

Adriana Filipa de Figueiredo Oliveira

Relatórios de Estágio e Monografia intitulada "Toxoplasmosis and Psychotic Disorders" referentes à Unidade Curricular "Estágio", sob orientação, respetivamente, do Dr. Paulo Monteiro, da Dra. Patrícia Flório e da Professora Doutora Maria do Céu Sousa e apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro 2017



UNIVERSIDADE DE COIMBRA

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Coimbra, 15 de setembro de 2017.

Adriana Filipa de Figueiredo Oliveira

(Adriana Filipa de Figueiredo Oliveira)

## Thanks

To my Mother and to my Father for the permanent support, strength, confidence, understanding and patience, for without them I could not have gotten here. There will never be words to describe what I feel or to thank for everything they have done for me.

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Thank you!

## Resumo

No âmbito da formação académica do Mestrado Integrado em Ciências Farmacêuticas está enquadrada a realização do Estágio Curricular e a elaboração de uma Monografia. Optei por realizar uma parte do Estágio Curricular na área de Indústria Farmacêutica e a outra parte em Farmácia Comunitária de forma a experienciar estas duas possíveis saídas profissionais e a executar, no futuro, uma escolha mais fundamentada. Os estágios referidos permitem aplicar e consolidar os conhecimentos teóricos e práticos adquiridos durante a formação académica, sendo que as atividades desenvolvidas no decorrer destes estágios serão aqui apresentadas num formato de análise SWOT, com descrição dos Pontos Fortes (Strengths), Pontos Fracos (Weaknesses), Oportunidades (Opportunities) e Ameaças (Threats). O tema escolhido para a Monografia foi “Toxoplasmose e Doenças Psicóticas” devido ao interesse que esta possível associação tem suscitado na comunidade científica.

A toxoplasmose e as doenças psicóticas apresentam elevada prevalência a nível mundial e constituem problemas de saúde pública. Com o aparecimento da hipótese da manipulação de comportamento exercida por *Toxoplasma gondii* nos hospedeiros, através de alterações comportamentais, de fenótipo e de personalidade, surgiram vários estudos a questionar esta associação e quais os mecanismos nela envolvidos. Surgiram, também, teorias acerca da possível relação entre a toxoplasmose e o aparecimento e evolução de doenças psicóticas, tais como esquizofrenia e doença bipolar.

Torna-se importante reunir a informação existente acerca destas possíveis associações de forma a compreender qual o papel que o parasita *T. gondii* exerce nos seus hospedeiros de forma a esclarecer os mecanismos relacionados com o aparecimento das doenças psicóticas. Além disso, através dos estudos efetuados poderão surgir novas alternativas terapêuticas para a toxoplasmose e/ou para as doenças psicóticas.

**Palavras-chave:** toxoplasmose, *Toxoplasma gondii*, doenças psicóticas, esquizofrenia, doença bipolar.

## **Abstract**

Within the scope of the academic training for the Integrated Master's Degree in Pharmaceutical Sciences, the Curricular Internship and the elaboration of a Monograph are required. I chose to do part of the Curricular Internship within the Pharmaceutical Industry sector and the other part in a Community Pharmacy, in order to experience these two possible career opportunities and to make a more informed choice in the future. The above mentioned internships allow for the application and consolidation of theoretical and practical knowledge acquired during the academic training, and the activities developed during these internships will be presented here in a SWOT analysis format, with a description of Strengths, Weaknesses, Opportunities and Threats. The theme chosen for the Monograph was "Toxoplasmosis and Psychotic Disorders" due to the interest that this possible association has aroused within the scientific community.

Toxoplasmosis and psychotic disorders are diseases with a high prevalence worldwide and constitute public health issues. Due to the emergence of the hypothesis of behaviour manipulation by *Toxoplasma gondii* in hosts, causing behavioural, phenotype and personality changes, several studies have appeared to question this association and the mechanisms involved in it. There have also been theories about the possible relationship between toxoplasmosis and the onset and evolution of psychotic disorders, such as schizophrenia and bipolar disorder.

It is important to gather existing information about these possible associations in order to understand the role that *Toxoplasma gondii* plays in its hosts and to clarify the mechanisms related to the onset of psychotic disorders. In addition, through the studies conducted, new clues and new therapeutic alternatives for toxoplasmosis and/or psychotic disorders may emerge.

**Key words:** toxoplasmosis, *Toxoplasma gondii*, psychotic disorders, schizophrenia, bipolar disorder.

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**I**

**Report on Community Pharmacy Internship**

**Farmácia S. José**

Supervisor: Dr. Paulo Monteiro

## **Abbreviations**

CD – Compounding Drug

CI – Curricular Internship

FPUC – Faculty of Pharmacy of the University of Coimbra

IMDPS – Integrated Master’s Degree in Pharmaceutical Sciences

SWOT – Strengths, Weaknesses, Opportunities and Threats

TT – Technical Team

## I Introduction

The Faculty of Pharmacy of the University of Coimbra (FPUC), which teaches the Integrated Master's Degree in Pharmaceutical Sciences (IMDPS), has the objective of training Pharmacists, who distinguish themselves positively and notoriously in the most diverse areas of which they will be part, and that constitute specialized professionals of medicines and health agents with technical skills and scientific knowledge, so that their intervention promotes the health and well-being of the citizen in general (ORDEM DOS FARMACÊUTICOS, 2015, UNIVERSIDADE DE COIMBRA, 2017). Thus, this master's degree consists of 5 years of theoretical and practical training in which the Curricular Internship (CI) in Community Pharmacy is inserted (UNIVERSIDADE DE COIMBRA, 2017). The internship allows students to apply and consolidate the knowledge acquired during their training and also establishes their connection within the labour market, since there is direct contact with patients in solving their problems and needs.

I chose “Farmácia S. José” as an internship place as it is a pharmacy with a lot of recognition due to the excellent quality of the Technical Team (TT) and the care, commitment and concern placed in each service and in the counselling and follow-up of the patient. In addition, this pharmacy has a very diverse range of services and products available to its public and a very friendly, dynamic and always ready to help team. I did my internship from April 3 to August 5 under the guidance of Dr Paulo Monteiro and the entire TT.

In this report, in SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis format, the activities carried out during my internship will be approached and analysed, critically and in relation to my experience as a trainee. Thus, the objective is to demonstrate the Strengths and Weaknesses in order to highlight the former and surpass the latter. And to present the Opportunities and Threats in order to take advantage of opportunities such as improvements and overcome threats.

## 2 SWOT Analysis

SWOT analysis is a very useful model for the diagnosis, evaluation and evolution of various activities and aspects, being used in a wide variety of areas, such as economics, business management and business negotiations, among others (IAPMEI, 2016). Thus, with this simple, practical and qualitative tool it is possible to analyse the various activities carried out during the CI, referring to the Strengths and Weaknesses belonging to the internal dimension, and the Opportunities and Threats related to the external dimension. The objective of this analysis is to identify opportunities for personal and professional improvement, taking advantage of strengths and overcoming weaknesses and threats.

### 2.1 Strengths

As mentioned above, the strengths have positively contributed to my curricular internship (IAPMEI, 2016).

#### 2.1.1 Location of the pharmacy

“Farmácia S. José” is located in Celas in a very busy and frequented area of Coimbra, since it is close to the Hospital Centre of the University of Coimbra, FPUC, a College Campus of Medicine of the University of Coimbra, a school, a hotel, clinics of the most varied specialities, being also surrounded by residential areas. For this reason, and also by the excellent TT and diversity of products and services available, is a place of passage and has a large number of patients with very heterogeneous characteristics, which contributed to my professional evolution.

#### 2.1.2 Technical Team

One of the factors that contributed most to the success of my internship and to the knowledge acquired was the sympathy, goodwill, hospitality, collaboration, experience and excellence of the TT of “Farmácia S. José”. They have always been very helpful for the clarification of doubts regarding both issues related to the pharmacy and its operation and the doubts that arose during the visits, clarifying how best to communicate, address or solve a particular situation. Thus, I felt integrated in the team and I was able to evolve a lot during the internship. As already mentioned, the experience of this team and the care that it puts into each service and the advice given to patients allows to differentiate “Farmácia S. José” from other pharmacies and these are essential elements for its high affluence, being that the importance of these values was transmitted to me early on as a member of the team.

### 2.1.3 Diverse public

The set of patients who attend “Farmácia S. José” is very heterogeneous and diversified, as already mentioned, and includes people of different age groups and belonging to different social, economic and literacy levels, part of which were already loyal customers to the pharmacy and people who just attend it on the one occasion. The loyal group consists largely of older people who visit the pharmacy several times a week; however, this group also contains younger people with different needs from the elderly.

Due to the existence of a very diverse public, the pharmacist needs to adapt to each patient and the situation presented to him/her, executing a personalized service. This factor, therefore, allowed me to grow personally and professionally and to develop confidence and skills in communication, adaptation, stress management, pressure and conflict, as well as acquiring essential tools to carry out personalized care and responsible counselling.

### 2.1.4 Opportunity to perform several roles

At the beginning of the internship period, the TT welcomed me through their explanation of the different areas in the pharmacy and the various roles performed in each of these areas, allowing me to get to know the functioning, organization and management of the pharmacy in general.

During the internship, I went through a range of functions, from order picking, prescriptions and invoices, to the storage of products in the robot, refrigerator, gondolas, linear, fast service drawers, drawers and sliding cabinets, also giving entry to some orders. In addition, I performed stock replenishment, management and regularization of returns, attendance to the public, determination of biochemical parameters and, also, preparation of compounding drugs (CD) with their respective supervision. In this way, I was able to integrate myself into the operation and organization of the pharmacy and thus increase my ability to perform the necessary functions in a virtually autonomous way.

### 2.1.5 Autonomy in Public Service

In a first phase, the contact with the public service is carried out by the observation of the interventions made by the TT in order to understand what clarifications to give to the patients in each situation and how best to communicate with these, asking the appropriate questions and clarifying doubts. This observation allowed me, in addition to the above, to realize the importance of personalized service and active listening.

Then, I was motivated and encouraged to perform independent and autonomous interventions, with the correct code of use for Sifarma 2000<sup>®</sup>, in which I put into practice the knowledge acquired in the IMDPS and developed communication skills, problem solving and autonomy. Whenever doubts arose, these were clarified by the most experienced pharmacists in order to transmit safety and confidence to the patients.

#### 2.1.6 Prescription Consultation

The consultation of prescriptions is a very important task in order to consolidate the necessary knowledge in this area and to check the points to bear in mind when dispensing the prescription, especially with manual prescriptions. In “Farmácia S. José” the prescription consultation is done in a first stage by the trainees, who organize the prescriptions by number in the month/series, lot and entity to which they belong, filling a sheet of their own with these data and with important observations so that, in a second phase, the pharmacist responsible for this function re-checks the prescriptions and heeds these observations.

The topics that need to be confirmed are the patient's beneficiary number, the doctor's sticker and the prescription location (where applicable), the reimbursement body, the number of prescription drugs and packs, the expiration date, the doctor's signature and the date of the prescription. The verification of the signature of the patient and the pharmacist or technician, the date of dispensing the prescription and the stamp of the pharmacy are also required. Participating in this task became extremely important in order to get to know the various reimbursement bodies and to understand the mistakes most often made during the service and prescription dispensation, thus trying to minimize them.

#### 2.1.7 Preparation of Compounding Drugs

A CD is any officinal preparation or master formula prepared and dispensed under the responsibility of a Pharmacist and which has as a main advantage the formulation of non-existent preparations in the market and the therapeutic adjustment of doses, very useful in pediatrics (IVO, 2005)

“Farmácia S. José” prepares on average one CD per day, having at its disposal laboratory materials, raw materials, a laboratory for the rigorous preparation of CD and also has SoftGaleno<sup>®</sup> Software, which facilitates the formulation and management of these medicines since, among other functionalities, it allows for the calculation of the price and the issuance of a preparation sheet of the CD. During my internship I had the opportunity to prepare some CD (see Annex I and II), always with the guidance of the responsible



pharmacists, which contributed to improving my understanding and technique in preparing these medicines and also to understand the quality and safety associated with its preparation.

#### 2.1.8 Robot

The fact that “Farmácia S. José” has a robot that stores the drugs in an organized way and according to its expiration date, that is, according to the rule "first to expire, first out", it allows a management of space and time which is quite efficient. During the internship, I noticed that, on the one hand, there is more space available to store other products and, on the other hand, it is possible to perform a more customer-focused service because the robot makes the medicines available for each counter, which is an advantage for trainees who can be more focused on the patient and their issues.

