Morphological evaluation of intestinal epithelial cells infected with *toxoplasma gondii*

Avaliação morfológica de células do epitelial intestinal infectadas com *toxoplasma gondii*

DOI:10.34117/bjdv8n9-136

Recebimento dos originais: 16/08/2022
Aceitação para publicação: 12/09/2022

Olga Maria de Jesus Souza
Graduação
Institution: Universidade do Estado do Rio de Janeiro (UERJ)
Address: Av. Manuel Caldeira de Alvarenga, 1203, Campo Grande, Rio de Janeiro - RJ, Brasil
E-mail: olgasouza23@yahoo.com.br

Gabriella Oliveira Alves Moreira de Carvalho
Mestrado
Institution: Universidade Federal Rural do Rio de Janeiro (UFRRJ)
Address: BR-465, Km 7, Seropédica - RJ, Brasil
E-mail: gabriellacarvalho_15@yahoo.com.br

Tiago Alexandre Silva Nascimento
Graduação
Institution: Universidade do Estado do Rio de Janeiro (UERJ)
Address: Av. Manuel Caldeira de Alvarenga, 1203, Campo Grande, Rio de Janeiro - RJ, Brasil
E-mail: tiagotasna@gmail.com

Elinéia de Oliveira Araujo Rodrigues
Graduação
Institution: Universidade do Estado do Rio de Janeiro (UERJ)
Address: Av. Manuel Caldeira de Alvarenga, 1203, Campo Grande, Rio de Janeiro - RJ, Brasil
E-mail: elineia.oar@gmail.com

Thiago Manchester de Mello
Mestrado
Institution: Universidade do Estado do Rio de Janeiro (UERJ)
Address: Av. Manuel Caldeira de Alvarenga, 1203, Campo Grande, Rio de Janeiro - RJ, Brasil
E-mail: thiagomanchester@gmail.com

Regina Coeli dos Santos Goldenberg
Pós-Doutorado
Institution: Universidade Federal do Rio de Janeiro (UFRJ)
Address: Av. Pedro Calmon, 550, Cidade Universitária, Rio de Janeiro - RJ, Brasil
E-mail: rcoeli@biof.ufrj.br

Sérgio Henrique Seabra
Doutorado
Institution: Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF)
Address: Av. Alberto Lamego, 2000, Parque Califórnia Campos dos Goytacazes – RJ, Brasil
E-mail: seabrash@gmail.com

Fabio da Silva de Azevedo Fortes
Doutorado
Institution: Universidade do Estado do Rio de Janeiro (UERJ)
Address: Av. Manuel Caldeira de Alvarenga, 1203, Campo Grande, Rio de Janeiro - RJ, Brasil
E-mail: fabiofortes@hotmail.com

ABSTRACT
Toxoplasmosis is a disease caused by intracellular obligate protozoan, *Toxoplasma gondii*. And it can be obtained mainly through the oral route, through the ingestion of oocysts or tissue cysts. For this reason, the intestinal epithelium is a cellular model that makes it possible to study the first line of defense against this type of infection. After infection, the protozoan invades intestinal cells, changing the physiology and functions of the intestinal epithelial barrier. In view of this, we analyzed the morphological changes and the microenvironment, *in vitro*, which are modified in the intestinal epithelial cell during the process of infection and evasion of *Toxoplasma gondii*.

Keywords: intestinal epithelial cell, IEC-6, cell culture, *Toxoplasma gondii*.

RESUMO
A toxoplasmose é uma doença causada pelo protozoário intracelular obrigatório, *Toxoplasma gondii*. E pode ser obtido principalmente por via oral, através da ingestão de oocistos ou cistos teciduais. Por isso, o epitélio intestinal é um modelo celular que permite estudar a primeira linha de defesa contra esse tipo de infecção. Após a infecção, o protozoário invade as células intestinais, alterando a fisiologia e as funções da barreira epitelial intestinal. Diante disso, analisamos as alterações morfológicas e do microambiente, *in vitro*, que são modificadas na célula epitelial intestinal durante o processo de infecção e evasão do *Toxoplasma gondii*.

Palavras-chave: célula epitelial intestinal, IEC-6, cultura de células, *Toxoplasma gondii*.

1 INTRODUCTION

In the intestinal lumen there are several microorganisms, which make up the microbiota of the intestine. These eating organisms, from food, live on the mucous surface

and act in order to compete with microorganisms with pathogenic potential. However, if these cohabiting microorganisms cross the intestinal barrier and fall into the circulation, they become highly harmful to the host (MUNIZ *et al*., 2012).

According to data from the Ministry of Health (2018), in Brazil, between 2000 and 2017, 12,503 Outbreaks of Foodborne Diseases (TD) were reported, however only 3,196 of the cases were laboratory confirmed. Where about 2,340,201 people were exposed to food contaminated with some kind of pathogen and 236,403 cases of diseases were recorded. Corroborating the foregoing, according to recent data from the Notifiable Diseases Information System (Sinan), an average of 700 outbreaks of DTA are reported per year, involving approximately 13,000 patients. Given this, the immune system cannot always fight these pathogens, thereby promoting millions of deaths worldwide for years (BRAZIL, 2020).

