Sociedade Brasileira de Infectologia

The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original Article

Bartonella henselae as a putative trigger for chronic type 2 leprosy reactions

Luciene Silva dos Santos [a,*], *Marina Rovani Drummond* [a], *Isabela Maria Bernardes Goulart* [b], *Andrea Fernandes Eloy da Costa França* [c], *Elemir Macedo de Souza* [c], *Paulo Eduardo Neves Ferreira Velho* [a,c]

[a] *Universidade Estadual de Campinas (UNICAMP), Faculdade de Ciências Médicas, Laboratório de Pesquisa Aplicada em Dermatologia e Infecção por Bartonella, Campinas, SP, Brazil*
[b] *Universidade Federal de Uberlândia, Centro Nacional de Referência em Dermatologia Sanitária e Hanseníase, Uberlândia, MG, Brazil*
[c] *Universidade Estadual de Campinas (UNICAMP), Faculdade de Ciências Médicas, Departamento de Medicina, Divisão de Dermatologia, Campinas, SP, Brazil*

ARTICLE INFO

Article history:
Received 10 July 2023
Accepted 31 October 2023
Available online 17 November 2023

Keywords:
Bartonella
Coinfection
Leprosy
Erythema nodosum

ABSTRACT

Leprosy reactions are an acute inflammatory phenomenon that can arise before diagnosis, during treatment, or after cure of leprosy. These reactions are considered one of the main diseases that cause physical disabilities. Immunosuppressive treatment for these immune responses makes these patients susceptible to coinfections, which can trigger new leprosy reactions. The main objective of this study was to evaluate the occurrence of infection by *Bartonella* sp. in blood samples from 47 patients who had untreatable episodes of type 2 leprosy reactions for more than six months, comparing them with a control group. Cultures and molecular methods (PCR) were used. Amplicons from species-specific reactions and sequencing showed a higher prevalence of *Bartonella henselae* infection in patients, 19/47 (40.4 %), compared to control, 9/50 (18.0 %), $p = 0.0149$. Five patients accepted treatment for coinfection, and all showed improvement in leprosy reactions with treatment for *B. henselae* infection. We conclude that these bacteria can trigger chronic reactions of type 2 leprosy and should be investigated in these patients.
Summary line: Patients who have chronic type 2 leprosy reactions are more susceptible to *Bartonella henselae* infection than controls: 19/47 (40.4 %) compared 9/50 (18.0 %), $p = 0.0149$.

Introduction

Leprosy is an age-old disease related to poverty because certain conditions of less favored populations considerably increase exposure to *Mycobacterium leprae* and *Mycobacterium*

* Corresponding author.
E-mail address: lucienesilvaflora@gmail.com (L.S.d. Santos).
https://doi.org/10.1016/j.bjid.2023.103701

lepromatosis.[1,2] The disease has a clinical spectrum closely related to the genetically inherited immune capacity of the affected individual. Patients with a good cellular immune response are more affected by localized forms such as tuberculoid, while those with more diffuse forms of the disease have a predominance of the humoral response, which is ineffective to combat intracellular infection in Borderline (BB), Borderline Lepromatous (BL) and Lepromatous (LL).[3,4]

Before, during, or even after the end of treatment, approximately 30 %–50 % of patients develop acute inflammatory reactions to mycobacterial antigens, often located in the peripheral nerves. These inflammatory reactions are responsible for most physical disabilities related to infectious diseases.[5,6] Studies show that infection by *Bartonella* sp. can also affect the nervous system. A mouse study suggests that *B. henselae* infection induces persistent mechanical hypersensitivity, and some reports show that *Bartonella* spp. may be a potential cause of chronic neurological and neurocognitive dysfunction, even in immunocompetent patients.[7,8] Another study that investigated the atypical manifestations of Cat Scratch Diseases (CSD) showed that, in the United States, neurological manifestations (neuritis or encephalitis) were one of the most frequent (13.8 %), as well as ocular manifestations (retinitis/neuroretinitis or conjunctivitis) (48.7 %) and hepatosplenic diseases (24.6 %).[9]

