UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

Ocorrência de *Coxiella burnetii* em ruminantes domésticos e selvagens no Brasil

Diego Carlos de Souza Zanatto
Biólogo

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Diego Carlos de Souza Zanatto
Orientador: Prof. Dr. Marcos Rogério André
Coorientador: José Maurício Barbanti Duarte

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DADOS CURRICULARES DO AUTOR

Diego Carlos de Souza Zanatto – nascido na cidade de Ribeirão Preto, São Paulo, em 8 de janeiro de 1994. Formado em Ciências Biológicas (Bacharelado) pela Universidade Estadual “Júlio de Mesquita Filho” – Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, Jaboticabal, São Paulo, no ano de 2016. Durante a graduação, foi bolsista PROEX como professor do Cursinho-Ativo, Jaboticabal. Em 2016 foi monitor da disciplina de Parasitologia Veterinária II, ministrada pelo Prof. Dr. Marcos Rogério André. Ingressou no curso de Pós-Graduação em Microbiologia Agropecuária na FCAV/UNESP em março de 2017, com bolsa da Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Aos meus pais, Carlos Roberto Zanatto e Mairce de Souza Zanatto

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Resumo

Coxiella burnetii é uma bactéria Gram-negativa intracelular obrigatória, que além de considerado agente zoonótico causador da Febre Q em vários países do mundo, foi classificado como um potencial agente de bioterrorismo. Bovinos, ovinos e caprinos representam as fontes de infecção mais frequentemente associadas à ocorrência da enfermidade em humanos, entretanto animais selvagens também podem atuar como importantes fontes de infecção. Desta forma, o presente estudo tem como objetivo investigar a ocorrência de *Coxiella burnetii* em ruminantes domésticos e selvagens no Brasil. Para tal, 188 amostras de sangue de cervídeos (143 *Blastocerus dichotomus*, 11 *Ozotocerus bezoarticus*, 27 *Mazama gouazobira,* 4 *M. bororo* e 3 *M. americanum*), capturados nos estados de MS, SP e MG, foram submetidas à extração de DNA e, subsequentemente, à nested (n)PCR para *C. burnetii* baseada no elemento de inserção repetitivo IS1111 do gene heat shock protein (*htpAB*). Além disso, 169 amostras de soro de cervídeos foram submetidas à Reação de Imunofluorescência para detecção de anticorpos IgG anti-*C. burnetii*. Amostras de soros de bovinos apresentando desordens reprodutivas foram submetidas às Reações de Vírus Neutralização para BoHV-1 e BVD, Soroaglutinação Microscópica para *Leptospira* spp., Reação de Imunofluorescência Indireta for *C. burnetii* e *Toxoplasma gondii*, e Ensaio de Imunoabsorção Enzimática Indireto para *Neospora caninum* e *Trypanosoma vivax*. Todas as amostras de sangue mostraram-se negativas na nPCR, evidenciando ausência de DNA circulante de *C. burnetii* nos cervídeos amostrados. Alternativamente, a concentração de DNA do agente poderia estar abaixo do limiar da técnica de nPCR utilizada. Na RIFI, 5,32% (9/169) dos cervídeos (7 *B. dichotomus* e dois *M. gouazoubira* amostrados no MS) mostraram-se positivos frente ao antígeno de *C. burnetii*, com títulos variando entre 256 e 16384. A soropositividade para *C. burnetii* em bovinos foi de 13,7%, com títulos de 128 a 131,072; 57,8% para BoHV-1, com títulos entre 2 a 1,024; 47,1% para BVDV-1a, com títulos de 10 a 5,120; 89,2% para *N. caninum*; 50% para *T. vivax*; 52,0% para *Leptospira* spp., com títulos entre 100 a 800 (sorovares: Tarassovi, Grippotyphosa, Canicola, Copenhageni, Wolffi, Hardjo, Pomona e Icterohaemorrhagiae); e 19,6% para *T. gondii* com título de 40. Este é o primeiro estudo que evidencia a soropositividade para *C. burnetii* associada à presença de anticorpos para o Vírus da Rinotraqueíte infecciosa bovina (BoHV) e da Diarreia Viral Bovina, *N. caninum*, *Leptospira* spp., *T. gondii* e *T. vivax* em bovinos. Ainda, trata-se do primeiro estudo a mostrar evidência sorológica de exposição à *C. burnetii* em cervídeos na América Latina. Conclui-se então que há evidências da circulação de *Coxiella burnetii* entre cervídeos de vida livre e bovinos no Brasil.

Palavras chave: Abortamento, Bovinos, Cervídeos, *Coxiella burnetii*, IS1111, Febre Q.

Occurrence of *Coxiella burnetii* in domestic and wild ruminants in Brazil

Abstract

Coxiella burnetii is an obligate intracellular Gram-negative bacterium, which, in addition to being considered a zoonotic agent that causes Q fever in several countries of the world, has been classified as a potential bioterrorism agent. Cattle, sheep and goats represent the most frequent sources of infection associated with the occurrence of the disease in humans, however, wild animals can also act as important sources of infection. In this way, the present study aims to investigate the occurrence of *Coxiella burnetii* in domestic and wild ruminants in Brazil. To this end, 188 cervus blood samples (143 *Blastocerus dichotomus*, 11 *Ozotocerus bezoarticus*, 27 *Mazama gouazobira*, 4 *M. bororo* and 3 *M. americanum*), captured in the states of MS, SP and MG, were subjected to DNA extraction and , subsequently to the nested (n) PCR for C. burnetii based on the heat shock protein (*htpAB*) gene IS1111 insertion element. In addition, 169 cervical serum samples were submitted to Immunofluorescence Reaction for the detection of anti-*C. burnetii* IgG antibodies. Adicionaly, samples of bovine sera presenting reproductive disorders were submitted to the Virus Reaction Neutralization for BoHV-1 and BVD, Microscopic Soroagglutination for *Leptospira* spp., Indirect Immunofluorescence Reaction for *C. burnetii* and *T. gondii*, and Indirect Enzyme Immunoabsorption Assay for *N caninum* and *T. vivax*. All blood samples were negative in nPCR, evidencing absence of circulating DNA of C. burnetii in the sampled cervids. Alternatively, the DNA concentration of the agent could be below the threshold of the nPCR technique used. In IFAT, 5.32% (9/169) of cervids (7 *B. dichotomus* and 2 *M. gouazoubira* sampled in MS) were positive against *C. burnetii* antigen, with titers varying between 256 and 16384. Seropositivity for *C. burnetii* in cattle was 13.7%, with titers from 128 to 131,072; 57.8% for BoHV-1, with titres ranging from 2 to 1,024; 47.1% for BVDV-1a, with titers from 10 to 5,120; 89.2% for *N. caninum*; 50% for *T. vivax*; 52.0% for *Leptospira* spp., with titres ranging from 100 to 800 (serovars: Tarassovi, Grippotyphosa, Canicola, Copenhageni, Wolffi, Hardjo, Pomona and Iterotoemorrhagiae); and 19.6% for *T. gondii* with a titer of 40. This is the first study to show the seropositivity to *C. burnetii* associated with the presence of antibodies to bovine infectious rhinotracheitis virus (BoHV) and Bovine Viral Diarrhea, *N. caninum, Leptospira* spp., *T. gondii* and *T. vivax* in cattle. Still, this is the first study to show serological evidence of exposure to C. burnetii in cervids in Latin America. It is concluded that there is evidence of Coxiella burnetii circulation between free-living cervids and cattle in Brazil.

Key words: Abortion, Cattle, Cervidae, *Coxiella burnetii*, IS1111, Q fever

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. INTRODUÇÃO

Coxiella burnetii é uma bactéria Gram-negativa intracelular obrigatória, que infecta diversos tipos celulares (principalmente fagócitos) de humanos e animais (VAN SCHAIK et al, 2013; ABDEL-MOEIN e HANZA, 2017; ELDIN et al., 2017). Este agente, além de considerado agente zoonótico causador da febre Q em vários países do mundo (MAURIN & RAOULT, 1999), foi classificado como um potencial agente de bioterrorismo pelo CDC (*Center for Diseases Control and Prevention*).

A inalação de aerossóis contaminados diretamente de fluidos de animais neonatos infectados mostra-se como a principal via de infecção para seres humanos. Adicionalmente, o consumo de leite e produtos lácteos, contato com a pele e transmissão de pessoa a pessoa são outras vias de transmissão da infecção (BOUVERY et. al., 2003). Ectoparasitas hematófagos também assumem grande importância na transmissão do agente entre animais (ELDIN et. al., 2017).

Dentre os mamíferos, bovinos, ovinos e caprinos representam as fontes de infecção mais frequentemente associadas à ocorrência da enfermidade em humanos (ELDIN et al., 2017). Adicionalmente, animais selvagens também podem atuar como importantes fontes de infecção (DAVOUST et al., 2014). Embora muitos estudos acerca da ocorrência e diversidade genética de *C. burnetii* tenham sido conduzidos em bovinos (AURÉLIEN *et al.*, 2016; NOKHODIAN *et al.*, 2016; ABDEL-MOEIN e HAMZA, 2017; ELDIN *et al.*, 2017)

e cervídeos (GONZÁLEZ-BARRIO et al., 2016; SAN-MIGUEL AYANZ et al., 2017; CANDELA et al., 2014; GONZÁLEZ-BARRIO et al., 2015A; RUIZ-FONS et al., 2008; CUMBASSÁ et al., 2015; EBANI et al., 2016) em diversas localidades no mundo, ainda são escassos os estudos acerca desse agente patogênico em ruminantes domésticos e selvagens no Brasil. Em ruminantes domésticos a infecção por este agente está associada à infertilidade, abortamento, natimortalidade, endometrite e mastite (TISSOT-DUPONT & RAOULT, 2008).

No Brasil, evidência sorológica de exposição ao agente já foi relatada em cães (15,3%; 2/14), ovinos (66,6%; 2/3) e caprinos (50%; 5/10) no estado do Rio de Janeiro (Mares-Guia et al., 2014) e em caprinos (2,1%; 9/412) e ovinos (2,2%; 9/403) no estado de Pernambuco (SOUZA et al., 2018). Evidência sorológica de exposição à *C. burnetii* foi relatada em seres humanos nos estados de Minas Gerais (3,9%; 17/437) e Rio de Janeiro (3,2%; 4/125; 100%; 1/1) (COSTA et. al., 2005; LAMAS et al., 2009; ROZENTAL et al., 2012). Ainda, o referido agente já foi molecularmente detectado em amostras de sangue de cães (14,3%; 2/14), caprinos (60%; 6/10) e roedores (4,6%; 6/131) (MARES-GUIA et al., 2014; ROZENTAL et al., 2017) e em amostra de soro de um paciente humano apresentando febre, mialgia e tosse seca (ROZENTAL et al., 2012) no estado do Rio de Janeiro.

Na Europa, cervídeos (principalmente *Cervus elaphus*) são considerados um dos principais reservatórios para *C. burnetii* (GONZALES-BARRIO et al., 2015A). Desta forma, hipotetizou-se que no Brasil, um país com grande biodiversidade de espécies, ruminantes domésticos (representados por bovinos) e cervídeos poderiam desempenhar importante papel na

epidemiologia deste parasita, tal qual fora observado em outras partes do mundo. Ainda, hipotetizou-se que *C. burnetii* estaria envolvida na etiologia de desordens reprodutivas em bovinos no Brasil, associada ou não a outros patógenos sabidamente causadores de abortamento (*Trypanosoma vivax, Leptospira sp., Toxoplasma gondii, Neospora caninum*, Vírus da Diarreia Viral Bovina (BVDV) e Herpesvírus bovino (BoHV)). Neste sentido, o projeto em tela teve como objetivo a detecção de *C. burnetti* em cervídeos de vida livre e bovinos de corte apresentando abortamento de várias regiões geográficas brasileiras, a fim de investigar a possível participação de ruminantes domésticos e selvagens na epidemiologia da Febre Q no Brasil.

2. OBJETIVOS

2.1 Geral

O presente estudo tem como objetivo investigar a ocorrência de *Coxiella burnetii* em ruminantes domésticos e selvagens no Brasil.

2.2 Objetivos específicos

1) Investigar a presença de DNA de *C. burnetii* e de anticorpos anti-*C. burnetii* em amostras de sangue e soro, respectivamente, de cervídeos de vida livre no Brasil, pertencentes às seguintes espécies *Blastocerus dichotomus, Mazama gouazoubira, Mazama bororo, Mazama americana* e *Ozotocerus bezoarticus*;

2) Investigar a soropositividade para *C. burnetii* em associação com BoHV (Herpes Vírus Bovino), BVDV (Vírus da Diarreia Viral Bovina), *N. caninum, Leptospira spp, Toxoplasma gondii* e *Trypanosoma vivax* em amostras de soro de bovinos com histórico de problemas reprodutivos, provenientes de quatro estados brasileiros (São Paulo, Minas Gerais, Goiás e Mato Grosso do Sul).

3 REVISÃO DE LITERATURA

3.1 *Coxiella burnetii*, agente etiológico da Febre Q

Coxiella burnetii é um bacilococos Gram-negativo pleomórfico pertencente à ordem Legionellales, família Coxiellaceae. Trata-se de uma bactéria intracelular obrigatória que pode infectar seres humanos e animais

(VAN SCHAIK *et al*, 2013; ABDEL-MOEIN e HANZA, 2017). Este agente foi identificado pela primeira vez em 1930, quase simultaneamente, em trabalhadores de abatedouros em Brisbane, Austrália, e em um laboratório de pesquisas de rickettsioses em Montana, nos Estados Unidos da América (EUA) (MAURIN e RAOULT, 1999).

O estudo conduzido na Austrália constituiu-se da observação de cortes de baço de camundongos corados por Hematoxilina-eosina (HE). Os camundongos foram experimentalmente infectados com amostras de sangue de trabalhadores de Brisbane. Já em Montana, a bactéria foi descoberta quando, ao objetivar estudar a ecologia de febre maculosa nos EUA, pesquisadores utilizaram cobaias (*Cavia porcellus*) para alimentar carrapatos obtidos em Nine Mile. As cobaias desenvolveram hepatoesplenomegalia e apresentavam estruturas intracelulares com características típicas de rickettsiae. Tais achados eram reproduzidos quando amostras de sangue infectado eram transferidas para outros animais por inoculação intraperitoneal. Em 1936, Herald Rea Cox se juntou ao grupo para caracterizar o agente. O agente foi nomeado, em 1938, *Coxiella burnetii*, em homenagem aos pesquisadores Marcfarlene Burnet, de Brisbane, e Herald Cox, de Montana, que identificaram o agente da Febre Q (Query Fever), zoonose causada pelo agente (MAURIN e RAOULT, 1999).

Desde então, a presença desta bactéria tem sido confirmada em todo o mundo, com exceção da Nova Zelândia (MAURIN e RAOULT, 1999; Eldin et al., 2017), sendo classificada como potencial agente de bioterrorismo pelo CDC (*Centers for Disease Control and Prevention*).

O patógeno em questão apresenta habilidade de sobreviver longos períodos no ambiente. Esta característica está aparentemente ligada à capacidade da bactéria em transitar por diferentes estágios de desenvolvimento. Enquanto a forma “Small Cell Variant” (SCV) mostra-se metabolicamente inativa e altamente resistente a ambientes diversos, a “Large Cell Variant” (LCV) é a forma metabolicamente ativa (COLEMAN *et al.* 2004, 2007). As SCVs apresentam cromatina condensada, cápsula espessa e um sistema de membranas internas incomum. Tais características podem estar relacionadas à sua excepcional sobrevivência no ambiente, apresentando alta resistência osmótica, química, mecânica, ao aquecimento e ao estresse por dessecação. Esta forma evolutiva pode sobreviver de 7 a 10 meses na pelagem dos animais em temperatura ambiente, mais de um mês em carne fresca e mais de 40 meses no leite (ELDIN *et al.*, 2017).

A SCV, ao entrar passivamente na célula eucariótica, incita a formação do vacúolo portador da *Coxiella* (CCV), acidificação do endossoma e diferenciação em LCV. Após a fusão do endossoma com o lisossomo e a acidificação do meio, a LCV multiplica-se por fissão binária e promove a maturação do CCV, com posterior formação de SCVs, as quais são liberadas para o meio extracelular (Figura 1) (VAN SCHAIK *et al*, 2013). Geralmente, as infecções ocorrem por meio da inalação de aerossóis contaminados diretamente de fluidos de animais recém-nascidos infectados. No entanto, o consumo de leite e produtos lácteos, contato com a pele e transmissão de pessoa a pessoa são outras vias de transmissão da infecção (BOUVERY et. al., 2003).

