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RESEARCH ARTICLE

Environmental, efface and filed study for source *Brucella* disease

Inas Saad Mohammed

Environmental Research Center, Microbiology and Toxicology Laboratory, University of Technology.

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Corresponding Author

Inas Saad Mohammed

Abstract

Objective: Diagnosis of brucellosis from patients suspects of infection and their casualty for brucellosis by serological methods (Rose Bengal test) and culture method e in diagnosis of human brucellosis.

Duration and place of study: Blood and serum samples were obtained from suspected and casualty brucellosis patients, referred to many hospitals in different city Baghdad province (Karkh and Rusafa), which include: {General hospital Mohammad Baqir Al-Hakim, Al-Shaheed Al-Sadder hospital, Al-Imam Ali (peace be upon him) hospital}, as well as access to statistics and maps in all Iraq provinces of from Ministry of Health / Communicable Disease Control Center, during the duration from (March to December 2014).

Methodology: A total of 117 peripheral blood samples were from patient's suspect of infection and their casualty for brucellosis. The diagnosis of brucellosis was established by clinical findings confirmed by serological test (Rose Bengal test) and culture and confirmed by used Gram staining and different biochemical test for diagnosis of brucellosis.

Results: A total of 117 peripheral blood samples, 70 (59.82%) samples were positive result by RBT and 59 (50.42%) samples were positive result by culture was applied to patient's blood.

Conclusions: These results indicate that patients were contact with infected livestock or suspected infection of *Brucella* like: sheep, goat, cow and buffalo located in epidemiological regions in Iraq especially in Baghdad province across the study period and showed blood culture method is important for the detection of brucellosis compared with serological methods (Rose Bengal test) for the diagnosis of brucellosis.

INTRODUCTION

Brucellosis is a recognized public health problem with worldwide distribution and one of the major causes of mortality and morbidity. It is also a disease of considerable economic and social importance. Brucellosis is one of the most important reemerging zoonosis in many countries. In endemic areas, brucellosis causes high economic loss and has serious public health consequences. Worldwide; *B. melitensis* is the most prevalent species causing human brucellosis [1-3].

Brucellosis is considered a professional hazard among laboratory technicians and veterinarians who work in areas where it is endemic [4]. Physicians who treat patients with brucellosis, however, are not regarded as having an increased risk, because person-to person

transmission of the disease is extremely uncommon. In the few proven cases of acquisition of the infection from human sources, mother-to-offspring transmission through the placental circulation, exposure to mother's fomites during delivery, breast-feeding [5,6], blood transfusion [7], bone marrow transplantation [8], and sexual contact [9] have been implicated.

The principal routes of infection for humans is food borne transmission via ingestion of contaminated unpasteurized milk or dairy products (fresh cheese) and occupational or environmental direct exposure (infected calves, placentas, amniotic fluids and other secretions and excrements of infected animals, either by contact with skin cuts and abrasions, conjunctival contamination or via inhalation of infectious aerosols [10,11,12-14]. It takes from 5 to 90 days (usually 14 days) from infection to the first sudden severe symptoms of the disease [15, 16]. Division of the genus into six classical species *Brucella*,namely *B. melitensis, B. abortus, B. suis, B. canis, B. ovis* and *B. neotomae*, is still widely used due to historical and clinical reasons [17]. *B. melitensis*, *B. suis* and *B. abortus* are considered the most pathogenic species for humans and have small ruminants, pigs and cattle as preferential hosts, respectively [18].

In addition, two recently identified *Brucella* species isolated from marine mammals, *B. ceti* and *B.pinnipedialis*, can also cause human brucellosis [19]. Importantly, *B. canis*, a pathogen of dogs, has a comparatively low zoonotic potential, while *B. neotomae* and *B. ovis* that infect desert rats and sheep and, respectively, are not associated with human disease [18].

Brucellosis in humans is known as "undulant fever" or "Mediterranean fever", "Malta fever" or "Bangs disease" [20, 21]. It is a systemic infection and may present in many atypical forms, from mild to severe acute infections in about half of the cases. Human brucellosis is considered as a life-threatening debilitating disease characterized by weakness, fever, malaise, arthritis, osteomyelitis, endocarditis or meningoencephalitis [22]. In domestic animals, the disease occurs as a chronic infection that results in placentitis and abortion in pregnant females [23,2 4] or orchitis and epididymitis in males [24].

