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# Serologic Evidence of Zoonotic Infections by Brucella canis in Southern Chile: A Neglected Emerging Disease

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## Evidencia serológica de infecciones zoonóticas por Brucella canis en Chile. Una enfermedad desatendida y emergente

***ABSTRACT***

*Brucella canis infections are poorly understood in humans and difficult to diagnose, with low blood culture yields. Serological diagnosis was introduced in Chile in 2017.* ***Aim:*** *To report a clinical series diagnosed by serological methods.* ***Methods:*** *Multicenter study in southern Chile of cases admitted in three hospitals in two regions. Diagnosis was made by Rapid Slide Agglutination Test with 2-mercaptoethanol (ME-RSAT).* ***Results:*** *Ten cases were identified between 2020 and 2024 (7 males, median age 54.5 years). Of these, 4 resided in rural areas, and 9 reported exposures to dogs. The cases presented as prolonged fever in 4 patients (40%), spondylodiscitis in 2 (20%), myopericarditis, meningoencephalitis, febrile hepatitis, and cervical lymphadenopathy with weight loss (10% each). Blood cultures were performed in 9 patients, all of which were negative (median incubation time 5 days). All 10 patients received treatment. Two were treated with doxycycline alone, while the remaining 8 received combination therapy. In 3 of the 10 cases, combination therapy was used to prevent relapse after a self-limiting episode of fever, myopericarditis or cervical lymphadenopathy. Histological analysis was available for 4 cases and 2 presented granulomas. The 7 patients who received treatment during the acute phase showed improvement. However, three of them developed chronic pain, and one patient required a disability pension. Additionally, the patient with hepatitis experienced three relapses. Human leucocyte antigen (HLA) typing showed a high relative frequency of the HLA B*07 allele. Epidemiological information reveals that most cases of brucellosis in Chile are due to B. canis, and are concentrated in southern regions.* ***Conclusions:*** *B. canis*

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*infections are emerging, appear to be pleomorphic, with prolonged morbidity and risk of relapse and sequelae. A zoonotic exposure to dogs, even in the past, can help to in suspecting them, and diagnosis is primarily serological. They respond to treatments recommended for other Brucella species and may be associated to some HLA alleles.*
***Keywords:*** *Brucella canis; Drug Therapy; Epidemiology; Serology; Zoonoses.*

***RESUMEN***
*Las infecciones por Brucella canis son poco conocidas en humanos y difíciles de diagnosticar, con un bajo rendimiento en hemocultivos. El diagnóstico serológico se introdujo en Chile en 2017.* ***Objetivo:*** *Reportar una serie clínica diagnosticada por métodos serológicos.* ***Métodos:*** *Estudio multicéntrico en el sur de Chile, con casos ingresados en tres hospitales de dos regiones. El diagnóstico se realizó mediante la prueba de aglutinación rápida en lámina con mercaptoetanol (ME-RSAT).* ***Resultados:*** *Se identificaron diez casos entre 2020 y 2024 (7 hombres, edad media 54,5 años). De estos, 4 residían en zonas rurales y 9 reportaron exposición a perros. Los casos se presentaron como fiebre prolongada en 4 pacientes (40%), espondilodiscitis en 2 (20%), miopericarditis, meningoencefalitis, hepatitis febril y linfadenopatía cervical con pérdida de peso (10% cada uno). Se realizaron hemocultivos en 9 pacientes, todos los cuales fueron negativos (mediana del tiempo de incubación de 5 días). Los 10 pacientes recibieron tratamiento. Dos solo fueron tratados con doxiciclina, mientras que los 8 restantes recibieron terapia combinada. En 3 de los 10 casos, la terapia combinada se utilizó para prevenir la recaída después de un episodio autolimitado de fiebre, miopericarditis y linfadenopatía cervical, respectivamente. El análisis histológico estuvo disponible para 4 casos y 2 presentaron granulomas. Los 7 pacientes que recibieron tratamiento durante la fase aguda mostraron mejoría. Sin embargo, tres de ellos desarrollaron dolor crónico que requirió analgésicos, y a un paciente requirió pensión de invalidez. Además, el paciente con hepatitis sufrió tres recaídas. La tipificación de antígenos leucocitarios humanos (HLA) mostró una alta frecuencia relativa del alelo HLA B*07. Actualmente, la mayor parte de los casos de brucelosis en Chile, están asociados a B. canis y están concentrados en las regiones del sur.* ***Conclusiones:*** *Las infecciones por B. canis tienen un patrón emergente, parecen ser pleomórficas, con morbilidad prolongada y riesgo de recaída y secuelas. Una exposición zoonótica a perros, incluso pasada, puede ayudar a sospecharlas, y el diagnóstico es principalmente serológico. Responden a los tratamientos recomendados para otras especies de Brucella. Pueden estar asociadas a algunos alelos HLA.*
***Palabras clave:*** *Brucella canis; Epidemiología; Serología; Terapia con Medicamentos; Zoonosis.*

