

## Molecular detection of *Brucella* species in rodents from Tehran livestock farms: Implications for zoonotic transmission and control strategies

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### Abstract

Brucellosis is an important zoonosis affecting public health and livestock, mainly caused by the genus *Brucella*. This study aimed to isolate and identify *Brucella* species from rodents associated with livestock farms in Tehran using polymerase chain reaction (PCR). A total of 31 rodent samples were collected over three months, 17 from cattle farms and 14 from sheep farms. Genomic DNA was extracted from spleen and liver tissues using the MBST nucleic acid extraction kit, followed by AMOS multiplex PCR targeting the bcs31 gene (223 bp for the *Brucella* genus) and species-specific primers (731 bp for *B. melitensis* and 498 bp for *B. abortus*). PCR analysis showed a positivity rate of 9.7% for *Brucella*, with all positive samples identified as *B. melitensis*. The positive samples were mainly from the cities of Islamshahr, Robat Karim and Shahriar. Statistical analysis revealed no significant differences in *Brucella* prevalence between the different housing systems or locations. However, all positive samples were from mature rodents, indicating a significant difference in age distribution ( $p < 0.05$ ). The results highlight the role of rodents as potential reservoirs for brucellosis and emphasize the need for accurate identification of *Brucella* species in epidemiological studies. This research helps to emphasize the importance of implementing effective control measures to mitigate the risks associated with this zoonosis.

### Introduction

Brucellosis, an important zoonotic illness, is triggered by *Brucella* species bacteria and affects both public health and livestock (1). It primarily affects cattle, sheep, goats and humans and leads to severe financial setbacks in animal farming, resulting from abortions, decreased dairy yield, and reproductive failures. In humans, infection usually occurs through exposure to diseased livestock or ingesting raw milk products, leading to clinical signs including persistent fever and long-term bacterial infections (2, 3). The dual effects of brucellosis on both veterinary and public health underscore its importance as a critical public health and veterinary issue (4).

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Recent studies have demonstrated that rodents may serve as potential reservoirs for *Brucella* species, contributing to the zoonotic transmission cycle. Dadar and Alamian (2024) reported 68.75% *Brucella* prevalence in rodents from Iranian farms (5). Rezaie et al. (2025) identified *Brucella abortus* in wild rodents (*Meriones persicus* and *Microtus qazvinensis*) in Iran, with all positive cases (0.48% prevalence) localized in Hamedan Province (6). This finding highlights the need to consider rodents in the epidemiology of brucellosis, particularly in endemic regions. The rodents inhabit agricultural environments and can spread the bacteria through contact with infected animals or contaminated surfaces. Certain *Brucella* species, such as *B. neotomae* and *B. microti*, have been found in rodents, which can also transmit the infection vertically to their offspring. This demonstrates the importance of understanding rodent populations in the control of brucellosis, as they can serve as silent carriers and vectors for future outbreaks (7, 8). This highlights the potential role of rodents as silent carriers and vectors in the epidemiology of brucellosis, emphasizing the need for further research into their contribution to disease dynamics.

While previous work focused on livestock surveillance, our molecular approach targeting rodents provides unique insights into wildlife-mediated transmission pathways in Tehran's ecosystem - a dimension overlooked in current control programs. Accurate identification of *Brucella* species is essential for implementing effective control and prevention strategies. Traditional diagnostic methods, such as bacterial culture, are often time-consuming and require specialized laboratory conditions that may not be feasible in resource-limited settings (9). Innovations in biomolecular tools, notably the polymerase chain reaction technique (PCR), have revolutionized the detection of *Brucella* DNA, offering a rapid, sensitive, and reliable alternative to conventional methods (10). These advancements have significantly improved the ability to diagnose and monitor brucellosis, particularly in regions where the disease is endemic.

This study addresses three critical unmet needs: (1) molecular characterization of rodent-borne *Brucella* strains in Tehran, (2) assessment of age-related susceptibility patterns, and (3) evaluation of farm-type-specific risks—all essential for targeted One Health interventions. Given the potential role of rodents in brucellosis transmission and the need for improved diagnostic tools, this study aims to detect and characterize *Brucella* spp. in rodent populations near livestock farms in Tehran using PCR. By focusing on rodents as potential reservoirs, this research provides valuable insights into brucellosis epidemiology and supports the development of more effective control strategies. The findings will underscore the importance of integrating rodent surveillance into brucellosis control programs and highlight the role of molecular diagnostics in managing this zoonotic disease.