#### 2.1.9 Sifarma 2000®

This very intuitive and practical computer program is an essential tool for the operation, management and organization of a pharmacy. During the internship I was able to have direct contact with its functionalities and with its relevance and intuitive character, which facilitated the functions performed by me. Through Sifarma 2000® and for back office activities, it is possible, among other things, to manage and place orders, manage and regularize returns, access lot and stock information, and organize and manage the prescriptions. Regarding the part of service, this allows access to the patient records, to the product records, which contains various information, namely scientific information (posology, therapeutic indications, adverse reactions, interactions and contraindications) and to proceed with instant orders, which facilitates the service process. With this program there is also the possibility of realizing different types of sales, such as sale with reimbursement, without reimbursement and suspended sale, so I observed the particularities of each one allowing me to attend to patients correctly.

#### 2.1.10 Range of available products and services

“Farmácia S. José” offers its patients a range of products including supplements, veterinary products and medical devices and an extraordinary range of childcare, cosmetic and cosmeceutical articles, which are strategically arranged to meet the needs of patients. In addition, in this pharmacy we can find pharmaceutical services, which include the determination of physiological and biochemical parameters (glycaemia, total cholesterol, triglycerides, blood pressure and weight) and the existence of nutrition and podiatry consultations, with the aim of promoting the well-being and health of the patients.

Due to the wide range of products mentioned I had the opportunity to expand my knowledge of these products, understanding their characteristics and particularities better and, in addition, I became aware of new products and brands. Thus the knowledge gained was quite important for the advice concerning these articles and for my training and experience as a future pharmacist. When performing the determinations of biochemical parameters I put into practice the knowledge already acquired, improving the technique of execution and understanding the operation of the devices.

## 2.2 Weaknesses

Weaknesses are part of the internal factors that contributed less positively to the internship (IAPMEI, 2016).

### 2.2.1 Insufficient knowledge in the areas of Dermocosmetics and Childcare

IMDPS offers its students a multidisciplinary and vast academic training, preparing them for the various possible professional opportunities. However, the knowledge regarding dermocosmetics and childcare have proved insufficient to respond autonomously to the practical cases that have been put to me by patients, needing help from the TT. This situation becomes a disadvantage to the image transmitted by the trainee and to the pharmacy, as these products grant a large profit margin. Knowing that these are areas increasingly sought after by the public, especially dermocosmetics, and there are numerous products and ranges on the market, it would be important to fill this gap at the academic level.

### 2.2.2 Number of trainees

Although the internship was extremely positive and exceeded my expectations, I consider that the high number of trainees was sometimes a less positive aspect since some tasks were carried out very quickly and there were no others that we could carry out soon after. Then, in addition, when there were more trainees in the pharmacy there were not enough service counters for all those who were already prepared to administer autonomous service to the public.

### 2.2.3 Manual prescriptions

Although electronic prescriptions constitute the majority of the dispensed prescriptions from the pharmacy, I noticed that there is still a large percentage of manual prescriptions that arrive at “Farmácia S. José”, and in this type of prescriptions I came across

the occurrence of a greater number of errors and before which I had greater difficulty. Since there are more details to be confirmed in these prescriptions, I found that my attention during service was focused mainly on the prescription, in order to reduce possible associated errors, rather than focusing on the patient and giving advice, which I consider being due to my lesser experience as a trainee. I have observed that the main mistakes that can be made are to give the wrong medication, dosage or package size, not to check the expiration date, the doctor's signature and the existence of risks or mistakes in writing, and also not to make the correct reimbursement mentioned in the prescription.

### 2.3 Opportunities

Opportunities are elements external to the internship and that integrate opportunities for improvement (IAPMEI, 2016).

#### 2.3.1 Electronic Prescription

Currently, prescription of medicines is almost exclusively performed by electronic prescription materialized or dematerialized, which is an advantage for doctors, pharmacists and patients. In the case of the pharmacy, electronic prescription makes it possible to reduce the occurrence of errors during dispense of the prescription, both in terms of the sold drugs and in terms of validation and confirmation of the prescriptions. In addition, there is a reduction of the time spent in the processes of attendance and of consultation of the prescriptions. As a trainee I verified the existence of these advantages in the course of the attending performed, allowing me to focus attention on the patient and providing the essential clarification and information.

#### 2.3.2 Training courses

The internship at “Farmácia S. José” gave me the opportunity to take part in various training courses, both held in the pharmacy itself by medical information delegates and carried out in other places by different brands and laboratories. The topics covered in these training courses were varied, including inhalation techniques, sun protection, food supplementation, face and body dermocosmetics, foot care, oral hygiene, intimate hygiene and sleep hygiene, among other topics. Through these training courses I acquired new knowledge, consolidated and updated the information already known and managed to clarify doubts directly with the representatives. All of these factors have proven to be very useful for product advice to patients and to better master the characteristics of each product.

## 2.4 Threats

Threats consist of situations outside the internship that could have had an adverse effect upon it (IAPMEI, 2016).

### 2.4.1 Frequent change in the price and reimbursement of medicines

Frequent change in the price and reimbursement of medicines are some of the factors that can affect the relationship between pharmacists and patients and lead to distrust on the part of the latter. Some patients question the reason why the price of medicines does not correspond to the price indicated in the treatment guide (choosing the cheapest generic), becoming uncomfortable and distrustful and considering that the increase of prices and the decrease of the reimbursement are the responsibility of the pharmacy. This non-correspondence may be due to the inexistence of the cheapest drug in the stock of the pharmacy or to changes in price and reimbursement by INFARMED, IP, the price on the guide being outdated, and it is therefore important to explain to the patient that the responsibility is not from the pharmacy, by trying to maintain their level of trust and fidelity.

### 2.4.2 High number of generic drug laboratories

It is known that when a prescription is processed, if the patient opts for a generic drug, his/her option may fall on several laboratories. However, if it is a continuation therapy, where it is advantageous to always take the same drug and from the same laboratory, many patients do not know the name of the laboratory of the drugs they usually take, which makes it difficult for the pharmacist to work when there is no record in the patient's file. Thus, due to the high number of existing laboratories, the time spent by the pharmacist in this service is higher, since the latter always tries to provide the best advice and solve these and other situations.

## 3 Practical Cases

### 3.1 Practical Case I

A young man, about 27 years old, came to the pharmacy and requested a product for tired and uncomfortable eyes. In order to have more information about this situation and to give responsible advice, I questioned whether these symptoms were associated with headaches, if he was a contact lens wearer, and if he spent a lot of time at the computer or other type of day-to-day screens. Faced with these questions, the young man replied that he

had no other associated symptoms and did not wear contact lenses. However, during his employment he remained for several hours at the computer.

With the information collected and after the patient informed me that he preferred a drop formulation, I opted for “Optrex® Colírio Refrescante” for tired eyes, which is suitable for daily use, refreshing and revitalizing tired and uncomfortable eyes. I advised the application of 1 to 2 drops in each eye, 2 to 3 times a day and with the head tilted back (RECKITT BENCKISER, 2014). In addition, I mentioned the importance of taking regular intervals from the computer monitor, for example, every 5 minutes to look away and focus on something farther away and every hour to take a 10 minute interval. I also informed that the expiration date of the product after opening was only 28 days. In case of persistent symptoms, I advised the patient to consult a doctor.

### 3.2 Practical Case 2

A woman, about thirty-five, went to the pharmacy and mentioned that her legs felt tired and heavy. After being questioned, the patient reported that she did not have any associated symptoms, such as pain, swelling, numbness and redness, that she was thus far unaware of having chronic venous insufficiency and that she spent a lot of time on her feet, also mentioning that these symptoms were especially common on warmer days.

Through this information, I advised the patient to use “Allestax® Gel Refrescante”, in order to provide relief of the symptoms mentioned and a feeling of freshness, resulting from the combination of red vine leaf extract, peppermint oil and menthol (GRAU et. al., 2016; BOEHRINGER INGELHEIM, 2017). I told her to apply the gel 2 times a day, or whenever she needed it throughout the day, massaging gently and firmly from the bottom up. In addition, I mentioned that this gel can be placed over resting socks or tights and that it can be stored in the refrigerator to give an even more refreshing effect (BOEHRINGER INGELHEIM, 2017). I also warned of the importance of non-pharmacological measures, which include physical exercise and non-direct exposure to heat, and to consult a physician in case of associated pain or any of the above symptoms.

### 3.3 Practical Case 3

One lady went to the pharmacy in the afternoon to ask for advice because she suspected the start of a urinary infection. There was a need to ask some questions to understand the symptoms of the patient, if this type of infection was frequent, the duration of the symptomatology and if the lady suffered from some pathology. The patient said that

since that morning she felt a pressure in the area of the bladder, needing to urinate many times and wanting to urinate after urination (CAR, 2006). In addition, she mentioned that it was not customary for her and that she had no other associated symptoms nor suffered from any pathology. In view of this information, I recommended the use of RoterCystiberry<sup>®</sup>, a medical device indicated for the treatment and prevention of urinary infections, which contains cranberry extract and anti-adherent properties, thus avoiding the adherence of the bacteria to the bladder wall (CAR, 2006; LIU et. al., 2008; ROTER, 2017). I advised taking 2 capsules a day (with a glass of water) every 12 hours for 15 days and warned the patient to consult a physician in the case of persistent symptoms or pain when urinating, blood in the urine and/or fever (ROTER, 2017). My counselling included non-pharmacological measures such as drinking plenty of water, wearing non-synthetic underwear and non-retention of urine.

#### **4 Final Considerations**

The internship at “Farmácia S. José” allowed me to apply not only the knowledge obtained during academic training but also to acquire new knowledge in the most varied areas. I acknowledge that some of the skills required of a pharmacist are not obtained in a theoretical context but in a practical context in the light of the situations presented to him/her, and thus the experience and professional practice acquired during this internship are important factors for my differentiation as a future pharmacist.

By monitoring the counselling provided by the members of the technical team of “Farmácia S. José” and analysing the counselling taken by myself, I realized the importance of giving care and advice to the patients in a careful, responsible and significant manner and understood better the role played by the Community Pharmacist in society.

I believe that this internship has exceeded my expectations, since it has given me greater interest in the area of community pharmacy and has enabled me to develop capacities for counselling, communication, trust and autonomy, as well as developing other relevant professional and personal qualities.

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## 6 Annexes

### Annex I Compounding Drug Preparation Sheet: "Álcool 60% Boricado à Saturação".

**REPÚBLICA PORTUGUESA**  
Saúde

FARMÁCIA S. JOSÉ

#### Ficha de Preparação do Manipulado

##### Álcool 60% Boricado à Saturação

Cliente: \_\_\_\_\_  
 Forma Farmacêutica: SOLUÇÃO  
 Data de Preparação: 09/06/2017 Prazo Validade: 08/08/2017  
 N° Lote: 10.VI.17 Registo Copiador: 1.339  
 Condições de Conservação: \_\_\_\_\_  
 Posologia: \_\_\_\_\_  
 Qtd. Total Medicamento: 1 X 30,00 ml  
 Director Técnico: Dr. Paulo Monteiro  
 Operador: Dra. Marta Abreu  
 Médico: \_\_\_\_\_

Honorários:	4,89 €	Valor Net:	21,53 €	<b>Valor PVP</b>
Factor Multiplicativo:	3,00	Valor IVA:	1,29 €	
		Valor Total:	22,82 €	

Materias Primas	Usar	N° Lote	Origem	Qtd. Usada	Unid	Preço Aq. s/ IVA	Factor Multiplic.	Preço Mat.prima
Ácido Bórico		151402-O-	Acofarma	1,20	g	0,01 €	2,20	0,03 €
Água Purificada		0010322	Maialab	5,30	g	0,01 €	2,20	0,12 €
<b>Subtotal</b>								<b>0,15 €</b>

Produto	Cod de Iva	% Iva	P.V.P	Preço
Álcool a 70 %	NOR	23,00	0,80 €	0,65 €

**Preparação**

Verificar o estado de limpeza do material a utilizar.

Colocar em proveta rolhada uma quantidade de álcool a 60% (24.7 mL de álcool a 70% + 5.3 mL de água para 30 mL) correspondente a cerca de 3/4 da quantidade total de solução a preparar.

Pesar o ácido bórico e adicionar, aos poucos, ao álcool a 60%, agitando fortemente durante 20 seg após cada adição.

Após a adição de todo o ácido bórico, completar o volume com álcool a 60% e agitar durante 20 seg.

Deixar a proveta em repouso 1 hora, agitando-a, durante 20 segundos, de 15 em 15 minutos.

Filtrar a solução obtida em 5.

Transferir para um frasco conta gotas, Fechar e rotular a embalagem.

Limpar e arrumar o laboratório.