Different species of the *Brucella* genus can affect humans through various animal reservoirs and routes. Most well-known and virulent species are *B. abortus* and B. *melitensis*. The clinical profile of these infections is pleomorphic, with diagnosis based on cultures –positive in approximately one-third of cases– and, primarily, by various serological tests, some of which are quantifiable, allowing for disease monitoring or relapse detection[1,2,3,4,5,6,7]. The clinical spectrum includes prolonged fever, spondylodiscitis, sacroiliitis, peripheral arthritis, hepatic abscesses, orchiepididymitis, uveitis, central nervous system involvement, and miscarriages or stillbirths in pregnant women. *B. melitensis* is the most common species in Latin America, primarily associated with the consumption of goat dairy products or agricultural exposure[1,2,3,4,5,6,7].

In contrast, human infections caused by *B. canis* are less known and more challenging to diagnose. They have been described in both children and adults, often associated with dog ownership, kennel workers or veterinary and laboratory personnel[8,9,10]. Human infections with *B. canis* have been characterized by acute or prolonged febrile syndromes, with or without focal involvement, as well as arthralgia, myalgia, diarrhea, vomiting, pneumonia, hepatitis, meningoencephalitis, Guillain-Barré syndrome, endocarditis, or an asymptomatic course[10,11,12,13,14,15,16].

Most cases described in the literature have shown positive blood cultures, although some reports have relied solely on serology[15,17]. Many of these cases involved exposure to dogs, either through pet ownership or occupational contact, and in some instances, to puppies or female dogs that had experienced miscarriages or stillbirths. Unlike other *Brucella* species, *B. canis* has a rough lipopolysaccharide that is not detected by the standard antibodies used for other *Brucella* species, necessitating the isolation of the agent or the use of specific tests for this species. Serological diagnosis was only introduced in Chile for human infections in 2017 at the Instituto de Salud Pública (ISP, the National Reference Laboratory), although the presence of *B. canis* has been recognized in the veterinary field since the 1980s[18,19]. In addition, human leukocyte antigens (HLA) alleles B39 and B27 have been identified as risk markers for joint involvement in patients with brucellosis due to *B. abortus* or *B. melitensis* but they have not been explored in cases of *B. canis* infection[20,21].

In dogs, *B. canis* causes epididymitis, orchitis, and sterility in males and abortions in females. It is associated with bacteremia and prolonged urinary, seminal, or vaginal *B. canis* excretion[22].

The purpose of this study is to present a series of cases with serological evidence of *B. canis* human infection in southern Chile, analyzing their clinical spectrum and progression, the regional epidemiology and HLA alleles association.

## Patients and Methods

Retrospective multicentric study of cases identified at three hospitals located in southern Chile (Puerto Montt, Osorno, Valdivia) and belonging to Los Ríos Region (Valdivia) and Los Lagos Region (Osorno, Puerto Montt). Cases were identified based on records from treating physicians or through epidemiological notifications from 2017 to 2024.

Cases were included if they had a serological diagnosis performed using the Rapid Slide Agglutination Test (RSAT) with 2-mercaptoethanol (ME-RSAT) at the ISP. This test is qualitative (positive or negative) and not quantifiable. The technique uses heat-inactivated colonies of a less mucoid strain (M-) of *B. canis*, first described in the late 1980s. The test includes 2-mercaptoethanol to improve specificity, achieving a sensitivity of 95% and a specificity of 88%[23]. This test has also been implemented in Argentina at Malbrán Institute, from where the diagnostic test kits are provided and quality controlled[24,25].

Clinical data were extracted from medical records using a standardized questionnary. Cases were included if they had at least one positive ME-RSAT test, while autoimmune cases or those with zoonotic coinfections were excluded to refine clinical interpretation. Additionally, histopathological information was included for cases where biopsies were performed.

HLA typing was included to investigate a possible association between osteoarticular involvement and the HLA-B*39 or B*27 allele. Blood samples were analyzed in one of the participating

centers using de LIFECODES® HLA SSO Typing kits in a Luminex 200 equipment.

A dual approach was used to estimate the regional frequency of this infection. First, total brucellosis cases and *B. canis* notified cases in the Los Ríos and Los Lagos regions from 2020 onward were analyzed (All brucellosis cases are of mandatory notification in Chile). Additionally, data on *B. canis*-reactive cases from the ISP were obtained from 2017. Information was obtained by request to regional epidemiological offices and to the ISP under the Transparency Act 20.285 that guarantees access to public information.

Ethical Considerations. This study was approved by the Scientific Ethics Committee of the Los Ríos Health Service.