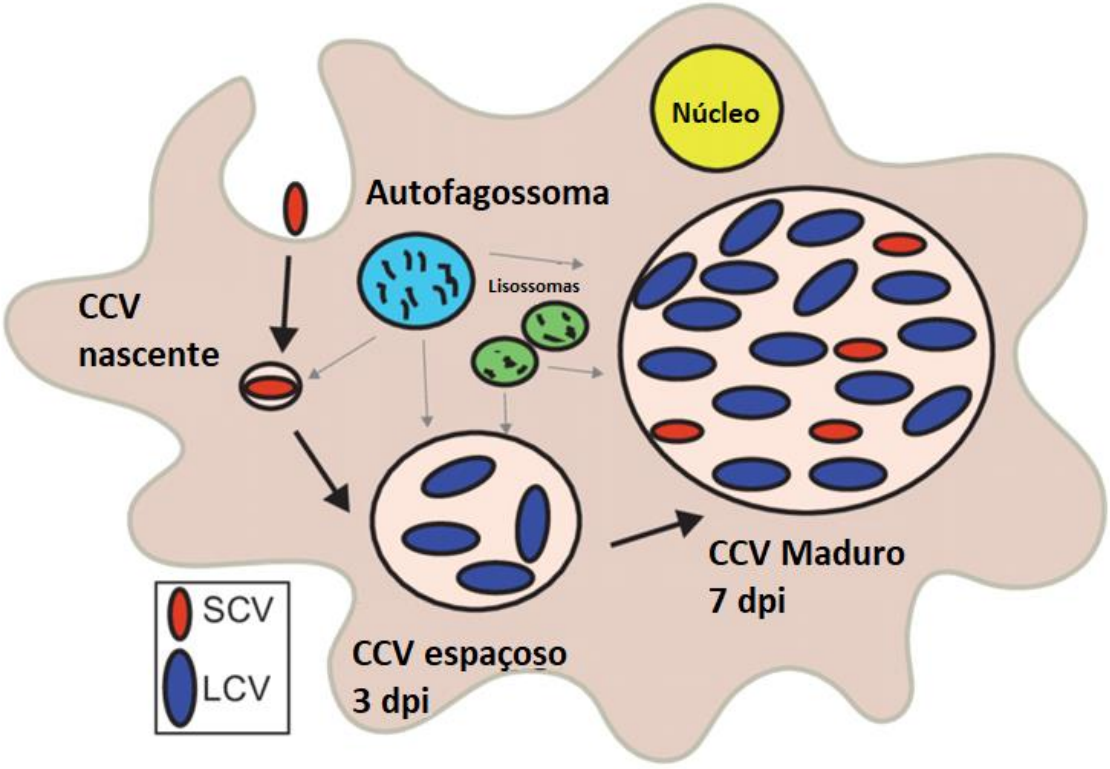

Figura 1. Representação esquemática da ação da bactéria *Coxiella burnetii* ao infectar uma célula.

O diagnóstico da infecção por *C. burnetii* vem sendo realizado por métodos sorológicos (RIFI) e moleculares (ELDIN *et al.*, 2017). Protocolos de PCR convencional podem utilizar oligonucleotídeos iniciadores baseados em diferentes regiões gênicas, tais como plasmídeos (MALLAVIA *et al.*, 1990), região intergênica 16S-23S RNA, gene da superóxido dismutase, e genes da proteína externa de membrana 1 (*com1*) e da sequência repetitiva *IS1111* (IBRAHIM *et al.*, 1997; STEIN *et al.*, 1997; VAIDYA *et al.*, 2008; FENOLLAR *et al.*, 2004; BODEN *et al.*, 2012; ABDEL-MEIN *et al.*, 2017). A PCR em tempo real (qPCR) com alvo na região *IS1111* vem mostrando alta sensibilidade (ELDIN *et al.*, 2017). Na Suíça, Jaton et al. (2013) padronizaram uma qPCR com base no gene *ompA, a* qual mostrou sensibilidade de 88% em amostras teciduais de válvulas cardíacas, 69% em amostras de sangue e 50% em

amostras de urina de seres humanos (JATON *et al.*, 2013). Além disso, Eldin *et al*. (2013) verificaram menor sensibilidade de um ensaio de qPCR baseado no gene *IS30A* quando comparado àquele baseado no gene *IS1111*. Edouard & Raoult (2016), por sua vez, mostraram que a sensibilidade da PCR baseada no gene *IS1111* pode ser otimizada quando se utiliza o processo de liofilização de amostras de DNA extraídas a partir de soro sanguíneo. A detecção de DNA nas amostras de soro passou a ser 100 vezes mais sensível (1 bactéria/mL) em comparação com o DNA não liofilizado (100 bactérias/mL).

O tratamento da Febre Q depende se da fase em que se encontra o paciente infectado (aguda ou crônica). Em casos de doença aguda, muitos indíviduos se recuperam sem tratamento, embora seja recomendado o tratamento com doxiciclina. Já em casos de doença crônica, é necessário tratamento à base de antibióticos por muitos meses, por meio da combinação de doxiciclina e hidroxicloroquina (CDC, 2019).

A primeira sequência completa do genoma de *C. burnetii*, oriunda da amostra de referência Nine Mile RSA 493, isolada em 1935 a partir de carrapatos *Dermacentor andersoni* infectados em Hamilton, Montana, EUA, foi disponibilizada em 2003 (MORI *et al.,* 2017). A sequência continha 1.995.275 pares de bases e foi obtida pelo método de *random shotgun* (SESHADRI et al., 2003). Recentemente, com o advento de técnicas de sequenciamento mais poderosas e modernas, o número de genomas sequenciados chega a mais de 40, dos quais 26 estão disponíveis publicamente (D'AMATO et al. 2014, 2015; KARLSSON et al. 2014; SIDI-BOUMEDINE et al. 2014; WALTER et al. 2014; HAMMERL et al. 2015).

Em relação à genotipagem da bactéria, as técnicas utilizadas que se destacam são: análise do número de variações do *locus* múltiplo de repetição em tandem (MLVA) (KLAASSEN et al. 2009; TILBURG et al. 2012a) e a genotipagem de polimorfismo de um único nucleotídeo (SNP) (HORNSTRA et al., 2011; HUIJSMANS et al., 2011).

O isolamento e a propagação do parasita a partir de amostras clínicas permitem a caracterização fenotípica e genotípica do agente utilizando técnicas moleculares, incluindo o sequenciamento (MORI *et al.*, 2017). Várias linhagens celulares suportam a replicação *in vitro* de *C. burnetii,* incluindo as linhagens de macrófagos (P388D1, J774, DH82), fibroblastos (L929, HEL) e linhagens epiteliais (Vero E6) (MAURIN e RAOULT, 1999; MEDIANNIKOV et al., 2010; SANTOS *et al*., 2012). A linhagem celular de fibroblasto embrionário humano (HEL) é a mais comumente utilizada, uma vez que é de fácil cultivo, preserva a integridade da cultura por longos períodos e mostra-se altamente susceptível à infecção (GOURIET et al. 2005; LAGIER et al., 2015). A linhagem celular de histiócitos malignos caninos - DH82 (ATCC CRL-10389) - vem sendo cada vez mais adotada para o sistema de cultivo *in vitro* de *C. burnetii* (MEDIANNIKOV et al. 2010; LOCKHART et al. 2012; SANTOS et al. 2012; CUMBASSA et al., 2015). As culturas *in vitro* são incubadas a 37°C com 5% de CO_2 atmosférico por dois meses, podendo estender este período para até cinco meses, com as devidas avaliações de crescimento da bactéria (MORI *et al*., 2017).

O isolamento *in vivo* de *Coxiella* tem se provado muito eficiente em modelos murinos, como camundongos (*Mus musculus*) e porquinhos-da-Índia (*Cavia porcellus*). A inoculação de uma amostra em hospedeiros vertebrados confere uma melhoria contra contaminação com microorganismos indesejados.

Além disso, modelos *in vivo* são essenciais para manter a forma virulenta nativa da *Coxiella*. A linhagem de ratos OF1 é frequentemente usada para o isolamento, pois é relativamente mais sensível do que as linhagens BALB/c ou C57/BL6 de camundongos. Baço, fígado e pulmão são os melhores orgãos para se fazer o monitoramento da infecção por *C. burnetii*, tanto por microscopia quanto por PCR em tempo real quantitativa (qPCR). Infecções por *C. burnetii* são tipicamente caracterizadas por esplenomegalia, devido à massiva propagação da bactéria (MORI *et al*., 2017).

3.2 Hospedeiros e transmissão de *C. burnetii*

Coxiella burnetii pode infectar uma vasta gama de vertebrados e invertebrados. A primeira amostra desta bactéria foi isolada a partir de carrapatos da espécie *Dermacentor andersoni* coletados em Montana, Estados Unidos (COX, 1938). A hematofagia é fator essencial para a aquisição do parasita e transmissão do mesmo entre artrópodes e animais. Estes últimos podem se infectar via vetores hematófagos, embora esta não seja a única forma de transmissão entre mamíferos. Outro aspecto importante para o ciclo da bactéria é a ausência de especificidade, haja vista que a mesma já fora detectada em 54 espécies de carrapatos, além de outros artrópodes, incluindo percevejos, moscas e ácaros. Recentemente, o parasita foi detectado em carrapatos (*Dermacentor marginatus, Haemaphysalis parva, Haemaphysalis sulcata, Ixodes gibbosus e Rhipicephalus sanguineus, Rhipicephalus bursa, Haemaphysalis punctata* e *Hyalomma marginatum*) na Grécia e Espanha. Experimentalmente, piolhos e pulgas mostram-se susceptíveis à infecção

(BABUDIERI, 1951 apud ELDIN *et al.*, 2017; Varela-Castro et al., 2017; Chaigiannis et al., 2018). Dentro de carrapatos, estes patógenos já foram encontrados em células epiteliais, intestino (SMITH, 1940, 1942 apud ELDIN *et al.*, 2017), hemócitos, glândulas salivares e ovários (VON WEYER, 1953 apud ELDIN *et al.*, 2017).

O agente foi detectado virtualmente em todo o reino animal (MAURIN e RAOULT, 1999). Um número crescente de animais tem sido identificados como hospedeiros da bactéria, incluindo mamíferos domésticos, selvagens, répteis e aves (ANDERSON et al., 2013). Dentre os mamíferos, bovinos, ovinos e caprinos representam as fontes de infecção mais frequentemente associadas à ocorrência da enfermidade em humanos (ELDIN *et al.*, 2017). Sendo assim, a identificação de animais infectados é de grande importância. Entretanto, tal identificação é desafiadora, uma vez que os animais podem atuar como portadores e eliminarem bactérias no ambiente. Os principais sinais clínicos associados à enfermidade em ruminantes são infertilidade, abortamento, natimortalidade, endometrite e mastite (TISSOT-DUPONT e RAOULT, 2008). Animais infectados podem eliminar grande quantidade de bactérias através da placenta, líquido amniótico, fezes, urina e leite (RODOLAKIS, 2009). Aerossóis contaminados com o parasita e a entrada de animais infectados no rebanho representam as principais formas de introdução de *C. burnetii* em propriedades rurais (NUSINOVICI *et al.*, 2015; ELDIN *et al.*, 2017).

Na França, Eldin et. al. (2013) testaram 103 produtos lácteos (queijos, iogurtes, cremes e manteiga) de supermercados e artesanais. No total, 64% de amostras mostraram-se positivas para *C. burnetii* com base na qPCR baseada no gene *IS1111*. DNA da bactéria foi identificado em 65% dos queijos, 70% dos

iogurtes, 55% dos cremes e 14% das manteigas analisadas. Dentre esses produtos, 66% dos produtos pasteurizados, 57% dos produtos não-pasteurizados e 100% dos produtos que passaram por tratamento térmico mostraram-se positivos na qPCR para *C. burnetii*. A análise estatística mostrou não haver diferença de positividade na qPCR para o agente sob estudo entre produtos pasteurizados e não-pasteurizados. Além disso, maior positividade foi verificada entre produtos industrializados quando comparada àquela verificada para produtos artesanais.

Animais selvagens também podem atuar como fontes de infecção de *C. burnetii*. Na Guiana Francesa houve um surto de febre Q em Tiger Camp, uma área residencial miliar, no ano de 2013. Acredita-se que a aquisição do patógeno tenha sido através do contato com a preguiça-de-três-dedos (*Bradypus tridactylus*). Foram coletadas amostras de diversos animais (13 cabras, 8 ovelhas, 7 morcegos, 34 aves, 2 gambás, 4 iguanas e 17 lagartixas), porém todos foram negativos. Em 2014 uma preguiça-de-três-dedos foi encontrada morta próxima à residência de um paciente com febre Q. Amostras de fezes e baço do animal mostraram-se positivas na qPCR baseada no gene IS1111, o que foi posteriormente confirmado por uma segunda qPCR baseada na sequência repetitiva IS30a. Ainda, 88% dos carrapatos (*Amblyomma geayi*) coletados da preguiça também mostraram-se positivos para *C. burnetii*. Apesar dos carrapatos terem se mostrado positivos, acredita-se que as infecções em humanos nesta região ocorreram por meio de aerossóis contaminados oriundos das fezes das preguiças (DAVOUST *et al*., 2014).

Recentemente, Rozental *et al.* (2017) conduziram estudo em oito cidades diferentes no estado do Rio de Janeiro, amostrando roedores das

espécies *Akodon cur*sor, *Mus musculus*, *Oligoryzomys nigripes* e *Oxymycterus dasytrichus*. DNA de *C. burnetii* foi detectado em amostras de baço de roedores nas cidades de Piraí e Valença, por meio de ensaios de nested PCR baseados na sequência repetitiva IS1111 do gene *htp*AB. Foi revelada a presença de DNA de *Coxiella* em 4,6% do total de roedores testados (6/131), representando 33,3% (2/6) dos animais capturados em Piraí e 66,6% (4/6) dos animais amostrados em Valença.

Interessantemente, na França, *C. burnetii* já foi detectada inclusive dentro de amebas da espécie *Acanthamoeba castellanii*, in vitro, tanto na forma de cisto quanto de trofozoíto, sugerindo a participação destes protozoários unicelulares na persistência da bactéria no ambiente (SCOLA e RAOULT, 2001).

3.3 *Coxiella burnetii* em seres humanos no Brasil

No que diz respeito à ocorrência de *C. burnetii* em humanos, Georgiev *et al.* (2013) sumarizou tais dados em uma revisão sobre o assunto, dividindo os indivíduos amostrados em inquéritos epidemiológicos de diversas localidades do mundo em três grupos. O primeiro grupo representou uma amostragem da população em geral; o segundo grupo correspondendo aquele de risco, o qual incluía veterinários, pessoas apresentando sinais clínicos, mulheres grávidas e trabalhadores rurais que relataram contato com os animais; já o terceiro grupo foi constituído por indivíduos que viviam em áreas onde ocorreram surtos de Febre Q. Para tal, realizaram-se ELISA (Ensaio imunoenzimático), RIFI (Reação de Imunofluorescência Indireta) e TFC (Teste de fixação do

Complemento). Para o primeiro grupo foram encontradas porcentagens de positividade de 2,4 a 24% nos Países Baixos, 1 a 4% na França, 22% na Alemanha, e 38% na Bulgária. Para o segundo grupo foram encontradas taxas de positividade de 15 a 18% para pacientes que apresentavam problemas cardiovasculares e pneumonia atípica na Bulgária; 2,6 a 71% entre mulheres grávidas, pacientes com problemas cardíacos, pessoas envolvidas em partos de caprinos e veterinários na França; 83,3% para veterinários de campo nos Países Baixos. Já para o terceiro grupo, foi encontrada positividade de 7,7% e 9,1% entre mulheres grávidas na Bulgária e Países Baixos, respectivamente; 14,7% na França para pessoas residentes em áreas nas quais foram relatados surtos epidêmicos e que não estavam sob estado de risco e de 22% entre trabalhadores da zona rural que possuíam contato com animais que abortaram na Alemanha.

A primeira descrição clínica da infecção por *C. burnetii* ocorreu em 1937 durante a investigação de um surto de uma doença febril entre trabalhadores de abatedouros em Queensland, Austrália (DERRICK, 1937). A infecção por *C. burnetii* pode manifestar-se através de uma ampla diversidade de sintomas e sinais clínicos. O período de incubação da infecção primária pode durar de 2 a 3 semanas e depende da severidade da infecção. Em grande parte dos pacientes a infecção primária pode ser assintomática (RAOULT et al., 2005). Em outros casos, pneumonia, hepatite, cefaléia e sinais semelhantes à gripe podem ser observados durante a fase aguda da doença. Em menor escala, a doença aguda também pode causar problemas cardíacos, como pericardite, miocardite e até endocardite (a qual está geralmente associado à fase crônica

da doença) e sinais neurológicos, como meningite e meningoencefalite (ELDIN et al., 2017).

Em 1949, Beck e Bell foram os primeiros a notar que: inúmeros indivíduos (300 casos em dois anos) foram encontradas com uma doença febril crônica que remontava a um surto de Febre Q" (BECK et al., 1949). Alguns anos depois, Marmion e colaboradores descreveram o que chamaram de “endocardite por *Rickettsia* subaguda”, uma endocardite negativa para hemocultura com altos níveis de anticorpos de fase I contra *C. burnetii* (MARMION et al., 1953, ANDREWS e MARMION et al., 1959). Nestes relatórios, o termo “febre Q crônica” foi cunhado pela primeira vez (ELDIN et al., 2017). Em sequência, foi estabelecido o ponto de corte de título sorológco de 1:200 para o diagnóstico de Febre Q crônica (SPICER, 1979). Posteriormente, a CDC oragnizou um guia para o diagnóstico da fase crônica da doença (Tabela 1).

Tabela 1. Critério para definição de “Febre Q crônica” formulado pelo CDC (*Centers for Disease Control and Prevention*).

Categoria	Descrição
Indicações	Endocardite com cultura negativa recentemente reconhecida (particularmente em um paciente com valvopatia prévia ou comprometimento do sistema imune), suspeita de infecção de aneurisma vascular ou prótese vascular, ou hepatite crônica, osteomielite, osteoartrite ou pneumonite na ausência de outra etiologia conhecida
Confirmação laboratorial	Um ou mais dos seguintes critérios: título de IgG de 1: 800 para antígeno de fase I de *C. burnetii* no teste de RIFI; detecção de DNA de *C. burnetii* em uma amostra por PCR, demonstração de *C. burnetii* em uma amostra por Imunohistoquímica (IHC); isolamento de *C. burnetii* em cultura
Suporte laboratorial	Títulos de IgG anti-*C. burnetii* de fase I ≥1:128 e <1:800
Febre Q crônica confirmada	Evidência clínica de infecção com confirmação laboratorial
Provável febre Q crônica	Evidência clínica de infecção com resultados de suporte laboratorial

Na década de 80, mostrou-se associação entre anticorpos (fases I e II) e o tipo de infecção causada. Enquanto anticorpos de fase I têm sido associados à infecções crônicas, anticorpos de fase II estão associados à infecções agudas (PEACOCK et al., 1983; DUPUIS et al., 1988).

