Brief Report

Absence of *Brucella canis* Detection in Dogs from Central Italy: Implications for Regional Surveillance and Zoonotic Risk

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Article

Absence of *Brucella canis* Detection in Dogs from Central Italy: Implications for Regional Surveillance and Zoonotic Risk

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Abstract

Background: *Brucella canis* is a zoonotic pathogen associated with reproductive disorders in dogs and represents an emerging public health concern. Dogs are the only known source of infection for humans, and transmission is often associated with close contact, particularly in occupational settings. Reports of canine and human infections in Europe are increasing, underscoring the need for integrated surveillance to assess the risk of introduction and spread. **Objectives:** This study aimed to estimate the prevalence of *B. canis* in dogs from Central Italy, a region potentially at risk due to several factors. **Methods**: A comprehensive serological, molecular, and bacteriological survey was conducted on 128 dogs of various origins, including strays, blood donors, breeding dogs, dogs accompanying refugees, and dogs previously linked to a *B. canis* outbreak. Blood samples were tested using bacterial culture, real-time PCR, serum agglutination test, complement fixation test, and indirect immunofluorescence antibody test. **Results**: All tested dogs were negative for *B. canis*, with an estimated maximum prevalence of 3.5% (95% confidence interval), suggesting a low likelihood of infection within the sampled population. **Conclusions**: Although no positive cases were detected, maintaining an active and continuous surveillance system is essential to prevent *B. canis* introduction, particularly through international dog movements and other high-risk scenarios. As dogs represent the only known source of human infection, veterinary surveillance plays a pivotal role in mitigating zoonotic risks. This study highlights the importance of regional surveillance, particularly in low-prevalence areas, as part of broader efforts to support evidence-based control strategies within a One Health framework.

Keywords: *Brucella canis*; brucellosis; zoonosis; dogs; public health

1. Introduction

Brucella canis, an emerging zoonotic pathogen, has been increasingly reported across Europe in humans and dogs, particularly in association with international dog movements [1–6]. This pathogen is a significant cause of reproductive failures in dogs and poses considerable public health risks due to its zoonotic potential [5–8]. Although human brucellosis due to *B. canis* is likely underdiagnosed, given its nonspecific clinical manifestations and the limited use of specific diagnostic tools, cases have been reported in both Europe and the Americas, especially in individuals with close or occupational contact with infected dogs [3,5,8–14]. In Latin America, particularly in Brazil and Argentina, human

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infections are more frequently reported, reflecting both higher canine prevalence and greater diagnostic awareness in some areas [6,24].

A recent outbreak in dogs in Italy, considered one of the largest in Europe, has highlighted the urgent need for regional surveillance, preventive measures, and international collaboration [15].

Worldwide, the growing globalization of pet movements, driven by diverse factors such as adoption campaigns, commercial breeding, and humanitarian crises, has facilitated the transboundary spread of *B. canis* [2,4,11,14,16–18]. In particular, the relocation of dogs accompanying refugees from conflict-affected regions has introduced additional biosecurity challenges, as these animals may originate from areas where *B. canis* is endemic. Countries like the United Kingdom, which have seen a rise in cases linked to imported dogs, have emphasized the importance of stricter import controls and risk assessments to identify high-risk countries and prevent the introduction of infected animals [17,19].

Surveillance data are essential for informing both veterinary and public health risk assessments and guiding preventive strategies, particularly in regions with limited baseline data. This study aims to address these global One Health concerns by investigating the prevalence of *B. canis* within the canine population of the Umbria Region in Central Italy, a region potentially vulnerable to introduction via national and international dog movements, to inform future public health and veterinary measures.

2. Materials and Methods

2.1. Study Design

A cross-sectional study was conducted between July 2023 and February 2024 in the Umbria region, involving collaboration among the Department of Veterinary Medicine of the University of Perugia, public veterinary services, the Istituto Zooprofilattico Sperimentale Umbria e Marche, the National Reference Centre for Brucellosis (Istituto Zooprofilattico Sperimentale of Teramo), and local veterinary practitioners. A sample size of 100 dogs was calculated to detect a minimum expected prevalence of approximately 5% with a 95% confidence level and a precision of ±10%, under the assumption of a binomial distribution [20]. To account for potential exclusions or unviable samples, a larger cohort was included.

2.2. Ethical Considerations

Samples were collected during routine veterinary procedures, requiring no specific ethical approval. Blood samples from donor dogs were anonymized and retrospectively analyzed. For the remaining groups, samples were collected during routine sanitary visits for diagnostic or surveillance purposes, so formal ethical approval was not required. Owners provided informed consent for the use of clinical samples for epidemiological investigation.

