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FONTE DE INFECÇÃO E GENOTIPAGEM DE *Toxoplasma gondii* DURANTE O SURTO DE TOXOPLASMOSE EM SANTA MARIA, BRASIL

Santa Maria, RS, Brasil
2020

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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração em Sanidade e Reprodução Animal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Medicina Veterinária**

Orientador: Fernanda Silveira Flores Vogel

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RESUMO

FONTE DE INFECÇÃO E GENOTIPAGEM DE *Toxoplasma gondii* DURANTE O SURTO DE TOXOPLASMOSE EM SANTA MARIA, BRASIL

AUTOR: Camila Encarnação Minuzzi

ORIENTADOR: Fernanda Silveira Flores Vogel

Toxoplasma gondii é altamente disseminado em todo o mundo, devido a sua alta capacidade de adaptação e diferentes formas de transmissão. Este parasita tem como hospedeiros intermediários uma ampla gama de espécies, nas quais ocorre a formação de cistos teciduais. Já os hospedeiros definitivos são os felídeos, que excretam oocistos em suas fezes. Os oocistos são uma importante fonte de infecção em humanos. Eles podem ser ingeridos em alimentos ou água contaminada. A toxoplasmose em humanos tem diferentes apresentações clínicas, porém ganha maior relevância quando se trata da infecção durante a gestação: toxoplasmose congênita. No ano de 2018 ocorreu um surto de toxoplasmose em humanos no munício de Santa Maria, sul do Brasil, o qual foi considerado o maior surto de Toxoplasmose já descrito no mundo. Esta tese foi escrita com intuito de colaborar na elucidação deste grande surto. No capítulo 1 encontra-se um estudo que apresenta a caracterização molecular de tecidos oriundos de placentas de pacientes com toxoplasmose aguda. Neste estudo foi realizado bioensaio em camundongos e a genotipagem dos isolados presentes. Com isso, pode-se verificar que se tratava de um genótipo atípico que nunca antes havia sido descrito. No estudo apresentado no capítulo 2 foi realizada a investigação da fonte de infecção do surto. Para isso foi feito um bioensaio com suínos que receberam água potencialmente contaminada oriunda de diferentes fontes da cidade. Foi realizada periodicamente a sorologia dos animais, e percebeu-se que ocorreu a soroconversão. Posteriormente um segundo bioensaio utilizando camundongos que foram inoculados com tecidos desses suínos, demonstrou que havia infecção ativa de *T. gondii* nos tecidos, sugerindo assim que a água foi uma potencial fonte de infecção neste surto.

Palavras-chave: Toxoplasmose, surto, caracterização molecular, genótipo atípico, sorolog água.

ABSTRACT

SOURCE OF INFECTION AND GENOTYPING OF *Toxoplasma gondii* DURING THE OUTBREAK OF TOXOPLASMOSIS IN SANTA MARIA, BRAZIL

AUTHOR: Camila Encarnação Minuzzi

ADVISER: Fernanda Silveira Flores Vogel

Toxoplasma gondii is highly widespread worldwide, due to its high adaptability and different forms of transmission. This parasite has a wide range of species as intermediate hosts , in which occurs the formation of tissue cysts . The definitive hosts are the felids, which excrete oocysts in their feces. Oocysts are an important source of infection in humans. They can be ingested in contaminated food or water. Toxoplasmosis in humans has different clinical presentations, but it gains more relevance when infection outcomes during pregnancy: congenital toxoplasmosis. In 2018 there was an outbreak of toxoplasmosis in humans in the municipality of Santa Maria, southern Brazil, which was considered the largest outbreak of Toxoplasmosis ever described in the world. This thesis was developed in order to collaborate in the elucidation of this great outbreak. Chapter 1 contains a study that presents the molecular characterization of T. gondii from placentas of patients with acute toxoplasmosis during the outbreak. In this study, bioassies were performed on mice and genotyping of the isolates . With this, it can be verified that it was an atypical genotype that had never been described before. In the study presented in chapter 2, the outbreak source of infection was investigated. For this, bioassaies were carried out with pigs that received water from different sources in the city. The animals' serology was periodically performed, and it was noticed that seroconversion occurred. Subsequently, a second bioassay using mice that were inoculated with tissues from these pigs, demonstrated that there was active infection of *T. gondii* in the tissues, thus suggesting that water was a potential source of infection in this outbreak.

Key words: *Toxoplasmosis, outbreak, molecular characterization, atypical genotype, serology, water.*

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1 – INTRODUÇÃO

Toxoplasma gondii é um parasita prevalente na maior parte do mundo, que pode provavelmente infectar todos os animais de sangue quente, incluindo humanos, e (DUBEY, 2008). Sua considerável disseminação ao redor do mundo se deve a alta capacidade de adaptação nos diferentes hospedeiros e facilidade de transmissão por diversas formas (DUBEY & SU, 2009). A infecção apresenta uma ampla gama de manifestações clínicas em mamíferos humanos, terrestres e marinhos, e várias espécies de aves (AKYAR, 2011).

T. gondii é protozoário formador de cistos teciduais, intracelular obrigatório, pertencente ao Filo Apicomplexa, classe Coccídea, Família Sarcocystidae, subfamilia Toxoplasmatinae, classificado como *Toxoplasma gondii* (NICOLLE e MANCEAUX, 1909). Este protozoário foi descrito pela primeira vez por NICOLLE & MANCEAUX (1908), que encontraram estágios de um parasita no fígado, baço e sangue de uma espécie de roedor africano, o gundi, (*Ctenodactylus gundi*). Em 1939, Sabin demonstrou que os isolados de *Toxoplasma* humanos e aqueles anteriormente obtidos de animais pertenciam à mesma espécie. Em 1948, a introdução do teste do corante azul de metileno por Sabin e Feldman permitiu estudos soroepidemiológicos em humanos, bem como em uma ampla gama de espécies animais que forneceu evidências para uma grande distribuição e alta prevalência de *T. gondii* em muitas áreas do mundo. Desde então, foi estimado que até um terço da população humana mundial tem sido exposta ao parasita (SABIN & FELDMAN, 1948).

T. gondii possui um ciclo de vida heteroxeno (TENTER et al., 2000), no qual os hospedeiros definitivos são os felídeos domésticos e silvestres, e os hospedeiros intermediários incluem uma grande variedade de espécies de animais de sangue quente, como aves, herbívoros e carnívoros terrestres (roedores, animais de caça, animais de produção e o homem), além de mamíferos marinhos (baleias e golfinhos) (DUBEY, 2002).

Três estágios de infecção constituem o ciclo de vida do *T. gondii*: taquizoítos, bradizoítos e oocistos (DUBEY & LINDSAY, 2006). Os taquizoítos são o estágio de multiplicação rápida do protozoário, responsáveis pela fase aguda da infecção. O estágio latente do parasito são os bradizoítos, esta fase marca o início da infecção crônica, na qual sua multiplicação diminui drasticamente e o parasito pode manter a infecção por um longo período (TENTER et al., 2000; DUBEY & LINDSAY, 2006). Já os oocistos são resultados da reprodução sexuada no intestino dos felídeos (hospedeiros definitvos), e liberados em suas fezes (HILL & DUBEY, 2002).