**Aparelhagem**

Balança electrónica \_\_\_\_\_

Espátula \_\_\_\_\_

Proveta \_\_\_\_\_

**Farmácia São José**  
 10.VI.17  
 Direcção Técnica  
 Paulo Jorge Silva Monteiro  
 € 22,82

*Álcool a 60% boricado à saturação 30mL*

*Dat. prep: 9/6/2017*  
*Praz. util: 8/8/2017*

Embalagem	Tipo	N° Lote	Fornecedor	Capac	Qtd	Preço	Fact. Mult.	Valor Net
Frasco Conta-Gotas Vidro	EMBAL		Plural	30 mL	1,00	1,03 €	1,20	1,24 €
<b>Subtot</b>								<b>1,24 €</b>

Ensaio	Especificação	Conforme	Utilizador	Assinatura
Cor	Incolor	<input checked="" type="checkbox"/>		
Odor	Inodoro	<input checked="" type="checkbox"/>		
Aspecto	Homogéneo	<input checked="" type="checkbox"/>		
Quantidade	30 mL ± 5%	<input checked="" type="checkbox"/>		

9/6/2017 *Marta*

Annex II Compounding Drug Preparation Sheet: "ATL gordo, Ácido Salicílico e Ácido Láctico".

FARMÁCIA S. JOSÉ

Pharmacia São José

### Ficha de Preparação do Manipulado

**ATL gordo, Ác. Salicílico e Ác. Láctico**

Cliente: \_\_\_\_\_  
 Forma Farmacêutica: POMADA  
 Data de Preparação: 25/05/2017  
 Nº Lote: 20.V.17  
 Condições de Conservação: Em recipiente bem fechado e à temperatura ambiente.  
 Posologia: \_\_\_\_\_  
 Qtd. Total Medicamento: 1 X 30,00 g  
 Director Técnico: Dr. Paulo Monteiro  
 Operador: Dra. Marta Abreu  
 Médico: \_\_\_\_\_

Prazo Validade: 21/11/2017  
 Registo Copiador: 1.325

Honorários:	4,89 €	Valor Net:	30,48 €	Valor PVP 32,31 €
Factor Multiplicativo:	3,00	Valor IVA:	1,83 €	
		Valor Total:	32,31 €	

Matérias Primas	Usar	Nº Lote	Origem	Qtd. Usada	Unid	Preço Aq. s/ IVA	Factor Multiplic.	Preço Mat.prima
Ácido salicílico (po fino)		150346-N-	Acofarma	1,50 g		0,03 €	2,20	0,11 €
Ácido Láctico		160970-N-	Acofarma	1,50 g		0,03 €	2,20	0,10 €
Subtotal								0,21 €

Produto	Cod de Iva	% Iva	P.V.P	Preço
ATL creme gordo 100g (Lt 160728)	NOR	23,00	10,50 €	8,54 €

Preparação

Verificar o estado de limpeza e conservação do material e laboratório.

Pesar o ácido salicílico e o ácido láctico e colocar no recipiente unguat α.

Juntar o creme gordo.

Homogeneizar.

Fechar e rotular.

Limpar e arrumar o laboratório.

Aparelhagem

Balança electrónica

Unguator B

Embalagem	Tipo	Nº Lote	Fornecedor	Capac	Qtd	Preço	Fact. Mult.	Valor Net
Unguator 50/70	EMBAL		Plural	50/70 m	1,00	1,67 €	1,20	2,00 €
Subtot								2,00 €

Ensaio	Especificação	Conforme	Utilizador	Assinatura
Cor	Esbranquiçada	<input checked="" type="checkbox"/>		
Odor	Inodoro	<input checked="" type="checkbox"/>		
Aspecto	Homogéneo	<input checked="" type="checkbox"/>		
Quantidade	30 g +- 5%	<input checked="" type="checkbox"/>		

FARMÁCIA S. JOSÉ Unip., Lda.



## Report on Pharmaceutical Industry Internship

Supervisor: Dra Patrícia Flório

## **Abbreviations**

FAAS – Flame Atomic Absorption Spectroscopy

HPLC – High Performance Liquid Chromatography

IMDPS – Integrated Master's Degree in Pharmaceutical Sciences

IPC – *In Process Control*

IR – Infrared Radiation

ISO – International Organization for Standardization

QC – Quality Control

SOP – Standard Operational Procedures

SWOT – Strengths, Weaknesses, Opportunities and Threats

TLC – Thin Layer Chromatography

UPLC – Ultra Performance Liquid Chromatography

UV – Ultraviolet

## I Introduction

The Integrated Master's Degree in Pharmaceutical Sciences (IMDPS), taught by the Faculty of Pharmacy of the University of Coimbra, is intended for the training of professional, free and competent Pharmacists (UNIVERSIDADE DE COIMBRA, 2017) at the scientific, technical and deontological level, taking into account that the Pharmacist is a health agent, a specialist in medicine and whose purpose it is to maintain the well-being and health of the patient in particular and citizens in general (ORDEM DOS FARMACÊUTICOS, 2015).

The pharmaceutical industry, being a place of research, development, production, quality control (QC) and issue of medication, is one of the specialties recognized by the Order of Pharmacists and is included in the range of possible professional outputs of the IMDPS (ORDEM DOS FARMACÊUTICOS, 2015). Thus, the execution of the curricular internship in this area provides for knowledge in the operation and organization of the pharmaceutical industry, as well as being able to observe the application of theoretical knowledge into the practical reality of the developed activities.

The factors mentioned above, coupled with the curiosity to personally experience the work performed within the pharmaceutical industry, were predominant in the choice of the accomplishment of this optional curricular internship. The industry in question is divided into four units, each of which is responsible for the production of certain pharmaceutical forms and/or classes of drugs and has a complex logistic. The curricular internship was carried out in the Physical and Chemical Quality Control Department of the Sterile Solutions Production Unit and the Solids and Semi-Solids Production Unit, analysing both semi-finished products and the finished product.

The purpose of this SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis is to outline the activities carried out along the internship and, through a critical analysis, to present the strengths and weaknesses felt, as well as the opportunities and threats encountered, at both a professional and personal level.

## 2 SWOT Analysis

The SWOT analysis model emerged due to the growing need to improve the performance, management and results of a particular company and increase its success in meeting internal and external demands and objectives and in the face of a progressive increase in competition. This type of analysis is divided into internal analysis (strengths and weaknesses) and external analysis (opportunities and threats) and is based on the identification and evaluation of strengths, weaknesses, opportunities and threats. Despite its use in areas of the economy, management and business of companies, this model is easily applied in other areas, such as the analysis of a curricular internship, due to its essentially qualitative and simplified nature (IAPMEI, 2016).

### 2.1 Strengths

The strengths include internal aspects that contributed positively to the realization of the curricular internship (IAPMEI, 2016)

#### 2.1.1 Observation of the operation of a pharmaceutical industry

The curricular internship allowed me to observe the practical reality of the pharmaceutical industry, verifying that it is divided into several units according to the set of activities and/or pharmaceutical forms and/or pharmacotherapeutic classes for which it is intended and, each one of these units is also segmented into several departments.

The Penicillin Production Unit is intended for the manufacture of powders for use in injectable solutions, the Sterile Solutions Production Unit produces sterile non-injectable solutions, medical devices, high volume injectable solutions (plastic) and small volume injectable solutions (plastic and glass). The Cephalosporins Production Unit manufactures powders for injectable solutions and the Solids and Semi-Solids Production Unit is for the production of tablets, capsules, ointments and creams. These units encompass actual production, IPC (In Process Control), QC and storage, under controlled conditions, in order to comply with Good Manufacturing Practices and to ensure the quality, safety and efficacy of all medication produced.

Having not had much contact with the activities of pharmacists in industry, they do have a responsibility to supervise and ensure the correct development, production, registration, QC and storage of medicines and medical devices.

### 2.1.2 Internship in the Quality Control department

In the Department of Physical and Chemical Quality Control of the Sterile Solutions Production Unit and the Solid and Semi-solid Production Unit, where the curricular internship was held, batches are examined for validation and release through the analysis of the raw materials used, semi-finished products and finished products of the respective batch.

The QC department aims to analyse the products according to their characteristics and critical parameters, presenting the results of these studies and ensuring that these products adhere and conform to predetermined specifications and meet the requirements of quality, safety and efficacy required by the industry. When I completed the internship in this department, I understood the importance of QC for the trust, success, differentiation and performance of the pharmaceutical industry in the face of compliance with standards and the existing market.

Although the internship took place in the QC department of the aforementioned units, I had the opportunity of being briefly shown the area of production of sterile solutions and solid and semi-solid products, the IPC associated with each one and also storage.

### 2.1.3 Application of theoretical knowledge

The educational institution transmits theoretical knowledge and some practical knowledge necessary to understand and execute various equipment, methods, procedures and techniques used in the pharmaceutical industry. However, the completion of the curricular internship allowed me to apply this knowledge to the daily reality of an industry and to perform the different tasks at a practical level, and therefore, understanding them better.

The theory taught within the teaching units for Pharmaceutical Technology, Analytical Chemistry, Instrumental Methods of Analysis and Management and Quality Assurance proved to be quite important in being compliant with laboratory procedures, since they involved methods of Polarimetry, Conductimetry, Ultraviolet (UV) Spectrophotometry, Infrared (IR) Spectrophotometry, High Performance Liquid Chromatography (HPLC), Ultra Performance Liquid Chromatography (UPLC), Thin Layer Chromatography (TLC) and Flame Atomic Absorption Spectroscopy (FAAS), among others. These methods were used in the measurement of pH and conductivity, in the determination of the active principle, excipients, related substances and/or impurities, in the identification of compounds, in particle counting, among other analysis.



In the QC of sterile solutions I examined batches for release and validation of semi-finished product using the analytical methods described above and also performed Precipitation Volumetric Titration by Argentometric Method, measurement of Density, Refractive Index, Extractable Volume of the Injectables and the Acidity and Alkalinity. In addition, I proceeded to identify ions, heavy metals and oxidizable substances, tested the solutions appearance, and calculated evaporation residues. In the QC of solids, in addition to the methods mentioned above, I performed procedures for Disintegration, Dissolution, Friability, Hardness, Thickness, Average Weight and Mass Uniformity.

The application of some analysis requires prior preparation of secondary reagents, primary and secondary standard solutions, mobile phases, wash solutions, impregnation and development solvents, and disintegration medium. Thus, contact with the preparation techniques of these reagents and with the preparation itself was possible.

#### 2.1.4 Learning and practical improvement of the laboratory procedures

In the execution of the QC analysis of semi-finished and finished products I was able to develop, perfect and improve some of the laboratory techniques and procedures known and taught in IMDPS classes as well as to know and learn other industry-specific procedures that I did not have contact with during the course. I also acquired greater skill, experience and assurance in laboratory execution and greater knowledge about the use of laboratory equipment and compliance with safety and waste regulations.

#### 2.1.5 Development of personal and professional characteristics

The autonomy, problem solving ability, teamwork, organization, responsibility, critical thinking and time and task management were some of the characteristics and competences developed and improved throughout the curricular internship, as I was given responsibility for performing some of the tasks and analyses that are part of the QC of the drugs for check and release batches.

In addition to the aforementioned competences, I developed skills in the English language in terms of reading and understanding, since some of the documentation consulted and used to carry out the required analyses was in English.

#### 2.1.6 Access to pharmaceutical documentation

Being responsible for the QC analysis of some products, I had to consult pharmaceutical documentation, namely, technical procedures, analysis bulletins, Standard

Operational Procedures (SOP), International Organization for Standardization (ISO) Standards and methods of analysis. The consultation of these documents allowed and aided a further understanding of the tasks, as well as their realization in the correct manner and in agreement with the policies and rules of the industry.

During the course of the IMDPS classes were provided on theoretical bases related to this type of documentation however, the curricular internship allowed for direct contact with it.

## 2.2 Weaknesses

The internal factors that contributed less positively to the realization of the internship constitute the weaknesses (IAPMEI, 2016).

### 2.2.1 Short-term internship

I consider the period of the internship to be short, since it takes some time to assimilate knowledge, adapt to different tasks and analyses, and also some personal adaptation to the team and methods used. In addition, the industry needs to assign and adapt the activities to the trainee so that the trainee performs competently and produce useful work for the company. Moreover, it would be more beneficial to pass through all departments and units, as it would make the internship experience more comprehensive providing for the acquisition of knowledge across the various areas.

