

Review Article

Current Laboratory Diagnostic Approaches to Brucellosis: An Evaluation of Conventional and Molecular Methods

Brusellozda Güncel Laboratuvar Tanı Yaklaşımları: Konvansiyonel ve Moleküler Yöntemlerin Değerlendirilmesi

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Abstract: Brucellosis is a zoonotic infectious disease caused by Gram-negative coccobacilli of the genus *brucella*, which is endemic worldwide, particularly in the Mediterranean basin, the Middle East, Central Asia, Africa, and Latin America. Because its clinical findings are nonspecific, definitive diagnosis relies largely on laboratory confirmation. This review covers conventional methods used in the laboratory diagnosis of brucellosis (blood/bone marrow culture, Rose Bengal test, standard tube agglutination test, Coombs test, complement fixation test, ELISA) as well as molecular diagnostic methods (conventional and multiplex PCR, real-time PCR, isothermal amplification techniques, high-resolution melting analysis, CRISPR-Cas-based tests, digital PCR, next-generation sequencing, and MALDI-TOF mass spectrometry) are discussed comparatively in light of the current literature. While conventional methods have limitations such as low sensitivity, long turnaround time, and cross-reactivity, molecular methods offer higher sensitivity and specificity, shorter turnaround time, and lower biosafety risk. Consequently, it is recommended that clinical findings, conventional serological tests, and molecular methods be used together within a stepwise diagnostic algorithm to ensure a reliable diagnosis of brucellosis.

Keywords: Brucellosis, *Brucella*, Laboratory Diagnosis, Serological Tests, Polymerase Chain Reaction, Molecular Diagnosis

Öz: Bruselloz, dünya genelinde özellikle Akdeniz havzası, Orta Doğu, Orta Asya, Afrika ve Latin Amerika'da endemik seyreden, *Brucella* cinsi Gram-negatif kokobasiller tarafından oluşturulan zoonotik bir enfeksiyon hastalığıdır. Klinik bulgularının özgül olmaması nedeniyle kesin tanı büyük ölçüde laboratuvar doğrulamasına dayanmaktadır. Bu derlemede brusellozun laboratuvar tanısında kullanılan konvansiyonel yöntemler (kan/kemik iliği kültürü, Rose Bengal testi, standart tüp aglütinasyon testi, Coombs testi, kompleman fiksasyon testi, ELISA) ile moleküler tanı yöntemleri (konvansiyonel ve multiplex PCR, gerçek zamanlı PCR, izotermal amplifikasyon teknikleri, yüksek çözünürlüklü erime analizi, CRISPR-Cas tabanlı testler, dijital PCR, yeni nesil dizileme ve MALDI-TOF kütle spektrometrisi) güncel literatür ışığında karşılaştırmalı olarak ele alınmıştır. Konvansiyonel yöntemlerin düşük duyarlılık, uzun sonuç süresi ve çapraz reaksiyon gibi sınırlılıkları bulunurken, moleküler yöntemler daha yüksek duyarlılık ve özgüllük, kısa sonuç süresi ve düşük biyogüvenlik riski sunmaktadır. Sonuç olarak, brusellozun güvenilir tanısı için klinik bulgular, konvansiyonel serolojik testler ve moleküler yöntemlerin birlikte, kademeli bir tanı algoritması içinde kullanılması önerilmektedir.

Anahtar Kelimeler: Bruselloz, *Brucella*, Laboratuvar Tanısı, Serolojik Testler, Polimeraz Zincir Reaksiyonu, Moleküler Tanı

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INTRODUCTION

Brucellosis is one of the world's most common zoonoses, affecting both humans and animals, and is caused by facultative intracellular Gram-negative coccobacilli belonging to the genus *Brucella* (1). The primary human pathogenic species are *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*, with *B. melitensis* being the species responsible for the highest number of human cases worldwide (2). The disease can be transmitted through the consumption of raw meat, milk, and dairy products from infected animals, direct contact, or via aerosols; it has been reported that the global incidence is currently on the rise again (3).

The clinical course of brucellosis is characterized by nonspecific symptoms such as fever, fatigue, night sweats, arthralgia, and myalgia; therefore, it can be confused with other febrile illnesses such as typhoid fever, tuberculosis, malaria, and tularemia (4). The lack of specificity in the clinical presentation necessitates that a definitive diagnosis relies almost entirely on laboratory confirmation (5). Advances in molecular biology over the past two decades have led to the adoption of polymerase chain reaction (PCR)-based methods and CRISPR-Cas-based next-generation molecular techniques for the diagnosis of brucellosis in both clinical and veterinary laboratories (6,7).

