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Comparison of Different Laboratory Methods for Clinical Detection of *Brucella* Infection

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The rapidity, accuracy, and detection abilities of different laboratory methods (tube agglutination test (SAT), indirect ELISA, fluorescence polarization test (FPA), and blood culture methods) to detect *Brucella* in the laboratory. The study included 95 patients with documented and 42 patients with suspected brucellosis and 56 healthy control subjects. For the tests, the positive rates of *Brucella* infection detection in the confirmed group were significantly higher than in group with suspected infection ($p<0.01$) and in healthy controls ($p<0.01$). There was no significant difference between indirect ELISA and FPA in detecting antibodies to *Brucella* in acute ($\chi^2=0.335$), subacute ($\chi^2=0.660$), and chronic cases ($\chi^2=5.332$). Among the detection methods, indirect ELISA showed the highest sensitivity (98.9%), specificity (100%), and Youden index (0.989). The sensitivity and specificity of FPA were 96.8 and 96.4%, respectively. In order to easily and rapidly diagnose brucellosis in clinical practice, a combination of detection methods is recommended, in which *Brucella* antibodies are screened by FPA and then confirmed by indirect ELISA.

Key Words: *tube agglutination test; indirect ELISA; fluorescence polarization test; brucellosis*

Brucellosis is a contagious disease caused by *Brucella* and is widespread and seriously harmful to humans and animals. It is one of the major zoonotic diseases in the world [10]. The clinical manifestations of this disease include fever, sweating, fatigue, and other symptoms. Brucellosis can damage joints, liver, spleen, lymph nodes, and other organs, and can therefore pose a serious threat to animal husbandry production and human health. Most people and animals infected with *Brucella* experience clinical manifestations that are diverse and non-specific, which hinders quick and accurate diagnosis of *Brucella* infection. Therefore, the development of a method enabling accurate and rapid detection of brucellosis is critical for the prevention and treatment of this disease. In China, the most popular methods currently used to detect *Brucella* are isolation and culturing of bacteria, tube agglutination test (SAT), and *etc*. However, indirect ELISA and fluorescence polarization test (FPA) are also applied in different countries to detect the *Brucella* infection.

Classical tests of brucellosis, such as culturing and phenotypic characterization, are laborious, time-consuming, and can pose the infection risk and generate discordant results. Isolation of the causative agent often fails in routine diagnosis. Therefore, serological tests have been developed and used for *Brucella* diagnosis in cattle and ruminants especially at the herd level; however, cross-reactions with other gram-negative bacteria become a major issue. Rose Bengal test (RBT), complement fixation test (CFT), and slow agglutination test (SAT) are widely used for the detection of antibodies to *Brucella* spp. The sensitivity of RBT fulfills the requirements for surveillance of free areas at the flock level, but it is known that only combination of RBT with CFT in infected flocks can obtain accurate individual sensitivity in test-and-slaughter programs. CFT is also recommended by World Organi-

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zation for Animal Health (OIE) as a test prescribed for international trade [7]; it is reliable when performed correctly, but it is cumbersome, time consuming and difficult to standardize [11]. Unfortunately, none of the above mentioned tests can distinguish between antibodies produced after vaccination and those due to infections [8]. Different ELISA systems were developed to overcome these problems. Additionally, ELISA could detect *Brucella* carriers which were seronegative by RBT, SAT and CFT [12]. Recently developed FPA can be used for serological diagnosis of *Brucella* infection. It is a rapid, homogenous, and species-independent assay, which was initially developed and validated for the detection of antibodies to *B. abortus* in cattle. FPA has many methodological advantages over the older, more established tests. It has yet to become established within the routine testing procedures of most National Brucellosis Reference Laboratories [9]. FPA requires minimal manipulations and can be completed in few minutes [4].

Our aim was to compare the rapidity, accuracy, and detection abilities of four tests for *Brucella* detection: blood culture methods, SAT, indirect ELISA, and FPA.

