UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

ISOLATION AND GENOTYPING OF *Bartonella henselae* IN DOMESTIC CATS FROM SÃO PAULO AND MINAS GERAIS STATES, SOUTHEASTERN BRAZIL

Maria Eduarda Chiaradia Furquim
Médica Veterinária

2021

UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

ISOLATION AND GENOTYPING OF *Bartonella henselae* IN DOMESTIC CATS FROM SÃO PAULO AND MINAS GERAIS STATES, SOUTHEASTERN BRAZIL

Maria Eduarda Chiaradia Furquim
Orientador: Prof. Dr. Marcos Rogério André

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Campus Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Medicina Veterinária (Patologia Veterinária).

2021

F989i

Furquim, Maria Eduarda Chiaradia
Isolation and Genotyping of Bartonella henselae in Domestic Cats From São Paulo and Minas Gerais States, Southeastern Brazil / Maria Eduarda Chiaradia Furquim. -- Jaboticabal, 2021
96 p.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal
Orientadora: Marcos Rogério André
Coorientadora: Rosangela Zacarias Machado

1. Bartonelose. 2. Análise Filogenética. 3. Análise de Distância. 4. Multilocus Sequence Typing (MLST). I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

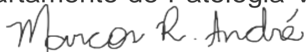
CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: ISOLATION AND GENOTYPING OF *Bartonella henselae* IN DOMESTIC CATS FROM SÃO PAULO AND MINAS GERAIS STATES

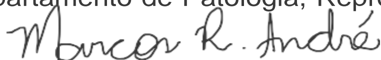
AUTORA: MARIA EDUARDA CHIARADIA FURQUIM
ORIENTADOR: MARCOS ROGÉRIO ANDRÉ
COORIENTADORA: ROSANGELA ZACARIAS MACHADO

Aprovada como parte das exigências para obtenção do Título de Doutora em MEDICINA VETERINÁRIA, área: Patologia Animal pela Comissão Examinadora:

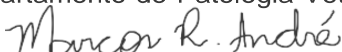
Prof. Dr. MARCOS ROGÉRIO ANDRÉ (Participaçao Virtual)
Departamento de Patologia Veterinária / FCAV / UNESP - Jaboticabal



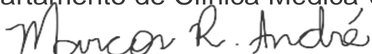
Prof. Dr. ESTEVAM GUILHERME LUX HOPPE (Participaçao Virtual)
Departamento de Patologia, Reprodução e Saúde Única / UNESP/Câmpus de Jaboticabal



Profa. Dra. DARCI MORAES BARROS-BATTESTI (Participaçao Virtual)
Departamento de Patologia Veterinária / FCAV-UNESP / Câmpus de Jaboticabal



Prof. Dr. PAULO EDUARDO NEVES FERREIRA VELHO (Participaçao Virtual)
Departamento de Clínica Médica-UNICAMP / Campinas/SP



Profa. Dra. ANANDA MÜLLER PEREIRA (Participaçao Virtual)
Ross University School of Veterinary Medicine / Basseterre/Ilha de São Cristovão/Caribe

Jaboticabal, 07 de junho de 2021

DADOS CURRICULARES DA AUTORA

Maria Eduarda Chiaradia Furquim – nascida em 29 de novembro de 1990, no município de Belo Horizonte, Minas Gerais, filha de Ligia Donizetti do Carmo Nascimento Furquim e Antonio Celso Furquim. Ingressou em fevereiro de 2009 na Faculdade de Medicina Veterinária da Universidade Federal de Uberlândia (UFU), Campus Umuarama, concluindo em dezembro de 2013. Realizou iniciação científica no período de 2010 a 2011, sobre a “Origem e inervação do nervo fibular comum na perna de ovinos sem raça definida”, sob orientação do Prof. Dr. Hudson Armando Nunes Canabrava. No período de 2011 a 2013, realizou iniciação científica sobre a “Soroepidemiologia da brucelose em equídeos abatidos em frigorífico exportador”, sob a orientação da Profa. Dra. Anna Monteira Correia Lima. Vencedora do II Encontro de Iniciação Científica e Tecnológicas da UFU na área de Agrárias com o trabalho “Investigação da brucelose em equídeos abatidos em frigorífico exportador”. Em 2016, concluiu o curso mestrado em Medicina Veterinária na área de concentração em Medicina Veterinária Preventiva, com a dissertação intitulada “Análise retrospectiva de exames sorológicos de leptospirose animal executados no Laboratório de Leptospirose e Brucelose da Unesp, campus Jaboticabal, de 2007 a 2015” na Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus de Jaboticabal, sob orientação do Prof. Dr. Luis Antonio Mathias. Em 2017, ingressou no curso de doutorado em Medicina Veterinária na área de concentração em Patologia Veterinária, na Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus de Jaboticabal, sob orientação do Prof. Dr. Marcos Rogério André.

Dedico

Ao meu pai, Antonio Celso Furquim, que sempre disse que a educação seria o bem mais precioso que ele me deixaria. Você está certo!

AGRADECIMENTOS

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Ao meu orientador, Prof. Dr. Marcos Rogério André, que mesmo diante de todas as adversidades encontradas nessa trajetória, sempre me estimulou a continuar e encontrar soluções aos problemas encontrados. Obrigada pela paciência e dedicação para com a minha formação.

À Profa. Dra. Rosângela Zacarias Machado, por todos os ensinamentos e auxílio para o desenvolvimento deste trabalho.

À Profa. Dra. Darci Moraes Barros-Battesti, pelo auxílio na identificação dos ectoparasitas amostrados.

Às minhas queridas Victória Valente e Amanda Garcia, que compartilharam comigo as angústias e felicidades da vida de pós-graduação. Vocês tornaram esses quatro anos mais especiais!

Aos amigos do Laboratório de Imunoparasitologia, Renan Amaral, Lívia Perles, Ana Calchi, Priscilla Ikeda, Luiz Gonçalves, Laryssa Borges, Jaqueline Camargo, Matheus Santana, Leidiane Duarte, Anna Mongruel, Ana Carolina Santiago, Lara Lopes, por compartilharem seus conhecimentos e por estarem sempre dispostos a ajudar.

Aos docentes e funcionários do Departamento de Patologia, Reprodução e Saúde Única, em especial à Mabel Custódio e Rafaela Beraldo, pela atenção e ajuda dispensada no decorrer do doutorado.

Aos meus pais, Ligia e Antonio Celso Furquim, pelo amor incondicional, por vibrarem a cada conquista e por proporcionarem todas as ferramentas para

que eu chegasse até aqui. Vocês são a principal razão de todo meu esforço e dedicação.

À minha irmã Daniela Albiach, por todo amor, pelo constante entusiasmo e por me dar minhas maiores alegrias, meus amados Augusto e Elis.

À Marcela Marconato, por estar ao meu lado e me apoiar durante toda minha trajetória. Serei eternamente grata a você.

À Marilaine Bonafin, Mariza Bonafim, Marileda Bonafim e Marisilvia Bonafim, pelo carinho, por me acolherem e se tornarem minha família em Jaboticabal.

À Profa. Dra. Anna Monteiro Correia Lima, minha primeira orientadora, por ser sempre um exemplo e por me auxiliar em todos os momentos da minha vida acadêmica. Obrigada por me incentivar até hoje.

À minha querida professora “Tia” Maria Cristina Cardoso e Cardoso, por me ensinar de forma tão entusiasmada a língua inglesa que se tornou parte do meu dia a dia. Obrigada por acreditar no meu potencial desde muito cedo.

Aos meus amigos Mariana Zeviani, Thiago Alves, Bruno Merenda, Bruno Scardoeli, Mariana Frizzas, Hemílio Casaletti, Guilherme Cunha, Vitor Stoque, Matheus Despezzi, Saulo Machado, Felipe Reis, que entre conversas construtivas e momentos descontraídos, me incentivaram a acreditar na minha capacidade e estiveram ao meu lado incondicionalmente. Obrigada por me ampararem nos momentos mais difíceis e me proporcionarem tantas alegrias.

CONTENTS

RESUMO - xii

ABSTRACT.. xiv

CHAPTER 1 – GENERAL CONSIDERATIONS 1

1. INTRODUCTION 1

2. LITERATURE REVIEW 3

2.1. Genus *Bartonella*... 3

2.2. Transmission of *Bartonella* henselae 8

2.3. Feline bartonellosis 11

2.4. Human bartonellosis... 13

2.5. Bartonella spp. infection diagnosis 17

2.6. Occurrence of *Bartonella* spp. in Brazilian cats 21

2.7. Multiple Locus Sequence Typing (MSLT) genotyping................................ 27

CHAPTER 2 – Genetic diversity and Multilocus Sequence Typing Analysis of *Bartonella henselae* in domestic cats from Southeastern Brazil........... 41

ABSTRACT... 41

1. INTRODUCTION.. 43

2. MATERIAL AND METHODS 46

2.1. Cats' blood and flea sampling 46

2.2. Molecular assays for *Bartonella* spp. and cloning 47

2.3. Pre-enrichment liquid culture and chocolate agar isolation of *Bartonella* spp. ……………………………………………………………………………………50

2.4. Conventional PCR assays (cPCR) for MLST for *Bartonella henselae* colonies genotyping... 51

2.5. Purification of amplicons, sequencing and phylogenetic analysis 52

2.6. Genetic diversity and genealogies within *Bartonella henselae* taxon 53

2.7. Multilocus Sequence Typing (MLST) Analysis 54

3. RESULTS... 54

3.1. Occurrence of *Bartonella* spp. in the cats' blood samples 54

3.2. Ectoparasites... 56

3.3. rpoB -cPCR assay and Cloning 58

3.4. Bartonella henselae isolation.. 58

3.4.1. BLASTn analysis 60

3.4.2. Phylogenetic analysis 60

3.4.3. Diversity and distance analysis 62
3.4.4. Multilocus Sequence Typing (MLST) 65
4. DISCUSSION 70
5. CONCLUSION 75
REFERENCES 75

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

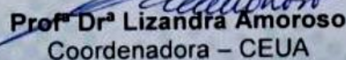
CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto intitulado **"Isolamento e genotipagem de *Bartonella* spp. em felinos domésticos domiciliados e errantes nos estados de São Paulo e Minas Gerais"**, protocolo nº 012017/17, sob a responsabilidade do Prof. Dr. Marcos Rogério André, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 03 de Agosto de 2017.

Vigência do Projeto	01/09/2017 a 06/12/2020
Espécie / Linhagem	*Felis catus*
Nº de animais	-
Peso / Idade	Variável
Sexo	Variável
Origem	Hospital Veterinário FCAV-UNESP; do Projeto de Castração realizado na universidade pela Associação Protetora dos Animais (APA) dos municípios de Jaboticabal-SP, Uberlândia-MG e Araguari-MG; de gatos resgatados das ruas por protetores(as); bem como clínicas veterinárias.

Jaboticabal, 03 de Agosto de 2017.



Profª Drª Lizandra Amoroso
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n - 14884-900 - Jaboticabal - SP - Brasil
tel 16 3209 2600 fax 3202 4275 www.fcav.unesp.br

ISOLAMENTO E GENOTIPAGEM DE *Bartonella henselae* EM GATOS DOMÉSTICOS NOS ESTADOS DE SÃO PAULO E MINAS GERAIS

RESUMO - A *Bartonella henselae* é o agente causador da Doença da Arranhadura do Gato (DAG), que pode ser fatal. Os felinos domésticos e selvagens são conhecidos por serem os seus principais reservatórios mamíferos. O presente estudo investigou a ocorrência e diversidade genética de *Bartonella* spp. em gatos amostrados nos estados de São Paulo (SP) e Minas Gerais (MG), sudeste do Brasil. Com base em um ensaio quantitativo de PCR em tempo real (qPCR), o fragmento do gene nuoG de *Bartonella* sp. foi detectado em 39,9% (122/306) das amostras de sangue (46/151 de SP; 76/155 de MG). As amostras de sangue foram submetidas a uma técnica de cultura de pré-enriquecimento que permitiu a detecção de 12 amostras positivas adicionais, que se revelaram negativas na qPCR das amostras de sangue correspondentes. Além disso, cinco isolados de *B. henselae* foram obtidos a partir de amostras qPCR-negativas tanto para cultura de sangue como para cultura de pré-enriquecimento. Sete das 24 pulgas de *Ctenocephalides felis* foram positivas para *Bartonella* spp. no ensaio qPCR; 4/7 pulgas positivas foram colhidas de gatos negativos para *Bartonella* spp. Vinte e três sequências clonadas do gene *rpoB* de *B. henselae* foram obtidas a partir de nove amostras de sangue de gatos, mostrando a ocorrência de 13 genótipos diferentes. A análise de *Median-joining network* e a análise à distância *SplitsTree* mostraram que as sequências obtidas representavam genótipos distintos de *B. henselae* quando comparadas com as anteriormente depositadas no *GenBank*. Foi encontrada diversidade intra-hospedeiro, uma vez que foram detectados

diferentes genótipos do gene *rpoB* de *B. henselae* em gatos individuais. Os isolados de *B. henselae* apresentaram dois perfis alélicos (ST37 em gatos do estado MG e ST9 no estado SP) pela análise de MLST (Multilocus Sequence Typing) baseada no sequenciamento de oito marcadores moleculares. O presente estudo é o primeiro relatório molecular de *Bartonella* sp. em gatos do estado de Minas Gerais. Em resumo, este trabalho mostrou a ocorrência de diferentes genótipos *B. henselae* do gene *rpoB* a um nível de hospedeiro intra-reservo. Com base no qPCR de amostras de sangue e de cultura líquida pré-enriquecimento e isolamento, a ocorrência de 33,1% (50/151) e 56,8% (88/155) de *Bartonella* sp. em gatos dos estados de SP e MG, respectivamente. Dois perfis alélicos diferentes de *B. henselae* foram encontrados em gatos dos estados de São Paulo (ST9) e Minas Gerais (ST37), sugerindo uma evolução clonal de bartonellae numa determinada região geográfica.

Palavras-chave: bartonelose; felino; análise à distância, genotipagem

ISOLATION AND GENOTYPING OF *Bartonella henselae* IN DOMESTIC CATS FROM SÃO PAULO AND MINAS GERAIS STATES, SOUTHEASTERN, BRAZIL

ABSTRACT - *Bartonella henselae* is the causative agent for the infectious disease Cat Scratch Disease (CSD), which can be fatal. Domestic and wild felines are known to be its main mammal reservoirs. The present study aimed to investigate the occurrence and genetic diversity of *Bartonella* spp. in cats sampled in São Paulo (SP) and Minas Gerais (MG) States, Southeastern Brazil. Based on a quantitative real-time PCR (qPCR) assay, a *Bartonella* sp. nuoG gene fragment was detected in 39.9% (122/306) of the blood samples (46/151 cats of SP; 76/155 cats of MG). The blood samples were submitted to a pre-enrichment culture technique that allowed the detection of 12 additional positive samples, which showed to be negative in the qPCR using DNA blood samples as templates. Furthermore, five *B. henselae* isolates were obtained from qPCR-negative samples for both blood and pre-enrichment culture. Seven out of 24 Ctenocephalides felis fleas were positive for *Bartonella* spp. in the qPCR assay; 4/7 positive fleas were collected from Bartonella-negative cats. Twenty-three rpoB *B. henselae* cloned sequences were obtained from nine cats' blood samples, showing the occurrence of 13 different genotypes. Median-joining network and SplitsTree distance analysis showed that the obtained sequences represented distinct *B. henselae* genotypes when compared to those previously deposited in GenBank. Intra-host diversity was found, since different rpoB genotypes of *B. henselae* were detected in individual single cats. *Bartonella henselae* isolates showed two allelic profiles (ST37 in cats from MG state and

ST9 in SP state) by MLST (Multilocus Sequence Typing) based on sequencing of eight molecular markers. The present study is the first molecular report of *Bartonella* sp. in cats from Minas Gerais State. In summary, this body of work showed the occurrence of different *B. henselae* rpoB genotypes at an intra-reservoir host level. Based on qPCR from blood samples and pre-enrichment liquid culture and isolation, occurrence of 33.1% (50/151) and 56.8% (88/155) for *Bartonella* sp. was found in cats from SP and MG states, respectively. Two different allelic profiles of *B. henselae* were found in cats from the states of São Paulo (ST9) and Minas Gerais (ST37), suggesting a clonal evolution of bartonellae in a certain geographical region.

Key-words: bartonellosis; feline; distance analysis, genotyping

CHAPTER 1 – GENERAL CONSIDERATIONS

1. INTRODUCTION

The advances of diagnostic methodologies allowed the improvement of the detection of species of the genus *Bartonella*, known to be responsible for several human and animal diseases and to have different vertebrate reservoirs and blood-sucking arthropods as their main vector. Among these agents, *Bartonella henselae* has felines as its primary host reservoirs and is the causative agent of the Cat Scratch Disease (CSD), a self-limiting disease characterized by lymph-node enlargement and fever. However, patients may develop atypical clinical signs of CSD, such as bacillary angiomatosis, bacillary peliosis, endocarditis, and encephalitis (BOULOUIS et al., 2005; CHOMEL; KASTEN, 2010; BREITSCHWERDT, 2017).

In Brazil, the detection of *Bartonella* spp. have been reported in cats in the states of Maranhão (BRAGA et al., 2012), Pernambuco (FONTALVO et al., 2017), Mato Grosso (MICELI et al., 2013; BRAGA et al., 2015), Mato Grosso do Sul (ANDRÉ et al., 2015), Rio Grande do Sul (STAGGEMEIER et al., 2010; STAGGEMEIER, 2014; MALHEIROS et al., 2016), Santa Catarina (PEDRASSANI et al., 2019), São Paulo (BORTOLI et al., 2012; ANDRÉ et al., 2014; DRUMMOND et al., 2018), and Rio de Janeiro (SOUZA et al., 2010; CRISSIUMA et al., 2011; SOUZA et al., 2017; SILVA et al., 2019b; RAIMUNDO et al., 2019), and the molecular occurrence of this agent varies from 1.6% to 97% within the Brazilian feline population.

Worldwide, studies aiming at genotyping *B. henselae* isolates by Multiple Locus Sequence Typing (MLST) have been used in cat and human biological samples from the USA, Argentina, Australia, United Kingdom, and several

European countries (Germany, Italy, France, Croatia, and Spain) (IREDELL et al., 2003; ARVAND et al., 2007; CHALONER et al., 2011; MIETZE et al., 2011; GIL et al., 2013; CICUTTIN et al., 2014; STEPANIĆ et al., 2019).

Despite the molecular detection of *Bartonella* spp. in cats from several Brazilian States to the best of authors' knowledge, neither study aiming at assessing the molecular occurrence of these agents in cats from Minas Gerais State nor genotyping *B. henselae* isolates from cats in Brazil with the MLST technique have been performed so far. Therefore, the present study aimed to investigate the occurrence and genetic diversity of *B. henselae* in cats from São Paulo and Minas Gerais States, Southeastern Brazil.

2. LITERATURE REVIEW

2.1 Genus *Bartonella*

The genus *Bartonella* (alpha-2 subgroup of the class Proteobacteria, order Rhizobiales, and family Bartonellaceae) comprises Gram-negative, fastidious and facultative intracellular pathogens, responsible for the infection of erythrocytes and endothelial cells primarily (HARMS; DEHIO, 2012). Additionally, bartonellae can potentially infect macrophage type-cells (microglia cells, dendritic cells), pericytes, monocytes, and CD34+ bone marrow progenitor cells (BREITSCHWERDT, 2017).

The first member of this genus was described in 1909 by Alberto Barton, namely *Bartonella bacilliformis*, known as the causative agent of both an acute febrile disease (Carrion's disease) and a chronic vasoproliferative disease (verruga peruana) (BREITSCHWERDT, 2014). Such agent remained as the only representative of the genus until 1993, when the unification of the genus *Rochalimaea* to the *Bartonella* genus (family Bartonellaceae) was proposed, as well as the removal of the later genus from the order Rickettsiales (BRENNER et al., 1993). In 1995, it was also proposed that the genus *Grahamella* (Bartonellaceae family) should also be integrated into the *Bartonella* genus (BIRTLES et al., 1995).

Over the last 25 years, several are the studies addressing the *Bartonella* genus and the number of species described has increased (BREITSCHWERDT, 2017; KOSOY et al., 2017). In 2017, Okaro et al. listed 45 *Bartonella* species, from which 14 have been implicated with human syndromes. However, 36 *Bartonella* species and three subspecies validly published are reported in the List of Prokaryotic names with Standing in

Nomenclature (LPSN – accessed on March 17, 2021: https://www.bacterio.net/genus/bartonella). Such agents present a complex natural cycle, in which several hematophagous arthropods play a role as vectors and many mammals as vertebrate reservoir hosts (HARMS; DEHIO, 2012) **(Table 1)**.

A noteworthy trait of this group of bacteria is the long lasting intra-erythrocytic bacteremia and endotheliotropic infection of infected animals, mostly without any association with concurrent disease. In fact, the detection of *Bartonella* species is frequent performed by blood culture and PCR in natural reservoirs. Such peculiarities are of great importance to healthcare professionals, once the identification of reservoir hosts for this genus is rising, particularly among rodent and bat species (BREITSCHWERDT, 2017).

Although the high complexity of the mechanisms that allow the persistence of *Bartonella* in the bloodstream is still unknown, previous works indicated that the primary niche of infection of these bacteria comprises endothelial cells. Only after their passage through this niche, such microorganisms are able to efficiently adhere and invade mature erythrocytes, and subsequently replicate (CHOMEL et al., 2009). The invasion of endothelial cells and the non-hemolytic intra-erythrocytes colonization are ingenious tools that ensure continuous vector infection as well as facilitate the bacterial spread within the infected host and their protection from the host immune response (BREITSCHWERDT, 2017).

Presently, 4 lineages are attributed to the *Bartonella* genus (**Table 2**) based on genomic and phylogenetic analysis (WOLF et al., 2014; HARMS et al., 2017). Accordingly, *B. bacilliformis* represents an ancient, deep-branching lineage (lineage 1) and presents itself isolated from other modern bartonellae

species, which are clustered into three different phylogenetic branches (lineages 2 to 4). Lineage 2 is limited to ruminant host pathogens, and collectively, lineages 3 and 4 comprises the majority of different reservoir hosts, which seems to be resulted from two adaptative parallel radiations that are connected with the acquisition of the VirB/D4 T4SS and the vast potential of its secreted effectors to manipulate host cell functions (HARMS et al., 2017). Within the lineage 4, which includes *B. henselae,* Trw type IV secretion (Trw T4SS) determines host specificity and red blood cells adherence (HARMS; DEHIO, 2012; WOLF et al., 2014).

Table 1. *Bartonella* species validly published and reported in the List of Prokaryotic names with Standing in Nomenclature (LPSN) distribution, main hosts, arthropod vectors, and possibility of human infection.