### 2.2.2 Adaptation of theoretical knowledge for each particular product

The theoretical knowledge acquired during the academic training is essential and provides for a very important basis for the execution of professional activities. However, in practice, tasks are not always as linear as in theory, some changes and adaptations of the general methods and procedures learnt are necessary according to the drug and/or compound that we aim to examine, so that the analysis is done in reasonable time, quality, safety and effectiveness for each type of product. This fact, on the one hand, allows for an increase of the acquired knowledge but, on the other hand, makes it difficult to execute each analysis due to the time spent in the research and adaptation for the different techniques used.

### 2.2.3 Only one compulsory internship period

The IMDPS comprises of only one compulsory curricular internship period with a duration of one semester included in the 5 years of theoretical training. I consider that the

practical component within a professional context is very small compared to the theoretical component, which makes it difficult for students to choose the internship they will undertake and also to manage their time, given the need to articulate the internship with the preparation of the traineeship report(s) and monograph. In addition, the completion of more than one internship throughout the duration of the course would be beneficial to students, both in terms of professional experience and in the assimilation of knowledge.

#### 2.2.4 Non-execution of work relative to Pharmacist

The activities that I performed during the internship do not belong directly to a Pharmacist, since it involves coordination, supervision and verification functions of the tasks performed within the department. However, the knowledge I gained in performing the analyses was quite useful in order to understand the whole process inherent in these and thus to be able to perform better the roles of a Pharmacist.

### 2.3 Opportunities

Opportunities are external elements to the internship which, nonetheless, constitute opportunities for improvement (IAPMEI, 2016).

#### 2.3.1 Optional Curricular Unit of Informatics, Pharmaceutical Software and Management

Throughout the internship I have verified that computer software and pharmaceutical software are essential tools in the day-to-day of an industry for the consultation of information and documentation, internal and external communication, equipment use and data processing. Thus, I consider that an optional curricular unit that covers these areas would be important in the IMDPS, since many students possess insufficient computer knowledge. I have also verified that the management of companies and management of materials and human resources are quite important in terms of the competencies of pharmacists, as they could occupy places of management and administration within pharmaceutical industries that today are occupied by professionals with a greater capacity at these levels. Therefore, I think it would be advantageous to include an optional curricular unit for management within the IMDPS.

#### 2.3.2 Curricular internships throughout the course

The completion of curricular internships throughout the course would be an advantage for IMDPS students. In addition to allowing for professional experience in the

various possible aspects of IMDPS (pharmaceutical industry, community pharmacy, hospital pharmacy, research, pharmaceutical distribution, clinical analysis, regulatory affairs, pharmaceutical marketing, clinical pharmacology, radio pharmacy and human genetics) (ORDEM DOS FARMACÊUTICOS, 2015), it would also allow for a sequential and chronological application, in practice, of the theory taught in class, with a better assimilation of knowledge and concepts. Thus, students and future pharmaceutical professionals could choose which area to pursue taking into account learning and personal experience.

## 2.4 Threats

Independent external situations relative to the accomplishment of the internship but that could have compromised this constitute the threats (IAPMEI, 2016).

### 2.4.1 Reduced number of Pharmacists

It is known that the number of pharmacists practicing in the pharmaceutical industry could be higher, since they are professional experts in medicine and have technical and scientific competence in this area. In addition, the vacancies for access of new professionals to this area are limited to the current demand that exists, which worries me as a future Pharmacist.

### 2.4.2 Competition and economic crisis

There is now a great competition between industries and, therefore, they must bet on safety, quality, efficiency, innovation and novelty in order to be able to stand out from the rest. In the case of a competitive area, it is also necessary to ensure the positions belonging to pharmacists and to ensure that they maintain their professionalism and differentiate themselves from each other in terms of technical, scientific and personal skills. In this way, as a future Pharmacist, I must also seek continuous training and improve my professional and personal skills.

### **3 Final Considerations**

The opportunity to train in a pharmaceutical industry, after 9 semesters of theoretical training in the IMDPS, was very important for my academic career, since it allowed me to experience one of the possible areas for professional practice, which is optional in Curricular Internship, and understand the daily reality of an industry at an organizational and functional level. In addition, I was able to better understand the theoretical content already addressed during the IMDPS, and to widen the range of knowledge in executing and understanding the quality control analyses required for the validation and release of a drug to the market.

As the Pharmacist is a specialist in medicine, it is very important that they know and experience all the stages inherent in the production cycle, from its development, through the production and quality control, to its release and advice to the patient. Thus, curricular stages throughout the course and in the different pharmaceutical areas would be of added value to IMDPS students and contribute not only to the assimilation of theoretical knowledge but also to highlight these future pharmacists in comparison to others.

The personal skills acquired during the internship, such as responsibility, autonomy, time and task management, problem solving and integration of knowledge allow us, as future pharmacists, to differentiate ourselves from other professionals, performing fundamental functions in the pharmaceutical sector and incorporating multidisciplinary teams.

I believe that this internship has allowed me to gain a real perception of the functioning of a pharmaceutical industry and the functions performed by pharmacists in this area, thus contributing positively to my personal and professional growth and to assist me in choosing the area to be followed in the future.

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III

**Toxoplasmosis and Psychotic Disorders**

Supervisor: Professor Dra. Maria do Céu Sousa

## Figures and Tables Index

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## Abbreviations

BD – Bipolar Disorder

CNS – Central Nervous System

IFN- $\gamma$  – Interferon-*gamma*

IL – Interleukin

NO – Nitric Oxide

OR – Odds Ratio

RhD – Rhesus D Protein

TNF- $\alpha$  – *Alpha*-Tumoral Necrotic Factor

## I Introduction

*Toxoplasma gondii* (*T. gondii*) is an intracellular parasite that affects mammals, including humans, and has high tropism for the muscles and brain where latency is established (SILVA and LANGONI, 2009; ZHU, 2009; DPDx, 2016; GARGATÉ et. al., 2016). It often causes severe central nervous system (CNS) lesions in immunocompromised individuals and in the foetus of pregnant women, giving rise to acute or latent toxoplasmosis or to congenital toxoplasmosis (SILVA and LANGONI, 2009; YOLKEN et. al., 2009). Toxoplasmosis is one of the most common human infections worldwide, infecting about a third of the world's population (HOUSE et. al., 2011; GARGATÉ et. al., 2016; GRANDE et. al., 2017). Thus, this parasitic infection is considered a public health problem and requires special attention and primary preventative measures by health professionals (SOUSA et. al., 2008; SILVA and LANGONI, 2009; FLEGR, 2013; GARGATÉ et. al., 2016).

In recent years, several studies have appeared suggesting that parasites, such as *T. gondii* can manipulate the host's behaviour, phenotype and personality in order to take some advantages such as continuing its lifecycle, a phenomenon called manipulation hypothesis or manipulation theory (WEBSTER, 2007; YOLKEN et. al., 2009; SILVA and LANGONI, 2009; FLEGR, 2013). The privileged position of *T. gondii* in the CNS may result in a change and manipulation of host behaviour in an intentional and beneficial manner to the parasite or in an unintentional and indirect manner (WEBSTER, 2007; FLEGR, 2013; WEBSTER et. al., 2013). Thus, *T. gondii* is one of the most studied parasites in the manipulation theory and is used as a model for human studies in this area due to its worldwide prevalence and the ease of obtaining empirical data (FLEGR, 2013; WEBSTER et. al., 2013). In addition to the behavioural changes, it is hypothesized that toxoplasmosis may be associated with psychotic disorders and constitute one of the risk factors for its onset and development, especially with regard to schizophrenia and bipolar disorder (BD) (ZHU, 2009; SUTTERLAND et. al., 2015; GARGATÉ et. al., 2016). There are pathophysiological mechanisms that appear to be present both in *T. gondii* infection and in psychosis, suggesting another association between these two diseases (WEBSTER et. al., 2006; ZHU, 2009; YOLKEN et. al., 2009; KAUSHIK et. al., 2012; HSU et. al., 2014; GRANDE et. al., 2017). Moreover, studies with antipsychotic drugs and mood stabilizers indicate that there is a possible therapeutic alternative for toxoplasmosis associated with psychosis.

The present monograph will, initially, address information regarding the *T. gondii* parasite, the infection caused by it and also the possible manipulation of behaviour on

animals and humans. Subsequently, data and studies indicating that toxoplasmosis may be associated and contribute to the onset and development of psychotic diseases will be reported and, finally, the impact of different drugs on the ability of *T. gondii* in the change of behaviours and psychoses will be presented. The aim of this study is to gather information about the aforementioned themes and thus to organize some of the already known data in order to better understand the possible impact of toxoplasmosis on human behaviour and psychotic disorders.

## 2 Toxoplasmosis

Toxoplasmosis is an infectious disease caused by *T. gondii*, an intracellular protozoan parasite of mammals and warm blooded animals, including humans (SILVA and LANGONI, 2009; ZHU, 2009; DPDx, 2016; GARGATÉ et. al., 2016). This parasite of the family *Apicomplexa* (WEBSTER, 2007; GARGATÉ et. al., 2016) is common within the worldwide population and is considered to be both an economic and public health issue among pregnant women and immunocompromised individuals (SILVA and LANGONI, 2009; FLEGR, 2013).

Members of family *Felidae*, mainly cats, constitute the only definitive hosts for *T. gondii*, mammals, especially birds and rodents, are the intermediate hosts and the secondary hosts are primarily humans (WEBSTER, 2007; SILVA and LANGONI, 2009; WEBSTER et. al., 2013; HSU et. al., 2014). Toxoplasmosis is an anthrozoosis (SILVA and LANGONI, 2009) characterized by tissue cysts usually formed in muscle, brain tissue and eyes in humans and intermediate hosts. These cysts can remain throughout the host's lifetime and their infection is commonly asymptomatic in immunocompetent individuals (KAUSHIK et. al., 2012; HSU et. al., 2014) causing only, in the majority of cases, an initial, benign and self-limiting acute infection. However, in immunocompromised individuals this infection can cause pneumonitis, lymphadenopathy, retinochoroiditis and encephalitis resulting from a CNS disseminated infection (HSU et. al., 2014; DPDx, 2016). Congenital toxoplasmosis, another infection manifestation, arises from an acute primary infection in pregnant mothers during pregnancy, where the incidence increases and the severity decreases with the time of gestation in which the infection is acquired (HSU et. al., 2014; DPDx, 2016; GRANDE et. al., 2017). When congenital infection occurs in the 1<sup>st</sup> or 2<sup>nd</sup> trimester the infection is widespread, being associated with foetal death, abortion, retinochoroiditis and psychomotor and mental retardation (YOLKEN et. al., 2009; SILVA and LANGONI, 2009; GARGATÉ et. al., 2016; GRANDE et. al., 2017). On the other hand, if the infection occurs in the 3<sup>rd</sup> trimester or during childbirth, it is usually asymptomatic and may lead to learning difficulties or retinochoroiditis in adolescence or adulthood (YOLKEN et. al., 2009). Although it is very unusual, cases in which latent toxoplasmosis infection has reactivated during pregnancy have already been reported, resulting in foetus congenital toxoplasmosis (HSU et. al., 2014).

The life cycle of *T. gondii* (Figure 1) starts in cats' intestines where it undergoes gametogenesis, sexual reproduction and non-sporulated (immature) oocyst formations, which are shed in cat's faeces (WEBSTER, 2007; SILVA and LANGONI, 2009; WEBSTER et. al., 2013; HSU et. al., 2014; DPDx, 2016). Oocysts' maturation in the environment makes

them sporulate and infectious toward the intermediate hosts, who can ingest them through contaminated food, water or soil (WEBSTER, 2007; WEBSTER et. al., 2013; DPDx, 2016). After ingestion, the parasite reproduces asexually through the transformation of oocysts into tachyzoites and, thereafter, of the slower division of these into bradyzoites. Bradyzoites encyst in tissues such as muscles, the heart and brain and may remain within them by establishing latent toxoplasmosis. Cats acquire toxoplasmosis by ingestion of sporulated oocysts or by hunting intermediate hosts infected with cysts. In humans, there are several routes through which they can be infected, such as ingesting undercooked or raw infected meat, consuming contaminated food or water, via transplacental, organ transplantation and blood transfusion (SILVA and LANGONI, 2009; HSU et. al., 2014; DPDx, 2016; GRANDE et. al., 2017).