The aim of the study is to analyze available data and present the frequency and distribution of brucellosis in humans in different region in Baghdad in the period March to December 2014 by gender, age and place of residence. In addition, the study intends to review the most important factors of the appearance and spread, and the approaches for control and eradication of *Brucella* infection in humans in different region in Baghdad

Materials and Methods:

A total of 117 peripheral blood specimens were collected from patients with high suspected and casualty of brucllosis, referred to General hospital Mohammad Baqir Al-Hakim, Al-Shahee Al-Sadder, Al-Imam Ali (peace be upon him) in Baghdad. The samples were taken from patient suspected to be with brucellosis before and after adequate antibiotic treatment and from casualty patients for brucellosis, during the period from March to December 2014.

The diagnosis of brucellosis was established by the presence of a compatible clinical picture [25] including undulant fever, night sweat and serological diagnosis was carried by positive Rose Bengal test titer of ≥1:160 and culture method, moreover demographic, occupational, clinical, and risk factor details were recorded for each patient.

The Statistical Analysis System- SAS [26] was used to effect of different factors in study parameters. Chi-square test was used to significant compare between percentages in this study.

Serological tests:

- Rose Bengal test (RBT):-

The RB test was performed, following the procedure described by Alton *et al*. [27]. The plates were shaken for 4 min and any agglutination that appeared within this time was recorded as a positive reaction.

Traditional test:

- Culture and biochemical test:-

All media were prepared according to the manufacturing company instructions; *Brucella* agar or Trypticase soy agar were used sterilized by autoclaving at 121ºC for 15 min, after cooling the media to 56 ºC, they were brought to antibiotics with 5% of fetal calf serum for *Brucella* nutrition and mixture with media [28] and put in petri dish. Otherwise the media were incubated at 37 ºC for 24 hours to ensure sterility. On the other hand, they were brought media (Blood agar) and brought Trypticase soy broth were prepared according to the manufacturers company instructions; and then sterilized by autoclaving at 121 ºC for 15 min.

Five milliliters of blood were taken from each patient and divided into identical parts. One part was collected in EDTA and the serum was separated from the second part, was aliquot and store at -20°C until processing. The first

part of the blood with anticoagulant was inoculated into: Blood agar, *Brucella* agar, trypticase soya agar and trypticase soya broth culture medium containing both a solid and a liquid phase [29]. Then it was subculture on duplicate agar plates and incubated one in air and the other in an atmosphere at 37°C in the presence of 5-10% CO_2 .After 7-30 days, colonies grown in the solid phase, were identified by inoculation into *Brucella* agar or trypticase soya agar and taken the growth of colonies by loop and spreaded on the surface of plates containing blood agar media and performance of biochemical tests [30].

Results:

A total of 117 peripheral blood specimens have been collected from suspected and casualty brucellosis patients. The diagnosis of brucellosis was established by clinical findings and used different tests like serological test:- Rose Bengal test and used culture and confirmed by Gram stain and different biochemical test.

The main serological test used for diagnosis of brucellosis is the Rose Bengal test (RBT), total of 117 samples, 70 (59.82%) samples were positive RBT and 47 (40.17%) samples were negative RBT, (Table 1).

Table 1: Relation between the different region in Baghdad and serum of patients determined using RBT (Positive & Negative).

No.	Hospitals in Baghdad	Number of sample	Positive		Negative		Chi-square- χ^2
			Sample	%	Sample	%	
1	Al-Shaheed Al-Saader	11	8	72.72	3	27.27	11.39 **
2	Al-Imam Ali (peace be upon him)	90	48	53.33	42	46.66	2.04 NS
3	General hospital Mohammad Baqir Al-Hakim	16	14	87.50	2	12.50	14.27 **
Total	-	117	70	59.82	47	40.17	6.71 **
** (P≤0.01).							

**** (P<0.01) = highly significant, ns: non-significant.**

Out of 117 (89%) serum samples were detected by RBT revealed 70 (59.82%) positive, whereas 59 (50.42%) samples were positive using conventional culture method. (Figure 1).