Em humanos, o parasita está associado à pneumonia, uma síndrome semelhante à gripe e hepatite; em alguns casos, foram observados prurido, pericardite, miocardite, encefalite e osteomielite (MAURIN & RAOULT, TISSOT-DUPONT & RAOULT 1999 e 2008). A forma crônica da infecção pode

causar endocardite, geralmente associada à doença cardíaca valvular e imunossupressão. Embora menos comumente, indivíduos podem desenvolver lesões granulomatosas nos ossos, articulações, fígado, pulmões, testículos e outros tecidos (RALPH et al. 2007, TISSOT-DUPONT & RAOULT 2008).

No Brasil, evidências da circulação do parasita já foram encontradas em seres humanos nos estados de São Paulo (BRANDÃO et al., 1953; VALLE et al., 1955; SICILIANO et al., 2015), Rio de Janeiro (LAMAS et al., 2009, 2013; LEMOS et al., 2011, 2018; ROZENTAL et al., 2012, 2018; MARES-GUIA et al., 2016), Minas Gerais (RIEMANN, et al., 1974; COSTA et al., 2005; COSTA et al., 2006) e Bahia (SICILIANO et al., 2008).

Costa *et al.* (2005) coletaram amostras de soro de 437 indivíduos saudáveis entre 5 e 92 anos no município de Piau, Minas Gerais, cuja economia está ligada à criação de gado e parte da população tem contato constante com animais de fazenda. Dezessete indivíduos (3,9%) mostraram-se soropositivos para *C. burnetii* pela RIFI.

No Rio de Janeiro, Lamas *et al.* (2009) encontraram soroprevalência de 3,2% em 125 indivíduos. Nenhum dos pacientes testados apresentavam sinais clínicos aparentes; entretanto, uma das 4 mulheres soropositivas apresentou três abortos espontâneos.

Rozental *et al.* (2012) registraram o caso de um homem de 33 anos que apresentava febre, mialgia e tosse seca e estava infectado por *C. burnetii.* O diagnóstico foi realizado pela RIFI e PCR convencional baseado no gene *heat shock protein* (*htp*AB).

Houve também um surto de febre Q recentemente descrito entre cadetes no estado do Rio de Janeiro, Sudeste do Brasil, cujo diagnóstico foi confirmado por testes sorológicos (LEMOS et al., 2018).

3.4 *Coxiella burnetii* em ruminantes domésticos

Coxiella burnetii é um patógeno de importância global e está frequentemente associado com infecções em bovinos, ovinos e caprinos (PORTER et. al., 2011). Na década passada este parasita causou grandes surtos de doenças humanas associadas a abortamentos em pequenos ruminantes (BOND et al., 2015; CDC, 2011; VAN DER HOEK et al., 2010).

Bovinos, ovinos e caprinos são os principais reservatórios para a infecção humana (GEORGIEV *et. al.,* 2013; NOKHODIAN et. al., 2017). Já foi notado que áreas com maior incidência de casos de febre Q são aquelas com maior número de ruminantes (FRANKEL *et. al.,* 2011). No setor agropecuário, *C. burnetii* é importante causadora de surtos de abortamentos em caprinos e ovinos, além de causar infertilidade e baixo peso ao nascimento em bovinos (BROOKS et. al., 2017; ELDIN et. al., 2017).

Um estudo feito em 2008 mostrou a ausência de diferença na disseminação de *C. burnetii* entre descargas vaginais, fezes ou leite de vacas leiteiras e, quando comparados, cabras. Além disso, este estudo também identificou que pelo menos 24% de animais soronegativos que não sofreram abortamento estavam disseminando ativamente o parasita (ROUSSET et. al., 2008). Em 2011, um estudo mostrou que a positividade para *C. burnetii* encontrada pela PCR de amostras de placentas estava associada à presença de anticorpos de fase II (encotrados durante a fase aguda da doença), embora

esta relação não tenha sido verificada para anticorpos de fase-I (associados à fase crônica da doença) (BOTTCHER *et al.,* 2011). Adicionalmente, *C. burnetii* já foi identificada em excretas (fezes, urina e líquidos liberados no parto) de rebanhos de bovinos e caprinos soronegativos (BERRI el. al., 2001; GUATTEO et. al., 2012). Em outro estudo conduzido em 2007 foram observados caprinos que disseminavam o parasita durante quase 4 meses após um surto na França (BERRI et al., 2007). No **Quadro 1** e **Figura 2** estão elencados estudos de ocorrência de *C. burnetii* em bovinos em diferentes países.

O agente em questão vem sendo molecularmente detectado em ruminantes domésticos em vários países da Europa, tais como Alemanha [26.87%], Bélgica [30%], Hungria [63%], Portugal [91%], França [14,98% - 38.9%], Espanha [10% - 32%], Suíça [11.6% - 27.6%], Itália [25%], Grécia [17.9%]), Egito (0,9%), Irã (8,6% - 17.9%), Tanzânia (5%), Senegal (4%), Nigéria (55%), Estados Unidos (94%), Coreia do Sul (17.8%) entre outros países (ADESIYUN *et al.,* 1984; KIM et al., 2005; RODOLAKIS et al., 2007; FRETZ et al., 2007; CZAPLICKI et al. 2009; KAMGA-WALADJO *et al.*, 2010; BOTTCHER et al., 2011; AURÉLIEN *et al.*, 2016; NOKHODIAN *et al.*, 2016; ABDEL-MEIN *et al.*, 2017; ELDIN et al., 2017; SEO et al., 2017; VARELA CASTRO et al., 2017; VIDAL et al., 2017; BIASE et al., 2018; CHALINGIANNIS et al., 2018). No que diz respeito a inquéritos sorológicos em bovinos, foram relatadas taxas de soroprevalência de 20,8% na Bulgária, 15% na França, 19,3% na Alemanha e 21% nos Países Baixos. Já entre caprinos, soroprevalência de 40% foi encontrada na Bulgária, 88,1% na França, 2,5% na Alemanha e 7,8% nos Países Baixos. Já entre ovinos, soroprevalência de

56,9% foi relatada na Bulgária, 20% na França, 8,7% na Alemanha e 3,5% nos Países Baixos (GEORGIEV *et al.,* 2013).

Embora *C. burnetii* seja um agente zoonótico associado à ocorrência de abortamento em ruminantes em todo o mundo, existem poucos estudos sobre a ocorrência desse agente em ruminantes domésticos no Brasil (TRAVASSOS et al., 1954; MARES-GUIA et al., 2014; GUIMARÃES et al., 2017; SOUZA et al., 2018; OLIVEIRA et al., 2018).

Em Itaboraí, estado do Rio de Janeiro, foram testadas amostras de sangue e soro de 14 cães, três ovinos e 10 caprinos quanto à presença de anticorpos anti-*C. burnetii* pela RIFI. Neste estudo foram encontrados 2 cães (14,3%), duas ovelhas (66,6%) e cinco cabras (50,0%) cabras soropositivas para *C. burnetii*. Além disso, amostras de sangue de dois cães (de um total de 2 cães), 6 amostras de leite e uma amostra de swab anal de cabras (de um total de 6 cabras) foram positivos na PCR convencional para *C. burnetii* baseada no gene transposon-like (MARES-GUIA *et al.*, 2014).

Guimarães et al. (2017) realizaram uma investigação sorológica para *C. burnetii* em caprinos e ovinos criados no entorno do Parque Nacional da Serra das Confusões (PNSC), localizado no estado do Piauí, região nordeste do Brasil. Amostras de soro de 202 caprinos e 153 ovinos foram testadas pela RIFI para detecção de anticorpos anti-*C. burnetii*. Enquanto soropositividade de 2% (3/153) foi encontrada entre os ovinos, com títulos variando de 64 a 4.096, todos os caprinos amostrados mostraram-se soronegativos frente ao antígeno de *C. burnetii*. Apenas um dos ovinos estava parasitado por *Rhipicephalus microplus*, não permitindo estabelecer correlação entre a presença do ectoparasita e a soropositividade para *C. burnetii*.

Similarmente, Oliveira et al. (2018) conduziram um estudo a fim de investigar a soroprevalência para *C. burnetii* em um rebanho de cabras leiteiras no estado de Alagoas, nordeste do Brasil. Amostras de soro de 312 cabras coletadas de um rebanho com aptidão leiteira e histórico de falha reprodutiva foram submetidas a um ELISA comercial para detecção de anticorpos IgG anti-*C. burnetii*. Amostras de cotilédones de 23 placentas foram analisadas por nested PCR para o agente sob estudo baseada no gene IS1111. A sororeatividade do teste ELISA encontrada foi de 55,1% (172/312) das amostras de soro analisadas. DNA de *C. burnetii* foi detectado em 8,7% (2/23) das amostras de placenta testadas, sendo ambos os animais também soropositivos.

Souza et al. (2018) investigaram os fatores de risco associados à presença de anticorpos anti-*C. burnetii* em 412 caprinos e 403 ovinos em Petrolina, no estado de Pernambuco, uma região semiárida do Nordeste do Brasil. Pela RIFI, 2,2% (9/412) dos caprinos e 2,1% (9/403) dos ovinos mostraram-se soropositivos para *C. burnetii*. A presença de anticorpos anti-*C. burnetii* foi estatisticamente associada com a área seca do Sequeiro (região norte do município de Petrolina), ovinos machos e criação intensiva de caprinos.

Quadro 1. Ocorrência sorológica e molecular de *Coxiella burnetii* em bovinos no mundo.

Referências	País	Animais	Número de amostras	Teste sorológico (antígeno)	Animais positivos (%)	Teste molecular (genes-alvo)	Número de amostras (tipo de amostra)	Animais positivos
Cetinkaya et al. (2000)	Turquia	Bovinos	416	RIFI	5,8% (para fase II)	-	-	-
Kim et. al. (2005)	Estados Unidos	Bovinos	-	-	-	nPCR (IS1111 *transpoase element*)	316 (tanques de leite)	94%
McQuiston et al. (2005)	Estados Unidos	Bovinos (Tanque de leite)	8600	RIFI	92% (para fase I) 38% (para fase II)	-	-	-
Rodolakis et al. (2007)	França	Vacas	-	-	-	cPCR (gene não especificado	90 (leite)	38,9%
Fretz et al. (2007)	Suíça	Bovinos	-	-	-	nPCR (*com1*)	27 (Tanques de leite)	27,6%
Czaplicki et al. (2009)	Bélgica	Bovinos (Tanques de leite)	1137	ELISA (amostra CbO1)	71,2%	qPCR (*IS1111 transpoase element*)	150 (Tanques de leite)	30%
Rahimi et al. (2009)	Iran	Bovinos	-	-	-	nPCR (*com1*)	210 (Tanques de leite)	17,9%
Ruiz-Fons et al. (2010)	Espanha	Ovelhas, cabras e vacas	1379, 115, 626	ELISA (ELISA Cox kit, (LSI-France) baseado na amostra CbO1)	11,8%, 8,7% e 6,7%	-	-	-
McCaughey et al. (2010)	Irlanda do Norte	Bovinos	5182	ELISA (Bommeli Chekit Q fever IgG kit baseado na amostra Nine-mile)	6,2%	-	-	-
Scolamacchia et al. (2010)	Camarões	Bovinos	1460	ELISA (amostra Nine-mile)	39,3%	-	-	-
Ryan et al, (2011)	Irlanda	Bovinos	1659 animais e 290 tanques de leite	ELISA (ELISA Cox kit, (LSI-France) baseado na amostra CbO1)	1,8% e 37,9%	-	-	-
Bottcher et al. (2011)	Alemanha	Vacas	21051	ELISA (*C. burnetii* de fase 1 e fase 2 inativas, juntas e separadamente)	14,8%	cPCR (**gene não especificado)**	67 (Placenta)	26,87%
Agger et. al. (2010)	Dinamarca	Bovinos (Tanques de leite)	100	ELISA (amostra Nine-Mile)	59%	-	-	-

Continua ...

Continuação …

Nokhodian et al., 2017	Iran	Caprinos, ovinos e bovinos	3334 (702 caprinos, 2293 ovinos e 339 bovinos)	ELISA (*C. burnetii* de fase 1 e fase 2 inativas)	33%, 27% e 17% em média	nPCR e qPCR para tanques de leite e amostras de sangue (*com1*, 16S rRNA e *IS1111 transpoase element*)	1852 (Tanque de leite) 87 (amostras individuais de sangue)	2,6%em caprinos, 2,54% em ovinos e 9,72% em bovinos (tanques de leite); 34,8% (caprinos) 40% (ovinos) (amostras de sangue, não especificadas por gene)
Gache et al. (2017)	França	Vacas, ovelhas e cabras	10040, 7776 e 5246	ELISA (*C. burnetii* de fase 1 e fase 2 inativas)	36%, 55,7% e 61%	-	-	-
Seo et al. (2017)	Coréia do Sul	Bovinos (Tanques de leite)	-	-	-	nPCR (16S rRNA)	108 (Tanques de leite)	17,8%
Vidal et al. (2017)	Suíça	Bovinos	249	ELISA (CHEKIT® Q fever antibody ELISA Test Kit)	15,9%	qPCR (IS1111 transpoase element)	242 (placenta) 57 (abomaso fetal) de um total de 249 casos	11,6% (placenta) e 12,1% (abomaso fetal)
Varela-Castro et al. (2017)	Espanha	Caprinos e carrapatos de carpinos	134	ELISA (Qfever Antibody Test Kit, IDEXX)	30%	cPCR (IS1111)	134 caprinos (Sangue, baço, e linfonodo submandibular) 669 Carrapatos (157 pools)	10% dos caprinos e 42,7% das pools
Biase et al. (2018)	Itália	Bovinos	-	-	-	One-tube PCR (gene não especificado)	40 (endométrio uterino)	25%
Chalingiannis el al. (2018)	Grécia	Carrapatos de animais domésticos (bovinos, ovinos e cães)	-	-	-	qPCR (IS1111)	179 (carrapatos inteiros)	17,9%
Klemmer et al. (2018)	Egito	Camelos e ruminantes domésticos	528 camelos, 840 bovinos, 304 búfalos, 716 ovinos e 311 caprinos	ELISA (IDEXX CHEKIT Q fever Antibody ELISA Test Kit)	40.7% camelos 19.3% bovinos 11.2% búfalos 8.9% ovinos 6.8% caprinos.	-	-	-
Ryan et al. (2018)	Irlanda	Tanques de leite	1484	Kit comercial de ELISA para *C. burnetii*	21,6%	-	-	-

3.5 *Coxiella burnetii* em ruminantes selvagens

O contato do ser humano com animais selvagens tem sido considerado um fator de risco para a infecção humana com *C. burnetii* (WHITNEY *et al.,* 2009). González -Barrio *et al.* (2016) mostraram a existência de mais de 15 genótipos de *C. burnetii* circulando em animais selvagens, dentre eles veados-vermelhos (*Cervus elaphus)* e coelhos (*Oryctolagus cuniculus*) na Península Ibérica.

Devido à sua distribuição (praticamente toda a Europa), densidade populacional (aproximadamente 70/km²), manejo (fazendas de produção e jogos de caça) e comportamento gregário, veados vermelhos foram considerados um dos reservatórios mais importantes para *C. burnetii* em toda a Europa (GONZÁLEZ-BARRIO et al., 2015a). O **Quadro 2** e **Figura 2** mostram a ocorrência do parasita em cervídeos em diferentes países.

Quadro 2. Ocorrência sorológica e molecular de *Coxiella burnetii* em cervídeos no mundo.

