Role of samples selection in diagnosis of Brucellosis in patients with localized joint pain in Najaf province by Q PCR

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Abstract

Background: Brucella species are Gram negative, coccobacilli bacteria, facultative, intracellular organism, environmental persistence for temperature, pH, humidity, Frozen and aborted materials, they are multiple species of brucella some of them are highly weaponizable like B. sues. Brucellosis can affect any organ or system and 20-60% of cases are presented with osteoarticular complications (Arthritis, spondylitis, & osteomyelitis). Polymerase chain reaction (PCR) is commonly used to diagnose infectious diseases. The use of PCR for pathogens detection has high sensitivity, and high specificity, in diagnosis. PCR is the most useful assays for the diagnosis of human brucellosis.

Objective: The aim of this review was to clarify existence of pathogen of security concern like brucella spp. in biohazard region, in addition to select the most appropriate samples in diagnosis by QPCR and their relation to chronic ache.

Methodology: This study was conducted in the period between Jan. 2015 and June. 2015 in Al-Najaf city-Iraq/Al-Najaf medical private lab. this data were analysed by Chi square, Study group include 49 patients with chronic localized ache from those patients we toke 63 samples include blood, and/or tissue biopsy from site of pain, and/or CSF, blood samples were collected as 5 ml → 2 ml in EDTA tube & 3 ml in serum tube, and CSF samples were collected in plane tube, while tissue biopsy submitted for DNA extraction as fresh samples immediately then these samples submitted for QPCR for diagnosis.

Results: the percentage of brucellosis in Najaf city’s patients with localized joint pain was about one quarter, both tissue and blood sample can be regarded as useful diagnostic tool for diagnosis of human brucellosis by RT-PCR.

Conclusion: Brucella is regarded as endemic in Iraq and both blood and tissue samples are best selective samples in diagnosis of brucella.

Recommendation: Brucella should be considered as a differential diagnosis in cases of localized ache.

Keywords: Human brucellosis; Brucella spp; QPCR
### INTRODUCTION

*Brucella* is a genus of Gram-negative bacteria\(^1\), they are small (0.5 to 0.7 by 0.6 to 1.5 µm), facultatively intracellular coccobacilli\(^2\), which lack capsules, flagellae, endospores or native plasmids. *Brucella* is the cause of human brucellosis which is emerging as a serious animal and public health issue in many parts of the world\(^3\). Transmission of brucellosis through conjunctiva or broken skin contacting infected tissues, blood, urine, vaginal discharges, aborted fetuses, placentas, or ingestion of raw milk & unpasteurized dairy products, also laboratory workers by inhalation, and rarely through undercooked meat,\(^4\)\(^5\)\(^6\) In Iraq there is a public Famous cheese which is homemade unpasteurized named arabadcheese is suspected to be the source of infection. Brucellosis can affect any organ or organ system, and 90% of patients have undulant fever, headache, weakness, arthralgia, depression, weight loss, fatigue, and liver dysfunction. About 20-60% of cases have osteoarticular complications - arthritis, spondylitis, or osteomyelitis, while 2-20% of cases can have genitourinary involvement and sometime the patients are presented with neurological symptoms include depression and mental fatigue, evencardiovascular system can be involvedlike endocarditis. Chronic brucellosis is hard to diagnosis while localized infection can occur frequently, and some cases are asymptomatic\(^6\). *Brucella* species have been found primarily in mammals\(^7\).