MATERIALS AND METHODS

Patients. The study included 95 patients with verified brucellosis infection examined at the Heilongjiang Provincial General Administration of Reclamation Department of Infectious Diseases from August 2014 to April 2015. The infection was diagnosed by clinical symptoms and antibody titers 1:100 (++) according to SAT data. The diagnosis of brucellosis was based on "*Brucella* disease diagnostic criteria" (WS 269-2007) published by the Ministry of Health of the People's Republic of China in 2007. The group with confirmed infection included 57 males and 38 females aged 1-74 years (mean 43 years). These confirmed patients were infected for different times, including 51 acute cases with a disease period <3 months, 10 subacute cases with a disease duration of 3-6 months, and 34 chronic cases with a disease period >6 months. The group of patients with suspected brucellosis (with clinical symptoms, but negative SAT) included 42 subjects, 25 males and 17 females, aged 9-69 years old (mean age 40 years). The control group comprised 56 healthy subjects, 30 males and 26 females, aged 19-69 years (mean 41 years). The study was approved by General Hospital of Heilongjiang Province Land Reclamation Bureau). Informed consent was signed by all patients and healthy subjects enrolled.

***Brucella* detection tests.** Blood culture, SAT, indirect ELISA, and FPA were utilized to detect either *Brucella* and/or *Brucella* antibody in the whole blood and serum samples. All experiments were carried out according to the reagent instructions and standard operating procedures. In the blood culture test, 10 ml of whole blood was collected and incubated for 7 days in aerobic flasks and anaerobic flasks (BD) containing resin, followed by biochemical and morphological examination. SAT was conducted following the procedure outlined in the Practical Clinical Brucellosis. SAT test results with a titer of 1:100 and higher were recognized as positive. The results of indirect ELISA were expressed as the ratio of optical density (OD) value to the cutoff value, the ratio <0.24 was interpreted as negative and >0.24 indicated positive results. The results of FPA test were expressed in milli-polarization units (mP); the detection values <72 mP were recognized as negative, 72-93 mP as suspicious, and >93 mP as positive results.

Reagent and instrument. *Brucella* tube agglutination antigen was obtained from the National Institute for Communicable Disease Control and Prevention in the Chinese Center for Disease Control and Prevention. The indirect ELISA kit and *Brucella* fluorescence polarization assay antibody detection kit were obtained from the Heilongjiang Province Pinghe Biotechnology Research Institute. The BACTEC 9050 automatic blood culture instrument (Labstar), ST-360 microplate reader (Shanghai Kehua Bioengineering Co., Ltd), and fluorescence polarization detector (FLUPO) were used in the experiments.

Statistical analysis. The results were processed statistically using SPSS 18.0 (IBM). Comparison of the positive rate of *Brucella* antibodies was conducted using the χ^2 test; the differences were significant at $p<0.05$. Performance evaluation:

Sensitivity=true positive/(true positive+false negative)×100%;

Specificity=true negative/(true negative+false positive)×100%;

Positive predictive value=true positive/(true positive+false positive)×100%;

Negative predictive value=true negative/(true negative+false negative)×100%;

Youden index=sensitivity+specificity-1.

RESULTS

The positive rates of *Brucella* antibody detected by SAT, indirect ELISA, FPA, and blood culture methods were significantly higher in the group of patients with confirmed than in group with suspected infection (χ^2=137.0,118.7,114.7, and 8.581; $p<0.01$) and healthy control group (χ^2=146.8, 146.8, and 130.4; $p<0.01$) (Table 1). Of note, the positive rate detected using the blood culture test was only 17.9% in the confirmed case group, yet a blood culture test was not conducted in the healthy control group.