## Results

A total of 10 cases were identified between 2020 and 2024 (7 males, 70%; median age 54.5 years, interquartile range [IQR] 41.75–61.5 years). Serology for other *Brucella* species were negative in all patients. Four resided in rural areas, and 9 reported exposures to dogs, not currently in 4. In 2 cases, there was exposure to canine puppies with litter mortality, and in another case, exposure to a canine miscarriage, all occurring in the past (Table 1). Only one patient had a significant comorbidity (lymphoma in complete remission under treatment with rituximab). No cases with occupational exposure were detected.

### *Clinical Presentation*

The cases presented as prolonged fever in 4 patients (40%), lumbago in 2 (due to spondylodiscitis, 20%), chest pain (due to myopericarditis), meningoencephalitis, febrile hepatitis, and cervical lymphadenopathy with weight loss (10% each) (Table 2). All patients were hospitalized (median 15.5 days; IQR 9.5–40.5 days). Four patients had anemia and/or lymphopenia, and one had leukopenia. C-reactive protein (CRP) had a median of 13.1 mg/dL (IQR 0.89–19.4; reference <0.5 mg/dL), and erythrocyte sedimentation rate (ESR) had a median of 41 mm/h (IQR 16.1–71).

Blood cultures were performed in 9 patients, all of which were negative median incubation time 5 days; IQR 5–14 days). Additionally, 2 patients underwent bone marrow or cerebrospinal fluid (CSF) cultures (both negative). Serology for *Coxiella burnetii* (Q fever) was tested in 8 patients (all negative), and *Bartonella henselae* IgG serology was performed in 7 patients (one positive at 1:256, considered not clinically relevant).

### *Details of Cases with Focal Involvement*

Spondylodiscitis. Two patients developed spondylodiscitis, one with psoas involvement (Figure 1A). Extensive testing, including 16S RNA sequencing, conventional cultures, and tuberculosis (TB) studies, yielded negative results. Histological analysis did not reveal granulomas (see further details below).

Myopericarditis. A 57-year-old woman presented with progressive chest pain over 20 days. ECG showed ST-segment elevation, and high-sensitivity troponin levels increased to 587 pg/mL (reference ≤14). Coronary angiography was normal. Cardiac MRI revealed focal hypokinesia in the inferoseptal segment of the left ventricle, with preserved contractility. T2 sequences showed transmural myocardial edema in the inferior septal segment (Figure 1B). Autoimmune, viral respiratory, Epstein-Barr virus, and cytomegalovirus studies (by PCR in blood) were negative. Serology for *Toxoplasma gondii, Mycoplasma pneumoniae,* and HIV was also negative. The patient experienced spontaneous recovery.

Meningoencephalitis. A 45-year-old male presented with a 4-day history of fever, headache, nausea, and vomiting. On admission, he was alert and oriented, with no focal neurological signs or meningeal symptoms. He reported a 2- to 3-month history of oral lesions and xerostomia. CSF analysis showed pleocytosis with 293 cells/mm³ (70% mononuclear predominance), normal glucose levels (126 mg/dL), and mildly elevated proteins (63 mg/dL). The BioMérieux meningitis-encephalitis panel detected no microorganisms, and cultures were negative. He recovered neurologically but continued to experience peripheral arthralgia and myalgia.

Febrile hepatitis. A 51-year-old male was hospitalized with fever, anorexia, myalgia, cough,

**Table 1.** Demographic, occupational, canine exposure and risk characteristics in 10 cases of human infections by *B. canis* in Southern Chile.

| Case | Gender*, age in years | Region | Residence or visit to rural areas | Agricultural activities | Current or previous dog exposure | Care or observation of puppies, litters with canine mortality or canine abortions |
|---|---|---|---|---|---|---|
| 1 | M, 74 | Los Ríos | No | No | Yes, previous | Puppies, litter mortality |
| 2 | F, 32 | Los Ríos | Yes | Unknown | Yes, previous | Puppies, litter mortality |
| 3 | M, 58 | Los Ríos | No | No | Yes, current and previous | No |
| 4 | M, 59 | Los Ríos | Yes | No | Yes, previous | No |
| 5 | F, 57 | Los Ríos | No | No | Yes, current and previous | Canine abortion |
| 6 | M, 51 | Los Lagos | Yes | Yes | Yes, previous | No |
| 7 | M, 52 | Los Lagos | Yes | Yes | Yes, current and previous | No |
| 8 | M, 45 | Los Lagos | Yes | Yes | Unknowm | Unknown |
| 9 | M, 28 | Los Lagos | Yes | Yes | Yes, current and previous | No |
| 10 | F, 69 | Los Ríos | No | No | Yes, current and previous | Puppies |

* M: Male; F: Female.