The purpose of this review is to comparatively evaluate conventional and molecular methods used in the laboratory diagnosis of brucellosis in light of current literature data, to highlight the advantages and limitations of each method, and to propose a rational diagnostic algorithm that can be followed in clinical practice.

Microbiological Characteristics and Epidemiology of The *Brucella* Genus

The genus *Brucella* consists of nonmotile, non-spore-forming, non-encapsulated, aerobic or facultative anaerobic, small Gram-negative coccobacilli. Twelve species have been identified within the genus based on host preference, metabolic characteristics, and cultural and antigenic differences: *B. melitensis* (sheep/goat), *B. abortus* (cattle), *B. suis* (pigs), *B. canis* (dogs), *B. ovis* (rams), and *B. ceti* and *B. pinnipedialis*, which were isolated from marine mammals, are among them (2). DNA-DNA hybridization studies indicate that these species are genetically very closely related and can, in fact, be considered evolutionary lineages that have adapted to different hosts (8).

Brucella species are among the most frequently reported microorganisms causing laboratory-acquired infections due to their low infectious doses and ability to spread easily via aerosols, and they require Biosafety Level 3 (BSL-3) laboratory conditions (9). It is emphasized that current estimates of global human brucellosis incidence are higher than in previous years and that the disease has re-emerged as a "neglected but resurgent" zoonosis (3). In a recent genomic analysis study conducted specifically in Türkiye, the phylogenetic diversity of *Brucella* strains isolated from animals and humans between 2010 and 2020 was revealed, and it was reported that the country continues to be a significant endemic region (10). Indeed, a regional clinical study conducted in Türkiye reported that cases monitored between 2022 and 2024 were concentrated particularly in the Eastern and Southeastern Anatolia regions, and that diagnosis was primarily based on the standard tube agglutination test and blood culture (11).

Challenges in Clinical Diagnosis and The Importance of Laboratory Confirmation

The clinical manifestations of brucellosis vary considerably depending on whether the disease presents in the acute, subacute, or chronic form. Symptoms such as fever, sweating, anorexia, weight loss, osteoarticular involvement, and hepatosplenomegaly are common, but these are not specific to the disease (4). This can lead to delayed or missed diagnosis; therefore, microbiological and/or serological confirmation based on clinical suspicion is crucial for initiating appropriate treatment and preventing complications such as spondylitis, endocarditis, and neurobrucellosis (1).

Conventional Laboratory Diagnostic Methods

Culture Methods

In the diagnosis of brucellosis, isolation of *Brucella* spp. from blood, bone marrow, and other sterile body fluids via culture is still considered the definitive diagnostic method and gold standard approach today, as it provides direct demonstration of the infection (8). The Ruiz-Castañeda two-phase culture system, widely used in the past, has been replaced in many centers by automated continuous monitoring culture systems such as BACTEC and BacT/ALERT (12). These automated systems allow for earlier detection of microorganisms, reducing workload and shortening turnaround time. However, due to the slow growth rate of *Brucella* species, the incubation period of cultures can range from a few days to three weeks, and in some cases, up to six weeks (2).

In contrast, the most significant disadvantage of the culture method is its limited sensitivity. Cultures can yield negative results in patients who have previously received empirical antibiotic treatment, in cases of chronic infection, and in situations where intracellular bacterial load is low, thus reducing diagnostic success (5,9). Furthermore, due to the high infectivity of *Brucella* spp., aerosols that may be generated during culture procedures pose a significant biosafety risk for laboratory personnel. Therefore, it is recommended that suspected clinical samples be processed under appropriate biosafety measures, preferably in BSL-3 laboratory conditions (8). Species-level identification of isolates obtained from cultures traditionally relies on time-consuming and labor-intensive phenotypic methods such as carbon dioxide requirements, hydrogen sulfide (H₂S) production, susceptibility testing to various dyes, and agglutination tests performed with monospecific antisera (2).

Serological Tests

Due to the low sensitivity of culture and the long turnaround time, serological tests remain the most used methods for the routine diagnosis of brucellosis. The Rose Bengal test (RBT) is a rapid, inexpensive, and easy-to-administer screening test based on the agglutination of a serum sample with stained *Brucella* antigen on a slide; while it has high sensitivity, its specificity is limited, and positive results must be confirmed (5).