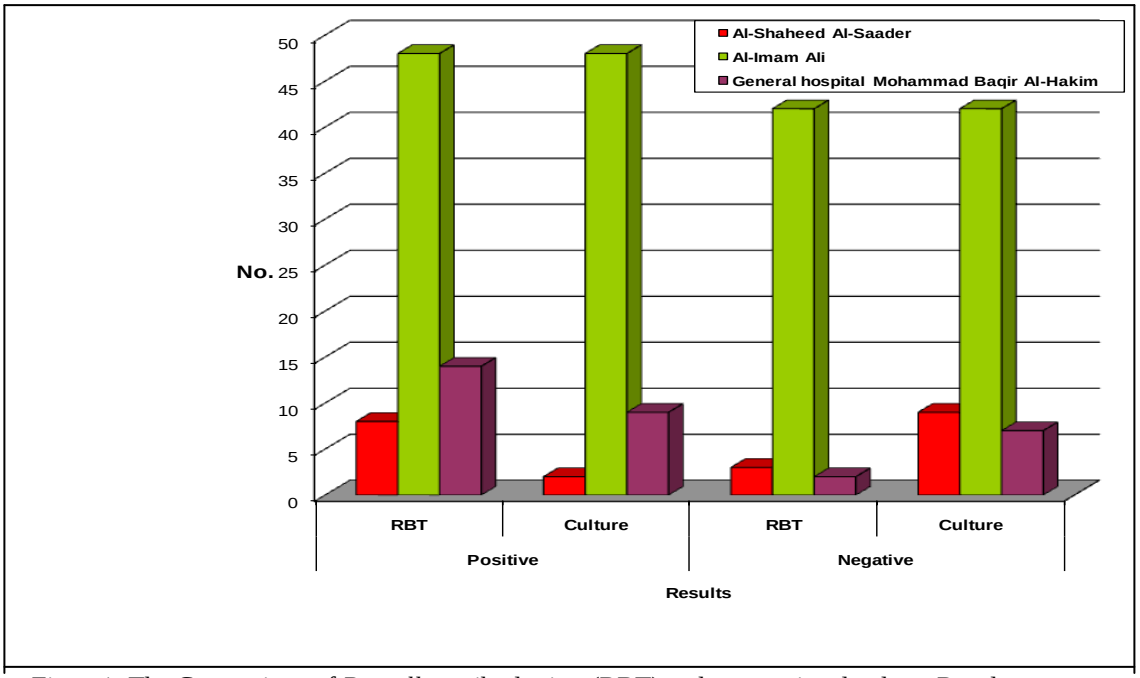

Figure1: The Comparison of *Brucella* antibody titer (RBT) and conventional culture Result.

For 59 patients (50.42 %), the diagnosis of brucellosis was established by isolated the pathogen in blood cultures. (Figure 2).

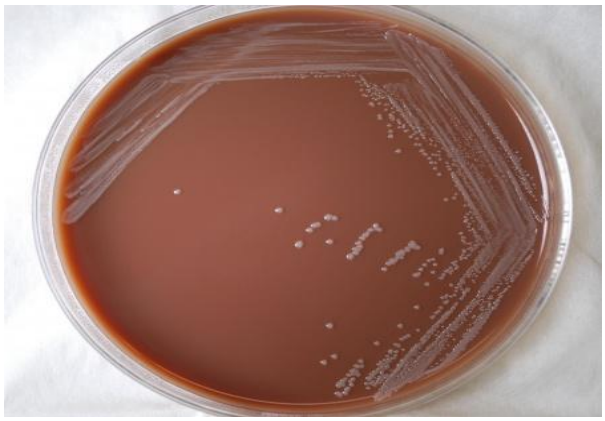

Figure 2: *Brucella* Culture on Blood Agar

The genus characterization were performed using Gram staining and identification by different biochemical tests. (Table 2).

Table 2: Biochemical Characters of *Brucella* Isolates.

No. of Test	Name of Tests	Isolates
1.	Oxidase	+
2.	Catalase	+
3.	Urease test	+
4.	Indole test	+
5.	Motility	-
6.	Production of H_2S	+

+ = Positive, - = Negative.

In present study evidence of the severity and incidence of *Brucella* in Iraq, explaining maps and statistics from Ministry of Health / Communicable Disease Control Center. (Table 3) and (Figure 3). [31]

Table 3: The scores concerted *Brucella* for different years in all Iraq provinces.