Referência	**País**	**Espécie(s) de cervídeos**	**Número de animais amostrados**	**Teste Sorológico (antígeno)**	**Animais positivos - Sorologia (%)**	**Teste Molecular (gene-alvo)**	**Animais positivos-PCR (%)**
Ruiz-Fons et al. (2008)	Espanha	Veados-vermelhos (*Cervus elaphus*), gamo (*Dama dama*), corça (*Capreolus capreolus*)	168	RIFI	29%, 0% e 15,4%, respectivamente	nPCR (*htpB*) para amostras de sangue	14,7% (*Cervus elaphus)*, 0%(*Dama dama*) e 0%(*Capreolus capreolus*)
Dorko et al. (2009)	Eslováquia	Gamo (*Dama dama*)	60	iELISA (antígeno bruto da amostra Nine mile)	5% (fase I) e 28,3% (fase II)	-	-

Castillo et al. (2010)	Península Ibérica (Espanha)	Veados-vermelhos (*Cervus elaphus*)	137	iELISA (Fie`vre Q serum monocupul, Institut Pourquier, Montpellier, 34090, France)	3,64%	-	-
Rijks et al. (2011)	Países Baixos	Corça (*Capreolus capreolus*)	79	-	-	qPCR (IS1111a) de pulmão, baço, medula óssea e rim	23% em um ou mais órgãos
Astobiza et al. (2011)	Espanha (região norte)	Corça (*Capreolus capreolus*)	106	-	-	cPCR (IS1111) de baço e pulmão	5,1% (não especificado de qual órgão)
Kirchgessner et al. (2012)	Estados Unidos	Veado-de-cauda-branca (*Odocoileus virginianus*)	1059	RIFI	14,64%	-	-
Kirchgessner et al. (2013)	Estados Unidos	Veado-de-cauda-branca (*Odocoileus virginianus*)	1069	RIFI	14,50%	-	-
Ohlson et al. (2014)	Suécia	Alce (*Alces alces*)	99	Teste de Fixação de Complemento (antígeno produzido em cultura de tecido de rim de macaco para anticorpos contra fase I e fase II)	0	-	-
Shin et al. (2014)	Coréia	Veado-d'água-chinês (*Hydropotes inermis*)	196	iELISA (ELISA CHEKIT Q-fever test (IDEXX Laboratories, Westbrook, ME, U.S.A.)	9,18%	qPCR (*IS1111*) de amostras de soro	6,63%

Candela et al. (2014)	França	Corça (*Capreolus capreolus*)	245	iELISA (CHECKIT Q fever IDEXX Diagnostics)	11,26%	-	-
Tavernier et al. (2015)	Bélgica (região de Flandres)	Corça (*Capreolus capreolus*	190	iELISA (iELISA kit LSI Fie`vre Q Ruminants Serum (Laboratoire Service International, Lissieu, France)	1,2%	-	-
Cumbassá et al. (2015)	Portugal	Veados-vermelhos (*Cervus elaphus*)	2	-	-	nPCR (IS*1111*) de amostras de baço e linfonodos	0%
Kreizinger et al. (2015)	Hungria	Veados-vermelhos (*Cervus elaphus*), gamo (*Dama dama*), corça (*Capreolus capreolus*)	91	-	-	qPCR (*IS1111*) de placenta	2.2% (do total), sendo apenas 2 (5,5%) *Cervus elaphus*
González-Barrio et al. (2015B)	Espanha (região sul)	Veados-vermelhos (*Cervus elaphus*)	482	iELISA (LSI Q fever ruminant serum/milk ELISA kit (Life Technologies, Grand Island, NY, USA)	36%	nPCR (*htpB*) de swabe vaginal	26,9%
Fernández-Aguilar et al. (2016)	França	Veados-vermelhos (*Cervus elaphus*), gamo (*Dama dama*), corça (*Capreolus capreolus*)	188	iELISA ((Q-Fever Antibody Test Kit; IDEXX, Westbrook, Maine, USA)	2,4%(*Cervus elaphus)*, 0% (*Dama dama*) e 0% (*Capreolus capreolus*)	-	-
Ebani et al. (2016)	Itália (região central)	Veados-vermelhos (*Cervus elaphus*)	60	-	-	cPCR (IS*1111a*) de amostras de sangue	10%

Candela et al. (2017)	Espanha	Veados-vermelhos (*Cervus elaphus*), gamo (*Dama dama*), corça (*Capreolus capreolus*)	878	iELISA (mixture of *C. burnetii* phase I and II antigens [Nine Mile strain])	1,4% (*Cervus elaphus*), 0,18% (*Dama dama*) e 0% (*Capreolus capreolus*)	-	-
San-Miguel Ayanz et al. (2017)	Espanha (região central)	Veados-vermelhos (*Cervus elaphus*)	389	iELISA (LSIVet Ruminant Q Fever commercial test (LSI, Lissieu, France)	0	-	-

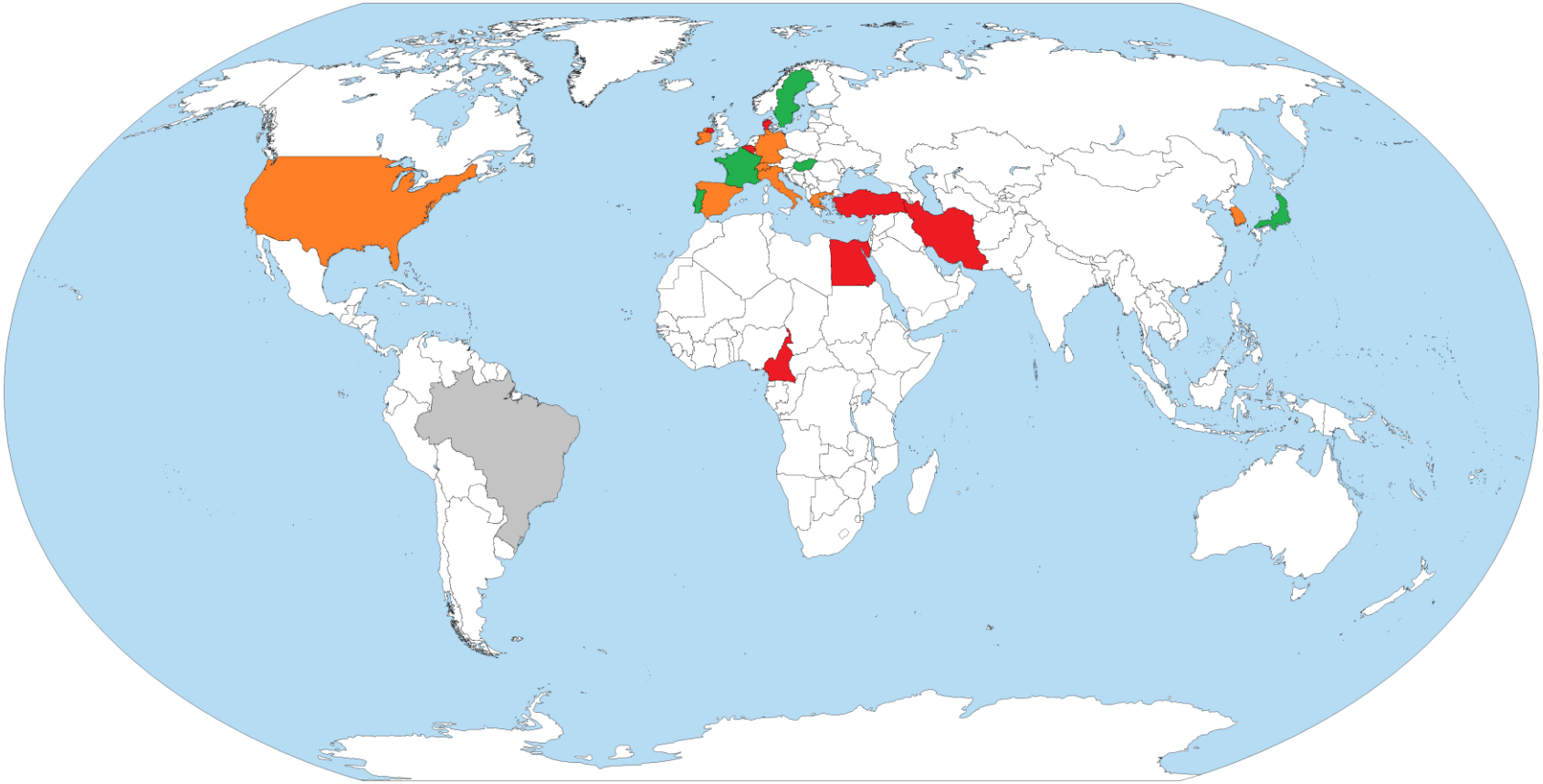

Figura 2. Mapa evidenciando a localização de estudos sobre ocorrência de *Coxiella burnetii* em bovinos e cervídeos no mundo. Verde: Estudos em cervídeos; Vermelho: Estudos em bovinos; Laranja: Estudos em cervídeos e bovinos; Cinza: Dissertação atual. Autoria: Diego Carlos de Souza Zanatto.

3.6 Abordagem *One Health*

A abordagem *One Health* visa otimizar a saúde pública, animal e ambiental (MORI et al., 2018). Neste sentido, a febre Q é um excelente exemplo da necessidade de aplicar este conceito: seres humanos podem ser infectados por aerossóis contaminados do ambiente gerados por secreções contaminadas (leite, fezes, urina ou materiais de nascimento), que por sua vez foram eliminados por mamíferos infectados (Angelakis e Raoult, 2010). Esse cenário pode favorecer as interações entre humanos (hospedeiros suscetíveis), animais (reservatórios) e o ambiente, possibilitando a transmissão de *C. burnetii* entre cervídeos, bovinos e humanos.

Antes de 2005, a bactéria *C. burnetii* era conhecida por circular entre os animais, não sendo incriminada como um agente etiológico causador de enfermidade significante em seres humanos. Após esse ano, 28 fazendas de cabras leiteiras e 2 fazendas de ovelhas leiteiras sofreram abortamentos causados por *C. burnetii* entre 2005 e 2009 na Holanda. Além disso, aproximadamente 4.000 casos de febre Q em humanos foram diagnosticados entre 2007 e 2010 no mesmo país (ROEST et al., 2011b). A genotipagem de *Coxiella burnetii* em amostras clínicas de cabras e humanos revelou que um genótipo havia sido transmitido de caprinos para seres humanos. Neste sentido, a ação colaborativa de médicos e pesquisadores das áreas de Medicina Humana e Veterinária foi um fator-chave no controle da doença (ROEST et al., 2011a; TILBURG et al., 2012). Este é um exemplo perfeito da necessidade do emprego da abordagem *One Health*, destacando o potencial que *C. burnetii* possui para causar surtos.

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Capítulo 2 - Evidence of exposure to *Coxiella burnetii* in neotropical free-living cervids in South America

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Abstract

Coxiella burnetii (order Legionellales, family Coxiellaceae), the etiological agent of Q fever, is a pleomorphic, obligate Gram-negative intracellular bacillococcus that can infect humans and animals. Among the mammals hosting this agent, both domestic and wild ruminants are of particular economic and public health importance. Ticks and other hematophagous arthropods or aerosols are incriminated in the transmission between reservoirs and

susceptible hosts. This study used serological and molecular methods to investigate the *C. burnetii* occurrence in blood samples from free-living deer (143 *Blastocerus dichotomus*, 27 *Mazama gouazoubira*, 4 *M. bororo*, 3 *M. americana,* and 11 *Ozotocerus bezoarticus*) sampled in Mato-Grosso do Sul, São Paulo, Goiás and Paraná states in Brazil. The DNA extracted from the blood samples of 188 cervids was submitted to nested (n) PCR for *C. burnetii* based on the IS1111 repetitive insertion element of the heat shock protein (*htpAB*) gene. Additionally, 169 serum samples were submitted to Indirect Immunofluorescence Assay (IFAT) to detect Ig antibodies to *C. burnetii*. The nPCR results indicated that all blood samples were negative, evidencing the absence of circulating *C. burnetii* DNA in cervids from the studied regions or, alternatively, the *C. burnetii* DNA concentration in the deer blood samples was below the threshold of the used PCR technique. On the other hand, 5.32% (9/169) of deer were seropositive for *C. burnetii* by IFAT, with titers ranging from 256 and 16384. In conclusion, the present work showed the first evidence of exposure of free-living deer to *C. burnetii* in Brazil.

Keywords: deer; *Blastocerus dichotomus*; *Mazama* spp., Q fever; serology; Brazil

1. INTRODUCTION

Q fever is a disease caused by *Coxiella burnetii*, a Gram-negative obligate intracellular bacterium that infects several cell types (mainly phagocytes) of humans and animals (Van Schaik et al, 2013; Abdel-Moein and Hanza, 2017; Eldin et al., 2017). Although inhalation of contaminated aerosols from infected neonates or contaminated excreta is considered the main

transmission mode, the vector transmission by hematophagous ectoparasites is also of great importance in the *C. burnetii* transmission (Eldin et al., 2017). Besides being a zoonotic agent in several countries around the world (Maurin & Raoult, 1999), *C. burnetii* was also classified as a potential bioterrorism agent by the Centers for Disease Control and Prevention (CDC).

In endemic areas, Q fever is usually associated with risk-related activities (work on farms, slaughterhouses or rural tourism) involving contact with animals (Eldin et al., 2017), since cattle, sheep, and goats are the main reservoirs associated with the occurrence of the disease in humans (Eldin et al., 2017).

Additionally, wild animals, including cervids, may also act as the reservoirs for *C. burnetii* (Davoust et al., 2014). In fact, in the Northern Hemisphere, the transmission potential of *C. burnetii* from cervids to humans and from livestock to humans is similar, supported by the fact that *C. burnetii* individual and population seroprevalence among red deer (*Cervus elaphus*) is similar to that of domestic ruminants in the United States of America and Spain (Alvarez et al., 2012, Astobiza et al. 2012; Hamlin et al., 2000). Since *C. burnetii* is most commonly transmitted to humans via inhalation of contaminated aerosols (Eldin et al., 2017), the risk of pathogen transmission from deer to livestock and humans depends on the contact between them. Thus, extensively reared domesticated ruminants, hunters and people involved with deer management and conservation would be at greater exposure risk (Gonzales-Barrio et al., 2015).

Q fever outbreaks can occur at the national level, as observed in the Netherlands, where approximately 4,000 people were infected between 2007 and 2010 (Delsing et al., 2010; Schimmer et al., 2008; Karagianis et al., 2007).

Among livestock, reproductive losses range from 5 to 50% in sheep herds and up to 90% in goat herds. Although reproductive losses may also occur in cattle, significant outbreaks have not yet been reported for this species so far. In Germany, 0.5-4% of all abortions in cattle were attributed to *C. burnetii*; however, female death due to this disease is uncommon in domestic ruminants (Center for Food Security and Public Health, 2017).

In Brazil, serological evidence of exposure to *C. burnetii* has been reported in dogs (15.3%, 2/14), sheep (66.6%, 2/3) and goats (50%, 5/10) in Rio de Janeiro, and in goats (2.1%, 9/412) and sheep (2.2%, 9/403) in Pernambuco (Souza et al., 2018). Besides, serological evidence of human exposure has been reported in Minas Gerais (3.9%, 17/437) and Rio de Janeiro (3.2%, 4/125, 100%, 1/1) (Costa et. al., 2005; Lamas et al., 2009; Rozental et al., 2012). Furthermore, *C. burnetii* has been already detected at the molecular level in blood samples from dogs (14.3%, 2/14), goats (60%, 6/10) and rodents (4.6%, 6/131) (Mares-Guia et al., 2014; Rozental et al., 2017) and in the serum sample of a human patient with fever, myalgia, and dry cough symptoms (Rozental et al., 2012) in Rio de Janeiro, southeastern Brazil.

Although several studies have been conducted on the occurrence and genetic diversity of *C. burnetii* in deer populations from several locations around the world (González-Barrio et al., 2016; San-Miguel et al., 2017; Candela et al., 2014; González-Barrio et al., 2015a,b; Ruiz-Fons et al., 2008; Cumbassá et al., 2015; Ebani et al., 2016), to the best of the authors' knowledge, there is no report on the occurrence of *C. burnetii* in wild ruminants in Brazil up to now. Since deer populations are important reservoirs of *C. burnetii* in Europe, the circulation of *C. burnetii* in Neotropical cervids was hypothesized. This work

aims at investigating the occurrence of *C. burnetii* in free-living cervids from several Brazilian regions, using serological and molecular methods, to determine the possible role of wild ruminants in an eventual Q fever epidemiology in Brazil.

2. MATERIAL AND METHODS

2.1 Ethical Statement

Deer blood sampling was conducted by Professor José Maurício Barbanti Duarte (IBAMA Registration number 263703), from the Department of Animal Sciences, FCAV – UNESP, Jaboticabal, with license number 10636-1 provided by IBAMA, between 1996 and 2011.

2.2 Studied sites and sampled animals

2.2.2 *Blastocerus dichotomus* capture and sampling

The Pantanal deer (*B. dichotomus*) was captured in the marginal floodplains of the Paraná River, between the mouths of the Aguapeí and Paranapanema rivers (in São Paulo and Mato Grosso do Sul), an area affected by the construction of the Sérgio Motta Hydroelectric Plant (former Porto Primavera) (SACCHI et al., 2012). The region tropical climate is marked by humid summer and dry winter, with high and low temperatures of 40 and 10ºC, respectively, high annual rainfall (between 1200 mm and 1400 mm), and about 80% relative humidity. This flooding region is an ecologically important area of the Paraná River Basin (Travassos, 2001).

During the development of the Pantanal deer Project in Porto Primavera, between 1998 and 2002, *B. dichotomus* specimens were captured following the methodology described by Duarte (2008). The animals were captured in the flooded region resulting from the dam construction, comprising the floodplain and ciliary forest areas adjacent to the Paraná River and its tributaries, such as Peixe, Verde, and Aguapeí rivers.

The animals were divided into four groups according to the capture local and time (Figures 1 and 2):

The region between the Quebracho and Bataguassú villages comprising the floodplain areas of the Parana river was the most affected by the lake formed during the construction of the “Sérgio Motta” Hydroelectric plant. In this work, the area is referred to as a) **MS01 (100 animals),** before the first flooding, and b) **MS02 (18 animals),** two years after filling the first flooding quota (Lemes et al., 2003; Andriolo et al., 2001).

The Peixe River region, referred to as **PX**, is formed by the Peixe river, one of the main tributaries of the Paraná river. The studied population (**seven animals**) refers to the first flood quota (quota 253 m) between November 1998 and December 2000 (Piovezan et al., 2001). This area originally consisted of floodplains already badly degraded due to cattle breeding in the region.

The Aguapeí River region, referred to as **WATER,** is formed by the Aguapeí river, a left bank tributary of Paraná river. This wildlife refuge shelters a Pantanal deer density of about 0.0043 animal/ha (Andriolo et al., 2001). A total of **18 animals** were captured two years after the first flood quota was filled. This region, monitored between April 2001 and November 2002 (Lemes et al., 2003), was not directly affected by the reservoir filling, although it could have been

indirectly affected by the animals migrating from the flooded areas to this region.

2.1.3. *Mazama* spp. and *Ozotocerus bezoarticus* sampling

Between 1996 and 2011, 34 and 11 blood samples were collected from *Mazama* spp. and *Ozotoceros bezoarticus*. Among these animals, 21 *M. gouazoubira* and 11 *O. bezoarticus* were captured in the Pantanal Sul Matogrossense (MS); 4 *M. gouazoubira* in the region of the Serra da Mesa Hydroelectric Power Plant (GO); 4 *M. bororo* and 2 *M. gouazoubira* in the Intervales State Park (SP); and 3 *M. americana* in the Iguaçu National Park (PR) (Figure 1).

2.1.4 Methodology for collecting deer blood samples

After chemical immobilization following the standards adopted by Nunes et al. (2001), each deer was tagged with the capturing site GPS coordinates. Blood was drawn from the animal jugular vein, using vacutainer-type tubes with and without anticoagulant. The blood samples collected in heparinized tubes were centrifuged (10 minutes, 1500 x G) and a Pasteur pipette was used to separate 1 mL of the impure leukocyte ring (with red blood cells). The leukocyte ring was then placed in a vial containing 4 mL of freezing medium. After homogenization, the material was transferred to vials and maintained at 4°C for four hours to stabilize, and then exposed to liquid nitrogen (N_2) for 30 minutes,

followed by further immersion and stored in the liquid nitrogen canister (-196°C) (Duarte et al., 1999).