## Materials and Methods

### Sample collection

A total of 31 rodents were captured over a three-month period from July to September 2023, coinciding with the season known for a higher prevalence of *Brucella*. Among these rodents, 17 were collected from farms dedicated to cattle farming, while 14 were from farms involved in sheep farming. The rodents were collected from 20 livestock farms situated in various counties of Tehran Province, including Islamshahr (n=15, 48.4%), Robat Karim (n=3, 9.7%), Shahriar (n=8, 25.8%), and Varamin (n=5, 16.1%), all of which had previously reported cases of *Brucella* infections in their livestock.

Rodent trapping was performed using standard mouse traps, ensuring strict adherence to biosafety protocols, including the use of protective gloves. Following humane euthanasia, the carcasses of the rodents were frozen at -19°C and conveyed to the testing facility with an unbroken cold chain to maintain sterility. Upon arrival at the laboratory, samples were aseptically collected from the lymphoid organs, specifically the liver, spleen, and kidneys, and were immediately processed for molecular analysis on the same day.

### DNA extraction

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Genomic DNA was isolated from tissue specimens employing the MBST nucleic acid extraction kit (Molecular Biological System Transfer, Iran) following the standard protocol. Briefly, 20-50 mg tissue fragments were homogenized in 180  $\mu$ L lysis buffer containing 20  $\mu$ L Proteinase K (20 mg/mL) and digested at 56°C for 30 minutes. Following complete lysis, 360  $\mu$ L binding buffer was added, and the mixture was incubated at 70°C for 10 minutes. Ethanol precipitation was then performed by adding 270  $\mu$ L of absolute ethanol and thoroughly mixing. The lysate was loaded onto silica-membrane columns and centrifuged at 8000  $\times$  g for 1 minute. After discarding the flow-through, the columns were washed twice with 500  $\mu$ L wash buffer (8000  $\times$  g, 1 minute each), followed by a final centrifugation at 12000  $\times$  g for 2 minutes to ensure complete ethanol removal. Purified DNA was eluted in 100-200  $\mu$ L pre-heated (70°C) elution buffer after 3 minutes of incubation at room temperature, with a final centrifugation at 8000  $\times$  g for 1 minute. DNA concentration and purity were verified spectrophotometrically before storage at -20°C for downstream molecular analysis.

#### Polymerase Chain Reaction (PCR)

The AMOS multiplex PCR assay, originally developed by the National Animal Disease Center for *Brucella* species differentiation, was employed to simultaneously detect and discriminate between *B. abortus*, *B. melitensis*, *B. ovis*, and *B. suis*. This method confirms the presence of *Brucella* and distinguishes between species. However, the primer for *B. suis* was not used in this study due to the prohibition of pig farming in our country.

The PCR process involved two stages: first, primers B4 and B5 were used to detect *Brucella*, followed by the AMOS method to identify species. Specific primers included RIS711 and FaborTus for *B. abortus*, and RIS711 and FmeliTensis for *B. melitensis*. The primer sequences and product sizes are summarized in Table 1 (11).

**Table 1.** Primers for *Brucella* AMOS-PCR with amplicon sizes (11)

Primer's name	Oligonucleotide sequences (5' to 3')	PCR product size (bp)
<b>B4</b>	TGGCTCGGTTGCCAATATCAA	223
<b>B5</b>	CGCGCTTGCCTTTCAAGGTCTG	
<b>RIS711</b>	TGCCGATCACTTAAGGGCCTTCAT	
<b>FaborTus</b>	AAATCGCGTCCTTGCTGGTCTGA	731
<b>FmeliTensis</b>	GACGAACGGAATTTTCCAATCCC	498

Following DNA extraction, the multiplex PCR reaction was conducted in a 25  $\mu$ L reaction mixture containing: 12.5  $\mu$ L of commercial PCR Master Mix (Amplicon, Denmark), 5.5  $\mu$ L nuclease-free water, 1  $\mu$ L each of forward and reverse primers (10  $\mu$ M), and 5  $\mu$ L template DNA. Thermal cycling conditions comprised: initial DNA denaturation (95°C, 3 min); 35 amplification cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 1 minute. Distilled water was used as the negative control, while *B. melitensis* (Strain 16M) and *B. abortus* (Strain 544) obtained from the microbial culture collection of the Faculty of Veterinary Medicine, University of Tehran, served as positive controls. Representative PCR amplicons were electrophoresed in 1% agarose gel prepared with Safe DNA stain (0.5  $\mu$ g/mL) (Yektatajhiz, Iran) using 0.5 $\times$  TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.3) at 80 V for 80 minutes, and visualized under UV transillumination (BTS-20 system, Japan).