A fase de taquizoítos ocorre dentro de 8 a 12 dias pós-infecção, com a invasão de vários tecidos, em especial músculo cardíaco, parênquima pulmonar, e encéfalo (TENTER et al., 2000). Em função da sua capacidade de atravessar barreiras como a hematoencefálica e placentária, ele pode chegar ao sistema nervoso central, e ao feto nos casos de mulheres grávidas (TENTER et al., 2000). O taquizoíto multiplica-se assexuadamente por divisões binárias repetidas até a célula hospedeira se romper. Depois de um número desconhecido de divisões, *T gondii* taquizoítos dão origem a outro estágio chamado cisto tecidual (DUBEY & LINDSAY, 2006), que protege o parasita do sistema imune do hospedeiro (DUBEY & LINDSAY, 2006). A partir da formação destes cistos, que estão repletos de bradizoítos, começa a fase crônica da infecção (DUBEY et al., 1998a). Os oocistos são resultado da fusão dos gamentas masculino e feminino no intestino dos hospedeitos definitivos, e já tiveram sua presença no solo relatada por vários autores, sendo que são necessárias condições ideais de umidade, oxigenação e temperatura para que ocorra a esporulação, podendo então o oocisto permanecer infectante por até 18 meses (FRENKEL, 1971).

Os felídeos tem um papel fundamental no ciclo de vida do *T. gondii*, por serem os únicos hospedeiros definitivos, ou seja, são os únicos hospedeiros que apresentam a forma sexuada do parasita, e dessa maneira são os únicos que excretam oocistos (DUBEY, 2008). Os oocistos são essenciais para o ciclo de vida de *T. gondii*, e dessa forma, a chave da epidemiologia da toxoplasmose está nos felídeos. Estes oocistos tem potencial para contaminar o ambiente, e quando esporulados são fontes duradouras de infecção (TENTER et al.,2000). O fato de os felinos cobrirem suas fezes, aumenta ainda mais as condições de sobrevivência do oocisto no ambiente. A liberação desses oocistos nas fezes dos felinos começa 3 a 10 dias após a ingestão de bradizoítos, sendo necessários, mais de 18 dias quando ingerem oocistos (DUBEY, 1998a). Geralmente, apenas cerca de 1% dos gatos em uma população são encontrados liberando grande quantidade de oocistos, além disso essa forma do parasita é eliminada por um curto período (1 a 2 semanas) na vida do gato (DUBEY & BEATTIE, 1988). Ainda assim, a grande quantidade de oocistos liberados nesse período assegura a contaminação generalizada do ambiente (HILL & DUBEY, 2002).

A infecção nos humanos e demais hospedeiros intermediários pode se dar pelas seguintes vias: ingestão de oocistos esporulados no ambiente; ingestão de cistos teciduais viáveis, presentes na carne ou em subprodutos destas, sobretudo cruas e mal cozidas; infecção transplacentária; além de transplante e transfusão sanguínea (FRENKEL, 1971; ELMORE et al, 2010).

A soroprevalência mundial em humanos varia dependendo do ambiente e condições socioeconômicas, incluindo hábitos alimentares e práticas de saúde, além do nível geral de higiene, suscetibilidade do hospedeiro, localização geográfica (geolatitude) e umidade do solo (TENTER et al., 2000; FURTADO et al, 2011; FLEGR et al., 2014).

A toxoplasmose clínica é muito rara em indivíduos imunocompetentes (TENTER et al., 2000), quando ocorrem sintomas, eles são inespecíficos e incluem mal-estar, febre, dor de garganta e mialgia (FLEGR et al., 2014). No entanto, em alguns casos, principalmente em pacientes imunocomprometidos, pode levar a apresentações graves como coriorretinite, linfadenite, miocardite e polimiosite, e até chegar óbito (WEISS & DUBEY, 2009; FLEGR et al., 2014). Em pacientes HIV-positivos encontra-se comumente a toxoplasmose cerebral, muitas vezes como resultado do diagnóstico e tratamento inapropriados (PEREIRA-CHIOCCOLA, 2009).

Embora toxoplasmose possa ser uma condição benigna para a maioria das pessoas que adquirem essa infecção, seu diagnóstico é vital em mulheres grávidas (infecção congênita) por causa do risco para o feto (HIIL & DUBEY, 2002). A toxoplasmose congênita geralmente é causada por contaminação transplacentária do feto com *T. gondii* após infecção primária da mãe durante a gravidez. Nesse caso, o parasita pode causar vários graus de danos que variam de formas subclínicas até morte fetal. (BOUGHATTAS et al., 2011).

A toxoplasmose congênita clássica é caracterizada pela tétrade descrita por Sabin em 1942: coriorretinite, hidrocefalia ou microcefalia, calcificações cerebrais e alteração neurológica. Outras complicações características da toxoplasmose congênita são: abortamento, restrição de crescimento fetal, parto pré-termo, morte neonatal, alterações hematológicas e déficit de desenvolvimento neurocognitivo (DUFF, 2012). Além disso, foi encontrada associação entre toxoplasmose crônica e doenças como esquizofrenia, epilepsia e outras doenças neuropsiquiátricas (WEBSTER, 2013).

A manifestação clínica depende principalmente do estágio da gestação, e do tempo da infecção, sendo que a doença fetal é mais grave quando a contaminação ocorre no início da gravidez e menos grave no segundo e terceiro trimestre (COOK et al., 2000). A gravidade da doença também pode ser influenciada pela cepa parasitária (BOUGHATTAS et al., 2011). Este fato torna a toxoplasmose ocular congênita mais grave no Brasil em comparação com a Europa, provavelmente devido à infecção por genótipos mais virulentos do parasita predominante no Brasil (GILBERT et al., 2008).

Além dos humanos, a toxoplasmose também causa prejuízos significativos em outros hospedeiros intermediários. Nos animais de produção, a toxoplasmose tem como principal característica os distúrbios reprodutivos, e gera perdas econômicas, principalmente em ovinos, caprinos e suínos, que são consideradas as espécies mais susceptíveis (GARCIA et al., 1999). Diversas espécies de aves também são infectadas pelo parasito, entretanto, na maioria das vezes, a infecção cursa de forma assintomática (DUBEY, 2002). No que diz respeito aos suínos a importância da doença está relacionada principalmente às perdas reprodutivas e às implicações em saúde pública, uma vez que a ingestão de cistos em carne crua ou mal cozida é uma importante via de transmissão de *T. gondii* para o homem (FIALHO & ARAÚJO, 2003). Além disso, em alguns casos a ingestão de embutidos artesanais preparados com carne suína é uma via de transmissão importante, não só para os indivíduos que ingerem, mas também para aqueles que estão envolvidos com a sua preparação (SPALDING et al., 2005).