Toxoplasmosis is a disease caused by *Toxoplasma gondii (T. gondii)*, a mandatory intracellular protozoan (MONTOYA & LIESENFELD, 2004; Ramírez-Flores et al., 2019). This parasitemia can be obtained in several ways, and among them the most common way to contract toxoplasmosis is through oral infection after ingestion of oocysts or tissue cysts (DUBEY *et al*., 2005).

In Brazil, the prevalence of toxoplasmosis is considered high, with about 50 to 80% of pregnant women and women of childbearing age infected and 4 to 5% at risk of becoming infected during pregnancy (REMINGTON *et al*., 2006). After oral infection, *Toxoplasma gondii* invades intestinal cells, altering the physiology and functions of intestinal epithelial barrier in some animal species (BURGER *et al*., 2018).

Although the invasion of parasites in host cells is a known event, the effects of *T. gondii* infection on the intestinal barrier aren't yet well established (WEIGHT *et al*., 2015; BRICEÑO *et al*., 2016). A study conducted in 2016 by Trevizan *et al*. showed that acute infection with *T. gondii* caused morphological changes in the intestinal wall of the duodenum in rats.

In view of this, the intestinal epithelium is a cellular model that provides to study the first line of defense against infections that can be acquired through the oral route, such as toxoplasmosis. Table 1 brings together several articles published in recent years that attempt to elucidate the interaction between the intestinal epithelium and the protozoan *Toxoplasma gondii.*

Therefore, given this, it is important to better understand the possible mechanisms of interaction between the intestinal epithelial cell and *Toxoplasma gondii* in order to

investigate the changes in the cellular microenvironment during the infection-inflammatory process and protozoan evasion according to temporality, besides being worth the morphological changes caused by the profile of parasite infection at different concentrations.

Table 1: Studies of the interaction between the intestinal epithelium and *Toxoplasma gondii.*

Age	Title of the article	Doi	Reference
1993	Rat intestinal epithelial cell line IEC-6 is activated by recombinant interferon-gamma to inhibit replication of the coccidian *Toxoplasma gondii*	10.1002 / eji.1830230435	DIMIER; I.H.; BOUT D.T. Rat intestinal epithelial cell line IEC-6 is activated by recombinant inferferon-γ to inhibit replication of the coccidian *Toxoplasma gondii*. **European journal of immunology**, v. 23, n. 4, p.981–983,1993.
1997	Inhibition of *Toxoplasma gondii* replication in IFN-gamma-activated human intestinal epithelial cells	10.1038/icb.1997.80	DIMIER, I.H., BOUT, D.T. Inhibition of *Toxoplasma gondii* replication in IFN-gamma-activated human intestinal epithelial cells. **Immunology and cell biology**, v. 75, no. 5, p. 511–514,1997.
2008	Regulation of toll-like receptors in intestinal epithelial cells by stress and *Toxoplasma gondii* infection	10.1111/j.1365-3024.2008.01055.x	GOPAL, R., BIRDSELL, D., MONROY, F.P. Regulation of toll-like receptors in intestinal epithelial cells by stress and *Toxoplasma gondii* infection. **Parasite immunology,** v. 30, n. 11-12, p. 563–576,2008.
2011	Modulation of early β-defensin-2 production as a mechanism developed by type I *Toxoplasma gondii* to evade human intestinal immunity	10.1128 / IAI.01086-10	MORAMPUDI, V., *et al*. Modulation of early β-defensin-2 production as a mechanism developed by type I *Toxoplasma gondii* to evade human intestinal immunity. **Infection and immunity**, v. 79 n. 5, p. 2043–2050, 2011.
2013	Regulation and migratory role of P-selectin ligands during intestinal inflammation	10.1371/journal.pone.0062055	HOFFMANN, U., *et al*. Regulation and migratory role of P-selectin ligands during intestinal inflammation. **PloS one**, v. 8, n. 4, E62055.
2014	Role of glucocorticoids and *Toxoplasma gondii* infection on murine intestinal epithelial cells	10.1016/j.parint.2014.05.005	JOHNSON, S.L., *et al*. Role of glucocorticoids and *Toxoplasma gondii* infection on murine intestinal epithelial cells. **Parasitology international**, v. 63 n. 5, p. 687–694, 2014.
2014	Spontaneous cystogenesis of *Toxoplasma gondii* in feline epithelial cells in vitro	10.14411 / fp.2014.017	MUNO, R.M. de *et al*. Spontaneous cystogenesis of *Toxoplasma gondii* in feline epithelial cells in vitro. **Parasitologic a Folia**, v.62, n.2, p.113-119, 2014.
2015	Elucidating pathways of *Toxoplasma gondii* invasion in the gastrointestinal tract: involvement of the tight junction protein occludin	10.1016/ j.micinf.2015.07.001	WEIGHT, C.M. *et al*. Elucidating pathways of *Toxoplasma gondii* invasion in the gastrointestinal tract: involvement of the tight junction protein occludin. **Microbes and infection**, v. 17, n. 10, p. 698-709, 2015.
2015	Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection	10.1016/j.immuni.2015.01.011	MUÑOZ, M., *et al*. Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection. **Immunity**, v. 42, n. 2, p. 321–331, 2015.
2017	P2X7 receiver-dependent tuning of gut epithelial responses to infection	10.1038/icb.2016.75	HUANG, S.W., *et al*. P2X7 receptor-dependent tuning of gut epithelial responses to infection. **Immunology and cell biology**, v. 95, n. 2, p. 178–188, 2017.
2017	Mechanisms and pathways of *Toxoplasma gondii* transepithelial migration	10.1080 / 21688370.2016.1273865	JONES, E.J.; KORCSMAROS, T.; CARDING, S.R.; Mechanisms and pathways of *Toxoplasma gondii* transepithelial migration. **Tissue Barriers.** v. 5, n. 1, e1273865, 2017.
2017	*Toxoplasma gondii* infection causes structural changes in the jejunum of rats infected with different inoculum doses	10.1016/j.lfs.2017.10.032	VINCENTIAN-VIEIRA, S L. *et al*. *Toxoplasma gondii* infection causes structural changes in the jejunum of rats infected with different inoculum doses. **Life sciences**, v. 191, p. 141–149, 2017.
2017	An in vitro model of intestinal infection reveals a developmentally regulated transcriptome of Toxoplasma sporozoites and an NF-κB-like signature in infected host cells	10.1371/journal.pone.0173018	GUITON, P.S., *et al*. An in vitro model of intestinal infection reveals a developmentally regulated transcriptome of Toxoplasma sporozoites and an NF-κB-like signature in infected host cells. **PloS one**, v. 12, n. 3, e0173018, 2017.