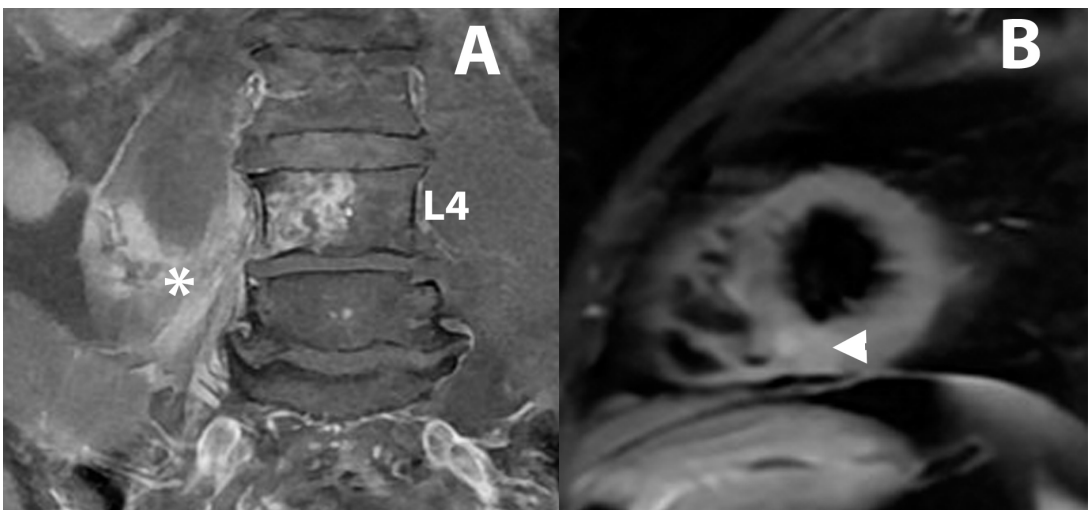


***Figure 1:*** *A. Coronal Spine MRI (T1-weighted gadolinium signal) showing bone abnormalities in the body of L4 with involvement of the right psoas (asterisk). B. Cardiac MRI showing transmural myocardial edema visible in the lower septal segment of the middle and apical third of the left ventricle (T2-weighted signal, white arrow).*

**Table 2.** Clinical and diagnostic features in 10 cases of human infections by *B. canis* in Southern Chile.

| Case | Clinical picture | Other studies | Laboratory parameters |
|---|---|---|---|
| 1 | Prolonged fever | Blood cultures, Q fever, Bone marrow biopsy, HLA typing: B40*; B51* | Anemia, ESR 62 mm/h |
| 2 | Prolonged fever | Blood cultures, *Bartonella* and Q fever serology HLA typing: B18*; B41* | Anemia, ESR 73 mm/h |
| 3 | Lumbago | Blood cultures, Bartonella and Q fever serology, vertebral biopsy. Tuberculosis PCR and culture. Ribosomal 16S RNA sequencing. HLA typing: B41*; B49* | ESR 22 mm/h |
| 4 | Lumbago | Blood cultures, Bartonella and Q fever serology, vertebral biopsy. Tuberculosis PCR and culture. HLA typing: B*07; B*15 | Anemia, ESR 41 mm/h |
| 5 | Chest pain | *Bartonella*, Q fever, and serology for other etiologies (see text). HLA typing: B07*; B51* | ESR 32 mm/h |
| 6 | Fever and Hepatitis | Blood cultures, Q fever serology, *Bartonella* IgG titer 1:256 HLA typing: B07*; B44* | Leukopenia, ESR 8 mm/h Hepatitis with a mixed pattern |
| 7 | Prolonged fever | Blood and bone marrow cultures, Q fever serology | Anemia |
| 8 | Meningoencephalitis | Blood, cerebrospinal fluid and bone marrow cultures, Q fever serology. | ESR 11 mm/h |
| 9 | Prolonged fever | Blood cultures, HLA typing: B07*; B39* | ESR 108 mm/h |
| 10 | Lymph node enlargement and weight loss | Blood cultures, Lymph node biopsy. Tuberculosis PCR. Q fever serology. HLA typing: B08*; B15* | ESR 69 mm/h |

vomiting, dark urine, pale stools, mild jaundice, and hepatitis, without biliary, pancreatic, or autoimmune abnormalities on laboratory testing. Notable findings included bicytopenia (white cell and platelet lineages) and significant mixed-pattern liver enzyme abnormalities, along with hepatosplenomegaly. Due to suspicion of zoonotic infection, doxycycline was initiated, later combined with rifampin for 6 weeks upon confirmation of serological results. The patient responded to treatment but experienced frequent relapses with sacroiliitis, recurrent febrile episodes, cytopenias, and hepatitis, which were attributed to secondary hemophagocytosis caused by the infection.

