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Detection of *Brucella* Infections from Clinically Suspected Cases of Brucellosis in Garmain City-Sulaimani Province Kurdistan- Iraq

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ABSTRACT

Brucellosis is a zoonotic disease transmitted between animals and humans by contaminated food, direct contact with an affected animal, or inhalation of aerosols. The research was for the period October 2019 to March 2020, 67vein blood samples of clinically suspected brucellosis (Malta fever) and 20 from healthy persons as control were tested. Control samples showed negative for brucellosis infection when serological testing was performed. while out of 67 suspected patients, 14 were significantly positive for Rose Bengal at titer (160), 39 positive for ELISA (37 for IgG and 2 for IgM). Out of 37 positive samples for IgG by ELISA, only seven samples were positive for Rose Bengal while two positive samples for IgM was negative for Rose Bengal.

Keyword: Brucellosis , IgG and IgM ,blood sample, ELISA, garmain city

1. INTRODUCTION

Brucellosis is a disease transmitted that can be communicated between animals, including livestock. It is thought to be one of the most prevalent bacterial zoonosis on a global scale[6]. It is produced by members of the genus Brucella, of which Brucella melitensis, Brucella abortus, Brucella suis, Brucella canis, Brucella ceti, and both have been found in humans, as well as in the animals they live with. [1-4]. Because of under-reporting and misdiagnosis, there is no accurate estimates of human incidence [5].

Brucellosis causes major financial damage to livestock production in endemic regions because to deaths, decreased milk production, and infertility, in addition to the public health cost [7]. Humans

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get the illness when they consume raw or undercooked milk and dairy products from sick animals, as well as when they come into direct touch with afterbirth and aborted materials. As a consequence, persons who interact with animals in endemic regions, such as farmers, slaughterhouse workers, shepherds, and veterinarians, have a significant risk of infection [9]. Human infection presents with a variety of symptoms, but the majority of individuals with the acute type have fever, malaise, anorexia, headache, arthralgia, and backache. The most prevalent clinical sign in sub-acute episodes is a persistent and recurring fever. Arthritis, endocarditis, spondylitis, sacroiliitis, osteomyelitis, and meningoencephalitis are all possible side effects[9, 10].

Because most human instances of brucellosis are caused by infected livestock, regulation of the illness in livestock is essential for human brucellosis prevention. Vaccination, testing, and killing of positive animals, as well as isolation and animal movement regulations, have all been used to varied degrees of effectiveness [11]. Brucellosis in cattle, which is mostly caused by B. abortus, has been eliminated. in a number of nations, including Japan, Canada, a few European countries, Australia, and New Zealand [12]. However, it seems that controlling B. melitensis in small ruminants is more difficult than controlling B. abortus may be due to its increased infectiousness [13] This disease is caused by a lot of things, like the way small ruminant populations move around more than big ruminant populations. [7, 12]. The United Nations Food and Agriculture Organization (FAO) has advised different control techniques based on flock/herd-level seroprevalence; consequently, accurate disease frequency estimations are critical for informing and monitoring the control program. In locations with a low frequency (2%), testing and killing of positive animals in conjunction with sanitary measures is suggested. The FAO recommends immunization of small adult animals that are not required to be vaccinated, and testing and killing of infected animals in areas with a frequency of between 2% and 10%. In areas with prevalence more than 10%, mass vaccination of all cattle is recommended as the ideal management technique until a sufficient drop in prevalence occurs and the plan may be updated. [11]. Furthermore, the right technique is determined by the economic context, the monitoring system in operation, the strategy adopted by relevant authorities, and the infection baseline level.[14]. Finally, Prioritizing brucellosis management above other illnesses should be prioritized based on human burden estimations. of diseases In disorder years of life, this is shown (DALYs) and financial impact, which includes monetary losses, associated with human sickness and decreased animal output. [7, 15]. The disease's ” possible burden, especially in