Reactions can be divided into Type 1 (T1R) and type 2 (T2R) leprosy reactions. T2R are known as a synonym for with Erythema Nodosum Leprosum (ENL) even though these reactions may present with other clinical manifestations such as erythema multiforme, erythematous plaques or even purpuric lesions, without necessarily coursing with erythema nodosum. The T2R are usually self-limited last for approximately two weeks. The T1R should last for few weeks to few month.[10] When patients have subsequent leprosy reactions, i.e., for more than six months, they are considered to have chronic reactions, and neural involvement is common. These reactions require corticosteroid treatment, often in high doses. In these cases, it is necessary to investigate factors that may act as triggers for these reactions, including coinfections, albeit subclinical.[11,12]

Immunity mediated by Th1 cells plays an important role in the evolution of intracellular bacterial infections and, therefore, in the evolution of leprosy and its reactions.[3] There is evidence that cell-mediated immunity is also closely related to the pathogenesis and control of infection by *Bartonella* spp.[13] These bacteria can cause asymptomatic infection and are responsible for emerging and reemerging diseases.[14] At least 17 species and subspecies of *Bartonella* are associated with human diseases, such as Carrión disease (Peruvian bartonellosis), trench fever, bacillary angiomatosis, endocarditis, and CSD. Among other clinical manifestations, erythema nodosum has been associated with *Bartonella* sp.-infections.[13,15,16] Often neglected, even when the diagnosis of these bacterial infections is considered, confirmation in routine microbiological laboratories is difficult. Being fastidious, they require enriched media and other special conditions to be isolated, in addition to presenting extremely slow growth.[17] Similar to other Gram-negative bacteria, they present with low bacteremia, making molecular diagnosis from blood samples difficult.[18]

Among *Bartonella* spp., *Bartonella henselae* is the species most frequently associated with human diseases.[19-21] Similar to other pathogenic species, it is transmitted by blood-sucking ectoparasites, particularly the flea (*Ctenocephalides felis*), with its main host being domestic cats.[22] Coinfection by *B. henselae* in a patient with chronic leprosy reactions monitored at Clinics Hospital of UNICAMP has already been described. In this patient, there was complete improvement of the reactions with the treatment for *B. henselae*.[23] Other cases of the association of leprosy with *B. henselae* have also been cited in an article on the detection of bacterial DNA in paraffin material.[24]

The main objective of this study was to evaluate and compare the occurrence of infection by *Bartonella* sp. in blood samples from patients from two reference centers for the treatment of leprosy in Southeastern Brazil with a control group of individuals without clinical complaints.

Material and methods

Because leprosy is a moderately endemic disease in the Southeastern Region of Brazil,[25] patients from two reference centers for leprosy treatment participated in the study: the Dermatology Outpatient Clinic of the Clinics Hospital of UNICAMP, Campinas-SP, Brazil and the National Reference Center for Leprosy and Sanitary Dermatology (CREDESH), Uberlandia-MG, Brazil. Many leprosy patients with chronic reactions are referred to these centers. The selected patients presented difficult-to-manage T2R with reactions for more than six months; the control group, students, and employees at UNICAMP, did not mention clinical symptoms at the time of sample collection. All study participants were older than 18 years and not pregnant.

The project was approved by the Institutional Research Boards of the State University of Campinas (UNICAMP), Campinas, SP (CAAE: 44,670,015.4.0000.5404) and the Federal University of Uberlandia (UFU), Uberlândia, MG (CAAE: 44,670,015.4.3001.5152), with both institutions located in the Southeastern region of Brazil (22°49′36.0″S 47°03′50.9″W and 18°53′36.2″S 48°17′45.5″W, respectively) (Fig. 1).

A volume of approximately 8 mL of blood without anticoagulant was collected in an aseptic manner in a tube from each participant and another 8 mL in a tube containing EDTA (ethylenediaminetetraacetic acid). The blood clot obtained from the dry tube after centrifugation at 3500 rpm for 15 min, and the tube containing blood with EDTA were frozen at −20 °C for at least 24 h.

After freezing, 1 mL of whole blood was inoculated into 4 mL of liquid medium specific for the growth of *Bartonella* spp.,[26] and the same was done with 1 mL of the clot. Both cultures were incubated in a shaker at 35 °C with 5 % CO_2 for 14 days.