Lymphadenopathy. A 69-year-old female presented with weight loss and bilateral palpable cervical lymphadenopathy, which was tender but without external inflammatory signs. She had a history of untreated pulmonary TB in childhood, leading to chronic obstructive pulmonary sequelae. Microbiological studies ruled out active pulmonary TB. Complementary CT scans did not reveal additional lymphadenopathy, and a PET scan showed metabolic activity associated with the cervical lymph nodes.

A biopsy revealed necrotizing granulomas (Figure 2, see below). TB PCR was negative. The patient experienced a spontaneous recovery.

***Histology***

Histological analysis was available for 4 cases. In two cases (bone marrow and lymph node), granulomas without caseation were observed, one with Langhans-type giant cells (Figures 2A and 2B). In the remaining two patients (vertebral biopsies), no granulomas were observed but they presented infiltrates with either an acute (neutrophils) or chronic (lymphocytes) inflammatory pattern.

***Treatment***

All 10 patients received treatment (Table 3), 7 in the acute phase and in the rest after a self-limiting episode of fever, myopericarditis, and cervical lymphadenopathy, respectively, to prevent relapses. In total, two were treated with doxycycline alone (for 2 and 6 weeks, respectively), while the remaining 8 received combination therapy, including: rifampin-doxycycline (5 cases, for 6–18 weeks; in 2 cases, initial gentamicin support was added for one week); doxycycline-trimethoprim/sulfamethoxazole (one case, for 4 weeks); doxycycline-hydroxychloroquine (two cases, for 6 weeks) (Table 3). In the 3 cases treated after the acute episode, combination therapy was used.

***Outcomes***

The seven patients who received treatment during the acute phase showed clinical and inflammatory improvement (Table 3). However, three of them developed chronic axial or peripheral musculoskeletal pain requiring pain medication, and one patient required a disability pension. Additionally, the patient with initial febrile hepatitis experienced three relapses (at 1, 7, and 12.5 months after the first treatment), one of which involved sacroiliitis. These episodes responded to prolonged treatment with the standard regimen and later with ciprofloxacin-hydroxychloroquine, respectively. Two patients experienced probable adverse reactions to the initial treatment or relapse therapies (one with renal-hepatic involvement and another with renal impairment, Table 3). In one case rifampin was discontinued.

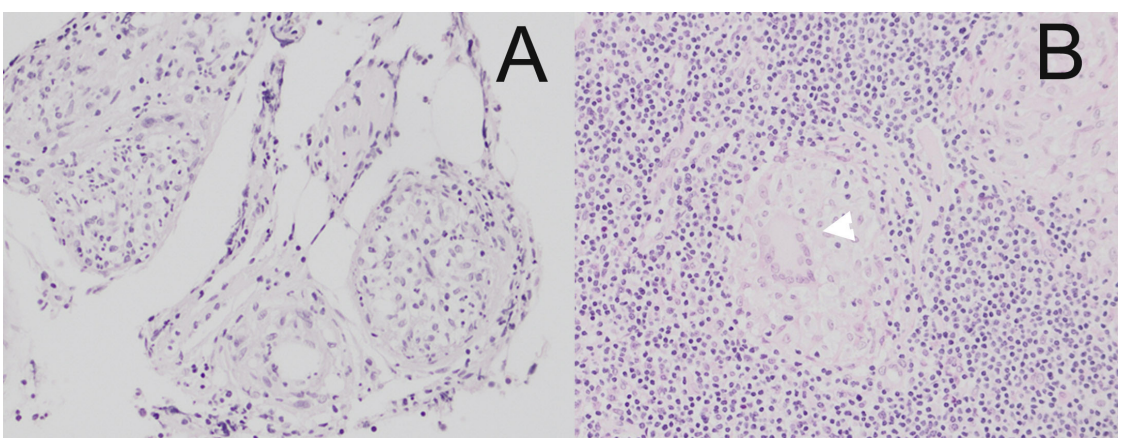


***Figure 2:*** *A. 20X hematoxylin-eosin stained micrograph: bone marrow tissue with two non-necrotizing granulomas, composed of numerous neutrophils, histiocytes, and lymphocytes. B. 20X hematoxylin-eosin stained micrograph: a necrotizing granuloma composed of epithelioid histiocytes, lymphocytes, and a Langhans-type giant cell (white arrow) in a lymphoid background.*