Os levantamentos soro-epidemiológicos têm relatado uma distribuição mundial de *T. gondii* em suínos, variando de menos de 1% a mais de 50% (PABLOS-TANARRO et al., 2018). No sul do Brasil, a frequência de anticorpos anti-*T.gondii* nessa espécie varia de 1,16% a 42,85% (FIALHO et al., 2009). As diferenças na soroprevalência dos suínos podem ser explicadas pela variação do risco e das fontes de infecção, diferenças nas condições de manejo em cada fazenda, diferentes regiões, e a presença ou não de gatos (GARCÍA-BOCANEGRA et al., 2010). Além disso, a fonte de água é uma importante via de contaminação, e o risco é maior quando se trata de água oriunda de poços (VILLARI et al. 2009). PABLOS-TANARRO et al., (2018), observaram em um estudo que suínos criados de forma extensiva apresentam maior soroprevalência para *T. gondii.* No entanto mesmo em criações semi-intensivas, e intensivas os suínos apresentaram anticorpos para o protozoário, sendo que as únicas exceções foram criações que recebiam apenas água tratada. Isso pode ser explicado pela facilidade com os suínos adquirem a infecção através de oocistos, já que apenas um oocisto é considerado suficiente para gerar infecção nessa espécie, fato que faz dos suínos uma ótima alternativa como sentinelas na infecção de *T. gondii* através da água (DUBEY & SU, 2009).

A grande disseminação de *T. gondii* ao redor do mundo se deve provavelmente a alta capacidade de adaptação nas diferentes regiões e facilidade de transmissão por diversas formas (DUBEY & SU, 2009). Nos últimos anos este protozoário tem se tornado um dos parasitas mais bem estudados devido à sua importância médica e veterinária, e sua adequação como modelo para biologia celular e estudos moleculares com organismo unicelular. (DUBEY, 2008). Embora este protozoário tenha uma distribuição mundial e

talvez a mais ampla gama de hospedeiros de qualquer parasita, *T. gondii* é a única espécie, no gênero Toxoplasma (DUBEY & LYNDSAY, 2006, DUBEY, 2008). No entanto, já se sabe que dentro da mesma espécie existem diferentes genótipos que vem sendo conhecidos e estudados nas últimas décadas (DUBEY, 2008). A diversidade genética de *T. gondii* segue uma típica distribuição geográfica. No hemisfério Norte do planeta existe uma população altamente clonal (DARDÉ, 2008), enquanto no hemisfério Sul predomina uma população não-clonal, ou seja, genótipos atípicos (SHWAB et al., 2014).

Os primeiros estudos de genotipagem sobre o *T. gondii*, realizados em um número limitado de cepas e isolados de laboratório principalmente da França ou dos Estados Unidos da América, levam à descrição de uma estrutura populacional clonal com três cepas principais, designados como tipo I, II e III (DARDÉ et al., 1988; HOWE & SIBLEY, 1995). Cepas do tipo I têm sido mais encontradas em humanos e assim, cepas tipo II e III predominam em animais (HOWE e SIBLEY, 1995).

Genótipos não pertencentes às três linhagens principais foram encontradas em continentes como América, África e Ásia, onde a estrutura da população *T. gondii* foi mais complexa, com maior diversidade genética do que inicialmente descrito. Esses genótipos recentemente descobertos, foram chamados de genótipos atípicos, exóticos, recombinantes ou não arquetípicos. A descrição desses genótipos atípicos oferece novas perspectivas na análise de virulência determinantes (DARDÉ, 2008).

Genótipos atípicos são formados através da recombinação genética dos genótipos já conhecidos, a qual ocorre no intestino dos hospedeiros definitivos. Eles são bastante descritos no Brasil (DUBEY & SU, 2009). Fato que está relacionado a vários fatores, como o clima tropical, fauna rica e diversas rotas de transmissão possíveis (FERREIRA et al., 2006). Quatro genótipos com ampla circulação e descritos em diferentes hospedeiros no Brasil foram propostos como Linhagens clonais brasileiras. Estas linhagens são denominadas BRI, BRII, BRIII e BRIV e são distintas das linhagens arquetípicas tipos I, II e III (PENA et al., 2008). Segundo Gilbert et al. (2008), no Brasil existe uma predominância de genótipos virulentos de *T. gondii* em comparação com a Europa. Os autores também relatam que a gravidade dos casos de toxoplasmose ocular é maior no Brasil do que na Europa (DARDÉ, 2008). Alguns estudos sugerem que cepas tipo I ou recombinantes do tipo I e III são mais prováveis de resultar em toxoplasmose ocular clínica (GRIGG et al., 2001).

O maior surto de toxoplasma descrito até o momento, no Brasil e no mundo ocorreu em Santa Maria, Rio Grande do Sus, em 2018. Segundo a pesquisa epidemiológica do Ministério da Saúde o pico de casos ocorreu entre março e abril do corrente ano. Houve

notificação de 2235 casos, dos quais 902 foram confirmados. Dentre estes 135 eram gestantes, e houveram três óbitos fetais, 9 abortos e 28 casos com toxoplasmose congênita. Suspeita-se que a água possa ser uma fonte de infecção devido ao grande número de pessoas infectadas, porém isso ainda precisa ser elucidado.

Tendo em vista a importância mundial da toxoplasmose, e o grande surto ocorrido no Brasil em 2018, este trabalho foi desenvolvido com intuito de: i) Isolar o protozoário *T. gondii* obtidos a partir de tecidos placentários de mulheres com toxoplasmose aguda infectadas durante o surto de toxoplasmose de Santa Maria, RS através de bioensaio em camundongos; ii) Determinar o genótipo presente nos isolados encontrados; iii) Investigar água como fonte de infecção do *T. gondii* na epidemia de Toxoplasmose em Santa Maria utilizando suínos como modelo experimental.

Esta tese está dividida em dois capítulos. Sendo o primeiro capítulo intitulado: "Isolation and molecular characterization of *Toxoplasma gondii* from placental tissues of pregnant women who received toxoplasmosis treatment during an outbreak in southern Brazil", e o segundo: "Contaminated water confirmed as source of infection by bioassay in an outbreak of toxoplasmosis in south Brazil".

2– CAPÍTULO 1 - Artigo científico

Este capítulo originou um artigo científico que foi publicado na revista: Plos One

Isolation and molecular characterization of *Toxoplasma gondii* from placental tissues of pregnant women who received toxoplasmosis treatment during an outbreak in southern Brazil

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Abstract

Toxoplasma gondii is a protozoan that has great genetic diversity and is prevalent worldwide. In 2018, an outbreak of toxoplasmosis occurred in Santa Maria, Brazil, which was considered the largest outbreak ever described in the world. This paper describes the isolation and molecular characterization of *Toxoplasma gondii* from the placenta of two pregnant women with acute toxoplasmosis who had live births and were receiving treatment for toxoplasmosis during the outbreak. For this, placental tissue samples from two patients underwent isolation by mice bioassay, conventional PCR and genotyping using PCR-RFLP with twelve markers. Both samples were positive in isolation in mice. The isolate was lethal to mice, suggesting high virulence. In addition, the samples were positive in conventional PCR and isolates submitted to PCR-RFLP genotyping presented an atypical genotype, which had never been described before. This research contributes to the elucidation of this great outbreak in Brazil.