The standard tube agglutination test (SAT, Wright test) is one of the classic reference methods for the serological diagnosis of brucellosis and measures the total agglutinating antibody titer in serum; titers of 1/160 or higher are considered significant when accompanied by clinical findings (4). However, the SAT may yield false-negative results in cases of chronic infection and in the presence of blocking antibodies; it may also produce false-positive results due to cross-reactions with microorganisms such as *Yersinia enterocolitica* O:9. The Coombs (antihuman globulin) test is used to detect blocking antibodies that cannot be detected by the SAT; recent studies have reported that the Brucellacapt test may serve as a practical alternative to the Coombs test because it can detect both agglutinating and non-agglutinating antibodies (4).

The enzyme-linked immunosorbent assay (ELISA) is a standardizable method that can be used for both screening and confirmation because it can detect IgM and IgG class antibodies separately. In a comprehensive study conducted on 3,587 serum samples collected at the French National *Brucella* Reference Center between 2012 and 2023, it was found that serological tests used alone (RBT, SAT, Brucellacapt, IgM/IgG ELISA) had good sensitivity and specificity but limited diagnostic accuracy; in contrast, an

algorithm combining RBT, Brucellacapt, and IgM/IgG ELISA increased sensitivity to 90.5% and specificity to 99.7% (13). Similarly, a study conducted in Iran reported that the combined use of IgG ELISA and the Brucellacapt test could significantly overcome the limitations of current serological diagnostic methods, achieving a sensitivity of 98.84% and a specificity of 93.65% (14). In the serological diagnosis of brucellosis (*B. canis*) in dogs, the comparative performance of ELISA and indirect fluorescent antibody tests continues to be evaluated in recent studies following the withdrawal of the commercial rapid agglutination test from the market (15).

In general, a common limitation of conventional serological tests is their inability to reliably distinguish between active and past infections, as well as the difficulty in interpreting results due to background seropositivity in endemic regions (4). For this reason, current guidelines recommend the combined use of at least two serological tests based on different principles (13).

Molecular Diagnostic Methods

Following the development of the polymerase chain reaction (PCR), nucleic acid-based methods have emerged as a rapid, sensitive, and reliable alternative in microbiological diagnosis (6). *Brucella*'s intracellular lifestyle, slow rate of reproduction, and the risk of laboratory-acquired infection associated with culture make molecular methods particularly valuable for this pathogen (16).

Conventional and Target-Gene-Based PCR

Among the most targeted genomic regions for the diagnosis of the *Brucella* genus are bcs₃₁ (the 31 kDa cell surface protein gene), the 16S rRNA gene, the IS711 insertion sequence, and the omp2a/omp2b outer membrane protein genes (2). While bcs₃₁-targeted PCR offers high sensitivity and specificity at the genus level, it cannot distinguish between species; therefore, additional tests are required for species-level differentiation (9).

Multiplex PCR: AMOS and Bruce-ladder

AMOS PCR is one of the first multiplex PCR methods capable of distinguishing between *B. abortus*, *B. melitensis*, *B. ovis*, and *B. suis* biovar 1 by utilizing the species-specific localization of the IS711 insertion sequence in the genome; however, it cannot detect other species such as *B. canis*, *B. neotomae*, *B. pinnipedialis*, and *B. ceti* (2). Developed to overcome this limitation, the Bruce-ladder test is a one-step multiplex PCR comprising eight primer pairs and can distinguish all classical *Brucella* species including the S19, RB51, and Rev.1 vaccine strains based on distinct band

profiles. In a multicenter evaluation study conducted with 625 *Brucella* strains across seven laboratories, it was reported that the test provided consistent and reliable results for strains obtained from different geographic and animal sources and demonstrated robust performance regardless of the DNA extraction method or thermal cycler used (17). The most significant limitation of the Bruce-ladder assay is its inability to distinguish between biovars within the same species (2).

Real-Time PCR (qPCR)

Real-time PCR is a method that enables simultaneous monitoring of amplification using fluorescence-based probe or dye systems; it reduces the risk of contamination because it operates in a closed-tube format and can provide quantitative results. Multiplex real-time PCR tests targeting markers such as bcs31 and IS711 have demonstrated high sensitivity and specificity both in the detection of *Brucella* spp. and in distinguishing between *B. abortus* and *B. melitensis*; it has been reported that sensitivity and specificity are close to 100% for genus-level detection and remain above 90% for species-level differentiation (18). A significant advantage of real-time PCR over conventional serological methods is that it poses a low biological risk and results can be obtained within a few hours (6).