2018	Immunocompetent host develops mild intestinal inflammation in acute infection with *Toxoplasma gondii*	10.1371/journal.pone.0190155	WATANABE P.D.S. *et al*. Immunocompetent host develops mild intestinal inflammation in acute infection with *Toxoplasma gondii*. **PLoS One.** V. v. 13, no. 1, E0190155, 2018.
2018	Interleukin-22-deficiency and microbiota contribute to the exacerbation of *Toxoplasma gondii*-induced intestinal inflammation	10.1038/s41385-018-0005-8	COUTURIER-MAILLARD, A., *et al*. Interleukin-22-deficiency and microbiota contribute to the exacerbation of *Toxoplasma gondii-induced*intestinal inflammation. **Mucosal immunology**, v. 11, no. Four p. 1181–1190, 2018.
2019	*Toxoplasma gondii* causes increased ICAM-1 and serotonin expression in the jejunum of rats 12h after infection	10.1016/j.biopha.2019.108797	PASTRE. M.J. *et al*. *Toxoplasma gondii* causes increased ICAM-1 and serotonin expression in the jejunum of rats 12 h after infection. **Biomedicine & pharmacotherapy.** v. 114, n. 108797, 2019.
2019	An Open-Format Enteroid Culture System for Interrogation of Interactions Between *Toxoplasma gondii* and the Intestinal Epithelium	10.3389/fcimb.2019.00300	LUU, L., *et al*. An Open-Format Enteroid Culture System for Interrogation of Interactions Between *Toxoplasma gondii* and the Intestinal Epithelium. **Frontiers in cellular and infection microbiology.** v. 9, n. 300, 2019.
2019	From Entry to Early Dissemination-*Toxoplasma gondii*'s Initial Encounter With Its Host	10.3389/fcimb.2019.00046	DELGADO BETANCOURT, E., *et al*. From Entry to Early Dissemination-*Toxoplasma gondii*'s Initial Encounter With Its Host. **Frontiers in cellular and infection microbiology**, v. 9 n. 46, 2019.
2020	Macrophage migration inhibitory factor (MIF) and pregnancy may impact the balance of intestinal cytokines and the development of intestinal pathology caused by *Toxoplasma gondii* infection	10.1016/j.cyto.2020.155283	MARCON, C.F., *et al*. Macrophage migration inhibitory factor (MIF) and pregnancy may impact the balance of intestinal cytokines and the development of intestinal pathology caused by *Toxoplasma gondii* infection. **Cytokine,** v. 136, n. 155283, 2020.

2 METHODOLOGY

2.1 INTESTINAL EPITHELIAL CELL CULTURE (IEC-6)

The intestinal epithelial cell lineage (IEC-6) is also provided by the Cell Bank of Rio de Janeiro. The cells were plated with an initial density of $1x10^6$ cells/mL, in the cover slips 13mm. The cultures were maintained in DMEM medium (Dulbecco´s modified Eagle´s medium), supplemented with 10% Bovine Fetal Serum (GIBCO-Life Technologies, Rockville, MD), 1Ul/mL of insulin, penicillin 1000IU/mL and streptomycin 100IU/mL (Sigma Chemical Company, St Louis, MO). The cells were kept at 37°C in a humid atmosphere at 5% CO_2 (Culture CO_2 Incubator, model CCL-170B-8, Sigapore).

2.2 OBTAINING TAQUIZOITE FROM *TOXOPLASMA GONDII*

Tachyzoite form of *T. gondii* (RH Strain) were provided by the Technology Laboratory in Cell Culture (LTCC) by UEZO, Brazil-RJ. These parasites were obtained through the number of the Ethics Committee of the State University of Northern Fluminense Darcy Ribeiro (ID 124396).

Tachyzoites of *T. gondii* (RH strain) were inoculated intraperitoneally into BALB/c mice. After 2-3 days, tachyzoites were harvested using intraperitoneal wash with phosphate buffered saline (PBS; pH 7.3) and then were counted.