***Bartonella* Species**	**Description**	**Distribution**	**Mammal reservoir**	**Arthropod Vector**	**Human Infection**
B. bacilliformis	Strong; Tyzzer; Sellards (1915)	Colombia, Ecuador, Peru	Human	Sandfly – *Lutzomyia verrucarum*	Yes
B. elizabethae	Brenner et al. (1993)	Worldwide	Rodents	Oriental Rat Flea - *Xenopsylla cheopis*	Yes
B. henselae	Brenner et al. (1993)	Worldwide	Felids	Cat flea - *Ctenocephalides felis*	Yes
B. quintana	Brenner et al. (1993)r	Worldwide	Human	*Pediculus humanis*	Yes
B. doshiae	Birtles et al. (1995b)	Europe, Afghanistan, Brazil, China	Rodents		Yes
B. grahamii	Birtles et al. (1995b)	North Hemisphere	Rodents	Rodent fleas	Yes
B. peromysci	Birtles et al. (1995b)	United Kington	Rodents		
B. taylorii	Birtles et al. (1995b)	Asia and Europe	Rodents	Fleas (?)	
B. talpae	Birtles et al. (1995b)	United Kington	Mole		
B. vinsonii subsp. berkhoffii	Kordick et al. (1995)	America, Europe, North Africa, Israel	Canids	Ticks (?)	Yes
B. vinsonii subsp. vinsonii	Kordick et al. (1995)	North America	Rodents	Vole ear mite - *Trombicula miroti*	
B. clarridgeaie	Lawson; Collins (1996)	Worldwide	Felids	Cat flea - *Ctenocephalides felis*	Yes
B. tribocorum	Heller et al.(1999)	Worldwide	Rodents		Yes
B. alsatica	Heller et al. (1999)	Europe, North America	Rabbits	Fleas (?) Ticks (?)	Yes
B. vinsonii subsp. arupensis	Welch et al. (1999)	Italy, North America, Thailand	Rodents	*Ixodes scapularis*	Yes
B. birtlesii	Bermond et al. (2000)	Europe	Rodents		
B. koehlerae	Droz et al. (1999)	Worldwide	Felids	Cat flea - *Ctenocephalides felis*	Yes
B. schoenbuchensis	Dehio et al. (2001)	China, Europe, South Korea, USA	Ruminants	Biting flies (?) Ticks (?)	Yes

B. bovis	Bermond et al. (2002)	Worldwide	Ruminants	Biting flies (?) Ticks (?)	
B. capreoli	Bermond et al. (2002)	Europe, USA, Japan	Ruminants	Biting flies (?) Ticks (?)	
B. chomelii	Maillard et al. (2004)	Europe, China, Algeria, Palestine	Ruminants	Biting flies (?) Ticks (?)	
B. rochalimae	Eremeeva et al. (2007)	North Hemisphere, South America	Canids	Fleas (?)	Yes
B. coopersplainsensis	Gundi et al. (2009)	Asia, Australia and Europe	Rodents		
B. queenslandensis	Gundi et al. (2009)	Asia, Australia, Africa, Canary Islands	Rodents		
B. rattaustraliani	Gundi et al. (2009)	Australia	Rodents		
B. japonica	Inoue et al. (2010)	Japan, China	Rodents		
B. silvatica	Inoue et al. (2010)	Japan	Rodents		
B. acomydis	Sato et al. (2013)	Japan, Isarael	Rodents		
B. callosciuri	Sato et al. (2013)	Japan	Rodents		
B. jaculi	Sato et al. (2013)	Japan	Rodents		
B. pachyuromydis	Sato et al. (2013)	Japan	Rodents		
B. florencae	Mediannikov et al. (2013)	France	Shrew		
B. senegalensis	Mediannikov et al. (2013)	Senegal	Unknown	Deer ked (?)	
B. ancashensis	Mullins et al. (2015)	Peru	Human		Yes
B. apis	Kesnerova, Moritz, Engel (2016)	Europe, USA, China	Honeybee		
B. fuyuanensis	Li et al. (2015)	China	Rodents		
B. heixiaziensis	Li et al. (2015)	China	Rodents		
B. kosoyi	Gutiérrez et al. (2020)	Israel	Rodents		Yes
B. krasnovii	Gutiérrez et al. (2020)	Israel	Rodents		

Table 2. *Bartonella* species distributed into the four lineages identified by genomic and phylogenetic analysis (adapted from (HARMS; DEHIO, 2012; WOLF et al., 2014).

Lineage	Members
1	*B. bacilliformis*
2	*B. bovis, B. schoenbuchensis, B. chomelii, B. capreolli*
3	*B. clarridgeiae, B. rochalimae,*
4	*B. birtlesii, B. doshiae, B. quintana, B. henselae, B. koehlerae, B. tribocorum, B. elizabethae, B. grahamii, B. taylorii, B.alsatica, B. vinsonii* subsp. *arupensis, B. vinsonii* subsp. *berkhoffii, B. vinsonii* subsp. *vinsonii*

2.2 Transmission of *Bartonella* henselae

The transmission of *Bartonella* species occurs, but are not limited to, through blood-sucking arthropod (LINS; DRUMMOND; VELHO, 2019), such as biting flies, ticks, fleas, and mites (**Table 1**), and over the years, several studies have been conducted to determine vectorial competence of these arthropods (GUPTILL, 2010).

Presently, the main known arthropod vector for *B. henselae* is the cat flea, *Ctenocephalides felis.* However, *B. henselae* DNA has been identified in numerous other blood-feeding arthropods (*Dermacentor* spp., *Ixodes* spp., *Haematobia* spp., *Stomoxys* spp.) (BOUHSIRA et al., 2013b) and further vectorial competence studies may be established towards this bacterium, once DNA evidence does not implies vectorial capacity (GUPTILL, 2010).

The potential mechanisms of transmission of *B. henselae* among cats includes the inoculation of contaminated flea feces through skin lesions, ingestion of infected fleas or infected feces. For humans, infection occurs with the inoculation of flea infected feces through cat scratch or bite (BOUHSIRA et al., 2013a), that due to cats' grooming habits accumulates flea feces under their nails or in the oral cavity.

The cat adult flea is an ectoparasite that spends almost its entire life on their host and hardly moves from one host to another. Female *C. felis* can gain up to 15 times their weight due blood ingestion and consume 13.6 µL of blood daily, and quickly after feeding, dejects small dark pellets of dried blood. Adult *C. felis* becomes infected after feeding on infected cats (BOUHSIRA et al., 2013a), in which 1 mL of blood present millions of *B. henselae* bacilli, substantially increasing the chances of acquiring this pathogen (CHOMEL et al., 1996). However, *Bartonella*-positive cats can harbor negative fleas for the agent, as well as the opposite (GUTIÉRREZ; NACHUM-BIALA; HARRUS, 2015).

The vectorial competence of *Ctenocephalides felis* for *B. henselae* was established by researchers that infested specific-pathogen-free (SPF) kittens with fleas collected from bacteremic cattery cats, and after two to six weeks all groups of infested SPF kittens became bacteremic. The same study also tried to prove direct *B. henselae* cat transmission, introducing the infected SPF kittens in a flea-free environment together with two SPF control kittens, but at that time no transmission occurred (CHOMEL et al., 1996).

Regarding *Bartonella* spp. replication in flea gut, a study demonstrated the presence of *B. quintana* as dense clusters along the digestive tract by immunohistochemistry (KERNIF et al., 2014). In another study, in which cat fleas were fed with *B. henselae* infected blood, bartonellae were observed in the flea gut from three hours to nine days after the feeding, using immunofluorescent antibody assay (IFA). On the 9th day, fluorescent bacteria were plentiful, suggesting the replication of such pathogens in the flea gut. By

performing culturing of flea guts and feces samples, authors were able to confirm the excretion of viable agents (HIGGINS et al., 1996).

An increase of the amount of *B. henselae* in the flea medium intestine was observed by Bouhsira et al. (2013b) seven days post artificial feeding with infected blood. A similar result was demonstrated by Finkelstein et al. (2002), who showed replication in flea guts six to eight days after infected blood feeding. In this later study, researchers verified that *B. henselae* does not replicate in feces in the environment but can survive in such conditions for up to three days.

Even though blood sucking associated with salivation is the usual route for vector transmission of infectious agents, research data evidenced that fleas transmission pathways are mainly via feces and not through their bite (LAROCHE; RAOULT; PAROLA, 2019). However, the presence of *B. henselae* DNA was showed in a primarily uninfected blood used to feed fleas previously fed by ingestion of *B. henselae*-infected blood, indicating a possible regurgitation during the flea blood meal (BOUHSIRA et al., 2013a).

Ticks are another arthropod vectors that have been suspected to potentially transmit *B. henselae.* A study has demonstrated that such bacteria may survive through molting of larvae to nymphs and from nymphs to adults of *Ixodes ricinus* and could be transmitted to blood through tick saliva (COTTÉ et al., 2008). A potential vector role has also been attributed to *Rhipicephalus sanguineus*, in which *B. henselae* DNA was detected in nymphs molted from larvae engorged on *B. henselae*-infected blood, suggesting transstadial transmission. Moreover, this work identified the presence of *B. henselae* DNA in the blood from the feeders used in the molted nymphs blood meal, supporting

the hypothesis that the bacteria are present in the tick salivary glands. However, the viability of these injected bacteria could not be evaluated since it could not be cultivated. Maybe due to a possible bacteria destruction by the tick immune system in the salivary gland, implying that, although the bacteria DNA can be harbored by ticks, not all arthropod vectors are fit to be efficient vectors due to *Bartonella* spp. selective adaptation (WECHTAISONG et al., 2020).

Blood transfusion may also be a route of *Bartonella* spp. transmission. A study conducted with 500 human blood donors' samples that were submitted to culture in liquid and solid medium with subsequent PCR analysis and resulted in 16 positive subjects. Later, *B. henselae* (15/16) and *B. clarridgeiae* (1/16) were identified within these samples. These findings indicate that asymptomatic blood donors can be infected by *Bartonella* spp. at the time of the blood donation, and since the ability of such bacteria of causing infection through the intravenous route has been described in animal models and in humans after needle sticks accidents, the existence of a potential risk for transmission through blood transfusion cannot be excluded (PITASSI et al., 2015)

2.3 Feline bartonellosis

Domestic cats are the main reservoirs for *B. henselae, B. clarridgeiae* and *B. koehlerae* (BOULOUIS et al., 2005), and may be infected by *B. quintana* and *B. bovis* (BREITSCHWERDT et al., 2010)*. Bartonella henselae* infection was firstly reported by Regnery, Martin and Olson (1992), in a study where the authors were able to grow this bacteria in vitro as well as detect its DNA via PCR/RFLP analysis from the same cat in two separate trials within three weeks.

In recent years, the number of studies involving paleomicrobiology are increasing, mostly because of the improvement of diagnostic techniques such as Polymerase Chain Reaction (PCR), used to retrieve DNA from numerous types of ancient samples (RIVERA-PEREZ; SANTIAGO-RODRIGUEZ; TORANZOS, 2016). Using such approach, researchers were able to detect *B. henselae* DNA from the dental pulp of three cats from the 13th, 14th, and 16th centuries from a burial site. This study showed that this agent has been associated with cats for at least 800 years (LA et al., 2004; FOURNIER et al., 2015).

Seroepidemiologic studies conducted worldwide indicated that cats are exposed to several *Bartonella* spp., particularly *B. henselae*, and the seroprevalence is higher in warm and humid climates, older and feral cats, and in those infested with fleas (GUPTILL, 2010).

Even though naturally infected cats are mostly asymptomatic, uveitis and endocarditis have been associated with *B. henselae* infection. Such clinical conditions may be related to multiple causes and the establishment of the causal agent for these manifestations is often difficult. Therefore, further epidemiological investigations are necessary to determine the association of *Bartonella* spp. to certain clinical conditions that may have multiple etiologies (GUPTILL, 2010).

Similarly, in cases of experimental infection, the majority of cats are asymptomatic and when clinical signs are present, they are mild and depend on the strain used. Among the listed clinical signs, there may be generalized or localized lymphadenomegaly, short periods of fever, lethargy and anorexia, mild neurological manifestations, muscular pain, cardiopathy, among others.

Hematological and biochemical evaluation of experimentally infected cats commonly does not show any significant alteration, although rare cases may present a subtle anemia, neutropenia and eosinophilia (GUPTILL, 2010; SOUZA et al., 2017).

2.4 Human bartonellosis

Bartonella sp. has the remarkable feature of causing acute or chronic infections and vascular proliferative or suppurative manifestations (ANGELAKIS; RAOULT, 2014). Among species that implicates human diseases, the most mentioned are *B. henselae*, *B. quintana* and *B. bacilliformis*, in which the latter two species have humans as their main reservoirs (ANGELAKIS; RAOULT, 2014; BREITSCHWERDT, 2017).

Bartonella henselae has been attributed as the causative agent of a disease named Cat Scratch Disease (CSD), since a group of researchers identified seroreactivity to *B. henselae* antigens in 88% of 41 patients with suspected CSD. Such data was posteriorly supported by the amplification of this agent DNA from lymph nodes samples in 21 of 25 (84%) patients with suspected CSD (BREITSCHWERDT, 2014). However, the first description of CSD occurred in 1950 (CHOMEL; KASTEN, 2010).

Typically, CSD denotes a self-limiting illness characterized by fever and lymphadenopathy, usually in the lymph nodes that drain the primary inoculation site (cat scratch or bite). No longer after the inoculation of this agent (7 to 12 days), an erythematous, non-pruritic papule develop in the scratch site and within 2-3 days it becomes vesicular and crusty and may persist for a few months. After 2-3 weeks, the lymph nodes that drain the area in which the

bacteria was inoculated gradually enlarge and may persist for months (ANGELAKIS; RAOULT, 2014; BREITSCHWERDT, 2014; LINS; DRUMMOND; VELHO, 2019). Other symptoms such as malaise, headache, and anorexia are also reported (BOULOUIS et al., 2005). Although cutaneous lesions are not the only clinical manifestations, the inoculation lesions should be accounted by physicians in their anamnesis, since they have been detected in 90% of the cases (LINS; DRUMMOND; VELHO, 2019)

Among other diseases assigned with *B. henselae* infections, there are Bacillary angiomatosis (BA) and Bacillary peliosis (BP), which are usually observed in immunocompromised individuals, particularly in those infected with the HIV virus (CHOMEL; KASTEN, 2010). BA is a proliferative disease of vascular epithelia displayed as a unique or multiple widespread papulonodular cutaneous lesions, with elevated edges as typical feature, friable, tending to bleed easily and profusely. This manifestation was first described in patients with HIV or in patients undergoing immunosuppressive therapy (cases of organ transplant recipients). BP is a rare condition characterized by multiple vascular hemorrhagic parenchymatous and cystic lesions that affects mainly the liver and may result from a wide spectrum of infectious and noninfectious diseases, being *B. henselae* one of the most infectious causative agent attributed to this pathology. Such pathology may result in liver failure or rupture and may even be fatal (OKARO et al., 2017; LINS; DRUMMOND; VELHO, 2019).

Nonetheless, up to 20% of the CSD cases develops atypical clinical manifestations (CANNETI et al., 2019), and a large set of these patients lacks the classical fever and lymphadenopathy associated with typical CSD. Therefore, the differential diagnosis for *Bartonella* spp. should be included in

cases of patients lacking fever, enlarged lymph nodes and history of cat exposure (BREITSCHWERDT, 2014, 2017).

Cases of atypical CSD may develop illnesses such as Parinaud's oculoglandular syndrome, encephalitis, hemolytic anemia, hepatosplenomegaly, glomerulonephritis, pneumonia, relapsing bacteremia, and osteomyelitis (CHOMEL; BOULOUIS; BREITSCHWERDT, 2004; BOULOUIS et al., 2005).

Due to persistent bacteremia triggered by the *Bartonella* genus, autoimmune and immune-mediated manifestations may occur as a component of the disease pathogenesis (BREITSCHWERDT, 2017), and a causative role has been attributed to *B. henselae* in Henoch-Schönlein purpura, an immune-mediated vasculitis characterized by excessive Ig A production, on the basis of serologic evidence (CHOMEL; BOULOUIS; BREITSCHWERDT, 2004; OKARO et al., 2017).

For many years, the severity of the infections caused by *B. henselae* were associated with the immune status of the patients (ANGELAKIS; RAOULT, 2014). Consequently, the typical manifestations of CSD were expected in immunocompetent patients (CHOMEL; BOULOUIS; BREITSCHWERDT, 2004). However, a study conducted with patients manifesting neurological CSD (NCSD) considered the immune status of the subjects and showed that the NCSD group were not composed by immunodeficient patients, in contrast with the non-neurological CSD (NNCSD) group, which was composed by 8.6% of immunocompromised individuals (CANNETI et al., 2019).

Over the years and with the improvement of diagnostic methods, that became more specific and sensitive (culture, PCR, enrichment culture/qPCR), the number of studies reporting atypical manifestations of CSD has increased,

implying that these cases occur more often than historically suspected (BREITSCHWERDT, 2014). Since the term "CSD" implies a self-limiting illness, and the spectrum of human clinical manifestations with bartonellae infections has increased over the years, the designation CSD lacks clinical, microbiologic, and zoonotic utility. A more inclusive term, namely 'Bartonellosis', has been proposed to facilitate enhanced future understanding of diseases caused by the members of the genus *Bartonella* (BREITSCHWERDT, 2008, 2014).

Recently, a spectrum of cutaneous lesions and neuropsychiatric symptoms, such as sleep disorders, mental confusion, irritability/rage, anxiety, depression, headache/migraine, among others, have been associated with bartonellae infection/exposure, a fact that should be brought to the attention of dermatologists, psychiatrists and neurologists. Breitschwerdt et al. (2020) carried out a cross-sectional study with 29 patients experiencing neurological symptoms that tested positive for *Bartonella* spp. either by serological or molecular techniques and identified that the majority of the patients developed cutaneous lesions (83%). Thus, the extension to which *Bartonella* spp. can provoke cutaneous lesions or neuropsychiatric symptoms is unclear.

Among neuropsychiatric diseases associated with infectious agents, schizophrenia (SCZ) stands out. This mental illness can be triggered by autoimmune encephalitis caused by infectious agents and there is also a well-established epidemiological association between SCZ and *Toxoplasma gondii* infection. Taking into account the association between cat-transmitted diseases and the potential association with infectious agents, Lashnits et al. (2021) aimed to determine the potential association between *Bartonella* sp. infections and SCZ cases. In that study, 11 (65%) out of 17 SCZ cases were *Bartonella*-

BAPGM/ddPCR positive and only 1 (13 – 8%) control case was positive. Within the SCZ cases group, any association between bartonellae infection and more severe symptoms was possible. Despite the lack of conclusion regarding a casual role for *Bartonella* in SCZ in this particular research, the authors recommend a thorough examination of the epidemiology of *Bartonella* sp. infection in schizophrenic patients, once this agent is known to cause encephalitis and trigger autoimmune processes that may be related to development of SCZ (LASHNITS et al., 2021).

2.5 Bartonella spp. infection diagnosis

At present, there is no single gold standard for bartonellosis diagnosis, and the association of multiple diagnostic methodologies has been indicated to both improve the diagnostic sensitivity and to prevent false negative results (PITASSI et al., 2015; DRUMMOND et al., 2018). Microbiological isolation and identification, enzyme immunoassay (ELISA) or IFA for detection antibodies to *Bartonella* spp., and PCR amplification of *Bartonella* sp. DNA fragments from patient biological samples all have significant limitations. Therefore, the confirmation of active infection is challenging (BREITSCHWERDT et al., 2010; BREITSCHWERDT, 2017).

In humans, the clinical diagnosis of CSD is based on the presence of enlarged lymph nodes and cutaneous lesions in the inoculation site. In cases of atypical CSD and other syndromes related with *B. henselae* infection, the clinical diagnosis is insufficient and laboratory diagnosis is needed. Serology is the major diagnostic tool used in human medicine, mainly IFA. IgG antibodies titers to *B. henselae* ≥ 1:64 are considered as positive, when the subjects are tested within three week after the suspected infection (BOULOUIS et al., 2005).

However, to an accurate interpretation of serological diagnosis, the understanding of the benefits and limitations of IgM and IgG antibodies detection by either IFA or ELISA is required (WOLF et al., 2014).

Among cat populations, serology has a limited diagnostic value as many cats are likely to be seropositive against *B. henselae*, particularly stray cats (BOULOUIS et al., 2005). False-negative results may also occur since 5-12% of the seronegative cats are bacteremic (GUPTILL, 2010).

A definitive diagnosis of any bacterial pathogen requires the growth and isolation of the organism from human or animal samples. However, due to the unique characteristics of the genus *Bartonella*, the isolation of such agent is rather difficult, since they are fastidious, slow-growing bacteria, and require specific nutritional elements. In addition, due to its stealth feature, these microorganisms are sequestered inside one or more immune-protected sites, such as erythrocytes. Thus, to increase the chances of a successful culture, some steps are necessary before adding blood samples into culture medium to release these intracellular bacteria, by either lysis combined with centrifugation or a 24-hour freeze-thaw cycle at -70ºC (WOLF et al., 2014; OKARO et al., 2017).

Maggi et al. (2005) developed a novel, chemically modified, insect-based liquid culture medium, namely *Bartonella* Alpha-Proteobacteria Growth Medium (BAPGM). Later, minor modifications were made by Duncan, Maggi, Breitschwerdt (2007) in BAPGM to facilitate the molecular detection and isolation of *Bartonella* spp.. Therefore, a new diagnostic approach was suggested combining preenrichment culture using BAPGM, followed by a PCR assay targeting sensitive genic regions, such as 16S-23S ITS region or *pap-31*

gene. This combined diagnostic methodology is laborious and time consuming, and yet necessary, since it may promote an increase in bacterial number to detectable levels (BREITSCHWERDT et al., 2010).

Using this new suggested methodology, Drummond et al. (2018) demonstrated an increased sensitivity of *Bartonella* sp. PCR detection after the use of liquid blood culture over PCR from DNA extracted directly from blood samples. The study showed that 15 out of 26 (13.4%) of the PCR blood-negative cats had molecular positive results after the liquid culture. Another study conducted by the same group showed positive results for *B. henselae* after liquid culture followed by a *ftsZ* nested PCR from a male patient that presented fever for two months duration, cough, and 13-kg weight loss with negative serology for *B. henselae* and *B. quintana,* as well as negativity for PCR from whole blood (DRUMMOND et al., 2019).

The nucleic acid amplification is one of the basis for diagnosing *Bartonella* sp. infections and plays an important role in fulfilling molecular Koch's postulates to associate *Bartonella* sp. with new syndromes (OKARO et al., 2017). Indeed, PCR amplification of targeted *Bartonella* gene fragments is commonly used in cases of unsuccessful culture and negative serology (BREITSCHWERDT, 2017).

The use of many gene targets has succeeded to detect and differentiate *Bartonella* at the genus and species levels, as well as to enhance the screening of these bacteria in several sample types, although no consensus on the best target to use has been achieved (BREITSCHWERDT et al., 2010). Among these gene targets, the citrate synthase gene (*gltA*) and the RNA polymerase β-subunit gene (*rpoB*) are commonly used to identify *Bartonella* species due to

their powerful discriminatory power, stability as housekeeping genes, and their extensive GenBank database (GUTIÉRREZ et al., 2017).

A qPCR assay targeting the *nuoG* gene and developed by André et al. (2015) showed specificity and sensitivity in the detection of *Bartonella* species. Out of 151 cat blood samples, 46 (54.4%) were positive in the *nuoG*-based qPCR for *Bartonella* species. Out of these 46 positive samples, only 18 (39.1%) samples were positive in subsequent cPCR targeting different *Bartonella* genes (ITS, *rpoB*, *gltA* and *pap-31*). This study demonstrated a better performance of this new qPCR assay over cPCR assays as well as showed to be successful when assessing samples with low bacteremia. So far, this qPCR showed to be able to amplify *Bartonella* DNA from dogs (MÜLLER et al., 2018), bats (ANDRÉ et al., 2019; IKEDA et al., 2020), American minks (*Neovison vison*) (SEPULVEDA et al., 2020), Xenarthra (CALCHI et al., 2020), cattle and buffaloes (GONÇALVES et al., 2020), and rodents (GONÇALVES et al., 2016).

Recently, Maggi et al. (2020) developed and validated a Digital Droplet PCR assay (ddPCR) based on 16S-23S intergenic region (ITS) that detects at least 16 *Bartonella* spp. and strains. The ddPCR is a water-oil emulsion-based technology that presents increased efficiency, a simplified absolute quantification that does not require a standard curve and can reduce the influence of inhibitory substances. The ddPCR assay was able to detect *Bartonella* sp. DNA from spiked blood DNA samples as low as 0.001 pg/µL and showed to be efficient to detect *Bartonella* sp. DNA in clinical samples, both from extracted blood samples and within 7-21day BAPGM enrichment blood culture. These results emphasize the advantages of this new methodology to shorten the time needed to produce a sensitive, specific and reliable *Bartonella*

spp. positive DNA result. Breitschwerdt et al. (2020) reported a substantial improvement of *Bartonella* spp. detection with the combination of enrichment blood culture and ddPCR in human blood samples spiked with *Bartonella* sp. DNA. Lashnits et al. (2021) demonstrated a better performance of BAPGM/ddPCR in detriment of BAPGM/qPCR, which did not amplify any *Bartonella* sp. DNA among the studied samples.

2.6 Occurrence of *Bartonella* spp. in Brazilian cats

The molecular occurrence of *Bartonella* spp. in Brazilian cats ranges from 1,6% and 97%, and the majority of studies have been conducted in the Southeast region of the country (**Table 3**). Such variation can be attributed to the cat population sampled, diagnostic technique applied, which in cases of molecular detection can also depends on the targeted gene, and/or geographic region.