Keywords: genotyping, bioassay, atypical genotype, acute toxoplasmosis, placenta.

Introduction

Toxoplasma gondii is a tissue cyst-forming protozoan capable of infecting warm-blooded animals, including humans, and is prevalent in most parts of the world [1]. It is one of the most studied coccidians due to its importance in animal and human health [2,3], as well as its suitability as a model in molecular studies [1].

Although *T. gondii* is the only species of the genus *Toxoplasma* [1,4], there are various genotypes [1]. The first genotyping studies of *T. gondii* led to the description of a clonal population structure with three main lines, designated as type I, II, and III [5, 6]. Currently, there are many known genotypes that do not belong to these three clonal lineages and are called atypical. They are generally considered more virulent [7]. They are formed by sexual reproduction between gametes of different genotypes which occur in the intestine of felids [1]. In Brazil, these atypical genotypes have been widely described [8]. There are studies showing the prevalence of *T. gondii* in animals and humans [9, 10, 11], and some studies have performed the isolation and genetic characterization from cases of congenital toxoplasmosis [12, 13].

T. gondii infection is generally asymptomatic in humans. However, it is potentially serious when acquired during pregnancy in immunocompetent individuals, as it carries the risk of fetal transmission [14]. When congenital toxoplasmosis occurs, the protozoan can cause lesions in the fetus that range from subclinical to neurological lesions, and even fetal death or miscarriage [15, 16]. The clinical manifestation varies according to the stage of pregnancy, infection time [17], and genotype [16]. The latter makes

congenital toxoplasmosis more serious in Brazil, due to infection with more virulent genotypes [18].

In 2018, an outbreak of toxoplasmosis occurred in Santa Maria, Rio Grande do Sul, with 809 confirmed cases. Of these, 114 were pregnant women who had 3 fetal deaths, 10 abortions, and 22 live births with congenital toxoplasmosis [19]. The objective of this study was to describe the isolation and molecular characterization of *T. gondii* from the placenta of two pregnant women with acute toxoplasmosis who delivered alive children and were receiving treatment for toxoplasmosis.

Materials and methods

Samples and clinical history

The placental tissue samples from two patients (patient 1 and patient 2) who delivered their babies at the University Hospital of Santa Maria during the toxoplasmosis outbreak in 2018, were referred to the Laboratory of Parasitic Diseases of the Federal University of Santa Maria (UFSM) for diagnostic purposes. Part of the tissue was intended for protozoan isolation, and another part for molecular tests.

According to their clinical history, both patients were positive for acute toxoplasmosis through the detection of anti-*T. gondii* IgM in Enzyme-linked Immunosorbent Assay (ELISA). The diagnosis of the two pregnant women occurred in the final trimester of gestation. Both patients received treatment and had alive children. The treatment protocol included a combination of Sulfadiazine, Pyrimethamine and Folinic Acid

(SPAF). Patient 1 started receiving treatment from 35 weeks of gestation, while patient 2 received treatment from the 36th week. Both patients received treatment for four weeks and thereafter gave birth.

Isolation through bioassay in mice

The placental tissues were subjected to peptic digestion individually, according to the technique described by Dubey, 1998 [20]. For digestion 50 g of placental tissue were used for peptic digestion. The digested material was resuspended in 5 mL of saline, and immediately after digestion, the mice were inoculated with 1 mL of the peptic digestion solution intraperitoneally. For each sample to be tested, four Swiss female mice were used, maintaining the fifth as a negative control. The animals were obtained from the Central Bioterium of the UFSM.

Mice were monitored daily for possible clinical signs of acute toxoplasmosis. When disease led to death, samples were collected from brain, heart, lung, and intraperitoneal fluid from all mice. The tissue was subjected to molecular analysis. Intraperitoneal fluid was also analyzed under a microscope with 40× magnification.

All procedures were approved by the Committee of Ethics in the Use of Animals of the Federal University of Santa Maria, under the protocol 7150250419.

DNA extraction

DNA extraction was performed from placental tissue samples from both patients, and from mouse tissues using Wizard Genomics DNA Purification kit (Promega), following

the manufacturer's instructions. In all cases, 20mg of tissues were used for DNA extraction.

Polymerase Chain Reaction (PCR)

The PCR amplification was performed with specific primers TOX4 (CGCTGCAGGGAGGAAGACGAAAGTTG) and TOX5 (CGCTGCAGACACAGTGCATCTGGATT) which amplified a 529 bp fragment from the *T. gondii* genome. The PCR was performed as described by Homan et al. 2000 [21]. As a positive control, tachyzoite DNA from the RH strain was used, and DNAase-free water was used as a negative control. A molecular marker of 100 bp (Brand - Ludwig Biotec) was used as the molecular standard size. Amplified products were visualized in the UV transilluminator after 1.5% agarose gel was stained with SYBR Safe DNA gel stain (Invitrogen).

Analysis of restriction fragment length polymorphism (RFLP)

The genotypic characterization was performed from mouse tissues that were positive for the TOX gene (529 bp) using twelve markers (SAG 1, 5' SAG2, 3' SAG2, Alt SAG2, SAG3, BTUB, GRA6, C22-8, C29-2, L358, PK1, APICO), according to the technique described by Su et al. 2010 [22]. To do so, the extracted DNA was amplified by nested-PCR (n-PCR) technique followed by PCR-RFLP analysis. DNA target sequences were first amplified by multiplex PCR, using external primers of all markers, followed by nested-PCR using internal primers for each marker. DNA samples from standard strains, RH, ME49 and VEG were used as controls for genotypes I, II, and III, respectively.

The polymorphism of each locus was analyzed by standard RFLP bands which was used to distinguish each strain type. For this, nested-PCR products were digested with appropriate restriction enzymes for each marker, according to Su et al. 2010 [22]. The controls were also digested using the same restriction enzymes. The negative control consisted of DNAase-free water. The results obtained were compared and classified according to the genotypes present in ToxoDB (http://toxodb.org/toxo/).

Results

T. gondii was isolated from the placental tissues of two patients. Within two weeks the mice presented signs indicative which acute toxoplasmosis such as apathy, bristly hair, photophobia, ascites, and death (Table 1). In addition, it was possible to identify a large amount of tachyzoites in the intraperitoneal fluid collected from the animals.

Table 1. Mouse bioassay.