2.3. Study Population and Sampling

The study population included dogs residing in the Umbria region of Italy. Blood samples were collected from a total of 128 dogs over two months of age divided into five groups: 1) 47 stray dogs housed in 3 different sanitary kennels of the Region, 2) 22 private breeding dogs, 3) 28 blood donor dogs affiliated with the University of Perugia Veterinary Teaching Hospital, 4) 23 dogs accompanying refugees from Ukraine with unknown *B. canis* health status, and 5) 8 dogs previously associated with a previous *B. canis* outbreak in Italy and currently under voluntary monitoring by their owners under the supervision of public veterinarians. Samples were stored and transported at 4 ± 2 °C to the National Reference Centre for Brucellosis for testing.

2.4. Diagnostic Methods

The study employed a comprehensive diagnostic approach, combining direct and indirect methods to ensure high sensitivity and specificity in detecting *B. canis*, also at different stages of

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infection. This strategy is particularly valuable for surveillance in low-prevalence regions, where subclinical cases may otherwise be missed [21,22]. Samples underwent both direct (bacterial culture and real-time PCR) and indirect (serological) diagnostic tests. Blood cultures were performed using Farrell's selective agar and enrichment broth, incubated at 37 ± 1 °C for up to one month, with weekly subcultures. Colonies indicative of *Brucella* spp. were subsequently identified via PCR. The serological tests included the serum agglutination test (SAT), the complement fixation test (CFT), and/or the indirect immunofluorescence antibody test (IFAT). These tests targeted antibodies specific to *B. canis*, utilizing sera of the animals and antigen preparations homologous to rough *Brucella* strains, ensuring high sensitivity and specificity. The SAT primarily detected IgM during early infection, while the CFT identified both IgM and IgG antibodies [21,22]. For blood donors, serological tests were performed using previously stored plasma. For dogs accompanying refugees, direct tests were conducted in all cases, while serological tests were feasible in only 8 samples, using IFAT.

3. Results

The study population consisted of diverse groups of dogs, varying in age, sex, and breed, representative of the regional population. Ages ranged from 2 months to 17 years (mean and median age of 3 years). The gender distribution was nearly equal, with 64 males, 62 females, and 2 individuals whose sex was unknown due to anonymization. Over 21 breeds were represented, with mixed breeds comprising 25.8% of the study population, followed by Golden Retrievers (18.5%) and Yorkshire Terriers (14.5%). Direct (culture, PCR) and indirect (SAT, CFT, IFAT) diagnostic methods were employed, with test selection tailored in some cases to the biological sample type and the potential infection phase (i.e., blood donors and dogs accompanying refugees). None of the tested dogs resulted positive for *B. canis*. Based on these findings, the estimated maximum prevalence in the sampled population is calculated to be up to 3.5% (95% confidence interval).

4. Discussion

The absence of positive cases in this study suggests that *B. canis* is not currently endemic in the Umbria region. However, sporadic infections cannot be ruled out due to the limited sample size relative to the total canine population, the proximity to a prior outbreak in a neighboring region, and the risks associated with international dog movements from endemic areas, particularly in Eastern Europe. Moreover, a recent study in the Veneto Region reported a prevalence of 1.95% [23], highlighting potential regional variability within Italy and underscoring the importance of continued surveillance across different areas to assess and mitigate the risk of *B. canis* introduction and spread.

Special attention should be directed to high-risk situations, such as cases of suspected infection in dogs exhibiting compatible clinical signs or the introduction of positive dogs into mixed populations [3,17]. These scenarios can facilitate the rapid spread of the infection, especially in groups with different ages, sexes, and origins [15,24]. Vigilance is therefore critical in such contexts, particularly where reproductive practices and international movements of dogs increase the likelihood of transmission [3,14,17,18]. Once introduced, *B. canis* may spread rapidly within canine populations, as demonstrated by an outbreak in a Dutch kennel following the introduction of a single infected dog [25]. This underscores the need for implementing early detection and containment strategies, particularly in high-density environments such as breeding kennels, shelters, or situations with high animal turnover.

Screening protocols should be strengthened and improved to ensure verification of *B. canis* status in dogs entering or moving within a region. Preparedness for implementing prophylactic measures in the event of a positive case is equally important. These strategies are integral to managing risks and safeguarding public and animal health [7,14].