In Maranhão state, a study conducted by Braga et al. (2012) found 4.5% (9/200) of positivity for *Bartonella* spp. in PCR assays targeting several housekeeping genes followed by sequencing, in which the species *B. henselae* and *B. clarridgeiae* were identified. However, in another study in Northeastern Brazil, in the state of Pernambuco, the cPCR technique based on the gene *ribC* were not able to amplify *Bartonella* sp. DNA among the cat blood samples, even though 15% of them were IFA positive and 53.3% of the ectoparasites (*C. felis felis)* retrieved from the sampled animals were PCR positive for *Bartonella* spp., later also identified as *B. henselae* and *B. clarridgeiae* (FONTALVO et al., 2017).

Among the studies conducted in Central-Western Brazil, the frequency of *Bartonella* spp. DNA found among cats ranged from 1.6% to 30.5% and the

identified species were *B. henselae* and *B. clarridgeiae.* The targeted region of these works were ITS, *rpoB, pap-31, ribC, gltA* and *nuoG* (MICELI et al., 2013; ANDRÉ et al., 2015; BRAGA et al., 2015).

In the Southern region of the country, the frequency of *Bartonella* spp. DNA found ranged from 13.3% to 25.5% and the identified species were *B. henselae, B. clarridgeiae* and *B. koehlerae* (STAGGEMEIER et al., 2010, 2014; MALHEIROS et al., 2016; PEDRASSANI et al., 2019). Using a multiplex SYBR® green qPCR, Staggermeier et al. (2014) detected *Bartonella* spp. DNA in 25.5% (12/47) blood samples and demonstrated a sensitivity increase using the aforementioned qPCR technique in relation to other study conducted using cPCR analysis (8/47) in the same blood samples. In this study, four additional samples were positive using the multiplex qPCR, which were also able to detect and differentiate two of the feline *Bartonella, B. henselae* and *B. clarridgeiae*. Other study detected 13.3% of *Bartonella* spp. DNA with a *nuoG* qPCR as a screening test, and with subsequent cPCR assays targeting the *gltA*, *rpoB* and *ftsZ* genes. After sequencing, *B. henselae, B. clarridgeiae* and *B.* koehlerae were identified among the positive samples (PEDRASSANI et al., 2019).

The Southeast region of the country comprises the majority of molecular detection of *Bartonella* spp. in cats, both in São Paulo and Rio de Janeiro States. In São Paulo State the frequency of *Bartonella* sp. DNA ranges from 4.3% to 90.2% and the identified species were *B. henselae* and *B. clarridgeiae* (BORTOLI et al., 2012; ANDRÉ et al., 2014; DRUMMOND et al., 2018). In the state of Rio de Janeiro, the frequency of *Bartonella* sp. DNA ranges from 24.7% to 97% and the identified species were *B. henselae, B. clarridgeiae* and *B. koehlerae* (CRISSIUMA et al., 2011; SOUZA et al., 2017; RAIMUNDO et al.,

2019; SILVA et al., 2019). In the most recent study conducted in Rio de Janeiro State, 39.9% (83/208) were positive for *Bartonella* spp. based on ITS and *gltA* gene-based PCR assays, and *Bartonella* sp. DNA were also detected in *C. felis* fleas retrieved from the infected cats. Out of these samples, 51 (208) were sequenced and confirmed that the cats were infected with *B. henselae* (68.6%, 35/51), *B. clarridgeiae* (23.5%, 12/51) and *B. koehlerae* (17.6%, 9/51). Coinfection was considered when the ITS and *gltA* sequences originated from the same cat, thus three (5.9%) cats had *B. henselae* and *B. clarridgeiae* coinfection, and two (3.9%) cats were infected with *B. henselae, B. clarridgeiae* and *B. koehlerae*, simultaneously (RAIMUNDO et al., 2019).

The only study conducted in Minas Gerais State, Southeast region, investigated the occurrence of IgG and IgM antibodies against *B. henselae* and *B. quintana*, among 437 serum samples from human subjects. A seropositivity of 13.7% (60/437) was found for *B. henselae* and 12.8% (56/437) for *B. quintana* (DA COSTA; BRIGATTE; GRECO, 2005). The lack of data regarding the occurrence of *Bartonella* sp. infection in this State in contrast with the above-mentioned data of high frequency of these bacteria among cat population in the Southeastern area makes research addressing these bacteria crucial in Minas Gerais State.

Table 3. Occurrence of *Bartonella* spp. among cats in Brazil, according to the studied region, sampled cat population, diagnostic method applied and detected species by molecular techniques.

Brazilian Region	State	Diagnostic Methods			*Bartonella* Species Identified	Reference
		Culture	Serology	PCR		
Northeast	Maranhão	-	-	ITS, *rpoB, pap-31, ribC, gltA, groEL* cPCR 4,5% (9/200)	*B. henselae* *B. clarridgeiae*	Braga et al. (2012)
	Pernambuco	-	IFA 15% (6/40)	*ribC* cPCR 0/40	*Bartonella* spp.	Fontalvo et al. (2017)
Central-West	Mato Grosso	-	-	cPCR - ITS, *rpoB, pap-31, ribC, gltA* 2.5% (4/163)	*B. henselae* *B. clarridgeiae*	Miceli et al. (2013)
		-	-	cPCR - *ribC* 1.6% (3/182)	*B. clarridgeiae*	Braga et al. (2015)
	Mato Grosso do Sul	-	-	qPCR – *nuoG* cPCR- ITS, *rpoB, pap-31, ribC, gltA* 30.5% (46/151)	*B. henselae* *B. clarridgeiae*	André et al. (2015)
South	Rio Grande do Sul	-	-	cPCR – *ribC* 17% (8/47)	*B. henselae* *B. clarridgeiae*	Staggemeier et al. (2010)
		-	-	qPCR – *gltA* 25.5% (12/47)	*B. henselae* *B. clarridgeiae*	Staggemeier et al. (2014)
		-	-	qPCR – *nuoG* 20% (6/30)	-	Malheiros et al. (2016)
	Santa Catarina			qPCR – *nuoG, gltA, rpoB* 13.3% (4/30)	*B. henselae* *B. clarridgeiae* *B. koehlerae*	Pedrassani et al. (2019)

Brazilian Region	State	Diagnostic Methods			*Bartonella* Species Identified	Reference
		Culture	Serology	PCR		
Southeast	São Paulo			cPCR - ITS, *rpoB, pap-31, ribC, gltA* 4.3% (2/23)	*B. henselae*	Bortoli et al. (2012)
		-	-	cPCR - ITS, *rpoB, pap-31, ribC, gltA* 30% (11/37)	*B. henselae* *B. clarridgeiae*	André et al. (2014)
		BAPGM + Solid Culture = 11 isolates	-	nPCR (sangue) – *fstZ* 76.8% (86/112) nPCR (BAPGM) – *fstZ* 45.5% (51/112) cPCR (BAPGM) – ITS 27.7% (31/112) Total 90.2% (101/112)	*B. henselae*	Drummond et al. (2018)
	Rio de Janeiro	-	IFA 68% (25/37)	cPCR – *groEL* 97% (36/37)	-	Souza et al. (2010)
		-	IFA 47% (19/40)	cPCR – *hrtA* 42% (17/40)	-	Crissiuma et al. (2011)
		-	-	cPCR – *gltA, rpoB* 75% (122/163)	*B. henselae*	SOUZA et al. (2017)
		-	-	cPCR – *gltA* 24.7% (22/89)	*B. henselae* *B. clarridgeiae*	Silva et al. (2019b)
		-	-	cPCR – *gltA* 39.9% (93/208)	*B. henselae* *B. clarridgeiae* *B. koehlerae*	Raimundo et al. (2019)

nuoG: nicotinamide adenine dinucleotide dehydrogenase gamma subunit; *gltA*: citrate synthase gene; *groEL*: heat shock protein gene; ITS: intergenic spacer region; *pap31*: bacteriophage-associated heme-binding protein gene; *ribC*: riboflavin synthase gene; *rpoB:* RNA polymerase beta subunit gene; *htrA*: 60kDa heat shock protein gene; *ftsZ:* Cell division protein ftsZ

2.7 Multiple Locus Sequence Typing (MSLT) genotyping

The profiling of pathogenic isolates is fundamental for the epidemiology of infectious diseases, as it provide important information to identify, track and intervene against disease outbreaks. Thus, the Multiple Locus Sequence Typing (MLST) was suggested as a nucleotide sequence-based method which could be used in several bacterial pathogens. The technique consists in targeting variations present at multiple housekeeping loci and all single sequence for a given locus are designated an allele number in order of detection. The alleles number at each MLST loci for a given isolate are then combined into an allelic profile and designated a sequence type (ST) (URWIN; MAIDEN, 2003).

To this end, the PubMLST.org website was created and harbor a collection of open-access, curated databases that embed population sequence data with origin and phenotype information for over 100 microbial species and genera. The website was created as part of the development of the first MLST scheme and uses the software Bacterial Isolate Genome Sequence database (BIgSdb), and allow the PubMLST to include all levels of sequence data, from single gene sequences to complete genomes (JOLLEY; MAIDEN, 2010; JOLLEY; BRAY; MAIDEN, 2018). The *B. henselae* PubMLST is based on a scheme described by Iredell et al. (2003), who characterized in this study 37 previously described *B. henselae* isolates from human and cats from Australia and other countries. For this, the researchers choose loci that included fragments of genes encoding housekeeping functions such as 16S rDNA, *eno*, *ftsZ*, *gltA*, *groEL*, *ribC* and *rpoB*, together with other less-well-defined proteins, *batR* and *nlpD.* Regarding nucleotide variation it was observed that the *eno*

gene presented no variation, while the *groEL* gene presented 97% of variation, and the number of different alleles varied between 1 (*eno*) and 4 (*batR*). With this observations, the *B. henselae* MLST scheme was structured with 16S, *batR, ftsZ, gltA, groEL, nlpD, ribC* and *rpoB*, excluding the *eno* gene from the analysis since it presented zero nucleotide variation within the loci.

Since then, genotyping *B. henselae* using the MLST technique has been used in cat and human biological samples from the USA, Argentina, Australia, United Kingdom and European countries (Germany, Italy, France, Croatia, and Spain) (IREDELL et al., 2003; ARVAND et al., 2007; CHALONER et al., 2011; MIETZE et al., 2011; GIL et al., 2013; CICUTTIN et al., 2014; STEPANIĆ et al., 2019).

Sequence Typing (ST) 1, 5 and 6 have been described in the United States, Europe and Australia suggesting a worldwide distribution of these STs (ARVAND et al., 2007). ST 7 was detected only in Europe and ST 1 was most frequently detected in the Mediterranean region. In Croatia, an isolate obtained from a blood sample from a cat, whose owner was a patient with CSD, belonged to ST 5, which in turn was described for the first time in Southeastern Europe (STEPANIĆ et al., 2019).

A MLST study conducted in Germany described 11 new ST (ST16 to ST 26) combinations within a low number of *B. henselae* isolates from cat, which suggests a clonal evolution between isolates from the same geographic region (MIETZE et al., 2011). Arvand and Viezens (2007) also described a new ST (ST 8) in a *B. henselae* isolated from cat blood originating from France. The other ST identified in the study were ST1, ST5, ST6 and ST 7 among isolates from Germany, France, Great Britain, Switzerland, USA, Australia and New Zealand.

In Spain, in a study carried out with 78 feline isolates and 52 human isolates. ST 5, ST 6 and ST 9 were associated with feline infection, while ST 1, ST 5 and ST 8 were associated with human infection. ST 5 accounted for 53.7% of infections by *B. henselae* in humans and 61.5% in felines (GIL et al., 2013).

A study conducted in the UK encountered 12 STs among 94 cat's *B. henselae* isolates, three of which were new (ST27, ST 28 and ST29). The most frequent STs were ST4, ST6, ST7 and ST8. This study also demonstrated a coinfection with different STs in the same cat, where one was coinfected with ST5 and ST7, while the other was coinfected with ST6 and ST8. Within human samples, ST1, ST2, ST4, ST5, ST7, ST8 were detected (CHALONER et al., 2011).

In Argentina, a total of seven *B. henselae* isolates and five blood samples from cats were characterized by MLST and ST1, ST5, ST6 and ST8 were identified (CICUTTIN et al., 2014). This study was the first that described *B. henselae* variants in South America and no other study was conducted in this continent so far.

REFERENCES

ANDRÉ, M. R.; BACCARIM DENARDI, N. C.; MARQUES DE SOUSA, K. C.; GONÇALVES, L. R.; HENRIQUE, P. C.; GROSSE ROSSI ONTIVERO, C. R.; LIMA GONZALEZ, I. H.; CABRAL NERY, C. V.; FERNANDES CHAGAS, C. R.; MONTICELLI, C.; ALEXANDRE DE SANTIS, A. C. G.; MACHADO, R. Z. Arthropod-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil. **Ticks and Tick-borne Diseases**, v. 5, n. 5, p. 545–551, 2014.

ANDRÉ, M. R.; DUMLER, J. S.; HERRERA, H. M.; GONCALVES, L. R.; DE SOUSA, K. C.; SCORPIO, D. G.; DE SANTIS, A. C. G. A.; DOMINGOS, I. H.; DE MACEDO, G. C.; MACHADO, R. Z. Assessment of a quantitative 5' nuclease real-time polymerase chain reaction using the nicotinamide adenine dinucleotide dehydrogenase gamma subunit (*nuoG*) for *Bartonella* species in domiciled and stray cats in Brazil. **Journal of Feline Medicine and Surgery**, v. pii: 10986, p. 1–9, 2015. Disponível em: <http://jfm.sagepub.com/lookup/doi/10.1177/1098612X15593787>.

ANDRÉ, M. R.; GUTIÉRREZ, R.; IKEDA, P.; DO AMARAL, R. B.; DE SOUSA, K. C. M.; NACHUM-BIALA, Y.; LIMA, L.; TEIXEIRA, M. M. G.; MACHADO, R. Z.; HARRUS, S. Genetic diversity of *Bartonella* spp. in vampire bats from Brazil. **Transboundary and Emerging Diseases**, v. 66, n. 6, p. 2329–2341, 2019.

ANGELAKIS, E.; RAOULT, D. Pathogenicity and treatment of *Bartonella* infections. **International Journal of Antimicrobial Agents**, v. 44, n. 1, p. 16–25, 2014. Disponível em: <http://dx.doi.org/10.1016/j.ijantimicag.2014.04.006>.

ARVAND, M.; FEIL, E. J.; GILADI, M.; BOULOUIS, H. J.; VIEZENS, J. Multi-locus sequence typing of *Bartonella henselae* isolates from three continents reveals hypervirulent and feline-associated clones. **PLoS ONE**, v. 2, n. 12, 2007.

ARVAND, M.; VIEZENS, J. Evaluation of pulsed-field gel electrophoresis and multi-locus sequence typing for the analysis of clonal relatedness among *Bartonella henselae* isolates. **International Journal of Medical Microbiology**, v. 297, n. 4, p. 255–262, 2007.

BENSON, D. A.; CAVANAUGH, M.; CLARK, K.; KARSCH-MIZRACHI, I.; OSTELL, J.; PRUITT, K. D.; SAYERS, E. W. GenBank. **Nucleic Acids Research**, v. 46, n. D1, p. D41–D47, 2018.

BERMOND, D.; BOULOUIS, H.; HELLER, R.; LAERE, V. G.; MONTEIL, H.; CHOMEL, B. B.; SANDER, A.; DEHIO, C.; PIÉMONT, Y. *Bartonella bovis* Bermond et al. sp. nov. and *Bartonella capreoli* sp. nov., isolated from European ruminants. **International Journal of Systematic and Evolutionary Microbiology**, v. 52, p. 383–390, 2002.

BERMOND, D.; HELLER, R.; BARRAT, F.; DELACOUR, G.; DEHIO, C.;

ALLIOT, A.; MONTEIL, H.; CHOMEL, B.; BOULOUIS, H. J.; PIÉMONT, Y. *Bartonella* birtlesii sp. nov., isolated from small mammals (*Apodemus* spp.). **International Journal of Systematic and Evolutionary Microbiology**, v. 50, n. 6, p. 1973–1979, 2000.

BIRKENHEUER, A. J.; LEVY, M. G.; BREITSCHWERDT, E. B. Development and Evaluation of a Seminested PCR for Detection and Differentiation of *Babesia gibsoni* (Asian Genotype) and *B. canis* DNA in Canine Blood Samples. **Journal of Clinical Microbiology**, v. 41, n. March, p. 4172–4177, 2003.

BIRTLES, R. J.; HARRISON, T. G.; SAUNDERS, N. A.; MOLYNEUX, D. H. Proposals to unify the genera *Grahamella* and *Bartonella*, with descriptions of *Bartonella talpae* comb. nov., *Bartonella peromysci* comb. nov., and three new species, *Bartonella grahamii* sp. nov., *Bartonella* taylorii sp. nov., and *Bartonella doshiae* sp. nov. **International Journal of Systematic Bacteriology**, v. 45, n. 1, p. 1–8, 1995.

BIRTLES, R. J.; RAOULT, D. Comparison of Partial Citrate Synthase Gene (*gltA*) Sequences for Phylogenetic Analysis of *Bartonella* Species. **International Journal of Systematic Bacteriology**, v. 46, n. 4, p. 891–897, 1996. Disponível em: <http://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/00207713-46-4-891>.

BORTOLI, C. P.; ANDRÉ, M. R.; SEKI, M. C.; PINTO, A. A.; MACHADO, S. de T. Z.; MACHADO, R. Z. Detection of hemoplasma and *Bartonella* species and co-infection with retroviruses in cats subjected to a spaying/neutering program in Jaboticabal, SP, Brazil. **Rev Bras Parasitol Vet, Jaboticabal**, v. 21, n. 3, p. 219–23, 2012. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/23070430>.

BOUHSIRA, E.; FERRANDEZ, Y.; LIU, M. F.; FRANC, M.; BOULOUIS, H. J.; BIVILLE, F. *Ctenocephalides felis* an in vitro potential vector for five *Bartonella* species. **Comparative Immunology, Microbiology and Infectious Diseases**, v. 36, n. 2, p. 105–111, 2013a. Disponível em: <http://dx.doi.org/10.1016/j.cimid.2012.10.004>.

BOUHSIRA, E.; FRANC, M.; BOULOUIS, H. J.; JACQUIET, P.; RAYMOND-LETRON, I.; LIÉNARD, E. Assessment of persistence of *Bartonella henselae* in *Ctenocephalides felis*. **Applied and Environmental Microbiology**, v. 79, n. 23, p. 7439–7444, 2013b.

BOULOUIS, H.-J.; CHAO-CHIN, C.; HENN, J. B.; KASTEN, R. W.; CHOMEL, B. B. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. **Veterinary Research**, v. 36, n. 3, p. 383–410, maio 2005. Disponível em: <http://www.edpsciences.org/10.1051/vetres:2005009>.

BRAGA, Í. A.; DE OLIVEIRA DIAS, I. S.; CHITARRA, C. S.; AMUDE, A. M.; AGUIAR, D. M. Molecular detection of *Bartonella clarridgeiae* in domestic cats from Midwest Brazil. **Brazilian Journal of Infectious Diseases**, v. 19, n. 4, p.

451–452, 2015. Disponível em: <http://dx.doi.org/10.1016/j.bjid.2015.05.002>.

BRAGA, M. do S. C. de O.; DINIZ, P. P. V. de P.; ANDRE, M. R.; BORTOLI, C. P. de; MACHADO, R. Z. Molecular characterisation of *Bartonella* species in cats from Sao Luis, state of Maranhao, north-eastern Brazil. **Memorias do Instituto Oswaldo Cruz**, v. 107, n. 6, p. 772–777, 2012.

BREITSCHWERDT, E. B. Feline bartonellosis and cat scratch disease. **Veterinary Immunology and Immunopathology**, v. 123, n. 1–2, p. 167–171, 2008.

BREITSCHWERDT, E. B. Bartonellosis: One health perspectives for an emerging infectious disease. **ILAR Journal**, v. 55, n. 1, p. 46–58, 2014.

BREITSCHWERDT, E. B. Bartonellosis, One Health and all creatures great and small. **Veterinary Dermatology**, v. 28, n. 1, p. 96-e21, 2017.

BREITSCHWERDT, E. B.; BRADLEY, J. M.; MAGGI, R. G.; LASHNITS, E.; REICHERTER, P. *Bartonella* associated cutaneous lesions (BACL) in people with neuropsychiatric symptoms. **Pathogens**, v. 9, n. 12, p. 1–19, 2020.

BREITSCHWERDT, E. B.; MAGGI, R. G.; CHOMEL, B. B.; LAPPIN, M. R. Bartonellosis: An emerging infectious disease of zoonotic importance to animals and human beings. **Journal of Veterinary Emergency and Critical Care**, v. 20, n. 1, p. 8–30, 2010.

BRENNER, D. O. N. J.; CONNOR, S. P. O.; WINKLER, H. H.; STEIGERWALT, A. G. Proposals To Unify the Genera *Bartonella* and *Rochalimaea* ,. **International Journal of Systematic Bacteriology**, v. 43, n. iv, p. 777–786, 1993.

CADENAS, M. B.; MAGGI, R. G.; DINIZ, P. P. V. P.; BREITSCHWERDT, K. T.; SONTAKKE, S.; BREITHSCHWERDT, E. B. Identification of bacteria from clinical samples using *Bartonella* alpha-Proteobacteria growth medium. **Journal of Microbiological Methods**, v. 71, n. 2, p. 147–155, 2007.

CALCHI, A. C.; VULTÃO, J. G.; ALVES, M. H.; YOGUI, D. R.; DESBIEZ, A. L. J.; AMARAL, R. B.; SANTI, M.; TEIXEIRA, M. M. G.; WERTHER, K.; MACHADO, R. Z.; ANDRÉ, M. R. Multi-locus sequencing reveals a novel *Bartonella* in mammals from the Superorder Xenarthra. **Transboundary and Emerging Diseases**, n. February, p. 1–14, 2020.

CANNETI, B.; CABO-LÓPEZ, I.; PUY-NÚÑEZ, A.; GARCÍA GARCÍA, J. C.; CORES, F. J.; TRIGO, M.; SUÁREZ-GIL, A. P.; RODRIGUEZ-REGAL, A. Neurological presentations of *Bartonella henselae* infection. **Neurological Sciences**, v. 40, n. 2, p. 261–268, 2019.

CHALONER, G. L.; HARRISON, T. G.; COYNE, K. P.; AANENSEN, D. M.; BIRTLES, R. J. Multilocus sequence typing of *Bartonella henselae* in the United Kingdom indicates that only a few, uncommon sequence types are associated

with zoonotic disease. **Journal of Clinical Microbiology**, v. 49, n. 6, p. 2132–2137, 2011.

CHOMEL, B. B.; KASTEN, R. W.; FLOYD-HAWKINS, K.; CHI, B.; YAMAMOTO, K.; ROBERTS-WILSON, J.; GURFIELD, A. N.; ABBOTT, R. C.; PEDERSEN, N. C.; KOEHLER, J. E. Experimental transmission of *Bartonella henselae* by the cat flea. **Journal of Clinical Microbiology**, v. 34, n. 8, p. 1952–1956, 1996.

CHOMEL, B. B.; BOULOUIS, H. J.; BREITSCHWERDT, E. B. Cat scratch disease and other zoonotic *Bartonella* infections. **Journal of the American Veterinary Medical Association**, v. 224, n. 8, p. 1270–9, 2004. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/15112775>.

CHOMEL, B. B.; BOULOUIS, H. J.; BREITSCHWERDT, E. B.; KASTEN, R. W.; VAYSSIER-TAUSSAT, M.; BIRTLES, R. J.; KOEHLER, J. E.; DEHIO, C. Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors. **Veterinary Research**, v. 40, n. 2, 2009.

CHOMEL, B. B.; KASTEN, R. W. Bartonellosis, an increasingly recognized zoonosis. **Journal of Applied Microbiology**, v. 109, n. 3, p. 743–750, 2010.

CICUTTIN, G. L.; BRAMBATI, D. F.; DE GENNARO, M. F.; CARMONA, F.; ISTURIZ, M. L.; PUJOL, L. E.; BELERENIAN, G. C.; GIL, H. *Bartonella* spp. in cats from Buenos Aires, Argentina. **Veterinary Microbiology**, v. 168, n. 1, p. 225–228, 2014.