Patients	*Number of inoculated mice*	*Number of positive mice in the bioassay*	*Mice life days*
1	4	4	11 – 13
2	4	4	12 – 15

As expected the samples of placental tissue as well as tissue samples from mice (brain, heart, and lung) submitted to conventional PCR showed an amplified product of 529

base pairs, confirming the presence of *T. gondii* DNA in the placenta of the evaluated patients, and in the bioassay mice.

In the genotypic characterization by the RFLP technique, the DNA analysis of *T. gondii* amplified from the tissues of mice submitted to the bioassay presented an atypical genotype, not yet described in ToxoDB. This result compared to other genotypes in table 2.

Table 2. Genotypic characterization of *T. gondii* isolates obtained from two patients during the Santa Maria toxoplasmosis outbreak compared to three other isolates [9,28].

Isolado	*Markers*											
	Sag1	5'Sag2	3'Sag2	Sag3	Gra6	BtuB	C22-8	C29-2	L358	PK1	Alt.SAG2	Apico
[a]*P. 1*	I	I	I	I	III	III	II	III	III	I	I	I
[a]*P. 2*	I	I	I	I	III	III	II	III	III	I	I	I
[b]*[28]*	I	I	I	I	III	III	II	III	III	I	I	III
[c]*BrI*	I	I	I	III	II	I	u-1	I	I	I	I	I
[c]*Br II*	I	I	I	III	III	III	I	III	I	II	II	III
[c]*Br III*	I	III	III	III	III	III	II	III	III	III	III	III
[c]*Br IV*	I	III	III	III	III	III	II	I	III	III	III	III

This is the Table 2 legend.

[a] Outbreak patients isolates

[b] Isolated recently described in Rio Grande do Sul

[c] Common isolates in Brazil

Discussion

Samples from animals have been widely used for isolation and genetic characterization of *T. gondii* [8]. However, in humans, this diagnosis is restricted [23], which makes it difficult to clarify the virulence of strains that infect humans and their genetic identity. In the present study conducted during the toxoplasmosis outbreak in Santa Maria, *T. gondii* was isolated from placental tissues of two patients with acute toxoplasmosis who received specific treatment in the third gestation trimester. This result is interesting since the success of *T. gondii* isolation is lower in cases of pregnant women receiving treatment [16, 24, 25]. The isolation of the protozoan species in the two patients in this study suggests that in both the cases the treatment protocol established did not prevent the protozoa from reaching the placenta, or that the congenital infection occurred even before the start of treatment.

In addition to confirming the presence of *T. gondii* in the placenta, the isolation in mice allows the virulence evaluation of genotypes present in the samples [26], since virulent strains usually cause acute infection with clinical signs in mice [3]. Signs which are characteristic of acute toxoplasmosis such as ascites, bristly hair, and photophobia were seen in all mice inoculated with placental samples from the patients in this study. In addition, the mice died within a maximum of 15 days, suggesting that the genotype present in the samples was quite virulent, although the amounts of inoculated tachyzoites can also interfere, since it was not estimated.

The genotype found in these samples was characterized as atypical, and is related to more severe forms of toxoplasmosis [27]. Atypical genotypes are not uncommon in Brazil, where the genetic diversity of *T. gondii* is large [8], but the genotype present in the samples of this research had not yet been described in ToxoDB. Recently, in

Southern Brazil, Vielmo et al., 2019 [28], also described an atypical genotype very similar to that found in the current study, capable of causing a chicken outbreak on a small rural property, suggesting that these two closely related genotypes are virulent to humans and animals. Although very similar to each other, both genotypes differ from the Brazilian clonal lineages, BrI, BrII, BrIII and BrIV [9], as shown in table 2.

In addition, it should be considered that the evaluated patients were diagnosed in the last gestational trimester and started receiving treatment after 30 weeks of gestation. This fact reaffirms the importance of diagnosis pregnant women through serology is essential for fast and efficient treatment to reduce cases of congenital toxoplasmosis [29, 30].

This study was funded by the Higher Education Personnel Improvement Coordination.

Conclusion

It was possible, by isolation and genotyping, to identify a new atypical *T. gondii* genotype, never described before, and with high virulence characteristics. This research contributes to elucidate the outbreak of toxoplasmosis in Santa Maria, Brazil.

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3 - CAPÍTULO 2 - Artigo científico

Este capítulo originou um artigo científico que foi submetido à revista: Transboundary and Emerging Diseases

Contaminated water confirmed as source of infection by bioassay in an outbreak of toxoplasmosis in south Brazil

Water as a source of toxoplasmosis infection in southern Brazil

The data that supports the findings of this study are available in the supplementary material of this article

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Abstract

The protozoan *Toxoplasma gondii* is a causative agent of toxoplasmosis, an important and widespread zoonotic disease. The transmission of this disease in humans includes ingestion of sporulated oocysts present in contaminated water or food. *T. gondii* oocysts are widely distributed and toxoplasmosis is considered a major food and waterborne pathogen worldwide, making drinking water containing sporulated *T. gondii* oocysts a major source of contamination for people. In the first half of 2018, an unprecedented outbreak of toxoplasmosis was reported in the city of Santa Maria, southern Brazil. The temporal and spatial distribution of the cases strongly suggested a waterborne infection. Thus, the aim of this study was to investigate a possible involvement of treated water as a source of the outbreak. For this, piglets received potentially contaminated water at will for 21 days and the infection was monitored by serology through IFAT and investigation of *T. gondii* DNA in tissues by PCR amplification of a 529 bp followed by mouse bioassays. All piglets receiving test water ad libitum for 21 days as well as positive controls seroconverted to *T. gondii*. *T. gondii* DNA was detected in 62.5% of the piglets that received test water. All mice inoculated with tissues from each positive piglet were PCR positive. These results strongly indicated the presence of viable oocysts in the test water administered to the animals during the study.

Keywords: Pigs, Mouse, Bioassay, Serology, PCR

Introduction

Toxoplasma gondii - a protozoa belonging to the subphylum Apicomplexa (Adl et al., 2012) and family Sarcocystidae – is the causal agent of toxoplasmosis, an important and widespread zoonotic disease. The protozoan life cycle involves domestic and wild felids as definitive hosts and a number of mammals, including humans, as intermediate hosts. Modes of *T.*

gondii transmission to human include: i. Ingestion of sporulated oocysts present in contaminated water or food; ii. Ingestion of bradizoites-containing cysts present in raw or under cooked animal meat and iii. Transplacentary transmission of tachizoites. Organ transplantation and blood transfusion have also been occasionally implicated in *T. gondii* transmission among people (Frenkel et al, 1970 ; Elmore et al., 2010). *T. gondii* oocysts are widely distributed and toxoplasmosis is considered a major water- and food-borne pathogen worldwide (Karanis et al., 2013). In Brazil, seroprevalence to *T. gondii* may reach up to 50% in children and 50-80% in pregnant women (Dubey et al., 2012; Bahia-Oliveira et al., 2003; Sobral et al., 2005; De Moura et al., 2006.