2.3 PARASIT-CELL INTERACTION

The cells were grown in cover slips and organized in the plate of 24 wells plates. *T. gondii* taquizoite was resuspended in DMEM medium and placed in contact with the cell in the proportion of five parasites for each cell (5:1) and ten parasites for each cell (10:1). After 1 hour of interaction in the greenhouse at 37°C, in humid atmosphere and 5% CO_2 (Culture CO_2 Incubator, model CCL-170B-8, Sigapore), the medium was discarded and replaced by supplemented DMEM and returned to the greenhouse within 24, 48 and 72 hours. After that, the cells were submitted to an evaluation of cell growth and its morphology by means of images obtained under inverted microscope (Axiovert 40 CFL, light field, phase contrast, ZEISS, Germany) at increases of 10x and 40x with the AxioVision program.

3 RESULTS

3.1 INFECTION WITH TWO PROPORTIONS *OF TOXOPLASMA GONDII:* CELL GROWTH AND MORPHOLOGICAL ASPECTS

The intestinal epithelial lineage IEC-6 was infected in the proportion of 5 parasites per cell and 10 parasites per cell in order to analyze how the morphological profile and the cellular microenvironment are altered in the face of acute infection generated by the parasite *T. gondii* for 24, 48 and 72 hours. The characteristics of the microenvironment can be observed in figures 1, 2 and 3 in the 10X lens. At the beginning of the infection process the changes in the microenvironment are subtle, however with the increase in incubation time the amount of parasites increases, leading to increased cell damage and cell death.

In these results we follow the progression of cellular changes that occur in the infection process, involving the maintenance of the survival of the parasite in the intracellular environment, always compared with control cells (without parasite).

In figure 1 we have the comparison between the microenvironment and morphological changes in 24 hours of infection highlighting the subtle increase in cell death, already in figure 2 this same comparison is made in 48 hours of infection highlighting the difference of the microenvironment between the concentrations of infection, finally figure 3 mark the time limit for the maintenance of the infected culture, being extreme 72 hours, by reducing the number of cells in culture.

Emphasizing that the alteration of the behavior of cells in relation to their occupation in the microenvironment and morphological changes in IEC-6 cells are evident with the progression of time and become evident from 48 hours, when compared with control cells.

Figure 1 - Phase contrast optical microscopy micrographs after 24 hours of infection using IEC-6 cells. Comparison of control cells (A) with cells infected with *Toxoplasma gondii* in the proportion 5:1 (B) and 10:1(C) (parasites per cell). It is possible to visualize the alteration of the behavior of cells in relation to their occupation and cell death in the microenvironment, when compared with the control cells in both proportions of infection, being more evident in the ratio 10:1 (C). Arrow in ellipse indicating cell *death.* Increases in 10X lens. It is possible to visualize the change in morphology with more turbid cells and the beginning of detachment of cells indicating the beginning of cell death, when compared with control cells in both proportions of infection, being more evident in the ratio 10:1 (C). Open arrow indicating morphological changes and arrows in ellipse indicating cell death. Increases in 40X lens.

