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Original Article

Enhanced diagnostic accuracy of combined serological and bacteriological tests for brucella infection

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Abstract: Objective: To analyze the effects and value of combining serological and bacteriological tests in diagnosing Brucella infection. Methods: In this retrospective study, patients suspected of having Brucella infection admitted to Lishui Second People's Hospital from January 2019 to December 2022 were assessed using serological, bacteriological, and combined (serological + bacteriological) tests. The diagnostic performance of each test was compared using previous clinical results as the gold standard. We also compared the acceptance rate and satisfaction with clinical diagnostic results for the different testing modalities. Results: The combined examination (serological + bacteriological test) showed higher diagnostic efficiency for Brucella infection compared to single serological and single bacteriological tests ($P < 0.05$). Similarly, the satisfaction rate for the clinical diagnostic effects of the combined examination was higher than for the serological and bacteriological tests alone ($P < 0.05$). Conclusion: The combination of serological and bacteriological tests provides superior diagnostic value for Brucella infection, with high diagnostic accuracy, sensitivity, and specificity. It is recommended as the preferred method for clinical diagnosis.

Keywords: Serological test, bacteriological test, Brucella infection, clinical diagnosis, effect evaluation

Introduction

Brucellosis, commonly referred to as Malta fever or undulant fever, is a zoonotic infectious disease caused by bacteria of the genus Brucella [1, 2]. This disease poses a significant public health threat globally, particularly in developing countries where it is endemic. Brucellosis affects various mammals, including livestock (cattle, goats, sheep) and wild animals [3]. Human infections are typically acquired through direct contact with infected animals or consumption of contaminated dairy products [4, 5]. The clinical manifestations of brucellosis in humans are highly variable and nonspecific, ranging from acute febrile illness to chronic debilitating conditions affecting multiple organ systems [6-9]. Given its nonspecific symptoms and variable presentation, accurate and timely diagnosis of brucellosis remains challenging, potentially leading to delays in treatment and complications [10-12].

Serological tests, which detect antibodies produced in response to Brucella infection, are commonly employed for diagnosing brucellosis [13, 14]. However, serological testing alone may yield inconclusive results due to false-positive reactions, particularly in endemic regions. Consequently, combining serological testing with bacterial detection methods, such as bacterial culture or molecular techniques, has been proposed to enhance diagnostic accuracy.

This study aims to evaluate the diagnostic performance of a combined approach using serological tests and bacterial detection methods for diagnosing Brucella infection. By comparing the results of different diagnostic methods, we seek to determine the optimal strategy for accurate and timely diagnosis of brucellosis, which is crucial for guiding appropriate treatment and preventing disease spread.

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Materials and methods

Baseline data

Retrospective data analysis was conducted on a cohort of 60 patients suspected of Brucella infection, admitted to Lishui Second People's Hospital from January 2019 to December 2022. The study adhered to the ethical guidelines approved by the Institutional Review Board of Lishui Second People's Hospital, and informed consent was waived due to the anonymous nature of data collection and analysis.

Inclusion and exclusion criteria

Inclusion criteria: (1) Diagnosis criteria for Brucella infection [17]; (2) With a history of living in an endemic area; (3) Complete clinical data records.

Exclusion criteria: (1) Other infectious diseases; (2) Mental illness; (3) Autoimmune diseases; (4) Breastfeeding or pregnant women.

Specimen collection

Prior to blood collection, patients were instructed to fast after 10:00 p.m. the night before. On the following morning at 8:00 a.m., 10 ml of blood was collected, including 2 ml anticoagulated with EDTA-K and 2.7 ml anticoagulated with sodium citrate. Additionally, 5 ml of venous blood was drawn into a yellow-capped vacuum tube containing separating gel. After coagulation, the blood was centrifuged at 3,000 rpm for 20 minutes, and the serum was retained for subsequent testing.