COSTA, P. S. G.; BRIGATTE, M. E.; GRECO, D. B. Antibodies to *Rickettsia rickettsii, Rickettsia typhi, Coxiella burnetii, Bartonella henselae*, *Bartonella quintana* and *Erhlichia chaffeensis* among healthy population in Minas Gerais, Brazil. **Memorias do Instituto Oswaldo Cruz**, v. 100, n. 8, p. 853–859, 2005.

COTTÉ, V.; BONNET, S.; LE RHUN, D.; LE NAOUR, E.; CHAUVIN, A.; BOULOUIS, H. J.; LECUELLE, B.; LILIN, T.; VAYSSIER-TAUSSAT, M. Transmission of *Bartonella henselae* by *Ixodes ricinus*. **Emerging Infectious Diseases**, v. 14, n. 7, p. 1074–1080, 2008.

CRISSIUMA, A.; FAVACHO, A.; GERSHONY, L.; MENDES-DE-ALMEIDA, F.; GOMES, R.; MARES-GUIA, A.; ROZENTAL, T.; BARREIRA, J.; LEMOS, E.; LABARTHE, N. Prevalence of *Bartonella* species DNA and antibodies in cats (*Felis catus*) submitted to a spay/neuter program in Rio de Janeiro, Brazil. **Journal of Feline Medicine and Surgery**, v. 13, n. 2, p. 149–151, 2011.

DEHIO, C.; LANZ, C.; POHL, R.; BEHRENS, P.; BERMOND, D.; PIÉMONT, Y.; PELZ, K.; SANDER, A. *Bartonella schoenbuchii* sp. nov., isolated from the blood of wild roe deer. **International Journal of Systematic and Evolutionary Microbiology**, v. 51, n. 4, p. 1557–1565, 2001.

DROZ, S.; CHI, B.; HORN, E.; STEIGERWALT, A. G.; WHITNEY, A. M.;

BRENNER, D. J. *Bartonella koehlerae* sp. nov., isolated from cats. **Journal of Clinical Microbiology**, v. 37, n. 4, p. 1117–1122, 1999.

DRUMMOND, M. R.; DOS SANTOS, L. S.; SILVA, M. N. Da; ALMEIDA, A. R. De; DE PAIVA DINIZ, P. P. V.; ANGERAMI, R.; VELHO, P. E. N. F. False Negative Results in Bartonellosis Diagnosis. **Vector-Borne and Zoonotic Diseases**, v. 19, n. 6, p. 453–454, 2019.

DRUMMOND, M. R.; LANIA, B. G.; DE PAIVA DINIZ, P. P. V.; GILIOLI, R.; DEMOLIN, D. M. R.; SCORPIO, D. G.; BREITSCHWERDT, E. B.; VELHO, P. E. N. F. Improvement of *Bartonella henselae* DNA detection in cat blood samples by combining molecular and culture methods. **Journal of Clinical Microbiology**, v. 56, n. 5, p. 1–8, 2018.

DUNCAN, A. W.; MAGGI, R. G.; BREITSCHWERDT, E. B. A combined approach for the enhanced detection and isolation of *Bartonella* species in dog blood samples: Pre-enrichment liquid culture followed by PCR and subculture onto agar plates. **Journal of Microbiological Methods**, v. 69, n. 2, p. 273–281, 2007.

EREMEEVA, M. E.; GERNS, H. L.; LYDY, S. L.; GOO, J. S.; RYAN, E. T.; MATHEW, S. S.; FERRARO, M. J.; HOLDEN, J. M.; NICHOLSON, W. L.; DASCH, G. A.; KOEHLER, J. E. Bacteremia, Fever, and Splenomegaly Caused by a Newly Recognized *Bartonella* Species. **New England Journal of Medicine**, v. 356, n. 23, p. 2381–2387, 2007.

EWING, B.; GREEN, P. Base-calling of automated sequencer traces using phred. II. Error probabilities. **Genome Research**, v. 8, n. 3, p. 186–194, 1998.
EWING, B.; HILLIER, L.; WENDL, M. C.; GREEN, P. Base-Calling of Automated Sequencer Traces Using Phred. I. Accuracy Assessment. **Genome Research**, v. 8, n. 3, p. 175–185, 1 mar. 1998. Disponível em: <http://genome.cshlp.org/lookup/doi/10.1101/gr.8.3.175>.

FINKELSTEIN, J. L.; BROWN, T. P.; O'REILLY, K. L.; WEDINCAMP, J.; FOIL, L. D. Studies on the growth of *Bartonella henselae* in the cat flea (Siphonaptera: Pulicidae). **Journal of Medical Entomology**, v. 39, n. 6, p. 915–919, 2002.

FOLMER, O.; BLACK, M.; HOEH, W.; LUTZ, R.; VRIJENHOEK, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. **Molecular marine biology and biotechnology**, v. 3, n. 5, p. 294–299, 1994.

FONTALVO, M. C.; FAVACHO, A. R. de M.; ARAUJO, A. de C.; SANTOS, N. M. dos; OLIVEIRA, G. M. B. de; AGUIAR, D. M.; LEMOS, E. R. S. de; HORTA, M. C. *Bartonella* species pathogenic for humans infect pets, free-ranging wild mammals and their ectoparasites in the Caatinga biome, Northeastern Brazil: a serological and molecular study. **Brazilian Journal of Infectious Diseases**, v. 21, n. 3, p. 290–296, 2017.
FOURNIER, P. E.; DRANCOURT, M.; ABOUDHARAM, G.; RAOULT, D. Paleomicrobiology of *Bartonella* infections. **Microbes and Infection**, v. 17, n.

11–12, p. 879–883, 2015.

GIL, H.; ESCUDERO, R.; PONS, I.; RODRÍGUEZ-VARGAS, M.; GARCÍA-ESTEBAN, C.; RODRÍGUEZ-MORENO, I.; GARCÍA-AMIL, C.; LOBO, B.; VALCÁRCEL, F.; PÉREZ, A.; JIMÉNEZ, S.; JADO, I.; JUSTE, R.; SEGURA, F.; ANDA, P. Distribution of *Bartonella henselae* Variants in Patients, Reservoir Hosts and Vectors in Spain. **PLoS ONE**, v. 8, n. 7, 2013.

GONÇALVES, L. R.; FAVACHO, A. R. de M.; ROQUE, A. L. R.; MENDES, N. S.; FIDELIS, O. L.; BENEVENUTE, J. L.; HERRERA, H. M.; D'ANDREA, P. S.; DE LEMOS, E. R. S.; MACHADO, R. Z.; ANDRÉ, M. R. Association of *Bartonella* species with wild and synanthropic rodents in different Brazilian biomes. **Applied and Environmental Microbiology**, v. 82, n. 24, p. 7154–7164, 2016.

GONÇALVES, L. R.; HARRUS, S.; GUTIÉRREZ, R.; HERRERA, H. M.; DE SOUZA RAMOS, I. A.; PORFÍRIO, G. E. de O.; NACHUM-BIALA, Y.; DE SOUSA, K. C. M.; DA SILVA, T. M. V.; CAMPOS, J. B. V.; LEMOS, W.; MORAES BARROS-BATTESTI, D.; MACHADO, R. Z.; ANDRÉ, M. R. Molecular detection and genetic diversity of *Bartonella* species in large ruminants and associated ectoparasites from the Brazilian Cerrado. **Transboundary and Emerging Diseases**, v. 67, n. 5, p. 1888–1897, 2020.

GUNDI, V. A. K. B.; TAYLOR, C.; RAOULT, D.; LA SCOLA, B. *Bartonella rattaustraliani* sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats. **International Journal of Systematic and Evolutionary Microbiology**, v. 59, n. 12, p. 2956–2961, 2009.

GUPTILL, L. Feline Bartonellosis. **Veterinary Clinics of North America - Small Animal Practice**, v. 40, n. 6, p. 1073–1090, 2010. Disponível em: <http://dx.doi.org/10.1016/j.cvsm.2010.07.009>.

GUTIÉRREZ, R.; NACHUM-BIALA, Y.; HARRUS, S. The relations between the presence and bacterial loads of *Bartonella* species in the cat and cat flea (*Ctenocephalides felis*), under natural conditions. **Applied and environmental microbiology**, v. 81, n. 16, p. 5613–5621, 2015. Disponível em: <http://aem.asm.org/content/81/16/5613.abstract?etoc>.

GUTIÉRREZ, R.; SHALIT, T.; MARKUS, B.; YUAN, C.; NACHUM-BIALA, Y.; ELAD, D.; HARRUS, S. *Bartonella* kosoyi sp. nov. and *Bartonella krasnovii* sp. nov., two novel species closely related to the zoonotic *Bartonella elizabethae*, isolated from black rats and wild desert rodent-fleas. **International Journal of Systematic and Evolutionary Microbiology**, v. 70, n. 3, p. 1656–1665, 2020.

GUTIÉRREZ, R.; VAYSSIER-TAUSSAT, M.; BUFFET, J.-P.; HARRUS, S. Guidelines for the Isolation, Molecular Detection, and Characterization of *Bartonella* Species. **Vector-Borne and Zoonotic Diseases**, v. 17, n. 1, p. 42–50, 2017. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/28055575%0Ahttp://online.liebertpub.co

m/doi/10.1089/vbz.2016.1956>.

HARMS, A.; DEHIO, C. Intruders below the Radar: Molecular pathogenesis of *Bartonella* spp. **Clinical Microbiology Reviews**, v. 25, n. 1, p. 42–78, 2012.

HARMS, A.; SEGERS, F. H. I. D.; QUEBATTE, M.; MISTL, C.; MANFREDI, P.; KÖRNER, J.; CHOMEL, B. B.; KOSOY, M.; MARUYAMA, S.; ENGEL, P.; DEHIO, C. Evolutionary dynamics of pathoadaptation revealed by three independent acquisitions of the VirB/D4 type IV secretion system in *Bartonella*. **Genome Biology and Evolution**, v. 9, n. 3, p. 761–776, 2017.

HELLER, R.; KUBINA, M.; MARIET, P.; RIEGEL, P.; DELACOUR, G.; CHRISTOPH, D.; MONTEIL, H.; CHOME, B.; PIEMONT, Y.; BOULO, H. Species Isolated From the Blood of Wild Rabbits. n. 1 999, p. 283–288, 1999.

HIGGINS, J. A.; RADULOVIC, S.; JAWORSKI, D. C.; AZAD, A. F. Acquisition of the Cat Scratch Disease Agent *Bartonella henselae* by Cat Fleas (Siphonaptera: Pulicidae). **Journal of Medical Entomology**, v. 33, n. 3, p. 490–495, 1996.

IKEDA, P.; TORRES, J. M.; PERLES, L.; LOURENÇO, E. C.; HERRERA, H. M.; DE OLIVEIRA, C. E.; MACHADO, R. Z.; ANDRÉ, M. R. Intra-and inter-host assessment of *Bartonella* diversity with focus on non-hematophagous bats and associated ectoparasites from Brazil. **Microorganisms**, v. 8, n. 11, p. 1–20, 2020.

INOUE, K.; KABEYA, H.; SHIRATORI, H.; UEDA, K.; KOSOY, M. Y.; CHOMEL, B. B.; BOULOUIS, H. J.; MARUYAMA, S. *Bartonella japonica* sp. nov. and *Bartonella silvatica* sp. nov., isolated from Apodemus mice. **International Journal of Systematic and Evolutionary Microbiology**, v. 60, n. 4, p. 759–763, 2010.

IREDELL, J.; BLANCKENBERG, D.; ARVAND, M.; GRAULING, S.; FEIL, E. J.; BIRTLES, R. J. Characterization of the Natural Population of *Bartonella henselae* by Multilocus Sequence Typing. **Journal of Clinical Microbiology**, v. 41, n. 11, p. 5071–5079, 2003.

JOLLEY, K. A.; BRAY, J. E.; MAIDEN, M. C. J. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications [version 1; referees: 2 approved]. **Wellcome Open Research**, v. 3, n. 0, p. 1–20, 2018.

JOLLEY, K. A.; MAIDEN, M. C. J. BIGSdb: Scalable analysis of bacterial genome variation at the population level. **BMC Bioinformatics**, v. 11, 2010.

KERNIF, T.; LEULMI, H.; SOCOLOVSCHI, C.; BERENGER, J. M.; LEPIDI, H.; BITAM, I.; ROLAIN, J. M.; RAOULT, D.; PAROLA, P. Acquisition and excretion of *Bartonella quintana* by the cat flea, *Ctenocephalides felis felis*. **Molecular Ecology**, v. 23, n. 5, p. 1204–1212, 2014.

KEŠNEROVÁ, L.; MORITZ, R.; ENGEL, P. *Bartonella apis* sp. nov., a honey bee gut symbiont of the class Alphaproteobacteria. **International Journal of Systematic and Evolutionary Microbiology**, v. 66, n. 1, p. 414–421, 2016.

KORDICK, D. L.; WILSON, K. H.; SEXTON, D. J.; HADFIELD, T. L.; BERKHOFF, H. A.; KORDICK, D. L.; WILSON, K. H.; SEXTON, D. J.; HADFIELD, T. E. D. L.; BERKHOFF, H. A.; BREITSCHWERDT, E. B. Prolonged *Bartonella* bacteremia in cats associated with cat-scratch disease patients . Prolonged *Bartonella* Bacteremia in Cats Associated with Cat-Scratch Disease Patients. v. 33, n. 12, p. 3245–3251, 1995.

KOSOY, M.; MCKEE, C.; ALBAYRAK, L.; FOFANOV, Y. Genotyping of *Bartonella* bacteria and their animal hosts : current status and perspectives. p. 1–20, 2017.

LA, V. D.; CLAVEL, B.; LEPETZ, S.; ABOUDHARAM, G.; RAOULT, D.; DRANCOURT, M. Molecular detection of *Bartonella henselae* DNA in the dental pulp of 800-year-old French cats. **Clinical Infectious Diseases**, v. 39, n. 9, p. 1391–1394, 2004.

LAROCHE, M.; RAOULT, D.; PAROLA, P. Insects and the transmission of bacterial agents. **Microbial Transmission**, n. January, p. 195–202, 2019.

LASHNITS, E.; MAGGI, R.; JARSKOG, F.; BRADLEY, J.; BREITSCHWERDT, E.; FROHLICH, F. Schizophrenia and *Bartonella* spp. Infection: A Pilot Case–Control Study . **Vector-Borne and Zoonotic Diseases**, v. XX, n. Xx, p. 1–9, 2021.

LAWSON, P. A.; COLLINS, M. D. Description of *Bartonella* clarridgeaie sp. nov. isolated from the cat of a patient with *Bartonella henselae* septicepmia. **Medical Microbiology Letters**, v. 5, p. 64–73, 1996.

LEIGH, J. W.; BRYANT, D. POPART: Full-feature software for haplotype network construction. **Methods in Ecology and Evolution**, v. 6, n. 9, p. 1110–1116, 2015.

LI, D. M.; HOU, Y.; SONG, X. P.; FU, Y. Q.; LI, G. C.; LI, M.; EREMEEV, M. E.; WU, H. X.; PANG, B.; YUE, Y. J.; HUANG, Y.; LU, L.; WANG, J.; LIU, Q. Y. High prevalence and genetic heterogeneity of rodent-borne *Bartonella* species on Heixiazi Island, China. **Applied and Environmental Microbiology**, v. 81, n. 23, p. 7981–7992, 2015.

LINARDI, P. M.; SANTOS, J. L. C. *Ctenocephalides felis* felis vs. *Ctenocephalides* canis: (Siphonaptera: Pulicidae): Algumas questões para identificar corretamente estas espécies. **Revista Brasileira de Parasitologia Veterinaria**, v. 21, n. 4, p. 345–354, 2012.

LINS, K. de A.; DRUMMOND, M. R.; VELHO, P. E. N. F. Cutaneous manifestations of bartonellosis. **Anais Brasileiros de Dermatologia**, v. 94, n. 5, p. 594–602, 2019. Disponível em:

<https://doi.org/10.1016/j.abd.2019.09.024>.

MAGGI, R. G.; DUNCAN, A. W.; BREITSCHWERDT, E. B.; CAROLINA, N.; AL, M. E. T.; ICROBIOL, J. C. L. I. N. M. Novel Chemically Modified Liquid Medium That Will Support the Growth of Seven *Bartonella* Species. **Journal of Clinical Microbiology**, v. 43, n. 6, p. 2651–2655, 2005.

MAGGI, R. G.; RICHARDSON, T.; BREITSCHWERDT, E. B.; MILLER, J. C. Development and validation of a droplet digital PCR assay for the detection and quantification of *Bartonella* species within human clinical samples. **Journal of Microbiological Methods**, v. 176, n. June, p. 106022, 2020. Disponível em: <https://doi.org/10.1016/j.mimet.2020.106022>.

MAILLARD, R.; RIEGEL, P.; BARRAT, F.; BOULLIN, C.; THIBAULT, D.; GANDOIN, C.; HALOS, L.; DEMANCHE, C.; ALLIOT, A.; GUILLOT, J.; PIÉMONT, Y.; BOULOUIS, H. J.; VAYSSIER-TAUSSAT, M. *Bartonella chomelii* sp. nov., isolated from French domestic cattle (Bos taurus). **International Journal of Systematic and Evolutionary Microbiology**, v. 54, n. 1, p. 215–220, 2004.

MALHEIROS, J.; COSTA, M. M.; DO AMARAL, R. B.; DE SOUSA, K. C. M.; ANDRÉ, M. R.; MACHADO, R. Z.; VIEIRA, M. I. B. Identification of vector-borne pathogens in dogs and cats from Southern Brazil. **Ticks and Tick-borne Diseases**, v. 7, n. 5, p. 893–900, 2016. Disponível em: <http://dx.doi.org/10.1016/j.ttbdis.2016.04.007>.

MEDIANNIKOV, O.; KARKOURI, K. El; ROBERT, C.; FOURNIER, P. E.; RAOULT, D. Non-contiguous finished genome sequence and description of *Bartonella florenciae* sp. nov. **Standards in Genomic Sciences**, v. 9, n. 1, p. 185–196, 2013.

MICELI, N. G.; GAVIOLI, F. A.; GONCALVES, L. R.; ANDRE, M. R.; SOUSA, V. R. F.; SOUSA, K. C. M. de; MACHADO, R. Z. Molecular detection of feline arthropod-borne pathogens in cats in Cuiabá, state of Mato Grosso, central-western region of Brazil. **Revista brasileira de parasitologia veterinaria = Brazilian journal of veterinary parasitology : Orgao Oficial do Colegio Brasileiro de Parasitologia Veterinaria**, v. 22, n. 3, p. 385–390, 2013.

MIETZE, A.; MORICK, D.; KÖHLER, H.; HARRUS, S.; DEHIO, C.; NOLTE, I.; GOETHE, R. Combined MLST and AFLP typing of *Bartonella henselae* isolated from cats reveals new sequence types and suggests clonal evolution. **Veterinary Microbiology**, v. 148, n. 2–4, p. 238–245, 2011.

MONTEIL, M.; DURAND, B.; BOUCHOUICHA, R.; PETIT, E.; CHOMEL, B.; ARVAND, M.; BOULOUIS, H. J.; HADDAD, N. Development of discriminatory multiple-locus variable number tandem repeat analysis for *Bartonella henselae*. **Microbiology**, v. 153, n. 4, p. 1141–1148, 2007.
MÜLLER, A.; SOTO, F.; SEPÚLVEDA, M.; BITTENCOURT, P.; BENEVENUTE, J. L.; IKEDA, P.; MACHADO, R. Z.; ANDRÉ, M. R. *Bartonella* vinsonii subsp. berkhoffii and B. henselae in dogs. **Epidemiology and Infection**, v. 146, n. 9,

p. 1202–1204, 2018.

MULLINS, K. E.; HANG, J.; JIANG, J.; LEGUIA, M.; KASPER, M. R.; VENTOSILLA, P.; MAGUIÑA, C.; JARMAN, R. G.; BLAZES, D.; RICHARDS, A. L. Description of *Bartonella* ancashensis sp. nov., isolated from the blood of two patients with verruga peruana. **International Journal of Systematic and Evolutionary Microbiology**, v. 65, n. 10, p. 3339–3343, 2015.

NORMAN, a F.; REGNERY, R.; JAMESON, P.; GREENE, C.; KRAUSE, D. C. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. **Journal of clinical microbiology**, v. 33, n. 7, p. 1797–1803, 1995.

OKARO, U.; ADDISU, A.; CASANAS, B.; ANDERSON, B. *Bartonella* species, an emerging cause of blood-culture-negative endocarditis. **Clinical Microbiology Reviews**, v. 30, n. 3, p. 709–746, 2017.

PAZIEWSKA, A.; HARRIS, P. D.; ZWOLIŃSKA, L.; BAJER, A.; SIŃSKI, E. Recombination Within and Between Species of the Alpha Proteobacterium *Bartonella* Infecting Rodents. **Microbial Ecology**, v. 61, n. 1, p. 134–145, 2011.

PEDRASSANI, D.; BIOLCHI, J.; GONÇALVES, L. R.; MENDES, N. S.; ZANATTO, D. C. de S.; CALCHI, A. C.; MACHADO, R. Z.; ANDRÉ, M. R. Molecular detection of vector-borne agents in cats in Southern Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 28, n. 4, p. 632–643, dez. 2019. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-29612019000400632&tlng=en>.

PITASSI, L. H. U.; DE PAIVA DINIZ, P. P. V.; SCORPIO, D. G.; DRUMMOND, M. R.; LANIA, B. G.; BARJAS-CASTRO, M. L.; GILIOLI, R.; COLOMBO, S.; SOWY, S.; BREITSCHWERDT, E. B.; NICHOLSON, W. L.; VELHO, P. E. N. F. *Bartonella* spp. Bacteremia in Blood Donors from Campinas, Brazil. **PLoS Neglected Tropical Diseases**, v. 9, n. 1, p. 1–8, 2015.

RAIMUNDO, J. M.; GUIMARÃES, A.; AMARO, G. M.; DA SILVA, A. T.; BOTELHO, C. F. M.; MASSARD, C. L.; DE LEMOS, E. R. S.; FAVACHO, A. R. M.; BALDANI, C. D. Molecular survey of *Bartonella* species in shelter cats in Rio de Janeiro: Clinical, hematological, and risk factors. **American Journal of Tropical Medicine and Hygiene**, v. 100, n. 6, p. 1321–1327, 2019.

REGNERY, R.; MARTIN, M.; OLSON, J. Naturally occurring “Rochalimaea henselae” infection in domestic cat. **The Lancet**, v. 340, n. 8818, p. 557–558, ago. 1992. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/0140673692917606>.

RENESTO, P.; GAUTHERET, D.; DRANCOURT, M.; RAOULT, D. Determination of the rpoB gene sequences of *Bartonella henselae* and *Bartonella* quintana for phylogenic analysis. **Research in Microbiology**, v. 151, n. 10, p. 831–836, 2000.

RIVERA-PEREZ, J. I.; SANTIAGO-RODRIGUEZ, T. M.; TORANZOS, G. A. Paleomicrobiology: a Snapshot of Ancient Microbes and Approaches to Forensic Microbiology. **Microbiology Spectrum**, v. 4, n. 4, p. 1–14, 2016.

SATO, S.; KABEYA, H.; FUJINAGA, Y.; INOUE, K.; UNE, Y.; YOSHIKAWA, Y.; MARUYAMA, S. *Bartonella* jaculi sp. nov., *Bartonella callosciuri* sp. nov., *Bartonella pachyuromydis* sp. nov. and *Bartonella acomydis* sp. nov., isolated from wild Rodentia. **International Journal of Systematic and Evolutionary Microbiology**, v. 63, n. PART 5, p. 1734–1740, 2013.

SILVA, B. T. G. da; SOUZA, A. M. de; CAMPOS, S. D. E.; MACIEIRA, D. de B.; LEMOS, E. R. S. de; FAVACHO, A. R. de M.; ALMOSNY, N. R. P. *Bartonella henselae* and *Bartonella* clarridgeiae infection, hematological changes and associated factors in domestic cats and dogs from an Atlantic rain forest area, Brazil. **Acta Tropica**, v. 193, n. February, p. 163–168, 2019. Disponível em: <https://doi.org/10.1016/j.actatropica.2019.02.026>.