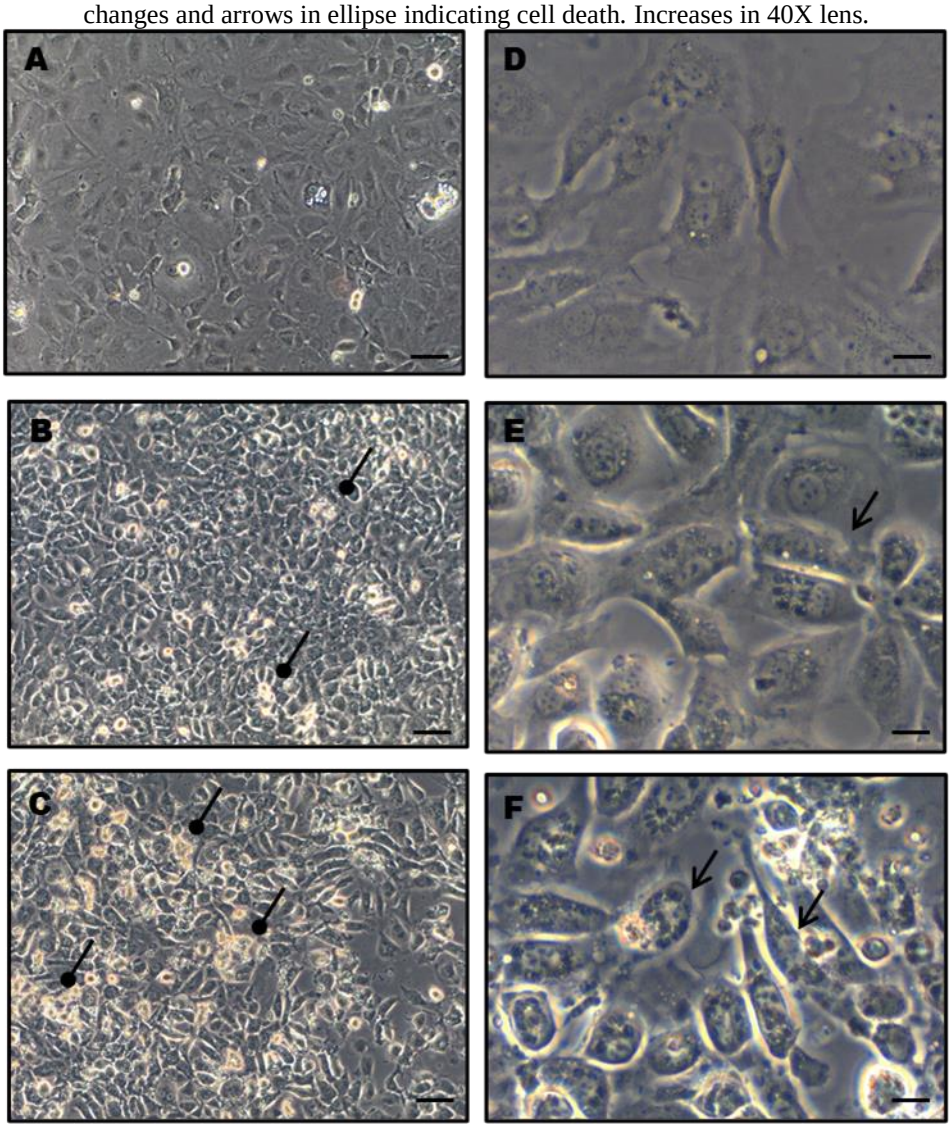


Figure 2 - Phase contrast optical microscopy micrographs after 48 hours of infection using IEC-6 cells. Comparison of control cells (A) with cells infected with Toxoplasma gondii in the proportion 5:1 (B) and 10:1(C) (parasites per cell). There is an increase in the number of cells in B due to cell growth, however in C we have increased cell death by the large number of parasites. Arrow in ellipse indicating cell death. Increases in 10X lens. It is possible to visualize cell morphology with discontinuous patterns due to the increase of intracellular parasites and a large amount of dead cells when compared with control cells in both proportions of infection. Open arrow indicating morphological changes and arrows in ellipse indicating cell death. Increases in 40X lens.

