Morphological evaluation of macrophage infected with *Toxoplasma Gondii*

Avaliação morfológica de macrófagos infectados com T*oxoplasma Gondii*

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ABSTRACT
Toxoplasma gondii is the parasite that causes toxoplasmosis, a neglected disease that still needs studies aimed at elucidating the relationship between parasitemia and the immune system. One of the main cells of the immune system are macrophages and these have immortalized cell lines that provide an accessible study model for *in vitro* experiments. With this, this work intends to emphasize the importance of the macrophage cell line J774G8 in studies with protozoa, highlighting the viability and morphological changes of the cell culture infected with *Toxoplasma gondii*.

Keywords: Macrophages, J774G8 cell line, cell culture, *Toxoplasma gondii.*

RESUMO
Toxoplasma gondii é o parasita causador da toxoplasmose, uma doença negligenciada que ainda necessita estudos voltados para elucidar a relação entre a paresitemia e o sistema imunológico. Uma das principais células do sistema imunológico são os macrófagos e estes possuem linhagem celulares imortalizadas que fornecem um modelo de estudo acessivel para experimentos *in vitro*. Com isto, este trabalho tem a intenção de enfatizar a importânca da linhagem celular macrofágica J774G8 em estudos com protozoários, destacando a viabilidade e alterações morfologicas da cultura celular infectada com *Toxoplasma gondii*.

Palavras-chave: Macrófago, J774G8 linhagem celular, cultura celular, *Toxoplasma gondii.*

1 INTRODUCTION

Toxoplasma gondii (*T. gondii*) is obligatory intracellular protozoan that causes Toxoplasmosis. This neglected disease affects up to a third of the world population, and

the serum prevalence in Brazil is 4 times higher than in the United States (DUBEY et al., 2012; MONTOYA and LIESENFELD, 2004).

This extremely widespread pathology still has mechanisms that need to be elucidated, highlighting the importance of understanding the relationship between parasitemia and the host's innate immune response (DELGADO BETANCOURT et al., 2019; SICA et al., 2015). For this, it is necessary to establish different technique studies and protocols with resident or established culture cells, but ethical and structural issues give strength to *in vitro* cell culture models since *T. gondii* is a protozoan parasite that can be grown both *in vivo* and *in vitro* (BUDDHIRONGAWATR et al., 2006; JABARI et al., 2018; EVANS et al., 1999; DÖŞKAYA et al., 2006).

Thus, this article highlights the importance of the J774G8 cell line for research with protozoa. This cell is widely used for experiments involving cell-parasite interaction due to its macrophage / monocytic characteristics, easy handling. The lineage of this study originated from the J774 cell lineage described in 1975 as to its similarity with resident immunological macrophages, highlighting its characteristic of adhesion, morphology, proliferative capacity and rapid pinocytosis. It was recloned by limiting dilution and described in 1979 (Unkless et. al, 1979). This cell line has a rapid cell cycle end the cells grow either in suspension or as adherent cells and differs from the original line in that it does not change in the binding of some membrane receptors.

Reports of the use of the macrophage cell line J774-G8 in experiments with protozoa started in 1980, has been used until then for interaction, maintenance or expansion of the culture of protozoa (CHANG, 1980).