SOUSA, K. C. M.; DO AMARAL, R. B.; HERRERA, H. M.; SANTOS, F. M.; MACEDO, G. C.; DE ANDRADE PINTO, P. C. E.; BARROS-BATTESTI, D. M.; MACHADO, R. Z.; ANDRÉ, M. R. Genetic Diversity of *Bartonella* spp. in Wild Mammals and Ectoparasites in Brazilian Pantanal. **Microbial Ecology**, v. 76, n. 2, p. 544–554, 2018.

SOUZA, A. M.; ALMOSNY, N. R. P.; FAVACHO, A. R. M.; ALMEIDA, D. N. P.; FERREIRA, R. F.; FERREIRA, E. O.; MOREIRA, N. S.; LEMOS, E. R. S. *Bartonella* spp. and hematological changes in privately owned domestic cats from Rio de Janeiro, Brazil. **Journal of infection in developing countries**, v. 11, n. 8, p. 591–596, 2017.

SOUZA, A. M. de; ALMEIDA, D. N. P. de; GUTERRES, A.; GOMES, R.; FAVACHO, A. R. de M.; MOREIRA, N. dos S.; MAIA, L. M. P.; ROZENTAL, T.; TORRES, R. de A.; CERQUEIRA, A. de M. F.; LEMOS, E. R. S. de; PEREIRA, A. N. R. Bartonelose: análise molecular e sorológica em gatos do Rio de Janeiro Brasil. **Revista Brasileira de Ciência Veterinária**, v. 17, n. 1, p. 7–11, 2010.

STAGGEMEIER, R.; PILGER, D. A.; SPILKI, F. R.; CANTARELLI, V. V. PCR em Tempo Real (qPCR) multiplex utilizando SYBR® Green para a detec ção e diferenciação de *Bartonella henselae* e *Bartonella* clarridgeiae em gatos. **Revista do Instituto de Medicina Tropical de Sao Paulo**, v. 56, n. 2, p. 93–95, 2014.

STAGGEMEIER, R.; VENKER, C. A.; KLEIN, D. H.; PETRY, M.; SPILKI, F. R.; CANTARELLI, V. V. Prevalence of *Bartonella henselae* and *Bartonella* clarridgeiae in cats in the south of Brazil: A molecular study. **Memorias do Instituto Oswaldo Cruz**, v. 105, n. 7, p. 873–878, 2010.

STEPANIĆ, M.; DUVNJAK, S.; REIL, I.; ŠPIČIĆ, S.; KOMPES, G.; BECK, R. First isolation and genotyping of *Bartonella henselae* from a cat living with a patient with cat scratch disease in Southeast Europe. **BMC Infectious**

Diseases, v. 19, n. 1, p. 1–6, 2019.

STRONG, R. P.; TYZZER, E. E.; SELLARDS, A. W. Oroya fever. Second report. **Journal of the American Medical Association**, v. 64, p. 806–808, 1915.

URWIN, R.; MAIDEN, M. C. J. Multi-locus sequence typing: A tool for global epidemiology. **Trends in Microbiology**, v. 11, n. 10, p. 479–487, 2003.

WECHTAISONG, W.; BONNET, S. I.; LIEN, Y. Y.; CHUANG, S. Te; TSAI, Y. L. Transmission of *Bartonella henselae* within rhipicephalus sanguineus: Data on the potential vector role of the tick. **PLoS Neglected Tropical Diseases**, v. 14, n. 10, p. 1–14, 2020. Disponível em: <http://dx.doi.org/10.1371/journal.pntd.0008664>.

WELCH, D. F.; CARROLL, K. C.; HOFMEISTER, E. K.; PERSING, D. H.; ROBISON, D. A.; STEIGERWALT, A. G.; BRENNER, D. J. Isolation of a new subspecies, *Bartonella* vinsonii subsp. arupensis, from a cattle rancher: Identity with isolates found in conjunction with Borrelia burgdorferi and Babesia microti among naturally infected mice. **Journal of Clinical Microbiology**, v. 37, n. 8, p. 2598–2601, 1999.

WOLF, L. A.; CHERRY, N. A.; MAGGI, R. G.; BREITSCHWERDT, E. B. In Pursuit of a Stealth Pathogen: Laboratory Diagnosis of Bartonellosis. **Clinical Microbiology Newsletter**, v. 36, n. 5, p. 33–39, mar. 2014. Disponível em: <http://dx.doi.org/10.1016/j.clinmicnews.2014.02.001>.

CHAPTER 2 – Genetic diversity and Multilocus Sequence Typing Analysis of *Bartonella henselae* in domestic cats from Southeastern Brazil

ABSTRACT

Bartonella henselae is the causative agent for the infectious disease Cat Scratch Disease (CSD), which can be fatal. Domestic and wild felines are known to be its main mammal reservoirs. The present study aimed to investigate the occurrence and genetic diversity of *Bartonella* spp. in cats sampled in São Paulo (SP) and Minas Gerais (MG) States, Southeastern Brazil. Based on a quantitative real-time PCR (qPCR) assay, a *Bartonella* sp. nuoG gene fragment was detected in 39.9% (122/306) of the blood samples (46/151 cats of SP; 76/155 cats of MG). The blood samples were submitted to a pre-

enrichment culture technique that allowed the detection of 12 additional positive samples, which showed to be negative in the qPCR using DNA blood samples as templates. Furthermore, five *B. henselae* isolates were obtained from qPCR-negative samples for both blood and pre-enrichment culture. Seven out of 24 Ctenocephalides felis fleas were positive for *Bartonella* spp. in the qPCR assay; 4/7 positive fleas were collected from Bartonella-negative cats. Twenty-three rpoB *B. henselae* cloned sequences were obtained from nine cats' blood samples, showing the occurrence of 13 different genotypes. Median-joining network and SplitsTree distance analysis showed that the obtained sequences represented distinct *B. henselae* genotypes when compared to those previously deposited in GenBank. Intra-host diversity was found, since different rpoB genotypes of *B. henselae* were detected in individual single cats. *Bartonella henselae* isolates showed two allelic profiles (ST37 in cats from MG state and ST9 in SP state) by MLST (Multilocus Sequence Typing) based on sequencing of eight molecular markers. The present study is the first molecular report of *Bartonella* sp. in cats from Minas Gerais State. In summary, this body of work showed the occurrence of different *B. henselae* rpoB genotypes at an intra-reservoir host level. Based on qPCR from blood samples and pre-enrichment liquid culture and isolation, occurrence of 33.1% (50/151) and 56.8% (88/155) for *Bartonella* sp. was found in cats from SP and MG states, respectively. Two different allelic profiles of *B. henselae* were found in cats from the states of São Paulo (ST9) and Minas Gerais (ST37), suggesting a clonal evolution of Bartonellae in a certain geographical region.

Key-words: bartonellosis; feline; distance analysis, genotyping

1. INTRODUCTION

The improvement of techniques for diagnosis of *Bartonella* infection increased the detection of such agents, which currently have been classified as (re)-emerging pathogens and attributed as a causative agent of several human diseases (HARMS; DEHIO, 2012). Among *Bartonella* species, *Bartonella henselae* is responsible for causing a self-limiting human disease denominated Cat Scratch Disease (CSD), mainly characterized by a benign regional lymphadenopathy and fever (BREITSCHWERDT et al., 2010). *Bartonella henselae* infected patients may develop atypical symptoms of CSD, such as prolonged fever, arthralgia, splenomegaly and Parinaud's Oculoglandular Syndrome (ANGELAKIS; RAOULT, 2014). This agent is also known to be responsible for vascular manifestations such as bacillary angiomatosis and peliosis hepatis, which are considered the most common symptoms in immunocompromised patients primarily infected with the human immunodeficiency virus (HIV). Besides that, neurological disorders (meningoencephalitis, myelitis polyradiculoneuritis, and optic neuritis) have been described in 1-7% of infected patients (BOULOUIS et al., 2005; CANNETI et al., 2019; CHOMEL; KASTEN, 2010).

While cats (*Felis catus*) are the main mammal reservoirs for *B. henselae*, the cat fleas (*Ctenocephalides felis*) play a role as its natural vectors, being responsible for shedding contaminated feces, which are inoculated via cat scratches or bites (HARMS; DEHIO, 2012).

The occurrence of *Bartonella* spp. among cats from Brazil ranges from 1.6% to 97% (BRAGA et al., 2015; SOUZA et al., 2010). *Bartonella henselae*

and B. clarridgeiae are the most frequent species identified among national studies in cats (ANDRÉ et al., 2015; DRUMMOND et al., 2018; SILVA et al., 2019). Indeed, these two *Bartonella* species were the most frequent species identified by molecular assays in cats from the state of São Paulo (ANDRÉ et al., 2014; BORTOLI et al., 2012; DRUMMOND et al., 2018). However, to the best of the authors' knowledge, no studies have addressed the occurrence of bartonellae in cats from Minas Gerais State so far.

Even though *B. henselae*, B. clarridgeiae, and B. koehlerae have been detected in cats from Brazil (ANDRÉ et al., 2015, 2014; PEDRASSANI et al., 2019; RAIMUNDO et al., 2019), the occurrence of multiple rpoB (RNA polymerase beta subunit) genotypes in cats has not been assessed so far, since most studies in the literature has focused on the analysis of genetic diversity by targeting different *Bartonella* housekeeping genes, as previously proposed (KOSOY et al., 2017).

Over the years, molecular investigations identified two genotypes of *B. henselae*, namely genotype I (*B. henselae* Houston-1 strain) and genotype II (Marseille) (MONTEIL et al., 2007). While Houston-1 strain I is the most reported genotype in humans presenting CSD, the Marseille genotype is frequently found among cats (BOULOUIS et al., 2005). Even though *B. henselae* has been extensively detected by molecular methods in cats around the world, few studies have been performed aiming at genotyping isolates (CHALONER et al., 2011; CICUTTIN et al., 2014; GIL et al., 2013; IREDELL et al., 2003; MIETZE et al., 2011; STEPANIĆ et al., 2019) by Multiocus Sequence Typing (MLST) (IREDELL et al., 2003) in order to gain a better understanding of Bartonellae population structure, diversity and evolution (AZZAG et al., 2012).

Therefore, the present study aimed to investigate the occurrence and genetic diversity of *B. henselae* in cats sampled in the states of São Paulo and Minas Gerais, Southeastern Brazil. For this purpose, two different strategies were used: i) assessment of the genetic diversity of cloned rpoB *B. henselae* genotypes at both intra and inter-vertebrate hosts levels; ii) MLST analyses of *B. henselae* isolates.

2. MATERIAL AND METHODS

2.1 Cats' blood and flea sampling

Three hundred and six (306) blood samples were obtained from stray and pet cats in Minas Gerais (155 samples – Uberlândia 19°00'39"S 48º05'45"W and Araguari 18º24'43"S 49º03'09"W) and São Paulo (151 samples – Jaboticabal 21º15'33"S 48º18'54"W) States, Southeastern Brazil **(Figure 1).** These samples were collected during neutering campaigns promoted by the Animal Protector Association Neutering Center, located at the School of Agricultural and Veterinarian Sciences (FCAV/UNESP, Jaboticabal, São Paulo State, southeastern Brazil) and Animal Populational Control Project a partnership by the Federal University of Uberlândia (UFU) and the city's Zoonosis Control Center (Uberlândia, Minas Gerais State) (IACUC FCAV/UNESP 012017/17).

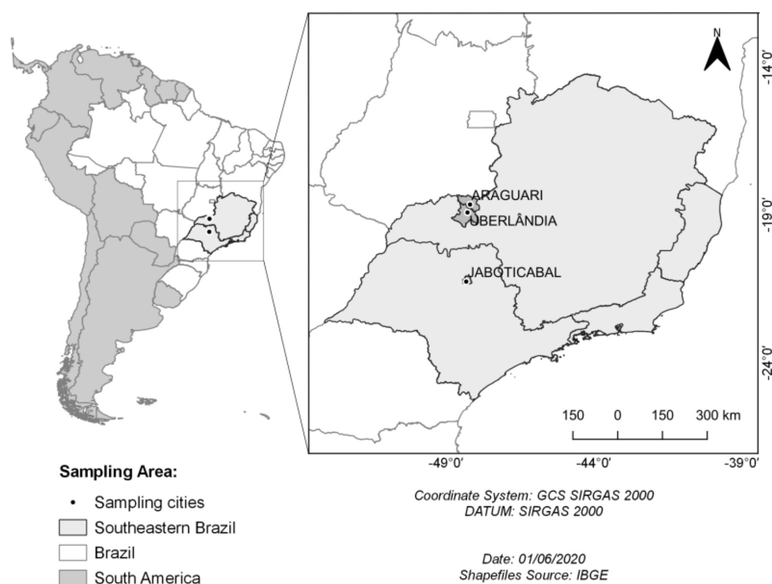


Figure 1. Map showing the Southeast region of Brazil and location of the cities of Jaboticabal (São Paulo State), and Araguari and Uberlândia (Minas Gerais State) where cats were sampled in the present study.

Blood samples were collected in tubes containing EDTA, transported in liquid nitrogen to the laboratory and stored in freezer (−70ºC) for subsequent culturing, DNA extraction and molecular analysis **(Figure 2)**. Fleas were also collected from sampled cats and stored in 70% ethanol (Merck, Darmstadt, Germany) for posterior identification and molecular analyses **(Figure 2)**. Flea identification was performed following previously described taxonomic keys (LINARDI; SANTOS, 2012).

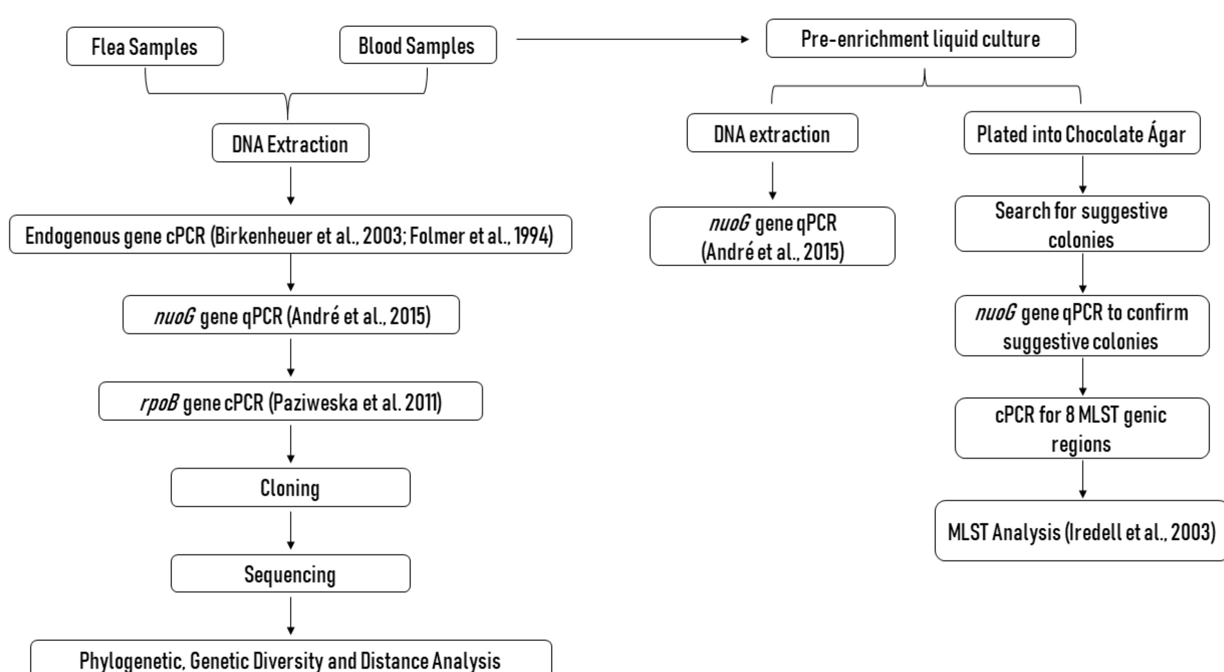


Figure 2. Flow chart of the diagnostic methods and molecular analyses applied in this study for genotyping *B. henselae*.

2.2 Molecular assays for *Bartonella* spp. and cloning

DNA was extracted from 250 µL from each blood sample according to protocol described by Kuramae-Izioka (1997). Fleas were individually subjected to DNA extraction using the Illustra Tissue and Cells genomicPrep Mini Spin Kit extraction kit (GE Healthcare, United Kingdom), following the manufacturer's

recommendations. The DNA concentration and absorbance ratio (260/280 nm) were measured using a Nano-Drop spectrophotometer (Thermo Scientific, Waltham, MA, USA).

The presence of amplifiable DNA in cat's blood samples and fleas was verified by conventional PCR assays targeting the mammal endogenous glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (BIRKENHEUER et al., 2003) and the cytochrome-c (cox-1) protein subunit 1 coding gene for invertebrates (FOLMER et al., 1994), respectively. The positive samples in these assays were submitted to a quantitative real-time PCR (qPCR) assay based on nuoG gene to detect and quantify the number of *Bartonella* DNA copies/μL in each sample (ANDRÉ et al., 2015). The qPCR assays were conducted in a low-profile multiplate unskirted PCR (BioRad, Hercules, California, United States) using a CFX96 thermal cycler (BioRad, Hercules, California, United States). Serial dilutions were performed to construct standard curves with different concentrations (2.0 × 107 to 2.0 × 100 copies) of a plasmid encoding a fragment of the 83 bp nuoG gene of *B. henselae* DNA (pIDTSMART; Integrated DNA Technologies, Coralville, Iowa, United States). The number of plasmid copies was determined according to the formula (XG/μL DNA/ [Plasmid Length (BP) × 660]) × 6. 22 × 1023 × plasmid copies/μL. All DNA samples were initially tested in duplicates. All duplicates whose Cq difference was higher than 0.5 were retested in triplicate. Amplification efficiency (E) was calculated from the slope of the standard curve in each run using the following formula (E = 10−1/ slope) (BUSTIN et al., 2009).

For molecular characterization, the qPCR assay positive samples were submitted to a conventional PCR (cPCR) assay based on rpoB gene (800 bp)

(PAZIEWSKA et al., 2011). *Bartonella henselae* DNA, previously detected in a naturally infected cat (ANDRÉ et al., 2015), and nuclease-free water (Promega, Madison, Wisconsin, United States) were used as positive and negative controls, respectively. The amplicons obtained in cPCR assays were submitted to ethidium bromide (Life Technologies, Carlsbad, California, United States)-stained agarose gels (1%) electrophoresis, at 100 V and 150 mA for 50 min. The gels were imaged under ultraviolet light (ChemiDoc MP Imaging System; Bio Rad, Hercules, California, United States) using the Image Lab software, version 4.1.

Subsequently, rpoB-cPCR positive samples were cloned using the pGEM-T Easy (Promega, Madison, Wisconsin, United States) vector, following the manufacturer's recommendations. The ligation reaction products were used to transform One ShotTM Mach1TM T1R Chemically Competent *E. coli* Cells (Invitrogen Cat # C8620-03) (109-1010 CFU/ng DNA) by thermal shocking. The 250 µL of transformed cells were added to each plate containing Luria Bertani solid medium prepared with 100 µg/mL ampicillin, 40 µL X-Gal (5-Bromo-4-chloro-3-indolyl-β-D-galactoside) (0.026%) and 20 µL IPTG (isopropylthio-β-galactoside) (0.82mM). The bacterial colonies containing the clones (white colonies) were transferred to 15 mL Falcon tubes containing 5 mL LB liquid medium and 100 µg/mL ampicillin. After incubation at 37 ºC for up to 24 hours, the plasmid DNA was extracted following the protocol previously established by Sambrook and Russel (2001). The samples were stored at -20ºC to be used later in PCR reactions to confirm the presence of rpoB inserts. Plasmid DNA extracted from the clones was submitted to PCR using the primers M13 F (5'-CGCCAGGGTTTTCCCAGTCACGAC-3') and M13 R (5'-

GTCATAGCTGTTTCCTGTGTGA-3') (LAU et al., 2010) that flank the cloning site of the plasmid pGEM T-easy and include, therefore, the rpoB inserts.

2.3. Pre-enrichment liquid culture and chocolate agar isolation of *Bartonella* spp.

A pre-enrichment liquid medium used was based on that previously described as Bartonella-Alphaproteobacteria Growth Medium. For this purpose, 500 mL of IPL-41 Insecta Medium (Sigma-Aldrich, St. Louis, Missouri, USA) was supplemented with 0.055 mg of NAD (Sigma-Aldrich, St. Louis, Missouri, USA), 0.69 mg of NADP (Sigma-Aldrich, St. Louis, Missouri, USA), 1.1 mg of ATP (Sigma-Aldrich, St. Louis, Missouri, USA), 1.1 mg sodium pyruvate (Sigma-Aldrich, St. Louis, Missouri, USA) and 1.1 g yeast extract (Sigma-Aldrich, St. Louis, Missouri, USA). Amino acid supplementation was performed by adding 35.1 mg of L-arginine HCl, 8.66 mg of L-cystine HCl, 11.64 mg of L-histidine, 14.6 mg of L-isoleucine and L-leucine each, 20.14 mg L-lysine, 4.16 mg L-methionine, 9.02 mg L-phenylalanine, 13,02 mg of L-threonine, 2.7 mg of L-tryptophan, 12 mg of L-tyrosine 2Na 2H2O and 13 mg of L-valine (Dinâmica, Indaiatuba, São Paulo, Brazil).

After the supplementation, the medium pH was adjusted to 6.2 and subsequently sterilized through a 0.2 µM filter (Corning, Corning, New York, United States). After filtration, the medium was also supplemented with 55 mL of defibrinated sheep blood (previously tested negative by qPCR for *Bartonella* sp.) and adjusted to a final concentration of 11% vol / vol. Two hundred microliters of the cat blood samples were then added to 2 mL of medium containing defibrinated sheep blood, placed into culture flasks (20 cm2, model

430639, Corning, Corning, New York, United States) and incubated for seven days, under constant movement, at 35 °C with 5% CO2 in a CO2 / O2 Water Jacketed incubator (NuAire, Plymouth, Massachusetts, USA) (DUNCAN et al., 2007; MAGGI et al., 2005). Two flasks of negative controls were also added for each batch of 20 tested samples: one containing only the pre-enrichment medium and another containing both the pre-enrichment medium and defibrinated sheep blood.

After seven days, 200 µL of the liquid culture were subjected to DNA extraction by the Illustra Tissue & Cells genomicPrep Mini Spin Kit, following the manufacturer's recommendations. Additionally, 200 µL of the content of each bottle were plated on enriched chocolate agar (Laborclin, Pinhais, Paraná, Brazil) and kept at 37 °C with 5% CO2 for up to 60 days in a CO2 / O2 Water Jacketed incubator (NuAire, Plymouth, Massachusetts, USA), being observed daily. Three negative controls were added for each batch of tested samples: two corresponding to the negative controls previously used in pre-enrichment liquid culture and another one represented by a chocolate agar plate without any sample. Colonies suggestive of Bartonellae were collected and subjected to DNA extraction by the boiling method (KEIM et al., 2000) and qPCR assay for *Bartonella* spp. based on the nuoG gene (ANDRÉ et al., 2015). Colonies confirmed to be *Bartonella* sp. were then submitted to three passages until isolated cultures were obtained.

2.4 Conventional PCR assays (cPCR) for MLST for *Bartonella henselae* colonies genotyping.

Colonies positive in the qPCR assay for *Bartonella* spp. were submitted to cPCR assays targeting eight genic regions for the MLST analysis, namely: gltA

(BIRTLES; RAOULT, 1996; NORMAN et al., 1995), rpoB (DINIZ et al., 2007), ftsZ (PAZIEWSKA et al., 2011), 16S RNA (PAZIEWSKA et al., 2011), 16-23S rRNA intergenic regions (ITS) (DINIZ ET al., 2007), groEL (PAZIEWSKA et al., 2011; ZEAITER et al., 2002), bartR and nlpD (IREDELL et al., 2003).

2.5 Purification of amplicons, sequencing and phylogenetic analysis

The rpoB-cloned products and the MLST's eight genic regions amplicons were purified using the ExoSAP- IT (Thermo Fisher Scientific, Waltham, Massachusetts, United States) and were sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States) and the ABI PRISM 310 DNA Analyzer (Applied Biosystems, Foster City, California, United States; SANGER; NICKLEN; COULSON, 1977) at the "Centro de Recursos Biológicos e Biologia Genômica (CREBIO -FCAV - UNESP)".

