Clinical and laboratory characteristics of childhood brucellosis in high-risk area of Western China

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SUMMARY  Childhood brucellosis present various non-specific clinical symptoms, and limited laboratory data exist for clinical diagnosis. A better understanding of these clinical and laboratory characteristics can avoid clinical misdiagnosis and mistreatment. In this case-series study, a total of 78 children with confirmed diagnosis of brucellosis were evaluated retrospectively. We observed that the incidence rate was higher in the first two quarters every year. The most common symptom was fever. Osteoarticular involvement was found in 44.87% of the patients. Laboratory tests showed that the values of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Hemoglobin (Hb), Neutrophils (NEU), Alanine aminotransferase (ALT) and Ferritin in childhood brucellosis with osteoarticular involvement had significant differences than those without osteoarticular involvement or control group ($P<0.05$). Childhood brucellosis without osteoarticular involvement often accompanied by decrease of NEU, increase of CRP and ALT compared with that control group ($P<0.05$). The Receiver Operating Curves (ROC) analysis revealed that NEU, CRP and ALT can be used as adjunct parameters in the differential diagnosis of childhood brucellosis. These data suggest that clinical and laboratory characteristics are very important for every clinician, which may have a complementary role in diagnosis of childhood brucellosis.

INTRODUCTION

Brucellosis is a zoonosis caused by the Brucella spp. Childhood brucellosis is a common public health problem in developing countries (1). Epidemiological investigation showed that the west pastoral area is the main epidemic area of
Brucellosis in China. The disease spreads to humans through raw dairy products, infected meat as well as via contacting with infected animals (2-4). The main transmission routes are skin, mucosal, respiratory and digestive tract contact. Childhood brucellosis has a high rate of complications, such as relapse, chronic infection and mortality. Therefore it is important for all children with brucellosis to have a rapid diagnosis and timely treatment.

Brucellosis is a multisystemic disease that can cause damage to many organs of the human body (5). In the acute period of the disease clinical manifestations are fever, fatigue, hyperhidrosis, joint pain, liver and spleen lymph nodes enlargement. Joint damage often appears in the chronic phase of the disease (6-7). The most common presenting signs of adult brucella infection are fever and arthritis. Different from adults, children with brucellosis present non-specific clinical symptoms compared with other diseases (8-9). In addition, the vague epidemiology of children often leads to misdiagnosis. Bacterial infectious diseases are often accompanied by changes in laboratory test indicators. As we all know, blood culture that can isolate the bacterium is indisputable evidence of the disease. But Brucella organisms detection hampered by the slow growing features and laboratory safety concerns (10). At present, other routine laboratory tests have not been investigated thoroughly. The purpose of this study is to determine the diagnostic significance of routine laboratory tests in childhood brucellosis.

METHOD

Materials
The medical records of children who were diagnosed of brucellosis based on manifestations and laboratory findings in the Xi’an Children Hospital (Western National Regional Medical Center for Children) between November 2015 and November 2020 were evaluated. The disease was diagnosed based on the presence of clinical signs, positive serum agglutination tests and bacterial culture. All kinds of blood cell counts were made in Sysmex XN-1500 (Japan) and CRP measures were held on Jet-iStar 3000 (China). The liver function tests were made in Power Processor of Beckman Coulter (USA). Blood cultures were performed using the BD BACTEC™ Blood Culture System (USA). Ethical approval to conduct this study was obtained from the institutional Review Board of Xi’an Children's Hospital. Written consent from the caregivers of the neonates could not be obtained due to the retrospective nature of the study. However, all the patient-related information was anonymized.

**Study design**

We evaluated retrospectively a total of 78 childhood brucellosis cases as case group. Besides, the same number of age-matched (3 months to 12 years old) children with non-BRU infection from fever clinic were selected as the control group. The case group was further divided into two groups, one with osteoarticular involvement and the other without it. Clinical and laboratory data including age, gender, milk products ingestion, physical symptoms and hematologic manifestations were obtained from patients’ follow-up folders and hospital records. Laboratory workups such as white blood cell count(WBC), Hemoglobin(Hb), Neutrophils(NEU), Erythrocyte
sedimentation rate (ESR), C-reactive protein (CRP), and liver function tests were performed for all patients. And the results interpreted based on the reference values specific for infancy and childhood. We also investigate the difference of laboratory datas between childhood brucellosis with osteoarticular infection group and that without osteoarticular infection group.