Protozoan	Age	Title of the article	Doi	Reference
Leishmania spp.	1980	Human cutaneous *Leishmania* in a mouse macrophage line: propagation and isolation of intracellular parasites	10.1126/science.7403880	CHANG, K. P. Human cutaneous L*eishmania* in a mouse macrophage line: propagation and isolation of intracellular parasites. **Science**, v. 209, n. 4462, p. 1240-1242, 1980.
	1981	Tubulin biosynthesis in the developmental cycle of a parasitic protozoan, *Leishmania* mexicana: changes during differentiation of motile and nonmotile stages.	10.1073/pnas.78.12.7624	Fong, D., Chang, K. P. Tubulin biosynthesis in the developmental cycle of a parasitic protozoan, *Leishmania mexicana*: changes during differentiation of motile and nonmotile stages. **Proceedings of the National Academy of Sciences**, v. 78, n. 12, p. 7624-7628, 1981.
	1981	Interaction of *Leishmania* with a macrophage cell line. Correlation between intracellular killing and the generation of oxygen intermediates	10.1084/jem.153.6.1690	MURRAY, H. W. Interaction of *Leishmania* with a macrophage cell line. Correlation between intracellular killing and the generation of oxygen intermediates. **The Journal of experimental medicine**, v. 153, n. 6, p. 1690-1695, 1981.
	1982	Antigenic changes during intracellular differentiation of *Leishmania mexicana* in cultured macrophages.	10.1128/IAI.36.1.430-431.1982.	Chang K.P, Fong D. Antigenic changes during intracellular differentiation of Leishmania mexicana in cultured macrophages. **Infection and immunity**, v. 36, n. 1, p. 430-431, 1982.
	1982	Surface antigenic change during differentiation of a parasitic protozoan, *Leishmania* mexicana: Identification by monoclonal antibodies.	10.1073/pnas.79.23.7366	Fong, D., Chang, K. P. Tubulin biosynthesis in the developmental cycle of a parasitic protozoan, *Leishmania mexicana*: changes during differentiation of motile and nonmotile stages. **Proceedings of the National Academy of Sciences**, v. 78, n. 12, p. 7624-762
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	Leishmania increases their binding to macrophages.		macrophages. **Proceedings of the National Academy of Sciences**, v. 89, n. 11, p. 4991-4995, 1992.
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1996	Surface Zn-proteinase as a molecule for defense of *Leishmania mexicana amazonensis* promastigotes against cytolysis inside macrophage phagolysosomes.	10.1128/IAI.64.12.5129-5137	Seay, M. B., Heard, P. L., Chaudhuri, G. Surface Zn-proteinase as a molecule for defense of *Leishmania mexicana amazonensis* promastigotes against cytolysis inside macrophage phagolysosomes. **Infection and immunity**, v. 64, n. 12, p. 5129-5137, 1996.
1997	Scavenger Receptor-Mediated Delivery of Antisense Mini-Exon Phosphorothioate Oligonucleotide to *Leishmania*-Infected Macrophages. SELECTIVE AND EFFICIENT ELIMINATION OF THE PARASITE	10.1016/s0006-2952(96)00763-0	CHAUDHURI, G. Scavenger receptor-mediated delivery of antisense mini-exon phosphorothioate oligonucleotide to *Leishmania*-infected macrophages: selective and efficient elimination of the parasite. **Biochemical pharmacology**, v. 53, n. 3, p. 385-391, 1997.
1998	*In vitro* Method for Isolation of Amastigote Forms of *Leishmania amazonensis* from J774G8 Macrophage Induced by Temperature Shifting	10.1590 / s0074-02761998000100017	MORENO, M. L. V.; MEIRELLES, M. N. L. *In vitro* method for isolation of amastigote forms of *Leishmania amazonensis* from J774G8 macrophage induced by temperature shifting. **Memórias do Instituto Oswaldo Cruz**, v. 93, n. 1, p. 99-102, 1998.
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	2013	The Effects of N-Butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide against Leishmania amazonensis Are Mediated by Mitochondrial Dysfunction	10.1155/2013/874367	Volpato H., et al.. The effects of N-butyl-1-(4-dimethylamino) phenyl-1, 2, 3, 4-tetrahydro-β-carboline-3-carboxamide against *Leishmania amazonensis* are mediated by mitochondrial dysfunction. **Evidence-Based Complementary and Alternative Medicine**, v. 2013, 2013.
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	2019	Asteraceae Plants as Sources of Compounds Against Leishmaniasis and Chagas Disease	10.3389/fphar.2019.00477	Moraes Neto, R. N.,et al. Asteraceae plants as sources of compounds against Leishmaniasis and Chagas disease. **Frontiers in pharmacology**, v. 10, p. 477, 2019.
Toxoplasma gondii	1985	Lymphokine Activation of J774G8 Cells and Mouse Peritoneal	10.1128 / IAI.49.3.760-764.1985.	SIBLEY, L. DAVID; KRAHENBUHL, J. L.; WEIDNER, E. Lymphokine activation of J774G8 cells and mouse peritoneal macrophages challenged with *Toxoplasma gondii*. **Infection and immunity**, v. 49, n. 3, p. 760-764, 1985.