The sequences obtained for the rpoB-cloned products were submitted to a quality-screening test using the software Phred-Phrap version 23 (EWING et al., 1998; EWING; GREEN, 1998), in order to evaluate the quality of the electropherograms and to obtain the consensus sequence from the alignment of the sense and antisense sequences (EWING et al., 1998). The BLASTn program (ALTSCHUL et al., 1990) was used to compare the obtained nucleotide sequences with previous sequences deposited in the GenBank database (BENSON et al., 2018).

The rpoB-cloned consensus sequences obtained in this study and those retrieved from GenBank were aligned using the Bioedit v. 7.0.5.3 software (HALL, 1999). The best evolutionary model was chosen using the jModelTest2

software (version 2.1.6) on XSEDE (DARRIBA et al., 2012) via the CIPRES Science Gateway (MILLER et al., 2010). The phylogenetic analysis was based on the Bayesian inference method (BI), performed using MrBayes 3.1.2 (RONQUIST; HUELSENBECK, 2003) via the CIPRES Science Gateway. Markov chain Monte Carlo (MCMC) simulations were run for 109 generations with a sampling frequency of every 100 generations and a burn-in of 25%. Additionally, the same alignment used for the phylogenetic analyses based on rpoB gene was also employed for distance analysis (Split-Network), using the SplitsTree program version 4.11.3 (HUSON; BRYANT, 2006). For this purpose, the ‘Neighbour-Net’ method was used. The support values were estimated by 1000 bootstrap replicates.

2.6 Genetic diversity and genealogies within *Bartonella henselae* taxon

For the analysis of the genetic diversity, the obtained rpoB *B. henselae* sequences were aligned with sequences previously deposited in the GenBank. The alignment was used to calculate the nucleotide diversity (π), polymorphism level (diversity of haplotypes [dh], number of haplotypes [h] and the average number of nucleotide differences [K]), using DnaSP v5 software (LIBRADO; ROZAS, 2009). The nucleotide sequences were submitted to the Median-Joining Network (BANDELT et al., 1999) and distance analysis based on Split-Network, inferred using the programs Population Analysis with Reticulate Trees (popART) and SplitsTree v 4.11.3 (HUSON; BRYANT, 2006; LEIGH; BRYANT, 2015), respectively.

2.7 Multilocus Sequence Typing (MLST) Analysis

The identified *B. henselae* sequences (ST) were assigned according to the online database *B. henselae* MLST (https://pubmlst.org/organisms/Bartonella-henselae) (IREDELL et al., 2003), and used the software GrapeTree to visualize and generate minimum spanning trees with the MLST data (https://pubmlst.org/bigsdb?db=pubmlst_bhenselae_isolates&page=plugin&name=GrapeTree) (ZHOU et al., 2018), which was also employed for distance analysis (Split-Network), using the SplitsTree program version 4.11.3 (HUSON; BRYANT, 2006).

3. RESULTS

3.1 Occurrence of *Bartonella* spp. in the cats' blood samples

The presence of amplifiable DNA was confirmed in all 306 feline samples by targeting the GAPDH gene and the concentration mean and absorbance ratio (260/280 nm) from the MG DNA samples extracted were 30.2 ng/µl (ranging from 1.0 to 567.8 ng/µl, standard deviation [SD] ± 100.4) and 2.0 (ranging from 0.78 to 3.9, SD ± 0.42) and from the SP samples DNA were 53.6 ng/µl (ranging from -1.6 to 426,9 ng/µl, SD ± 51,55) and 1.9 (ranging from -4.39 to 3.76, SD ± 0.76). One hundred twenty-two out of 306 (39.9%) cats' blood samples were positive in the qPCR assay for *Bartonella* spp. targeting the nuoG gene. 49.9% (76/155) and 30.4% (46/151) of the samples from MG and SP States were positive in the qPCR assay, respectively.

The number of copies of the *Bartonella* spp. nuoG gene fragment per microliter ranged from 6.02 x 10-2 a 1.63 x 103, with an average of 5.93 x 101 copies/µL of DNA (**Table 1**). The efficiency, R2, slope and Y-intercept of the qPCR assays

ranged from 98.7% to 102.5% (mean = 99.6%), 0.836 to 0.997 (mean = 0.98), -3.252 to -3.354 (mean = -3.34) and 33.645 to 37.035 (mean = 37.54), respectively, following the MIQE guideline recommendations (BUSTIN et al., 2009).

Table 1. Quantification of *Bartonella* sp. DNA (number of copies of a fragment of the *nuoG* gene of *Bartonella* sp. per microliter) assessed by qPCR in cat positive blood samples.

Bartonella-nuoG **qPCR assay**			
Samples	**Quantification**	**Samples**	**Quantification**
MG03	3.05×10^{-1}	**MG113**	3.94×10^{-1}
MG04	9.49×10^{-1}	**MG125**	2.55
MG07	4.75×10^{-1}	**MG126**	5.26×10^{2}
MG09	3.26×10^{-1}	**MG127**	1.67
MG11	1.07×10^{1}	**MG131**	4.67
MG16	3.72×10^{-1}	**MG138**	7.02
MG17	3.72×10^{-1}	**MG139**	1.51×10^{1}
MG19	3.13×10^{1}	**MG141**	1.63×10^{3}
MG20	1.88	**MG143**	2.31×10^{1}
MG23	5.22×10^{2}	**MG145**	1.12
MG24	1.38×10^{3}	**MG146**	9.46
MG25	5.77×10^{-1}	**MG147**	3.01×10^{1}
MG26	2.21	**MG148**	2.53×10^{1}
MG27	1.79	**MG149**	3.12×10^{-1}
MG28	5.87×10^{-1}	**MG153**	1.34×10^{1}
MG29	5.46	**SP04**	5.03×10^{-1}
MG30	1.7×10^{-1}	**SP05**	1.08×10^{1}
MG32	1.03×10^{3}	**SP07**	1.69
MG34	4.23×10^{-1}	**SP09**	1.45
MG36	2.87×10^{-1}	**SP24**	1.26×10^{1}
MG37	4.43×10^{-1}	**SP33**	2.28×10^{-1}
MG38	8.28×10^{-1}	**SP39**	8.32
MG39	1.29×10^{1}	**SP40**	1.32
MG40	3.32×10^{2}	**SP41**	2.43
MG41	1.30×10^{3}	**SP44**	5.09
MG42	5.82×10^{1}	**SP46**	4.21
MG43	7.71	**SP48**	1.18
MG44	1.22	**SP55**	1.44×10^{1}
MG45	2.11	**SP64**	1.64×10^{1}

MG46	1.00	**SP66**	2.25×10^{1}
MG47	6.06×10^{-1}	**SP67**	2.85
MG48	2.37	**SP68**	2.75×10^{1}
MG49	1.13×10^{1}	**SP76**	7.81×10^{-1}
MG50	5.80×10^{-1}	**SP78**	8.28×10^{-1}
MG51	2.32×10^{-1}	**SP87**	9.82×10^{-1}
MG54	1.15	**SP94**	1.03×10^{2}
MG58	1.71×10^{1}	**SP96**	4.38
MG60	7.11×10^{2}	**SP99**	2.03×10^{1}
MG61	6.02×10^{-2}	**SP100**	8.26×10^{2}
MG62	5.14	**SP101**	3.06
MG63	7.35×10^{-1}	**SP103**	3.29
MG66	1.27	**SP106**	8.80×10^{-1}
MG69	3.88×10^{2}	**SP109**	1.74
MG70	3.67×10^{1}	**SP111**	9.09
MG71	4.81×10^{1}	**SP114**	2.84×10^{-1}
MG72	2.30×10^{1}	**SP115**	2.25×10^{-1}
MG73	1.28	**SP117**	6.11×10^{-1}
MG75	1.27×10^{-1}	**SP119**	6.02×10^{-1}
MG78	1.08×10^{1}	**SP120**	1.31
MG80	8.63×10^{-1}	**SP121**	1.79
MG81	4.36	**SP122**	4.42×10^{1}
MG86	3.91×10^{2}	**SP123**	1.1
MG88	8.73×10^{-1}	**SP125**	5.92×10^{2}
MG89	4.84×10^{-1}	**SP126**	3.06
MG92	2.70×10^{-1}	**SP127**	6.03×10^{1}
MG96	1.12	**SP128**	3.10×10^{1}
MG97	1	**SP134**	2.45
MG99	1.5	**SP137**	2.73×10^{-1}
MG106	1.16×10^{1}	**SP145**	1.03
MG111	1.05×10^{2}	**SP146**	7.85×10^{-1}
MG112	5.65	**SP147**	7.85×10^{-1}
Mean	**5.93×10^{1} copies/µL**		

3.2 Ectoparasites

A total of 38 (1-3 per/animal) Ctenocephalides felis fleas were collected from 23/306 (13 from SP; 10 from MG) infested domestic cats sampled. Twenty-four fleas (9 from MG; 15 from SP) were positive in the cox-1 gene cPCR. Fourteen (36,8%) negative samples for the endogenous gene were excluded from the subsequent analyses.

Seven (29.2%) out of the 24 flea samples were positive in the qPCR assay for *Bartonella* spp. targeting the nuoG gene (3 from MG; 4 from SP). The number of copies of the *Bartonella* spp. nuoG gene fragment per microliter ranged from 1.17 x 100 a 3.9 x 108, with an average of 5.93 x 101 copies/µL of DNA. The efficiency, R2, slope and Y-intercept of the reactions ranged from 99.3%, 0.996, -3.338 e 37.276, respectively, following the MIQE guideline recommendations (BUSTIN et al., 2009). None of the qPCR positive fleas were positive in the rpoB-based cPCR, precluding additional *Bartonella* speciation.

Four (57.15%) positive fleas for *Bartonella* spp. were collected from Bartonella-negative cats in the blood sample qPCR (**Table 2**). Out of 17 negative fleas in the qPCR for *Bartonella* spp., 3 (17.6%) were removed from *Bartonella* spp. positive cats. Unfortunately, *Bartonella* qPCR-positive fleas were negative in cPCR assays based on gltA and rpoB genes (data not shown), precluding the speciation of bartonellae from these ectoparasites.

Table 2. *Bartonella nuoG*-qPCR results for *Ctenocephalides felis* specimens in comparison with those found in the respective host blood samples, liquid culture and isolated colonies.

State	Sample	qPCR quantification (number of copies of *Bartonella nuoG* / µL)			
		C. felis	Host Blood Sample	Liquid Culture	Isolated colonies
São Paulo	01	5.96×10^0	Negative	Negative	Negative
	55.2	1.17×10^0	1.44×10^1	Negative	Negative
	57.2	3.90×10^8	Negative	Negative	Negative
	104.2	2.50×10^0	Negative	Negative	Positive
Minas Gerais	32.1	9.00×10^1	1.03×10^3	4.23×10^4	Positive
	32.2	1.24×10^2			
	110	2.11×10^0	Negative	Negative	Negative

3.3 *rpoB* -cPCR assay and Cloning

Out of 122 nuoG-qPCR positive samples, 22% (27/122) samples were positive for the rpoB gene-based PCR. Nine samples (2 samples from Minas Gerais State and 7 from São Paulo State) showed rpoB positive clones, producing a total of 23 clones.

3.4 *Bartonella henselae* isolation

Two hundred and ninety (n=290) samples were submitted to the pre-enrichment liquid culture and 29 (10%) were positive after seven days at the nuoG gene qPCR assay. The number of copies of the *Bartonella* sp. nuoG gene fragment per microliter ranged from 2.47 to 1.4 x 106. with an average of 2.51 x 101 copies/µL of DNA. The efficiency, R2, slope and Y-intercept of the reactions ranged from 91.4% to 103% (mean = 97.2%), 0.995 to 0.999 (mean = 0.997), -3.252 to -3.547 (mean = -3.395) and 38.571 a 405 (mean = 37.54), respectively, following the MIQE guideline recommendations (BUSTIN et al., 2009).

Out of the 29 positive samples post pre-enrichment liquid culture, 17 (58.6%) were also qPCR-positive when DNA blood samples were used as templates. However, 12 (41.4%) of the culture-positive samples were previously negative when DNA blood samples were used as templates (**Table 3**).

Table 3. Comparison of *Bartonella-nuoG* qPCR quantification values between pre-enrichment liquid cultures and their respective blood samples.

Samples	qPCR (Number of copies / µL)	
	Liquid Culture	**Blood**
MG15*	2.37×10^4	Negative
MG19*	7.75×10^4	3.13×10^1
MG29*	2.24×10^4	5.46
MG30*	2.53×10^4	1.7×10^{-1}
MG32*	4.23×10^4	1.03×10^3
MG40*	2.38×10^5	3.32×10^2
MG44*	1.66×10^4	1.22
MG48*	1.71×10^4	2.37
MG67*	Negative	Negative
MG69	1.25×10^5	3.88×10^2
MG78	1.07×10^6	1.08×10^1
MG81	5.41×10^5	4.36
MG86	8.09	3.91×10^2
MG93	3.24×10^1	Negative
MG94	2.41×10^1	Negative
MG101	1.26×10^6	Negative
MG102	6.77×10^5	Negative
MG106*	$\mathbf{1.15 \times 10^5}$	$\mathbf{1.16 \times 10^1}$
MG111*	$\mathbf{1.94 \times 10^1}$	$\mathbf{1.05 \times 10^2}$
MG113	2.47	3.94×10^{-1}
MG115	1.01×10^1	Negative
MG117	1.61×10^1	Negative
MG118	4.74	Negative
MG121	5.01	Negative
MG124	4.81	Negative
MG126	1.4×10^6	5.26×10^2
MG131	5.07×10^5	4.67
MG139*	Negative	Negative
MG140*	Negative	Negative
MG147*	3.53×10^3	3.01×10^1

SP018*	**1.37 x 10^2**	**Negative**
SP79*	Negative	Negative
SP104*	Negative	Negative
SP116	9.91	Negative

*Samples for which *Bartonella* isolates were obtained in chocolate agar plates.

Only 17 (5.9%) plates presented suggestive *Bartonella* spp. colonies and were confirmed as *Bartonella* spp. positive after nuoG gene qPCR assay. Five (5/17 – 29.4%; 2 from SP and 3 from MG) of the isolates were originated from both blood and pre-enrichment culture samples that showed to be negative in nuoG-based qPCR assay. Seventeen (58.6%) (4 from SP and 13 from MG) blood samples negative in the nuoG-based qPCR assay were positive for *Bartonella* spp. either after pre-enrichment liquid culture or isolation (**Table 3**).

3.4.1 BLASTn analysis

BLASTn analysis of rpoB-gene clone sequences showed 99.2% to 100% identity with *B. henselae* sequences (CP020742.1; FJ832093.1; KX499341.1; MF314831.1), with query coverage between 98% and 100%.

3.4.2 Phylogenetic analysis

The phylogenetic tree inferred by Bayesian analysis and TIM+I+G evolutionary model based on the rpoB gene positioned all 23 sequences in a clade containing rpoB sequences of *B. henselae* previously deposited in GenBank, with 100% posterior probability (**Figure 3**). The sequences were deposited in the GenBank under the following accession numbers MW854833 to MW854855.

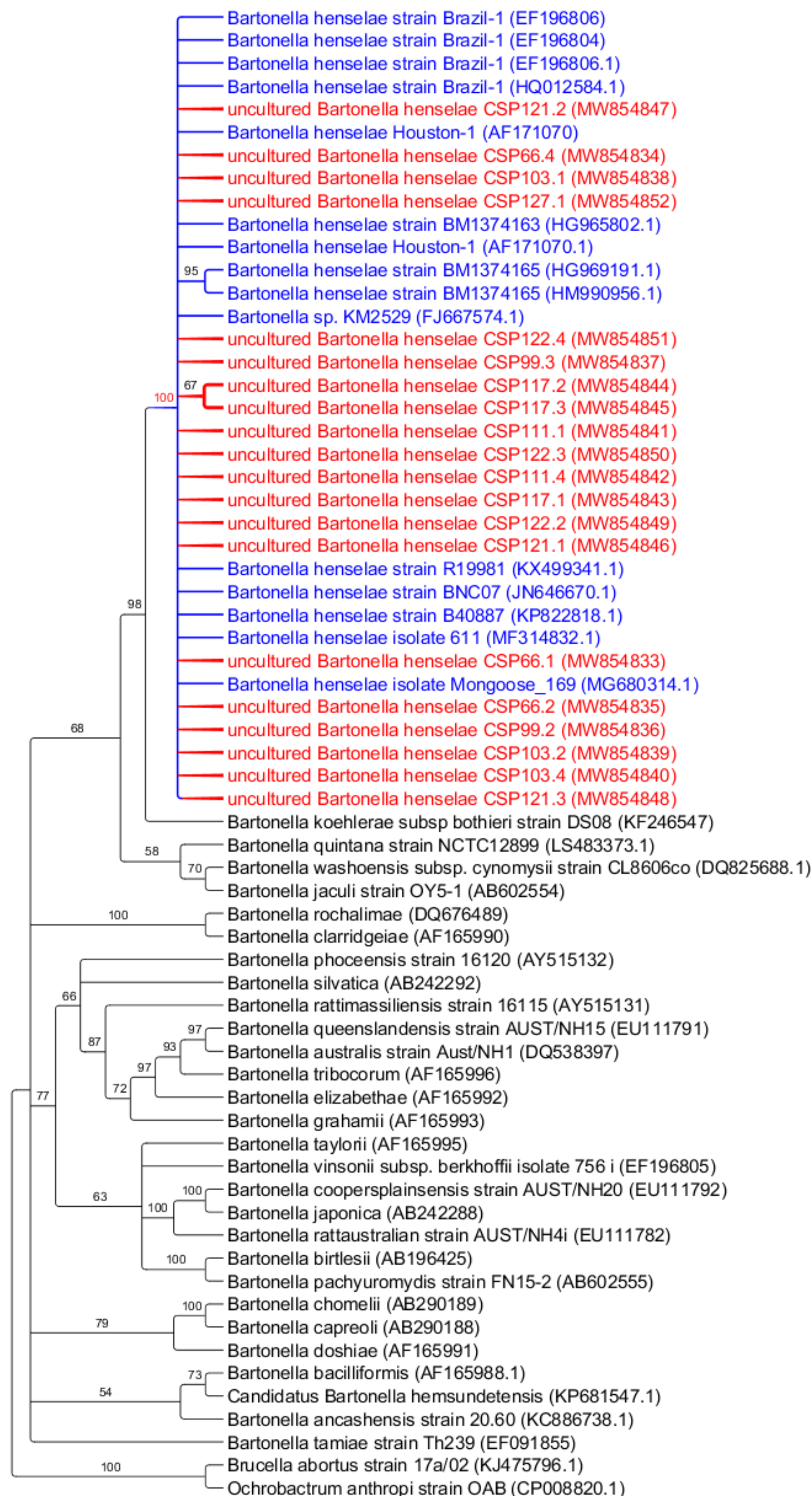


Figure 3. Phylogenetic analysis of *rpoB* sequences (500 pb) based on the topology generated by the BI method with the TIM+I+G evolutionary model. The numbers at the nodes correspond to posterior probability accessed with 1,000 replicates. *Brucella abortus* and *Ochrobactrum anthropic* were used as outgroups.

3.4.3 Diversity and distance analysis

Among the 23 sequences obtained for the rpoB gene in the present study, 13 genotypes were identified with π = 0.00268, dh = 0.818 (± 0.082) and K = 1.209. Based on the analysis of Median-joining network (**Figure 4**) a clear separation was observed between the genotype #1 and the other genotypes. It was also possible to observe the existence of different rpoB genotypes of *B. henselae* in the same blood sample of domestic cats from MG and SP States.

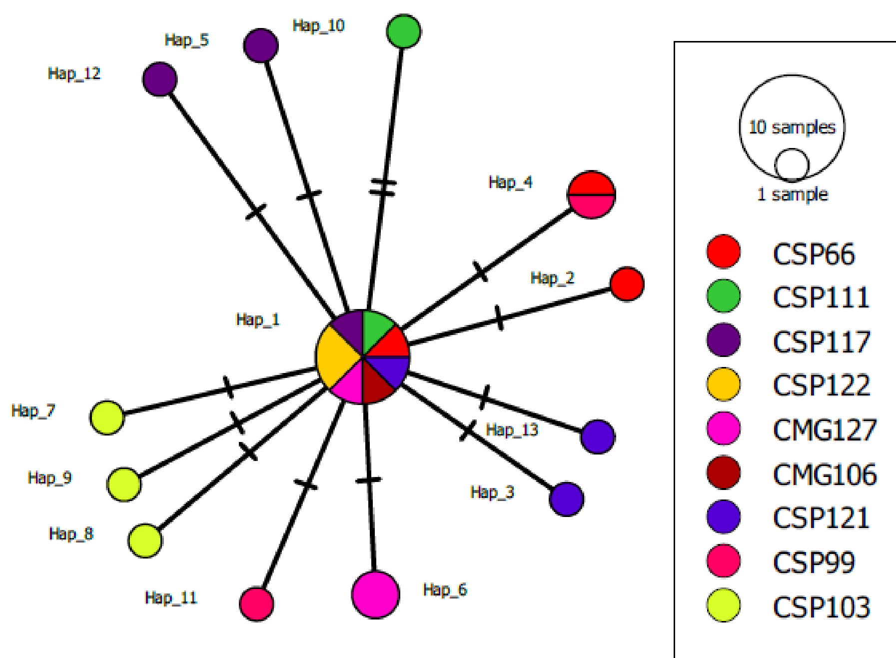


Figure 4. Median-joining network analysis based on the *rpoB* genotypes of *B. henselae* detected in domestic cats from the States of Minas Gerais and São Paulo, southeastern Brazil.

When compared with 34 additional sequences from nine countries retrieved from GenBank, 14 genotypes were identified, with π = 0.00195. dh = 0.660 (± 0.072) and K = 0.879. Most of the *B. henselae* rpoB sequences found

in the present study separated from genotype #2, which represents rpoB sequences from 9 different countries. In contrast, genotype #1, detected in 8 of the 18 clones evaluated, was also detected in the following countries: Germany, Australia, France, Guatemala, New Caledonia, New Zealand, Taiwan, and Zimbabwe (**Figure 5**). The SliptsTree distance analysis corroborated the Median-joining network analysis since all sequences were separated from each other in a distinct way (**Figure 6**).

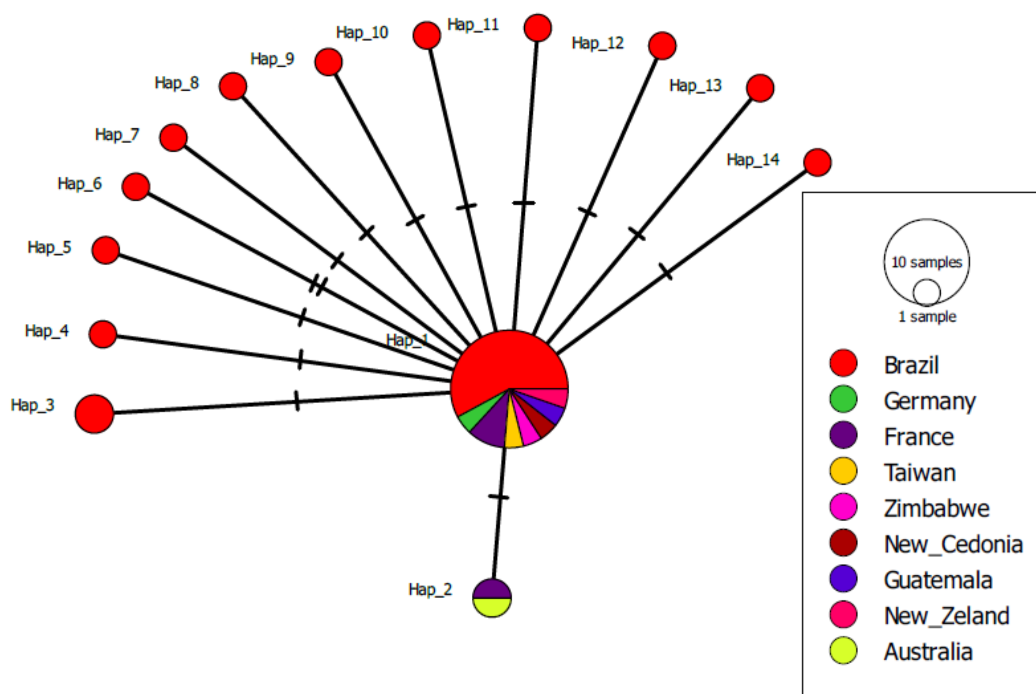


Figure 5. Median-joining network analysis based on *rpoB* genotypes of *B. henselae* detected in domestic cats from the Brazilian States of Minas Gerais and São Paulo, and worldwide.

