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APPLICATION OF ROSE BENGAL TEST FOR SURVEILLANCE HUMAN BRUCELLOSIS IN ERBIL GOVERNORATE KURDISTAN REGION IRAQ

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ABSTRACT: This study was conducted to monitoring human brucellosis among Erbil Governorate. A total of 280 blood samples were collected from male (146) and female (134). Information about persons was recorded, including sex, age, and place of habitation. These samples comprised 155 and 125 blood samples from rural and urban area respectively. The overall prevalence of brucella antibodies was (8.6%).The highest rate was found in female (9.7%), while the lowest rate was from the male (7.5 %). The prevalence rate among male was high (10.3 %) in age group 31- 40 years, while in females were high (19.2%) in age group 21- 30 years. Out of 280 blood samples, only (5.7%) brucella isolates were found. The isolation of *Brucella abortus* were (57.1%) and (66.7 %), while (42.9%) and (33.3%) were *Brucella melitensis* from male and female respectively. The relation between result of RBT and isolation of Brucella species achieved that (7.5 %) and (9.7%) from male and female were positive according to RBT, compared with (4.8%) and (6.7%) gave isolates of Brucella species respectively. According to the habitation of the participants, high prevalence rate (9.7%) among Rural area, whereas (7.2%) among urban area. The highest rate were found in September (12.0%), while the lowest rate were in July (6.7%).This study clarified that brucellosis is still one of a significant public health challenge for Iraqi Kurdistan. We recommend that the RBT play significant role in monitoring of human brucellosis.

KEYWORDS: RBT, Surveillance, Human Brucellosis, Erbil Governorate, Kurdistan Region, Iraq.

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1. INTRODUCTION

Human brucellosis is one of the most common a cosmopolitan bacterial debilitating disease that affects humans, various species of wild and domestic animals especially food-producing animals, rodents, and marine animals. It is a very old zoonosis and modern indication from Egyptian ancient skeletons has shown that this disease has been present since no less than 750 BC [1,2]. .Brucellosis also known by other names such as “Undulant fever”, “Mediterranean fever” or “Malta fever” is a highly emerging infectious disease (EID) and one of the most important reemerging zoonosis particularly in industrialized countries where the disease was previously endemic but is now mainly associated with returning travelers and the global map of human brucellosis has significantly changed over the past few years, because of a complex multifactorial set of changing circumstances such as lack of various sanitary conditions, the standard of socio-economic activities, and political reasons, together with increased moving of persons, animals, and their products around the world [3,4].It is an important human disease world widespread, particularly Mediterranean Basin Countries, the Middle East including Iraq and Iraqi Kurdistan Region, the Arabian Peninsula, Indian subcontinent, Countries of Europe, Africa, Asia, Central and South America, Mexico, and yet it is often unrecognized and frequently goes unreported. There are a few countries in the world that are officially free of the disease although cases still occur in people returning from endemic countries. This disease has a history from 1937 in Iraq when the microorganism was first isolated via an Iraqi clinician [5,6].Brucella species are generally transmitted to human through the consumption of unpasteurized milk and dairy products, inhalation of dust containing the organism in the laboratory or place of animal husbandry, direct contact with infected carcasses. Meat may also be a significant source of infection, especially in cultures where the eating of raw or undercooked meat products is favored. The organism penetrates the skin or mucous membranes and travels to the lymph nodes, which become haemorrhagic, leading to bacteremia and the dissemination of the bacteria throughout the body, as well as by contacting with infected domestic animals such as cattle, sheep, and goats. The occasional person-to-person transmission has been reported, including transmission to infants via breastfeeding, blood transfusion, bone marrow transplant, needle sharing in drug users, and also intrauterine transmission through the placenta, there is a little evidence for sexual transmission of brucellosis [7, 8].The onset of clinical manifestations can be insidious or acute, beginning within 2 to 4 weeks following infection in human. The general symptoms are often vague and similar to the flu, they include, intermittent or remittent fever, headache, back pain, body-wide aches and pains, abdominal pain, cough, anorexia and weight loss, night sweats, weakness, malaise, and prostration. Compared with the symptoms, there are often few signs in the physical examination. Mild lymphadenopathy and splenomegaly or hepatomegaly may be seen in a portion of patients [9,10]. According to World Health Organization (WHO), there are approximately 500.000 new cases of human brucellosis reported globally each year. Many investigators mentioned that this figure

