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ORIGINAL ARTICLE

# Brucellosis in a Camel Herd: The Role of Missed Vaccination and Illegal Imports

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## Abstract

This study aimed to detect the presence of *Brucella* spp. DNA in aborted camel fetuses from a dairy herd in Meybod, Yazd Province, Iran, using genus-specific PCR. The broader objective was to assess the epidemiological importance of camel brucellosis in a region with limited surveillance and no targeted vaccination programs for camels. A total of 50 aborted fetuses from a single Camelus dromedarius herd were examined. Fetal abomasal contents were collected and tested using Modified Ziehl-Neelsen staining, followed by molecular confirmation via polymerase chain reaction (PCR) targeting the IS711 gene of *Brucella* spp. DNA was extracted using a commercial tissue DNA kit, and PCR conditions were optimized for specificity. Positive and negative controls were included in each PCR run to ensure reliability and prevent contamination. Modified Ziehl-Neelsen staining revealed *Brucella*-like organisms in 82% (41/50) of the samples. PCR confirmed the presence of *Brucella* DNA in 92% (46/50) of the fetal tissues, indicating a high prevalence of infection in the herd. While 72% of the samples tested positive in both Modified Ziehl-Neelsen staining and PCR assays, 2% were negative by Modified Ziehl-Neelsen staining but positive by PCR. This study reveals an unusually high rate of *Brucella* spp. in aborted fetuses from a single camel herd, suggesting an active outbreak likely exacerbated by illegal livestock movement and lack of camel surveillance. Molecular detection confirms infection, although species-level identification was not performed. These findings highlight the urgent need for targeted camel vaccination, enhanced molecular surveillance, and stricter control over cross-border animal trade.

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## Introduction

Brucellosis remains one of the most significant zoonotic diseases affecting both livestock productivity and public health in many developing regions, including the Middle East and North Africa. In camels (*Camelus dromedarius*), *Brucella* infection leads to abortion, infertility, and reduced milk yield, posing a substantial risk of transmission to humans, particularly through raw milk consumption or direct contact with infected tissues (1,2). While *Brucella melitensis* and *Brucella abortus* are traditionally associated with small ruminants and cattle, respectively, they are also recognized as the primary etiological agents of brucellosis in camels (3).

Infected camels often show few clinical signs until reproductive failures become apparent. The disease in animals is characterized by late-term abortions, retained placentas, stillbirths, and decreased reproductive performance. Bulls may exhibit orchitis or epididymitis. Notably, infected animals can shed the bacteria in vaginal discharges, aborted materials, urine, and particularly milk, posing a continuous source of environmental contamination and risk to other animals and humans (4). In humans, brucellosis typically presents as a febrile illness with non-specific symptoms such as undulating fever, malaise, night sweats, joint and muscle pain, and fatigue. If not diagnosed and treated appropriately, it may progress to a chronic condition with complications involving the bones, joints, cardiovascular system, or central nervous system. Human infection occurs through several routes: ingestion of unpasteurized milk or dairy products; direct contact with infected animal secretions or tissues, especially during birthing or slaughter; inhalation of aerosols in laboratory or farming environments; and accidental inoculation in veterinary or laboratory settings (5, 6). *B. melitensis* is considered the most pathogenic *Brucella* species for humans and is commonly transmitted via raw milk from infected animals (7).

Despite the economic and epidemiological importance of camel brucellosis, control programs in several endemic countries remain underdeveloped, and diagnostic efforts are often limited to serological methods with suboptimal sensitivity and specificity in camels (4, 8). In Iran, where camels are primarily reared in the arid central and southeastern regions, including Yazd Province, the disease remains underdiagnosed and underreported. Notably, camels are not included in Iran's national *Brucella* vaccination program, which focuses on small ruminants and cattle. This omission has contributed to the persistence of infection in mixed and camel-only herds (6, 9).

Moreover, the unregulated and sometimes illegal trans boundary movement of camels, particularly across Iran's eastern borders with Afghanistan and Pakistan, has been implicated in the introduction and dissemination of various infectious agents, including *Brucella* spp. (10). The movement of untested and quarantined animals undermines local control strategies and facilitates the spread of genetically diverse *Brucella* strains into previously unaffected herds.

Although previous studies in Iran have primarily focused on *Brucella* detection in cattle, sheep, and goats, there is a lack of molecular evidence concerning active infection in camels, especially in cases of abortion. This study aimed to detect the presence of *Brucella* spp. DNA in aborted camel fetuses from a herd in Meybod, Yazd Province, Iran, using genus-specific PCR, and to provide insight into the epidemiological risks associated with camel brucellosis in this region.