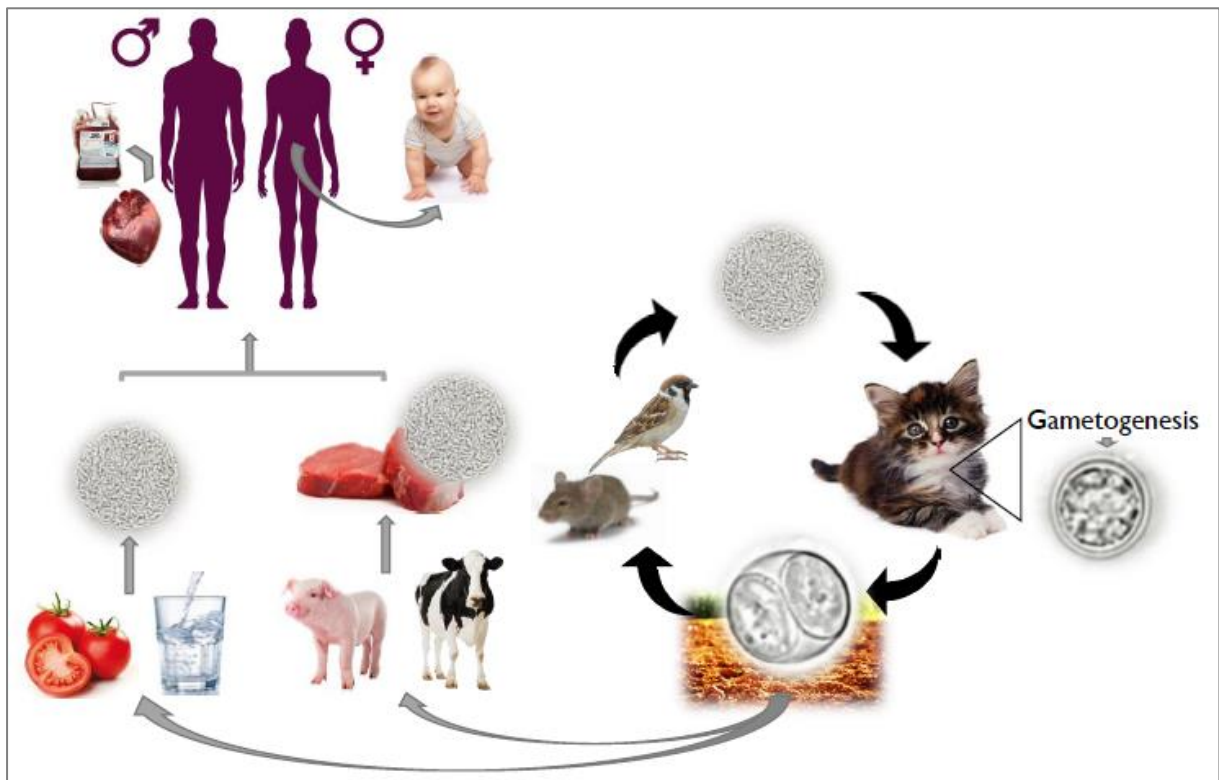


Figure 1: Life cycle of *Toxoplasma gondii* demonstrating the possible routes of infection and the different forms of parasite's evolution (adapted from CDC, DPDx: <https://www.cdc.gov/dpdx/toxoplasmosis/index.html>)

Depending on the cultural and feeding factors, contact with cats, type of work and geographical area, about 15% to 85% of the adult population has a chronic infection with *T. gondii* (SILVA and LANGONI, 2009; KAUSHIK et. al., 2012). Feeding habits and food preparation seem to be one of the main causes of the difference in the prevalence of this infectious disease in different countries throughout the world (GARGATÉ et. al., 2016).

Thus, both the incidence of latent and congenital toxoplasmosis varies from country to country according to the seroprevalence of *T. gondii* in those same countries, with France and the USA being among the countries with the highest prevalence. The seroprevalence of toxoplasmosis in pregnant or fertile women also varies in the distinct countries worldwide (10% to 60 %), and there is a great heterogeneity in Europe. Thus, it is quite difficult to estimate and predict cases of congenital toxoplasmosis.

In Portugal, the seroprevalence of antibodies IgG anti-*T. gondii* was 22% in the year 2013 and it is estimated that only about 18% of Portuguese women are immunized (GARGATÉ et. al., 2016). According to data obtained between 2000 and 2006 on the seroprevalence of *T. gondii* in the Centre of Portugal, only 27.3% of women of childbearing age had IgG antibodies against toxoplasmosis, in other words, they were immunized (SOUSA et. al., 2008). It is possible to infer that the majority of women of childbearing age in Portugal are not immunized, so they are more likely to develop a primary infection during pregnancy, thus placing the foetus at risk of developing congenital toxoplasmosis. Taking this into account, it is necessary to take primary preventative measures to avert cases of congenital toxoplasmosis (SOUSA et. al., 2008; GARGATÉ et. al., 2016). Thus, in Portugal, toxoplasmosis is a notifiable disease and all pregnant women are submitted to medical evaluation in order to verify the presence or absence of this infection, and the surveillance of susceptible pregnant women is done in every three months (DGS, 2011; GARGATÉ et. al., 2016). Although there is a lack of epidemiological data on the number of cases of toxoplasmosis in Portugal, unpublished data refer to the existence of about three cases of congenital toxoplasmosis per year in Portugal (GARGATÉ et. al., 2016).

In animals, the prevalence is also high with about 50% in dogs, rabbits, sea otters and farmed animals, 60% in rodents and birds and 70% in cats, bears and deer, depending on exposure to *T. gondii* in different types of feeding and environments (TENTER et. al., 2000; WEBSTER, 2007; KAUSHIK et. al., 2012).

Diagnosis of toxoplasmosis is determined mainly through serology where the presence of *T. gondii*-specific IgG and IgM antibodies is tested and, in the case of congenital toxoplasmosis, amniocentesis plays an important role in prenatal diagnosis by detecting parasite DNA (DPDx, 2016). The first line of treatment for toxoplasmosis in pregnant women is spiramycin (antibiotic, macrolide) and, in case of positive prenatal diagnosis, the treatment is carried out with a sulphonamide such as sulfadiazine. Congenital toxoplasmosis is treated in the first 12 months of life with pyrimethamine (antimalarial drug) associated

with sulfadiazine and folinic acid, and the latter treatment can also be used in cases of acute infection in immunocompromised individuals (HSU et. al., 2014).

The parasite *T. gondii* presents three main genotypes, Type I, Type II and Type III (WEBSTER, 2007; HSU et. al., 2014; SUTTERLAND et. al., 2015). Type II is apparently the most frequent in nature and in Europe, however it is not known whether this genotype is altered and therefore it is difficult to understand which strain is responsible for the majority of the infections in humans and animals in different regions.

### **3 Host-parasite interaction and behaviour manipulation**

As said before, several studies have emerged in recent years suggesting that parasites, especially *T. gondii*, can manipulate the host phenotype, personality and behaviour in order to be able to continue their life cycle (WEBSTER, 2007; SILVA and LANGONI, 2009; FLEGR, 2013; HSU et. al., 2014). It is possible that different genotypes of this parasite spread through different brain zones and cause different behavioural changes (WEBSTER, 2007). According to several studies, the location of the cysts seems to be preferential in the amygdala and prefrontal cortex, zones that are related to the regulation of the behaviour towards fear, being nevertheless found in other zones of the brain (VYAS et. al., 2007; BERENREITEROVÁ et. al., 2011). In accordance with studies carried out in rats, cysts also seem to have preference for limbic system regions, which are involved both in the innate defence and in reproductive behaviour, that is, in the avoidance and approach behaviours towards predator stimuli or sexual stimuli (HOUSE et. al., 2011). But there are other studies, including neurological studies, which claim the existence of cysts in all areas of the brain, presenting a variable density in different brain areas depending on the individual (FABIANI et. al., 2015; DUBEY et. al., 2016).

*T. gondii* spreads in the CNS through the invasion of different nerve cells such as astrocytes, neurons and microglia cells and causes damage to the neurological tissues of immunocompromised individuals (SILVA and LANGONI, 2009; GRANDE et. al., 2017). Nevertheless, mainly microglial cells, by preventing the growth and replication of the parasite, are believed to constitute a defence against toxoplasmosis (SILVA and LANGONI, 2009; SUTTERLAND et. al., 2015).

The parasite, upon infecting a host, triggers an immune response, which is very important to control and prevent *T. gondii* replication, reactivation of pre-existing cysts and invasion to different organs (SILVA and LANGONI, 2009; HSU et. al., 2014; GRANDE et. al.,

2017). Several cytokines, among which interferon-gamma (IFN- $\gamma$ ), interleukin (IL)-12, alpha-tumoral necrotic factor (TNF- $\alpha$ ), IL-1, IL-15, IL-6 and IL-10 and nitric oxide (NO) are produced by dendritic cells, lymphocytes T CD4+ and T CD8+, neutrophils, macrophages and natural killer cells. At times this exaggerated alteration of the inflammatory mediators can lead to the emergence of aggressiveness and psychotic symptoms due to the downstream effect on the neurotransmitters (GRANDE et. al., 2017). An example is the association between increased pro-inflammatory cytokines (TNF- $\alpha$  and IL-6) in toxoplasmosis and increased suicide attempts and self-directed violence.

There is some discussion about the effect of anti and pro-inflammatory cytokines, however, a balance between them is beneficial, on the one hand to avoid pathological changes caused by an exacerbated immune response to the parasite and, on the other hand, to the host survival during acute infection (SILVA and LANGONI, 2009).

*T. gondii*, as already mentioned, is also transmitted through the predation of intermediate hosts by definitive ones, explaining why the behavioural alteration of the hosts seems to have the objective of increasing the transmission of the parasite (KAUSHIK et. al., 2012). In this way, it is believed that the parasite causes changes both physically and mentally, leading, on the one hand, to the modification of sensory and response capacities of hosts in a dangerous situation and, on the other hand, causing an alteration of physical coordination, which may reduce the ability to escape from potential predators or increase the possibility of being attacked by them. Nonetheless, the change in behaviour observed in natural intermediate hosts (rodents) is believed to be the result of specific manipulation whereas in secondary hosts (humans) it may result from an indirect by-product of the infection, since the parasite, in the case of humans, will not continue the sexual reproduction in its life cycle (KAUSHIK et. al., 2012; WEBSTER et. al., 2013).

There are descriptions of two types of behavioural changes associated with chronic infection with *T. gondii*, the simple alterations which can be confused with toxoplasmosis side effects, such as prolongation of reaction time, and the complex alterations, for example the loss of fear (FLEGR, 2013). The latter coupled with the fact that the intensity of some behavioural changes increase with increase in the time span from the beginning of the infection, supporting the hypothesis of host manipulation by the parasite (FLEGR, 2013).

The mechanisms by which *T. gondii* causes behavioural manipulation in hosts is not yet known, but there are some assumptions and hypothesis about them (WEBSTER, 2007; YOLKEN et. al., 2009; KAUSHIK et. al., 2012). Immunological, neuromodulatory and



histopathological mechanisms may be involved. At the immunological level, due to the immune response triggered, there can be a dysregulation of cytokine levels, which may influence the neuromodulatory mechanism. In relation to neurotransmitters, there is a change in dopamine, homovanillic acid and norepinephrine concentrations, which are involved in the regulation of mood, locomotor activity, learning and memory. According to results observed in studies conducted in mice that were infected with the *T. gondii* C56 strain (genotype III), those concentrations may be increased or decreased depending on whether it is a chronic or acute infection. Concerning dopamine levels, there is an increase due to the high release of this neurotransmitter by dopaminergic cells infected by *T. gondii*, due to the influence of cytokines produced during host immune response and, moreover, due to their production in the cerebral cysts by the enzyme tyrosine hydroxylase, which is encoded by genes of the parasite itself (KAUSHIK et. al., 2012; HSU et. al., 2014). In addition, there appears to be a blocker in the *N*-methyl-D-aspartic acid receptors and a supply of serotonin receptors that decrease cat aversion by rats (WEBSTER, 2007). The position of the cysts in the various cerebral regions and their density can lead to inflammation, deposition of necrotic material and even occlusions, thus constituting a histopathological mechanism of action (WEBSTER, 2007). This location and density may be preferred or may be random and depend on accessibility to certain cerebral areas (BERENREITEROVÁ et. al., 2011).

Latent toxoplasmosis can cause neurodegeneration and neuroinflammation in hosts due to possibly altered neuronal function, ventricular dilatation and neuronal lesions (HERMES et. al., 2008). Thus, functional and structural disturbances in the corticolimbic circuits may explain some behavioural changes, since this circuit is related to the control of aggressive and impulsive behaviour (COCCARO et. al., 2011).

There is, therefore, an association of different mechanisms that may be involved in the behavioural change triggered by toxoplasmosis, indirect mechanisms related to host immune response and direct mechanisms related to parasite action in the organs and concentration of neurotransmitters (KAUSHIK et. al., 2012).

