Correspondence

Improvement and Advancement of Early Diagnosis of Human Brucellosis in Window Period

TO THE EDITOR—Brucellosis is an infectious disease prevalent in many countries, with half a million new cases reported each year [1, 2]. Brucellosis can easily become chronic, resulting in great suffering and health burden for the patient due to treatment failure and relapse. Timely and accurate diagnosis is a prerequisite for efficient treatment and prevention of chronic infection [3]. However, because typical characteristics are absent, brucellosis is often delayed and/or misdiagnosed, particularly in nonendemic regions [4]. Currently, human brucellosis is diagnosed based on clinical symptoms, exposure history, and serological conversion. Serologically negative cases with clinical symptoms and an exposure history are defined as suspected or negative cases and usually do not receive treatment. Improvement in accurate and early diagnosis will significantly contribute to timely treatment and prevent chronic infection.

Epidemiological information and serum samples of 350 patients with suspected brucellosis was collected from brucellosis clinics. Antibodies were detected using the standard tube agglutination test (SAT).

Antibody titers >1:100 were defined as positive. Persons with suspected infection and antibody titer <1:100 were asked to return and undergo retesting 1–3 months after the first diagnosis. Of the 350 patients, 140 patients (40.0%) with acute infections had both clinical symptoms and previous exposure, but were negative by SAT. These patients underwent retesting, and 76 patients (54.3%) showed serological positivity. This implied that there was a window period for human brucellosis and that incidence of misdiagnosis by the present single-time-point serum test was high.

Next, we tested the possibility of using highly sensitive nucleic acid detection (NAD) for accurate and faster diagnosis. Genomic DNA was extracted from blood samples with QIAamp DNA Blood Mini Kit (Qiagen). *Brucella* DNA was detected using a *Brucella* Isothermal Amplification Diagnostic Kit (Ustar Biotechnologies) that had a sensitivity of 2–5 copies per reaction as determined by simulated serum sample detection. A total of 196 serum samples collected from a separate group of patients with suspected acute infection were then assessed by both NAD and SAT. Of the patients, 59.2% and 70.9% had positive results with SAT and NAD, respectively ($\chi^2 = 0.264, P > .05$).

Furthermore, 43.9% had positive results in both tests, and 11.7% showed positive results in only NAD (Table 1). Patients with positive results in only NAD were followed up and tested for antibody conversion; 50.9% and 79.2% showed SAT positivity at 1 month and 3 months, respectively.

The present single-time-point test has a high rate of misdiagnosis. Similar to other chronic infectious diseases, [5] human brucellosis has a window period (the time from symptom onset to seroconversion). This window period plays an important role in misdiagnosis [4]. Retesting of serologically negative patients 1–3 months later could improve the diagnosis. *Brucella* DNA can be detected during the window period. The addition of sensitive NAD may increase the positive detection rate and allow early diagnosis. Thus, retesting 1–3 months later, or using a combination of NAD and SAT, will improve and accelerate the diagnosis of human brucellosis, thereby reducing the possibility of false-negative diagnoses.

### Table 1. Comparison of the Serum Agglutination Test and Nucleic Acid Detection Test Results for Diagnosing Acute Brucellosis

<table>
<thead>
<tr>
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<th>NAD</th>
<th>SAT</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>+</td>
<td>86 (42.9%)</td>
<td>30 (15.3%)</td>
<td>116 (59.2%)</td>
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<tr>
<td>−</td>
<td>53 (27.0%)</td>
<td>27 (13.8%)</td>
<td>80 (40.8%)</td>
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<tr>
<td>Total</td>
<td>139 (70.9%)</td>
<td>57 (29.1%)</td>
<td>196 (100.0%)</td>
</tr>
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</table>

Abbreviations: NAD, nucleic acid detection; SAT, serum agglutination test.

Notes

Author contributions. Z. C. and L. H. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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