		Macrophages Challenged with *Toxoplasma gondii*		
	2010	Activation of the P2X7 receptor triggers the elimination of *Toxoplasma gondii* tachyzoites from infected macrophages.	10.1016/j.micinf.2010.03.004	CORRÊA, G. et al. Activation of the P2X7 receptor triggers the elimination of *Toxoplasma gondii tachyzoites* from infected macrophages. **Microbes and infection**, v. 12, n. 6, p. 497-504, 2010.
Trypanosoma cruzi	1988	Activity of P536, a UDP-glucose analog, against *Trypanosoma cruzi.*	10.1128/aac.32.9.1412	Alcina A., Fresno M., Alarcón B. Activity of P536, a UDP-glucose analog, against *Trypanosoma cruzi*. **Antimicrobial agents and chemotherapy**, v. 32, n. 9, p. 1412-1415, 1988.

Within the host cell, the parasite has several evasion mechanisms and set up mechanisms of host subversion (CLOUGH and FRICKEL, 2017). Infection with *T. gondii* promotes a high innate immune response, however, this protozoan has effector proteins for evasion strategies (LIMA and LODOEN, 2019), that allow control of transcription host gene (ONG, REESE and BOOTHROYD, 2010; RAZAEL et al., 2018), deregulation of signaling pathways that result in modulation of adhesion and cell migration, secretion of immunoregulatory cytokines (MATTA et al., 2018), production of microbicidal molecules (WILSON, TSAI and REMINGTON, 1980) and apoptosis (GOEBEL, GROSS and LÜDER, 2001; KELLER et al., 2006; MOLESTINA, EL-GUENDY and SINAI, 2008) to establish infection. Among the changes inside the cell we also have the modification of the positioning of cellular organelles (GUIMARÃES, DE CARVALHO and SANTOS BARBOSA, 2008) and alteration of the behavior of cell culture.

Therefore, the objective of this work is to describe the behavior, main morphological changes, and alterations in the proliferative activity of the macrophage J774G8 cells infected with *T. gondii* in an acute parasitic infection study model.

2 MATERIALS AND METHODS

Cell culture of macrophage lineage J774-G8

The lineage of macrophage cell J774-G8 the cells were plated, with an initial density of $1x10^5$ cells/ml in plastic bottles of 25cm². The culture were kept in medium DMEM (Dulbecco's modified eagle's medium) supplemented with 10% of fetal bovine serum (v/v) (GIBCO-Life Technologies, Rockville, MD), penicillin 1000 UI/mL and streptomycin 100uI/mL (Sigma Chemical Company, St Louis, MO). The cells were kept at 37°c, in humid atmosphere to 5% of CO_2 (Culture CO_2 Incubator, model CCL-170B-8, Sigapore).

Obtaining Tachyzoite

Tachyzoite form of *T. gondii* (strain RH) 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 Universidade Estadual do Norte 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.

Cell Parasite Interaction

The cells were plated in plastic bottles of 25 cm² and after 48 hours, the culture reached the confluence. The tachyzoite of *T. gondii* was resuspended in medium DMEM and placed in contact with the cell in the proportion of 10 parasites for each cell. This was kept at 37°c, in humid atmosphere to 5% of CO_2 (Cuture CO_2 Incubator, model CCL-170B-8, Sigapore), by 1 hour. Then, the medium was discarted and replaced for DMEM (Dulbecco´s modified Eagle´s medium) supplemented with 10% fetal bovine serum (GIBCO-Life Technologies, Rockville, MD), penicillin 1000Ul/ml and streptomycin 100Ul/ml (Sigma Chemical Company, St Louis, MO), were kept to 37°c in humid atmosphere to 5% CO_2 in the period of 24, 48 and 72 hours.