Isothermal Nucleic Acid Amplification Techniques

Non-cyclic (isothermal) amplification methods developed in recent years include loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and saltatory rolling circle amplification (SRCA). These methods offer rapid, field-friendly alternatives suitable for point-of-care applications, as they can operate at a constant temperature without requiring a thermal cycler. A LAMP-LFIA (lateral flow immunoassay) test targeting the BME11661 gene has been reported to demonstrate high sensitivity and specificity in the diagnosis of *B. melitensis* (19). Similarly, it has been demonstrated that a LAMP technique combined with a lateral flow biosensor (LAMP-LFB) can identify all *Brucella* species in a simple, rapid, and reliable manner, and may serve as a potential diagnostic tool for basic, clinical, and field applications (20). It has also been reported that a recombinase-based amplification (RAA) test developed for canine brucellosis (*B. canis*) demonstrates promising performance in rapid diagnosis. However, optimizing primer and probe design for these methods can be challenging, and due to their high sensitivity, they may carry a risk of false positives resulting from contamination (16).

High-Resolution Melting (HRM) Analysis

High-resolution melting (HRM) analysis is a rapid, closed-tube molecular genotyping method that allows for the identification of nucleotide sequence changes by evaluating small differences in the melting curves of amplicons obtained after real-time PCR (21). Recent studies have shown that multiplex real-time PCR-HRM applications targeting the omp2a/omp2b, vdc, int-hyp, and glk gene regions provide higher diagnostic sensitivity and more consistent results compared to PCR-restriction fragment length polymorphism (PCR-RFLP) in differentiating *B. melitensis* from *B. abortus* in clinical blood samples. One of the most important advantages of the HRM method is that species differentiation can be performed within the same closed reaction system without the need for gel electrophoresis or restriction enzymes after amplification. However, reliable results require high-quality DNA isolation and a level of technical expertise to ensure accurate interpretation of melting curves (2).

CRISPR-Cas-Based Detection Systems

CRISPR-Cas-based diagnostic platforms are next-generation molecular methods that recognize target nucleic acid sequences via species-specific crRNAs to generate colorimetric or fluorescent signals, typically combined with isothermal pre-amplification. It has been demonstrated that a CRISPR-Cas12a test strip (CRISPR/CAST) kit, developed for the detection of *Brucella* in infected animals under field conditions, can provide visual results with high sensitivity when combined with isothermal amplification (22). In more recent studies, a system combining CRISPR/Cas12b with multiple cross displacement amplification (MCDA) has been shown to detect the *Brucella* genus and *B. melitensis* with ultra-high sensitivity and high specificity (7), an RPA-CRISPR/Cas12a-based test provides a detection limit of 1 copy/μL in a fluorescent format—10 times more sensitive than real-time PCR—and shows 100% agreement with serological results in clinical serum samples (23), it has also been reported that a CRISPR/Cas13a-based, RNA-targeted, portable test enables the sensitive and practical detection of *Brucella* nucleic acid (24). However, the fact that crRNA design depends on specific protospacer-adjacent motif (PAM) regions limits its flexibility, and it is emphasized that further research is needed for the comprehensive detection of different *Brucella* species and vaccine strains (22).

Digital PCR

Droplet digital PCR (ddPCR) is based on the principle of dividing the reaction mixture into thousands of microdroplets and performing amplification separately in each one, thereby enabling the absolute quantification of the target nucleic acid. It has been reported that this feature allows for higher sensitivity compared to real-time PCR in

samples with low bacterial loads; however, the cost of equipment and consumables poses a significant limitation (6).

Next-Generation Sequencing and Metagenomic Approaches

Metagenomic approaches based on next-generation sequencing (NGS) enable the direct detection of pathogens from clinical samples in a culture-independent manner. A recently published case series reported that five atypical brucellosis cases in a non-endemic region, where the causative agent could not be detected by standard diagnostic methods, were identified using metagenomic next-generation sequencing (mNGS). This finding demonstrates that mNGS can provide a significant diagnostic contribution, especially in cases where brucellosis is not considered clinically or conventional diagnostic methods are insufficient (25). NGS is also used for strain-level epidemiological surveillance via whole-genome single nucleotide polymorphism (SNP) analysis; this approach was preferred in the genomic analysis of isolates collected in Türkiye between 2010 and 2020 (10). The most important limitations of this method are its high cost, the need for specialized bioinformatics infrastructure, and long analysis time (25).