Water borne diseases are frequently reported in under- or developing countries due to the poor quality of drinking water (WHO/UNICEF, 2010). In this sense, drinking water containing sporulated oocysts of *T gondii* is considered a major source of contamination for people (Benenson, Takafuji, Lemon, Greenup & Sulzer, 1982; Jones & Dubey, 2010) and water-borne toxoplasmosis cases/outbreaks have been described in several countries (Benenson et al., 1982; Bowie et al., 1997) and also in Brazil (Bahia-Oliveira et al., 2003; De Moura et al., 2006).

In the first semester of 2018, an unprecedent outbreak of toxoplasmosis – probably the largest ever described - was reported in the city of Santa Maria, Southern Brazil, with the peak of notified cases between March and April. The outbreak investigation began when physicians reported an expressive increase in the number of cases of a clinical syndrome characterized by fever, myalgia, cefaleia, rash and mild gastrointestinal and respiratory signs. As of November 30th, 2018, 902 cases had been laboratory confirmed and 260 remained under investigation. Out of confirmed cases, 135 (15%) were pregnant women, from which three fetal deaths, nine abortions and 28 cases of congenital toxoplasmosis were diagnosed. A number of pregnancies, congenital toxoplasmosis, abortions and fetal deaths still remain under investigation (Minuzzi et al., 2020).

The temporal and spatial distribution of cases strongly suggested a water borne or, perhaps, a food borne infection through oocysts present in drinking water or, indirectly, in hydropony-growth vegetables (Minuzzi et al., 2020). Hence, the objective of this study was to investigate a possible involvement of treated drinking water as the source of the outbreak. For this, piglets were giving potentially contaminated water *at libitum* during 21 days and the infection was monitored by serology and investigation of *T. gondii* DNA in tissues, followed by bioassays in mice.

Material and methods

Experimental design was t he investigation of drinking water as the source of the outbreak involved the exposure of twelve specific pathogen free (SPF) piglets to water samples potentially contaminated. Water to be tested with *T. gondii* oocysts were giving *at libitum* to pigs eight during 21 days and the infection was monitored by serology and investigation of protozoan DNA by PCR in tissues of the piglets and mouse bioassay. Figure 1 indicates the flowchart of the activities carried out in this work. The project was approved by the Animal Ethics Committee under CEUA nº7150250419.Experimental animals and facilities.

Twelve specific pathogen free (SPF) piglets, approximately 30 days-old, free of T. gondii and specific antibodies, were purchased from Genetica Agroceres. The experiment was conducted at a private experimental facility (SAMITEC – Instituto de Soluções Analíticas, Microbiológicas e Tecnológicas Ltda, Santa Maria, RS). The piglets were housed in individual pens, in a room with controlled temperature (30 +- 1º C) and gas exsaustion. Prior to the experiment, the room floor and equipments were washed with water, neutral soap and disinfected with a 2% NAOH solution, followed by formaldeyde at 5%. All equipments were also washed and disinfected with a 2% formaldeyde solution followed by quaternary amonium. All procedures for disinfection followed the recommendations by Sesti et al. (1998). Rodent control

is carried out by a company qualified for this activity. The control of raw materials for the realization of mixtures of rations is carried out periodically by SAMITEC.

Inoculum and bioassay in piglets

The inoculum for the piglets consisted of water collected out of the following sources: i. water tank/tower of households of patients diagnosed positive for acute toxoplasmosis; ii. Water from reservoirs of the Water and Sewage Company (CORSAN) and iii. Samples of water collected from fountains. Positive controls (# consisted of two piglets inoculated intraperitoneally with 1.5×10^7 taquizoites of the RH strain produced in cultured cells; negative control piglets (#1 and #2) received ultra pure water (Milli-Q) water. Bioassay was done according to the description of Dubey JP, 2010.

The experimental animals (#3 to #10) received test water *at libitum* during 21 days (daily mean consumption = 3,5 liters/animal), and thereafter received ultra pure water (Milli-Q) up to the end of the study. Animals #11 and #12, positive controls received ultra pure water (Milli-Q) up to the end of the study. Animals # 3 to 6 received household/public water tanks and animals #7 to 10 received households/fountains water.

Clinical monitoring and sample collection

The experimental animals were monitored clinically in a daily basis and blood for serology was collected at days 0, 7, 15, 21 (during test water administration) and days 28 and 35.

Serology

Antibodies to *T. gondii* in the sera of inoculated piglets were investigated by fluorescent antibody assay (IFAT) essentially as described by (Garcia et al., 2000) Briefly, IFAT was performed in glass slides containing *T. gondii* RH tachizoites, by incubating two-fold serum

dilutions (starting at 1:16) as primary antibody for 1h followed by washings and incubation with a FITC-conjugated anti-swine IgG antibody (Sigma, Inc.). Serum samples positive and negative for *T. gondii* antibodies were used as positive and negative controls, respectively. Positive samples were diluted to 1:64 to determine the maximum reaction titer.

Tissue collection

Inoculated piglets were euthanized in a slaughterhouse at day 60 post-infection for tissue collection. Brain, pulmonary parenchyma and myocardium were obtained from the experimental animals for further analysis (PCR and mouse bioassays).

Mouse bioassays

The bioassays for *T. gondii* were performed in 18 six-weeks-old female Swiss mice. The mice were obtained from the Central Biotery of UFSM and maintained in appropriate cages, receiving food and water at libitum during the experiment. The mice were allocated into groups of three animals each and each group was inoculated IP with digested tissues obtained from the eight inoculated piglets according to methodology described by Dubey JP (2010).

Inoculated mice were monitored in a daily basis for clinical manifestations. Animals developing signs of acute toxoplasmosis (photophobies and ascites) were euthanized with Tipental being used at 100mg / kg (and those dying after acute disease) and submitted to peritoneal washing for investigation of tachyzoites. The mice that remained healthy were euthanized at day 45 pi for tissue collection. Fragments of the brain, lungs and hearts were submitted to DNA extraction for PCR. Brain fragments were also compressed between slides and coverslips for microscopic investigation of cysts.

DNA extraction and PCR

Three tissue fragments (approximately 25 mg each) from each brain, lung and heart of inoculated mice were submitted to DNA extraction independently. Total DNA was extracted with the *Wizard Genomics DNA purification kit* (Promega) according to the manufacturer's instructions.

The PCR amplification was performed with specific primers TOX4 (CGCTGCAGGGAGGAAGACGAAAGTTG) and TOX5 (CGCTGCAGACACAGTGCATCTGGATT) which amplified a 529 bp fragment from the *T. gondii* genome. The PCR was performed as described by Homan et al. (2000). DNA extracted from RH strain tachizoites and ultrapure water were used as positive and negative controls, respectively. A 100 pb ladder molecular weight marker (Marca – Ludwig Biotec) was used as the molecular mass standard. PCR products were visualized under UV light after electrophoresis in a 1.5% agarose gel and stained with *SyBr safe dna gel stain* (Invitrogen).