The micrographs were obtained using the Axiovert 40 CFL Microscope (ZEISS, Germany) with the aid of the AxioVision program. The cells were counted with the aid of the Image J program to obtain the data plotted in the statistical program Graphpad Prism v6.0.

Statistical Analysis

To compare the analysis of variance was used one-way Anova with the pos test of turkey. All statistics were made through the software Graphpad Prism v6.0. The values of significance were less than 0,001 ($p < 0,001$).

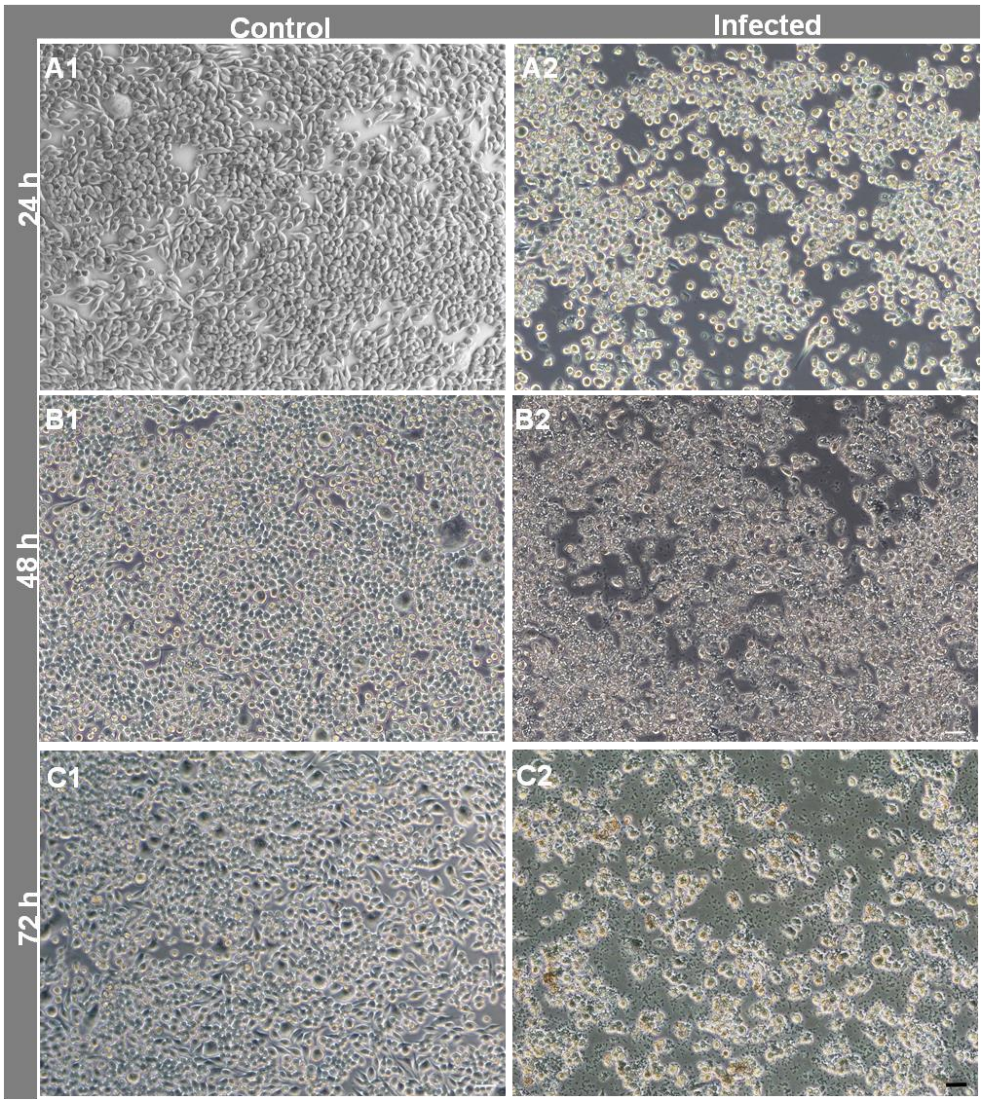
3 RESULTS

The behavior of the cell culture was evaluated using phase contrast microscopy in order to monitor the cell behavior. The macrophage cell lineage J774-G8 was infected at the rate of 10 parasites per cell in order to analyze how the junction communication profile is altered in the acute infection microenvironment generated by the parasite *T. gondii* during 24, 48 and 72 hours. In this, it is possible to observe the cellular changes that occur in the infection process, involving the maintenance of the parasite's survival in the intracellular environment, compared with the control cells (without parasite).

Figure 1 shows phase contrast micrographs of the infection of J774-G8 cells at 24, 48 and 72 hours in cultures of the control group (Figure 1 A1, B1 and C1) and infected

(Figure 1 A2, B2 and C2). Over time we can observe the increasing damage caused by increased parasitemia and parasite-cell interaction. All micrographs were taken in 100X magnification, in order to present an overview of the general damage of the crop.

Figure 1: Micrographs in optical microscopy (phase contrast) using cells of the macrophage lineage J774-G8 control (number 1) and infected with *T. gondii* in the proportion of ten parasites by cell (number 2). In the schedules of 24, 48 and 72 hours (respectively A, B and C) it's possible observe the change of the behavior of infected cells regarding to your occupation of the environment, being in lumps (On the bottom of the bottle or in the supernatant) or detaching from the culture bottle (A2, B2 and C2) when compared to control cells. Calibration bar 20 µm. Increases of 100x (Objective of 10x).