MALDI-TOF Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a technology that provides reliable microorganism identification in a short time, based on comparing protein fingerprint profiles obtained from bacterial colonies or positive blood culture bottles with reference databases. Studies have shown that *Brucella* isolates can be identified with high accuracy at the genus level from colonies obtained from solid media and experimentally induced positive blood cultures. However, the success of species-level differentiation largely depends on the scope and spectral diversity of the reference database used (12).

Although MALDI-TOF MS significantly speeds up the identification process, it cannot eliminate the main disadvantages such as the long incubation time and limited sensitivity of the culture, which are due to the slow growth characteristic of *Brucella*, as it is a culture-based method. Furthermore, due to the high infectivity of *Brucella* species, it is crucial to fully implement appropriate inactivation protocols prior to analysis to minimize the risk of laboratory-acquired contamination (8).

Comparative Summary of Molecular Methods

Table 1 shows the main methods used in the molecular diagnosis of brucellosis, their target genomic regions, and their main advantages and limitations.

Table 1. Comparison of the molecular methods used in the molecular diagnosis of brucellosis.

Method	Target Region(s)	Essential Advantage	Basic Limitation/Reference
Conventional PCR	bcs31, 16S rRNA, IS711, omp2a/omp2b	Simple, widely accessible infrastructure	Species differentiation is limited (9)
Multiplex PCR (Bruce ladder, AMOS)	IS711, omp25, omp31, wboA vb.	Differentiation of species and vaccine strain in a single reaction	It cannot distinguish between biovars (2,17)
Real Time PCR (qPCR)	bcs31, IS711, omp2a/omp2b	Fast, quantitative, low risk of contamination	The device is expensive (18)
Isothermal amplification (LAMP, RPA)	IS711, bcs31, omp25, BMEI1661	Fast results at a constant temperature; suitable for field use	Difficult to optimize (19,20)
High-Resolution Melting Analysis (HRM)	omp2a/omp2b, vdce, int-hyp, glk	Rapid species separation in a closed-tube system	Requires high-quality DNA (21)
CRISPR-Cas-based tests	Species-specific crRNA targets	Ultra-sensitive detection without amplification or with minimal amplification	Standardization is in its early stages (7,22-24)
Digital PCR (ddPCR)	bcs31, IS711	Absolute quantification, high sensitivity at low copy numbers	Costly, with a relatively slow workflow (6)
Next-generation sequencing/metagenomics (mNGS)	Whole genome/metagenome	Culture-independent diagnosis, diagnostic accuracy in rare/atypical cases	Cost and bioinformatics infrastructure (25)
MALDI-TOF MS	Protein/peptide mass profile	Identification at the species level from culture within minutes	Culture-dependent, an expanded database is needed at the species level (12)

Comparison of Conventional and Molecular Methods

Traditional and molecular methods used in the laboratory diagnosis of brucellosis should be considered complementary approaches rather than alternatives to each other. Culture methods maintain their position as the gold standard for definitive diagnosis due to their ability to directly isolate the causative agent and allow for antimicrobial susceptibility testing. However, their low diagnostic sensitivity, the need for long incubation periods due to the slow growth of *Brucella* species, and the biosafety risks they pose to laboratory personnel limit their use as a sufficient diagnostic method alone (8).

Serological methods, on the other hand, hold a significant place in routine diagnosis due to their ease of application, wide laboratory accessibility, and relatively low cost. However, cross-reactions with other bacteria and the inability to always distinguish between active and past infections serologically necessitate the evaluation of these test results in conjunction with clinical and epidemiological data (13).

Molecular diagnostic methods are gaining increasing importance today due to their high sensitivity and specificity, rapid results, lower biosafety risk compared to culture, and their ability to differentiate between *Brucella* species and strains. However, factors such as the lack of complete standardization among methods, variable performance of different commercial kits, high cost, the need for advanced laboratory infrastructure, and the need for experienced personnel are among the main elements limiting the widespread use of molecular tests (2,16). Table 2 compares the two approaches in terms of their key characteristics.

Table 2. Comparison of the conventional and molecular methods used in the diagnosis of brucellosis.