After 14 days, 1 mL of the liquid culture was seeded in a solid medium as previously described.[18] The culture flasks were kept at 35 °C with 5 % CO_2 in a water-saturated atmosphere for up to 42 days. Evaluations verifying growth were performed weekly. When there was bacterial growth, a colony was collected and stained using the Gram technique. If Gram-

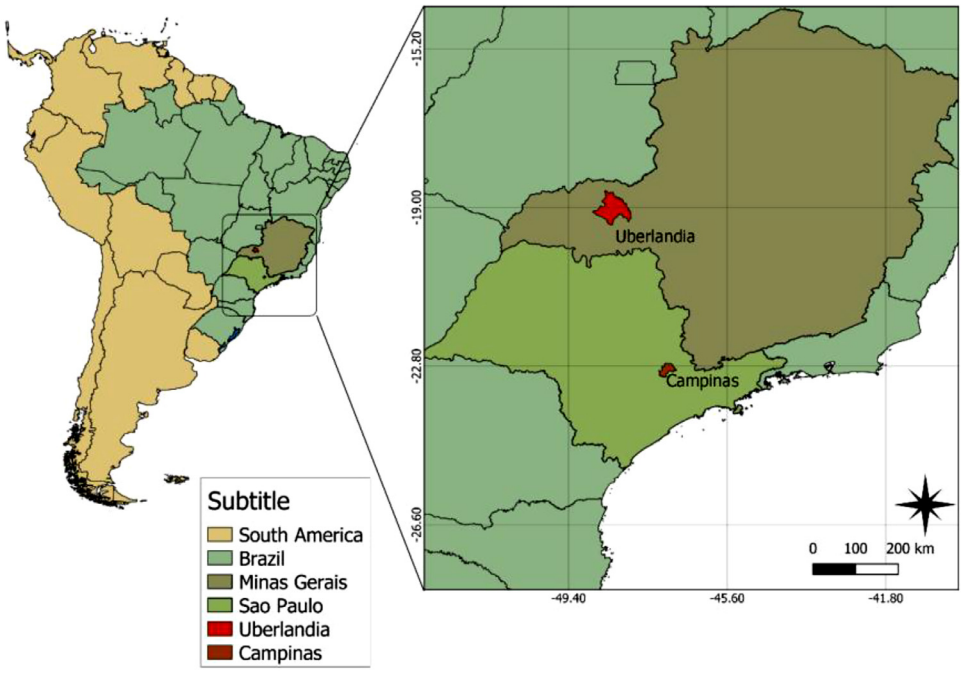

Fig. 1 – Map showing the Southeast region of Brazil and location of the cities of Campinas (São Paulo State) and Uberlandia (Minas Gerais State).

negative bacteria suggestive of *Bartonella* sp. were observed, samples were collected for subsequent molecular analysis.

DNA extraction was performed with the commercial QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's recommendations using whole blood, liquid culture (whole blood and clot), and solid culture isolates.

All extracted samples, except for the isolates, were tested with PCR for amplification of the gene fragment GAPDH (glyceraldehyde-3-phosphate dehydrogenase) related to the production of an enzyme expressed by mammalian cells for the purpose of verifying the quality of the extracted DNA and to rule out the existence of amplification inhibitors.[27]

The DNA of *Bartonella* sp. was amplified by PCR from whole blood, blood liquid culture, clot liquid culture and subculture isolates in solid medium from liquid cultures. Three types of reactions were used: conventional PCR, nested PCR and qualitative real-time PCR.

In conventional PCR, primers for the genus *Bartonella* were used, directed to the Intergenic Spacer region (ITS) 16S-23S of the Rrna.[28] In nested PCR, primers were used for the target gene which encodes the ftsZ protein involved in bacterial cell division, which is species-specific for *B. henselae*.[29] In real-time PCR, primers were used for the target gene which encodes an enzyme which encodes the citrate synthase (*gltA*) in the SYBR Green system, also specific for *B. henselae*, and these results were used only qualitatively, considering the results as positive or negative.[30]

All PCR products were analyzed by electrophoresis in a 2 % agarose gel stained with GelRed® and visualized under ultraviolet light. The flowchart of the procedures is shown in Fig. 2. The obtained amplicons that showed quality for sequencing were sent for comparison with previously deposited genetic material.

Results

The sensitivity of the reactions was 50 equivalent genomic copies per tube for conventional, gender-specific PCR, and 10 for species specific, nested PCR and real-time PCR. A total of 47 patients participated in the study, of which 20 were undergoing treatment at UNICAMP and 27 at CREDESH. The control group consisted of 50 volunteers, students or employees of UNICAMP. DNA of *B. henselae* was detected in at least one sample of 19 of the 47 patients (40.4 %), while in the control group, it was detected in 9 of the 50 volunteers (18,0 %). There was a statistically significant difference ($p = 0.0149$) using the chi-square test (Table 1).