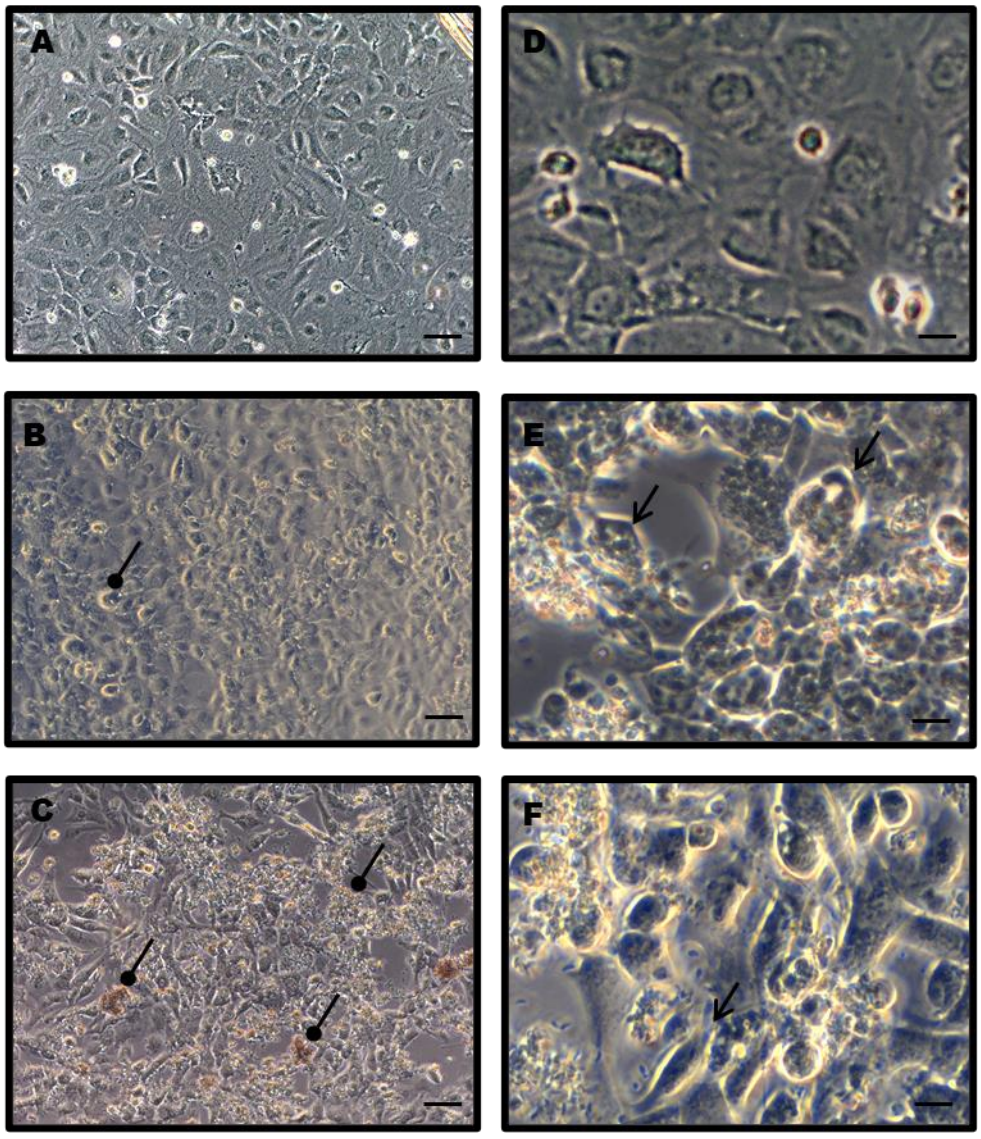
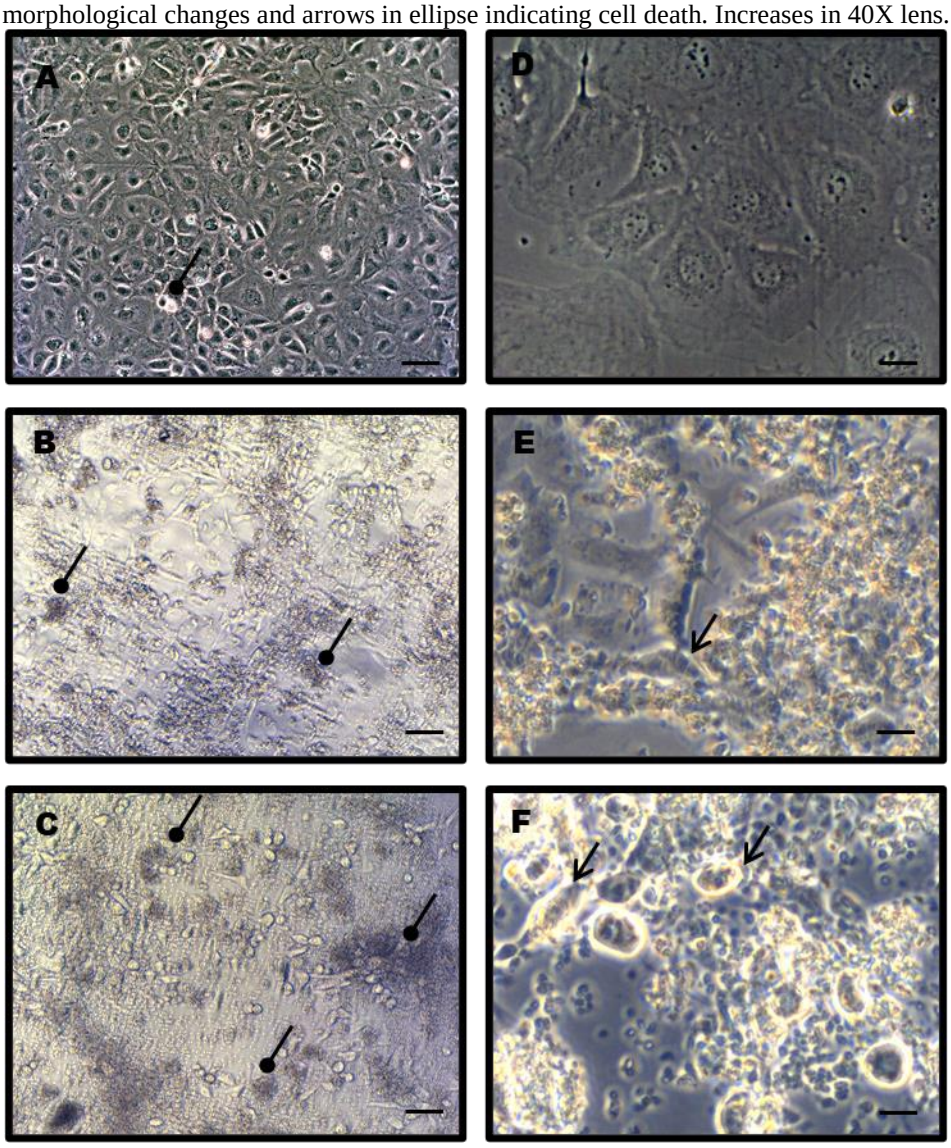


Figure 3 - Micrographs under phase contrast optical microscopy after 72 hours of infection using IEC-6. Comparison of control cells (A) with cells infected with Toxoplasma gondii in the proportion 5:1 (B) and 10:1(C) (parasites per cell). In A we have the increase in the number of cells by the continuity of the cell cycle (arrow in rand), while in B and C we have the increase of cell death by the large number of parasites, and in B we observed a microenvironment with more viable cells when compared with C. Arrow in rare go showing the increase in the number of cells and arrows in ellipse indicating cell death. Increases in 10X lens. Comparison of control cells (A) with cells infected with Toxoplasma gondii in the proportion 5:1 (B) and 10:1(C) (parasites per cell). It is possible to visualize completely altered cell morphology in both proportions and a large amount of dead cells when compared to control cells and it is also possible to observe the increase of the extracellular parasite due to lysis of infected cells, being more evident in the 10:1 (C) ratio. Open arrow indicating morphological changes and arrows in ellipse indicating cell death. Increases in 40X lens.