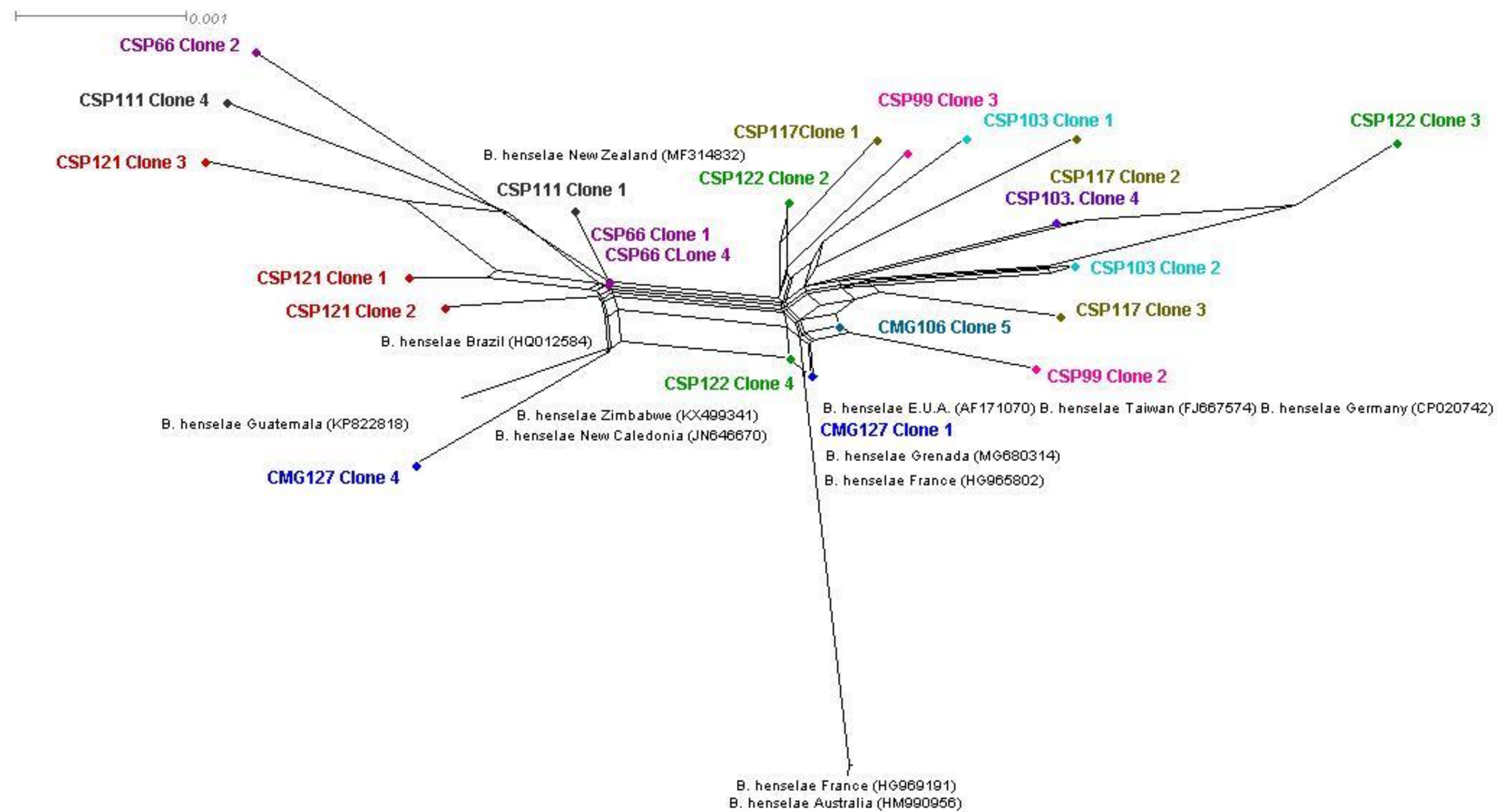


Figure 6. SplitsTree distance analysis based on the cloning products for the *B. henselae rpoB* gene detected in domestic cats in the states of São Paulo and Minas Gerais, southeastern Brazil.

3.4.4 Multilocus Sequence Typing (MLST)

When comparing the allelic profile of *B. henselae* isolates with other profiles described worldwide, the isolates obtained in the present study differed from the STs previously described in the literature (**Table 4**). The allelic profile found in MG State samples was similar to ST9 and ST34 previously described, differing only in the bartR gene for ST9 and for the 16S gene for ST34. This new sequence typing was designated as ST37 on the online database *B. henselae* MLST (https://pubmlst.org/organisms/Bartonella-henselae) (Iredell et al., 2003). On the other hand, the allelic profile of the SP State isolate sample belonged to ST9 (**Table 4**). Based on these results, it is possible to notice a difference between the allelic profiling found among isolates from SP in comparison to isolates from MG (**Table 4**). The relatedness of the *B. henselae* STs found in the present study (#9 and #37) with those previously detected around the world was assessed by GrapeTree and SplitsTree analyses (**Figures 7, 8, and 9**). The ST37 and ST9 found in the present study were positioned near the ST5, and closely to STs 4, 12, 14, 20, 23, 24, 28, and 35 (**Figure 7**). The *B. henselae* STs from Brazil were positioned closely to those previously detected in Germany, USA, Spain, and Romania (**Figure 8**). Based on Splistree distance analysis, while ST9 grouped closely to STs 35, 5, 12, 24 and 4, ST 37 grouped more closely to ST 23, 20, and 28 (**Figure 9**).

Table 4. Comparison between the allelic profiles of *B. henselae* isolates from cats from Brazil and the allelic profiles ST 9 and ST 34 previously detected in the USA, Spain, Romania, and Germany.

				Brazilian Isolates MLST								
ID	**Isolate**	**Source**	**Country**	**16S**	***batR***	***ftsZ***	***gltA***	***groEL***	***nlpD***	***ribC***	***rpoB***	***ST***
	106A	Feline	Brazil	2	6	1	1	1	1	1	1	37
	106B	Feline	Brazil	2	6	1	1	1	1	1	1	37
	106C	Feline	Brazil	2	6	1	1	1	1	1	1	37
	111A	Feline	Brazil	2	6	1	1	1	1	1	1	37
	111B	Feline	Brazil	2	6	1	1	1	1	1	1	37
	111C	Feline	Brazil	2	6	1	1	1	1	1	1	37
	18A	Feline	Brazil	2	1	1	1	1	1	1	1	9
	18B	Feline	Brazil	2	1	1	1	1	1	1	1	9
	18C	Feline	Brazil	2	1	1	1	1	1	1	1	9
				Worldwide Isolates MLST								
156	H075040029	Human	Romania	2	**1**	1	1	1	1	1	1	9
158	Bart-1-6	Human	Spain	**1**	6	1	1	1	1	1	1	34
173	Ber-K143	Feline	Germany	2	1	1	1	1	1	1	1	9
292	Mew Mew	Feline	USA	2	1	1	1	1	1	1	1	9
295	Toby	Feline	USA	2	1	1	1	1	1	1	1	9
301	Timothy	Feline	USA	2	1	1	1	1	1	1	1	9
436	I53	Feline	Spain	2	1	1	1	1	1	1	1	9
438	I86	Feline	Spain	2	1	1	1	1	1	1	1	9
439	I96	Feline	Spain	2	1	1	1	1	1	1	1	9
440	I103	Feline	Spain	2	1	1	1	1	1	1	1	9

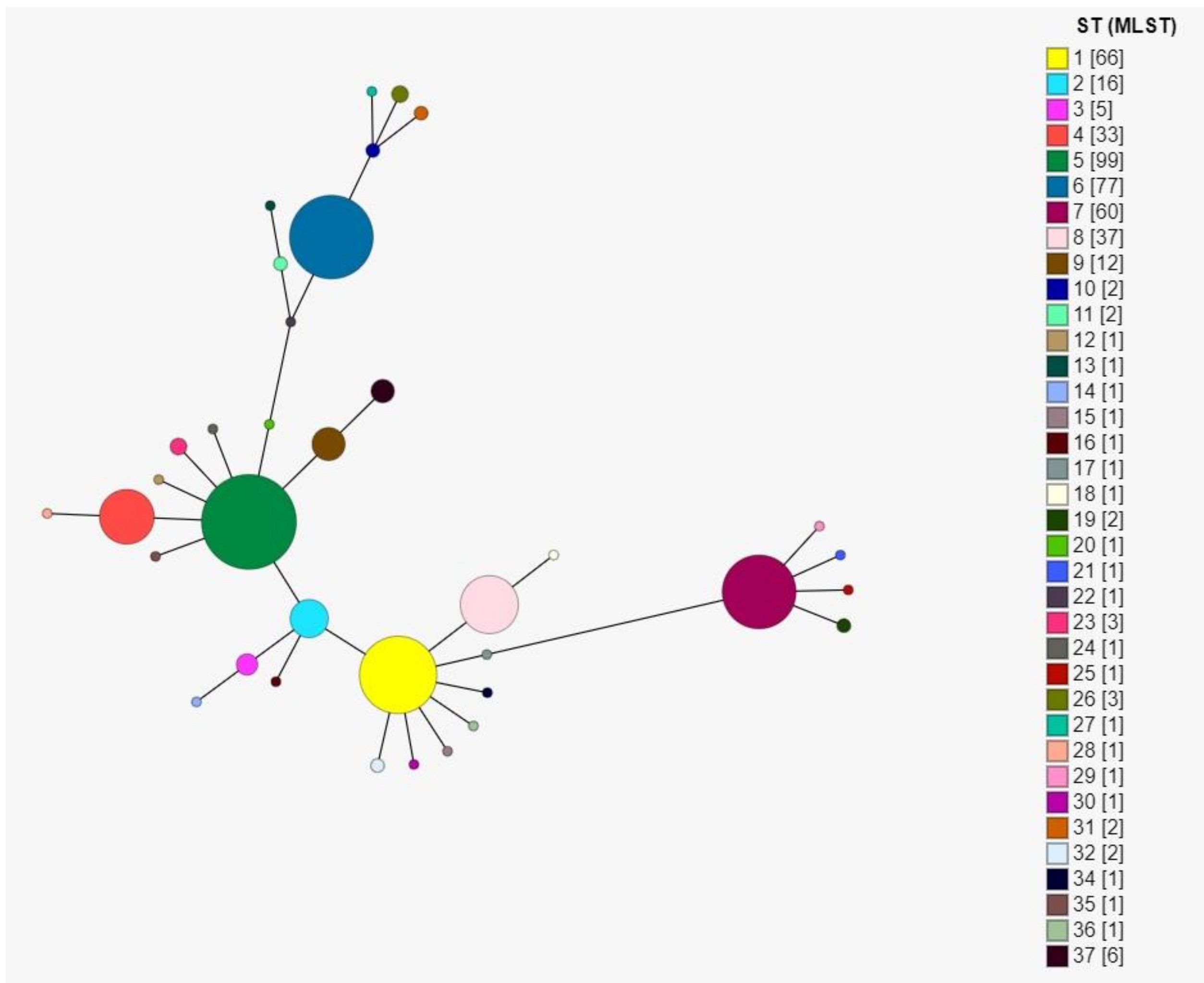


Figure 7 – GrapeTree clustering of 37 STs available in the *Bartonella henselae* PubMLST database (https://pubmlst.org/organisms/*Bartonella*-henselae). Each node corresponds to a single ST marked with an individual color.

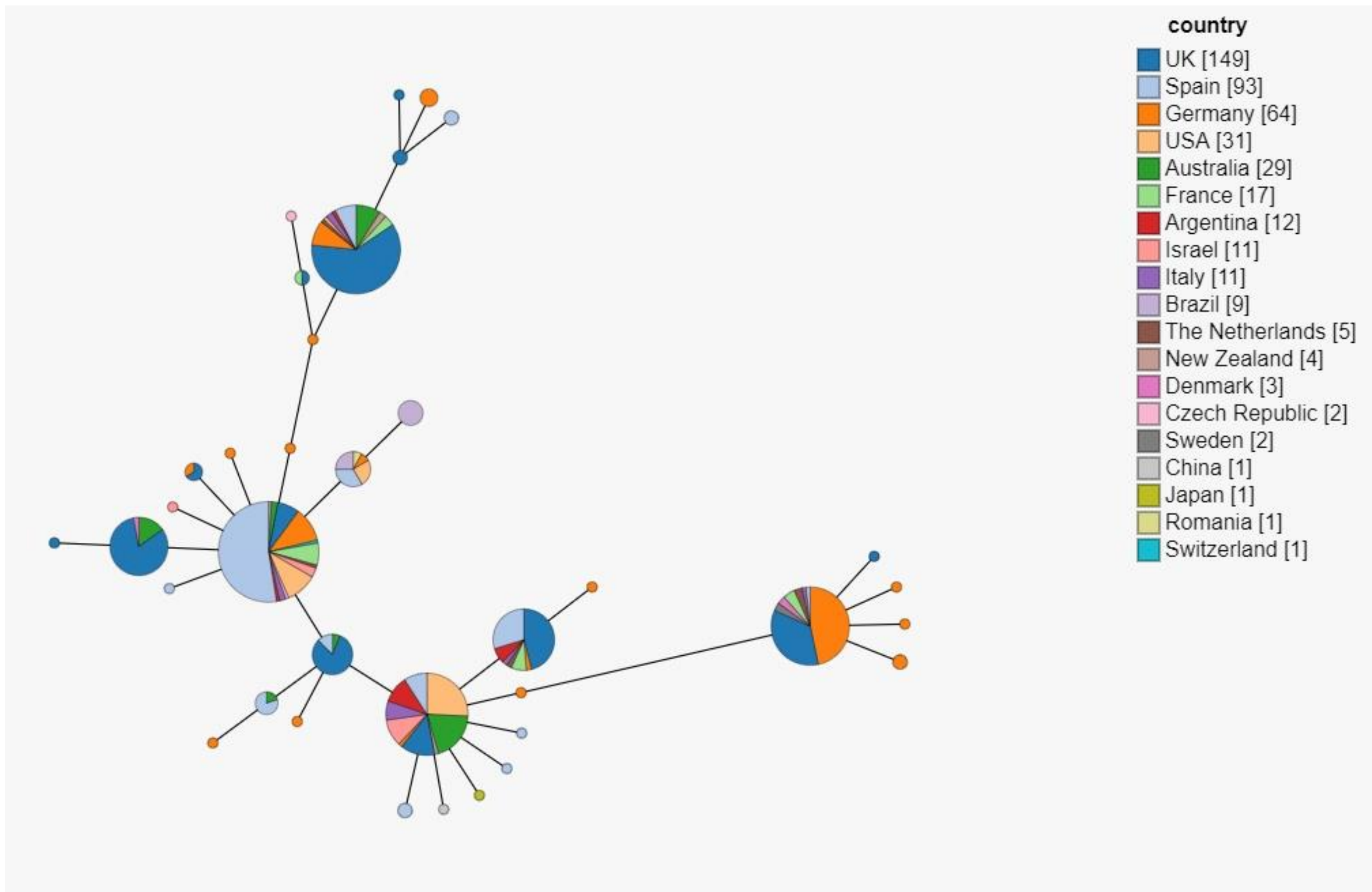


Figure 8 – GrapeTree clustering of 37 STs available in the *Bartonella henselae* PubMLST database (https://pubmlst.org/organisms/*Bartonella*-henselae) according to the countries where the STs were detected, which are marked with an individual color.

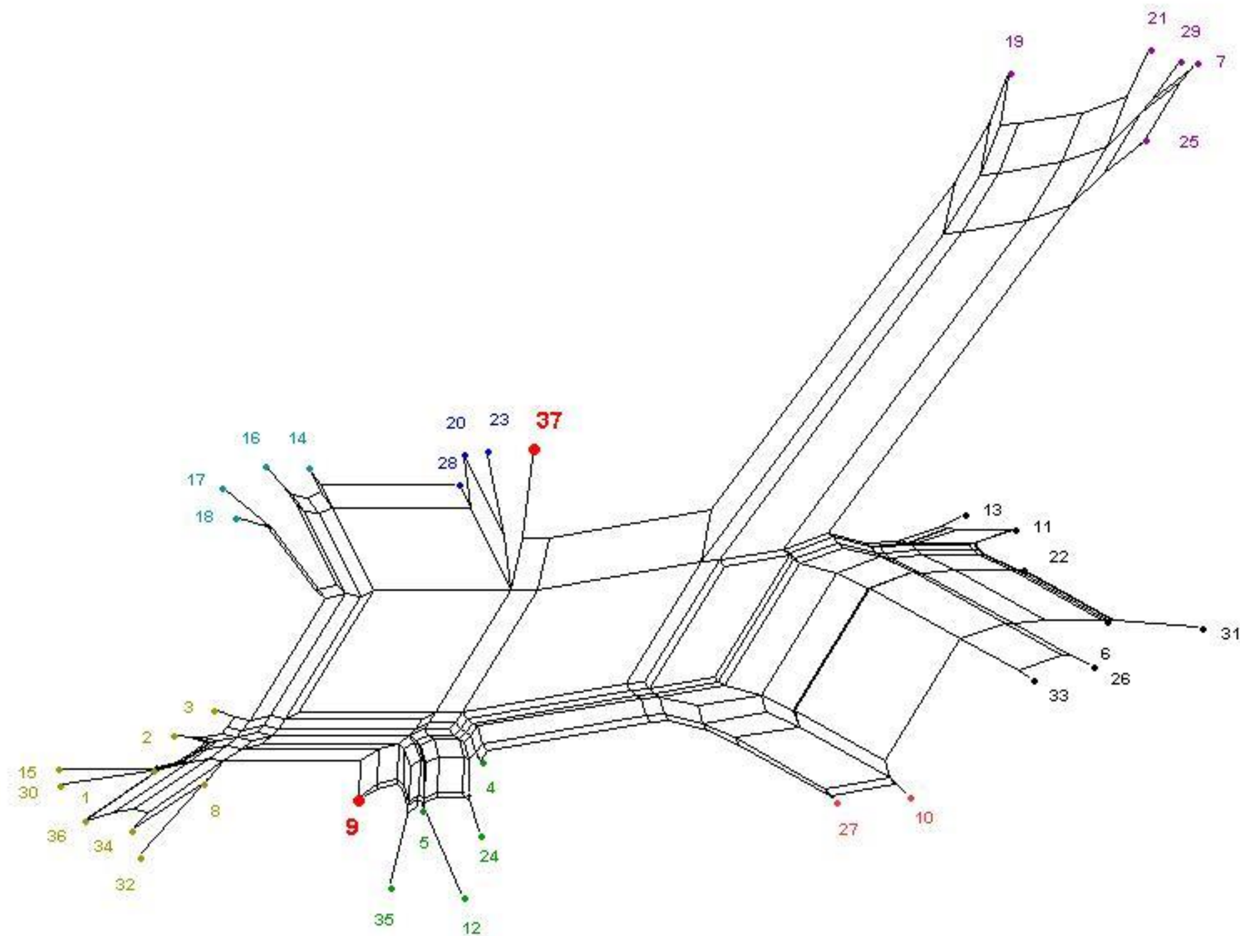


Figure 9 – SliptsTree analysis of 37 STs available in the *Bartonella henselae* PubMLST database (https://pubmlst.org/organisms/*Bartonella*-henselae). ST 9 and ST 37 presented in this study are marked in red.

4. DISCUSSION

An occurrence of 57.4% (89/155) and 33.1% (50/151) of positivity for *Bartonella* spp. was found in cats from the states of MG and SP, respectively, based on the qPCR results of both blood samples and pre-enrichment liquid culture, and bacterial isolation. Previous studies reported a molecular occurrence ranging from 4.3% to 90.2% of *Bartonella* sp. in cats from SP state (BORTOLI et al., 2012; DRUMMOND et al., 2018). This variation can be attributed to the cat population sampled, diagnostic technique applied, and/or geographic region. Furthermore, to the best of authors' knowledge, the present study reported the first molecular survey of *Bartonella* spp. in domestic cats in MG state as well as the first MLST of *B. henselae* conducted in Brazil. To this date, only serological evidence of exposure to *Bartonella* spp. has been reported in humans in the state of MG State (COSTA et al., 2005). The difference of occurrence of *Bartonella* between cats from SP and MG may be associated with the characteristics of the two sampled populations of cats in the present study.

In the present study, the cloning of a fragment of rpoB gene revealed the *Bartonella* species found in cats in the states of SP and MG shared a high identity with *B. henselae*, which was corroborated by Bayesian Inference-based phylogenetic analysis. The targeted gene chose for assessing the phylogenetic positioning and diversity of *B. henselae* in the present study, namely rpoB, is highly conserved, since it is critical for the beginning of any life event, being responsible for the catalysis of RNA polymerization (RENESTO et al., 2000). The Median-joining network analysis showed the presence of 13 genotypes among the cloned sequences detected in this study. This diversity could be a

result of the infection by different *B. henselae* genotypes mediated by the arthropod vector, namely C. felis fleas. The bacteria multiplication in the digestive system of the cat flea, the main arthropod vector of *B. henselae* among cats, may allow interactions with other genotypes as well as with other species of the same genus. As a result, the bacterial multiplication process in the arthropod vector can play an important role on its genetic diversity (DIAZ et al., 2012). Herein, based on the Median-joining network analysis, we showed that a unique cat can harbor different rpoB genotypes of *B. henselae* at any given time, as previously shown in a study conducted in California, USA, that revealed two (2/3) co-infected kittens with *B. henselae* genotypes I and II (HUWYLER et al., 2017). The biological implications of this finding, both from the point of view of the bacteria persistence in the vertebrate host and the immune response prompted by this event, are still unknown.

Herein, qPCR-positive pre-enrichment liquid cultures were obtained from 12 blood cat blood samples that showed to be negative in qPCR assay for *Bartonella* spp. Moreover, 5 *Bartonella* spp. isolates were obtained from blood/pre-enrichment liquid culture samples negative in the qPCR for *Bartonella* spp. Indeed, the values of quantification of a fragment of *Bartonella* nuoG gene by qPCR increased after pre-enrichment liquid culture in 93% of the samples, showing the importance of a culture-based diagnosis, which can improve the detection of *Bartonella* sp. in molecular assays. These findings support the proposed combination of diagnostic methods for *Bartonella* detection, i.e., the association of qPCR from blood and pre-enrichment liquid medium samples with bacterial isolation (CADENAS et al., 2007; DUNCAN et al., 2007). Similarly, a previous study performed with cats sampled (n=112) in the city of

Campinas, SP, showed that the combination of different diagnostic methods promotes an increased sensitivity in the diagnosis of infections by *Bartonella* spp. (90.2% - 101/112). In that study, pre-enrichment liquid culture increased the detection of *Bartonella* sp. by PCR in relation to PCR assays performed using DNA obtained from blood samples (76.8 % - 86/112), since 13.4% (15/26) of the samples that were negative in nested-PCR from blood DNA samples were positive in nested-PCR after being subjected to liquid culture (DRUMMOND et al., 2018).

Studies using the MLST technique for *B. henselae* genotyping in Brazil have not yet been performed so far. When analyzing the allelic profile found among our *B. henselae* isolates, a new allelic combination (ST37) was observed among the isolates obtained from MG State, whereas the obtained isolates from cats from SP belonged to ST9. This approach has been already used for genotyping *B. henselae* in cat and human biological samples from the USA, Argentina, Australia, European countries (United Kingdom, Germany, Italy, France, Croatia, Spain and Germany) (CHALONER et al., 2011; CICUTTIN et al., 2014; GIL et al., 2013; IREDELL et al., 2003; MIETZE et al., 2011; STEPANIĆ et al., 2019).