2.2. Indirect immunofluorescence assay (IFAT)

Serum samples from 129 *B. dichotomus* serum samples (86 MS01, 18 MS02, 18 WATER, and 7 PX), 25 *Mazama guazoubira*, 3 *M. bororo,* and 9 *Ozotoceros bezoarticus* were analyzed by IFAT for detecting IgG antibodies (anti-phase I) to *C. burnetii*, using slides covered with the At12 strain crude antigen (PACHECO *et al*., 2013).

Serum samples were diluted 1:64 in phosphate buffered saline, PBS pH 7.4 (130 mM NaCl, 2.7 mM KCl, 5.6 mM Na_2HPO_4, 1.0 mM KH_2PO_4, 0.8 mM NaH_2PO_4). A 20 µL aliquot of each diluted serum sample was deposited in each well of the slides containing *C. burnetii* (At12 strain) antigens (Faculdade de Medicina Veterinária e Zootecnia, São Paulo, São Paulo, Brazil). The controls used were goat sera previously known to be positive and negative for *C. burnetii* in the IFAT (Souza et al., 2018). The slides were incubated at 37°C for 30 minutes in a humid chamber, washed in buffer solution (buffered phosphate saline, PBS pH 7.4 + 1% Triton), and dried. Subsequently, 20 µL of fluorescein isothiocyanate-labeled conjugates and diluted as directed by the manufacturer were added to each slide (Sigma-Aldrich®, St. Louis, Missouri, USA) (anti-deer IgG, diluted 1:10 for test samples and anti-goat IgG, diluted 1: 1,000 for positive and negative controls). The slides were then incubated at 37ºC in a humid chamber for an additional 30 minutes. During the washes, 1.5 mL of Evans Blue was added to the solution. After further washing and drying, the slides were

analyzed under ultraviolet light emission microscopy (Olympus BX60). The reaction was positive when fluorescence was observed in the cells, compared to positive and negative control samples.

2.3. DNA extraction from deer buffy coat samples

DNA samples from 143 *B. dichotomus* (100 MS01, 18 MS02, 18 WATER, and 7 PX), 27 *Mazama guazoubira*, 3 *M. americana*, 4 *M. bororo,* and 11 *Ozotocerus bezoarticus* were extracted from 200 µL buffy coats containing red blood cells, using the QIAamp DNA Blood Mini Kit (QIAGEN) and following the manufacturer's specifications. The DNA samples were properly labeled and submitted to NanoDrop (Thermo Scientific®, San Jose, CA, USA) spectrophotometric analysis of the DNA concentration, as well as the 260/280 and 260/230 ratios. The DNA samples were stored at -20°C.

2.4. PCR endogenous control

To eliminate false negative results due to the presence of inhibitors, *the* DNA samples were submitted to a conventional PCR to amplify the mammals-*gapdh* gene (Birkenheuer et al., 2003).

2.5. Amplification of *C. burnetii* DNA by the nested (n)PCR based on the IS1111 repetitive element of the heat shock protein gene (htpAB)

DNA samples were submitted to nested PCR assay targeting the *htpAB*

gene (485 bp and 266 bp in the first and second reactions, respectively), following a previously described protocol (Fenollar *et al.,* 2004a). The *C. burnetii* DNA positive control was kindly provided by Prof. Dr. Renato Arruda Mortara (Universidade Federal de São Paulo, São Paulo, SP, Brazil).

The amplified products were submitted to horizontal electrophoresis in 1.0% agarose gel stained with Ethidium Bromide (0.5 µL/mL) in TEB run buffer at pH 8.0 (44.58 M Tris-base; 44 M boronic acid, 12.49 mM EDTA). Electrophoresis was performed at 90 V/150 mA for 60 minutes. A 100bp molecular weight marker (Life Technologies®) was used to determine the size of the amplified products. The results were visualized and analyzed using an ultraviolet light transilluminator (ChemiDoc MP Imaging System, BIO RAD®).

2.6. Statistical analysis

The frequency of animals seropositive for *C. burnetii* was analyzed by the Fisher exact test of the R Project for Statistical Computing software (R version 3.4.4). A *p-value* <0.05 indicated a significant difference in the number of seropositive animals in each region.

3. RESULTS

The IFAT results indicate that 5.32% (9/169) of the sampled deer was seropositive for *C. burnetii*, with titers ranging from 256 to 16384. The seropositive animals were distributed as follows: 8.0% (2/25) *M. gouazoubira,* both individuals from the Nhumirim farm in the Pantanal (MS); and 5.43% (7/129) *B. dichotomus*, of which 5 (5.81%) from the MS1 region, 1 (14.28%)

from the PX region, and 1 (5.55%) from the WATER region (Table 1; Figure 4). The seropositivity rate among the deer population sampled in each of the studied regions was not statistically different (P = 0.7237).

Even though all DNA samples were positive in the endogenous control PCR for mammals-*gapdh* gene, evidencing that the DNA extraction procedure worked properly, all sampled animals were negative for *C. burnetii* according to the nPCR results.

4. DISCUSSION

This study showed that 5.43% of the neotropical deer sampled in four different geographical regions of Brazil were seropositive to *C. burnetii*, constituting the first evidence of neotropical deer exposure to *C. burnetii* in South America. Among the five deer species sampled, *M. gouazoubira* and *B. dichotomus* specimens had antibodies to *C. burnetii*.

The found results corroborate the *C. burnetii* seropositivity rates reported among deer populations sampled in Europe (0.18% - 36%), albeit slightly lower than that found among deer in Korea (9.18%) (Shin et al., 2014) and in the United States (14.5% - 14.64%), but well below than that found among deer in Japan (56% - 69%) (Ejercito et al., 1993; Ruiz-Fons et al., 2008; Dorko et al., 2009; Castillo et al., 2010; Kirchgessner et al. 2012, 2013; Candela et al., 2014; Gonzales-Barrio et al., 2015b; Tavernier et al., 2015; Fernández-Aguilar et al., 2015; Candela et al., 2017).

Deer are considered one of the most important reservoirs of *C. burnetii* in Europe due to its wide geographic distribution, high demographic density (70

individuals/km²), gregarious behavior, and the fact that these mammals are targeted by hunters (Gonzáles-Barrio et al., 2016; Ruiz-Fons et al., 2008; Dorko et al., 2009; Castillo et al., 2010; Rijks et al., 2011; Astobiza et al., 2011; Ohlson et al., 2014; Candela et al., 2014; Tavernier et al., 2015; Cumbassá et al., 2015; Kreizinger et al., 2015; Gonzáles-Barrio et al., 2015 a,b; Ebani et al., 2016; Fernández-Aguilar et al., 2016; Candela et al., 2017; San-Miguel Ayanz et al., 2017). However, it is noteworthy that in Brazil, the *B. dichomotus* population density ranges from 0.35 to 1.90 individuals/km² in São Paulo, Mato Grosso do Sul and Paraná (Andriolo et al., 2005; Tiepolo et al., 2010; Andriolo et al., 2013; Peres et al., 2017); therefore, this low population density is probably associated with the low number of seropositive deer observed in the present study.

The *Mazama guazoubira* deer seropositive to *C. burnetii* was from the Nhumirim farm, in southern Pantanal in Mato Grosso do Sul, a region dominated by extensive cattle breeding. Since *C. burnetii* can be dispersed via aerosols to distances greater than 30 km (Eldin et al., 2016) and is capable of surviving as small cell variant (SCV) in the environment for long periods (Eldin et al., 2017), it is very likely that this zoonotic agent is distributed in regions close to the areas sampled in the present study. Furthermore, the seropositive deer were found in the Paraná River (PX and WATER regions) and Pantanal (Fazenda Nhumirim) wetlands, environments characterized by wet and frequently flooded areas that could favor the agent maintenance in the environment and its transmission. The Parana River surrounding areas (PX and WATER) and the Pantanal floodplains are characterized by seasonal floods that determine specific ecosystem processes, with the presence of plants and animals adapted to the annual habitat shrinkage and expansion due to the

seasonal hydrological regime (Alho, 2011). The small cell variant (SCV) provides bacteria stability in the environment, conferring high osmotic, mechanical, chemical, heat and desiccation resistance (Eldin et al., 2017). Therefore, the annual average temperatures of 24°C (Marengo et al., 2015) and high humidity in the flood periods also favor the agent maintenance in the environment. In this sense, future studies on bovine populations reared in the vicinity of the sites where deer were captured in this study should be conducted to determine how widespread the agent is among domestic ruminants in Brazil.

Although less than 10% of the tested animals were seropositive to *C. burnetii* (5.43%), the majority (88.88%) of the seropositive deer had high phase I-IgG antibodies titers (512 to 16384), suggesting that these mammals were chronically infected (Peacock et al., 1986).

Herein, IFAT was the serological assay used to investigate the presence of phase I-IgG antibodies to *C. burnetii* in deer blood samples. Ideally, the analysis of both IFAT phase I and II IgG and IgM antibodies is recommended to discriminate between current and past infections. ELISA usually show similar performance to IFAT when it comes to detection of IgM antibodies to *C. burnetii*. Therefore, a proper diagnosis of infection by *C. burnetii* should preferably combine clinical and epidemiological data, serology (both IFAT and ELISA) and PCR, aiming at achieving a comprehensive algorithm (Meekelenkamp et al., 2011).

All sampled deer were negative for *C. burnetti* in nPCR assays based on *the IS1111* repetitive element of the heat shock protein gene (*htpAB*)**.** It has been previously demonstrated that targeting repeated multicopy gene sequences in PCR assays has higher sensitivity than targeting a single copy

gene, without altering its specificity (Fenollar et al., 2004b, Willians et al., 1994). The use of the *htpAB*-associated repetitive element, which exists in 20 copies in the *C. burnetii* genome, has been successfully used for the molecular diagnosis of *C. burnetii* (Willians et al., 1994). Therefore, considering that such a highly sensitive PCR protocol was used in this study, the negative results found in the molecular analysis might be associated with the lack of circulating *C. burnetii* DNA in deer buffy coats at the time of sampling. Besides, based on both IFAT (presence of phase I IG antibodies) and PCR (negativity in nPCR assays) results, we could suggest that the sampled deer were probably chronically infected at the time of blood sampling or, alternatively, might have been exposed to *C. burnetii* in the past. Previous studies reported the detection of *C. burnetii* DNA in deer serum samples, spleen, lung, and placenta tissues and vaginal swabs (Astrobiza et al., 2011; Shin et al., 2014; Kreizinger et al., 2015; González-Bárrio et al., 2015).

One of the possible *C. burnetti* transmission routes is through arthropod vectors. Ticks are important *C. burnetii* vectors among free-living animals (Maurin and Raoult, 1999; Kazar, 2005). *Amblyomma sculptum* and *Ornithodoros rostratus* tick species, which occur in the sampled biome, have been found to be positive for *C. burnetti* in previous studies conducted in Panama and Brazil (Pantanal Sul-Matogrossense), respectively (De Rodaniche and Rodaniche, 1949; Almeida et al. al., 2012). Considering that *A. sculptum* tick infestation has already been reported in deer in the Brazilian Pantanal (Cançado et al., 2017; Onófrio et al., 2007), future studies to detect this agent in ticks parasitizing deer should be conducted in Brazil.

Although *C. burnetii* infections in domestic ruminants have been associated with infertility, abortion, stillbirth, endometritis, and mastitis (Tissot-Dupont and Raoult, 2008; Eldin et al., 2017), little is known about its effects on deer. Thus, the impact of *C. burnetii* infection on the endangered *B. dichotomus* and *M. gouazoubira* deer populations is unknown.

Q fever outbreaks in humans have been mostly associated with the contact with domestic ruminants (Roest et al., 2011a; Georgiev et al., 2013), still, the bacterium ability to infect free-living wild hosts (Ruiz-Fons 2012; Babudier 1959) and its high resistance to the environment (Angelakis & Raoult 2010) make deer a potential reservoir for *C. burnetii*. Based on this, wild hosts can maintain and transmit the pathogen to another free-living wild (González-Barrio et al., 2015A) and domestic animals (Jado et al., 2012), and humans (Tozer et al., 2014). In the Brazilian Pantanal region, due to the extensive animal husbandry practiced in the region (SACCHI et al., 2012), wild and domestic animals are in contact, probably favoring the agent transmission from wild to domestic animals and vice-versa, and consequently to humans.

The One Health approach aims at optimizing the public, animal, and environmental health (Mori et al., 2018). In this sense, Q fever is an excellent example of the need to apply this concept: humans can be infected by contaminated aerosols from the environment generated by contaminated secretions (milk, feces, urine or birth materials), which in turn were eliminated by infected mammals (Angelakis and Raoult, 2010). The studied regions comprise areas of intense anthropic actions. The flooding region scenario has a high ecological value for the Paraná River Basin (Travassos, 2001). Additionally, the tourist potential of the region is widely explored on the Parana

River banks, especially for sport fishing and water sports tournaments (Travassos, 2001). This scenario may favor the interactions among humans (susceptible hosts), animals (reservoirs) and the environment, enabling the transmission of *C. burnetii* among deer, livestock, and humans.

Before 2005, *C. burnetii* was known to be present among animals but was not incriminated as a significant disease etiological agent. After that year, 28 dairy goat farms and 2 dairy sheep farms suffered from abortions caused by *C. burnetii* between 2005 and 2009 in the Netherlands. Besides, approximately 4,000 cases of Q fever in humans were diagnosed between 2007 and 2010 in the same country (Roest et al., 2011b). *Coxiella burnetii* genotyping in clinical samples from goats and humans revealed that one strain had been clonally spread from goats to humans. In this sense, collaborative action from both human and veterinary medicine practitioners and researchers was a key factor in controlling the disease (Roest et al., 2011a; Tilburg et al., 2012). This is a perfect example of the need for employing the One Health approach, highlighting the potential that *C. burnetii* has to start outbreaks.

5. CONCLUSION

This study shows, for the first time, serological evidence of *C. burnetii* exposure in deer in Latin America. Future studies should be conducted to investigate how widespread *C. burnetii* is in wildlife and the real role of neotropical deer in eventual epidemiology of Q Fever in Brazil. The findings presented in the present work are of Public Health concern, highlighting the importance of using the One Health approach to establish collaborative

investigations by veterinarians, physicians and researchers, aiming at moving forward to untangling the epidemiology of *C. burnetii* and Q fever in Brazil.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure captions

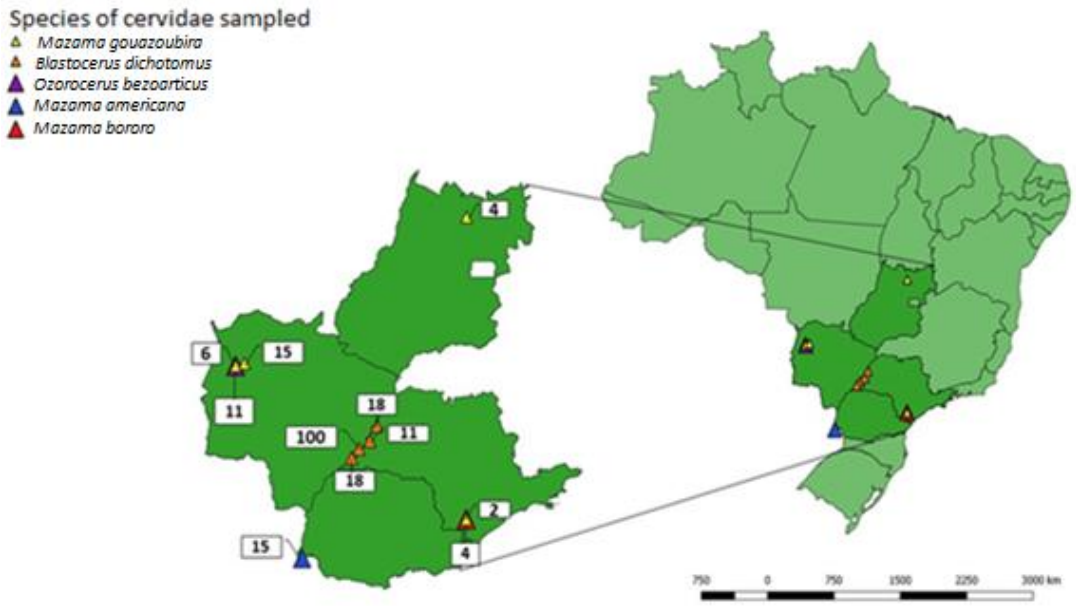

Figure 3. Map showing the location (Brazil) and quantity of biological samples (Cervids) collected, as well as deer species.