4 DISCUSSION

4.1 MORPHOLOGY AND MICROENVIRONMENT

Toxoplasma gondii is a parasitic pathogen transmitted mainly through oral consumption of contaminated food and water, so the gastrointestinal tract is its main route of entry into the host. To promote infection, the parasite has developed several strategies to quickly overcome the intestinal epithelial barrier (JONES *et al*., 2017). The ability of

Toxoplasma gondii to spread in the host depends on its ability to avoid a sequence of antimicrobial responses that are initiated rapidly after infection (GREGG *et al*., 2013). This invasion of the host cell parasite occurs around 30 seconds (Sibley & Andrews, 2000). According to Howe and Sibley (1995), the RH strain, the same used in the previous study, is the most virulent, thus being able to replicate rapidly promoting infection.

It is known that toxoplasmosis is initially established in the body through infection in the small intestine being widely reinforced the luminal pathway, however the exact part is not well described. To date it is established that the small intestine ileum is the main replication site of *Toxoplasma gondii* as pointed out by Dubey (1997), Speer (1998) and Barragan; Brossier & Sibley (2005), however Courret *et al.* (2006) conducted a study using quantitative analysis demonstrating high parasitic loads present in jejunum and Góis *et al.* (2015) highlighted lesions in the jejunum of rats after infection with *T. gondii.* This data was reinforced by Coombes *et al.* (2013) through the microscopy assay that reported several marking points in the small intestine before the spread of the infection. Based on this, the cell IEC-6 characterized by Quaroni *et al.* (1979) is one of the viable models for in vitro experiments because it is a cell line derived from the rat jejunum crypt (*rattus norvergicos*), for studies of cellular interaction with *Toxoplasma gondii* in order to evaluate changes in this cellular microenvironment

It was found that the infection performed in 10:1 presented more significant changes when compared to the 5:1 infection. This data can be evidenced already from 24 hours of infection as shown in Figure 1, being possible to visualize a greater number of dead cells when the cells are infected in the proportion of 10:1 (figure 1C). When observing figure 1, with an increase in the 40x lens, also in 24 hours of infection, the onset of morphological changes is noticeable when the infected cells (figure 1E and 1F) are compared with the control (figure 1D), however these changes are considerably higher in the proportion of infection of 10:1 (figure 1F), with more turgid cells due to parasitemia.

Changes in cell morphology and microenvironment become more pronounced with increased infection time. In 48 hours (figure 2), it is possible to visualize a large increase of parasites both in the intracellular environment showing that there was replication of the parasite by the endodiogeny process, as well as the increase of *Toxoplasma gondii* in the extracellular medium, due to the disruption of some infected cells, leading to the process of reinfection of the surviving cells (DUBEY *et al*., 1998). Because of this, infected cells acquire discontinuous morphological patterns, such changes are even more significant in the proportion of infection of 10 parasites per cell.

When commencing the 72-hour times of infection (figure 3), it's possible to observe in both proportions the decrease of viable cells. However, in the proportion of infection 5:1 it's possible to highlight that there are more viable cells in the microenvironment despite morphological changes, allowing to monitor the changes in the microenvironment as seen by Briceño *et al.* (2016). The proportion of infection 10:1 was used in other strains as seen in the work of De Carvalho *et al.* (2020), and this proportion was also used in the IEC-6 strain by Muno *et al.* (2014) with the ME-49 strain for 10 days, however we observed that this proportion of infection in the RH strain presented a significant amount of dead cells and consequently the reduction of viable cells, corroborating the data of Bernstein *et al.* (2020), but this model may be relevant to evaluate acute infection in vitro in a short period of time. Another notorious fact attributed to excess lysed cells is the increase of the extracellular parasite more evident in the 10:1 ratio when compared to previous times, due to reproductive cycle of about 6 to 12h (MENG *et al.* 2009). Therefore, it was possible to observe that the proportion of infection of 10 parasites by cells is not the most appropriate for studies in a period of more than 48h.

When analyzing the studies involving the interaction of IEC-6 with *Toxoplasma gondii* such as Jones's (2017) and Muno's (2014) there are no reports of research and follow-up of morphological and microenvironment alterations caused by parasitemia under phase contrast microscopy in the description of the infection process by temporality. With this, the present work presents for the first time in the literature these data.

5 CONCLUSION

In view of the results presented, it was concluded that the cells of the intestinal epithelial lineage IEC-6 infected with the parasite *Toxoplasma gondii* presented changes in the microenvironment and morphological changes in both proportions of infection, however in the 1:5 infection process there were viable cells at all times despite the cellular damage caused by parasitemia, concluding that the 1:5 infection ratio is the most appropriate for future studies requiring temporal follow-up because the 1:10 ratio only remained viable up to 48h.

REFERENCES

BARRAGAN, Antonio; BROSSIER, Fabien; SIBLEY, L. David (2005) Transepithelial migration of Toxoplasma gondii involves an interaction of intercellular adhesion molecule 1 (ICAM-1) with the parasite adhesin MIC2. **Cellular microbiology**, v. 7, n. 4, p. 561-568.

BERNSTEIN, Mariana *et al*. (2020) Evaluation of biological behavior of Toxoplasma gondii atypical isolates 14 and 163. **Experimental parasitology**, v. 211, p. 107860.

BRICEÑO, M.P.; *et al*. (2016) *Toxoplasma gondii* Infection Promotes Epithelial Barrier Dysfunction of Caco-2 Cells.; **J Histochem Cytochem**.; V.64, n. 8, p. 459-69.