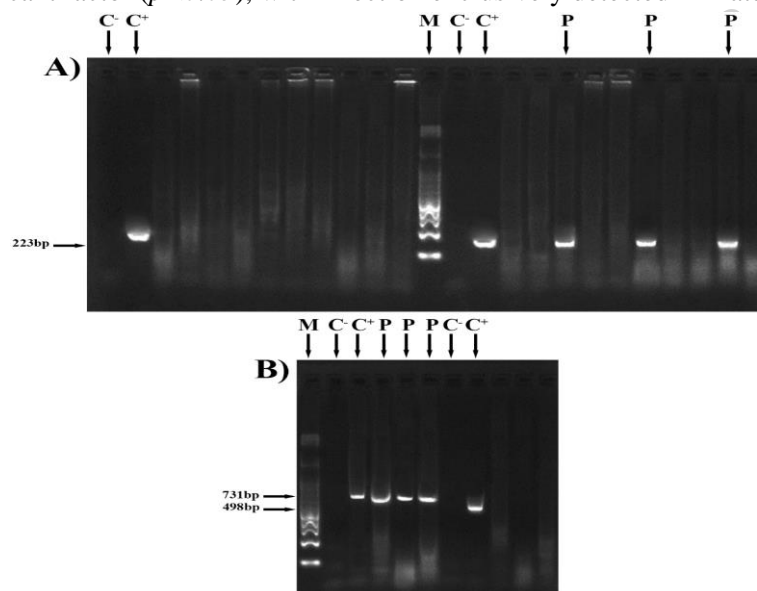
#### Statistical analysis

Data analysis was conducted using SPSS version 21 software. The Kruskal-Wallis test was utilized to evaluate significant differences in the prevalence of *Brucella* species across various livestock farms. Inter-group comparisons were performed using the non-parametric Mann-Whitney U test with Bonferroni adjustment to

identify specific farms that exhibited statistically significant differences in *Brucella* prevalence. Statistical significance was established at  $p < 0.05$ , reflecting significant intergroup variations.

## Results

PCR analysis of 31 rodent samples from Tehran Province livestock farms revealed a 9.7% (3/31) *Brucella* prevalence, with all positive cases identified as *B. melitensis*. Geographic distribution showed one positive case each in Islamshahr, Robat Karim, and Shahriar (Figure 1). Prevalence differed between farm types: 5.9% (1/17) in cattle farms versus 14.3% (2/14) in sheep farms, though this difference was not statistically significant ( $p > 0.05$ , Chi-square test). Molecular characterization confirmed all positives as *B. melitensis* (731 bp amplicon), with no detection of *B. abortus* (498 bp) or other species (Figure 1). The 223 bp universal *Brucella* marker validated all positives. Samples were collected from various farming systems and locations, as summarized in Table 2. The captured rodents were classified by species, gender, and age, as shown in Table 3. While no significant associations were found with farming systems, locations, or host demographics (all  $p > 0.05$ ), age emerged as a significant factor ( $p < 0.05$ ), with infection exclusively detected in mature rodents (Table 3).



**Fig. 1.** *Brucella* detection by 1% agarose gel electrophoresis. (A) Genus-specific band (223 bp). (B) Species-specific bands: *B. melitensis* (731 bp) and *B. abortus* (498 bp). M: DNA ladder; C-: negative control; C+: positive control; P: positive samples.

## Discussion

Rodents serve as *Brucella* reservoirs, transmitting infection to livestock and humans (7). Detecting *Brucella* in rodents is crucial for disease control. The present investigation was designed to detect and characterize *Brucella* spp. circulating among rodent populations in Tehran's farms. Our findings underscore the prevalence of *Brucella* in rodents coming into contact with livestock in Tehran province, Iran, indicating a potential risk for transmission within this environment. Our analyses revealed that *Brucella* was isolated from 9.7% of the rodent samples collected, all of which were identified as belonging to the *B. melitensis* species. This result is consistent with previous reports from different regions of the world highlighting rodents as important vectors for *Brucella*. Geographic variability in rodent-associated *Brucella* is evident, as demonstrated by Rezaie et al. (2025), who reported *B. abortus* exclusively in *Meriones persicus* and *Microtus qazvinensis* (0.48% prevalence) in Hamedan Province (6), contrasting with our detection of *B. melitensis* in Tehran's peri-domestic rodents. While Hammerl

et al. (2017) reported 14.2% *Brucella* prevalence in German rodents (12), our lower rate (9.7%) may reflect differences in reservoir species or livestock contact patterns. Notably, all our positives were *B. melitensis*, contrasting with *B. microti* in Russian rodents (13) and *B. abortus* in Iranian wild rodents (6), underscoring regional strain diversity. The study conducted by Dadar and Alamian (2024) found that 68.75% of rodents tested from seropositive livestock farms in Iran carried *Brucella* spp. indicating a significant presence of these bacteria in rodent populations (5). In addition, the identification of *B. abortus* biovar 3 and *B. melitensis* biovar 1 in these rodents emphasizes their potential role as reservoirs for the transmission of brucellosis.