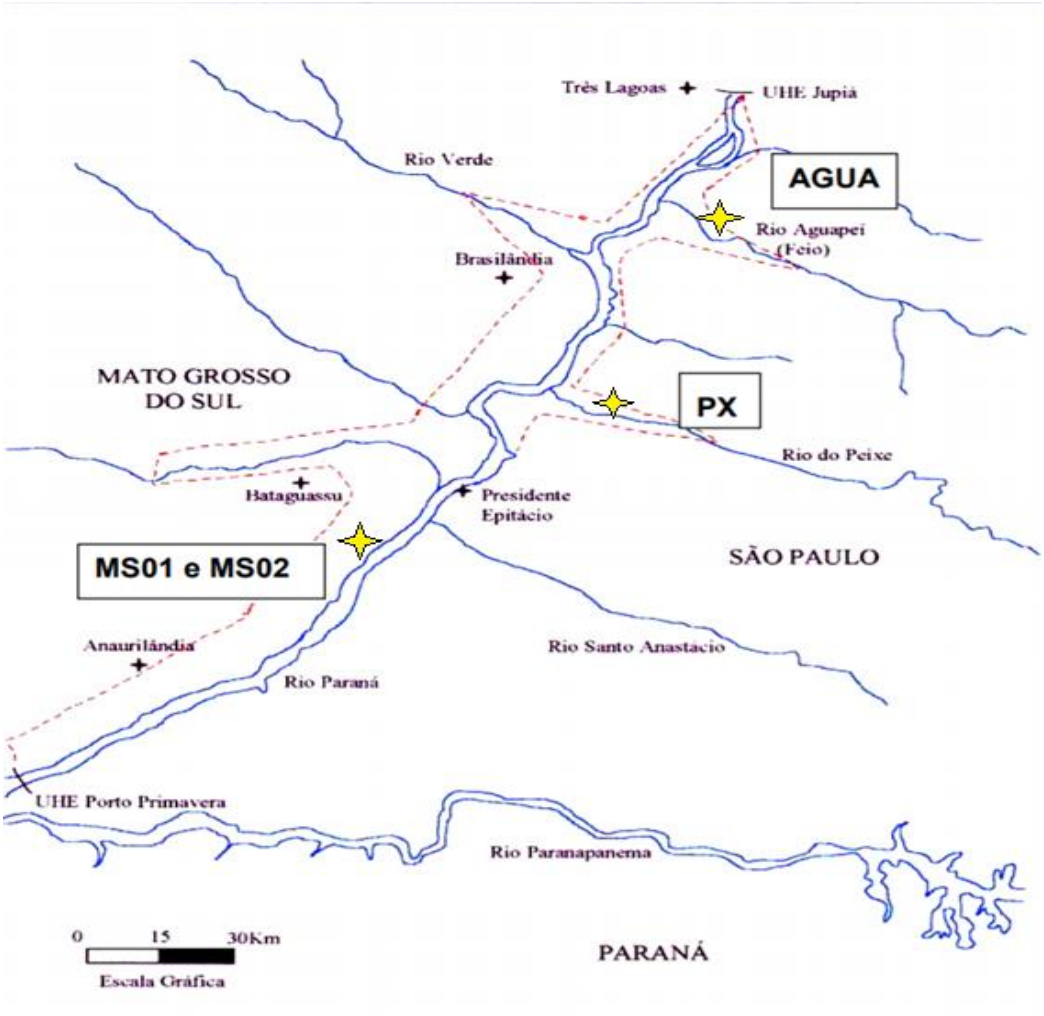

Figure 4. Schematic map showing the study area and capture sites of *Blastocerus dichotomus* deer sub-populations (MS01, MS02, PX01, and WATER), from 1998 to 2002. Source: SACCHI, 2009. Stars show the location of seropositive animals. MS01 is the location *B. dichotomus* specimens were sampling before flooding.

Table

Table 2. Number and origin of deer seropositive in the IFAT assays for detection of IgG antibodies to *Coxiella burnetii* in Brazil.

SAMPLING REGION	NUMBER OF SAMPLED DEER	DEER SPECIES	NUMBER OF SEROPOSITIVE DEER (%)	POSITIVE DEER SPECIES	IGG ANTIBODIES TITRES TO *C. BURNETII*
MS01 (between MS AND SP STATES)	86	*Blastocerus dichotomus*	5 (5.8%) [a*]	5 *B. dichotomus*	512 - 16384
MS02 (between MS AND SP STATES)	18	*Blastocerus dichotomus*	0	-	-
PX (between MS AND SP STATES)	7	*Blastocerus dichotomus*	1 (14.3%)[a]	5 *B. dichotomus*	1024
AGUA (between MS AND SP STATES)	18	*Blastocerus dichotomus*	1 (5.5%)[a]	5 *B. dichotomus*	512
Pantanal – Mato grosso do Sul state (MS)	32	21 *Mazama gouazoubira* *11 Ozotocerus bezoarticus*	2 (6,2%)[a]	2 *M. gouazoubira*	256-512
Serra da Mesa – Goiás state (GO)	4	*Mazama gouazoubira*	0	-	-
Intervales State Park – São Paulo state (SP)	6	2 *Mazama gouazoubira* 4 *Mazama bororo*	0	-	-
Iguazu National Park – Paraná STATE (PR)	3	*Mazama americana*	0	-	-

*Same letters in the same column represent no statistical differences between the sites where seropositive deer to *C. burnetii* were found. Statistical nalyses were performed using Fisher Exact test (P=0.7237).

Capítulo 3 - *Coxiella burnetii* associated with BVDV (Bovine Viral Diarrhea Virus), BoHV (Bovine Herpesvirus), *Leptospira* spp., *Neospora caninum, Toxoplasma gondii* and *Trypanosoma vivax* in reproductive disorders in cattle

Coxiella burnetii associada ao BVDV (Vírus da Diarreia Viral Bovina), BoHV (Vírus do Herpes Bovino), *Leptospira* spp., *Neospora caninum*, *Toxoplasma gondii* e *Trypanosoma vivax* em distúrbios reprodutivos em bovinos

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Abstract

This is a cross-sectional study to assess the presence of antibodies in ruminants against selected pathogens associated with reproductive disorders in

cattle in four Brazilian states, including the zoonotic agent *Coxiella burnetii*. The used tests were Virus Neutralization Assay for IBR and BVD, Microscopic Agglutination Test for *Leptospira* spp., Indirect Fluorescent Antibody Test (IFAT) for *C. burnetii* and *Toxoplasma gondii*, and Enzyme-Linked Immunosorbent Assay for *Neospora caninum* and *Trypanosoma vivax*. Seropositivity for *C. burnetii* was 13.7% with titers from 128 to 131,072; 57.8% for BoHV-1, with titers between 2 and 1,024; 47.1% for BVDV-1a, with titers from 10 to 5,120; 89.2% for *N. caninum*; 50% for *T. vivax*; and 52.0% for *Leptospira* spp., with titers between 100 to 800 (the following serovars were found: Tarassovi, Grippotyphosa, Canicola, Copenhageni, Wolffi, Hardjo, Pomona and Icterohaemorrhagiae); 19.6% for *T. gondii* with titer of 40. This is the first study that has identified *C. burnetii* in cattle associated with BoHV and BVDV, *N. caninum*, *Leptospira* spp., *T. gondii* and *T. vivax*. Thus, future studies should be conducted to investigate how widespread this pathogen is in Brazilian cattle herds.

Key Words: *Coxiella burnetii*, abortion, Q Fever, cattle.

Resumo

Este é um estudo transversal para avaliar a presença de anticorpos em ruminantes contra patógenos selecionados associados a distúrbios reprodutivos em bovinos de quatro estados brasileiros, incluindo o agente zoonótico *Coxiella burnetii*. Os testes utilizados foram Teste de Vírus-Neutralização para BoHV e BVDV, teste de Aglutinação Microscópica para *Leptospira* spp., Reação de Imunofluorescência Indireta for *C. burnetii* e

Toxoplasma gondii, e Ensaio de Imunoabsorção Enzimática para *Neospora caninum* e *Trypanosoma vivax*. A soropositividade para *C. burnetii* foi de 13,7% com títulos de 128 a 131.072; 57,8% para BoHV-1, com títulos entre 2 a 1.024; 47,1% para BVDV-1a, com títulos de 10 a 5.120; 89,2% para *N. caninum*; 50% para *T. vivax*; e 52,0% para *Leptospira* spp., com títulos entre 100 a 800 (sorovares encontrados: Tarassovi, Grippotyphosa, Canicola, Copenhageni, Wolffi, Hardjo, Pomona e Icterohaemorrhagiae) 19,6% para *T. gondii* com título de 40. Este é o primeiro estudo que evidencia a participação de *C. burnetii* em bovinos associada ao Vírus da Rinotraqueíte bovina infecciosa e da diarreia viral bovina, *N. caninum*, *Leptospira* spp., *T. gondii* e *T. vivax* em bovinos. Desta forma, futuros estudos devem ser conduzidos a fim de investigar o quão disseminado se encontra este patógeno em rebanhos bovinos brasileiros.

Palavras-Chave: *Coxiella burnetii*, abortamento, Febre Q, gado.

1. Introduction

Both Brazilian beef and dairy cattle production are prominent in economic scenario worldwide. However, reproductive disorders are a serious obstacle to excellence in production (LUCY, et al., 2001; ROYAL, et al., 2016), and abortion is considered to be one of the biggest causes of economic losses in cattle farming (REICHEL et al., 2013). Considering that infections involving the reproductive system can reduce fertility, the pathogenicity involved infectious agents has been associated with both intrinsic and extrinsic factors (PELLERIN, el al., 1994; STENLUND et al., 2003; GROOMS, et al., 2004), including

management practices, such as vaccination and introduction of new animals into the herd (CHI et al., 2002).

In the center-west of the United States, it was found that approximately 30% of abortions were caused by infectious agents; half of them involved pathogenic bacteria as etiologic agents (KIRKBRIDE, 1992). Infectious-parasitic agents associated with reproductive disorders in ruminants include *Coxiella burnetii,* Bovine Herpesvirus (BoHV) and bovine viral diarrhea (BVD) virus, *Neospora caninum* (KIKBRIDE, 1992), *Leptospira* spp, (MURRAY, 1990), *Toxoplasma gondii* (BÁRTOVÁ et al., 2009) and *Trypanosoma vivax* (SILVA et al., 1996, 1998).

Coxiella burnetii, a Gram-negative obligatory intracellular bacterium (VAN SCHAIK et al, 2013; ABDEL-MOEIN & HAMZA, 2017), was reported for the first time in 1930 and has been detected worldwide ever since, except in New Zealand (MAURIN & RAOULT, 1999; ELDIN et al., 2017). This parasite is the zoonotic agent that causes Q fever (MAURIN & RAOULT, 1999). It was also considered as a potential bioterrorism agent by the Center for Disease Control and Prevention (CDC, 2018). Although the main route of infection for humans is via aerosols (MAURIN & RAOULT, 1999; PARKER et al., 2006; TISSOT-DUPONT & RAOULT, 2008), ingestion of contaminated food and infected ticks may also represent alternative routes of transmission of the parasite (ELDIN et al., 2017).

In humans, this agent has been associated with pneumonia, a syndrome similar to influenza and hepatitis; in some cases, pruritus, pericarditis, myocarditis, encephalitis, and osteomyelitis have also been observed (MAURIN & RAOULT, 1999; TISSOT-DUPONT & RAOULT, 2008). The chronic form of

the infection can cause endocarditis, which is usually associated with valvular heart disease and immunosuppression. Although less commonly, individuals can develop granulomatous lesions in the bones, joints, liver, lungs, testicles and other tissues (RALPH et al. 2007, TISSOT-DUPONT & RAOULT, 2008).

The first serological evidence of exposure to *C. burnetii* infection in Brazil occurred in the 1950s in humans in the state of São Paulo (BRANDÃO et al., 1953) and in cattle in the state of Rio de Janeiro (TRAVASSOS et al., 1954 apud Oliveira et al., 2018). Ever since, serological and molecular evidence of circulation of the parasite has been found in humans in São Paulo (BRANDÃO et al., 1953; VALLE et al., 1955; SICILIANO et al., 2015), Rio de Janeiro (LAMAS et al., 2009, 2013; LEMOS et al., 2011, 2018; ROZENTAL et al., 2012, 2018; MARES-GUIA et al., 2016), Minas Gerais (RIEMANN et al., 1974; COSTA et al., 2005, 2006) and Bahia (SICILIANO et al., 2008).

The main clinical signs associated with this infection in ruminants are infertility, abortion, stillbirth, endometritis and mastitis (TISSOT-DUPONT & RAOULT, 2008). Although *C. burnetii* is a zoonotic agent associated with the occurrence of abortion in ruminants all over the world, there are few studies about the occurrence of this agent in ruminants in Brazil (TRAVASSOS et al., 1954 apud Oliveira et al., 2018; MARES-GUIA et al., 2014; GUIMARÃES et al., 2017; OLIVEIRA et al., 2018; SOUZA et al., 2018). Therefore, the objective of this study was to investigate the frequency of antibodies against ruminant-selected pathogens, namely *C. burnetii*, BoHV-1, BVDV, *N. caninum*, *Leptospira* spp, *T. gondii* and *T. vivax* in serum samples from cattle with a history of reproductive problems from four Brazilian states.

2. Materials and Methods

2.1 Sample selection

This is a cross-sectional study to assess the presence of antibodies to ruminant-selected pathogens associated to reproductive disorders in cattle from four Brazilian states, including the zoonotic *C. burnetii*. The total of 12 proprieties (**Supplementary Table**) were conveniently selected from the Reproductive Viruses Center (São Paulo State University, Unesp, Jaboticabal - São Paulo, Brazil). The total of 102 serum samples were obtained from cows showing a history of reproductive disorders. All sample sets were located in the central-western and southeastern regions of Brazil.

2.2 Eligibility criteria

Study animals consisted of adult cows (> 24 months) from productions (milk and breeding) (i.e., commercial cattle production). All samples were obtained from the sampled farms between December, 2014 and November, 2015.

2.3 Bovine serum samples

Between 2014 and 2015, serum samples collected from 102 cattle presenting reproductive disorders were selected by convenience from four Brazilian states, São Paulo (José Bonifácio, Urânia and Torrinha), Minas Gerais

(Espera Feliz, Patos de Minas, Lagoa Grande and Coromandel), Mato Grosso do Sul (Anastácio) and Goiás (Nova Caixás and Goiatuba). While the proprieties A, B, G and L were composed by beef cattle, the proprieties C, D, E, F, H, I, J and K were composed by dairy cattle (**Suplementary Table**). Serum samples were stored at -20°C until all serological assays were performed.

2.4 Virus neutralization (VN) assay for detection of BVDV-1 and BoHV-1

The selected bovine serum samples were tested by virus neutralization (VN) assay for the detection of antibodies to BVDV-1 and BoHV-1, as recommended by the "*Manual of Diagnostics Tests and Vaccines for Terrestrial Animals*" (OIE, 2018a,b), with modifications. For this purpose, "Madin-Darby bovine kidney (MDBK)" cell line and cytopathic strains of BVDV-1a (Singer) and BoHV-1 (Nebraska), were used. All seropositive samples were tested twice in order to estimate the geometric mean of titer (GMT) values. In addition to the internal controls, positive and negative controls were also used (GATTO et al. 2018). A sample was considered positive when the total neutralization of 100 $TCID_{50}$ occurred in the serum and no cytopathic effect (CPE) was observed in the cell layer in serum dilutions higher than 1:10 (BVDV) and 1:2 (BoHV-1) (OIE, 2018a, b).

BVDV-1

The serum samples were heat-inactivated for 30 minutes at 56°C. All serum samples were tested in duplicate at the same time and were subjected to successive dilutions ranging from 1:10 to 1:5120, using cell culture medium as

diluent. At each dilution of serum, for each sample one well was left without virus, aiming at monitoring for evidence of sample toxicity that could mimic viral cytopathology or interfere with virus replication. An equal volume (50 µL) of a stock of cytopathic strain of BVDV, which contained 100 $TCID_{50}$ (50%) tissue culture infective doses, was added to each well. The plate was incubated for 1 hour at 37°C. Fifty microliters of the cell suspension at 2×10^5 /mL were added to each well. The plate was incubated at 37°C for 4–5 days, either in a 5% CO_2 atmosphere.

BoHV-1

The serum samples were heat-inactivated for 30 minutes at 56°C. All serum samples were tested in duplicate and were subjected to successive dilutions ranging from 1:2 to 1:1024, using cell culture medium as diluent. At each dilution of serum, for each sample, one well was also left without virus in order to monitor the evidence of sample toxicity that could mimic viral cytopathology or interfere with virus replication. An equal volume (50 µL) of a stock of cytopathic strain of BoHV-1, which contained 200 $TCID_{50}$ (50%) tissue culture infective doses, was added to each well. The plate was incubated for 24 hours at 37°C. One hundred microliters of the cell suspension at 3×10^5 cells per well were added to each well. The plate is incubated at 37°C for 3–5 days, either in a 5% CO_2 atmosphere.

2.5 Microscopic Agglutination Test (MAT) for detection of IgG antibodies to *Leptospira* spp.

The *Leptospira* spp. antigens used in serological tests were obtained from bacteria subcultured weekly in liquid EMJH culture medium (Ellighausen, McCullough, Johnson and Harris), with 10% of the medium volume used to seed cultures that were maintained in a bacteriological incubator at 28°C ± 1°C (OIE, 2018c).

The MAT was used to identify the serogroups/serovars. The 24 *Leptospira* serovars used can be found in **Table 1**. Serum samples were diluted in saline, at an initial dilution of 1:50. Aliquots (25 µL) of serum were placed in polystyrene plates with a flat bottom, with an equal quantity of antigens, resulting in a dilution of 1:100. The serum-antigen mixture was homogenized gently and incubated in an environmental incubator at a temperature of 28 °C for 40 to 120 minutes.

Results were read by dark field microscopy with 10× objective, directly from the plate wells. Samples shoeing 50% agglutination were considered to be reactive. Samples reactive at the initial dilution were assayed with serial twofold dilutions from the original 1:100 dilution, as recommended by the OIE (2014). Positive tests were defined as MAT results ≥1:100 for at least one of the 24 serovars (**Table 1**).

2.6 Indirect Fluorescent Antibody Test (IFAT) for detection of IgG anti-*C. burnetii* and anti-*T. gondii* antibodies

The detection of anti-*C. burnetii* IgG antibodies was performed with crude antigen of strain At12 of *C. burnetii*, phase-1 reactive (PACHECO et al., 2013). The serum samples were first diluted at 1:64 in phosphate-buffered saline solution, PBS pH 7.4 (130 mM NaCl; 2.7 mM KCl; 5.6 mM Na_2HPO_4; 1.0 mM KH_2PO_4; 0.8 mM NaH_2PO_4). After that, 20 µL of each diluted serum sample was deposited in wells of slides containing the antigen of *C. burnetii*. The slides were incubated at 37°C for 30 minutes, in a moist chamber. Afterwards, they were washed with Washing Buffer solution (phosphate-buffered saline solution, PBS pH 7.4 + 1% Triton) and then dried. Each slide received 20 µL conjugate (bovine anti-IgG diluted at 1:200) marked by fluorescein isothiocyanate (Sigma-Aldrich®, St. Louis - Missouri, USA) for test samples as well as positive and negative controls. The slides were then incubated for a further 30 minutes at 37°C in a moist chamber. After being washed (this time was added 1.5 ml of Evans Blue on each wash in the washing buffer solution) and dried again, the slides were evaluated through ultraviolet light microscopy. The reaction was considered to be positive when cells were fluorescent at the dilution of 1:64 (PACHECO et al., 2013), according to the protocol previously described by Reeves et al. (2006). Bovine serum samples previously tested for *C. burnetii* and considered non-reactive and reactive were used as negative and positive controls, respectively (GUIMARÃES et al., 2017).