This study serves as a model for regional surveillance of *B. canis*, emphasizing the importance of continuous monitoring to assess infection dynamics over time and promptly identify potential zoonotic threats. This is especially important in regions affected by previous outbreaks, international

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migration, including routes from conflict-affected areas, or the movement of reproductive animals. Proactive monitoring can help mitigate risks and establish a strong basis for infection control [3,14,17,26]. Although the absence of positive cases in Umbria suggests a low risk at present, the detection of a 1.95% prevalence in Veneto demonstrates that *B. canis* infections, while sporadic, can still occur [23]. Surveillance efforts remain crucial in low-prevalence regions, where undetected subclinical cases and sporadic introductions may pose a risk for pathogen establishment and spread. Regional differences in *B. canis* prevalence may be influenced by various factors, including variations in dog population density, stray dog management practices, and the extent of international dog movements. This is particularly important in Italy, where health service organization is managed at the regional level: the availability of region-specific data becomes essential to support evidence-based decision-making and to tailor veterinary public health strategies to local epidemiological contexts.

Given that canine infection is the sole source of human exposure to *B. canis*, the absence of positive cases in dogs indicates a negligible zoonotic risk in the study area. This underscores the critical role of veterinary surveillance as a frontline measure for preventing human infections and supports the integration of animal data into One Health risk assessment frameworks [6,26].

A significant strength of this study lies in its comprehensive diagnostic approach, which combined direct methods, such as culture and PCR, with indirect serological tests, including SAT, CFT, and IFAT. This multimodal strategy ensures reliable detection across various stages of infection, from active bacterial presence to antibody responses. By compensating for the limitations of individual tests, this approach provides a robust framework for detecting infections, even in challenging scenarios such as intermittent bacteremia or past exposure without active shedding [21,22].

This study adds valuable data to the limited body of knowledge on *B. canis* in Europe. The absence of positive cases aligns with findings from similar low-prevalence regions but contrasts with reports from high-risk areas, underscoring the heterogeneity of epidemiological patterns. The regional focus, though limited, provides critical insights for targeted surveillance and reinforces the need for standardized diagnostic and reporting protocols across Europe.

Globally, the rise in *B. canis* cases underscores the urgent need for international guidelines on infection control and management in pets. Countries like the United Kingdom, which are implementing risk-based import controls and diagnostic screening for *B. canis*, provide valuable examples of how to mitigate the risk of introduction and spread [17]. Expanding these measures globally, especially in or near endemic regions, is essential. Some countries adopt extreme measures such as culling infected animals to prevent the spread of *B. canis*, reflecting the seriousness of its zoonotic potential and its impact on public and animal health [2,14,16,27,28].

In addition to regulatory measures, education and awareness for the general public, particularly targeting high-risk groups, are important. Veterinary professionals, kennel operators, and breeders must be equipped with the knowledge and tools to effectively manage infection risks. Public awareness campaigns targeting dog owners and adopters can also play a crucial role in reducing the likelihood of introducing infected animals [14,26,29].

5. Conclusions

While no evidence of *B. canis* circulation was found in the surveyed canine population, the risk of sporadic introduction persists due to ongoing international movements and regional variability. Until robust international guidelines are established, *B. canis* will remain an underestimated threat to animal and public health. These findings underscore the importance of proactive, regionally tailored surveillance and comprehensive diagnostic protocols as key tools for early detection and control. Integrating veterinary data into One Health frameworks is essential for anticipating zoonotic threats and informing public health preparedness.

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Institutional Review Board Statement: Ethical review and approval were waived for this study because all blood samples were obtained as part of routine clinical, diagnostic, or health monitoring procedures, with no additional interventions performed for research purposes. No experimental treatment or manipulation was applied, and the study was conducted using residual samples collected within standard veterinary care, in accordance with national and international regulations on the ethical use of animals in research.

Informed Consent Statement: Informed consent was obtained from all dog owners at the time of sample collection for clinical or surveillance purposes, except for blood donor dogs whose samples were retrospectively analyzed in anonymized form, in accordance with institutional policies and the principles of the EU General Data Protection Regulation (Regulation (EU) 2016/679). No human participants were involved in the study, and no identifying personal data were collected or processed.

Data Availability Statement: No datasets were generated or analyzed beyond those presented in the current study. All relevant data are included within the article.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

SAT	Serum Agglutination Test
CFT	Complement Fixation Test
IFAT	Indirect Immunofluorescence Antibody Test
PCR	Polymerase Chain Reaction

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