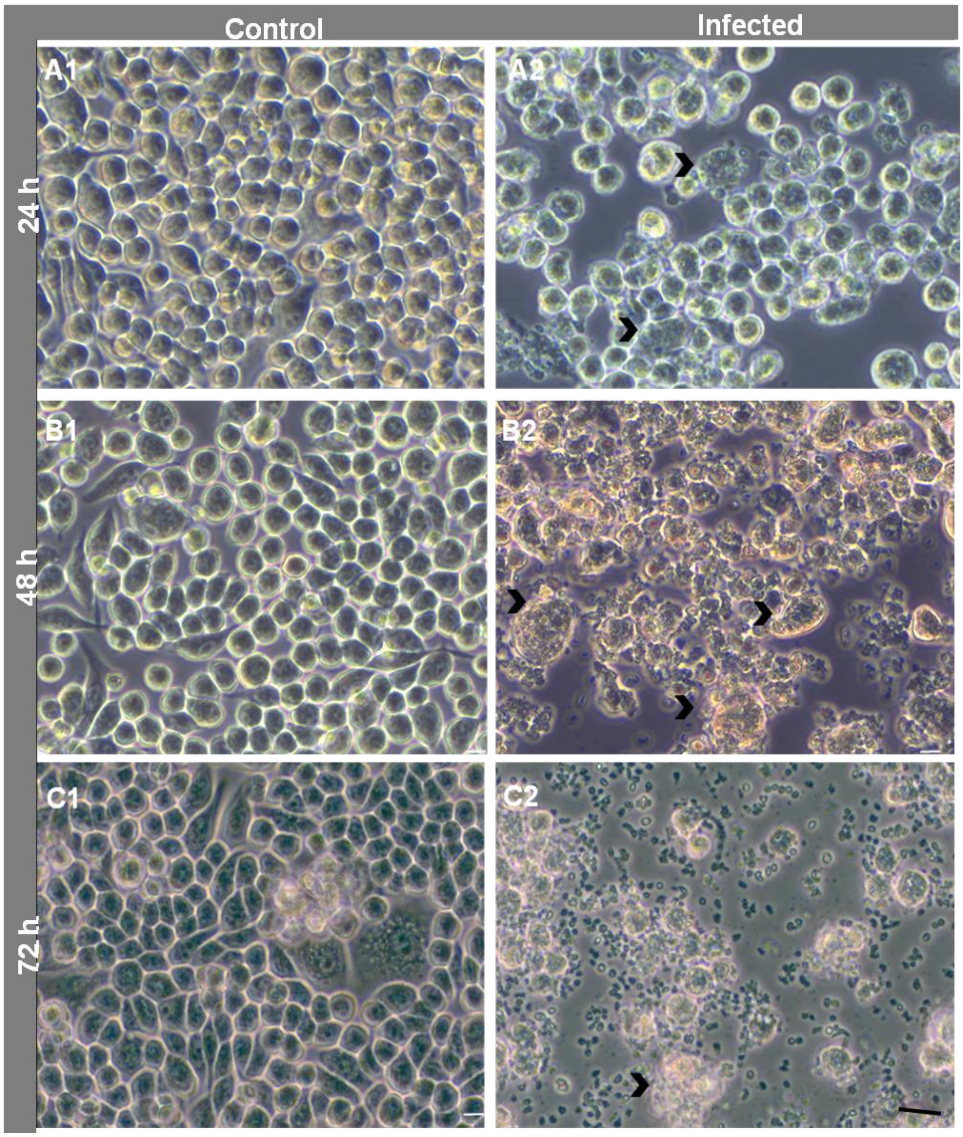


We observed that there were changes in all incubation times, with the formation of cell clumps and the detachment of cells from the culture bottle, leading to a decrease in the number of cells in culture and an increase in the amount of free parasites by cell lysis. During the interaction, in the period of 72 hours, the greatest damage was observed when compared to other times of interaction and to the control, since the infected cells

were lysed by the parasite thus increasing the amount of this free in culture.

