

## Diagnosis of *Brucella abortus* infections in patients suffer from PUO

### In Basrah governorate

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### Abstract

A total number (130) of patients whom suffering from pyrexia unknown origin blood samples were collected, 20 sera sample from healthy individuals (blood donors) were taken and used as control group.

Out of the total number of samples 11(8.4%) were showed growing isolate of *Brucella abortus* on Castaneda Di-phasic medium which were diagnosed by biochemical tests. Enzyme Linked Immune Sorbent Assay (ELISA) was done for screening IgM and IgG, the results were revealed from 50 samples, 25(50%) positive for IgM in acute infections and 7(14%) positive for IgG in chronic infections. All 20 control samples were negative for the previous tests.

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Key words: Brucellosis, Diagnostic tests, ELISA, Serologic tests.

## Introduction

Human brucellosis is a potentially life-threatening multisystem disease, its zoonotic disease of bacterial origin (Sauret and Vilissova, 2002) caused by members of the genus *Brucella*. In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs, human and animal brucellosis share the persistence of the bacteria in tissues of the mononuclear phagocyte system, including the spleen, liver, lymph nodes, and bone marrow (Manteconet *et al.*, 2008). Most cases are caused by occupational exposure to infected animals or the ingestion of unpasteurized or contaminated dairy products, also by inhalation of contaminated dust or aerosols (Atluriet *et al.*, 2011). In human the disease can come in acute and chronic forms. Acute symptoms include fever, chills as well as an aching head and back. In its chronic form joint pain and fatigue can also result from the disease.

Brucellosis results from infection by various species of *Brucella*, a Gram negative, facultative intracellular coccobacillus or short rod in the family Brucellaceae. Six named species occur in animals: *Brucella abortus*, *Brucella melitensis*, *Brucellaisuis*, *Brucellaovis*, *Brucella canis* and *Brucella neotomae* (The center of food security and public health, 2009 ;Roushan *et al.*, 2010 ).

Laboratory diagnosis of human brucellosis is based on the isolation of *Brucella* spp. from blood cultures and on the demonstration of the presence of specific antibodies through the use of serological tests (e. g. slide or tube agglutination, Brucella capt, immunochromatographic lateral flow, enzyme linked immunosorbent assay (ELISA) and the indirect fluorescent antibody test) (Poester *et al.*, 2010 ; Curry *et al.*, 2011). Laboratory diagnosis of brucellosis is frequently based on demonstration of the presence of serum antibodies (Araj, 2010

;Vancelic *et al.*, 2008). An enzyme linked immunosorbent assay (ELISA) mostly used for detection and quantitation of antibodies against *Brucella* (Ertek *et al.*, 2006 ; Arabaci and Oldacay, 2012) and assessment the diagnostic value of *Brucella* ELISA IgG and IgM in patients with brucellosis (Gomiz *et al.*, 2008).

**Aim of this study:** the present study deals with usefulness of ELISA test in the diagnosis of human brucellosis in Basrah governorate, and significance of ELISA to determine the levels of *Brucella* specific IgG and IgM to determine the acute and chronic infections.

#### **Patients and clinical specimens**

A total of one hundred-thirty adult patients whom suffering from PUO (pyrexia unknown origin) were admitted to al-Mawani General Hospital between June 2007 to June 2008. Patients ranges between 20- 69 years, forty-three females and eighty-seven males.

Ten milliliter blood samples were obtained, 5ml were subjected to blood culture, the other were extracted sera and frozen at  $-20^{\circ}\text{C}$  until processing for Enzyme Linked Immune Sorbent Assay (ELISA) test that used for screening the presence of specific antibodies. Totally 20 sera sample from healthy individuals (blood donors) were taken and used as control group.

#### **Bacteriological and serological methods**

Blood culture: 5ml of blood were inoculated on Castaneda Di-phasic medium slants, incubated at  $37^{\circ}\text{C}$  for 21 days. The grown colonies on Castaneda Di-phasic medium slants were re-cultivated on *Brucella* agar, MacConkey agar, blood agar and chocolate agar, incubated at  $37^{\circ}\text{C}$  for 3-5 days to confirm diagnosis, then diagnosed by biochemical tests (Goerge *et al.*, 2005). While the other 5ml was centrifuged to get

serum that subjected to ELISA test(Joung, 2000).

### Results and Discussion

From total number of 130 patients that suspected with brucellosis, 11(8.4%) patients were infected with *Brucella abortus* that isolated from Castaneda Di-phasic medium slants (Table 1).The re-cultivated colonies on,

*Brucella* agar, MacConkey agar, blood agar and chocolate agar were appeared as small, slow growing white colonies, non-haemolytic, non lactose and glucose fermenter, microscopically were appeared as Gram negative non motile coccobacilli. The previous results were confirmed with further diagnosis by biochemical tests (Table 2).

**Table (1). Results of blood samples cultivation on Castaneda Di-phasic medium**

The patients age	Number (percentage)	Male	Female
20-29	1 (0.7%)	0	1 (9%)
30-39	6 (4.6%)	5 (45.4%)	1 (9%)
40-49	2 (1.5%)	2 (18.1%)	0
50-59	0 (0%)	0	0
60-69	2 (1.5%)	2 (18.1%)	0

**Table (2).Biochemical tests applied on *Brucella abortus***

Isolated bacteria	CO <sub>2</sub> requirement	Iron Kligler	Urease production	Citrate utilization	Catalase test	Oxidase test
<i>Br.abortus</i>	+	+	+	-	+	+

Cultural features of colonies and Gram's stain were gave an indicator for the presence of *Brucella spp.*, but prolonged incubation period and costly culture media used in isolation made the culture un dependence way in diagnosis, beside that in most cases the patients were received bulk of antibiotics that affect the results of culture.

Diagnosis of brucellosis requires isolation of the causal agent. Blood culture is the method of choice, but specimens need to be obtained early, and culture often need long periods of incubation. In addition, failure to detect the pathogen is a frequent occurrence, beside that the blood culture not have the capacity to specify the acute and chronic infections, these limitations make serology the most useful tool for laboratory diagnosis of *Brucella* infections (Lopez-Goni and O'Callaghan, 2012 ; Serra and Vinas, 2004), its based on demonstration of

the presence of serum antibodies, various laboratory tests are used for this purpose. Rose Bengal test is commonly used to screen for brucellosis infections (Aliskan, 2008), but the agglutination techniques may have limitations in sensitivity due to prozone phenomena, which produce false negative results. These techniques may also results in non specific agglutination reactions due to the presence of antibodies against bacteria with antigenic determinant common with *Brucella spp.* such as *Yersinia enterocolitica*, *E. coli*, *Salmonella urbana*, *Campylobacter fetus*. The non specific agglutination produce false-positive results affecting the specificity of the tests (Uzalet *al.*, 1995).

### **ELISA test**

In 130 patients were suspected with brucellosis the diagnostic values of IgG and IgM have been evaluated. From the total number of patients 50 serum samples were tested by ELISA

for IgG and IgM antibodies (including the 11 patients with positive blood culture) and the results were revealed that 25(50%) IgM which represented

acute infections, 7(14%) IgG which represented chronic infections. All 20 controls were negative for IgG and IgM (Table 3).

**Table (3).Evaluation of IgM and IgG values by ELISA test**

<b>Patients according to age</b>	<b>IgM</b>	<b>IgG</b>
20-29	2(8%)	1(14.2%)
30-39	12(48%)	4(57.1%)
40-49	4(16%)	0
50-59	4(16%)	0
60-69	3(12%)	2(28.5%)

ELISA test demonstrate a high specificity in this study, its reliable and sensitive test in the diagnosis of brucellosis, the results of this study showed that ELISA is an excellent method for screening large population for *Brucella* antibodies, and have the sensitivity and specificity to differentiate between acute and chronic phases of the disease depending on the presence of IgG and IgM values. The obtained results were

compatible with the studies of (Peeridogahah *et al.*, 2013; Pabuccuoglu *et al.*, 2011 ; Araj *et al.*, 1986 ; Osoba *et al.*, 2001 ; Diaz *et al.*, 2011).

According to the results of the current study the ELISA is accurate rapid diagnostic assay and it can be useful for the determination of acute and chronic infections in human brucellosis. So, the present study recommended to adopt the ELISA test

in all health institutions in our country.

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## تشخيص الأصابات الناتجة عن جرثومة البروسيلا في مرضى يعانون من حمى غير مشخصة في محافظة البصرة

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### الملخص

تم جمع عينات دم من 130 مريض يعانون من حمى غير مشخصة، استخدمت 20 عينة دم من اشخاص اصحاء كمجموعة سيطرة . اظهرت نتائج العزل الجرثومي 11(8.4) عينة موجبة عند زرع عينات الدم على وسط كاستانيدا ثنائي الطور، إذ تم عزل جرثومة البروسيلا من هذه العينات واكمل تشخيص العزلات الناتجة بالأختبارات الكيمياحياتية. اجري اختبار معايرة الأمتصاص المناعي المرتبط بالأنزيم (الأليزا) للكشف عن الأضداد IgG و IgM في مصول الأشخاص المصابين ، واطهرت النتائج من مجموع 50 عينة أن 25 (50%) عينة موجبة لفحص IgM وتمثل الأصابات الحادة و 7 (14%) عينة موجبة لفحص IgG وتمثل الأصابات المزمنة. اظهرت العينات المأخوذة من مجموعة السيطرة نتيجة سالبة للأختبارات السابقة.