**Table 3.** Treatment and outcome in 10 cases of human infections by B. canis in Southern Chile.

| Case | Complications and treatment | Evolution and outcome |
|---|---|---|
| 1 | No complications<br>Acute phase treatment with doxycycline 6 weeks (anticoagulant treatment) | No relapses at 24 months |
| 2 | No complications<br>Treatment to prevent relapse with doxycycline + hydroxychloroquine for 6 weeks | No relapses at 21 months |
| 3 | Spondylodiscitis, paraspinal abscess<br>Acute phase treatment with rifampicin + doxycycline for 12 weeks and gentamicin for 1 week | Chronic pain, disability pension |
| 4 | Spondylodiscitis<br>Acute phase treatment with rifampicin + doxycycline for 18 weeks and gentamicin for 1 week | Chronic pain with slow resolution |
| 5 | Myopericarditis<br>Treatment to prevent relapse with rifampicin + doxycycline for 6 weeks | No relapses at 12 months |
| 6 | Hepatitis + Sacroiliitis<br>Acute phase treatment with rifampicin + doxycycline for 6 weeks | Relapses at 1, 7, and 12.5 months, one with sacroiliitis, which responded to prolonged treatment with a standard regimen and then with ciprofloxacin-hydroxychloroquine, respectively. Hepatoxicity by rifampicin with a mixed pattern |
| 7 | No complications<br>Acute phase treatment with cotrimoxazole forte for 6 weeks + gentamicin for 2 weeks, then combined with doxycycline for 4 weeks | Nephrotoxicity<br>No relapses at 56 months. |
| 8 | Neurobrucellosis<br>Acute phase treatment with doxycycline 2 weeks | Chronic joint pain with myalgias |
| 9 | No complications<br>Acute phase treatment with rifampicin + doxycycline for 6 weeks | No relapses at 12 months |
| 10 | No complications<br>Treatment to prevent relapse with doxycycline + hydroxychloroquine for 6 weeks (anticoagulant treatment) | No relapses at 24 months. |

### *Epidemiology*

The incidence rate of total notified brucellosis cases showed a marked increase in the Los Ríos Region during 2023–2024 that was almost exclusively linked to *B. canis* (Figure 3A). In Los Lagos Region, no recent increase was observed (3B) but for both regions, almost all recent cases of brucellosis were associated to *B. canis*. In addition, we explored the total number of cases diagnosed by the ISP since the introduction of the ME-RSAT in 2017, observing also a progressive increase in cases (Figure 4). The Los Ríos Region's contribution to the total number of cases has augmented in parallel but cases out of these 2 regions are also being diagnosed.

HLA Typing. HLA-B allele analysis was performed in 8 out of 10 patients; however, only one of them carried the HLA-B39 allele, which was not associated with axial or peripheral joint involvement. The HLA-B27 allele was not detected. Unexpectedly, allele B*07 was overrepresented (4 out of 8 running) (Table 2).

## Discussion

The results of this study suggest that *B. canis* infections have been increasing in Chile in recent years at the expense of 2 southern regions but still at a low rate. This emerging phenomenon can be partially explained by the incorporation of the ME-RSAT test at Chile's ISP at the year 2017, and possibly by increased clinical suspicion. It remains unknown whether this rise is due to a higher prevalence/incidence of *B. canis* infections in the canine population, greater human exposure, or an increase in the total number of canines in these regions. None of these questions can be answered with the currently available data.

Serological studies conducted in 1978 in the city of Valdivia, which is part of this study, revealed a *B. canis* seroprevalence of 16% in the canine population at that time[18]. More recently, in a nearby city (Temuco), seroprevalence was much lower, reaching only 1%, although this may be due to differences in the applied techniques[26]. Another study conducted in the Metropolitan Region of central Chile between 2018 and 2019 reported a prevalence of 7% in different canine populations based on culture or serology[27].

Thus, there is a zoonotic risk of acquiring *B. canis* through contact with dogs in various regions of Chile, and it is possible that recent or past exposure to puppies or adult dogs–observed in almost all our cases– represents the source of infection. Anamnestic data suggest that exposure may occur in both urban and rural settings and that some cases could result from reactivation