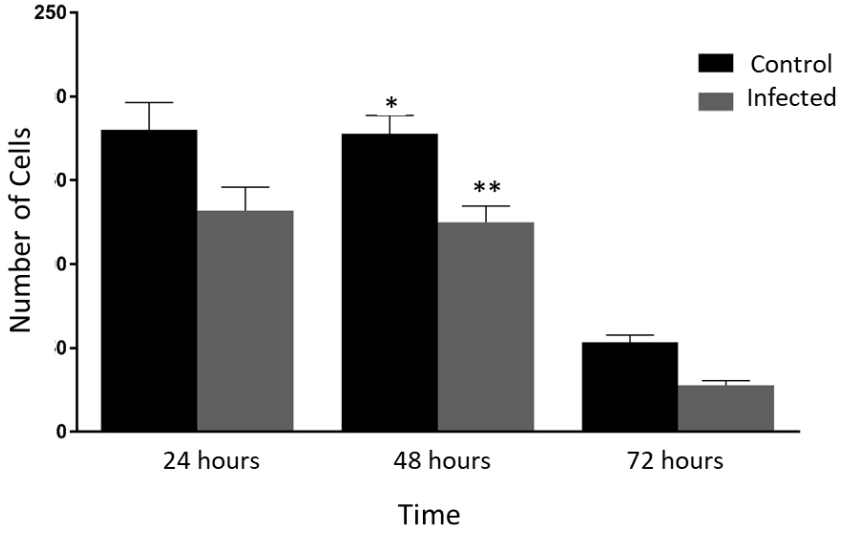
In figure 2, the phase contrast micrographs show the morphological aspects of J774-G8 cells in the control (Figure 2 A1, B1 and C1) and infected (Figure 2 A2, B2 and C2) groups, after the parasite-cell interaction for 24, 48 and 72 hours. Comparing the control and treated groups, we observed that there were morphological changes and cell detachment with the progression of time, as well as an increase the number of free parasites in the culture. Magnifications of 400X (40X objective) were used.