For *T. gondii*, the tachyzoites of the RH strain were used as an antigen, according to a protocol previously described (OLIVEIRA et al. 2008; ANDRÉ et al., 2010). Samples were considered positive when titration was above 40. The

used protocol was similar to that one used for detection of IgG antibodies for *C. burnetii*, with some modifications, such as following: i.) PBS pH 7.4 was used in all washing procedures; ii.) Evans Blue at 10% to the conjugate solution was used instead of the washing solution. IFAT used a serial dilution of the test sera to the log base 2 for titration of antibodies. The positive and negative controls used in this serological assay were serum samples from cattle known to be positive and negative for *T. gondii*, provided by IMUNODOT Diagnósticos (Jaboticabal - São Paulo, Brazil).

2.7 Indirect Immunoassay (iELISA) for detection of IgG antibodies anti-*Neospora caninum* and anti-*Trypanosoma vivax*

The detection of IgG antibodies anti-*T. vivax* was performed with the indirect ELISA (iELISA), according to the protocol established by Machado et al. (1997) and Aquino et al. (1999), with minor adaptations. After purification of the trypomastigote forms, as described by González et al. (2005), and sonication in a 750w Ultrasonic Processor (Coleparmer – Montréal – Quebec, Canada), the protein concentration of the soluble antigen was determined by the bicinchoninic acid method (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, San Diego – California, USA). The total antigen of *T. vivax* was diluted in carbonate-bicarbonate buffer 0.5M and pH 9.6 and its optimal concentration was adjusted to 0.1 µg/mL. After incubation for 12 hours at 8 ºC, blocking was performed with PBS Tween 20 (pH 7.2), and 6% normal rabbit serum was added. The plates (Nunc Maxisorp®, Thermo Fisher Scientific, São Paulo - São Paulo, Brazil) sensitized with *T. vivax* antigen were incubated for 90

minutes at 37ºC, in a moist chamber. After three washes with PBS-Tween 20 buffer, reference positive and negative sera (FIDELIS et al., 2016) and test sera were added to the ELISA plate. All serum samples were diluted 1:400 solution in PBS-Tween 20 plus 5% of skimmed milk powder (Molico®, Nestlé, São Paulo - São Paulo, Brazil). The plates were incubated again at 37ºC for 90 minutes. After three washes with PBS-Tween 20 buffer, the ELISA plate received the anti-bovine IgG antibody conjugate linked to alkaline phosphatase (Sigma®, St. Louis - Missouri, USA) at a dilution of 1:30000 in PBS-Tween 20 plus 5% of skimmed milk powder (Molico®, Nestlé, São Paulo - São Paulo Brazil), with subsequent incubation and washing. Finally, the substrate of the enzyme alkaline phosphatase, p-nitrophenyl phosphate (Sigma®, St. Louis - Missouri, USA), diluted to 1 mg/mL at pH 9.8 buffer diethanolamine (Sigma®, St. Louis - Missouri USA) was added.

The total antigen of *N. caninum* (IMUNODOT®, Jaboticabal - São Paulo, Brazil), at optimal concentration of 0.1 µg/mL, was diluted in carbonate-bicarbonate buffer 0.5M and pH 9.6. After incubation for 12 hours at 8 ºC, blocking was performed with PBS Tween 20 (pH 7.2), and 6% of skimmed milk powder (Molico®, Nestlé, São Paulo - São Paulo, Brazil). The plates (Nunc Maxisorp®, Thermo Fisher Scientific, São Paulo - São Paulo, Brazil) were sensitized with the antigen of this parasite and then were incubated for 60 minutes at 37ºC in a moist chamber. After three washes with PBS-Tween 20 buffer, the ELISA plate received the reference positive and negative sera, supplied with the kit, and tested sera, which were diluted at 1:200 in PBS-Tween 20 solution. The plates were incubated again at 37ºC for 60 minutes. After three washes with PBS-Tween 20, the ELISA plate received the anti-

bovine IgG antibody conjugate linked to alkaline phosphatase (Sigma®, St. Louis - Missouri, USA) at a dilution of 1:30000 in PBS-Tween 20, with subsequent incubation and washing. Finally, the substrate of the enzyme alkaline phosphatase, p-nitrophenyl phosphate (Sigma®, St. Louis - Missouri, USA), diluted at 1 mg/mL at pH 9.8 diethanolamine buffer (Sigma®, St. Louis - Missouri, USA) was added.

The plates were sealed with aluminum foil and incubated for 45 minutes at ambient temperature. Reading was performed in ELISA plate reader (B.S.-100; Embrabio, São Paulo - São Paulo, Brazil), with a 405nm filter. The cut-off point was calculated as 2.5 times the average absorbance of negative control sera (MACHADO et al., 1997).

3. Results

The seropositivity for *C. burnetii* was 13.7% (14/102), with titers ranging from 128 to 131072; 57.8% (59/102) for BoHV-1, with titers ranging from 2 to 1024; 47.1% (48/102) for BVDV-1a, with titers ranging from 10 to 5120; 19.6% (20/102) for *T. gondii*, with titers of 40; 89.2% (91/102) for *N. caninum,* with optical densities ranging from 0.309 to 1.646 (cutoff point: 0.309); 50% (51/102) for *T. vivax,* with optical densities ranging from 0.406 to 2.597 (cutoff point: 0.393); and 52.0% (53/102) for *Leptospira* spp., with titers ranging from 100 to 800 (serovars found: Tarassovi, Grippotyphosa, Canicola, Copenhageni, Wolffi, Hardjo, Pomona and Icterohaemorrhagiae).

Antibodies for *C. burnetii* were found in two animals in the state of Goiás (10%, 2/20), four animals in the state of São Paulo (12.5% 4/32), seven animals in the state of Minas Gerais (17.9%, 7/40) and only one animal in Mato Grosso do Sul (10%, 1/10). All seropositive animals for *C. burnetii* also showed

antibodies to at least one more agents. There were significant differences for the presence of antibodies against *Leptospira* spp., *T. gondii* and BVDV-1 between the animals sampled in the four selected states (**Table 2**).

Among the selected animals presenting reproductive disorders in the present study, a higher seropositivity for *N. caninum* was found. In this sense, antibodies to *N. caninum* were found in 100% (10/10) of the animals sampled in the state of Mato Grosso do Sul, 85% (17/20) of the animals sampled in the state of Goiás, 93.7% (30/32) of the animals sampled in the state of São Paulo, and 85% (34/40) of the animals sampled in the state of Minas Gerais. Six animals showed to be seropositive only for *N. caninum* (**Table 2 and Figure 3).**

4. Discussion

Although several pathogens interfere with the reproductive performance of cattle, e.g., the bovine viral diarrhea virus (BVDV), bovine herpesvirus (BoHV), *Brucella abortus* (occasionally *B. melitensis*), *N. caninum, C. burnetii, Campylobacter fetus venerealis* or *Campylobacter fetus fetus*, *Leptospira* spp., *Tritrichomonas foetus* and *Chlamydia abortus* (AGERHOLM, 2013; BUTZLER, 2004; DAMMAN et al., 2015; DÍAZ APARICIO, 2013; CERQUEIRA-CÉZAR et al., 2017), there is still not enough information on the occurrence and the epidemiological impact of some pathogens on reproductive disorders in ruminants.

Despite the low rate of seropositivity found for *C. burnetii* (13.7%, 14/102) among the sampled bovine population with reproductive disorders, it should be noted that most (71.4%, 10/14) of the seropositive animals showed high antibody titers, ranging from 512 to 131072. Even though high antibody titers

are suggestive of recent infection (GUIMARÃES et al., 2017; OLIVEIRA et al., 2018), the antigen used in the present study is only reactive to phase-1 antibodies, found only in the chronic phase of the disease (PEACOCK et al., 1983). Therefore, it is possible that the seropositive animals were in the chronic phase of the disease at the time of sampling. Additionally, despite the high titers found in the present study, we can't rule out the occurrence of serological cross-reaction with other agents, such as *Legionella* sp. and *Bartonella* sp. (MUSSO & RAOULT, 1997; SCOLA & RAOULT, 1996). Although a previous study reported the occurrence of antibodies to *C. burnetii* in cattle in Brazil (BRANDÃO et al., 1953), the present work showed high antibody titers against this agent in this country.

Although serological evidence of exposure to *C. burnetii* has already been reported in cattle in Europe (CZAPLICKI et al., 2009; AGGER et al., 2010; BOTTCHER et al., 2011; GACHE et al., 2017; RYAN et al., 2018; VARELA-CASTRO et al., 2018; VIDAL et al., 2017; SOFTIC et al., 2018), Asia (CETINKAYA et al., 2000; NOKHODIAN et al., 2016), North America (MCQUISTON et al., 2005) and Africa (KAMGA-WALADJO et al., 2010; SCOLAMACCHIA et al., 2010), there are few data on seroprevalence to this agent in ruminants in Brazil. In this regard, while Guimarães et al. (2017) found a seropositivity rate of 2% for *C. burnetii* among sheep sampled in the state of Piauí, northeast of Brazil, Oliveira et al. (2018) found a seroprevalence of 55.1% in a herd of goats with history of reproductive disorders in the state of Alagoas, Northeast of Brazil. Recently, Souza et al. (2018) found 2.2% of the goats and 2.1% of the sheep seropositive to *C. burnetii* in the state of Pernambuco, also in northeastern Brazil. In southeastern Brazil, Mares-Guia et al. (2014) found

66.6% seropositivity in sheep and 50% seropositivity in goats in the state of Rio de Janeiro.

According to Mori et al. (2018), vaginal swabs samples from cows that aborted within 8 days should preferably be collected aiming at the direct detection of *C. burnetii* by PCR. However, if the abortion occurred a longer time ago or if there is any history of reproductive problem in the herd, serologic tests can be used to verify if there was any contact with *C. burnetii* in the herd. This approach justifies the use of IFAT on cattle serum samples for investigating the occurrence of *C. burnetii* in the cattle showing reproductive disorders in the present study. Unfortunately, additional information regarding the abortion in each sampled property was not available.

In the present study, the seropositive samples were originated from both beef and dairy cattle herds. The ELISA test in milk storage tanks has proved very effective in sorting lactating animals seropositive for *C. burnetii* (VAN DEN BROM et al., 2012; GUATTEO et al., 2007) as well as have produced results concordant with those found from individual serum samples because of the transfer of immunoglobulins from blood to milk (NIELSEN et al., 2011). Thus, future large-scale studies should be conducted in Brazil using this approach aiming at investigating the exposure of dairy cows to the agent under study. The detection of DNA of *C. burnetii*, but not of viable bacteria in dairy products (cheese, yoghurt, butter and creams) in France (ELDIN et al., 2013) emphasizes the need for additional studies in Brazil to investigate the prevalence of this agent in dairy cattle.

Although the main clinical signs associated with Q fever in ruminants are linked to reproduction (infertility, abortion, stillbirth, endometritis and mastitis)

(TISSOT-DUPONT & RAOULT, 2008; ELDIN et. al., 2017), seronegative cows that do not have medical abortion are also capable of spreading the parasite through vaginal discharges (RODOLAKIS et al., 2007). Considering that *C. burnetii* can be dispersed via aerosols at distances greater than 30 Km (ELDIN et al., 2016) and survives for long periods in the environment as a form of resistance (*Small Cell Variant*) (ELDIN et al., 2017), this agent is very likely to be widely distributed in regions close to the areas sampled in this study. Because *C. burnetii* is an agent that can infect a wide range of hosts, from unicellular beings (such as amoeba) to invertebrates, reptiles, birds and mammals (CUTLER et al., 2007; NASPHV et al., 2013; ELDIN et al., 2017), the dispersion of this pathogen in extensive areas seems to be easily achieved. In this sense, the data presented in this study were relevant, both from an economic point of view and for public health, considering that cattle are one of the main reservoirs for human infection (GEORGIEV et al*.,* 2013; NOKHODIAN et. al., 2016). An outbreak of Q fever has been recently described among cadets in the state of Rio de Janeiro, Southeastern Brazil, whose diagnosis was confirmed by serologic tests (LEMOS et al., 2018). Additionally, future studies should be conducted about the participation of ticks in the transmission of *C. burnetii* between animals and humans in Brazil.

In the present study, we have also reported the occurrence of antibodies against BoHV-1, BVDV and *Leptospira* spp. In Brazil, previous seroepidemiological studies have indicated a significant dissemination of BVDV, BoHV-1 (AFFONSO et al., 2010; FINO et al., 2012; FLORES et al., 2013), and *Leptospira* sp. in herds (FÁVERO et al., 2017). While prevalence rates ranging from 22.2% to 85.4% (FLORES et al., 2013; QUINCOZES et al., 2007;

ALMEIDA et al., 2013) have been reported for BVDV, seropositivity rates between 18% and 90% were found for BoHV-1 in non-vaccinated herds in different geographic regions of Brazil (LOVATO et al., 1995, TAKIUCHI et al., 2001, DIAS et al., 2013). The *Leptospira* spp. serovars Hardjo, Wolffi and Grippotyphosa, are the most frequently associated with the occurrence of abortions in cattle (MINEIRO, et al., 2007), which corroborates the results of the present study. The serological results of this study for BVDV, BoHV-1 and *Leptospira* sp. support the evidence that these three etiological agents are disseminated in Brazil and may directly favor the occurrence of reproductive disorders in cattle, thus participating as primary infectious agents or worsening clinical co-infection conditions.

The highest rate of seropositivity (89.2%, 91/102) in the present study was found for the apicomplexan *N. caninum*. Our data corroborate those found previously in other Brazilian states, in which *N. caninum* has been reported as the main causative agent of abortion in cattle. In this sense, seropositivity rates of 23-91.7% to this agent were described in cattle presenting abortions in the states of Rio Grande do Sul, Minas Gerais, Paraná, Rondônia, Mato Grosso and Amazonas (CERQUEIRA-CÉZAR et al., 2017). In Brazil, seroprevalence to *N. caninum* in cattle ranges from 6.7 to 91.7%, depending on the geographic region and cattle breed. Cattle can be infected by ingesting oocysts of the parasite eliminated through feces of Canidae (horizontal transmission) or via the placenta (vertical transmission) (CERQUEIRA-CÉZAR et al., 2017). Transplacental transmission was incriminated the main *N. caninum*'s route of transmission in cattle in the state of Pernambuco, northeastern Brazil, allowing the maintenance of this agent in production systems in the study region

(RAMOS et al., 2016). In this sense, farms that use embryo recipients should take preventive measures to avoid economic losses and perpetuation of the parasite on farms.

While *N. caninum* stands out as one of the most important parasites associated with reproductive problems in cattle, such animals are considered to be resistant to *T. gondii* (CERQUEIRA-CÉZAR et al., 2017), in spite of its worldwide distribution and its association with abortions in small ruminants (WYROSDICK & SCHAEFER, 2015). Toxoplasmosis in animal production is serious because it poses potential risks to public health, as a result of the consumption of non-pasteurized milk and raw or underdone beef, associated with economic losses caused by abortion in small ruminants (TENTER et al., 2000; WYROSDICK & SCHAEFER, 2015). Herein, a seroprevalence of 19.2% (20/102) was found to *T. gondii*, which is lower than that others reported in previous studies (29.1-32.9%) in Brazil (TILAHUN et al., 2018). This fact corroborates results of previous studies that indicate low pathogenicity of *T. gondii* in cattle, with little or no influence on the occurrence of abortion in herds (CERQUEIRA-CÉZAR et al., 2017).

Trypanosoma vivax infects a large number of species of domestic and wild ungulates and, in South America, it is the main etiologic agent of trypanosomiasis in cattle, hence it causes great economic losses (JONES & DÁVILA, 2001). In Brazil, the transmission occurs mechanically by Tabanidae (horseflies) and *Stomoxys calcitrans* (stable fly) (PAIVA et al., 2000; RODRIGUEZ-BATISTA et al., 2005; CADIOLI et al., 2012), or iatrogenically by fomites (CADIOLI et al., 2012). The present study reported a seroprevalence of 50% (51/102) for this agent among the cattle population with reproductive

disorders. This protozoan is currently associated with the occurrence of abortions in different Brazilian regions (DÁVILA & SILVA, 2000; LINHARES et al., 2006; CADIOLI et al., 2012). Previously considered as an endemic agent in cattle herds in the Pantanal region in the Brazilian states of Mato Grosso (SILVA et al., 1996) and Mato Grosso do Sul (PAIVA et al., 1997, 2000), *T. vivax* has been recently causing outbreaks of the disease in several Brazilian states, e.g., Maranhão (FEITOSA et al., 2004), Tocantins (LINHARES et al., 2006), Paraíba (BATISTA et al., 2007), Minas Gerais (CARVALHO et al., 2008), Rio Grande do Sul (SILVA et al., 2009), Pernambuco (PIMENTEL et al., 2012), São Paulo (CADIOLI et al., 2012), Alagoas (NETO, 2015), Santa Catarina (FÁVERO et al., 2016), Goiás (BASTOS et al., 2017), Sergipe (VIEIRA et al., 2017) and Piauí (LOPES et al., 2018). Our study did not find significant differences regarding the presence of antibodies against *T. vivax* across the cows sampled in the four states, which suggests that the parasite is being scattered across the country. Such expansion of its geographical distribution in Brazil is mainly due to the movement of cattle from endemic regions to those where there is a favorable epidemiological situation (climatic conditions favoring the presence of hematophagous dipterans, animals without prior immunity to the parasite in question and long-standing practice of sharing needles contaminated with blood [fomites] during vaccination and application of oxytocin) (CADIOLI et al., 2012; BASTOS et al., 2017).