**Statistical analyses**

The data were analyzed using SPSS version 22.0. Chi-Square test was used for statistical significance of categorical variables. Data corresponding to an abnormal distribution were expressed in median (minimum–maximum). These abnormally distributed numerical datas were compared using the non-parametric Mann-Whitney U test.

**RESULTS**

**Seasonal Characteristics**: In this study, 78 children with brucellosis were analyzed. The frequency of childhood brucellosis was recorded in 2016 (N= 6, 7.69%), 2017 (N= 13, 16.67%), 2018 (N= 18, 23.08%), 2019 (N= 20, 25.64%) and the highest rates were observed in 2020 (N= 21, 26.92%). The results of time series analysis showed that the occurrence of the disease presented periodic fluctuation (Fig 1). The incidence of childhood brucellosis has been on the rise in recent five years. And more cases were confirmed in the first two quarters than the last two quarters of these study years.

**Clinical Characteristics**: Various clinical characteristics of the children with brucellosis are shown in Table 1. According to the study, the age distribution of the whole sample ranged from 3 months to 12 years, with most cases concentrated
between 1 and 6 years (59/78, 75.64%). The results showed that 35 children with osteoarticular symptoms were assigned to group with osteoarticular involvement. Among all patients, 42 (53.85%) were male, and 36 (46.15%) were female. The most common symptom were fever (71, 91.03%), fatigue (34, 43.59%) and sweating (26, 33.34%). Some patients have gastrointestinal symptoms such as lack of Appetite (16, 20.51%) and vomiting (6, 7.69%). While a few patients have cough symptoms (8, 10.26%). Four children had hepatosplenomegaly (5.13%) and two patients only had splenomegaly (2.56%). These symptoms did not differ significantly between the two groups. A total of 69 of the children (88%) lived in the countryside. Habitual drink of raw dairy products, especially goat milk, was present in 53% (n=41) of the cases. About 21% (n=16) of the patients had family members engaged in animal husbandry or suffered the disease. The remaining 27% (n=21) patients had no clear epidemiological history.

**Bacterial Culture:** Blood cultures is used as gold standard for laboratory diagnoses. As is known to all blood culture is a routine test for febrile patients. This study found that pathogenic bacteria were detected in blood culture in only 73 (93%) patients. And the positive alarm time was concentrated in 1.98~3.12 d (Table 2). The positive rate of bone marrow culture was lower than that of blood culture. These bone marrow cultures have a long warning time. Although blood culture is the ideal diagnostic method, the slow growth of the bacteria reduce the sensitivity of the assay.

**Laboratory Analysis Characteristics:** Laboratory analysis results of the case group with or without osteoarticular involvement and control group were compared by
Mann-Whitney U test (Table 3). The study found there was no difference in white blood cells (WBC) among three groups. However, there were significant differences in NEU, CRP and ALT. At the same time, we found differences in Hb, ESR, and ferritin only between the osteoarticular involvement group and the control group, but not between the without osteoarticular involvement group and the control group.

NEU in the case group was lower than that in the control group \( (P<0.05) \), especially in patients with osteoarticular involvement. However, the levels of CRP and ALT in case group were higher than those in control group \( (P < 0.05) \). The median values of ESR and Ferritin were increased remarkably in the group with osteoarticular involvement than the other two groups \( (P < 0.05) \). Laboratory results showed that patients with bone involvement were often accompanied with more serious with anemia. ROC curves were drawn to compare the predictive values of the laboratory test results in the study (Fig 2). The analysis revealed that AUC for NEU, CRP and ALT were greater than 0.5. And the p-values for laboratory test were less than 0.05 \( (\text{NEU } P=0.016, \text{CRP } P=0.002, \text{ALT } P=0.039) \).

**Discussion**

Brucellosis is one of the most widespread zoonotic infectious disease which remains endemic in developing countries(11-13). Now the zoonotic infectious disease that remains endemic in Western China. Especially in recent years, the incidence of the disease has a rising trend. Owing to its clinical symptoms were not specific, and lack of awareness among clinicians, the disease is often diagnosed lately or misdiagnosed in children(14,15). Children undiagnosed frequently lead to delay in
treatment, which could potentially harm children’s health. In this study, data of children with brucellosis from 2015 to 2020 were collected, and the results showed that incidence of the disease was on the rise. Combined with the medical history, it was found that the high incidence was related to some people's belief that raw milk had high nutritional value. So drinking unpasteurized milk that has been contaminated with brucella can increase the risk of infection in children. These results are consistent with previous studies(16,17). Most cases were admitted in the first and second quarters, and the lower rate of cases was detected in the third and fourth quarters of the year. It is well known that spring to summer is the goat's birth season and contaminated goat milk is easily brought to the table, which may be related to the seasonal differences in incidence.