DNA from *B. henselae* was detected in 40.0 % of UNICAMP patients and in 40.7 % of CREDESH patients. There was no significant difference in the detection of bacterial DNA between the two groups ($p = 0.9591$).

Considering that chronic leprosy reactions are a rare event[6] and using Fisher's exact test to establish the sample calculation of the study and setting the significance level at 5 % (alpha or type I error) with a sample power of 80 % (beta or type II error of 20 %), based on the data obtained from the UNICAMP patients, it can be established that the minimum sample for the results to have statistical significance would be 42 patients.

Of the 28 individuals, patients and healthy volunteers, who had *B. henselae*-DNA detected in the molecular tests, five had *B. henselae*-DNA detected in species-specific reactions but could not have their amplicons sequenced, and 23 had at least one of their samples with sequenced amplicons. All sequenced samples showed 99 % to 100 % similarity for *B. henselae*. The access code corresponding to GenBank® was

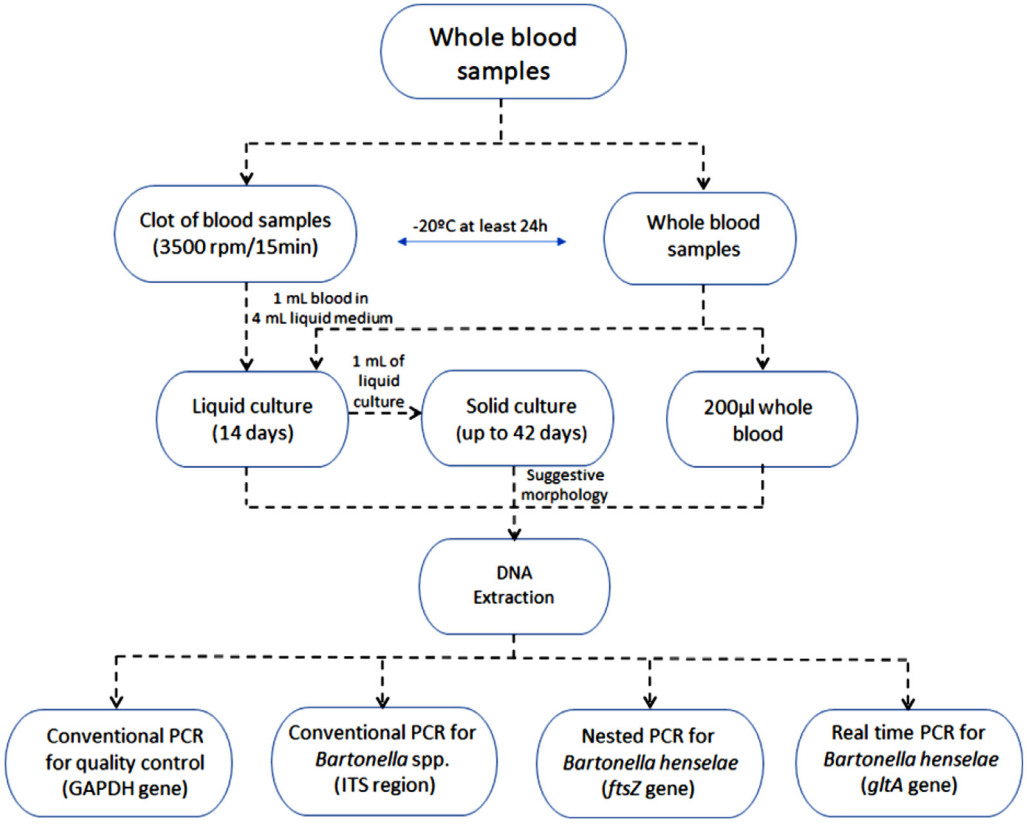

Fig. 2 – Flowchart of the procedures for the detection of *Bartonella* sp.

KT945243.1 in the *gltA* gene, HG965802, in the *ftsZ* gene and CP020742.1 in the ITS region. The bacteria were isolated in four patients and in two controls.