Feature	Conventional Methods	Molecular Methods
Result time	3–21 days for culture (sometimes up to 6 weeks); a few hours for serological tests	1–4 hours for PCR/qPCR; 15–60 minutes for isothermal methods (LAMP, RPA)
Analytical sensitivity	Low to moderate in culture; variable in serology depending on the type of test	Generally high; DNA can be detected at levels of a few copies per μ L using real-time PCR
Analytical specificity	Risk of false positives due to microorganisms that cross-react	High specificity through target-specific primer/probe design; species differentiation is possible
Biosafety risk	The risk of laboratory-acquired infection is high during culture and manipulation (BSL-3 required)	Since the work involves inactivated samples or extracted DNA, the risk is lower
Species/biovar distinction	Differentiation limited to labor-intensive phenotypic tests such as Faj lysis and dye sensitivity	High-resolution differentiation using Bruce-ladder, HRM, MLVA, and whole-genome sequencing
Distinguishing Between Active and Past Infections	Partially possible with IgM/IgG ELISA and titer monitoring; interpretation is difficult	Conventional molecular methods cannot do this directly; quantitative charge tracking is still in the research phase
Cost and Infrastructure	Relatively low-cost and applicable in most laboratories	Higher equipment and reagent costs; require qualified personnel
Standardization	Methods that have been in use for a long time and have defined reference ranges	Lack of standardization across laboratories; variable performance of commercial kits

Diagnostic Approach and Recommended Algorithm

Current literature supports an approach that integrates clinical findings with multiple laboratory methods in a stepwise manner, rather than relying on a single method for the reliable diagnosis of brucellosis (4,13). In line with this, the recommended general algorithm can be summarized as follows:

- In cases of clinical suspicion, perform rapid screening with the Rose Bengal test as the first step.
- Confirm Rose Bengal-positive cases with SAT and/or IgM/IgG ELISA, and, if possible, with the Brucellacapt test (13).
- Collect simultaneous blood and bone marrow cultures in clinically appropriate cases and use automated continuous monitoring systems.
- In cases of positive culture, the isolate should be confirmed at the species level using molecular methods (Bruce-ladder, real-time PCR) and, if possible, rapidly identified using MALDI-TOF MS (12,17).
- In cases of chronic, recurrent, or seronegative disease where clinical suspicion is high, performing PCR/qPCR directly on clinical specimens and, when necessary, evaluating CRISPR-Cas-based tests, digital PCR, or metagenomic sequencing (7, 25).

- Prioritizing molecular testing on cerebrospinal fluid (CSF) or tissue samples in specific clinical presentations such as neurobrucellosis or endocarditis.

This stepwise approach both improves diagnostic accuracy and ensures the rational use of resources; particularly in endemic regions such as Türkiye, the early use of molecular methods when clinical suspicion is high is important for preventing diagnostic delays (10).

CONCLUSION

Brucellosis is a zoonotic infectious disease of global importance, whose diagnosis largely depends on laboratory tests due to its mostly non-specific clinical manifestations and its ability to mimic many infectious diseases. Therefore, accurate and timely diagnosis requires clinical evaluations to be supported by appropriate laboratory methods (1,3). While conventional culture and serological tests have been used for many years and are accessible and relatively low-cost methods, they have significant limitations, such as low sensitivity, long turnaround times, cross-reactions, and the risk of laboratory-acquired infections (9). Molecular methods particularly real-time PCR, multiplex PCR (Bruce-ladder), and next-generation isothermal amplification along with CRISPR-Cas-based technologies are playing an increasingly central role in the diagnosis of brucellosis by offering higher sensitivity and specificity, shorter turnaround times, and lower biosafety risks (7,23,24).

However, for molecular methods to be fully integrated into clinical practice, it is necessary to ensure interlaboratory standardization, expand performance comparisons of commercial kits, conduct cost-effectiveness analyses, and develop tests that are affordable, suitable for field conditions, and applicable in a point-of-care, format particularly in resource-limited regions with high prevalence (16). Furthermore, the development of quantitative molecular biomarkers capable of distinguishing active infection from past infection is among the priority areas for future research (4). In conclusion, the use of conventional and molecular methods in a complementary manner, within a stepwise algorithm that takes the clinical context into account, forms the basis of today's evidence-based approach for the reliable and timely diagnosis of brucellosis.

Declarations

This manuscript is a narrative review derived exclusively from previously published studies. It does not include any experiments involving humans or animals, nor does it contain any patient-identifiable data. For this reason, obtaining ethical approval was not necessary.

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