Previously, a MLST study conducted in Germany described 11 new ST combinations from *B. henselae* isolates from cat blood samples (MIETZE et al., 2011). Sequence Typing (ST) #1, #5 and #6 have been described in the United States, Europe and Australia, suggesting a worldwide distribution of these STs (IREDELL et al., 2003). While ST7 has been so far only detected in Europe, ST1 has been most frequently detected in the Mediterranean region. In Croatia, an isolate obtained from a blood sample from a cat, whose owner was a patient

presenting Cat Scratch Disease, belonged to ST5, which in turn was described for the first time in southeastern Europe (STEPANIĆ et al., 2019). In Spain, while ST5, ST6 and ST9 were associated with feline infection, ST1, ST5 and ST8 were associated with human infection. ST5 accounted for 53.7% of infections by *B. henselae* in humans and 61.5% in felines (GIL et al., 2013)

Despite the low number of isolates obtained in the present study, it was possible to find an allelic combination described as ST37 among samples from MG state in the MLST database for *B. henselae* (JOLLEY et al., 2018). Based on GrapeTree and Splitstree analysis, it is notorious the difference between the allelic profiles between MG and SP States, indicating that isolates from the same geographic region have clonally evolved, corroborating with MIETZE et al. (2011).

Regarding the analyzed fleas, 16.6% of qPCR-negative fleas for *Bartonella* spp. were collected from positive cats for Bartonella. According to Gutiérrez et al. (2015), Bartonella-positive cats can harbor negative fleas for the agent, as well as the opposite. In a study performed in the state of Pernambuco, 53.33% (24) of the C. felis fleas collected from cats were positive in the cPCR (ribC gene) for *Bartonella* spp. and later identified as B. clarridgeiae and *B. henselae* (FONTALVO et al., 2017).

When analyzing the estimate of *Bartonella* nuoG quantification found in the feline blood samples and fleas assessed by qPCR, the quantification values found in feline blood samples were lower than that found in the collected fleas. According to SOUSA et al. (2018) the vertebrate-host blood hemoconcentration in the digestive system of arthropods after blood meal may increase the molecular diagnosis sensitivity for *Bartonella* spp. Based on an experimental

infection of C. felis fleas using an artificial feeding system, BOUHSIRA et al. (2013) found an increase in the amount of *B. henselae* in the flea medium intestine 7 days post-infection. These findings suggested the possible replication of *B. henselae* in fleas during this period. In fact, these data corroborate those of HIGGINS et al. (1996) and FINKELSTEIN et al. (2002), whose works demonstrated *B. henselae* replication in cat flea intestine. Therefore, the number of copies of *Bartonella* DNA will depend on the period when the flea was collected from the animal, which is strictly related to the period of time after ingestion of blood containing Bartonella. Similarly, the quantification of *Bartonella* in cats' blood varies depending on the fluctuating bacteremia in these hosts.

In fact, study aiming to evaluate the presence and bacterial load of *Bartonella* in cats and their fleas, demonstrated that there is a quantitative relationship between the presence of the agent in the host and associated fleas when both vertebrate and invertebrate hosts were infected by the same *Bartonella* species. Fleas infected by the same *Bartonella* species as cats harbored more bacteria than fleas infected by species other than those found in vertebrate hosts or fleas collected from animals without bacteremia (GUTIÉRREZ et al., 2015).

5. CONCLUSION

The present study showed the occurrence of different *B. henselae* rpoB genotypes at an intra-mammal reservoir host level. At least two different allelic profiles of *B. henselae* (namely ST9 and ST37) circulate in cats from the states of São Paulo and Minas Gerais, suggesting a clonal evolution of Bartonellae in a certain geographical region.

REFERENCES

ALTSCHUL, S. F.; GISH, W.; MILLER, W.; MYERS, E. W.; LIPMAN, D. J. Basic local alignment search tool. **Journal of Molecular Biology**, v. 215, n. 3, p. 403–410, 1990.

ANDRÉ, M. R.; BACCARIM DENARDI, N. C.; MARQUES DE SOUSA, K. C.; GONÇALVES, L. R.; HENRIQUE, P. C.; GROSSE ROSSI ONTIVERO, C. R.; LIMA GONZALEZ, I. H.; CABRAL NERY, C. V.; FERNANDES CHAGAS, C. R.; MONTICELLI, C.; ALEXANDRE DE SANTIS, A. C. G.; MACHADO, R. Z. Arthropod-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil. **Ticks and Tick-borne Diseases**, v. 5, n. 5, p. 545–551, 2014.

ANDRÉ, M. R.; DUMLER, J. S.; HERRERA, H. M.; GONCALVES, L. R.; DE SOUSA, K. C.; SCORPIO, D. G.; DE SANTIS, A. C. G. A.; DOMINGOS, I. H.; DE MACEDO, G. C.; MACHADO, R. Z. Assessment of a quantitative 5' nuclease real-time polymerase chain reaction using the nicotinamide adenine dinucleotide dehydrogenase gamma subunit (*nuoG*) for *Bartonella* species in domiciled and stray cats in Brazil. **Journal of Feline Medicine and Surgery**, v. pii: 10986, p. 1–9, 2015. Disponível em: <http://jfm.sagepub.com/lookup/doi/10.1177/1098612X15593787>.

ANGELAKIS, E.; RAOULT, D. Pathogenicity and treatment of *Bartonella* infections. **International Journal of Antimicrobial Agents**, v. 44, n. 1, p. 16–25, 2014. Disponível em: <http://dx.doi.org/10.1016/j.ijantimicag.2014.04.006>.

AZZAG, N.; HADDAD, N.; DURAND, B.; PETIT, E.; AMMOUCHE, A.; CHOMEL, B.; BOULOUIS, H. J. Population structure of *Bartonella henselae* in Algerian urban stray cats. **PLoS ONE**, v. 7, n. 8, p. 1–13, 2012.

BANDELT, H. J.; FORSTER, P.; ROHL, A. Median-joining networks for inferring intraspecific phylogenies. **Molecular Biology and Evolution**, v. 16, n. 1, p. 37–

48, 1999. Disponível em: <https://academic.oup.com/mbe/article-lookup/doi/10.1093/oxfordjournals.molbev.a026036>.

BENSON, D. A.; CAVANAUGH, M.; CLARK, K.; KARSCH-MIZRACHI, I.; OSTELL, J.; PRUITT, K. D.; SAYERS, E. W. GenBank. **Nucleic Acids Research**, v. 46, n. D1, p. D41–D47, 2018.

BIRKENHEUER, A. J.; LEVY, M. G.; BREITSCHWERDT, E. B. Development and Evaluation of a Seminested PCR for Detection and Differentiation of *Babesia gibsoni* (Asian Genotype) and *B. canis* DNA in Canine Blood Samples. **Journal of Clinical Microbiology**, v. 41, n. March, p. 4172–4177, 2003.

BIRTLES, R. J.; RAOULT, D. Comparison of Partial Citrate Synthase Gene (*gltA*) Sequences for Phylogenetic Analysis of *Bartonella* species**. International Journal of Systematic Bacteriology**, v. 46, n. 4, p. 891–897, 1996. Disponível em: <http://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/00207713-46-4-891>.

BORTOLI, C. P.; ANDRÉ, M. R.; SEKI, M. C.; PINTO, A. A.; MACHADO, S. de T. Z.; MACHADO, R. Z. Detection of hemoplasma and *Bartonella* species and co-infection with retroviruses in cats subjected to a spaying/neutering program in Jaboticabal, SP, Brazil. **Revista Brasileira de Parasitologia Veterinária**, Jaboticabal, v. 21, n. 3, p. 219–23, 2012. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/23070430>.

BOUHSIRA, E.; FRANC, M.; BOULOUIS, H. J.; JACQUIET, P.; RAYMOND-LETRON, I.; LIÉNARD, E. Assessment of persistence of *Bartonella henselae* in *Ctenocephalides felis*. **Applied and Environmental Microbiology**, v. 79, n. 23, p. 7439–7444, 2013.

BOULOUIS, H.-J.; CHAO-CHIN, C.; HENN, J. B.; KASTEN, R. W.; CHOMEL, B. B. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. **Veterinary Research**, v. 36, n. 3, p. 383–410, maio 2005. Disponível em: <http://www.edpsciences.org/10.1051/vetres:2005009>.

BRAGA, Í. A.; DE OLIVEIRA DIAS, I. S.; CHITARRA, C. S.; AMUDE, A. M.; AGUIAR, D. M. Molecular detection of *Bartonella clarridgeiae* in domestic cats from Midwest Brazil. **Brazilian Journal of Infectious Diseases**, v. 19, n. 4, p. 451–452, 2015. Disponível em: <http://dx.doi.org/10.1016/j.bjid.2015.05.002>.

BREITSCHWERDT, E. B.; MAGGI, R. G.; CHOMEL, B. B.; LAPPIN, M. R. Bartonellosis: An emerging infectious disease of zoonotic importance to animals and human beings. **Journal of Veterinary Emergency and Critical Care**, v. 20, n. 1, p. 8–30, 2010.

BUSTIN, S. A.; BENES, V.; GARSON, J. A.; HELLEMANS, J.; HUGGETT, J.; KUBISTA, M.; MUELLER, R.; NOLAN, T.; PFAFFL, M. W.; SHIPLEY, G. L.; VANDESOMPELE, J.; WITTWER, C. T. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. **Clinical Chemistry**, v. 55, n. 4, p. 611–622, 2009.

CADENAS, M. B.; MAGGI, R. G.; DINIZ, P. P. V. P.; BREITSCHWERDT, K. T.; SONTAKKE, S.; BREITHSCHWERDT, E. B. Identification of bacteria from clinical samples using *Bartonella* alpha-Proteobacteria growth medium. **Journal of Microbiological Method**s, v. 71, n. 2, p. 147–155, 2007.

CANNETI, B.; CABO-LÓPEZ, I.; PUY-NÚÑEZ, A.; GARCÍA GARCÍA, J. C.; CORES, F. J.; TRIGO, M.; SUÁREZ-GIL, A. P.; RODRIGUEZ-REGAL, A. Neurological presentations of *Bartonella henselae* infection. **Neurological Sciences**, v. 40, n. 2, p. 261–268, 2019.

CHALONER, G. L.; HARRISON, T. G.; COYNE, K. P.; AANENSEN, D. M.; BIRTLES, R. J. Multilocus sequence typing of *Bartonella henselae* in the United Kingdom indicates that only a few, uncommon sequence types are associated with zoonotic disease. **Journal of Clinical Microbiology**, v. 49, n. 6, p. 2132–2137, 2011.

CHOMEL, B. B.; KASTEN, R. W. Bartonellosis, an increasingly recognized zoonosis. **Journal of Applied Microbiology**, v. 109, n. 3, p. 743–750, 2010.

CICUTTIN, G. L.; BRAMBATI, D. F.; DE GENNARO, M. F.; CARMONA, F.; ISTURIZ, M. L.; PUJOL, L. E.; BELERENIAN, G. C.; GIL, H. *Bartonella* spp. in cats from Buenos Aires, Argentina. **Veterinary Microbiology**, v. 168, n. 1, p. 225–228, 2014.

COSTA, P. S. G.; BRIGATTE, M. E.; GRECO, D. B. Antibodies *to Rickettsia rickettsii, Rickettsia typhi, Coxiella burnetii, Bartonella henselae, Bartonella quintana* and *Erhlichia chaffeensis* among healthy population in Minas Gerais, Brazil**. Memórias do Instituto Oswaldo Cruz**, v. 100, n. 8, p. 853–859, 2005.

DARRIBA, D.; TABOADA, G. L.; DOALLO, R.; POSADA, D. JModelTest 2: More models, new heuristics and parallel computing. **Nature Methods**, v. 9, n. 8, p. 772, 2012.

DIAZ, M. H.; BAI, Y.; MALANIA, L.; WINCHELL, J. M.; KOSOY, M. Y. Development of a novel genus-specific real-time PCR assay for detection and differentiation of *Bartonella* species and genotypes. **Journal of Clinical Microbiology**, v. 50, n. 5, p. 1645–1649, 2012.

DINIZ, P. P. V. D. P.; MAGGI, R. G.; SCHWARTZ, D. S.; CADENAS, M. B.; BRADLEY, J. M.; HEGARTY, B.; BREUTSCHWERDT, E. B. Canine bartonellosis: Serological and molecular prevalence in Brazil and evidence of co-infection with *Bartonella henselae* and *Bartonella vinsonii* subsp. berkhoffii. **Veterinary Research**, v. 38, n. 5, p. 697–710, 2007.

DRUMMOND, M. R.; LANIA, B. G.; DE PAIVA DINIZ, P. P. V.; GILIOLI, R.; DEMOLIN, D. M. R.; SCORPIO, D. G.; BREITSCHWERDT, E. B.; VELHO, P. E. N. F. Improvement of *Bartonell henselae* DNA detection in cat blood samples by combining molecular and culture methods. **Journal of Clinical Microbiology**, v. 56, n. 5, p. 1–8, 2018.

DUNCAN, A. W.; MAGGI, R. G.; BREITSCHWERDT, E. B. A combined approach for the enhanced detection and isolation of *Bartonella* species in dog blood samples: Pre-enrichment liquid culture followed by PCR and subculture onto agar plates. **Journal of Microbiological Methods**, v. 69, n. 2, p. 273–281, 2007.

EWING, B.; GREEN, P. Base-calling of automated sequencer traces using phred. II. Error probabilities. **Genome Research**, v. 8, n. 3, p. 186–194, 1998.

EWING, B.; HILLIER, L.; WENDL, M. C.; GREEN, P. Base-Calling of Automated Sequencer Traces Using Phred. I. Accuracy Assessment. **Genome Research**, v. 8, n. 3, p. 175–185, 1 mar. 1998. Disponível em: <http://genome.cshlp.org/lookup/doi/10.1101/gr.8.3.175>.

FINKELSTEIN, J. L.; BROWN, T. P.; O'REILLY, K. L.; WEDINCAMP, J.; FOIL, L. D. Studies on the growth of *Bartonella henselae* in the cat flea (Siphonaptera: Pulicidae). **Journal of Medical Entomology**, v. 39, n. 6, p. 915–919, 2002.

FOLMER, O.; BLACK, M.; HOEH, W.; LUTZ, R.; VRIJENHOEK, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. **Molecular marine biology and biotechnology**, v. 3, n. 5, p. 294–299, 1994.

FONTALVO, M. C.; FAVACHO, A. R. de M.; ARAUJO, A. de C.; SANTOS, N. M. dos; OLIVEIRA, G. M. B. de; AGUIAR, D. M.; LEMOS, E. R. S. de; HORTA, M. C. *Bartonella* species pathogenic for humans infect pets, free-ranging wild mammals and their ectoparasites in the Caatinga biome, Northeastern Brazil: a serological and molecular study. **Brazilian Journal of Infectious Diseases**, v. 21, n. 3, p. 290–296, 2017.

GIL, H.; ESCUDERO, R.; PONS, I.; RODRÍGUEZ-VARGAS, M.; GARCÍA-ESTEBAN, C.; RODRÍGUEZ-MORENO, I.; GARCÍA-AMIL, C.; LOBO, B.; VALCÁRCEL, F.; PÉREZ, A.; JIMÉNEZ, S.; JADO, I.; JUSTE, R.; SEGURA, F.; ANDA, P. Distribution of *Bartonella henselae* Variants in Patients, Reservoir Hosts and Vectors in Spain. **PLoS ONE**, v. 8, n. 7, 2013.

GUTIÉRREZ, R.; NACHUM-BIALA, Y.; HARRUS, S. The relations between the presence and bacterial loads of *Bartonella* species in the cat and cat flea (*Ctenocephalides felis*), under natural conditions. **Applied and environmental**

microbiology, v. 81, n. 16, p. 5613–5621, 2015. Disponível em: <http://aem.asm.org/content/81/16/5613.abstract?etoc>.

HALL, T. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. **Nucleic Acids Symposium Series**, 1999. Disponível em: <http://jwbrown.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf>.

HARMS, A.; DEHIO, C. Intruders below the Radar: Molecular pathogenesis of *Bartonella* spp. **Clinical Microbiology Reviews**, v. 25, n. 1, p. 42–78, 2012.

HIGGINS, J. A.; RADULOVIC, S.; JAWORSKI, D. C.; AZAD, A. F. Acquisition of the Cat Scratch Disease Agent *Bartonella henselae* by Cat Fleas (Siphonaptera: Pulicidae). **Journal of Medical Entomology**, v. 33, n. 3, p. 490–495, 1996.

HUSON, D. H.; BRYANT, D. Application of phylogenetic networks in evolutionary studies. **Molecular Biology and Evolution**, v. 23, n. 2, p. 254–267, 2006.

HUWYLER, C.; HEINIGER, N.; CHOMEL, B. B.; KIM, M.; KASTEN, R. W.; KOEHLER, J. E. Dynamics of Co-Infection with *Bartonella henselae* Genotypes I and II in Naturally Infected Cats: Implications for Feline Vaccine Development. **Microbial** Ecology, v. 74, p. 474–484, 2017.

IREDELL, J.; BLANCKENBERG, D.; ARVAND, M.; GRAULING, S.; FEIL, E. J.; BIRTLES, R. J. Characterization of the Natural Population of *Bartonella henselae* by Multilocus Sequence Typing**. Journal of Clinical Microbiology**, v. 41, n. 11, p. 5071–5079, 2003.

JOLLEY, K. A.; BRAY, J. E.; MAIDEN, M. C. J. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications [version 1; referees: 2 approved]. **Wellcome Open Research**, v. 3, n. 0, p. 1–20, 2018.
KEIM, P.; PRICE, L. B.; KLEVYTSKA, A. M.; SMITH, K. L.; SCHUPP, J. M.; OKINAKA, R.; JACKSON, P. J. Multiple-Locus Variable-Number Tandem Repeat Analysis Reveals Genetic Relationships within Bacillus anthracis. **Journal of Bacteriology,** v. 182, n. 10, p. 2928–2936, 2000.

KOSOY, M.; MCKEE, C.; ALBAYRAK, L.; FOFANOV, Y. Genotyping of *Bartonella* bacteria and their animal hosts: current status and perspectives. ***Parasitology***, v. 145, p. 1–20, 2017.

LAU, A. O. T.; CERECERES, K.; PALMER, G. H.; FRETWELL, D. L.; PEDRONI, M. J.; MOSQUEDA, J.; MCELWAIN, T. F. Genotypic diversity of merozoite surface antigen 1 of Babesia bovis within an endemic population.

Molecular and Biochemical Parasitology, v. 172, n. 2, p. 107–112, 2010. Disponível em: <http://dx.doi.org/10.1016/j.molbiopara.2010.03.017>.

LEIGH, J. W.; BRYANT, D. POPART: Full-feature software for haplotype network construction. **Methods in Ecology and Evolution**, v. 6, n. 9, p. 1110–1116, 2015.

LIBRADO, P.; ROZAS, J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. **Bioinformatics**, v. 25, n. 11, p. 1451–1452, 2009.

LINARDI, P. M.; SANTOS, J. L. C. *Ctenocephalides felis felis* vs. *Ctenocephalides canis*: (Siphonaptera: Pulicidae): Algumas questões para identificar corretamente estas espécies. **Revista Brasileira de Parasitologia Veterinária**, v. 21, n. 4, p. 345–354, 2012.

MAGGI, R. G.; DUNCAN, A. W.; BREITSCHWERDT, E. B.; CAROLINA, N.; AL, M. E. T.; ICROBIOL, J. C. L. I. N. M. Novel Chemically Modified Liquid Medium That Will Support the Growth of Seven *Bartonella* Species. **Journal of Clinical Microbiology**, v. 43, n. 6, p. 2651–2655, 2005.

MIETZE, A.; MORICK, D.; KÖHLER, H.; HARRUS, S.; DEHIO, C.; NOLTE, I.; GOETHE, R. Combined MLST and AFLP typing of *Bartonella henselae* isolated from cats reveals new sequence types and suggests clonal evolution. **Veterinary Microbiology**, v. 148, n. 2–4, p. 238–245, 2011.

MILLER, M. A.; PFEIFFER, W.; SCHWARTZ, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. **2010 Gateway Computing Environments Workshop**, GCE 2010, 2010.

MONTEIL, M.; DURAND, B.; BOUCHOUICHA, R.; PETIT, E.; CHOMEL, B.; ARVAND, M.; BOULOUIS, H. J.; HADDAD, N. Development of discriminatory multiple-locus variable number tandem repeat analysis for *Bartonella henselae*. **Microbiology**, v. 153, n. 4, p. 1141–1148, 2007.

NORMAN, A. F.; REGNERY, R.; JAMESON, P.; GREENE, C.; KRAUSE, D. C. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. **Journal of Clinical Microbiology**, v. 33, n. 7, p. 1797–1803, 1995.

PAZIEWSKA, A.; HARRIS, P. D.; ZWOLIŃSKA, L.; BAJER, A.; SIŃSKI, E. Recombination Within and Between Species of the Alpha Proteobacterium *Bartonella* Infecting Rodents. **Microbial Ecology**, v. 61, n. 1, p. 134–145, 2011.

PEDRASSANI, D.; BIOLCHI, J.; GONÇALVES, L. R.; MENDES, N. S.; ZANATTO, D. C. de S.; CALCHI, A. C.; MACHADO, R. Z.; ANDRÉ, M. R. Molecular detection of vector-borne agents in cats in Southern Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 28, n. 4, p. 632–643, dez. 2019.

Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-29612019000400632&tlng=en>.

RAIMUNDO, J. M.; GUIMARÃES, A.; AMARO, G. M.; DA SILVA, A. T.; BOTELHO, C. F. M.; MASSARD, C. L.; DE LEMOS, E. R. S.; FAVACHO, A. R. M.; BALDANI, C. D. Molecular survey of *Bartonella* species in shelter cats in Rio de Janeiro: Clinical, hematological, and risk factors. **American Journal of Tropical Medicine and Hygiene**, v. 100, n. 6, p. 1321–1327, 2019.
RENESTO, P.; GAUTHERET, D.; DRANCOURT, M.; RAOULT, D. Determination of the rpoB gene sequences of *Bartonella henselae* and *Bartonella quintana* for phylogenic analysis. **Research in Microbiology**, v. 151, n. 10, p. 831–836, 2000.

RONQUIST, F.; HUELSENBECK, J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. **Bioinformatics**, v. 19, n. 12, p. 1572–1574, 2003.

SANGER, F.; NICKLEN, S.; COULSON, A. R. DNA sequencing with chain-terminating inhibitors. **Proceedings of the National Academy of Sciences**, v. 74, n. 12, p. 5463–5467, 1977. Disponível em: <http://www.pnas.org/cgi/doi/10.1073/pnas.74.12.5463>.

SILVA, B. T. G.; SOUZA, A. M. de; CAMPOS, S. D. E.; MACIEIRA, D. de B.; LEMOS, E. R. S. de; FAVACHO, A. R. de M.; ALMOSNY, N. R. P. *Bartonella henselae* and *Bartonella* clarridgeiae infection, hematological changes and associated factors in domestic cats and dogs from an Atlantic rain forest area, Brazil. **Acta Tropica**, v. 193, n. February, p. 163–168, 2019. Disponível em: <https://doi.org/10.1016/j.actatropica.2019.02.026>.

SOUSA, K. C. M.; DO AMARAL, R. B.; HERRERA, H. M.; SANTOS, F. M.; MACEDO, G. C.; DE ANDRADE PINTO, P. C. E.; BARROS-BATTESTI, D. M.; MACHADO, R. Z.; ANDRÉ, M. R. Genetic Diversity of *Bartonella* spp. in Wild Mammals and Ectoparasites in Brazilian Pantanal. **Microbial Ecology**, v. 76, n. 2, p. 544–554, 2018.

SOUZA, A. M. de; ALMEIDA, D. N. P. de; GUTERRES, A.; GOMES, R.; FAVACHO, A. R. de M.; MOREIRA, N. dos S.; MAIA, L. M. P.; ROZENTAL, T.; TORRES, R. de A.; CERQUEIRA, A. de M. F.; LEMOS, E. R. S. de; PEREIRA, A. N. R. Bartonelose: análise molecular e sorológica em gatos do Rio de Janeiro Brasil. **Revista Brasileira de Ciência Veterinária**, v. 17, n. 1, p. 7–11, 2010.

STEPANIĆ, M.; DUVNJAK, S.; REIL, I.; ŠPIČIĆ, S.; KOMPES, G.; BECK, R. First isolation and genotyping of *Bartonella henselae* from a cat living with a patient with cat scratch disease in Southeast Europe**. BMC Infectious Diseases**, v. 19, n. 1, p. 1–6, 2019.

ZEAITER, Z.; FOURNIER, P. E.; OGATA, H.; RAOULT, D. Phylogenetic classification of *Bartonella* species by comparing groEL sequences. International **Journal of Systematic and Evolutionary Microbiology**, v. 52, n. 1, p. 165–171, 2002.