Figure 2: Micrographs in optical microscopy (phase contrast) using cells of the macrophage lineage J774-G8 control (number 1) and infected with *T. gondii* in the proportion of ten parasites by cell (number 2). In the schedules of 24, 48 and 72 hours (respectively A, B and C) morphological changes in cells J774-G8 are evident, as can be seen in the image A2, B2 and C2 (Arrowhead highlighting) with increased infection time the amount of parasites increased what may explain cell damage and cell death calibration bar 20µm. Increases of 400 X (40x objective).



In experiments with phase contrast, is possible to observe changes in all incubation times, with the formation of cell clumps and the detachment of cells from the culture bottle, leading to a decrease in the number of cells in culture and an increase the number of free parasites by cell lysis. As seen in Figure 3, the interaction in the 48-hour period presents a significant difference between the number of cells in the control and infected group, showing that the parasitemia has altered the cell behavior in a more expressive way. However, in 72 hours of infection, we observed the greatest damage when compared to other times of interaction, since the infected cells were lysed by the parasite, thus increasing the amount of this free in culture.

Figure 3: Graph of cell growth of the J774G8 control and infected with *Toxoplasma gondii* in the 24h, 48h and 72h. In the graph is observed of the progression of infection of the J774G8 strain compared to the uninfected control cell with the reduction in the number of cells in culture at all times compared. In the 24h period, no significant difference was observed between the number of control and infected cells. In 48h the number of cells contained is chronic greater than the number of infected cells and in the 72h column the decline in the number of cells in the supply parameters is observed. P <0.001.



4 DISCUSSION

Parasitic infections are a global public health problem (TALABANI et al., 2010). We sought to study the changes related to cell growth and the morphological aspects associated with macrophages (Cell Lineage J774-G8), infected with *Toxoplasma gondii*.

It was possible to observe a severe decrease in cell growth and survival throughout the infection process (24, 48 and 72 hours) (Figure 1 and 2 - A2, B2 and C2), associated to the maintenance of the parasite survival in the intracellular environment, when compared to the cells (uninfected) showing a significative difference between the number of control and infected cells within 48h (Figure 3).

These modifications are in agreement with the findings associated to the induction of alterations in the biochemical and cellular pathways promoted by the parasite, so that the same can maintain its survival. One of these actions is associated with the inhibition of nitric oxide (NO) production in peritoneal macrophages and macrophage lineage J774-A1, after infection with *T. gondii* (SEABRA et al., 2004; DA CRUZ PADRÃO et al., 2014). This deactivates its microbicidal ability to destroy the pathogen. Lüder et al. (2017) demonstrated that *T. gondii* has the ability to inhibit the activation of macrophages.

Increasingly, we observed the formation of cell clumps and the detachment of cells from the culture bottle, associated with morphological changes caused by increased parasitemia and consequently of parasite-cell interaction, thus causing a decrease in the number of viable cells in culture (Figure 3).

Different changes in the cell microstructure are highlighted in the literature, which can justify the changes in the cell structure and its size as seen in Figure 2, such as the change in the organization of the cell organelles in the presence of the parasite, bringing them closer to the parasitophore vacuole.

Thus, we observed that a J774G8 cell culture shows satisfactory resistance for experiments of acute infection model with *T. gondii* in the maximum 10:1 parasite-cell ratio. Observing in 24h the reduction of cell growth due to infection and the beginning of lysed cells in 48h of infection that led to reduced the number of cells in culture, however in the maximum period of 72h of interaction despite the observation of a low amount of viable cells, they were present even with morphological changes.

4 CONCLUSION

Considering the results obtained in our studies, we can conclude that J774-G8 macrophagic line infected with the parasite *Toxoplasma gondii* showed not only changes in cell growth, but also demonstrated morphological changes, accompanied by cell death.

However, these cells present a satisfactory resistance to experiments that mimic an acute phase of infection with *T. gondii* even with a high concentration of protozoa interacting with the cells.

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