low-income areas, is not appropriate to the level of attention it receives from healthcare systems globally, and as a consequence, brucellosis has been added to the World Health Organization's list of Zoonotic Diseases That Have Been Neglected [6]. Brucellosis is a significant health of the general public concern in portions of the Middle East, Mediterranean region, Asia, Africa, and Latin America[12, 16, 17]. In this work, we concentrate on the Middle East, where brucellosis is believed to be one of the most prevalent zoonoses [18]. Bahrain, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, and the United Arab Emirates comprise the area.[19]. The majority of these regions have a great deal in common when it comes to livestock management techniques, ecological factors, and culture [18]. Brucellosis is gaining traction in the Middle East; some nations, such as Egypt and Oman, are conducting vaccination programs for small and big ruminants, while others, such as Iran, Iraq, and Israel, are launching mass immunization programs for ruminants of little size. The purpose of this study was to investigate the function of the two most widely serological testing were performed to diagnose human brucellosis, the Rose Bengal test and the indirect enzyme linked immunoassay assay (ELISA).

2. Materials and methods

2.1. Method

2.1.1. Study samples

A total of 87 patients suffering from brucellosis were studied peripheral vein blood specimens were collected including 20 controls and the other 67 samples were obtained from patients with high suspicion outpatients of

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Brucellosis at Kalar General Hospital Laboratory& Rzgari General Hospital Laboratory. The period of sample collection was from October 2019 to March 2020. Patients were divided into both sexes in different ages, and all tests carried on in the medical laboratory technology Dept. Kalar Technical College

2.1.2. Specimens collection

Patients had 4 mL of blood taken from their peripheral veins. With a brucellosis diagnosis, under aseptic technique, and put into plane tube for serum separation, the serum stored at -20º C for serological diagnostic examination.

Rose Bengal examination

Antibodies against Brucella were detected in all sera use the Rose Bengal plate test kit that is readily available (Institute-Pourquie/France), according to the manufacturer's guidelines. To summarize, 30L of undiluted serum was poured onto and combined with an equal number of white glossy ceramic tiles of Rose Bengal test antigen. After 4 minutes at room temperature, the serum and antigen were well mixed, and any visible agglutination was interpreted as a positive result. Otherwise, the outcome was considered negative. [19–20].

ELISA test

Sera from patients suspected of clinical brucellosis (n=67) were tested for anti-Brucella IgG and IgM Abs concentrations using ELISA kits from VirCell (VirCell, Spain), Novagnost (Novatec, Germany) and Antigen Discovery (Antigen Discovery, USA). The results of the ELISA tests were examined, in parallel studies, in comparison to those used in agglutination tests in the presence and absence of b2-mercaptoethanol (CAPT). All the ELISA assays were evaluated for linearity and imprecision according to CLSI: EP15:A2 & EP6-A and the reference ranges were verified. The cut-off value for a Brucella agglutination test positive result was 1/160 [21].

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3. Results and Discussion

The main way to diagnose this disease is to look for specific antibodies with different serological methods.[22].

3.1. Serological test

3.1.1. Rose Bengal test

We collected a total of 87 peripheral blood samples from Kalar& Rzgari General Hospital Laboratory, 20 of these were healthy individuals using as control and the other 67 were patients clinically suspected to be brucellosis. Out of 67 samples, 14(20.9 %) were diagnosed as brucellosis as showed Rose Bengal titer (1/160) which is considered positive according to [23]. Out of 14 samples, 6(8%) were males and 8 (18%) were females, while all the 20 healthy controls were negative for this test (no agglutination) (table, 4-1).

Table (4-1): Results of Serum titers by Rose Bengal test for 67suspected brucellosis patients.