**Table 2.** *Brucella* prevalence in rodents from cattle and sheep farms

Parameter	Count (%)	Positive samples (%)	<i>p</i> -value
Total samples	31 (100)	3 (9.7)	
Cattle	17 (54.8)	1 (5.9)	0.620
Sheep	14 (45.2)	2 (14.3)	0.431

**Table 3.** *Brucella* infection rates by rodent species, sex and age group

	Parameters					
	Species		Gender		Age	
	<i>Rattus norvegicus</i>	<i>Rattus rattus</i>	Male	Female	Mature	Immature
Count (%)	24 (77.4)	7 (22.6)	18 (58.1)	13 (41.9)	11 (35.5)	20 (64.5)
<i>p</i> -value	0.969		0.751		<0.05	

It is noteworthy that all positive samples in our study came from mature rodents, suggesting that age plays a crucial role in susceptibility to infection. Mature rodents may be more frequently exposed to contaminated environments or infected livestock, which can lead to a higher infection rate.

The lack of significant differences in *Brucella* prevalence between the different livestock systems and locations suggests that environmental factors, such as common farming practices or similar exposure to infected animals, contribute to a uniform risk of transmission within the regions studied. This uniformity means that preventive measures should be comprehensively applied in the different farming systems to mitigate the risk of brucellosis effectively. In support of this notion, Al Dahouk *et al.* (2005) investigated the sporadic occurrence of *B. suis* biovar 2 in domestic pigs across Germany, emphasizing the ongoing potential for zoonotic transmission from wildlife (14). The findings of Godfroid *et al.* (2013) also reinforce the concept that wildlife acts as a key contributor to the spread of *Brucella* species and emphasize the urgent need for thorough surveillance of both wildlife and domestic animal populations (15).

There are several methods for identifying *Brucella*, each with its own advantages. Recent studies emphasize the importance of diagnostic tools and control strategies in the control of brucellosis in agriculture. Among these methods, the AMOS-PCR technique (11) allows rapid identification of *Brucella* species, while Bruce-Ladder PCR (16) efficiently detects multiple strains in a single reaction. In addition, Vemulapalli *et al.* (1999) developed a PCR assay specifically designed to distinguish the RB51 strain of *B. abortus* from other strains, which improves detection capabilities (17). In our study, we utilized polymerase chain reaction (PCR) using the AMOS multiplex technique as a diagnostic tool, which was found to be effective and efficient. The AMOS multiplex PCR technique facilitated the differentiation between species and provided valuable information about the specific strains present in the rodent population.

In total, this study highlights the crucial role of rodents in the epidemiology of brucellosis and emphasizes the importance of accurate species identification using advanced molecular techniques such as PCR. Based on the findings of this study, implementing integrated rodent control programs in Tehran's livestock farms appears essential. Control measures should primarily target mature rodents around stables and parturition areas, as this demographic poses the highest transmission risk. Improving feed storage hygiene through the use of orodent-proof netting and sealed containers can reduce environmental attractants. Furthermore, periodic molecular surveillance of rodent populations in high-prevalence areas and during peak risk seasons is strongly advised. These combined strategies could effectively diminish the disease reservoir within rodent populations while supporting brucellosis prevention efforts.

### Conclusion

This study demonstrates the efficacy of AMOS-PCR in detecting *B. melitensis* in Tehran's rodents (9.7% prevalence), with higher rates in sheep versus cattle farms (14.3% vs. 5.9%). The exclusive infection of mature rodents ( $p < 0.05$ ) suggests age-related susceptibility. However, three limitations must be acknowledged: (1) sample size constraints ( $n=31$ ) may have limited statistical power to detect farm-type differences, (2) single-season sampling could miss temporal prevalence variations, and (3) absence of bacterial isolation prevents strain characterization beyond PCR amplicon size. These findings nonetheless underscore the need to integrate rodent surveillance into brucellosis control programs, particularly targeting mature populations near sheep farms.

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### Conflict of Interest

None declared.

### Ethical approval

The study was conducted in accordance with ethical guidelines for research involving animals and was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Islamic Azad College of Tabriz, Iran (Ethical Approval Code: IR.IAU.TABRIZ.REC.1403.098).

### Artificial Intelligence Statement

The authors declare that no artificial intelligence tools were used in the preparation of this manuscript.

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