Discussion

Our data show the association of the *B. henselae*-bloodstream infection and chronic T2R. This infection is not consistently self-limiting, and a subset of asymptomatic individuals can be bacteremic, potentially for long, but poorly defined periods. Bartonelloses can also presented by atypical manifestations other than CSD, bacillary angiomatosis/pellioses, Peruvian bartonellosis, or endocarditis and are neglected diseases once neglected diseases can be potentially controlled, prevented and even eradicated using feasible and effective measures. Bartonelloses are not included in the World Health Organization list of Neglected Diseases, but, similar to leprosy, they are supposedly to be more prevalent in vulnerable populations.[31] *Bartonella* sp. can cause subclinical infection and thus, theoretically, trigger leprosy reactions, which are associated with physical disabilities resulting from these reactions.[14]

Table 1 – Results of DNA detection of *Bartonella* sp. per analyzed group.

Groups	Patients		Controls	*p*-value
	UNICAMP	CREDESH		
Total	20	27	50	–
DNA detection	8	11	9	–
Detection rate (%)	40.0 %	40.7 %	18.0 %	0.0149

UNICAMP, Patients followed up at the Clinical Hospital of the University of Campinas, Campinas-SP; CREDESH, National Reference Center for Leprosy and Sanitary Dermatology, Uberlandia-MG.

The prevalence of *Bartonella* sp. infection in asymptomatic humans has been evaluated by molecular tests in some studies and ranges from 1.8 % to 23 %,[14,32-36] but different methodologies have been used, and these results cannot be compared to each other. Portillo et al. analyzed samples from 97 health professionals using several molecular tests from whole blood, liquid and solid cultures in addition to sequencing. With this combination of diagnostic tests, they obtained the DNA of *Bartonella* spp. was amplified by 21.6 %.[37] This percentage of molecular detection in asymptomatic individuals is very similar to Drummond et al. whose study analyzed 500 blood donors and the agent was detected or isolated in 23 % of the analyzed blood donors.[36] In the present study, *B. henselae*-DNA was detected in nine of the 50 (18.0 %) volunteers, who did not report symptoms, in at least one of the molecular reactions.

There are few studies that have evaluated the detection of *Bartonella* sp. in groups of patients with other diseases. In patients with rheumatic symptoms, *Bartonella* sp. infection was observed by PCR analysis of blood, serum and BAPGM liquid culture samples and isolates in 41.1 % of 296 participants.[38] The risk of developing *Bartonella* sp. infection was

22 times higher in patients with endocarditis, 45 times higher in patients with arrhythmias and 40 times higher in patients with chagasic myocarditis than in a group of asymptomatic volunteers.[32] There was no statistically significant difference between a small group of patients with psoriasis and healthy individuals, although *B. henselae*-DNA was detected in 6/30 and 3/30 individuals, respectively.[34] A recent study using digital PCR with patients with schizophrenia showed a statistically higher prevalence (p = 0.0024) compared to healthy volunteers, with detection in 11 of the 17 patients and in one of the 13 individuals in the control group.[33] Patients with primary livedoid vasculopathy had twice as much *B. henselae* infection, 4/16 (25 %) compared to the control group, 4/32 (12.5 %), but the difference was also not statistically significant (p = 0, 24).[39] These data demonstrate that not all individuals colonized by *B. henselae* present clinical manifestations, typical or not, and that the chronic inflammation caused by the presence of the bacteria can be one of the pathogenic mechanisms in the triggering of diseases such as the chronic leprosy reaction or other immune mediated diseases.

Of the two services researched in the Southeastern region of Brazil, there was no significant difference between the group of patients with chronic leprosy reactions. In this region, the rate of patients with permanent sequelae disabilities is greater and higher than in regions of the country where the rates of leprosy detection are higher.[23,25] Infection with *B. henselae* may be indirectly associated, among other factors, with physical disabilities in patients with leprosy by triggering leprosy reactions.

B. henselae was isolated from patients and controls. Although *B. henselae* is the species most associated with diseases in humans,[21] in the present study, all samples were tested with three reactions, but just the conventional PCR was genus-specific, which has lower sensitivity than the two species-specific reactions used (nested and real-time). This factor could skew the results for the detection of *B. henselae*-DNA exclusively in all individuals with the *Bartonella* sp.-DNA detection.

The previously described patient with *M. leprae* and *B. henselae* coinfection had two blood samples collected during retreatment with MDT for leprosy with detection of *Bartonella* sp.-DNA in both samples.[23] In this case of coinfection, the patient had been treated for leprosy with 24 doses of the MDT regimen (in which dapsone had been replaced by ofloxacin by anemia), maintained chronic T2R for 33 months after the end of this first treatment and had started retreatment with the same regimen for six months when *B. henselae* infection was effectively treated. The patient completed 12 doses for his multibacillary leprosy and had no recurrence of leprosy, even after 60 months of follow-up after *B. henselae* treatment.[23]

The need for retreatment occurs in 11.9 % of cases of CREDESH, 25.45 % of UNICAMP patients and 15.1 % of the cases in Campinas in 2018.[40,41] The role of *B. henselae* coinfection in the need for retreatment for leprosy needs to be better evaluated in due course.