Results and discussion

All piglets (# 3 – #10) receiving test water *ad libitum* during 21 days – as well as the positive controls (#11 and 12) - seroconverted to *T. gondii* by day 21 pi as determined by IFAT assay. *T. gondii* antibodies were first detected in 6/8 (75%%) of the animals at day 7 pi and in 100% (8/8) at day 21 and reached a maximum titer of 32. No clinical signs were observed in experimental animals during the observation period. The negative control piglets (#1 and #2) remained seronegative. These results demonstrate a serologic response of piglets to *T. gondii* antigens upon continuous ingestion of test water. As the negative controls (receiving ultra pure Milli-Q water) remained seronegative, these data indicate that the test water contained infective forms of the protozoa. Figure 2 indicates the kinetics of the IgG Anti-Toxoplasma gondii antibody in the experiment. In addition, these results also showed that piglets may be adequate for bioassays designed to investigate water contamination by *T. gondii.* As to confirm whether the seroconversion resulted from infection by infective forms of *T. gondii*, the piglets receiving

test water were euthanized at day 60 for investigation of the presence of protozoan DNA in tissues (Table 1). *T. gondii* DNA – as determined by PCR amplification of a 529 bp - was detected in at least one organ/tissue of five out of eight (62.5%) piglets receiving test water. Piglet #6 was PCR positive in brain, lungs and heart whereas the other animals harbored *T. gondii* DNA in heart and lungs (# 3 and 8) or only in the lungs (#4 and 10). The lung was the organ/tissue more consistently positive (5/5) whereas the brain was found positive only in one animal (#6). The uneven distribution of the protozoan DNA in the tested organs/tissues may be explained by the acute character of the infection. All three organs of the positive controls (#11 and 12) were PCR positive. No protozoan DNA was detected in tissues of 3/8 inoculated piglets nor in tissues of the negative controls (#1 and 2) (Table 1). These results indicate that the piglets receiving test water were actively infected by the protozoan, likely due to the presence of viable oocysts in the water. Taken together with the results of serology, these results strongly indicated the presence of viable oocysts in the test water administered to the animals during the study.

To investigate the presence of infective forms of *T. gondii* in tissues of piglets receiving test water, mice were inoculated with homogenates of tissues of these piglets. Upon IP inoculation, *T. gondii* DNA was detected in tissues of mice receiving material from 5 out of 8 piglets (ID #3, #4, #6, #8 #10). These same piglets have been tested positive for *T. gondii* DNA in their tissues (Table 1). All three mice inoculated with tissues from each positive piglet were PCR positive. Tissues from mice inoculated with homogenates of control piglets – in addition to mice inoculated with homogenates of piglets #5, #7 and #9 - were PCR negative. These data demonstrate the infectivity of the protozoa in swine tissues since the infection could be experimentally transmitted to mice as demonstrated by DNA detection in mouse tissues. Piglets 5, 7 and 9 may not have amplified the DNA in the PCR because the DNA is not present in the tissue portion analyzed or its replication was not enough.

The mouse bioassay has been shown to be a valuable tool for the diagnosis of toxoplasmosis yet it presents a low sensitivity to detect the protozoa in water samples due to the usually low concentration of oocysts and/or their low viability in water (Isaac-Renton et al.,

1998; Dubey JP 2010). Thus, the development of bioassays in other animal species would be very useful to detect *T. gondii* oocysts and to determine their infectivity as well (Wells et al., 2015). In this sense, our results demonstrate that piglets represent an attractive model for such assays due to their high susceptibility to *T. gondii* (Villena et al., 2002). The high volume of water consumption by pigs would certainly contribute for detection of low concentration of oocysts in the water and increase the sensitivity of the assay.

The detection of *T gondii* DNA in tissues associated with seroconversion of piglets receiving test water represent important findings since they associate the transmission to the suspected source of infection during the outbreak. Epidemiological data gathered during and after the outbreak have already pointed out for this association: i. A high number of confirmed cases during a limited period of time (point epidemics) suggested a common source of infection; ii. The spatial distribution of cases reinforced this hypothesis; iii. A case control study indicated the consumption of tap water and washed vegetables as risk factors for the infection. Our findings reinforce the hypothesis as the drinking water as the major source of the Santa Maria's outbreak.

The water samples collected for this study were collected during the outbreak. The water collection was carried out for 45 days, every two days, and the collections were carried out in the same places. The risk of *T. gondii* infection through drinking of contaminated water has been described previously (Jones & Dubey, 2010; Vieira et al., 2015), including in outbreaks of toxoplasmosis in Brazil (Bahia -Oliveira et al, 2003) and elsewhere (Karanis et al., 2013). In this sense, *T. gondii* has been repeatedly detected by active surveillance in water samples in Brazil (Galvani et al., 2019) and elsewhere (Gallas-Lindemann et al., 2013; Villena et al., 2004; Mahmoudi et al., 2015; Weels et al., 2015). Contamination of water with oocysts probably occurs during floods occurring mainly in rainy seasons (Bahia-Oliveira et al., 2003; Galvani et al., 2019).

T. gondii oocysts remain viable in the environment for long periods (Dubey JP, 2010) and can resist to several chemical processes routinely used for decontamination of w ater

supplies (Dubey JP, 2010). Water contamination and *T. gondii* infection has been associated with consumption of non-filtered water in Brazil (Bahia-Oliveira et al., 2003; Heukelbach et al., 2007). In this sense, water floculation and filtration are usually effective in removing *T. gondii* contamination (Jones & Dubey, 2010; Galvani et al., 2019). Regardless, decontamination may not be always effective since Villena et al., (2002) reported the oocyst resistance to disinfection processes. As a consequence, *T. gondii* infective forms have been repeatedly detected in treated water (Bahia-Oliveira et al., 2003; Villena et al., 2004; Sroka et al., 2006; e Hernandez-Cortazar et al., 2017). In any case, Karanis et al., (2013) suggested that the epidemiological impact of *T. gondii* transmission through sporulated oocysts in water is underestimated.

The Santa Maria outbreak reported herein (809 confirmed cases) overcomes a previous outbreak described in Santa Isabel do Ivaí (Almeida, 2011) in 2002 in which 426 people were infected, likely by ingestion of oocysts-containing water from a public reservoir contaminated with feline feces (Bahia-Oliveira et al., 2003; De Moura et al., 2006). In Santa Maria, of the 809 cases, 114 were pregnant women. There were 3 fetal deaths, 10 abortions and 22 born with congenital toxoplasmosis (Minuzzi et al., 2020). Of these, patients had fever, headache and / or myalgia. They also had lymphadenopathy, weakness, arthralgia or impaired vision (Arquilla et al., 2019).

In sum, the results reported herein strongly support that the water administered to the piglets contained infective forms of *T. gondii*, leading to infection and seroconversion. These findings were confirmed by the mouse bioassay. These results also call attention for the need of continuous monitoring of *T. gondii* contamination in public water treatment plants.

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Conflict of interest

I declare that there are no conflicts of interest.