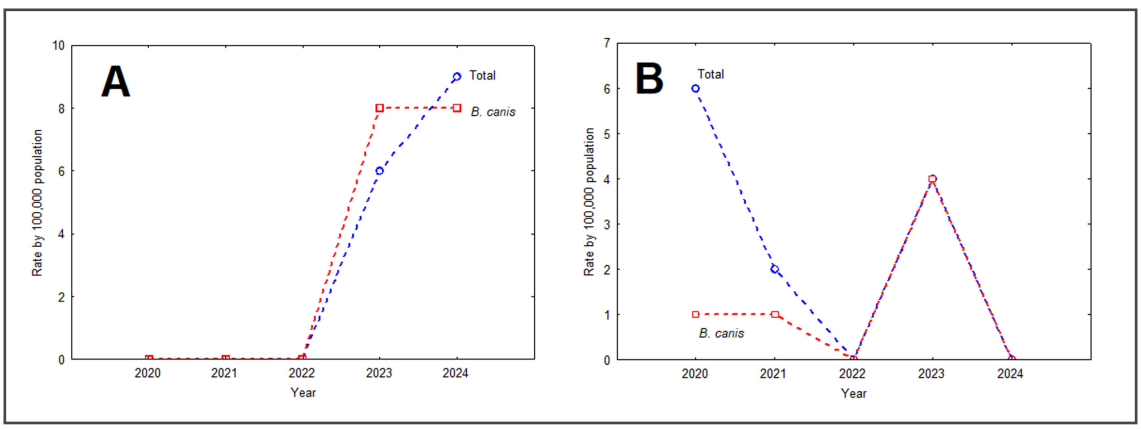


***Figure 3:*** *Trends in B. canis and total Brucellosis rates in the Los Ríos (A) and Los Lagos (B) regions according to notifications to the MINSAL (Ministry of Health) between 2020 and 2024. Brucellosis is a zoonotic disease of mandatory notification in Chile. Not all notified cases are included in this study.*

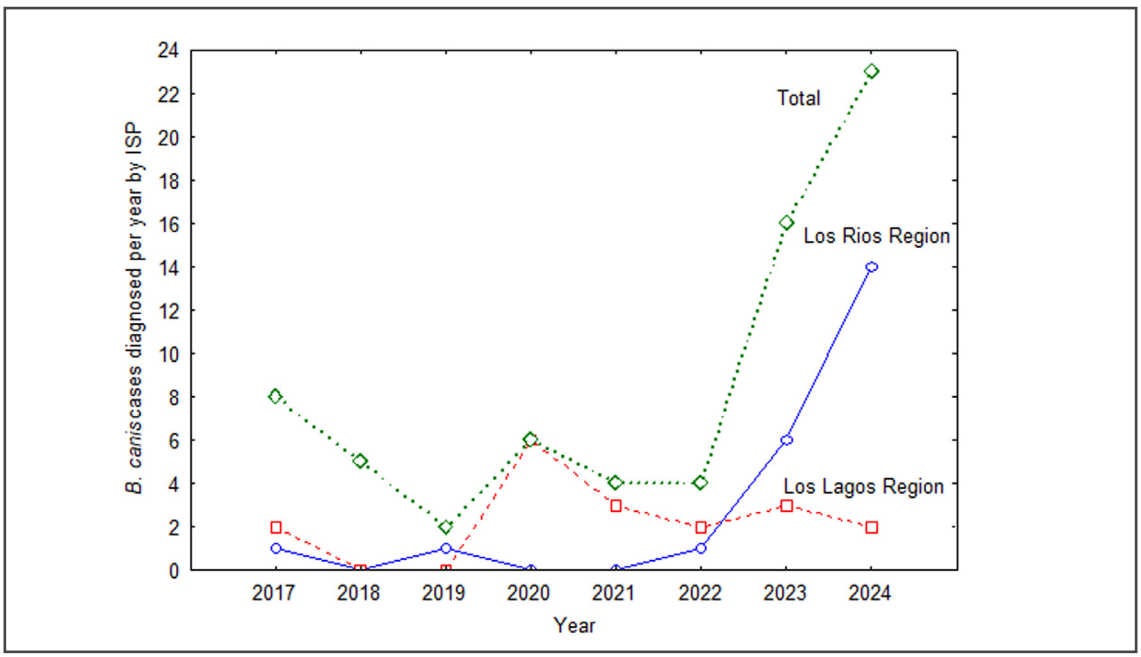


***Figure 4:*** *Cases of B. canis infections diagnosed at the Instituto de Salud Pública (National Reference Laboratory) by MERSAT, per year.*

rather than recent infection. Unfortunately, we were unable to study the involved dogs to confirm the presence of an active reservoir in the environment of our patients.

There are no previously reported cases of human infections by *B. canis* in Chile, and our series, although lacking confirmed cases and consisting only of presumptive ones, suggests an emergent problem that should be considered. Currently, there is no standardized diagnostic approach, veterinary regulations, or mandatory reporting of brucellosis for the canine population. In Europe, *B. canis* is considered an emerging and unregulated issue[28].

In Chile, it is estimated that there is one pet dog for every 2.5 people in urban areas and one for every 1.7 people in rural areas, indicating greater exposure to dogs in non-urban regions. Additionally, half of pet dogs sleep inside home[29]. These data suggest that human infections by B. canis will likely increase in the coming years, regardless of the diagnostic approach used.

The clinical spectrum of human infections caused by *B. canis* is diverse and does not necessarily follow a benign course, as evidenced by the need of hospitalizations, sequelae, relapses, and the occurrence of extra-articular clinical manifestations in our cases. This underscores the need of a high degree of clinical suspicion, the use of blood cultures with extended incubation periods, and access to improved serological diagnostic techniques. Additionally, it highlights the need for a parallel veterinary study.