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. melitensis</em></td>
<td>Goats and sheep</td>
</tr>
<tr>
<td><em>B. abortus</em></td>
<td>Cattle</td>
</tr>
<tr>
<td><em>B. canis</em></td>
<td>Dogs</td>
</tr>
<tr>
<td><em>B. suis</em></td>
<td>Pigs</td>
</tr>
<tr>
<td><em>B. ovis</em></td>
<td>Sheep</td>
</tr>
<tr>
<td><em>B. neotomae</em></td>
<td>desert woodrat (Neotomaepida)</td>
</tr>
<tr>
<td><em>B. pinnipedialis</em></td>
<td>Seal</td>
</tr>
<tr>
<td><em>B. ceti</em></td>
<td>dolphin, porpoise, whale</td>
</tr>
<tr>
<td><em>B. microti</em></td>
<td>common vole (Microtus arvalis)</td>
</tr>
<tr>
<td><em>B. inopinata</em></td>
<td>Unknown</td>
</tr>
<tr>
<td><em>Brucella</em> sp. NVSL 07-0026</td>
<td>Baboon</td>
</tr>
</tbody>
</table>

* B. melitensis: Accounts for most human cases in the Mediterranean and Middle East, up to 78 cases/100,000 people/year, and represent 20% seroprevalence in Arabic Peninsula\(^8\), while B. abortus is worldwide Notifiable disease in many countries most cases were presented as Fever of Unknown Origin and because of lack of recognition poor surveillance and reporting was common\(^9\).
Diagnosis in Humans

Isolation of organism (Culture on Castaneda medium) from blood, bone marrow, or other tissues\textsuperscript{(10)}. Serum agglutination with a titer of $> 1:160$ in the presence of a compatible illness supports the diagnosis of brucellosis. Demonstration of a four-fold greater increase or decrease in agglutinating antibodies over four to 12 weeks provides even stronger evidence for the diagnosis. ELISA is probably the second-most common serologic method. Immunofluorescence test (indirect) is widely used in diagnosis of brucellosis. As for other fastidious pathogens, molecular methodology offers an alternative way of diagnosing brucellosis, and define the optimal clinical specimen of human origin for this test. PCR, characterized by high sensitivity and specificity and short time can overcome the limitations of conventional methodology\textsuperscript{(11)}.

Q PCR (Real time PCR)

Real-time PCR is a valuable technique in determining the quantification of nucleic acids in clinical specimen of human origin. Recently, real-time PCR for the rapid detection and differentiation of \textit{Brucella} species in clinical samples has recently been developed, targeting 16S-23S internal transcribed spacer region (ITS) and the genes coding omp25 and omp31\textsuperscript{(12)}, BCSP31\textsuperscript{(13)(14)(15)(16)}.

**METHODOLOGY**

Subjects:
This study was conducted in the period between Jan. 2015 and June. 2015 in Al-Najaf city-Iraq/Al-Najaf medical private lab.
Study group include 49 patients with chronic localized ache (small joint, localized backache in vertebra and/or headache) from those patients we take 63 samples include blood, and/or tissue biopsy from site of pain, and/or CSF, because not all of patients were agree to give more than one sample or submitted for surgical biopsy or CSF puncture. Blood samples were collected as 5 ml $\rightarrow$ 2 ml in EDTA tube & 3 ml in serum tube, and CSF samples were collected in plane tube, while tissue biopsy submitted for DNA extraction as fresh samples immediately.

PCR Kit:

**Bosphore Brucella Detection Kit v1**

Bosphore\textsuperscript{TM} Brucella Detection Kit v.1 detects Brucella DNA, encompassing all the major Brucella genotypes (\textit{B. abortus}, \textit{B. melitensis}, \textit{B. canis}, \textit{B. suis}, \textit{B. ovis} and \textit{B. microt}). A region within the \textbf{BCSP31 gene} of Brucella genome is amplified and fluorescence detection is accomplished using the FAM filter. The kit contains an internal control which checks PCR inhibition. The amplification data of the internal control is detected with the Cy5 filter. The internal control can be added during PCR step.