Serological test

The tube agglutination test is employed to detect Brucella-specific antibodies in serum. Blood samples are centrifuged at 3000 rpm for 10 minutes. Following centrifugation, the serum is extracted and inspected for protein clots or hemolysis. If the serum is clear, it is diluted with 0.5% physiological saline. Subsequently, Brucella smooth lipopolysaccharide antigen is added to the serum, and the mixture is agitated. The serum is then incubated at 37°C for 22-24 hours. The result is determined based on the degree of serum agglutination If the Brucella-specific antibody in the serum (mainly in the IgG) reaches 1:100 (++), it can be considered as positive [15].

Bacteriological examination

Bacterial isolation and culture are performed on collected blood samples. The resulting solution is plated onto dry agar medium. The presence of bacterial colonies indicates a positive result. Blood specimens are inoculated onto dry agar culture medium, incubated at 35°C for 18-24 hours, and observed for colony formation. Positive cultures are then isolated and identified. Throughout the testing process, personnel must adhere strictly to the prescribed regulations and procedures, with at least two personnel conducting the tests. In case of inconsistent results, a second test is performed to enhance test accuracy [16].

Outcome measures

A positive diagnosis is confirmed when a 1:100 antigen titer and an agglutination grade of (+)(+) or higher are observed in the test tube. Following the evaluation of positive cases from serological and bacteriological tests, as well as their combined results, the diagnostic sensitivity, specificity, and accuracy of each test are calculated and analyzed for consistency with the final diagnosis.

Statistical analysis

All pertinent research data were analyzed using IBM SPSS Statistics 21.0 (IBM, Armonk, NY, USA). Count data were described as "percentages", and the χ^2 test was performed. A statistically significant difference was considered when $P < 0.05$.

Results

General information

The study included 60 subjects, comprising 49 males and 11 females, resulting in a male-to-female ratio of 4.45:1. Occupationally, farmers and herdsmen were the most prevalent, accounting for 43 farmers (71.67%), followed by urban residents (5.00%), herdsmen (5.00%), veterinarians (6.67%), fur processors (3.33%), butchers (3.33%), professional deer breeders (3.33%), and one student (1.67%) (**Table 1**).

Table 1. Gender and occupational distribution

	Cases	Percentage (%)
Male	49	81.67
Female	11	18.33
Farmer	43	71.67
Citizen	3	5.00
Herdsman	3	5.00
Veterinarian	4	6.67
Fur processing	2	3.33
Slaughter	2	3.33
Raising deer	2	3.33
Student	1	1.67

Table 2. Distribution of infectious sources

Infectious sources	Cases	Percentage (%)
Sheep	43	71.67
Cattle	11	18.33
Dog	3	5.00
Deer	2	3.33
Horse	1	1.67

Regarding the source of infection, sheep and cattle were the most common, totaling 54 cases (90% of the total). Among the patients, 43 (71.67%) had a history of contact with sheep, 11 (18.33%) with cattle, 3 (5%) with dogs, 2 (3.33%) with deer, and 1 (1.67%) with horses (**Table 2**).

Clinical manifestations and complications

All 60 patients with brucellosis presented with fever. Notably, 71.67% (43 cases) experienced high fever during the illness, and an equal percentage (43 cases) exhibited excessive sweating following defervescence. Additionally, 21.67% (13 cases) suffered from moderate fever, while 68.33% (41 cases) displayed intermittent fever. Other symptoms included chills (21.67%, 13 cases), muscle aches (30%, 18 cases), headaches (28.33%, 17 cases), gastrointestinal distress (26.67%, 16 cases), lymph node enlargement (28.33%, 17 cases), liver enlargement (46.67%, 28 cases), spleen enlargement (48.33%, 29 cases), and arthritis (51.67%, 31 cases). Of these arthritis cases, 28.33% (17 cases) were peripheral, 13.33% (8 cases) hip arthritis, and 8.33% (5 cases) spinal arthritis. Hepatitis was prevalent, affecting 61.67% (37 cases), while epididymitis and orchitis occurred in 8.33% (5 cases), pneumonia in 5% (3 cases), meningitis in 3.33% (2 cases), and nephritis in 1.67% (1 case) (**Table 3**).