BURGER, E. *et al*. (2018) Loss of Paneth cell autophagy causes acute susceptibility to *Toxoplasma gondii*-mediated inflammation. **Cell host & microbe**, v. 23, n. 2, p. 177-190.

COOMBES, J.L. *et al.* (2013) Motile invaded neutrophils in the small intestine of *Toxoplasma gondii*-infected mice reveal a potential mechanism for parasite spread. **Proceedings of the National Academy of Sciences of the United States of America.** v.110, n. 21.

COURRET, N., *et al*. (2006) CD11c- and CD11b-expressing mouse leukocytes transport single *Toxoplasma gondii* tachyzoites to the brain. **Blood, v.**107, n.309.

DE CARVALHO, G.O.A.M. *et al*. (2021) Morphological evaluation of macrophage infected with *Toxoplasma gondii*. **Brazilian Journal of Development**, v. 7, n. 1, p. 4035-4050.

DUBEY, J.P. *et al.* (2005) Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: risk assessment to consumers. **Journal of Parasitology**, v. 91, n. 5, p. 1082-1093.

DUBEY, J.P. *et al.* (1997) Bradyzoite-induced murine toxoplasmosis: stage conversion, pathogenesis, and tissue cyst formation in mice fed bradvzoites of - different strains of *Toxoplasma gondii*. **Journal of Eukaryotic Microbiology**. v. 44, p. 592–602.

DUBEY, J.P.; LINDSAY, D.S.; SPEER, C.A. (1998) Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. **Clinical microbiology reviews**, v. 11, n. 2, p. 267-299.

GÓIS, Marcelo Biondaro. (2015) Toxoplasma gondii causa alterações na túnica mucosa e no plexo submucoso do jejuno e do cólon proximal de ratos. Ph.D. Tese em Ciências Biológicas. Universidade Estadual de Maringá, Maringá (PR) Brasil.

GREGG, B. *et al*. (2013) Replication and distribution of *Toxoplasma gondii* in the small intestine after oral infection with tissue cysts. **Infection and immunity,** v. 81, n. 5, p. 1635-1643.

HOWE, D.K.; SIBLEY, L.D.; (1995) *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. **The Journal of Infectious Diseases**, v:172, p:1561-1566.

JONES, E.J.; KORCSMAROS, T.; CARDING, S.R. (2017) Mechanisms and pathways of *Toxoplasma gondii* transepithelial migration. **Tissue Barriers**, v:5, n:1.

MENG, Xiao Li *et al*. (2009) Kinetic observation on the invasion into and proliferation in IEC-6 cells of Toxoplasma gondii RH strain tachyzoites in vitro. **Zhongguo ji sheng chong**

xue yu ji sheng chong bing za zhi Chinese journal of parasitology & parasitic diseases, v. 27, n. 1, p. 39-42.

MINISTÉRIO DA SAÚDE (Brasil). **Surtos de Doenças Transmitidas por Alimentos no Brasil**. Disponível em: <http://portalarquivos2.saude.gov.br/images/pdf/2018/janeiro/17/Apresentacao-Surtos-DTA-2018.pdf>. Publicado em jan. 2018. Acesso em: 08 de mar. 2021.

MINISTÉRIO DA SAÚDE (Brasil). **Doenças Transmitidas por Alimentos**. Disponível em: <https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z-1/d/doencas-transmitidas-por-alimentos>. Publicado em 16/11/2020 - Atualizado em 27/04/2021. Acesso em: 08 de mai. 2021.

MONTOYA, J. G.; LIESENFELD, O. (2004) Toxoplasmosis. **The Lancet**, v. 363, p. 1965–1976.

MUNIZ, L.R. *et al*. (2012) Intestinal antimicrobial peptides during homeostasis, infection, and disease. **Frontiers in immunology** v. 3 p. 310.

MUNO, R. M. *et al*. (2014) Spontaneous cystogenesis of *Toxoplasma gondii* in feline epithelial cells in vitro. **Folia Parasitologica,** v.62, n.2, p.113-119.

QUARONI, A. *et al*. (1979) Epithelioid Cell Cultures from Rat Small Intestine Characterization by Morphologic and Immunologic Criteria. **The Journal of cell biology**, v. 80, n. 2, p. 248-265.

RAMÍREZ-FLORES, C. J. *et al.* (2019) Characterization of metalloproteases and serine proteases of *Toxoplasma gondii* tachyzoites and their effect on epithelial cells. **Parasitology research**, v. 118, n. 1, p. 289-306.

REMINGTON J. S. *et al*. (2006) Infectious diseases of the fetus and newborn infant. In:Toxoplasmosis. **Elsevier Saunders.** 6º edição. p.947-1091.

SIBLEY, L.D.; ANDREWS, N.W. (2006) Cell Invasion by un-palatable parasites. **Traffic,** v:1 p:100-106.

SPEER, C. A.; DUBEY, J. P. (1998) Ultrastructure of early stages of infections in mice fed *Toxoplasma gondii* oocysts. **Parasitology**, v. 116, n. 1, p. 35-42.

WEIGHT, C.M. *et al*. (2015) Elucidating pathways of *Toxoplasma gondii* invasion in the gastrointestinal tract: involvement of the tight junction protein occludin. **Microbes and infection**, v. 17, n. 10, p. 698-709.