There are several reports supporting that therapeutic elimination of *Bartonella* spp. it is difficult and questionable.[42,43] In Carrión disease caused by *Bartonella bacilliformis*, therapeutic failures and persistent bacteremia have been reported, and successful treatment of Oroya fever with antibiotic does not eliminate the patient's risk for development of the *verruga Peruana*.[44] There are reports of cases of recurrence of trench fever, caused by *Bartonella quintana*, years after treatment of the infection.[45] and doxycycline and were treated with azithromycin orally for six weeks. The eradication of *Bartonella* sp. from the human body with antibiotic treatment is questionable.

There are no studies on the ideal treatment for diseases caused by *Bartonella* sp. other than CSD, for which the use of azithromycin orally for five days is indicated.[46] Spach suggests treatment for endocarditis by *Bartonella* sp. with gentamicin and doxycycline for two and six weeks, respectively. The alternative use of azithromycin for up to six months is cited by the same author.[47] Future studies are needed to define the best treatment for *B. henselae* infection in coinfection with *M. leprae*.

Conclusions

Chronic T2R patients may be more related to B. henselae-DNA detection than asymptomatic individuals.

Funding

Doctoral scholarship by The Brazilian National Council for Scientific and Technological Development (CNPq) 170501/2018-3 (Santos, LS); Postdoctoral Scholarship by The São Paulo Research Foundation (FAPESP) 2018/12565-6 (Drummond, MR); Productivity Grant by The Brazilian National Council for Scientific and Technological Development (CNPq) 306970/2018-0 (Velho, PENF). Research Grant by the São Paulo Foundation against Leprosy 193/2018. Support Fund for Dermatology of São Paulo (FUNADERSP). Fund for Support to Teaching, Research and Outreach Activities (FAEPEX) 3184/18.

Conflicts of interest

The authors declare no have conflicts of interest.

Acknowledgments

We thank the National Council for Scientific and Technological Development (CNPq), the São Paulo Research Foundation (FAPESP), the São Paulo Foundation against Leprosy and Fund for Support to Teaching, Support Fund for Dermatology of São Paulo (FUNADERSP), and Research and Outreach Activities (FAEPEX) for their financial support.

REFERENCES

1. Nery JS, Ramond A, Pescarini JM, Alves A, Strina A, Ichihara MY, et al. Socioeconomic determinants of leprosy new case detection in the 100 Million Brazilian Cohort: a population-based linkage study. Lancet Glob Health. 2019;7:e1226–36.