TABLE 1. Comparison of Four Methods in Detecting the Positive Rate of *Brucella* in the Serum of Patients with Confirmed and Suspected Infection

Group	SAT		Indirect ELISA		FPA		Blood culture	
	abs.	%	abs.	%	abs.	%	abs.	%
Control (*n*=56)	1.8	1	0	0	3.6	2	—	—
Patients with confirmed infection (*n*=95)	100	95	98.9	94	96.8	92	17.9	17
Patients with suspected infection (*n*=42)	0	0	7.1	3	4.8	2	0	0

Note. All values significantly ($p<0.01$) differ from the control (for patients with confirmed infection) or from patients with confirmed infection (for patients with suspected infection). Blood culture test was not conducted in the control group.

To further investigate the accuracy of the diagnostic capabilities of indirect ELISA, FPA, or blood culture methods to detect *Brucella* antibodies, we examined the detection results in confirmed patients with acute, subacute, or chronic cases. No significant differences in the detection of *Brucella* antibodies in the acute, subacute, and chronic cases were found for all methods tested (χ^2=0.335, 0.660, and 5.332 for ELISA, FPA, and blood culture, respectively; $p>0.05$ for all methods) (Table 2).

We also found that indirect ELISA and FPA had substantially higher sensitivity and specificity capabilities than the blood culture method (Table 3). Hence, indirect ELISA and FPA methods are more preferable for detection of *Brucella* antibodies in patients than blood culturing techniques.

During the last decade, the incidence of brucellosis in animals or humans increased by ~10% per year, and the trend of human brucellosis has changed significantly, especially in non-professional populations [3,13]. The diversity of clinical manifestations has made it inherently difficult for clinicians to identify brucellosis infection early and accurately and can easily lead to misdiagnosis [10]. At present, laboratory testing methods include separation culture and serological detection methods. However, these methods have various drawbacks and limitations.

In this study, human serum samples from infected and healthy subjects were investigated by blood culture, SAT, indirect ELISA, and FPA methods. We found that the positive rate of *Brucella* infection in the confirmed group was significantly higher than in the suspected group and the healthy controls. In China, the SAT test is most widely used to confirm brucellosis. In our study, the patients exhibiting clinical symptoms and *Brucella* antibody titers above 1:100 (++) as indicated using the SAT method, were labeled as confirmed cases. In contrast, patients labeled as suspected cases showed clinical symptoms, but no positive antibody titers detected by SAT. The SAT method is influenced by human factors as an overnight incubation is needed, results can be obtained only after 24 h, and the turbidity results have to be classified by the naked eye. It was reported that 3 patients showed false negative results when the SAT method was performed due to insufficient amounts of *Brucella* antibodies in serum samples [1].

In the suspected group, 3 and 2 cases were positive for *Brucella* antibodies according to indirect ELISA and FPA tests, respectively. These 5 patients all exhibited symptoms of fever, joint pain, and others. The SAT re-test results of these five patients were 1:100 (++) after 2 weeks and was 1:800 (++) after 4 weeks, which indicates that these 5 suspected cases suffered from brucellosis infection and that the SAT test misdiagnosed these patients.

We found that blood culture method showed positive rate of brucellosis in only 17.9% patients of the confirmed group and in 0% patients in the suspected group, which suggests that the blood culture method is a reliable and accurate method, but the rate of positive samples detected by this method was very low. Bearing in mind that such a diagnosis is expensive and requires

TABLE 2. Detection of *Brucella* Antibodies by Indirect ELISA, FPA, and Blood Culture Tests in Serum Samples from Patients with Confirmed Acute, Subacute, and Chronic Brucellosis Cases

Group	Indirect ELISA		FPA		Blood culture	
	abs.	%	abs.	%	abs.	%
Acute infection (*n*=51)	98	50	96.8	49	19.6	10
Subacute infection (*n*=10)	100	10	90	9	10	1
Chronic infection (*n*=34)	97.1	33	94.1	32	0.09	3

TABLE 3. Comparison of the Positive Rates of Detection of *Brucella* Antibodies in Human Serum

Method	Sensitivity, %	Specificity, %	Youden index	Prognostic value, %	
				positive	negative
Indirect ELISA	98.9 (94/95)	100 (56/56)	0.989	100 (94/94)	98.2 (56/56+1)
FPA	96.8 (92-95)	96.4 (54/56)	0.932	97.9 (92/92+2)	94.7 (54/54+3)
Blood culture*	17.9 (17/95)	—	—	—	—

Note. *Blood culture test was not conducted in the control group.

high biosecurity level, wide application of the blood culture method for routine diagnosis was limited.