Brucellosis has a rich clinical manifestation, especially in children, which is indistinguishable from other diseases. From our analyzed data, it shows that the main clinical manifestations of childhood brucellosis are fever, arthralgia, fatigue and sweating. Long-term fever and arthralgia are important signs for physicians to make early consideration for brucellosis(18). A few children with chronic period have hepatosplenomegaly. More than one third (44.87%) of children with brucellosis presented major clinical symptom is arthralgia. These children often go to orthopedic department for the first time see the doctor, so the orthopedic surgeon should be aware of brucellosis. A few children with brucellosis had hepatomegaly, splenomegaly, and enlargement of lymph nodes. The result of present study is almost consistent with that reported by previous studies(19,20). According to the study,
brucellosis was more frequent in preschool-aged children (1-6 years old), which was similar to the study of Ghadi Mohammed Al Hashan et al(21). Children of this age group have low immunity and frequent exposure to dairy products, which might be the important reason for high incidence. Most of the cases lived in the countryside. The results may be related to poor rural conditions and many children drink fresh dairy products directly after weaning.

In view of the fact that the symptoms of childhood brucellosis is complex, and epidemiological history of fuzzy, which often lead to misdiagnosis and delayed treatment(22,23). When the possibility of the disease is not considered, Brucella-specific serodiagnostic assays and nucleic acid amplification tests are not requested, and thus, the diagnosis can be overlooked(24-26). In this survey, the diagnostic value of routine laboratory testing items to brucellosis were evaluated. Blood culture, as part of the routine workup of a nonspecific febrile syndrome, is the gold standard for laboratory diagnoses of brucellosis(27). The results of the study found that the positive rate of Brucella in blood culture was 93.59% , and the alarm time of blood culture was concentrated in 1.98~3.12 d, which are generally longer than other common bacteria. Meanwhile, 7 patients had bone marrow culture and the positive rate was 42.86%. The isolated strains were confirmed as B.melitensis by the CDC laboratory, which was consistent with the survey data from China CDC Weekly. Long generation time and low concentration of circulating bacteria can affect test results and delay diagnosis. Although routine laboratory testing do not have definite diagnostic value, the changes in results can prompt clinician to carry on the further
brucella-specific serodiagnostic assays and nucleic acid amplification tests. In our study, there was no significant difference in white blood cell count between case group and control group, which similar to the study conducted by Kazanasmaz (28). But we found neutrophils count in case group was significantly reduced. Other indicators in patients with bone involvement, such as ESR, CRP and ALT values, were higher than control group. Furthermore, we found that Hb is significantly different in bone involvement group from control group. However, Hb, ESR and Ferritin only changed in osteoarticular involvement group and there was no difference between group without osteoarticular involvement and control group. The ROC analysis revealed that NEU, CRP and ALT may have a complementary role in the differential diagnosis of childhood brucellosis. These abnormal laboratory findings were accordance with the results of other studies (29-31). Clinical and laboratory characteristics of pediatric brucellosis patients with osteoarticular involvement are differ from cases without skeletal complications. Patients with osteoarticular involvement presented more frequently with fever and myalgia than those without involvement. Additionally, most important abnormal laboratory findings, such as ESR, CRP, ALT and Ferritin appears to be increased in children with osteoarticular involvement. Patients with joint involvement often develop more severe anemia, which was similar to the study of Çiftdoğan DY (32).

In conclusion, we found that the main clinical symptoms of childhood brucellosis are fever, fatigue, arthralgia, sweating. Therefore, diagnosing brucellosis based on nonspecific symptoms is still an issue for physicians, especially working in
non-epidemic areas. As is known to all that suspicion was important to approach diagnosis. In the multivariate analysis, we found a significant correlation between routine laboratory parameters such as ESR, Hb, Ferritin, NEU, CRP, and ALT to childhood brucellosis with ostéoarticular involvement. Among them, ESR, Hb and Ferritin are more meaningful for diagnosis of childhood brucellosis without ostéoarticular involvement. The results of these routine laboratory tests and main clinical manifestations can prompt the clinician to conduct further tests by ideal diagnostic method such as serology and the polymerase chain reaction assay. In addition to the blood culture, declining indicators (NEU, Hb) and rising indicators (CRP, ESR, ALT, Ferritin) may have a complementary role in diagnosis of childhood brucellosis. Ostéoarticular involvement should be considered as a key role in children infected with brucella. As noted above, the results of this study can help physicians to make early consideration and diagnosis of childhood brucellosis, so that the disease can be treated in time.