2. Pescarini JM, Strina A, Nery JS, Skalinski LM, Andrade KVF, Penna MLF, et al. Socioeconomic risk markers of leprosy in high-burden countries: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12:e0006622.
3. Mi Z, Liu H, Zhang F. Advances in the immunology and genetics of leprosy. Front Immunol. 2020;11:567.
4. Goulart IM, Goulart LR. Leprosy: diagnostic and control challenges for a worldwide disease. Arch Dermatol Res. 2008;300:269–90.
5. Antunes DE, Ferreira GP, Nicchio MV, Araujo S, Cunha ACR, Gomes RR, et al. Number of leprosy reactions during treatment: clinical correlations and laboratory diagnosis. Rev Soc Bras Med Trop. 2016;49:741–5.
6. Pocaterra L, Jain S, Reddy R, Muzaffarullah S, Torres O, Suneetha S, et al. Clinical course of erythema nodosum leprosum: an 11-year cohort study in Hyderabad, India. Am J Trop Med Hyg. 2006;74:868–79.
7. Vieira-Damiani G, Almeida AR, Silva MN, Lania BG, Soares TCB, Drummond MR, et al. Bartonella henselae infection induces persistent mechanical hypersensitivity in mice. Rev Inst Med Trop Sao Paulo. 2020;62:e79.
8. Breitschwerdt EB, Maggi RG, Nicholson WL, Cherry NA, Woods CW. Bartonella sp. bacteremia in patients with neurological and neurocognitive dysfunction. J Clin Microbiol. 2008;46:2856–61.
9. Nawrocki CC, Max RJ, Marzec NS, Nelson CA. Atypical manifestations of cat-scratch disease, United States, 2005-2014. Emerg Infect Dis. 2020;26:1438–46.
10. Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. Clin Microbiol Rev. 2006;19:338–81.
11. Kumar B, Dogra S, Kaur I. Epidemiological characteristics of leprosy reactions: 15 years experience from north India. Int J Lepr Other Mycobact Dis. 2004;72:125–33.
12. Walker SL, Lebas E, Doni SN, Lockwood DNJ, Lambert SM. The mortality associated with erythema nodosum leprosum in Ethiopia: a retrospective hospital-based study. PLoS Negl Trop Dis. 2014;8:e2690.
13. Breitschwerdt EB. Bartonellosis, one Health and all creatures great and small. Vet Dermatol. 2017;28:96–e21.
14. Pitassi LH, de Paiva Diniz PP, Scorpio DG, Drummond MR, Lania BG, Barjas-Castro ML, et al. Bartonella spp. bacteremia in blood donors from Campinas, Brazil. PLoS Negl Trop Dis. 2015;9:e0003467.
15. Okaro U, Addisu A, Casanas B, Anderson B. Bartonella species, an emerging cause of blood-culture-negative endocarditis. Clin Microbiol Rev. 2017;30(3):709–46.
16. Velho PE, Cintra ML, Uthida-Tanaka AM, Moraes AM, Mariotto A. What do we (not) know about the human bartonelloses? Braz J Infect Dis. 2003;7:1–6.
17. Duncan AW, Maggi RG, Breitschwerdt EB. 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. J Microbiol Methods. 2007;69:273–81.
18. Drummond MR, Lania BG, de Paiva Diniz PPV, Gilioli R, Demolin DMR, Scorpio DG, et al. Improvement of Bartonella henselae DNA detection in cat blood samples by combining molecular and culture methods. J Clin Microbiol. 2018;56. e01732-17.
19. Kaiser PO, Riess T, O'Rourke F, Linke D, Kempf VAJ. Bartonella spp.: throwing light on uncommon human infections. Int J Med Microbiol. 2011;301:7–15.
20. Anderson BE, Neuman MA. Bartonella spp. as emerging human pathogens. Clin Microbiol Rev. 1997;10:203–19.
21. Harms A, Dehio C. Intruders below the radar: molecular pathogenesis of Bartonella spp. Clin Microbiol Rev. 2012;25:42–78.
22. Guptill L. Feline bartonellosis. Vet Clin North Am Small Anim Pract. 2010;40:1073–90.
23. Santos LSD, Drummond MR, França AFED, et al. Chronic type 2 reaction possibly triggered by an asymptomatic Bartonella henselae infection in a leprosy patient. Rev Inst Med Trop Sao Paulo. 2022;64:e17.
24. Santos LS, Drummond MR, da Costa França AFE, Pavan MHP, Stelini RF, Cintra ML, et al. Paraffin-embedded tissue: an alternative to Bartonella sp. infection diagnosis. J Dtsch Dermatol Ges. 2018;16:1147–8.
25. Ministério da Saúde B. Hanseníase. Boletim epidemiológico 2018. Brasil; 2018. Available from: http://portalarquivos2.saude.gov.br/images/pdf/2018/janeiro/31/2018-004-Hanseniasepublicacao.pdf [Last Accessed; 10-FEB-2020].
26. Drummond MR, Visentainer L, Almeida AR, Angerami RN, Aoki FH, Velho PENF, et al. Bartonella henselae bacteremia diagnosed post-mortem in a myelodysplastic syndrome patient. Rev Inst Med Trop Sao Paulo. 2019;61:e50.
27. Birkenheuer AJ, Levy MG, Breitschwerdt EB. Development and evaluation of a seminested PCR for detection and differentiation of Babesia gibsoni (Asian genotype) and B. canis DNA in canine blood samples. J Clin Microbiol. 2003;41:4172–7.
28. Diniz PP, Maggi RG, Schwartz DS, Cadenas MB, Bradley JM, Hegarty B, et al. Canine bartonellosis: serological and molecular prevalence in Brazil and evidence of co-infection with Bartonella henselae and Bartonella vinsonii subsp. berkhoffii. Vet Res. 2007;38:697–710.
29. Kawasato KH, de Oliveira LC, Velho PE, Yamamoto L, Del Negro GM, Okay TS. Detection of Bartonella henselae DNA in clinical samples including peripheral blood of immune competent and immune compromised patients by three nested amplifications. Rev Inst Med Trop Sao Paulo. 2013;55:1–6.
30. Staggemeier R, Pilger DA, Spilki FR, Cantarelli VV. Multiplex SYBR® green-real time PCR (qPCR) assay for the detection and differentiation of Bartonella henselae and Bartonella clarridgeiae in cats. Rev Inst Med Trop Sao Paulo. 2014;56(2):93–5.
31. Brouqui P. Arthropod-borne diseases associated with political and social disorder. Annu Rev Entomol. 2011;56:357–74.
32. Corrêa FG, Pontes CL, Verzola RM, Mateos JCP, Velho PENF, Schijman AG, et al. Association of Bartonella spp bacteremia with Chagas cardiomyopathy, endocarditis and arrhythmias in patients from South America. Braz J Med Biol Res. 2012;45:644–51.
33. Lashnits E, Maggi R, Jarskog F, Bradley J, Breitschwerdt E, Frohlich F. Schizophrenia and bartonella spp. infection: a pilot case-control study. Vector Borne Zoonotic Dis. 2021;21:413–21.
34. Santos LS, Drummond MR, Magalhães RF, Silva MN, Ferreira PAR, Velho PENF. Prevalence of infection by Bartonella spp. in patients with psoriasis. An Bras Dermatol. 2021;96:107–10.
35. Oteo JA, Maggi R, Portillo A, Bradley J, García-Álvarez L, San-Martín M, et al. Prevalence of Bartonella spp. by culture, PCR and serology, in veterinary personnel from Spain. Parasit Vectors. 2017;10:553.
36. Drummond MR, Santos LS, Almeida AR, Lins KA, Barjas-Castro ML, Diniz PPVP, et al. Comparison of molecular methods for Bartonella spp. detection in blood donors. PLOS Neglected Tropical Diseases. 2023;17:e0011336.
37. Portillo A, Maggi R, Oteo JA, Bradley J, García-Álvarez L, San-Martín M, et al. Bartonella spp. prevalence (serology, culture, and PCR) in sanitary workers in La Rioja Spain. Pathogens. 2020;9:189.
38. Maggi RG, Mozayeni BR, Pultorak EL, Hegarty BC, Bradley JM, Correa M, et al. Bartonella spp. bacteremia and rheumatic symptoms in patients from Lyme disease-endemic region. Emerg Infect Dis. 2012;18:783–91.