## Materials and Methods

### Sample Collection

During November 2023, a total of fifty aborted camel fetuses were submitted for diagnostic investigation from a single dromedary camel (*Camelus dromedarius*) herd located in Meybod, Yazd Province, Iran (Figure 1). The herd comprised approximately 500 camels and was managed as an industrial farming operation primarily focused on the production of milk and dairy products. Abortions were reported during the late gestation period, and there was no recent history of vaccination against *Brucella* spp. in the herd. Fetal abomasal contents were aseptically collected and transported under cold chain conditions to the microbiology laboratory at the Faculty of Veterinary Medicine, Ardakan University, Ardakan, Iran for molecular analysis.

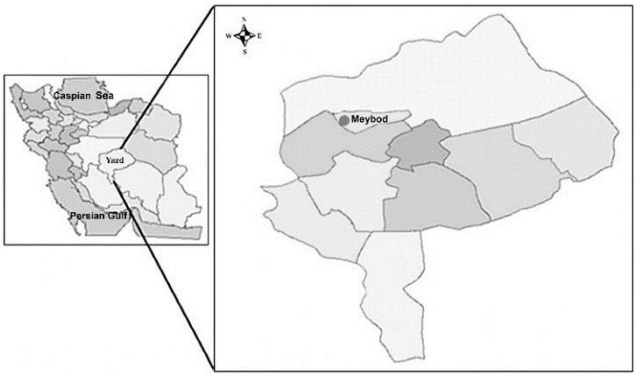


**Figure 1:** Meybod, Yazd province, Iran

## Preliminary Detection via Modified Ziehl-Neelsen Staining

As a preliminary diagnostic step, all tissue samples obtained from aborted camel fetuses were subjected to Modified Ziehl-Neelsen (MZN) staining for the presumptive detection of *Brucella* spp. This technique, a modification of the classical Ziehl-Neelsen method, is specifically designed to visualize weakly acid-fast bacteria such as *Brucella* species.

Briefly, smears were prepared from tissue impressions, air-dried, and heat-fixed on clean glass slides. The slides were then flooded with hot carbol fuchsin stain for 5 minutes and gently heated to steaming without boiling. After rinsing with tap water, slides were decolorized using 1% acetic acid for 10–15 seconds, instead of the stronger acid-alcohol used in standard protocols. This milder decolorization allows retention of the primary stain by weakly acid-fast organisms (11). Subsequently, slides were counterstained with methylene blue for 30 seconds, rinsed again, and air-dried.

Microscopic examination was performed under oil immersion at 1000× magnification. *Brucella* organisms appeared as small, red-stained coccobacilli against a blue background (12, 13). This method provided rapid initial screening for the presence of *Brucella* spp. in the collected samples. PCR analysis was performed subsequently to confirm the presence of *Brucella* DNA and enhance diagnostic specificity (14).

## DNA Extraction

Approximately 25 mg of each tissue sample was homogenized and used for DNA extraction using the Blood & Tissue Extraction DNA Kit (Parstous, Iran), following the manufacturer's instructions. The concentration and purity of the extracted DNA were measured using a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

## PCR Detection of *Brucella* spp.

A genus-specific polymerase chain reaction (PCR) targeting the IS711 gene of *Brucella* spp. The specific primers (Pishgam Biotechnology Co., Iran) used for amplification were as follows: forward primer (IS 711-F): 5′-GAGAATAAAGCCAACACCCG-3′ and reverse primer (IS711-R): 5′-GATGGACGAAACCCACGAAT-3′ (15). Each PCR run included a positive control (DNA from a *B. abortus* strain), a negative control (PCR-grade water), and appropriate blank extractions. All procedures were conducted in designated pre-PCR and post-PCR areas using filter tips and dedicated pipettes to minimize the risk of contamination.

The expected amplicon size was 317 base pairs. Each 25 µL PCR reaction contained 12.5 µL of 2× PCR Master Mix (Amplicon, Iran), 1.5 µL of each primer (10 µM), 5 µL of DNA template, and 6 µL of nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 minutes; followed by 35 cycles of: denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds; and a final extension at 72°C for 5 minutes. Amplified products were visualized by electrophoresis on a 1.2% agarose gel stained with ethidium bromide and examined under UV illumination.

## Results

Out of the 50 aborted camel fetuses examined from the industrial dairy camel herd in Meybod, Yazd Province, Iran, initial screening using Modified Ziehl-Neelsen staining revealed that 41 samples (82%; 95% CI: 71.4%–92.6%) were positive for *Brucella* spp., indicated by the presence of characteristic red-stained coccobacilli against a blue background.

Subsequent molecular confirmation using PCR targeting the IS711 gene of *Brucella* spp. yielded positive amplification in 46 of the 50 samples (92%; 95% CI: 84.5%–99.5%), producing the expected 317 bp amplicon. Gel electrophoresis of the PCR products confirmed the presence of the specific band in positive samples when visualized under UV light. The positive control consistently exhibited the expected band, while no amplification was observed in the negative and blank controls, thereby verifying the accuracy and reliability of the PCR results (Figure 2).

In the analysis of 50 aborted camel fetuses, MZN staining and PCR targeting the IS711 gene of *Brucella* spp. exhibited strong agreement, with PCR showing enhanced sensitivity in select samples. A comprehensive summary of the diagnostic comparison is provided in Table 1.

Based on the data distribution and standard statistical analyses, there is a statistically significant association between the two methods, indicating that PCR and MZN results are not independent and tend to concur, with PCR demonstrating slightly higher sensitivity.

## Discussion

In Iran, brucellosis is endemic in many provinces, including Yazd, with *B. melitensis* being the predominant species affecting both small ruminants and humans (16, 17).

**Table 1.** Comparison of MZN staining and PCR for detecting *Brucella* spp. in aborted camel fetuses

| Category | Count | Percentage (%) | 95% CI (%) |
|---|---|---|---|
| **PCR-negative / MZN-negative** | 7 | 14.0 | 6.95-26.19 |
| **PCR-negative / MZN-positive** | 5 | 10.0 | 4.35-21.36 |
| **PCR-positive / MZN-negative** | 2 | 4.0 | 1.10-13.46 |
| **PCR-positive / MZN-positive** | 36 | 72.0 | 58.33-82.53 |

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Although national vaccination campaigns using the *B. melitensis* Rev.1 strain in sheep and goats and *B. abortus* S19 in cattle have been implemented for decades, camels have not been included in these programs (18). This exclusion has contributed to the persistence of infection in camel herds, particularly in desert and semi-desert areas where camels hold significant economic and cultural importance. Several studies have suggested that camels are frequently exposed to *Brucella* spp. in endemic areas, and that their infections are often underdiagnosed due to low clinical visibility and limited molecular diagnostic tools (19, 20).

Our findings are consistent with previous reports from Iran. For example, Golshani et al. (2018) used real-time PCR to detect *Brucella* DNA in aborted ruminant tissues and highlighted the need for molecular confirmation in clinically ambiguous cases (21). In southeastern Iran, Moghaddas et al. (2015) detected *Brucella* spp. in camel serum using ELISA and suggested cross-species transmission in mixed herding systems (22). Internationally, similar molecular detection studies have confirmed the role of camels in brucellosis transmission. In Saudi Arabia, Al-Khalaf et al. (2020) used PCR to detect *B. melitensis* in dromedary camels, reporting a high seroprevalence in border areas (23). Likewise, in Sudan, Musa et al. (2008) reported abortion storms in camel herds due to *B. abortus* and emphasized the lack of targeted vaccination in camel populations (24). Variations in diagnostic techniques, sample sizes, animal origins, husbandry practices, and environmental conditions may account for discrepancies between the findings of this study and other research.

In epidemiological terms, an outbreak refers to the occurrence of disease cases in excess of what would normally be expected in a defined community, geographical area, or season (Centers for Disease Control and Prevention (25). Based on our findings, the detection of *Brucella* spp. in 82% of MZN-stained samples and 92% of PCR-confirmed samples from aborted camel fetuses in a single industrial dairy herd strongly suggests an active outbreak of brucellosis within that specific herd. This unusually high infection rate, far exceeding baseline prevalence rates reported in other studies, indicates a sudden cluster of infections leading to widespread reproductive loss.