### **3.1 Animals**

Since rodents are the natural intermediate hosts of *T. gondii*, it is expected that most of the studies carried out to evaluate the hypothesis of manipulation are performed in these mammals. They are used as an experimental model for humans at the level of the immune system and CNS, since they have neurochemical and structural similarities (KAUSHIK et. al.,

2012). Many of the studies presented results consistent with the manipulation hypothesis since the parasite needs to be transmitted from the intermediate host to the definitive host in order to be able to reproduce sexually and complete its life cycle (WEBSTER, 2007; SILVA and LANGONI, 2009; HOUSE et. al., 2011; HSU et. al., 2014).

When specific tests, such as Y-shaped maze, running wheels and others, were performed in mice inoculated with *T. gondii*, it was found that mice exhibit decreased learning and memory capacity and increased activity compared to uninfected controls (WEBSTER, 2007; YOLKEN et. al., 2009). Moreover, infected mice showed preference for more exposed areas of the test apparatus and spend less time grooming in comparison with uninfected mice, which demonstrates their unconcerned behaviour (WEBSTER, 2007). However, mice have been shown to be more susceptible to *T. gondii* infection with severe signs of acute infection. Thus, it was necessary to carry out the experiments in more resistant rodents, such as rats, and that has served as a better model for extrapolation of the results to humans.

In order to study the effects of *T. gondii* on the level of activity, risk perception and behaviour of rats infected, tests were carried out observing their exploratory behaviour in 1x1 m pens with plastic nest-boxes containing four distinct odours, among them, the odour of cat urine (WEBSTER et. al., 2006). Comparing the infected and uninfected rats, the former were more likely to remain in the cat-smelling area, spending more time in this area, which demonstrated the characteristic of “feline attraction”. Besides that, infected rats showed increased activity and increased likelihood of grooming and remaining unconcerned in exposed areas.

Other studies carried out on rats with toxoplasmosis also showed an increase in activity, a preference for more exposed areas and a decrease in neophobia towards new stimuli compared to uninfected rats (WEBSTER, 2007; YOLKEN et. al., 2009; KAUSHIK et. al., 2012; HSU et. al., 2014). In addition, there seems to be a decrease in learning and attention capacity and a change in anxiety levels, which may be related to the modification of dopamine and norepinephrine concentrations, to the release of metabolites by the cysts or to the direct effect of these on the CNS (SILVA and LANGONI, 2009; HSU et. al., 2014). It was also found that some brain areas involved in defensive behaviour, fear and unconditioned anxiety, such as ventral hippocampus, medial and basolateral amygdala, have a higher cyst density than that found in other areas of the brain (SILVA and LANGONI, 2009).

The decrease in rodent fear to odour, sound or image related to a predator was demonstrated in several studies (WEBSTER, 2007; YOLKEN et. al., 2009; SILVA and LANGONI, 2009; FLEGR, 2013; KAUSHIK et. al., 2012). Typically, odour of cat or cat urine causes defensive responses and innate aversion in rodents, however, in mice and rats infected with *T. gondii*, this odour aversion is blocked and, unlike expected, rodents appear to be attracted by these odours, depending on concentration and time since the harvest. The cat urine odour attraction was further verified in another study comparing the behaviour of rats with toxoplasmosis and uninfected rats when exposed to a cat urine odour and to oestrous female rat odour (HOUSE et. al., 2011). At the level of the limbic system, in the uninfected rats, the route responsible for the defensive behaviour was activated when they were exposed to the cat urine odour and the path responsible for reproduction and approach was activated when exposed to the oestrous female odour. On the other hand, in rats with toxoplasmosis, there seems to be a dysregulation in the activated pathways, since they spend more time in the cat's urine odour exploration but show no change in the odour behaviour towards oestrous female odour when compared to uninfected rats. In the presence of cat urine odour, the dysregulation of the different limbic pathways caused by toxoplasmosis leads to an activation of the approach pathway and to an incomplete inhibition of the avoidance route, and the normal functioning of this system would lead to an inhibition of the first route and to an activation of the second. The two pathways involved in these behaviours are anatomically close and thus the establishment of cysts in these areas and the increase of their density can cause damage and dysregulation in the neuronal pathways and, consequently, cause behavioural changes in the host.

In hormonal terms, experiments performed in rats with toxoplasmosis demonstrated increased expressions of genes involved in the production of testosterone, a steroid that increases sexual attraction in males and reduces fear (LIM et. al., 2013; COCCARO et. al., 2016).

According to all these results it can be inferred that both rats and mice infected with *T. gondii* are more predisposed to predation, since cats are attracted by exposed, inattentive and moving prey (WEBSTER et. al., 2006; WEBSTER, 2007; SILVA and LANGONI, 2009; KAUSHIK et. al., 2012).

Whales and sea otters are an example of other mammals affected by behavioural manipulation since these infected animals have irregular movements that diminish their ability to escape and attract the attention of sharks (WEBSTER, 2007; SILVA and LANGONI,

2009). In a study in California that carried out the genotyping of *T. gondii* isolated from sea otters with toxoplasmic encephalitis, a fourth genotype was observed, Type X, associated to sites with higher mortality (MILLER et. al., 2004 In WEBSTER, 2007). In this study it was found that sea otters with toxoplasmic encephalitis were 3.7 times more likely to be hunted by sharks than sea otters without encephalitis.

### 3.2 Humans

At humans, the personality profile of individuals infected with *T. gondii* was evaluated in several studies over the years and there were differences and similarities between the results on men and women (Table 1). Infected women demonstrated high scores on superego strength, lower scores on protension, weak instinct for self-preservation and presented a more warm-hearted, outgoing and easy-going personality than uninfected women, while men presented the opposite characteristics, such as low superego strength, pretension and cold, introvert and inflexible personality (SILVA and LANGONI, 2009; KAUSHIK et. al., 2012; FLEGR, 2013). Another difference between infected men and women is related with altruism and sociability, with women being more sociable and more selfless than men (SILVA and LANGONI, 2009; FLEGR, 2013). Beyond that, men infected with *T. gondii* has low self-esteem, unstable temper, disrespect for social rules and group dependence, being also more jealous and distrustful, while women demonstrate high self-esteem and respect for social rules and are more intelligent, sentimental, affective, independent and loyal (SILVA and LANGONI, 2009; YOLKEN et. al., 2009). On the other hand, both men and women showed a decrease in conscientiousness and in the score of novelty seeking, which is associated with high levels of dopamine and an increase in self-transcendence (KAUSHIK et. al., 2012; FLEGR, 2013). Besides that, both genders reported lower diplomacy, slow and passive behaviour towards danger, decreased resilience and increased anxiety (SILVA and LANGONI, 2009; FLEGR, 2013).

According to a study, differences in behaviour presented by women and men infected with toxoplasmosis may be due not to the direct effect of the parasite on the individuals' brains, but to their response to stress and anxiety, which means that there is no gender specificity (SUTTERLAND et. al., 2015). Notwithstanding, further studies on gender specificity and behavioural differences need to be performed on larger samples with homogeneous characteristics.

Toxoplasmosis is known to affect behaviour, personality and other traits of human phenotype. A reaction time test demonstrated that *T. gondii* causes an increase in reaction

time in affected individuals due to the action of this parasite on the long-term concentration capacity, and may also result in an increased risk of work-related injury and traffic-accidents (SILVA and LANGONI, 2009; YOLKEN et. al., 2009; FLEGR, 2013). However, according to some studies, these changes have only been verified in Rhesus D protein (RhD) negative individuals, which may indicate a protective effect on latent toxoplasmosis conferred by the RhD protein in individuals positive for this protein, but more studies are needed to prove this possible protective effect (KAUSHIK et. al., 2012; FLEGR, 2013; SUTTERLAND et. al., 2015).

Table 1: Differences and similarities in the personality profile of women and men with toxoplasmosis (based on YOLKEN et. al., 2009; SILVA and LANGONI, 2009; KAUSHIK et. al., 2012; FLEGR, 2013).

<b>Personality Profile</b>			
<b>Women</b>		<b>Men</b>	
Warm-hearted and outgoing	Weak self-preservation	Cold and introverted	Pretension
Sociable and selfless	High self-esteem	Unsociable and selfish	Unstable temper
High superego strength	Independent and intelligent	Low superego strength	Low self-esteem
Sentimental, affective and loyal	Respect for rules	Jealous and distrustful	Dependent
Honest	Easy-going		Disrespect for rules
			Inflexible
Low conscientiousness Low diplomacy High anxiety Low resilience High self-transcendence Slow and passive behaviour towards danger			

The influence of toxoplasmosis on the human cognitive part was evaluated in some studies by measuring cognitive function and levels of IgG antibodies against *T. gondii* in individuals without a history of psychosis. It was verified that the individuals with high levels of antibodies i.e. with serological evidence of toxoplasmosis, present worse results in the tests of immediate and delayed memory, as in other areas, however these results did not show change in relation to variables such as the level of education and age (YOLKEN et. al., 2009). There is also evidence that behavioural manipulation by *T. gondii* can result in signs of aggression and impulsivity and decreased levels of intelligence quotient (WEBSTER et. al., 2006; GRANDE et. al., 2017).

At the hormonal level there are some contradictions. Some studies report an increase in testosterone concentration in *T. gondii*-infected male students, who are taller and have more masculine and dominant faces, and a decrease in infected female students (KAUSHIK et. al., 2012; FLEGR, 2013; GRANDE et. al., 2017), while other studies report an increase in testosterone in both men and women (SHIRBAZOU et. al., 2011 *In* FLEGR, 2013; VYAS, 2013 *In* FLEGR, 2013). Other studies have been carried out on infected individuals from two independent sample populations (immunology clinic patients and male soldiers) in whom testosterone levels were not increased (KAŇKOVÁ et. al., unpublished *In* FLEGR, 2013). The reason for the different results in testosterone levels is not yet fully understood and may be due to the characteristics of each individual, such as the gender, the susceptibility to infection or the population and geographical area where they were created (KAUSHIK et. al., 2012; FLEGR, 2013).

The foetal development and motor development of the children of mothers infected with toxoplasmosis, and also the mechanisms involved in controlling their development without anomalies are diminished (KAŇKOVÁ et. al., 2012; FLEGR, 2013). Such facts appear to be associated with immunosuppression and can result in abortion and developmental disabilities. Thus, it is extremely important to monitor pregnancy in mothers who may develop the infection in order to reduce cases of congenital toxoplasmosis.

As with rodents, experiments with cat urine and from other species were also carried out with humans to evaluate the phenomenon of “feline fatal attraction” (KAUSHIK et. al., 2012; FLEGR, 2013). Compared with uninfected individuals, men infected with toxoplasmosis rated the odour of cat urine as more pleasant and contrary the infected women that rated it as less pleasant (FLEGR et. al., 2011; FLEGR, 2013). A similar but less intense result was found for hyena urine however, the attraction phenomenon reported in

men was not observed for tiger urine. This fact is curious since the tigers belong to the feline family but can be explained by the presence of a less concentration of certain feline pheromones in tigers when compared with levels present in cats. The reaction to the odour of human urine was also tested and it was observed that men infected with *T. gondii* classified the smell of male urine and of female urine in fertile phase of the menstrual cycle as more pleasant (FLEGR, 2013).

Since humans are not the main intermediate hosts of *T. gondii*, the parasite's hypothesis of manipulating behaviour in humans seems unlikely and behavioural changes may be the result of indirect and non-specific adaptive modifications (KAUSHIK et. al., 2012). However, due to the high prevalence of this infectious disease worldwide, both in its latent and congenital forms, and due to the doubts and the inconclusive results of studies carried out so far, it is necessary to execute several studies in order to understand the influence of the hormonal part, the gender and the RhD phenotype, among others, and clarify the role of this parasite in the brain and in the human organism.

#### 4 Toxoplasmosis and psychotic disorders

Psychotic disorders are those in which episodes of psychosis occur, which are characterized by the appearance of various mental and behavioural disorders and distortions of perception and thought. These disorders include delusions, hallucinations, apathy, excitement and hyperactivity or inactivity (WHO/EUROPE, 2017). These diseases are a part of mental disorders, which have a very high prevalence in Europe with data indicating that about 27% of the adult population, 83 million people, had suffered at least one mental disorder in the last year (WHO/EUROPE, 2017).

Since it was discovered that *T. gondii* can be associated with behaviour manipulation, several assumptions emerged about the possible association between this parasite and psychotic disorders namely schizophrenia and bipolar disorder (BD) (ZHU, 2009; SUTTERLAND et. al., 2015; GARGATÉ et. al., 2016). This association appears to increase with increasing levels of antibodies against *T. gondii* (SUTTERLAND et. al., 2015). It is also reported that toxoplasmosis increases dopamine levels and alters the glutamate, serotonin and gamma-aminobutyric acid pathways in the host brain so, considering that the concentration of these neurotransmitters is one of the main factors associated with psychosis and aggressive behaviour, the probable relationship between this parasitic infection and psychotic disorders is discussed (ZHU, 2009; YOLKEN et. al., 2009; KAUSHIK et. al., 2012; HSU et. al., 2014; GRANDE et. al., 2017).