In confirmed cases of acute, subacute, and chronic *Brucella* infection, no significant difference between the results of antibody detection in serum samples by indirect ELISA, FPA, or blood culture tests were revealed. This result suggests that the positive rates detected by these three different methods are not affected by progression of brucellosis. However, this observation needs to be further investigated before it can be verified by assaying more human serum samples.

The Youden index is commonly used to reflect the authenticity of a diagnostic test in the clinic. In this study, we examined the sensitivity, specificity, the Youden index, the positive predictive value, and the negative predictive value to evaluate the indirect ELISA, FPA, and blood culture methods. It was previously reported [2] that the sensitivity of *Brucella* detection conducted by the indirect ELISA method was >95%, suggesting that indirect ELISA test was effective in diagnosing brucellosis. Our study showed similar results. We found that the sensitivity of *Brucella* detection was 98.9%. Moreover, the specificity and Youden index of indirect ELISA were the highest among the three methods employed in this study. In contrast, the sensitivity of *Brucella* detection conducted by the blood culture method was the lowest. A microplate reader was used to read the results of indirect ELISA tests in this study, which is an improvement over the traditional serological methods as the interference of human factors such as artificial dilution and determination of the results by the naked eye, could influence the results. Thus, we found that indirect ELISA test is a rapid and accurate detection method for brucellosis.

FPA is a simple method to detect the interaction between antigen and antibody. According to the criteria of the World Organization for Animal Health (OIE), FPA is recognized as one of the primary detection methods of brucellosis in the international trade as a simple, fast, and high throughput method. In addition, the FPA method can be run with a serum of 40 μl, takes about 5 min to complete, and has been demonstrated to be an accurate test for the detection of antibodies to *B. abortus*, *B. melitensis*, and *B. suis*. It could be adapted for clinical laboratories and blood banks [6]. The FPA method is also quite suitable for the detection of *Brucella* in patients and for the screening and monitoring of the humans at risk for brucellosis, since washing the plate is an unnecessary step and 92 samples can be screened for *Brucella* antibody within 15 minutes. In our study, the sensitivity and specificity of *Brucella* antibody detection by the FPA method was 96.8 and 96.4%, respectively, which is consistent with the previous report [6] on 96.1% sensitivity and 97.9% specificity of *Brucella* antibody detection by the FPA method.

Quantitative detection is one of the main advantages of the FPA method. This is reflected by its clinical importance in the diagnosis and treatment of brucellosis, as it has been applied in early diagnosis, for monitoring of curative effects, and in prognosis evaluation to improve the diagnostic techniques and the cure rate of brucellosis.

Indirect ELISA can be advocated for use in diagnosis of brucellosis, which can compensate for the drawbacks of routine SAT method and therefore reduce the number of misdiagnoses. However, our study advocates for the combination of indirect ELISA and FPA, as using both would likely improve the diagnoses of brucellosis in a more comprehensive and objective manner much more rapidly. Overall, using indirect ELISA and FPA methods together would provide the crucial technical support needed to improve the diagnosis and treatment of brucellosis.

B. Suo and J. He contributed equally in this study.

B. Suo, J. He, and D. Wang conceived the study, study design, and participated in literature search. B. Suo, J. He, and C. Wu collected and evaluated the data. B. Suo and J. He performed data analysis, data interpretation, and wrote the manuscript. D. Wang revised the manuscript. All authors agree to publish this work under the current authorship. The original data are available from the corresponding author under request. The authors have declared that they have no conflicts of interest. All authors read and approved the final manuscript.

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