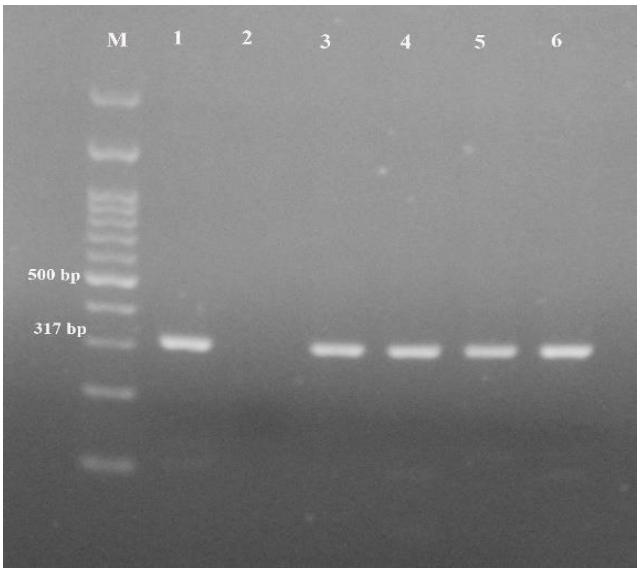


**Figure 2:** garose gel electrophoresis of PCR products targeting *Brucella* spp. DNA. Lanes 3–6 show positive amplification with expected band size (~317 bp). Lane M: DNA ladder (100 bp). Lane 2: negative control (no template DNA). Lane 1: positive control (DNA from a *Brucella abortus* strain).

While the term "outbreak" typically implies temporal clustering, the concentration of positive cases within a short surveillance period in one herd aligns with outbreak characteristics (26). Therefore, this investigation does not merely reflect sporadic endemic transmission but rather points to an acute and possibly ongoing outbreak event, likely exacerbated by the absence of vaccination in camels, undocumented animal movement, and weak surveillance in the region.

This study had several limitations. First, although PCR provided high sensitivity and specificity for detecting Brucella spp., species-level identification (e.g., *B. melitensis vs. B. abortus*) was not performed, limiting insights into the specific epidemiological risks. Second, bacterial isolation and culture, considered the gold standard, were not conducted due to biosafety and resource constraints. Additionally, the study was restricted to a single industrial herd, which may not reflect infection dynamics across different camel populations or management systems. Despite these limitations, the findings offer important preliminary evidence of a possible outbreak and highlight

the urgent need for enhanced molecular surveillance and vaccination strategies in camels.

Despite ongoing control efforts, several factors continue to hinder the eradication of brucellosis in Iran and similar regions. These include incomplete vaccination coverage, lack of regular testing in camels and other less-monitored species, poor movement control, and the illegal importation of livestock (18, 27). In particular, camels are frequently imported—both legally and illegally—from neighboring countries such as Pakistan and Afghanistan, where brucellosis control programs are either weak or nonexistent (28). For instance, studies in Pakistan have reported seroprevalence in camels ranging from 3.4% to 18%, with molecular detection of *B. abortus* in multiple herds (29, 30, 31). These imported animals often bypass formal quarantine procedures, thereby introducing new *Brucella* strains into native herds and undermining local biosecurity.

Iran is home to several endemic camel populations, most notably the "Arvana" (or Iranian dromedary), which are well-adapted to the central desert climate and primarily raised for meat and transport (32). In contrast, camels imported from Pakistan (such as the Sindhi and Makrani breeds) are introduced for their higher meat yield or as part of traditional livestock trade networks. However, these animals often originate from high-risk areas with endemic brucellosis and may be asymptomatic carriers. The lack of genetic compatibility and differing immune responses between native and imported breeds may further complicate disease dynamics.

## Conclusion

This study confirms that camels in Iran can suffer reproductive losses due to brucellosis even in the absence of obvious clinical symptoms, posing significant risks to both animal and human health given the close human–camel interactions and traditional consumption practices. Molecular diagnostic methods like PCR are invaluable for rapid and sensitive detection, particularly when serological testing is insufficient. However, PCR alone does not allow for species or strain-level differentiation. Future studies should incorporate sequencing-based approaches, such as multilocus sequence typing (MLST) or whole genome sequencing (WGS), to identify *Brucella* species and track transmission patterns more precisely. These methods can improve our understanding of epidemiological links between local cases and imported infections, and support more targeted control strategies (33, 34). Therefore, it is imperative to include camels in Iran's national brucellosis control programs through targeted vaccination, movement restrictions, molecular surveillance, and stricter regulation of cross-border camel trade. Failure to implement these measures will likely hinder eradication efforts and continue to threaten livestock productivity and public health.

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## Author Contributions

**Zeinab Abiri:** Conceptualization, investigation, supervision, writing, review & editing. **Mohammad Sadegh Safaee Firouzabadi:** Formal analysis, methodology.

## Data Availability

All data generated or analyzed during this study are included in this published manuscript.

## Ethical Approval

This article does not contain any studies with human participants or animals.

## Conflict of Interest

The authors declare no conflict of interest.

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