Comparison of serological tests for brucellosis detection

Among 60 suspected patients, 50 were confirmed. The serological test yielded a positive detection rate of 68% (34 cases) and a negative rate of 32% (26 cases) (**Table 4**).

Comparison of bacteriological test for brucellosis detection

Among 60 suspected patients, 50 cases were finally diagnosed. In the bacteriological test, 40 cases were detected as positive and 20 cases as negative (**Table 5**).

Comparison of combined approach for brucellosis detection

Among 60 suspected patients, 50 cases were finally diagnosed. Utilizing a combined examination, 49 cases were detected as positive and 11 cases as negative (**Table 6**).

Comparison of diagnostic efficacy of serological, bacteriological tests and the combination examination

The diagnostic accuracy of the combined examination (serological test + bacteriological test) for Brucella infection stood at 95.00%, with a sensitivity of 96.00%, specificity of 90.00%, positive predictive value of 97.96%, and negative predictive value of 81.82%. This diagnostic efficiency significantly surpassed that of serological and bacteriological tests alone ($P < 0.05$) (**Table 7**).

Comparison of clinical diagnosis satisfaction rate of serological, bacteriological tests and the combination examination

The patients' satisfaction rate with the combined examination was significantly higher than with serological and bacteriological tests alone ($P < 0.05$) (**Table 8**).

Discussion

Brucellosis, a zoonotic disease, is clinically prevalent due to Brucella's robust reproductive capacity and resilience [18]. This bacterium can survive for extended periods in feces and

Table 3. Clinical manifestations and complications

Clinical features	Cases	Percentage (%)
Symptoms and signs		
Fever	60	100.00
High fever during the course of the disease	43	71.67
Moderate heat	13	21.67
Accompanied by a chill	13	21.67
The fever is intermittent	41	68.33
Heat relieves excessive sweating	43	71.67
Fatigue	17	28.33
Digestive symptoms	16	26.67
Headache	17	28.33
Muscle soreness	18	30.00
Hepatomegaly	28	46.67
Splenomegaly	29	48.33
Lymph node enlargement	17	28.33
Complications		0.00
Arthritis	31	51.67
Peripheral arthritis	17	28.33
Spinal arthritis	5	8.33
Osteoarthritis	8	13.33
Meningitis	2	3.33
Epididymitis	5	8.33
Hepatitis A	37	61.67
Interstitial pneumonia	3	5.00
Nephritis	1	1.67

Table 4. Comparison of serological test for brucellosis detection

Gold standard	Serological test	
	Positive	Negative
Positive	30	20
Negative	4	6

Table 5. Comparison of bacteriological test for brucellosis detection

Gold standard	Bacteriological test	
	Positive	Negative
Positive	35	15
Negative	5	5

Table 6. Comparison of combined approach for brucellosis detection

Gold standard	Joint inspection (serological test + bacteriological test)	
	Positive	Negative
Positive	48	2
Negative	1	9

dead organs, demonstrating a degree of variability [19, 20]. Once Brucella enters the human immune system, it readily causes secondary blood infection, significantly affecting patients' work capabilities [21, 22]. Therefore, early diagnosis and treatment are crucial for improving patients' life safety and quality of life.

The results of this article show that among the studied group, 40 patients were identified with Brucella infection through bacteriological testing, yielding a positive rate of 87.5%. Serological testing detected 34 positive cases, representing a positive rate of 88.24%. When the two methods were combined, 49 patients were accurately diagnosed with Brucella infection, achieving a remarkably high positive rate of 97.96%. This finding underscores the importance of combining serological and bacteriological approaches to enhance the accuracy of Brucella detection in clinical settings.

In clinical practice, various methods are used for Brucella detection, including bacterial isolation and culture, tube agglutination test, and complement fixation test [23, 24]. However, the agglutination test can lead to non-specific agglutination in patients' sera, resulting in false positives, while the complement fixation test requires a significant amount of blood and is cumbersome, exhibiting instability and low accuracy [25, 26].