Provinces	2009	2010	2011	2012	2013
Dohuk	**295**	**393**	**230**	**129**	**40**
Erbil	**772**	**644**	**261**	**210**	**71**
Sulaymaniyah	**1077**	**1370**	**1245**	**1058**	**976**
Ninawa	**1097**	**1036**	**1027**	**567**	**191**
Kirkuk	**669**	**604**	**511**	**420**	**385**
Salahuddin	**1709**	**1241**	**1223**	**889**	**167**
Diyala	**124**	**348**	**227**	**261**	**144**
Baghdad /Al-Rusafa	**159**	**240**	**31**	**100**	**89**
Baghdad /Al-Karkh	**123**	**109**	**177**	**41**	**25**

Al-Anbar	**305**	**498**	**591**	**686**	**482**
Babil	**66**	**108**	**87**	**63**	**70**
Wasit	**78**	**135**	**49**	**107**	**112**
Karbala	**56**	**64**	**62**	**20**	**35**
Al-Najaf	**36**	**109**	**19**	**8**	**1**
Al-Dewania	**71**	**77**	**114**	**146**	**71**
Al-Muthana	**198**	**275**	**227**	**274**	**49**
Dhiqar	**4**	**12**	**39**	**19**	**11**
Maysan	**101**	**105**	**46**	**64**	**64**
Al-Basra	**7**	**34**	**57**	**3**	**8**
Total	**6947**	**7402**	**6223**	**5065**	**2991**

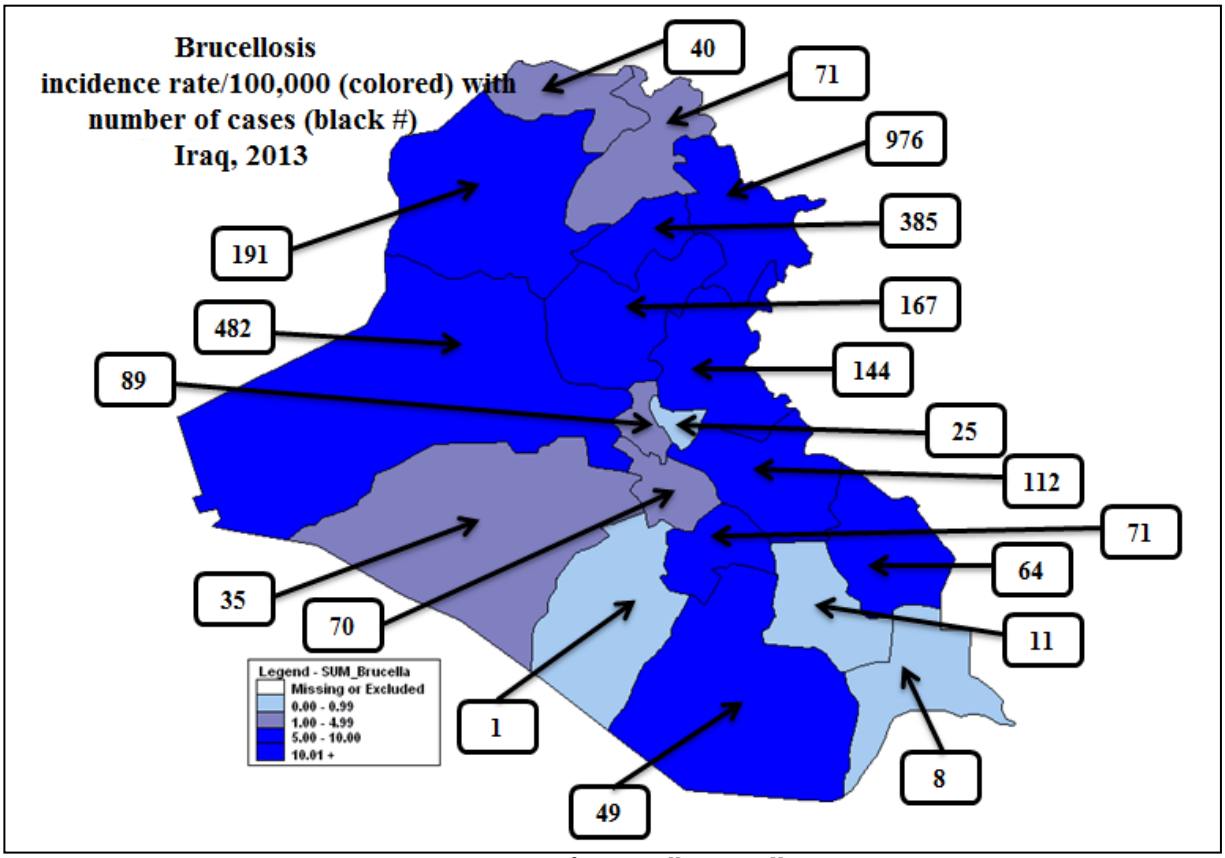

Figure 3: Map for Brucellosis in all Iraq provinces

Discussion:

Brucellosis continues to be a serious public health issue in Iraq, especially in epidemiological region in Baghdad because population who consumes unpasteurized dairy products like:- milk, cheese and also population who contact with infected animals. High, endemic level ever since. Awareness about the disease among physicians, however, is low, and in a substantial fraction of patients, diagnosis of brucellosis is only made after the causative organism is unexpectedly detected in cultures of blood or exudates specimens [32] or serological test like: Rose Bengal test.

In serological diagnosis of brucellosis in humans, The Rose Bengal test appears to have its main value in epidemiological surveys to delineate potential risk of infection in various population groups [33, 34]. False-positive results for Rose Bengal test or patients suffering from typhoid fever can occur because of cross-reactions with

antigens from other organisms, especially *Yersinia enterocolitica* O9 and to a lesser degree with other bacteria with LPS-rich outer membranes, such as *Escherichia coli* and *Vibrio cholerae* [35, 36]. The presence of 4-amino, 4, 6 dideoxymannose in the LPS is also responsible for the antigenic cross-reactivity with *Escherichia hermanni* and *Escherichia coli* O: 157, *Salmonella* O: 30, *Stenotrophomonas maltophilia*, *Vibrio cholerae* O: 1, and *Yersinia enterocolitica* O: 9 LPS (37). Therefore, the diagnosis is wrong in some cases, and that suspected typhoid fever not Malta fever.

Despite the fact that the clinical course of the disease in the herein described pregnant woman was characterized by prolonged fever and hepatic involvement, common manifestations of brucellar infections in humans [38,39], the true etiology of her illness was not suspected, and the laboratory investigation did not include either blood cultures or *Brucella* serologic tests. Moreover, the patient was regularly checked during the course of her pregnancy and was even hospitalized for a prolonged period, but the opportunity to correctly diagnose the disease and administer her specific antibiotic therapy was repeatedly missed. The fact that the antibody tests performed retrospectively on the serum samples collected many weeks before delivery were consistent with an active *Brucella* infection indicates that the diagnosis of the disease could have been made at an early stage and the congenital infection (as well as the nosocomial outbreak) could have been avoided by timely administration of appropriate antimicrobial therapy. Because of the serious associated obstetric pathology and premature delivery, it is unknown whether the death of the neonate could have also been prevented. The borderline anti-*Brucella* screening test result obtained for the mother shortly after delivery is explained by dilution of the antibody concentration by profuse bleeding and replacement of blood loss by blood products devoid of specific antibodies, whereas serum samples collected a few weeks earlier and 1 month after delivery exhibited titers that were consistent with an active infection.

Statistical analysis showed that the 70 (59.82%) patients revealed positive result by RBT and 47 (40.17%) patients negative result for RBT out of 117 patients. In this study occurs in the epidemiological region. The prevalence found in children, men, women and also pregnant women RBT of < 1/160 is problematic in areas of enedmicity, since low RBT titers may be present in healthy people who previously suffered the disease [40], in patients during the first stage of the infection [41], and in patients suffering chronic brucellosis or a relapse [42], and also for patients suffering joint pain and an increase in Erythrocyte Sedimentation Rate(ESR) and also for presence of appropriate signs and symptoms, a presumptive diagnosis of brucellosis is usually defined serologically as a RBT titer of 1/160 or greater [43]. Hence statistic showed that seropositive of brucellosis by RBT 70 (59.82%) and it is increase comprised with culture, thus 59 (50.42%) samples reported that culture was positive. The explanation for the low yield of conventional culture in present study appears to be related more to the low number of pathogen in the blood sample and use of different antibiotic treatments for various diagnostic suspicions in the other clinical sector, before samples are taken from hospitals and health centers, than to the technical difficulty of isolation *Brucella* spp. from clinical samples.

As well as the statistical analyses in this study are aware that the incidence in certain cities of Baghdad, more than others and also in certain provinces of Iraq more than others.

Considering the difficulties mentioned above, it is clear that the association of direct and indirect laboratorial tests with clinical and epidemiological data is essential to perform a definitive diagnosis of brucellosis.

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