39. Drummond M, Santos L, Souza L, et al. Detecção do DNA de Bartonella henselae no sangue de pacientes com vasculopatia livedoide. An Bras Dermatol. 2023;98:472–9.
40. Brasil MdS. Acompanhamento dos dados de Hanseníase. Brasília; 2018.
41. Nascimento ACMD, Dos Santos DF, Antunes DE, Gonçalves MA, Santana MAO, Dornelas BC, et al. Leprosy relapse: a retrospective study on epidemiologic, clinical, and therapeutic aspects at a Brazilian referral center. Int J Infect Dis. 2022;118:44–51.
42. Sykes JE, Lindsay LL, Maggi RG, Breitschwerdt EB. Human coinfection with Bartonella henselae and two hemotropic mycoplasma variants resembling Mycoplasma ovis. J Clin Microbiol. 2010;48:3782–5.
43. Psarros G, Riddell J, Gandhi T, Kauffman CA, Cinti SK. Bartonella henselae infections in solid organ transplant recipients: report of 5 cases and review of the literature. Medicine (Baltimore). 2012;91:111–21.
44. Rolain JM, Brouqui P, Koehler JE, Maguina C, Dolan MJ, Raoult D. Recommendations for treatment of human infections caused by Bartonella species. Antimicrob Agents Chemother. 2004;48:1921–33.
45. Brouqui P, Lascola B, Roux V, Raoult D. Chronic Bartonella quintana bacteremia in homeless patients. N Engl J Med. 1999;340:184–9.
46. Bass JW, Freitas BC, Freitas AD, Sisler CL, Chan DS, Vincent JM, et al. Prospective randomized double blind placebo-controlled evaluation of azithromycin for treatment of cat-scratch disease. Pediatr Infect Dis J. 1998;17:447–52.
47. Spach D. Endocarditis caused by Bartonella. 2019. Available from: https://www.uptodate.com/contents/endocarditis-caused-by-bartonella#H11 [Last Accessed; 13-October-2020].