cells with salivary proline-rich proteins via GrpE chaperone protein[J]. J Biol Chem, 2012, 287(26): 22266-22275.
[14] 周长平,陈莹,李媛,等. 鸡毒支原体热休克蛋白GrpE粘附特性的鉴定[J]. 中国预防兽医学报, 2017, 39(1): 28-33.
[15] Zhang WZ, Zhang SJ, Wang QY, et al. Outbreak of macrolide-resistant *mycoplasma pneumoniae* in a primary school in Beijing, China in 2018[J]. BMC Infect Dis, 2019, 19(1): 871.
[16] Lin M, Shi L, Huang A, et al. Efficacy of levofloxacin on macrolide-unresponsive and corticosteroid-resistant refractory *Mycoplasma pneumoniae* pneumonia in children[J]. Ann Palliat Med, 2019, 8(5): 632-639.
[17] 黄晓慧,王炜,王倩,等. 生殖支原体P110蛋白的生物信息学分析[J]. 中国病原生物学杂志, 2021, 16(06): 639-643, 650.
[18] Tang Y, Guo F, Lei A, et al. GrpE immunization protects against *Ureaplasma urealyticum* infection in BALB/C mice[J]. Front Immunol, 2020(11): 1495.
[19] Kim WS, Kim JS, Kim HM, et al. Comparison of immunogenicity and vaccine efficacy between heat-shock proteins, HSP70 and GrpE, in the DnaK operon of *Mycobacterium tuberculosis*[J]. Sci Rep, 2018, 8(1): 14411.

【收稿日期】 2022-05-08 【修回日期】 2022-07-22