Although the found results presented information about serological evidence of exposure of cattle to the selected pathogens, it is not possible to determine which one was the real causative agent of the abortion, or if there was an association of agents that could possibly have potentialized the final

outcome of a reproductive disorder. The present study was the first to show *C. burnetii* as a pathogen associated with bovine rhinotracheitis and bovine viral diarrhea viruses, *N. caninum*, *Leptospira* spp., *T. gondii* and *T. vivax* in cattle with abortion in Brazil. Future studies should be conducted to investigate how widespread *C. burnetii* is in Brazilian cattle herds and its real role in the etiology of reproductive problems in cattle in South America, as the single agent causing abortion or in co-infections with other agents.

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Tables subtitles

Table 3. *Leptospira* antigens used in MAT.

Table 4. Seropositive samples for selected bovine pathogens associated to abortion in cattle in Brazil.

Table 5. Seropositivity for parasites associated with abortion in cattle in Brazil according to place of origin.

Supplementary Table. Detailed serological results for selected bovine pathogens associated to abortion in cattle in Brazil.

No	Serogroup	Serovar
1	Australis	Australis
2	Australis	Bratislava
3	Autumnalis	Autumnalis
4	Autumnalis	Butembo
5	Ballum	Castellonis
6	Bataviae	Bataviae
7	Canicola	Canicola
8	Celledoni	Whitcombi
9	Cynopteri	Cynopteri
10	Grippotyphosa	Grippotyphosa
11	Hebdomadis	Hebdomadis
12	Icterohaemorrhagiae	Copenhageni
13	Icterohaemorrhagiae	Icterohaemorrhagiae
14	Javanica	Javanica
15	Panama	Panama
16	Pomona	Pomona
17	Pyrogenes	Pyrogenes
18	Sejroe	Hardjo
19	Sejroe	Wolffi
20	Shermani	Shermani
21	Tarassovi	Tarassovi
22	Andamana	Andamana
23	Seramanga	Patoc
24	Djasiman	Sentot

	% OF SOROPOSITIVITY IN EACH STATE				
PATHOGENS	**Goiás (20)**	**São Paulo (32)**	**Minas Gerais (40)**	**Mato Grosso do Sul (10)**	**Total (102)**
Coxiella burnetii	10% (2)	12.5% (4)	17.5% (7)	10% (1)	13.7% (14)
Leptospira spp.	65% (13) ab	37.5% (12) b	47.5% (19) b	90% (9) a	52.0% (53)
BVDV-1a (Singer)	50 % (10) ab	25% (8) b	62.5% (25) a	50% (5) ab	47.1% (48)
BoHV-1 (Nebraska)	60% (12)	68.7% (22)	50% (20)	50% (5)	57.8% (59)
Neospora caninum	85% (17)	93.7% (30)	85% (34)	100% (10)	89.2% (91)
Toxoplasma gondii	10% (2) b	40.6% (13) a	12.5% (5) b	0% b	19.6% (20)
Trypanosoma vivax	55% (11)	34.4% (11)	55% (22)	70% (7)	50.0% (51)

Different lowercase letters on the same row represent a significant difference ($P < 0.05$), a>b

	Goiás	São Paulo	Mato Grosso do Sul	Minas Gerais	Total
	20	32	10	40	
N.c	1 (0.98%)	1 (0.98%)	-	-	2 (1.96%)
BoHV-1	-	-	-	1 (0.98%)	1 (0.98%)
C.b. + T.g.	-	-	-	1 (0.98%)	1 (0.98%)
N.c. + T.g	-	1 (0.98%)	-	-	1 (0.98%)
N.c. + T.v.	-	-	-	3 (2.94%)	3 (2.94%)
N.c. + BVDV-1	1 (0.98%)	-	-	1 (0.98%)	2 (1.96%)
N.c. + BoHV-1	-	2 (1.96%)	-	1 (0.98%)	3 (2.94%)
***N.c. + L.* sp.**	2 (1.96%)	2 (1.96%)	2 (1.96%)	1 (0.98%)	7 (6.86%)
T.v. + BVDV-1	-	-	-	1 (0.98%)	1 (0.98%)
BVDV-1 + BoHV-1	-	-	-	1 (0.98%)	1 (0.98%)
C.b. + N.c. + T.v.	1 (0.98%)	-	-	-	1 (0.98%)
C.b. + T.g. + BoHV-1	-	1 (0.98%)	-	-	1 (0.98%)
C.b. + N.c. + BVDV-1	-	-	1 (0.98%)	-	1 (0.98%)
***C.b. + N.c. + L.* sp.**	-	-	-	1 (0.98%)	1 (0.98%)
N.c. + T.v. + BVDV-1	-	-	-	2 (1.96%)	2 (1.96%)
N.c. + T.g. + T.v.	-	1 (0.98%)	-	-	1 (0.98%)
N.c. + T.g. + BoHV-1	-	2 (1.96%)	-	-	2 (1.96%)
***N.c. + T.v. + L.* sp.**	1 (0.98%)	-	1 (0.98%)	1 (0.98%)	3 (2.94%)
N.c. + T.v. + BoHV-1	1 (0.98%)	5 (4.90%)	-	1 (0.98%)	7 (6.86%)
N.c. + BVDV-1 + BoHV-1	-	-	-	2 (1.96%)	2 (1.96%)
***N.c. + T.g. + L.* sp.**	1 (0.98%)	-	-	1 (0.98%)	2 (1.96%)
***N.c. + BVDV-1 + L.* sp.**	-	1 (0.98%)	-	1 (0.98%)	2 (1.96%)
T.v. + BVDV-1 + BoHV-1	-	-	-	1 (0.98%)	1 (0.98%)
***BVDB-1 + BoHV-1 + L.* sp.**	1 (0.98%)	-	-	-	1 (0.98%)
***C.b. + T.v. + BVDV-1 + L.* sp.**	-	-	-	1 (0.98%)	1 (0.98%)
***C.b. + N.c. + BoHV-1 + L.* sp.**	-	1 (0.98%)	-	-	1 (0.98%)
***C.b. + N.c. + BVDV-1 + L.* sp.**	-	1 (0.98%)	-	1 (0.98%)	2 (1.96%)
C.b. + N.c. + T.g. + T.v.	-	1 (0.98%)	-	-	1 (0.98%)
C.b. + N.c. + BVDV-1 + BoHV-1	-	-	-	1 (0.98%)	1 (0.98%)
C.b. + N.c. + T.v. + BoHV-1	-	-	-	1 (0.98%)	1 (0.98%)
***N.c. + T.g. + BoHV-1 + L.* sp.**	-	2 (1.96%)	-	-	2 (1.96%)
***N.c. + T.g. + BVDV-1 + L.* sp.**	-	-	-	1 (0.98%)	1 (0.98%)
***N.c. + T.g. + T.v. + L.* sp.**	-	-	-	2 (1.96%)	2 (1.96%)
***N.c. + T.v. + BoHV-1 + L.* sp.**	-	-	1 (0.98%)	2 (1.96%)	3 (2.94%)
N.c. + T.g. + T.v. + BoHV-1	-	1 (0.98%)	-	-	1 (0.98%)
N.c. + T.v. + BVDV-1 + BoHV-1	2 (1.96%)	1 (0.98%)	-	4 (3.92%)	7 (6.86%)
***N.c. + T.v. + BoHV-1 + L.* sp.**	2 (1.96%)	1 (0.98%)	2 (1.96%)	1 (0.98%)	6 (5.88%)
***N.c + BVDV-1 + BoHV-1 + L.* sp.**	3 (2.94%)	2 (1.96%)	-	3 (2.94%)	8 (7.84%)
N.c. + T.g. + BVDV-1 + BoHV-1	-	3 (2.94%)	-	-	3 (2.94%)
***T.v. + BVDV-1 + BoHV-1 + L.* sp.**	1 (0.98%)	-	-	-	1 (0.98%)
***N.c. + T.g. + T.v. + BoHV-1 + L.* sp.**	-	1 (0.98%)	-	-	1 (0.98%)
***N.c. + T.v. + BVDV-1 + BoHV-1 + L.* sp.**	2 (1.96%)	-	3 (2.94%)	2 (1.96%)	7 (6.86%)
***C.b. + N.c. + BVDV-1 + BoHV-1 + L.* sp.**	-	-	-	1 (0.98%)	1 (0.98%)
***C.b. + N.c. + T.g. + T.v. + L.* sp.**	1 (0.98%)	-	-	-	1 (0.98%)

C.b.: Coxiella burnetii; N.c. : Neospora caninum; T.g.: Toxoplasma gondii;

T.v.: Trypanosoma vivax; L. sp.: Leptospira sp.

Table 6 supplemental. Detailed serological results for selected bovine pathogens associated to abortion in cattle in Brazil.

Sample Set ID	State/ Farm	Sample ID	CB	Lepto	Serovar	BVD	IBR	NC	TG	TV
1	Goiás A (beef)	1		200/200	Tarassovi/Grippotyphosa		1024	0.404		0.678
		2		200/100	Wolffi/Grippotyphosa	40	1024			
		3				20		0.433		
		4		200/200	Wolffi/Grippotyphosa	160	2	0.793		
		5		400/400	Canicola/Copenhageni	40	512	0.584		0.56
		6		200	Grippotyphosa			0.42		0.916
		7					1024	0.384		0.542
		8		400	Grippotyphosa	320	256	0.37		
		9		400	Grippotyphosa	80	128	0.873		
		10		800/400	Wolffi/Hardjo	1280	64	0.584		0.448
2		11				80	512	0.49		0.819

	Goiás B (beef)	12	4096	200/100	Wolffi/Hardjo			0.928	0.492
		13		100	Grippotyphosa			0.453	
		14		400	Pomona	40	1024		0.422
		15		200	Wolffi		256	1.847	0.895
		16		100	Icterohaemorrhagiae			0.561	
		17				40	64	0.467	0.777
		18	128					0.503	0.668
		19						0.973	
		20							
		21	4096	200	Grippotyphosa	20		0.634	
3	São Paulo C (milk)	22	32798					1.646	0.559
		23		800/200	Wolffi/Grippotyphosa			0.445	
		24						1.595	40

		25					0.965	40	0.62
4	São Paulo D (milk)	26			320	64	0.833	40	
		27				256	0.554	40	0.523
		28			40	128	0.332	40	
		29				128	0.479		1.299
5	São Paulo E (milk)	30	800/200	Copenhageni/Wolffi		256	0.329	40	0.406
		31	200/200	Wolffi/Grippotyphosa	40	128	0.622		
		32	200/200	Pomona/Wolffi	80		0.465		
		33	800/200	Pomona/Icterohaemorrhagiae		512	0.807		0.495
		34	200	Canicola			0.711		
6	São Paulo F (milk)	35				256	1.036		0.545
		36			40	128	0.735		0.53
		37				512	0.71		0.59

								3		2
		38						0.678		
		39					256	0.542		
		40					4	0.873		0.431
		41	4096				128		40	
		42					64	0.354		
		43					512	0.48	40	
		44				10	256	0.572	40	
		45					8	0.904	40	
		46					512	0.511		0.434
		47	2048	200/100	Wolffi/Hardjo		128	0.345		
		48		400	Wolffi	10	128	0.477		
7	São Paulo									
	G (beef)	49		200/100	Wolffi/Hardjo		128	0.408	40	
		50								

		51	400/200	Wolff/Tarassovi			0.32	
		52	800/800	Wolffi/Hardjo		8	0.411	40
		53	200	Pomona	640		0.415	1.646
		54	400	Icterohaemorrhagiae	320	1024	0.333	
		55			640	1024	0.358	1.933
		56			640	128	0.399	1.289
		57			320	128	0.41	0.702
8	Minas Gerais H (milk)	58	100	Hardjo	5120	1024	0.316	0.613
		59	800/400	Wolffi/Hardjo	640	128	0.492	
		60	200	Hardjo	640	512	0.441	
		61			640	1024	0.432	1.096
		62			640	128	0.309	
9		63	200/100	Pomona/Hardjo			0.389	40

	Minas Gerais I (milk)	64		400/200	Icterohaemorrhagiae/Pomona			0.633	0.927
		65	128					40	
		66						0.531	0.448
		67		200/100	Wolffi/Hardjo			1.033	0.757
		68				20	128	0.446	
		69		200/100	Wolffi/Hardjo			0.899	0.459
		70		800/400	Wolffi/Pomona	10		0.34	40
		71		800/800	Wolffi/Hardjo	20		0.792	
		72		800/400	Wolffi/Hardjo			0.327	
		73		100	Wolffi	160	8	0.527	0.546
		74	256	100/100	Wolffi/Hardjo			0.322	
10	Minas Gerais J (milk)	75					2	0.569	0.613
		76				80		0.524	0.626
		77	512	200/100	Wolffi/Hardjo	40		0.49	

		78					32	0.642	
		79	512	100	Wolffi	320			1.112
		80				80		0.723	
		81	2048	100	Wolffi	1280	256	0.529	
		82				80		0.701	0.582
		83						0.456	0.553
		84	131072				256	0.537	1.975
		85		800/800	Wolffi/Hardjo		256	0.513	1.06
		86						0.56	2.597
11	Minas Gerais K (milk)	87		400	Tarassovi	40		0.406	2.493
		88				20	8		
		89	128			80	32	0.415	
		90				20			0.924

		91				10	2	1.107
		92					16	
		93		100/100	Wolffi/Hardjo	20		0.318	0.498
		94		200	Wolffi	20	128	0.726	0.61
		95		200/100	Wolffi/Hardjo	40	32	0.456	0.625
		96		100	Wolffi			0.661	
		97		200/100	Wolffi/Hardjo		128	0.478	0.455
12	Mato Grosso do Sul L (beef)	98		800/200	Wolffi/Hardjo			0.366	
		99		800/100	Wolffi/Hardjo	10	64	0.527	0.471
		100	512			20		0.343	
		101		200	Wolffi		4	0.506	0.624
		102		100/100	Wolffi/Hardjo			0.704	0.634

Capítulo 4 - CONSIDERAÇÕES FINAIS

É axiomático o papel da *Coxiella burnetii*, bactéria causadora da zoonose conhecida como Febre Q (MAURIN e RAOULT, 1999). Não obstante, faz-se necessário conscientizar agropecuaristas, veterinários, biológos e trabalhadores rurais em geral acerca da 'Febre Q' e da própria aplicação do conceito *One Health* (ANGELAKIS e RAOULT, 2010).

As regiões estudadas no presente trabalho compreendem de áreas de intensas ações antrópicas. No que diz respeito aos locais onde foram amostrados parte dos cervídeos, o cenário é uma região de alagamento, apresentando incontendível valor ecológico na Bacia do Rio Paraná (TRAVASSOS, 2001). Ademais, o potencial turístico da região é amplamente explorado às margens do Rio Paraná, principalmente por meio da pesca esportiva e torneios de esportes aquáticos (TRAVASSOS, 2001). Partindo desses pressupostos, é evidente a interação entre o ambiente, seres humanos e animais, possibilitando o intercâmbio de *C. burnetii* entre cervídeos, bovinos de criações extensivas e seres humanos. De fato, na região do Pantanal onde foram encontrados cervídeos soropositivos é predominante a criação extensiva de gado de corte, possibilitando o contato de animais de produção com o agente sob estudo (**Figura 5**).

Figura 5. Possível cadeia epidemiológica de *Coxiella burnetii*, tendo cervídeos como fontes de infecção para bovinos e seres humanos, e bovinos como fontes de infecção para cervídeos e seres humanos.

A forma de resistência de *Coxiella burnetii*, SCV, pode também representar uma preocupação se levarmos em consideração produtos lácteos artesanais no Brasil. Na Europa, ainda que produtos artesanais possuam matéria prima de alta qualidade, DNA da bactéria em questão foi detectado em tais amostras (ELDIN et al., 2013). Já no Brasil, a fiscalização da matéria prima é ineficaz e nem sempre possui alta qualidade como aquela encontrada na Europa. Tais considerações tornam possível sugerir que tais produtos possam representar um fator de risco para infecção por *C. burnetii* em seres humanos e devem ser investigados quanto à presença de bactérias viáveis.

Os dados apresentados no presente trabalho deixam evidente a importância do trabalho em conjunto entre profissionais da Medicina Humana, Veterinária e agropecuaristas. Salienta-se também a importância da educação em saúde a respeito de *C. burnetii* entre produtores e trabalhadores rurais, veterinários, professionais da Saúde e turistas das regiões nas quais o agente foi detectado.

Os resultados do presente estudo evelam que os cervídeos brasileiros apresentam pouco contato com o parasita quando comparados a cervídeos amostrados no continente europeu (RUIZ-FONS et al., 2008; DORKO et al., 2009; GONZÁLEZ-BARRIO et al., 2015b), evidenciando que seu papel como reservatório não seja tão relevante quanto aquele verificado em cervídeos na Europa.

No tocante aos bovinos, os ensaios sorológicos conduzidos no presente estudo demonstraram a possibilidade de contato efetivo com o parasita em todos as localidades estudadas, o que salienta sua ampla distribuição. Entretanto, não foi possível determinar qual foi a real causa de abortamento, visto que não houveram animais soropositivos exclusivamente para *Coxiella burnetii.*

Este estudo oferece dados inéditos no que diz respeito à circulação de *Coxiella burnetii* em cervídeos (grupo considerado como relevante reservatório na Europa) e bovinos (animais de produção que possuem contato direto com o ser humano) em diversos estados brasileiros. Em ambos os grupos de animais, a despeito da baixa tava de soropositividade, fora também possível depreender alta titulação de anticorpos anti-*C. burnetii*. No mais, salienta-se a falta de estudos que visem avaliar a verdadeira representatividade do parasita no Brasil, tópico de extrema relevância que deve ser melhor explorado, a fim de preservar a saúde dos animais e dos seres humanos que estão em contato com esses.