Serum titer	No. and percentage(%) of positive suspected samples
161:1	3 (4.5)
163:1	2 (3.0)
16:1	9 (13.4)
No titer	53 (79.1)
Total	67 (111)

While the Rose Bengal test has a high sensitivity, it has a poor specificity. [22]. Although the Rose Bengal test has an overall sensitivity of 92.9 percent, false-positive results can also be caused by other illnesses such as salmonellosis, tularemia, and cholera, as well as cross-reactions with other gram-negative bacteria such as Yersinia enterocolitica

O:9, Escherichia coli O:157, Francisella tularensis, Salmonella urbana O:30, and Vibrio cholerae. [24-25] therefore Out of the 14 cases, 7 showed positive result with IgG, and for diagnose brucellosis other serological tests are used for confirmation and to avoid false positive result, therefore, suggested that for an accurate and foolproof diagnosis of brucellosis, ELISA and the Rose Bengal test are used together. should be followed, [23] has reported that false positive result does not pose any problem if other serological tests are used for confirmation.

3.1.2. ELISA test

After Appling ELISA test, results have revealed that all healthy controls were negative with IgG and IgM, while in suspected patients' results have showed (2) positive case of IgM and (37) cases positive for IgG Table (4-2). Antibodies to IgG are detected in the serum of patients. who are in the final stages of the disease, and they are also found in the serum of recurring individuals.[24].

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Table (4-2): ELISA results for 67 clinically suspected brucellosis patients.

Antibody type	No. of positive samples	No. of negative samples
IgG	37	30
IgM	2	65

ELISA is a serological test that measure antibodies against specific *Brucella* proteins, which is found in order not to generate a cross-reaction issue with other Gram negative bacteria [27]. Because it is based on primary interaction, ELISA is more sensitive and offers for the identification of acute and chronic brucellosis, a profile of immunoglobulin classes was created. [28].may be helpful indicators of current brucellosis [29].and may simply be standardized using a smooth lipopolysaccharide antigen and a monoclonal antibody. [30]. The enzyme-linked immunosorbent assay (ELISA) is used to distinguish between specific IgM and IgG antibodies. and to provide a general estimate of the stage of sickness. SAT (Serum Agglutination Test) and 2-Mercaptoethanol (2-Mercaptoethanol) tests (2-ME). Are also performed and the results, although they are less effective. Finally, ELISA is thought to be a more sensitive and quick method of detecting brucellosis, but it is considerably more costly, particularly in endemic regions where the test would be performed regularly. [31].

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4- CONCLUSIONS

Brucella spp. detected from patients in Kalar and Rzgari with brucellosis by using Rose Bengal test. Some of the samples which were negative for Rose Bengal test were positive by ELISA test and vice versa. ELISA test is very accurate procedure for diagnosing human brucellosis. The accurate assessment of IgG and IgM antibody levels is facilitated by the identification of IgG and IgM antibody levels. identification of the illness at any stage of the disease's evolution.

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Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

الحمى المالطية هو مرض حيواني المنشأ ينتقل بين الحيوانات والبشر عن طريق الطعام الملوث، أو الاتصال المباشر مع حيوان مصاب ، أو استنشاق الهباء الجوي. تم إجراء البحث في الفترة من أكتوبر 2019 إلى مارس 2020، وتم اختبار 67 عينة دم من داء البروسيلا المشتبه به سريريًا (حمى مالطا) و 20 عينة من الأشخاص الأصحاء كعينة تحكم. أظهرت عينات المراقبة سلبية الإصابة بداء البروسيلات عند إجراء الاختبارات المصلية. بينما من بين 67 مريضًا مشتبهًا، كان 14 مريضًا إيجابيًا بشكل ملحوظ لـــ Rose Bengal عند عيار (160) ، و 39 إيجابيًا لـــ ELISA (37 لـــ IgG و 2 لـــ IgM). من بين 37 عينة إيجابية لـــ IgG بواسطة ELISA ، كانت سبع عينات فقط إيجابية لـــ Rose Bengal بينما كانت عينتان إيجابيتان لـــ IgM سلبية لـــ Rose Bengal.

الكلمة المفتاحية:

الحمى المالطية، IgGو IgM ، عينة الدم ، ELISA، مدينة الجارمين

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