Positive control $Ct=33(+/-2)$

Sensitivity : $7.5 \times 10^0 \text{copy/ml}$
The Agilent Mx3005P (stratagene 3005P) QPCR Systems from Agilent Technologies is the most flexible—and reliable—instruments for pathogen detection. Agilent’s qPCR software, MxPro, provides users with an intuitive interface, quick experiment design, powerful data analysis and easy report generation. (Made in Germany)

Thermal profile

N.B: Number of PCR cycles=50

RESULTS

Table (1): Demographical Characteristics distributed in the patients age groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Samples</th>
<th>Age Groups/years</th>
<th>Frequency</th>
<th>Number of Positive cases</th>
<th>Percent of positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Groups</td>
<td>PCR</td>
<td>1-20</td>
<td>12</td>
<td>1</td>
<td>8.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21-40</td>
<td>21</td>
<td>6</td>
<td>28.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41-60</td>
<td>11</td>
<td>3</td>
<td>27.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61-80</td>
<td>5</td>
<td>2</td>
<td>4.0</td>
</tr>
</tbody>
</table>

This table show that the majority of positive cases were higher in older age.

Table (2): Samples types in the patients Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Samples</th>
<th>Groups</th>
<th>Positive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>QPCR</td>
<td>Sixty three sample From 49 patients</td>
<td>Blood (33 sample)</td>
<td>8</td>
<td>24.24 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tissue (16 sample)</td>
<td>4</td>
<td>25 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSF (14 sample)</td>
<td>0</td>
<td>0 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total patient’s number=49</td>
<td>12/49</td>
<td>24.48 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total samples=63</td>
<td>12/63</td>
<td>19.04 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of patients with two different samples simultaneously= (14)</td>
<td>6/14 (3 tissue+ 3 blood)</td>
<td>42.85 %</td>
</tr>
</tbody>
</table>

$\times c$ Contingency Table: Results(Blood, Tissue & CSF)
The results of a contingency table $X^2$ statistical test
chi-square = 4.24
degrees of freedom = 2
probability = 0.120
Note: One patient has 2 tissues sample first from inflamed portion and second from the adjacent part, the former one was positive.

Fig. 1 Percentage of Brucella in patient with localized pain
This figure is show approximately one quarter of patients with localized joint pain have brucella.

Fig. 2 Percentage of Brucella in Blood and CSF
There is a significant p value between the selections of blood or tissue sample versus CSF for QPCR detection of brucellosis.

Fig. 3 Percentage of Brucella in Tissue and CSF

\[ r \times c \] Contingency Table:
Results(Tissue & CSF)
chi-square = 4.04
degrees of freedom = 1
probability = 0.044
DISCUSSION

The majority of positive cases were higher in older age groups, which is consistent with Cetinkaya et al. (17), so peaks of brucella appear in elderly, due to the ‘immunosenescence in elderly individuals (>65 years old). So loss function of immune system has been associated with increased susceptibility to diseases. In other hand the Brucellosis can occur at any age and also common in adolescents and young adults, where the majority of the population usually consume unpasteurized dairy products like cheese collected from rural area (18).

So this study show the percentage of brucellosis in patients with localized joint pain was 24.48% so about one quarter of those patients in Najaf were infected, this indicate the incidence of brucella is high in Iraq, unfortunately there is no precise statistical study about the incidence of brucellaspp in our country, but most of studies consider brucella as endemic disease in countries of the Mediterranean basin, Arabian Peninsula, and Arabian Gulf (19).

This study indicate the CSF samples have poor role in diagnosis of brucellaspp in those patients even whose had had vertebral brucellosis, and this results are inconsistent with (13), while 25% of positive cases of brucella diagnosed through tissue samples, in other aspect about 24.24% of blood samples were positive, that is mean both tissue (20) and blood sample can be regarded as useful diagnostic tool for diagnosis of human brucellosis by PCR detection, so the RT-PCR is a confirmatory test for accurate diagnosis of individuals infected with brucellosis (21).

CONCLUSIONS

1. Brucella is endemic in Iraq and is pathogen of security of concern so the degree of awareness should be increased.
2. Blood and tissue biopsies are recommended samples for diagnosis.
3. Tissue biopsy from inflamed portion increase the reality of test
4. CSF are poor selective sample in diagnosis of brucella.

RECOMMENDATIONS

Brucella should be considered as a differential diagnosis in cases of localized ache.

Increase awareness level for persistance of Brucella in biohazard regions because it is considered as pathogen of security of concern.
REFERENCES:


