Enrofloxacina e toltrazuril são capazes de diminuir a transmissão transplacentária de *Toxoplasma gondii* em roedores *Calomys callosus*

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ANNA BEATRIZ MACHADO VERCESI

ENROFLOXACINA E TOLTRAZURIL SÃO CAPAZES DE DIMINUIR A TRANSMISSÃO DE *TOXOPLASMA GONDII* EM ROEDORES *CALOMYS CALLOSUS*

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RESUMO

Introdução: O tratamento padrão da toxoplasmose congênita é uma associação de ácido folínico em conjunto com sulfadiazina e pirimetamina, drogas que causam efeitos teratogênicos quando administradas durante a gestação além de efeitos adversos para a gestante. A busca por tratamentos alternativos é de extrema importância. Estudos realizados anteriormente demonstraram que toltrazuril e enrofloxacina foram capazes de reduzir a infecção por *Toxoplasma gondii* em fibroblasto humano (linhagem HFF) e em roedores *Calomys callosus.* Portanto, o objetivo deste estudo foi avaliar o efeito dos fármacos na transmissão congênita de *T. gondii* em roedores *C. callosus.*

Metodologia: Fêmeas de roedores *C. callosus*, após detecção da rolha vaginal, foram infectadas com cistos da cepa ME-49 de *T. gondii* e divididas em grupos experimentais. Cada um dos grupos recebeu ou não tratamento (enrofloxacina, toltrazuril ou associação) e no 19º dia as fêmeas de todos os grupos foram eutanasiadas para coleta de amostras. As amostras de encéfalo foram analisadas por imuno-histoquímica para contagem de cistos e as demais amostras foram analisadas por qPCR para avaliação da carga parasitária.

Resultados: Houve redução significativa na transmissão transplacentária do parasito nos grupos experimentais que foram tratados com enrofloxacina ou toltrazuril, assim como associação, quando comparados ao grupo controle positivo (fêmeas somente infectadas). Ambos os medicamentos apresentaram também menor taxa de aborto quando comparados ao tratamento com associação. Em adição, houve diferença estatística significativa no número de cistos teciduais nas amostras de encéfalo materno apenas entre o grupo experimental tratado com enrofloxacina e o grupo tratado com associação.

Discussão: Ambos os fármacos propostos apresentaram efeito positivo no controle da transmissão transplacentária do parasito, entretanto a taxa de aborto observada deve ser estudada, podendo estar relacionada a produção de citocinas próinflamatórias devido a infecção por *T. gondii.*

Conclusão: Enrofloxacina e toltrazuril foram capazes de reduzir a transmissão transplacentária do parasito em roedores *C. callosus*.

Palavras-chave**:** *Toxoplasma gondii*; *Calomys callosus;* tratamento; enrofloxacina; toltrazuril; toxoplasmose congênita.

1. Introdução

Toxoplasma gondii é um protozoário do filo Apicomplexa, agente etiológico da toxoplasmose. A infecção por *T. gondii* acomete inúmeras espécies de vertebrados homeotérmicos, incluindo o homem [1, 2]. Estima-se que atualmente cerca de um terço da população global esteja infectada cronicamente pelo parasito [3, 4], e, embora a doença seja conhecida, surtos de infecção por *T. gondii* não são incomuns, sendo uma das infecções parasitárias de maior ocorrência [3].

A toxoplasmose pode ser assintomática, no caso da maioria dos indivíduos imunocompetentes, ou causar manifestações clínicas que variam desde uma doença leve auto-limitada até corioretinite, meningoencefalite e infecção congênita [3, 4]. A toxoplasmose congênita representa uma das formas mais graves da doença, podendo causar em recém nascidos cegueira, atraso no desenvolvimento e problemas neurológicos tal como a epilepsia. [5 - 7]. Nos EUA, cerca de 400 à 4000 crianças são diagnosticadas com toxoplasmose congênita anualmente [8], e a infecção por *T. gondii* também está relacionada com casos de aborto [7, 8]. O diagnóstico precoce e o uso de medicamentos podem resultar no decréscimo da taxa de transmissão para o feto e da gravidade nos casos de infecção [4].

O tratamento padrão para toxoplasmose congênita é baseado na administração de sulfadiazina e pirimetamina com suplementação de ácido folínico. Estes medicamentos, quando utilizados em associação, apresentam efeito na diminuição da replicação de *T. gondii* [9, 10], entretanto o uso da pirimetamina durante o primeiro trimestre de gestação não é indicado por ser um medicamento que apresenta efeito teratogênico, podendo ainda prejudicar a atividade da medula óssea [11 - 13]. Dessa maneira, quando ocorre infecção materna no primeiro trimestre de gestação e não há evidências de infecção fetal, o tratamento durante a fase aguda da infecção é realizado com

espiramicina para reduzir o risco de transmissão vertical de *T. gondii* [13 - 15]. Essa droga não oferece risco ao feto, uma vez que não atravessa a barreira placentária [13 - 15].

Devido à toxicidade e aos efeitos adversos, tanto para a gestante quanto para o feto, causados pelos medicamentos utilizados no tratamento padrão, é importante a busca por novos tratamentos igualmente ou mais eficazes, que consigam reduzir a transmissão transplacentária do parasito e, ao mesmo tempo, diminuir os efeitos indesejados que os fármacos causam.

Enrofloxacina é um antibiótico da classe das fluoroquinilonas, muito utilizado na medicina veterinária devido ao seu amplo espectro de ação contra bactérias gram-negativas e gram-positivas [16, 17]. O mecanismo de ação de enrofloxacina é caracterizado por inibir a DNA girase, uma enzima que participa da replicação do DNA, não permitindo a formação de forquilhas de replicação e transcrição, o que leva a destruição da bactéria [17 - 20]. Estudos recentes demonstraram que este medicamento, além da atividade antibacteriana, também possui efeito positivo no controle de protozoários como, por exemplo, *Trypanosoma congolense* [21] e *Neospora caninum* [22], um parasito também pertencente ao filo Apicomplexa que é altamente semelhante ao *T. gondii*. Além disso, estudos prévios realizados pelo nosso grupo de pesquisa demonstraram que a enrofloxacina teve efeito positivo no controle da invasão e replicação de *T. gondii* em células fibroblasto (linhagem HFF), células BeWo e explantes de vilos placentários humanos em comparação ao tratamento padrão da toxoplasmose congênita, bem como foi capaz de diminuir o número total de parasito e a imunopatologia em encéfalos de *C. callosus* [17, 29].

Toltrazuril é um medicamento também amplamente utilizado na medicina veterinária, indicado no tratamento de coccidioses [23, 24]. Estudos recentes mostraram

que toltrazuril é altamente eficaz para o controle da infecção por *T. gondii*, por agir nas diferentes formas evolutivas do parasito [25, 26]. Além disso, esse medicamento foi capaz de induzir dano mitocondrial e inibir a divisão celular em taquizoítas em células intestinais do hospedeiro definitivo [26, 27]. A infecção por outros parasitos do filo Apicomplexa, como *Eimeria* sp, *Isospora* sp e *N. caninum* também foi controlada utilizando toltrazuril [25, 26, 28].

Recentemente, nosso grupo mostrou que tanto a enrofloxacina quanto o toltrazuril foram capazes de controlar o parasitismo por *T. gondii* em modelo *in vitro* utilizando células BeWo e explantes de vilos de placenta humana de terceiro trimestre, sem causar efeito citotóxico [29]. Sendo assim, o objetivo do presente estudo foi avaliar o efeito do tratamento com enrofloxacina e toltrazuril na transmissão congênita de *T. gondii* em roedores *C. callosus*.

2. Materiais e métodos

2.1. Animais

Machos e fêmeas de roedores *C. callosus* (linhagem Canabrava) foram mantidos em criatório para pequenos animais no Laboratório de Histologia e Embriologia da Universidade Federal de Uberlândia. Os animais foram mantidos com ciclos de 12 horas claro e 12 horas escuro, em condições livres de patógenos e dieta *ad libitum*, que é composta por ração apropriada e água filtrada. Os procedimentos experimentais de manejo e experimentação com estes animais foram conduzidos em acordo com os princípios éticos adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA, 1991) e pelo Comitê de Ética no Uso Animal (CEUA) da Universidade Federal de Uberlândia (número de aprovação pelo Comitê de Ética 144/13).

2.2. Manutenção da cepa ME-49 de T. gondii

O protocolo para manutenção da cepa ME-49 de *T. gondii* foi realizado de acordo com Barbosa e colaboradores (2007). Brevemente, machos previamente infectados por *T. gondii* foram anestesiados com solução de xilazina 2% (Syntec, Hortolândia, SP, Brasil) e cetamina 10% (Syntec) e eutanasiados por deslocamento cervical. Os encéfalos dos animais foram removidos em condições assépticas, lavados, macerados e homogeneizados em solução de PBS estéril (pH 7.0) por aspiração utilizando uma seringa e agulha. Em seguida, foi realizada centrifugação a 1000*g* durante 10 minutos em temperatura ambiente. Alíquotas de 20µL dessa solução foram observadas sob lâmina e lâminula em microscópio de luz para contagem de cistos teciduais contendo bradizoítas no interior. Feita a contagem dos cistos, um volume de 100 µL de PBS estéril contendo 20 cistos teciduais foi preparado para inoculação via oral de novos machos de *C. callosus* com a finalidade de manutenção da cepa. Esse mesmo volume de PBS estéril contendo 20 cistos teciduais foi utilizado para inoculação via oral das fêmeas dos grupos experimentais. A inoculação via oral foi realizada com a utilização de uma seringa adaptada com cânula gástrica, com a finalidade de garantir que ocorra a infecção adequada dos animais [31]. No total, 15 machos de *C. callosus* foram utilizados para a manutenção da cepa ME-49 de *T. gondii*.

2.3. Grupos experimentais

Para a realização dos diferentes procedimentos experimentais, __ fêmeas virgens de *C. callosus* com idade entre 2 e 3 meses e peso corporal em torno de 30g foram colocadas para acasalar com machos, na proporção de duas fêmeas para cada macho. Diariamente foi verificada a presença de rolha vaginal. A data em que a presença da rolha vaginal foi detectada é considerada como o primeiro dia de gestação. Após a detecção da rolha vaginal, as fêmeas foram infectadas oralmente com 20 cistos da cepa

ME-49 de *T. gondii*. Todas as fêmeas infectadas, e tratadas ou não, foram divididas num total de quatro grupos experimentais (grupos I, II, III e IV). Além dos grupos com animais infectados, algumas fêmeas foram colocadas para acasalar e após a detecção da rolha vaginal, estas foram apenas tratadas ou não com enrofloxacina ou toltrazuril (grupos V, VI e VII). A eutanásia foi realizada no 19º dia de gestação/infecção.

As fêmeas do grupo experimental I prenhes e infectadas foram tratadas com enrofloxacina (Bayer Healthcare, São Paulo, SP, Brasil) (16,7 mg/kg) de acordo com Gottstein e colaboradores (2005). O tratamento foi administrado na água de beber, na concentração de 10mg de enrofloxacina em 100 mL de água durante sete dias consecutivos a partir do 10º dia de gestação/infecção.

As fêmeas do grupo experimental II, prenhes e infectadas, a partir do 10º dia de gestação/infecção foram tratadas com toltrazuril (Bayer Healthcare, São Paulo, SP, Brasil) por um período de sete dias consecutivos. Para o tratamento foi feita a diluição de 31,5mg de toltrazuril num volume de 100 mL de água, tendo uma media diária de consumo correspondente a 5mL para uma fêmea de 30g [22].

As fêmeas do grupo experimental III, prenhes e infectadas, foram tratadas com a combinação de sulfadiazina (Laboratório Catarinense S.A., Joinville, SC, Brasil) (1,5 mg a cada 12 horas), pirimetamina (Farmoquímica S/A., Rio de Janeiro, RJ, Brasil) (0,025 mg a cada 12 horas) e ácido folínico (Neo Química S/A., Rio de Janeiro, Brasil) (0,0075 mg a cada 24 horas) (SPFA), de acordo com Costa e colaboradores (2009). As doses administradas foram baseadas nas doses recomendadas para mulheres gestantes [32, 33], porém adaptadas para a massa corporal de *C. callosus* [34]. A combinação SPFA foi diluída em 0,5mL de PBS estéril e administrada por via oral usando seringa adaptada com cânula intragástrica para garantir o tratamento adequado das fêmeas. Este

tratamento foi iniciado no 14º dia de gestação/infecção, sendo realizado diariamente até o dia da eutanásia [34].

As fêmeas do grupo experimental IV, prenhes e infectadas, não receberam qualquer tipo de tratamento (grupo controle positivo).

As fêmeas do grupo V e VI não foram infectadas e foram tratadas seguindo o mesmo regime de tratamento das fêmeas I e II, respectivamente. Já as fêmeas do grupo VII representa o controle de fêmeas grávidas, sem infecção e tratamento (grupo controle negativo).

A eutanásia das fêmeas de todos os grupos foi realizada no 19º dia de gestação/infecção. Amostras de sangue foram coletadas do plexo orbital e as fêmeas foram laparatomizadas para a coleta do encéfalo materno, placenta e fetos. O número de fetos e os pontos de reabsorção foram avaliados. Algumas amostras de feto e placenta foram processadas para PCR em tempo real, e outras, assim como os encéfalos maternos, foram fixadas em formol tamponado 10% sendo posteriormente submetidos ao processamento em parafina para realização da análise em microscopia de luz.

2.4. PCR em tempo real

A reação em cadeia da polimerase em tempo real (qPCR) foi utilizada para avaliar a quantidade de DNA de *T. gondii* nas amostras de placenta e fetos que foram coletadas das fêmeas de quatro grupos experimentais descritos (grupo I, II, III e IV). DNA total foi extraído a partir de 20 mg de tecido usando *Wizard Genomic DNA Purification Kit* (PROMEGA), de acordo com as instruções do fabricante. Todas as reações foram realizadas utilizando o sistema de detecção SYBR Green PCR Master Mix (Applied Biosystems, Inc.) com 10µL de volume em cada reação (25ng de DNA "template"; 2,5pmol de cada primer e 5µL de SYBR Green). Os ciclos foram realizados de acordo

com as instruções do fabricante. Os primers utilizados para amplificação do DNA são correspondentes ao gene B1 de *T. gondii* (amplicon 50pb): forward, 5'-TTCAAGCAGCGTATTGTCGA-3', e reverse, 5'- ATGAACGGATGCAGTTCCT-3'. Adicionalmente, os ensaios de quantificação da carga parasitária nos tecidos foram realizados por uma máquina de PCR em tempo real, *StepOnePlus™ Real Time PCR System (*Applied Biosystems, USA).

Os dados foram analisados usando o software *Data Analysis and Technical Graphics* (Origin version 6.0; Microcal Software, Inc., Northampton, MA) [35].

2.5. Análise morfógica e imuno-histoquímica para detecção de T. gondii

Cortes histológicos de placentas, fetos e encéfalo das fêmeas de todos os grupos experimentais foram analisados em microscopia de luz. As amostras foram fixadas em formol a 10%, desidratadas em concentrações crescentes de álcool e incluídas em parafina. Utilizando um micrótomo foram obtidos os cortes histológicos com 4 μm de espessura e estes foram depositados em lâminas de vidro para a análise morfológica e imuno-histoquímica [30, 36].

Para a reação de imuno-histoquimica foi realizado primeiramente a reidratação dos cortes, onde eles passaram por uma bateria de xilol, seguida de uma bateria decrescente de álcoois. Posteriormente, foi realizado o resgate antigênico com tampão citrato e o bloqueio da fosfatase endógena, utilizando solução de ácido acético 5%, seguido do bloqueio dos sítios inespecíficos com soro de cabra 2,5%. Após essa etapa, as lâminas foram incubadas *overnight* a 4ºC, em seguida foi adicionado soro de *C. callosus* anti *T. gondii* (1:100) por 1 hora a 37ºC, e depois o anticorpo antimouse biotinilado (1:600) (Sigma-Aldrich, St. Louis, MO, USA). A reação foi amplificada utilizando o sistema avidina-biotina-fosfatase alcalina (ABC kit, PK-4000; Vector Laboratories, Inc.,

Burlingame, CA, USA) e desenvolvida com fast red-naftol (Sigma). As lâminas foram contra coradas utilizando hematoxilina de Harris e analisadas em microscópio de luz. Para determinação do número total de parasitos foi realizada a contagem do número de marcações específicas para *T. gondii* em cada corte.

As lâminas utilizadas para imuno-histoquímica foram escaneadas em aumento 40x e 100x para obtenção de imagens, utilizando o sistema VS120 da Olympus em um microscópio BX 61. O processamento e análise dessas imagens foram feitos através do programa Fiji [37].

2.6. Análise estatística

A análise foi feita considerando todos os dados como média e erro padrão, utilizando o programa GraphPad *Prisma versão 5.0* (GraphPad Software, Inc., San Diego, EUA). Os dados foram analisados pelo teste Kruskal-Wallis e pós teste de Dunns. As diferenças estatísticas foram consideradas significantes quando $P<0,05$.

3. Resultados

3.1. Sucesso gestacional

Com o objetivo de avaliar se os tratamentos com enrofloxacina e toltrazuril causariam efeitos teratogênicos, fêmeas *C. callosus* que apresentaram rolha vaginal, infectadas ou não, foram submetidas aos tratamentos com os dois medicamentos. Nossos dados indicam que fêmeas não infectadas e tratadas com enrofloxacina ou toltrazuril apresentaram 100% de sucesso gestacional (grupos V e VI), ou seja, todas as fêmeas apresentaram fetos viáveis (Tabela 1). Esses resultados são semelhantes aos dados obtidos do grupo de fêmeas grávidas não submetidas à infecção e tratamento, o qual apresentou 100% de sucesso gestacional (grupo VII). Entretanto, nos grupos os

quais as fêmeas foram infectadas e tratadas com enrofloxacina e toltrazuril (grupos I e II) podemos ver que ocorreu uma diminuição do sucesso gestacional (42,8%), ou seja, foi observada maior taxa de aborto se comparado com o grupo controle positivo (100%) e o grupo não infectado e tratado (100%). Além disso, o grupo III, infectado e tratado com associação (pirimetamina, sulfadiazina e ácido folínico), apresentou baixo sucesso gestacional (40%). Por fim, ao avaliarmos o número de fetos por fêmea com sucesso gestacional observamos que todos os grupos apresentaram uma média semelhante, em torno de 5 ou 4 fetos por fêmea.

3.2. Enrofloxacina e toltrazuril são capazes de diminuir a transmissão congênita de T. gondii em C. callosus

A análise da carga parasitária de *T. gondii* por qPCR nos fetos mostrou que os grupos experimentais que receberam os tratamentos com enrofloxacina, toltrazuril ou associação (grupos I, II e III) apresentaram uma diminuição significativa da taxa de DNA de *T. gondii*, quando comparados ao grupo IV (somente infectado e não tratado), (P<0,05; Fig. 1A), porém, não houve diferença estatística na taxa de parasitismo nos fetos entre os diferentes tratamentos (Fig. 1A).

Com relação a carga parasitária em amostras de placentas, nossos dados demostraram que houve diminuição significativa nos grupos experimentais que receberam os tratamentos com toltrazuril ou associação (grupos II e III) quando comparados ao grupo apenas infectado (grupo IV) (P<0,05; Fig. 1B). Entretanto, não houve diferença significativa entre o tratamento com enrofloxacina e o grupo controle positivo. Quando comparado os diferentes tratamentos, associação foi mais eficiente no controle do parasitismo nas amostras de placenta do que o tratamento com enrofloxacina (P<0,05; Fig. 1B).

Fotomicrografias representativas são evidenciadas nas Fig. 1C-J, onde é possível evidenciar um maior número de parasitos nos cortes dos fetos e placentas, respectivamente, dos grupos controle positivo (Fig 1C e 1G), em comparação com os fetos e placentas dos grupos que receberam os tratamentos com toltrazuril (Fig 1D e 1H), enrofloxacina (Fig 1E e 1I) e associação (Fig 1F e 1J).

3.3. Enrofloxacina foi capaz de controlar o parasitismo em encéfalos de fêmeas de C. callosus

Além de avaliarmos o parasitismo na placenta e no feto, nós também analisamos o efeito de enrofloxacina e toltrazuril no cérebro das fêmeas somente infectadas ou infectadas e tratadas com os diferentes medicamentos (grupos I, II, III e IV) através da imunolocalização de *T. gondii* por imuno-histoquímica. Nossos dados demonstram que enrofloxacina diminuiu significativamente o parasitismo no cérebro das fêmeas se comparado com o controle positivo ($P<0,05$, Fig. 2A). No entanto, toltrazuril e associação não apresentaram diferença significativa em relação ao controle. (Fig. 2A).

Fotomicrografias representativas são evidenciadas nas Fig. 2B-E, onde é possível evidenciar um maior número de parasitos nos corte de encéfalos do grupo controle positivo (Fig. 2B), em comparação com cortes de encéfalos dos grupos que receberam os tratamentos com toltrazuril (Fig. 2 C), enrofloxacina (Fig. 2D) e associação (Fig. 2E).

4. Discussão

A Toxoplasmose congênita é uma das formas mais graves da toxoplasmose, podendo levar a sérias consequências ao feto dependendo da idade gestacional em que ocorreu a infecção materna, ou se houve ou não tratamento durante a gestação [4, 6, 7].

Porém, o tratamento clássico, que é a associação de pirimetamina e sulfadiazina, apesar de ser eficaz na diminuição de replicação de *T. gondii* apresenta efeitos adversos que são prejudiciais tanto para a mãe quanto para o feto [9, 10]. Sendo assim, é de extrema importância a busca por tratamentos alternativos que sejam tão ou mais eficazes quanto o tratamento convencional e não causem efeitos adversos. No presente trabalho, nós avaliamos a eficácia de enrofloxacina e toltrazuril contra a infecção por *T. gondii* em fêmeas grávidas de *C. callosus,* pois esses dois medicamentos foram eficientes no controle de *T. gondii* em células BeWo e em explantes de vilos placentários humanos, levando ao aumento de citocinas proinflamatórias, como IL-6 e MIF [29].

Primeiramente, foi avaliado se os tratamentos com enrofloxacina e toltrazuril poderiam desencadear abortos nas fêmeas de *C. callosus*. Nossos resultados mostraram que tanto a enrofloxacina quanto o toltrazuril não causaram abortos, ou seja, todas as fêmeas que foram apenas tratadas com os dois medicamentos tiveram uma taxa de sucesso gestacional de 100%, sugerindo que os fármacos não são prejudiciais para a gestação. Porém, quando infectamos as fêmeas grávidas e tratamos com enrofloxacina e toltrazuril, nós observamos que houve uma maior taxa de aborto nesses dois grupos (grupos I e II). Gottstein e colaboradores (2005) mostraram que toltrazuril protegeu as fêmeas grávidas (linhagem C57BL/6) contra abortos desencadeados pela infecção por *N. caninum,* por outro lado, enrofloxacina não foi eficaz na diminuição da taxa de abortos em decorrência da neosporose. Sugere-se então que o tratamento, quando acompanhado da infecção, levou a diminuição do sucesso gestacional.

Sabe-se que a infecção por *T. gondii* leva a uma maior produção de citocinas proinflamatórias, como TNF-α e IFN-γ, sendo prejudicial para a gestação por resultar em um desequilíbrio no perfil imunológico materno-fetal [38]. Já foi demonstrado que a expressão de mRNA dessas citocinas em fêmeas de camundongo BALB/c e C57BL/6,

prenhes e infectadas por *T. gondii* (cepa RH), foi predominante durante a infecção [39]. Além do mais, estudos do nosso grupo mostraram que o tratamento de células BeWo com enrofloxacina e toltrazuril aumentaram os níveis de MIF e IL-6, que são citocinas potencialmente proinflamatórias [29]. Assim, o perfil de citocinas desencadeado pela infecção e pelo tratamento pode explicar a redução do sucesso gestacional quando o tratamento é realizado em fêmeas infectadas.

Embora tenhamos conseguido uma baixa porcentagem de sucesso gestacional nos grupos I e II, observamos que as fêmeas que apresentaram fetos viáveis no final do período analisado, apresentaram uma menor taxa de transmissão vertical de *T. gondii* após o tratamento com enrofloxacina e toltrazuril. Nossos resultados corroboram com dados prévios do nosso grupo em que ambos os medicamentos diminuíram o parasitismo em células BeWo e vilos placentários humanos [29], assim como de outros grupos, onde foi observado que o toltrazuril é capaz de controlar o parasitismo em infecções por *T. gondii* [25, 26] e em *N. caninum* [22].

Em relação ao número marcações específicas para *T. gondii* identificados por imuno-histoquímica nas amostras de encéfalos das fêmeas dos grupos I a IV, verificamos que apenas no grupo tratado com enrofloxacina ocorreu uma redução do parasitismo em relação ao grupo controle. Entretanto, não houve diferença estatística entre os grupos tratados (I, II e III). Esse resultado corrobora com trabalhos prévios do nosso grupo, onde o tratamento com enrofloxacina diminuiu o número de parasitos totais e a imunopatologia em encéfalos de *C. callosus* [17]. Por outro lado, em nossos dados, toltrazuril não foi eficiente na redução do parasitismo nesse tecido. Em estudos anteriores Gottstein e calobarodores (2005) mostraram que toltrazruil foi mais eficaz do que enrofloxacina para diminuir a infecção por *N. caninum* em encéfalos de fêmeas gestantes. Dessa maneira, sugerimos que para uma melhor análise da taxa de

parasitismo as amostras sejam analisadas por qPCR, devido a alta sensibilidade da reação, ao invés de contagem de marcações específicas de *T. gondii.*

Concluímos então que, embora nossos dados mostrem que as doses usadas para enrofloxacina e toltrazuril promoveram maior taxa de aborto em animais infectados, esses dois medicamentos mostraram ser efetivos no controle do parasitismo quando houve sucesso da gestação. Assim, é de extrema importância que seja testado outras doses ou um regime de tratamento mais tardio para avaliar os efeitos destas duas drogas nos animais, como também seria interessante para estudos futuros realizar, em conjunto com as diferentes concentrações dos fármacos, as dosagens de TNF-α e IFN-γ, MIF e IL-6.

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Figuras

Figura 1

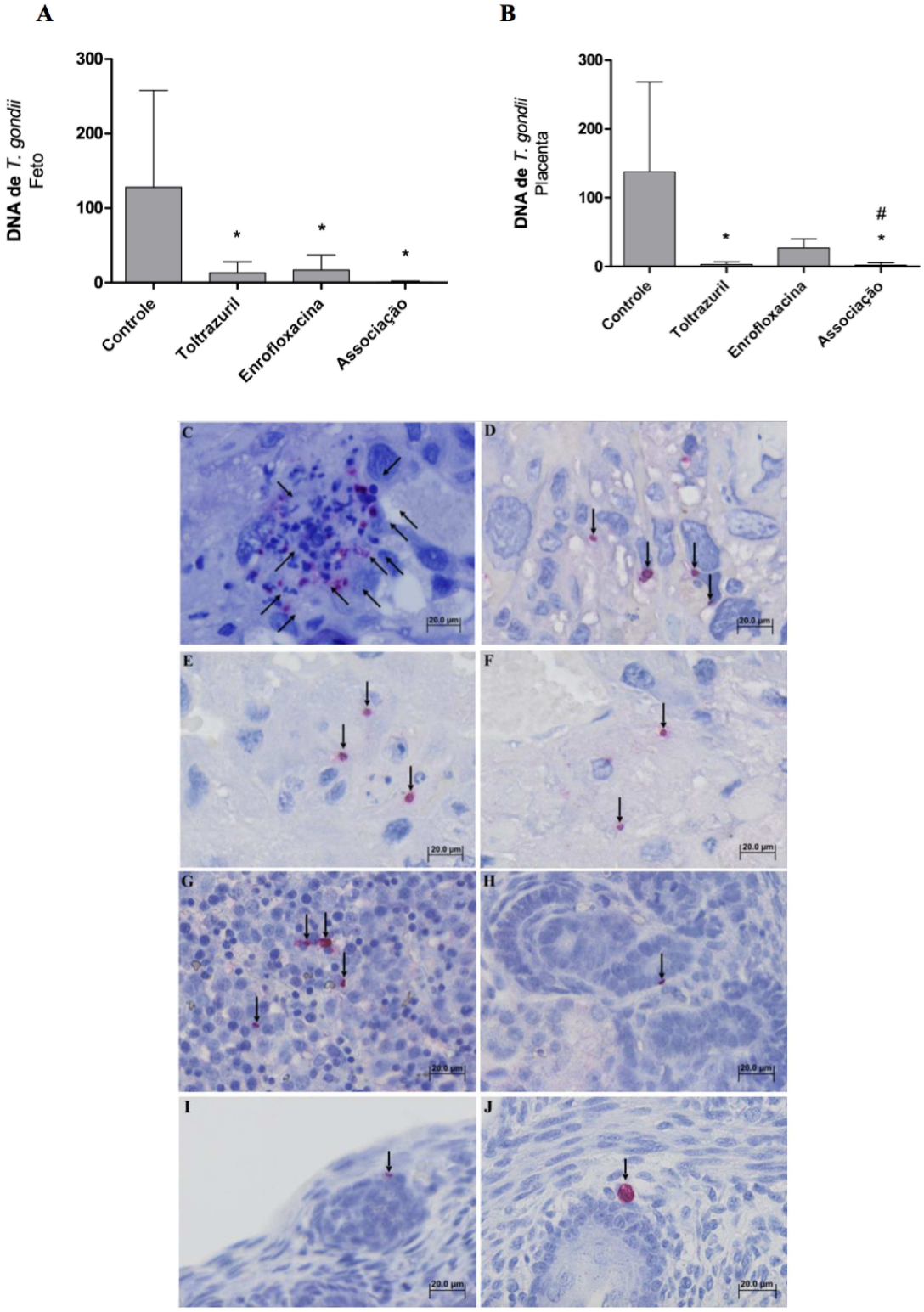

Fig.1: Comparação da carga parasitária em amostras de feto e placenta de fêmeas de *C. callosus* após a infecção com cistos da cepa ME-49 de *T. gondii* e tratamento com enrofloxacina, toltrazuril e associacão. Fêmeas dos 4 grupos experimentais foram infectadas oralmente com 20 cistos da cepa ME-49. Os animais foram eutanasiados no 19º dia de gestação/infecção e as amostras coletadas para análise por qPCR. **(A)** Amostras de fetos de fêmeas infectadas, tratadas ou não com os diferentes medicamentos; **(B)** Amostras de placentas de fêmeas infectadas, tratadas ou não com os diferentes medicamentos. Diferença significativa em relação ao controle (* $P < 0,05$); enrofloxacina (#$P < 0,05$). (Teste Kruskal-Wallis e Teste de Comparação Múltipla Dunn). Fotomicrografias representativas de amostras de fetos e placentas de fêmeas infectadas e não tratadas (**C e G**), ou tratadas com enrofloxacina (**D e H**), toltrazuril (**E e I**) ou associação (**F e J**). Cortes histológicos submetidos ao ensaio de imuno-histoquímica e contra corados com Hematoxilina de Harris. Setas indicam a imunolocalização do parasita por fast red naftol. Escala: 20.0 μm.

Figura 2

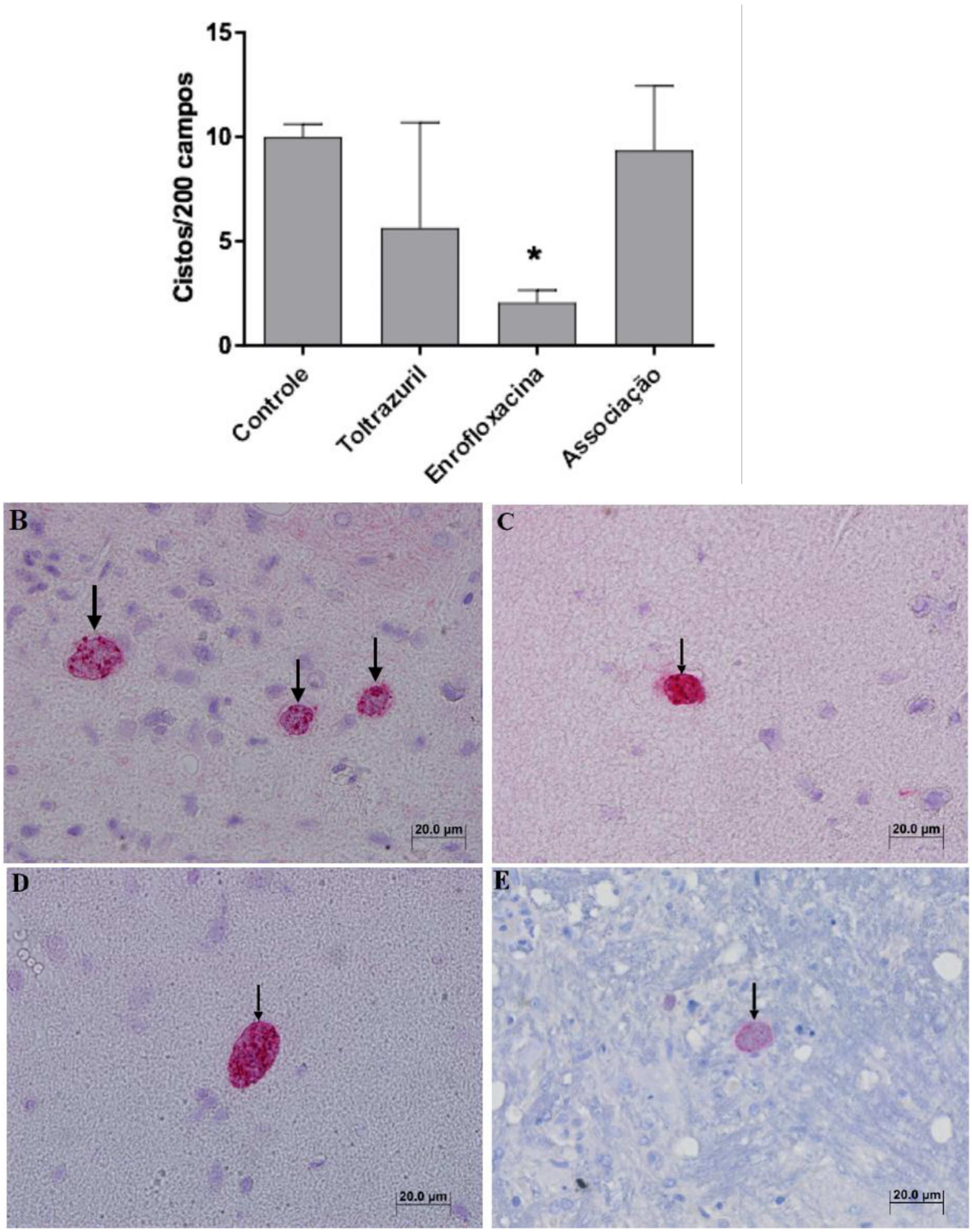
Cistos/200 campos
15
10
5
0
*
Controle
Toltrazuril
Enrofloxacina
Associação
B
C
D
E
20.0 µm
20.0 µm
20.0 µm
20.0 µm

Fig. 2: Número de cistos encontrados em cortes histológicos de encéfalos de fêmeas de *C. callosus* infectadas. A infecção oral foi realizada com 20 cistos da cepa ME-49 e os animais foram eutanasiados no 19º dia de gestação/infecção. Os cortes histológicos de encéfalos das fêmeas foram analisados por microscopia de luz após imuno-histoquímica para detecção de *T. gondii* (**A**). O número total de cistos foi apresentado em relação a 200 campos analisados. Diferença significativa em relação ao controle (*$P < 0,05$). (Teste Kruskal-Wallis e Teste de Comparação Múltipla Dunn). Fotomicrografias representativas de amostras de encéfalos de fêmeas infectadas e não tratadas (**B**), ou tratadas com enrofloxacina (**C**), toltrazuril (**D**) ou associação (**E**). Cortes histológicos submetidos ao ensaio de imunohistoquímica e contra corados com Hematoxilina de Harris. Setas indicam a imunolocalização do parasita por fast red naftol. Escala: 20.0 μm.

Tabelas

Tabela 1

Resultados de sucesso gestacional de fêmeas de *Calomys callosus* pertencentes a cada um dos grupos experimentais. Todas as fêmeas foram eutanasiadas no 19° dia de gestação. N: gravidez a termo com fetos; N: número total de fêmeas.

Grupos	Infecção/ Tratamento	Sucesso Gestacional n/N (%)	Abortos	N° de fetos Média
Grupo I	**Enrofloxacina**	**3/7 (42,8%)**	**4/7 (57,2%)**	**5**
Grupo II	**Toltrazuril**	**3/7 (42,8%)**	**4/7 (57,2%)**	**5**
Grupo III	**Associação**	**2/5 (40%)**	**3/5 (60%)**	**5**
Grupo IV	**Controle positivo (apenas infecção)**	**7/7 (100%)**	**0/7 (0%)**	**5**
Grupo V	**Enrofloxacina (sem infecção)**	**4/4 (100%)**	**0/4 (0%)**	**4**
Grupo VI	**Toltrazuril (sem infecção)**	**3/3 (100%)**	**0/3 (0%)**	**5**
Grupo VII	**Sem infecção e tratamento**	**4/4 (100%)**	**0/4 (0%)**	**4**

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