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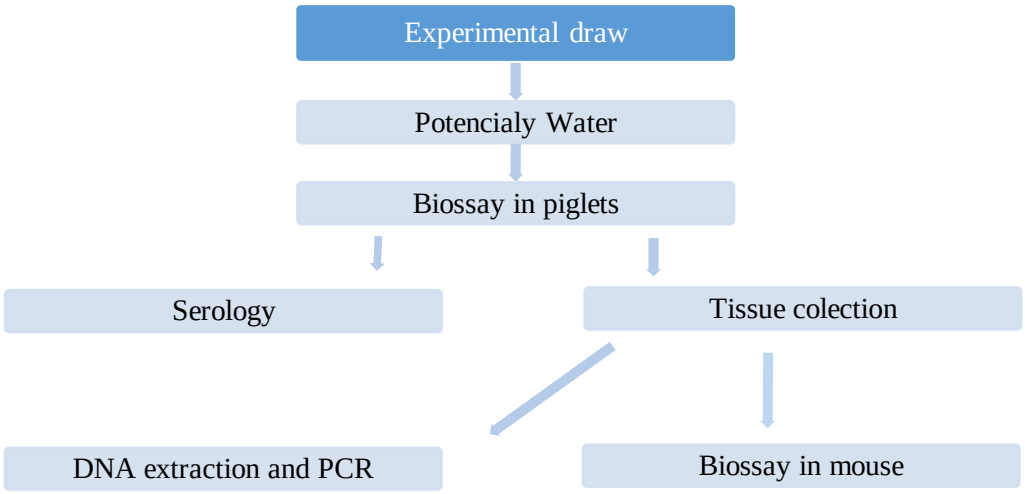

FIGURE 1 Indicates the flowchart of the activities

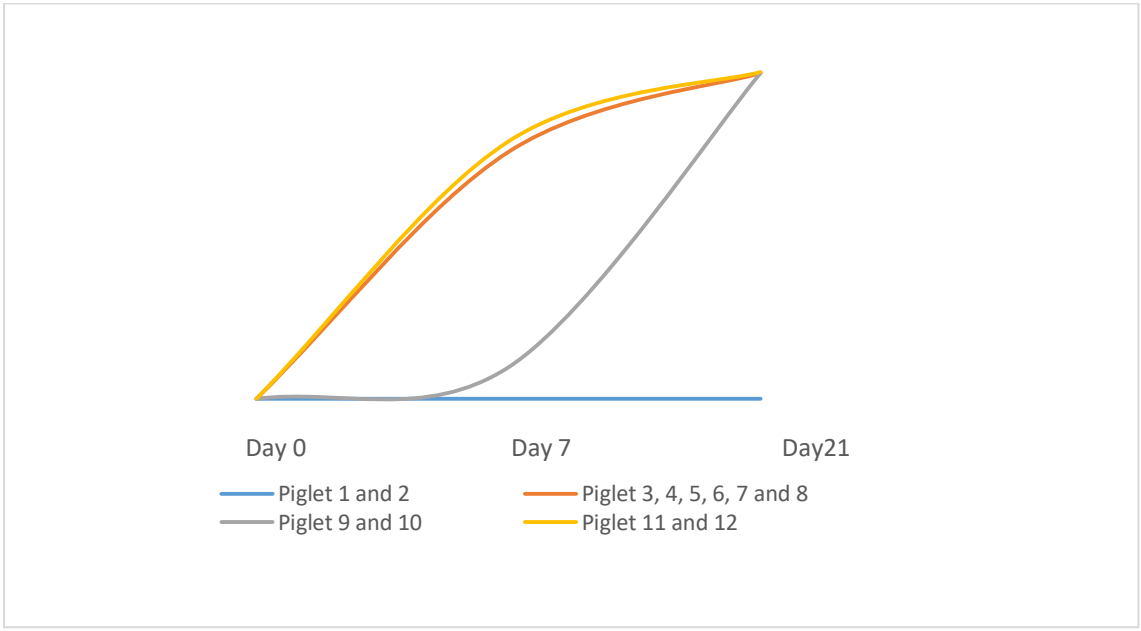

FIGURE 2 IgG Antibody Anti- *Toxoplasma gondii* Kinetics

Animal/tissue	PCR for *Toxoplasma gondii* gene																	
Piglet ID	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**#1***		**#2***		**#11****			**#12****		
Brain	-	-	-	+	-	-	-	-	-		-		+			+		
Heart	+	-	-	+	-	+	-	-	-		-		+			+		
Lung	+	+	-	+	-	+	-	+	-		-		+			+		
Mouse ID	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**#1***	**#1***	**#2***	**#2***	**#11****	**#11****	**#11****	**#12****	**#12****	**#12****
Brain	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+
Heart	+	+	-	+	-	-	-	+	-	-	-	-	+	+	+	+	+	+
Lung	+	+	-	+	-	+	-	+	-	-	-	-	+	+	+	+	+	+

*- Negative control

**- Positive control

TABLE 1 Investigation of the presence of protozoan DNA in tissues

4 – DISCUSSÃO GERAL

Em um primeiro momento foi realizado o bioensaio em camundongos a partir dos tecidos placentários de pacientes com sorologia positiva para *T. gondii*. Essa técnica permitiu confirmar a presença do protozoário nas placentas no momento que os camundongos apresentaram sinais clínicos após a inoculação, além de permitir-nos considerar que a cepa apresentava alta virulência. A partir disso foi possível realizar a genotipagem dos isolados através da RFLP, e então identificou-se a presença de um genótipo atípico de *T. gondii*, o qual até então não havia sido descrito. Consideramos que esse fato pode ter sido um fator de grande influência para a ocorrência do surto em Santa Maria.

No capítulo 2, os suínos submetidos ao bioensaio que receberam água de reservatórios de Santa Maria, sob suspeita de contaminação com oocistos de *T. gondii*, passaram a apresentar anticorpos para o protozoário. O que sugere fortemente a ocorrência da infecção através da ingestão de água contaminada com oocistos. Posteriormente camundongos foram submetidos a um bioensaio no qual foram inoculados com tecidos destes suínos. Embora estes camundongos não tenham apresentado sinais clínicos, houveram resultados positivos nos tecidos submetidos à PCR, fortalecendo assim a hipótese de que havia ocorrido a infecção nos suínos.

5 – CONCLUSÃO

Nas amostras das pacientes infectadas durante o surto de toxoplasmose em Santa Maria foi detectado um genótipo atípico e virulento. Já nas amostras provenientes do bioensaio com água não foi possível realizar a genotipagem. A comparação entre os dois genótipos seria extremamente relevante no esclarecimento do surto, porém ainda assim podemos indicar fortemente a infecção através da água contaminada com oocistos.

A partir dos dois estudos apresentados nessa tese acreditamos que foi possível colaborar com a elucidação deste grande surto de Toxoplasmose identificando o genótipo presente no surto, bem como sugerindo fortemente uma provável fonte de infecção.

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