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Authors’ contributions Wei Wang and Wang Lin designed the study. Wei Wang analyzed the data and wrote the draft of the manuscript. Wang Zengguo, Jia Kai and Tang Jianyong collected the blood samples and performed laboratory test. All authors contributed to revising the manuscript and approved the submitted version.
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**Conflict of interest** All authors have declared that no potential conflict of interest relevant to this article.

**REFERENCES**


Fig 1 The trend of childhood brucellosis detection in different quarters of the study period
Fig 2 Comparison of specificity and sensitivity with receiver operating characteristic graph

Table 1 Comparison of clinical characteristics of patients with/without osteoarticular involvement

Table 2 The positive alarm of blood culture and bone marrow culture in children with brucellosis

Table 3 Comparison of laboratory characteristics among patients with/without osteoarticular involvement and control group
### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Childhood brucellosis (N=78)</th>
<th>with osteoarticular involvement (N=35)</th>
<th>Without osteoarticular involvement (N=43)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (1-6 years old)</strong></td>
<td></td>
<td></td>
<td></td>
<td>59 (75.64)</td>
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<td></td>
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<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td>42 (53.85)</td>
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<tr>
<td><strong>Fever</strong></td>
<td></td>
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<td>71 (91.03)</td>
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<tr>
<td><strong>Fatigue</strong></td>
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<td>34 (43.59)</td>
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<tr>
<td><strong>Sweating</strong></td>
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<td></td>
<td>26 (33.34)</td>
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<tr>
<td><strong>Myalgia</strong></td>
<td></td>
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<td>24 (30.77)</td>
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<tr>
<td><strong>Lack of Appetite</strong></td>
<td></td>
<td></td>
<td></td>
<td>16 (20.51)</td>
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<td></td>
<td></td>
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<tr>
<td><strong>Vomiting</strong></td>
<td></td>
<td></td>
<td></td>
<td>6 (7.69)</td>
</tr>
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<tr>
<td><strong>Cough</strong></td>
<td></td>
<td></td>
<td></td>
<td>8 (10.26)</td>
</tr>
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<tr>
<td><strong>Hepatomegaly</strong></td>
<td></td>
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<td></td>
<td>4 (5.13)</td>
</tr>
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<tr>
<td><strong>Splenomegaly</strong></td>
<td></td>
<td></td>
<td></td>
<td>2 (2.56)</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Bacterial culture</th>
<th>No. of bacterial culture cases</th>
<th>No. of positive bacterial culture cases</th>
<th>Mean positive alarm time Median (Min-Max), day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>78</td>
<td>73</td>
<td>2.16 (1.98-3.12)</td>
</tr>
<tr>
<td>Bone marrow culture</td>
<td>7</td>
<td>3</td>
<td>2.55 (2.24-2.67)</td>
</tr>
<tr>
<td>Blood culture (reviewed after treatment)</td>
<td>62</td>
<td>5</td>
<td>4.19 (3.86-7.31)</td>
</tr>
</tbody>
</table>
Table 3

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Childhood brucellosis (N=78)</th>
<th>Control group (N=78)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with osteoarticular involvement (N=35)</td>
<td>without osteoarticular involvement (N=43)</td>
<td>Total</td>
</tr>
<tr>
<td>WBC, x10^9/L</td>
<td>7.39 (2.37-12.76)</td>
<td>6.32 (2.42-13.18)</td>
<td>7.12 (2.37-13.18)</td>
</tr>
<tr>
<td>NEU, x10^9/L</td>
<td>1.93 (0.19-6.55)$#$</td>
<td>3.07 (0.56-14.41)$#$</td>
<td>2.86 (0.19-14.41)</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>99 (89-134)$#$</td>
<td>125 (101-137)$#$</td>
<td>104 (89-137)</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>25 (9-48)$#$</td>
<td>9 (5-22)$#$</td>
<td>21 (5-48)</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>17.12 (2.42-57.69)$#$</td>
<td>9.67 (2.17-23.69)$#$</td>
<td>10.99 (2.17-57.69)</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>45 (26-291)$#$</td>
<td>22 (10-154)$#$</td>
<td>41 (10-291)</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>187.03 (19.08-251.35)$#$</td>
<td>102.00 (10.56-206.13)$#$</td>
<td>142.53 (10.56-251.35)</td>
</tr>
</tbody>
</table>

#: p<0.05 (between control and patients with osteoarticular involvement or those without it)
$#: p<0.05 (between patients with osteoarticular involvement and those without it)