The parasites can cause or increase the probability of occurrence of psychotic disorders either directly or indirectly (YOLKEN et. al., 2009; GRANDE et. al., 2017). Directly through neuronal or cerebral damage, and indirectly through increased stimulation of the immune response with release of possibly neurotoxic substances (ZHU, 2009; HSU et. al., 2014; GRANDE et. al., 2017). As already mentioned above in behavioural manipulation, toxoplasmosis infection associated with other factors, such as stress, causes the activation of microglia cells and the release of pro-inflammatory cytokines by mast cells. This exacerbated activation and release may lead, in the foetus or new born, to a delay in brain development and an increased likelihood of subsequent psychotic disturbance and, in the adult, to psychoses with cerebral inflammation (GRANDE et. al., 2017). Thus, knowing that dysregulation of immune system activity is a susceptible factor for psychotic disorders and knowing that inflammatory processes and autoimmune responses are described in BD and schizophrenia, the inflammatory brain environment caused by activation of microglia cells and release of cytokines may be the link between toxoplasmosis and subsequent susceptibility to psychoses (GRANDE et. al., 2017).

In the studies carried out on the association between toxoplasmosis and psychosis, it is important to consider the possible moderators of heterogeneity, that is, the factors that may contribute to the heterogeneity of the results obtained (SUTTERLAND et. al., 2015). The age, serointensity, *T. gondii* strain most prevalent in a region, the region itself, disease phase and the types of antibodies produced are some of the moderators to be taken into account when comparing results between the control and the patient groups.

#### 4.1 Schizophrenia

Schizophrenia is a severe psychotic disorder that affects the brain, neurons and glia causing change in its functions and structures (YOLKEN et. al., 2009). It usually begins in early adulthood or late adolescence and is characterized by distortion of emotions, language, thoughts, perception and behaviour, delusions and hallucinations (WHO, 2017). Schizophrenia may follow a recurrent or chronic course without complete social and symptomatology recovery or, in a smaller percentage of cases, may follow a full recovery of symptoms and social characteristics (WHO, 2001). This disease is considered a socioeconomic and health problem since it affects between 0.5 % and 1 % of the world population (YOLKEN et. al., 2009; FLEGR, 2013).

At first it was believed that the increased risk for the development of schizophrenia was due to genetic factors, however, considering the genes that would be responsible for



this predisposition are not yet clear, the possibility arose that some cases of schizophrenia were caused by infectious agents (YOLKEN et. al., 2009). Thus, similarities and differences between schizophrenia and toxoplasmosis were analysed. The similarities are related to the average age of onset of both diseases taking into account the individual's symptoms and gender (men develop diseases earlier) and are related to household and socioeconomic conditions (increased prevalence in poorer conditions). Furthermore, they are still associated with the increase of stillbirths in both patients and with the possibility of a genetic predisposition for both diseases although the responsible genes are not clear (YOLKEN et. al., 2009). The differences refer to the origins of the patients (urban or rural), where there are quite dissenting results regarding toxoplasmosis, and to the decrease in the seroprevalence of *T. gondii* not associated with the decrease of schizophrenia. However, this set of differences and similarities is limited and there are still some contradictions and lapses that will have to be evaluated in future studies (YOLKEN et. al., 2009).

As the effects of latent toxoplasmosis are also strongly related to the risk of schizophrenia and there are studies demonstrating the high prevalence of *T. gondii* in patients with schizophrenia, the hypothesis that this psychotic disorder could be caused by *T. gondii* infection was reinforced (SILVA and LANGONI, 2009; KAUSHIK, 2012; FLEGR, 2013). *T. gondii* can cause lesions in astrocytes, activating them and leading to increased production of kynurenic acid in the brain, which is elevated in individuals with schizophrenia (SCHWARCZ and HUNTER, 2007). Cognitive impairment in schizophrenics may be due to increased levels of kynurenic acid since it causes inhibition of nicotine and glutamine neurotransmitter receptors.

Despite what has been said about not needing direct contact with cats for *T. gondii*'s transmission, some studies were performed to evaluate this contact during pregnancy and childhood in individuals with schizophrenia (YOLKEN et. al., 2009). In the first study, it was found that schizophrenic or BD patients had a higher percentage of being exposed to cats at different periods (during pregnancy, from birth to 1<sup>st</sup> year, from 1<sup>st</sup> to 5<sup>th</sup> year and from 6<sup>th</sup> to 10<sup>th</sup> year) than the control individuals and the period in which the percentage differences were greater was from 6 to 10 years (TORREY and YOLKEN, 1995). Also in the second study, results similar to the first one were verified, however only two periods were considered (during pregnancy and from birth to the 13<sup>th</sup> year) and the comparison between being a cat or a dog owner was also made. Significant results were found in schizophrenic or BD individuals who had cats between the ages of birth and 13 years (TORREY et. al., 2000).

Most studies have investigated the presence of anti-*T. gondii* IgG antibodies in the blood of the individuals with chronic schizophrenia and control subjects and some studies have performed this research on cerebrospinal fluid, obtaining a substantial adjusted odds ratio (OR) of 1.43 for the association toxoplasmosis and schizophrenia (SUTTERLAND et. al., 2015). This association has been more moderate in Europe and North America compared to other continents/regions such as Africa, Asia, Middle East and South America, which had a higher association. The risk of schizophrenic patients presenting antibodies against *T. gondii* is 2.73 times higher than in non-schizophrenic individuals and this association between toxoplasmosis and schizophrenia appears to be more common in women (SILVA and LANGONI, 2009; YOLKEN et. al., 2009; HSU et. al., 2014). It was also observed that mothers who developed symptoms of schizophrenia, babies of schizophrenic mothers and untreated individuals with recent onset of schizophrenia are at increased risk of having antibodies IgG against *T. gondii* (SILVA and LANGONI, 2009; HSU et. al., 2014; GRANDE et. al., 2017).

However, although less frequent, some studies have evaluated the presence of IgM antibodies against *T. gondii* in schizophrenics and in individuals with recent onset of this psychosis (MONROE et. al., 2015; SUTTERLAND et. al., 2015). In one of the studies, there was a significant association between the presence of anti-*T. gondii* IgM antibodies and the presence of acute toxoplasmosis in individuals with chronic schizophrenia and episodes of acute psychosis in comparison with control subjects (MONROE et. al., 2015). In the other study the results were found to be non-significant i.e. not many individuals with this type of antibodies were observed (SUTTERLAND et. al., 2015). Due to this contradictory results, it is important to carry out studies that relate IgM antibodies against *T. gondii* with relapse in chronic schizophrenic patients and also with IgG levels (MONROE et. al., 2015). Thus, besides being possible to establish a temporal and causal nexus, it would also be possible to distinguish between acute, chronic or reactivated infection and to infer about the course of this psychotic disorder.

Prenatal exposure to parasites with CNS tropism, such as *T. gondii*, can trigger serious sequelae and consequences, including later development of psychotic disorders (WEBSTER et. al., 2006; YOLKEN et. al., 2009; HSU et. al., 2014; SUTTERLAND et. al., 2015; GRANDE et. al., 2017). Thus, some studies have observed that the offspring of mothers with high levels of anti-*T. gondii* antibodies, resulting from increased activation of the immune system during pregnancy, would be at higher risk of developing schizophrenic disorders in adults.

Due to the different studies already performed, it is known that schizophrenic patients have a higher rate of anti-*T. gondii* antibodies compared to the control subjects, which may be due to an exacerbated immune system response or a more active infection. In addition, toxoplasmosis may precede schizophrenia and the association of these two diseases becomes stronger with the evolution of psychosis. Studies were then carried out in order to assess the levels of antibodies against toxoplasmosis in individuals likely to develop schizophrenia i.e. before the onset of the disease itself (YOLKEN et. al., 2009; SUTTERLAND et. al., 2015). It was found that individuals predisposed to schizophrenia had a higher level of IgG antibodies and an OR of 1.30 indicating the elevated risk of toxoplasmosis before the onset and development of schizophrenia.

According to different studies, when comparing schizophrenic patients infected with *T. gondii* and uninfected schizophrenic patients, it was found that in the former there is an increase in the severity of symptoms and, in terms of morphology, there is a decrease in grey matter density in some areas of the brain and an increased size of ventricles (SILVA and LANGONI, 2009; KAUSHIK et. al., 2012; FLEGR, 2013). This evidence together with the fact that toxoplasmosis increases dopamine levels and alters other neurotransmitters in the host brain and that the parasite contains genes for tyrosine hydrolase, an enzyme involved in dopamine synthesis, suggest that infection with *T. gondii* can precede and lead to schizophrenia and may play an important role in the progress of this disease (YOLKEN et. al., 2009; KAUSHIK et. al., 2012; FLEGR, 2013; HSU et. al., 2014). Data indicate that the mortality rate in schizophrenic individuals infected with toxoplasmosis is five times higher than in uninfected individuals (SILVA and LANGONI, 2009).

In addition, toxoplasmosis infection has also been associated with aggressive and suicidal behaviour in young schizophrenics, with levels of IgG antibodies against *T. gondii* being higher in these individuals (HSU et. al., 2014; GRANDE et. al., 2017).

Although additional and more in-depth studies on this relationship between toxoplasmosis and schizophrenia are needed, another contribution to this association is related with the induction of the pro-inflammatory response by the parasite with consequent alteration of T-helper lymphocyte response and C-reactive protein levels (KAUSHIK et. al., 2012). This dysregulation, as well as that verified in the dopaminergic and serotonergic systems, is verified in the organisms of schizophrenic individuals. Thus, *T. gondii* mimicking what happens in these individuals may cause an acceleration of psychotic symptoms and the onset of schizophrenia in individuals already vulnerable to the disease.

## 4.2 Bipolar disorder

Bipolar disorder is a chronic psychiatric disease characterized by marked mood disorders and a chronic and dysregulated inflammatory state both at the cerebral and peripheral levels, affecting about 60 million people worldwide (GRANDE et. al., 2017; WHO, 2017). In the long term, BD is mainly manifested by depressive and manic episodes and symptoms, which are related to the risk of suicide and to high levels of disability (KERNER, 2014; MUNEEER, 2016; ROSENBLAT and MCINTYRE, 2016; GRANDE et. al., 2017). This disease presents a multifactorial origin, involving factors of genetic heredity and environmental factors among which the dysfunction of the immune system and where infectious attacks are evidenced.

When analysing the results of different studies on the prevalence of IgG antibodies against *T. gondii* in psychotic disorders, a significant OR of 1.52 was found for the association among toxoplasmosis infection and BD (SUTTERLAND et. al., 2015) and individuals seropositive for toxoplasmosis are about 3.6 times more likely to have BD (GRANDE et. al., 2017). In addition, anti-*T. gondii* antibody levels and seropositivity were clearly high in patients with BD.

Antibody levels of IgG and IgM anti-*T. gondii* in the serum of hospitalized individuals with manic events were compared with these antibody levels in individuals with BD or schizophrenia and with healthy controls. It was found that patients with manic episodes have increased levels of IgM anti-*T. gondii* and individuals with recent onset of psychosis (BD or schizophrenia) have high levels of IgG anti-*T. gondii* (DICKERSON et. al., 2014 In GRANDE et. al., 2017). It was hypothesized that subjects with elevated levels of IgM would have been infected or had a reaction to *T. gondii* at the time of their manic episode hospitalization, as increased levels of IgM antibodies against *T. gondii* were observed after re-infection or reactivation.

According to the results by the Third National Health and Nutrition Examination Survey, individuals already exposed to toxoplasmosis were about 2.3 times more likely to have BD type I associated with depressive and manic symptoms when compared to individuals who did not have anti-*T. gondii* antibodies (PEARCE et. al., 2012; GRANDE et. al., 2017). This association between toxoplasmosis and BD may result from a greater predisposition to infection in individuals with BD due to increased exposure related to their behaviours or due the acceleration of depressive symptoms in subjects with BD type I resulting from infection.

It is important to mention the existence of a study with contrasting results to those presented above. In Iran, a country with a high prevalence of toxoplasmosis, the difference in IgG antibodies anti-*T. gondii* in health subjects and patients with BD type I was not significant (KHADEM VATAN et. al., 2013). Therefore, more research and studies on the association between toxoplasmosis and BD are needed to clarify the contradictory results (GRANDE et. al., 2017).