The results of this study indicate that both serological and bacteriological tests can diagnose Brucella infection, but the combination examination exhibits higher accuracy. Therefore, in clinical testing, to ensure the accuracy of diagnosis, the combined examination is the preferred choice. It is noteworthy that serological detection may yield false positives or negatives. This occurs when Brucella transitions from acute to chronic or recurrent stages, as the number of bacteria in the blood decreases due to phagocytosis by immune cells [27]. During this period, testing blood

Table 7. Comparison of diagnostic efficacy of serological, bacteriological tests and the combination examination

Way	Accuracy	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Serology test	60.00 (36/60)	60.00 (30/50)	60.00 (6/10)	88.24 (30/34)	23.08 (6/26)
Bacteriological test	66.67 (40/60)	70.00 (35/50)	50.00 (5/10)	87.50 (35/40)	25.00 (5/20)
Combined examination (serological test + bacteriological test)	95.00 (57/60)	96.00 (48/50)	90.00 (9/10)	97.96 (48/49)	81.82 (9/11)

Table 8. Comparison of clinical diagnosis satisfaction rate of serological, bacteriological tests and the combination examination

Type	Satisfy	Satisfied	Dissatisfied	Satisfaction rate (%)
Serology test	33 (55%)	20 (33.3%)	7 (11.7%)	88.33
Bacteriological test	31 (51.7%)	20 (33.3%)	9 (15%)	85.00
Combined examination (serological test + bacteriological test)	39 (65%)	20 (33.3%)	1 (1.7%)	98.33
X^2				6.756
P				0.034

samples may result in missed detections, leading to false positives or negatives.

In our study, we observed that the combination of serological and bacteriological tests significantly improved the diagnostic accuracy for Brucella infection. This combined approach offers several advantages that contribute to patients' satisfaction rates.

First, the combined examination provides a more comprehensive and accurate understanding of the patient's condition. By utilizing both serological and bacteriological tests, we can achieve a more precise diagnosis, which aids in formulating more targeted treatment plans. This, in turn, gives patients a greater sense of security and confidence in the diagnosis and treatment process [28]. Second, the combination examination shortens the time for diagnosis. By combining the two testing methods, we can quickly identify the source of the infection, allowing patients to receive timely treatment. This reduces patients' waiting time and anxiety [29]. Third, the use of multiple testing methods enhances the professional image and reliability of the medical team. Patients perceive this approach as a demonstration of the carefulness and professionalism of the medical staff, making them feel that they are receiving thorough and meticulous care [30]. Fourth, the combination examination leads to better treatment outcomes. With a more accurate diagnosis, the treatment is more targeted, potentially improving the patient's prognosis and recovery. This, in turn, increases patients' satisfaction with the overall treatment process.

Lastly, good communication and explanation during the testing process are crucial. By thoroughly explaining the importance and necessity of the combined examination, we can help patients better understand the value of these tests, further enhancing their acceptance and satisfaction. Overall, the combination of serological and bacteriological tests for Brucella infection offers numerous benefits, not only in terms of diagnostic accuracy but also in improving patients' satisfaction rates.

Our study still has a few limitations. Firstly, serological testing alone may yield inaccurate results due to false positives and negatives caused by cross-reactivity with other pathogens or variations in antibody levels. Secondly, bacterial testing also poses challenges, including the difficulty of culturing Brucella bacteria and the possibility of bacteria being absent in collected samples. Moreover, molecular testing methods like PCR may not detect low bacterial levels reliably. Interpreting serological and bacterial test results is complex, requiring expertise to differentiate between active, past infections, and vaccination statuses, especially in vaccinated regions.

In summary, the combination of serological and bacteriological tests for Brucella infection offers high diagnostic value, accuracy, sensitiv-

ity, and specificity, making it a preferred choice for clinical diagnosis.

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Signed written informed consent was obtained from the patients and/or guardians.

Disclosure of conflict of interest

None.

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