The diagnosis of *B. canis* infections is challenging due to the low sensitivity of blood cultures and the absence of optimal serological tests. The low sensitivity of blood cultures can be attributed to the slow bacterial replication rate, the need for prolonged incubation periods of up to four weeks, variations in sample volume, the possible inhibitory effect of sodium polyanetholesulfonate (SPS), and the stage of disease progression (with

higher detection rates during the acute phase)[30]. The low sensitivity of blood cultures (<20%) has also been reported in studies on canines, where serology has been used to determine infection prevalence[18,27].

Additionally, the rough phenotype of *B. canis* prevents the use of serological tests and diagnostic algorithms typically applied for other *Brucella* species[30]. Currently, diagnosis relies on tests developed by various research centers using different methodologies, the most commonly applied being the ME-RSAT and ELISA tests. At present, the only test available at the ISP is ME-RSAT.

Nonetheless, ME-RSAT has shown high sensitivity and specificity in the canine population (sensitivity: 86–95%, specificity: 88–97%)[23,31,32], although some authors have reported lower sensitivity in acute infections (42.9%)[33]. The positivity of this test remains prolonged, lasting several months in bacteremic dogs and often exceeding one year[23].

There are few studies in humans providing diagnostic performance data for ME-RSAT. These studies indicate a sensitivity of 95–100% for cases confirmed by blood cultures[24,34]. Specificity ranges from 90–100% for infections not associated with the *Brucella* genus[24,31]. However, specificity is lower in cases of infections caused by other *Brucella* species in humans. The ISP panel includes tests directed to *B. abortus, B. melitensis* and *B. canis*, allowing for the exclusion of cross-reactivity. In our patients, none tested positive for other *Brucella* species. Thus, despite the lack of bacteriological confirmation, the test's characteristics support the evidence for these infections.

Commercial PCR-based diagnostic tests also exist varying in the sequences they amplify. The molecular targets include genes encoding surface proteins, outer membrane proteins, insertion sequences, among others[30]. However, amplification attempts in some of our patients were unsuccessful. The lack of amplification could be due to the absence of the pathogen in the tested fluid, the presence of amplification inhibitors, or genetic variations that limit the effectiveness of certain primers. Multiple reports of human infections have relied solely or primarily on serological testing, underscoring the widespread challenges in diagnosing *B. canis* infections and the erroneous underestimation of its epidemiological significance[15,17]. Additionally, ME-RSAT serology does not allow for titer quantification, particularly for monitoring treatment response and/or relapses.

The treatment of brucellosis due to *B. canis* follows recommendations for other types of brucellosis. Treatment involves prolonged combination of antimicrobial regimens to achieve bacterial eradication, control morbidity, and prevent relapses. For uncomplicated cases, oral doxycycline for six weeks is recommended, combined with streptomycin for two weeks. However, since streptomycin is difficult to access, it can be replaced with gentamicin[35].

The use of rifampin with doxycycline for six weeks avoids the need for parenteral agents but has been associated with higher failure and relapse rates[36]. In cases of complicated brucellosis, adding a third drug and extending treatment to 4–6 months is recommended.

In our series, parenteral aminoglycosides and rifampin had to be avoided in two cases due to concurrent anticoagulant therapy. Additionally, in some cases, hydroxychloroquine was used to alkalinize the phagolysosome, enhancing the bactericidal effect of doxycycline as in Q fever[37].

Interestingly, the self-limiting course observed in some cases, raised the question of whether preventive treatment may be necessary to avoid potential future relapses. As of the completion of this study, none of these patients have experienced a relapse.

The predominant histological finding in cases of brucellosis has been the presence of granulomas, particularly of the histiocytic type, with a predominance of lymphocytic infiltrates[38,39]. The presence of giant cells, caseous necrosis, and neutrophilic infiltrates has been occasionally described[38,39]. Our series showed findings consistent with these descriptions, highlighting the value of histological analysis in the diagnosis of challenging cases.

In our study, we also investigated a possible association between axial involvement and specific HLA alleles. Such associations have been described

in rheumatologic diseases, adverse drug reactions, and post-infectious joint complications[20]. Both the HLA-B39 and HLA-B27 alleles have been identified as risk markers for joint involvement in patients with brucellosis[20,21]. However, we were unable to confirm this association, in agreement with other authors[40]. We found a wide clinical spectrum associated with the HLA B*07 allele. Its frequency reached 50% of the studied patients in contrast to the 7-11% reported for kidney allograft donors[41], hematopoietic stem cells donors at local level (L. Carrasco, personal communication) or Latin American habitants[42] This finding is suggestive of an association for *B. canis* infection but requires more research.

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