Another curious fact is that the number of suicides and self-directed violence among patients with BD appears to increase in toxoplasmosis-infected women due to latent toxoplasmosis associated with other suicidal elements (LING et. al., 2011; PEDERSEN et. al., 2012). With regard to prenatal exposure and although there is a study demonstrating the increased risk of developing BD with psychotic traits in the offspring of mothers infected with *T. gondii* (XIAO et. al., 2009), it was found that the association between prenatal exposure to *T. gondii* and the risk of BD in adults is not significant (GRANDE et. al., 2017).

According to some studies, it is believed that the BD maniac phase and depressive phase, may be related to a certain inflammatory state triggered by the increase of pro-inflammatory cytokines, especially TNF- $\alpha$  and IL-6 (HAMDANI et. al., 2015; BARROS et. al., 2017; GRANDE et. al., 2017). The increase of these two cytokines occurs mainly in depressive episodes and associated with *T. gondii* infection, leading to discontinuation of neurogenesis and, consequently, to cognitive impairment in patients with BD and also with schizophrenia (HAMDANI et. al., 2015; SUTTERLAND et. al., 2015; BARROS et. al., 2017; GRANDE et. al., 2017). Thus, IL-6 levels may serve as advantageous immunological biomarkers in predicting cognitive decline in toxoplasmosis-infected BD patients, as well as possibly being crucial in the development of new drugs for the treatment of these diseases (GRANDE et. al., 2017). Consistent with the above, treatment of BD patients with mood stabilizers resulted in a return to euthymic status, a decrease in pro-inflammatory cytokines and the return of IL-6 to its basal levels (HAMDANI et. al., 2015; GRANDE et. al., 2017).

As reported for schizophrenia, there are also references to increased levels of dopamine and immunological changes in individuals with BD and infected with toxoplasmosis (KAUSHIK et. al., 2012; HAMDANI et. al., 2015; SUTTERLAND et. al., 2015; BARROS et. al., 2017). The increase in dopamine occurred mainly in the limbic region (which is altered in individuals with BD) and could be involved in the production and efficacy of infection and in the conversion of *T. gondii*, from tachyzoites to bradyzoites, in the brain (PRANDOVSKY et. al., 2011; STROBL et. al., 2012). The parasite has two genes that encode enzymes with

tyrosine hydroxylase activity (a limiting enzyme of dopamine production) and one of the genes is induced throughout bradyzoites formation, increasing the production of dopamine (GASKELL et. al., 2009). Besides that, this enzyme is found in the intracellular tissue of the cysts (PRANDOVSKY et. al., 2011). These facts could explain the neurochemical mechanism involved in the increase of this neurotransmitter in individuals with toxoplasmosis and the induction of psychotic and behavioural alterations by *T. gondii*. (PRANDOVSKY et. al., 2011; GRANDE et. al., 2017). In a study performed on brain sections of *T. gondii*-infected mice and on infected-mammalian dopaminergic cells there was an increase in dopamine metabolism in neural cells and the increase in release of this neurotransmitter being proportional to the amount of infected cells (PRANDOVSKY et. al., 2011). Additionally, increased levels of kynurenic acid and kynurenine have been observed not only in schizophrenic patients but also in bipolar patients with a history of psychosis, as evidenced by post-mortem studies (MILLER et. al., 2006) and studies using human fibroblast cell lines obtained from individuals with BD and schizophrenic individuals (JOHANSSON et. al., 2013).

## 5 Impact of drugs on ability of *Toxoplasma gondii* to change host behaviour

Patients undergoing treatment for schizophrenia showed, in some studies, a decrease in anti-*T. gondii* antibody levels when compared to untreated individuals (WEBSTER et. al., 2006; YOLKEN et. al., 2009; GRANDE et. al., 2017).

Several studies showed the *in vitro* antiprotozoal activity of antipsychotic drugs used in schizophrenic and BD patients (WEBSTER et. al., 2006; GRANDE et. al., 2017). Besides that, studies also examine the effect of these drugs in ability of *T. gondii* to alter its capacity for infection, dissemination and replication and host behaviour (WEBSTER et. al., 2006; WEBSTER, 2007; YOLKEN et. al., 2009; SILVA and LANGONI, 2009; HSU et. al., 2014; GRANDE et. al., 2017). The overall results show that particularly the antipsychotics can reduce *T. gondii* replication and the behavioural alterations caused by this parasite.

The haloperidol and valproic acid (antipsychotic and mood stabilizer for schizophrenia treatment) were tested in rats infected with *T. gondii* and compared with pyrimethamine/dapsone (anti-toxoplasmosis drugs) in order to assess their activity against toxoplasmosis and the inhibition of cognitive and behavioural changes in those animals (WEBSTER et. al., 2006; WEBSTER, 2007; HSU et. al., 2014). The study demonstrated a reduction in the behavioural alterations and suicidal attraction in rats treated with haloperidol (a dopaminergic D2 antagonist), compared with pyrimethamine/dapsone and

valproic acid treatments. However, treatment with haloperidol, valproic acid or pyrimethamine/dapsone in non-infected rats has been found to elicit behaviours similar to those reported by infected but untreated rats, such as increased activity and feline attraction (WEBSTER et. al., 2006). Such side effects may be due to the action of these drugs on the neurotransmitters and therefore further studies are needed to establish their role in individuals with toxoplasmosis and its behavioural effect. After fluorescence staining of postmortem brain sections, haloperidol treatment also demonstrated a reduction in the frequency of *T. gondii*-exposed animals exhibiting immunohistochemically positive glial cells and neurons i.e. there is less likelihood of neurons and glia cells, from treated animals, contain the parasite (WEBSTER et. al., 2006; WEBSTER, 2007).

In another study, the activity of various mood stabilizers and antipsychotics in inhibiting cell invasion and tachyzoites replication was evaluated and compared with trimethoprim (drug used to treat toxoplasmosis) (JONES-BRANDO et. al., 2003). Haloperidol, valproic acid and sodium valproate (antiepileptic and anticonvulsant used in the treatment of BD) demonstrated the stronger inhibition capacity followed by 9-OH-risperidone and fluphenazine (antipsychotics used in the treatment of schizophrenia). Valproic acid and its salt, sodium valproate, were found to have a very similar therapeutic indices to trimethoprim, and showed inhibition against low concentrations of *T. gondii* and synergistic inhibitory activity with the drugs trimethoprim and haloperidol.

The results show that haloperidol and valproic acid have efficacy against *T. gondii* replication and can be used satisfactorily in the treatment of chronic toxoplasmosis associated with psychotic disorders (WEBSTER et. al., 2006; SILVA and LANGONI, 2009; GRANDE et. al., 2017). Their mechanism of action may be related to calcium-inhibiting properties, since these drugs function as calcium ion channel blockers and tachyzoites need calcium to invade host cells. Haloperidol, in addition to the mechanism of action referred above, also has activity as a dopamine D2 antagonist which allows for a decrease in dopamine levels (WEBSTER et. al., 2006; HSU et. al., 2014; GRANDE et. al., 2017).

In a population of patients with schizophrenia or BD infected with toxoplasmosis the effect of treatment with psychotropic drugs was evaluated (FOND et. al., 2015). Haloperidol, valproate, risperidone, fluphenazine, loxapine (antipsychotic for BD and schizophrenia) showed anti-*T. gondii* effect, whereas lithium carbonate (lithium for BD treatment), quetiapine and olanzapine (antipsychotics for schizophrenia and BD), carbamazepine (antiepileptic and anticonvulsant) have no or insignificant anti-*T. gondii* effect.

The results also showed that treatment with anti-toxoplasmosis drugs does not reduce the episodes associated with mania and psychoses but seem to reduce depressive episodes. Since only significant results were found in individuals with BD and seropositive for toxoplasmosis, it was concluded that serological levels of *T. gondii* could be used as a biomarker in the prevention of bipolar depressive episodes by treatment with drugs with anti-*T. gondii* activity in those individuals.

The possibility was raised that anti-infective drugs, azithromycin, pyrimethamine-sulfadiazine and trimethoprim-sulfamethoxazole, used in the treatment of toxoplasmosis could also be used in patients with schizophrenia associated with *T. gondii*. However, the results for schizophrenic patients having the disease for many years were not significant (YOLKEN et. al., 2009). A clinical trial of adjuvant azithromycin (broad-spectrum antibiotic, macrolide) treatment in schizophrenic patients with toxoplasmosis was performed and the results demonstrated that azithromycin also had no benefit or effect (DICKERSON et. al., 2009). Larger studies were conducted in individuals with a more recent onset of schizophrenia, including studies using antimalarial drugs, in order to better understand the effects of these drugs on the possible treatment of toxoplasmosis associated with schizophrenia (DICKERSON et. al., 2009). The artemisinin (antimalarial drug) derivatives have demonstrated inhibition of tachyzoites replication and inhibition of invasion of host cells, and may be a therapeutic alternative for the treatment of toxoplasmosis (D'ANGELO et. al., 2009).

Notwithstanding all the results presented, it will be useful to conduct further studies about the activity and mechanism of action of haloperidol, valproic acid and other antipsychotics on the behaviour and psychotic episodes presented by individuals infected with toxoplasmosis (WEBSTER et. al., 2006; SILVA and LANGONI, 2009; GRANDE et. al., 2017).



## 6 Conclusion

The change in humans' behaviour does not seem to be a result of manipulation by *T. gondii*, but rather as side effects generated by *T. gondii* infection, causing changes in anxiety, attention, personality and phenotypic profile (KAUSHIK et. al., 2012; WEBSTER et. al., 2013). The main mechanisms that may be involved in the behavioural change include the modification of the levels of several neurotransmitters, such as dopamine and norepinephrine, the immune response triggered by the infection, which promotes the release of mediators and pro and anti-inflammatory cytokines, and the direct action of cysts in the brain and neuronal pathways (WEBSTER, 2007; YOLKEN et. al., 2009; KAUSHIK et. al., 2012).

In the future, further studies on the causal relationship between toxoplasmosis and the behavioural and phenotypic alterations presented by infected hosts will be necessary and it would be important to deepen the mechanisms involved. In addition, when analysing data and results obtained, it is necessary to be critical and take into account the heterogeneity, genetic polymorphism, environmental factors, sample size and all the characteristics (time of exposure, duration of infection, state of immune system, parts of the brain infected and others) that may influence the study population since the influence of toxoplasmosis on the same personality or phenotypic factor may vary in different populations (WEBSTER et. al., 2006; WEBSTER, 2007; FLEGR, 2012).

Regarding the possible role of toxoplasmosis in the onset and development of psychotic disorders such as schizophrenia and BD, the existence of factors compatible with this association were observed, namely the increase of anti-*T. gondii* antibodies in these individuals and behavioural and personality changes (ZHU, 2009; SUTTERLAND et. al., 2015; GARGATÉ et. al., 2016). In the case of schizophrenia, increased dopamine levels, dysregulation of other neurotransmitters, inflammatory response and some morphological changes appear to be the link between this disease and toxoplasmosis. In BD, in addition to the factors mentioned for schizophrenia, there is still an increase in IL-6 levels, which appear to be caused by *T. gondii* and lead to cognitive alterations (YOLKEN et. al., 2009; KAUSHIK et. al., 2012; FLEGR, 2013; HSU et. al., 2014; HAMDANI et. al., 2015; GRANDE et. al., 2017).

Since the data on the association of toxoplasmosis with psychotic disorders are not conclusive and there are still many doubts and contradictions in these areas it is important to carry out more in-depth studies using adequate profiles to able comparison of results and

obtain accurate and useful conclusions. In addition, since the studies already reveal some mechanisms by which *T. gondii* leads to altered behaviour in mammals and the possible onset and development of psychotic disorders, more research is needed to understand why some individuals are asymptomatic and others develop forms more or less severe of behavioural changes or psychoses and to elucidate the different factors that may influence this phenomenon (WEBSTER et. al., 2006; YOLKEN et. al., 2009). It is also very important to understand all the roles played by *T. gondii* in the development of psychotic disorders.

Finally, the studies have demonstrated that antipsychotics and mood stabilizers, especially haloperidol, appear to have a positive anti-*T. gondii* potential, inhibiting parasite replication and consequently, inhibiting its effects on hosts (WEBSTER et. al., 2006; YOLKEN et. al., 2009; GRANDE et. al., 2017). However, we must take into account the side effects that may be associated with this alternative therapy. Thus, further studies and experimental trials with different patient groups need to be carried out, both at the level of antipsychotics and mood stabilizers as well as other possible candidates, so that their prophylactic or therapeutic use in toxoplasmosis and psychotic disorders is performed safely and effectively (WEBSTER et. al., 2006; YOLKEN et. al., 2009).

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