underestimates the size of the problem, and some of them expected that the number of human brucellosis cases may be up to 26 times higher than the above number of cases [11, 12]. The disease is caused by different species of the genus brucella, which tend to be host-specific. Brucellae are gram-negative coccobacilli or short rods, facultative intracellular, non – motile, non-spore forming, urease^{+} ive, Brucella members are aerobic, but some strains require an atmosphere containing 5 to 10% Co2 added for growth, especially on primary isolation and non-capsulated [13, 14].Currently, there are eleven species described in the genus Brucella, every one may infect different host species, but each Brucella species has a favorite for its host species. Six classical out of eleven species include *Brucella abortus*, *Brucella melitensis*, *Brucella ovis*, *Brucella canis*, *Brucella suis*, *Brucella neotomae* and five novel species of Brucellainclude*Brucellaceti*,*Brucellapinnipedialis*,*Brucellamicroti*,*Brucellainopinata*,*Brucel la papionis*[15-17]. The prevalence of human brucellosis in Iraqi Kurdistan are yet higher than reported from bordering countries, and it has been recorded from all three Iraqi Kurdistan Governorates [18]. He indicated that the occurrence rate in Erbil city was 10.7% in 2012, in Dohuk was 6.36% in 2011, and 976 cases were recorded in Sulaimani Governorate in 2013. Perfect and quick diagnosis of human brucellosis is very important because the delay or misdiagnosis leads to failure in treatment, retrogression, chronic courses, and complications in just one organ or throughout the body including liver, heart, reproductive system, and central nervous system. Presently, diagnosis is based on clinical observation which has never been straightforward, complemented by serology, so the serological diagnosis still have a main role in the routine diagnosis of brucellosis due to their low price, ease in handling, and high sensitivity [19,20]. Rose Bengal Test (RBT) is timely diagnostic tool, simple, high sensitivity, easy, cheap, and accurate. Rapid slide-type agglutination assay performed with a stained *Brucella abortus* suspension at pH 3.6–3.7 and plain serum, which was designed originally for screening use in veterinary medicine, but nowadays is widely used for identifying human brucellosis [21,22].Therefore, the objectives of this work were to monitoring the human brucellosis according to gender, age, and residence (Rural or Urban), by application of RBT in Erbil Governorate ,and to determine the relationship between occurrence of brucellosis in humans with months during the period of study . Also high light on the hazard of brucellosis help the peoples to understand the different ways of infection with Brucella in order to protect themselves from this prevalent disease.

2. MATERIAL AND METHODS

2.1. Study Design and Sampling

Two hundred and eighty (280) human blood samples were collected among Erbil Governorate, during the period from July 2017 to December 2017. Samples were collected from male (146) and female (134), ranged in age from eleven (11) years to sixty (60) years. These samples comprised 155 blood samples from rural area and 125 blood samples from urban area. Twelve (12) ml blood samples were

collected from every individual, and each sample divided into two tubes (6 ml into vacutainer tube without anticoagulant and 6 ml into vacutainer tube containing anti-coagulant). The samples collected were forwarded by sterile container to the Microbiology Laboratory / Department of Pathological Analysis / College of Science / Knowledge University. In laboratory the first part of blood allowed to clot and centrifuged, the serum was separated for used to detect brucella antibody, the second part of blood was used for isolation of Brucella species [23].

2.2. Personal information

Information about persons were recorded, including sex, age, and place of habitation.

2.3. Detection of Brucella antibodies in Blood

In laboratory, the detection of brucella antibodies in blood was done by using Rose Bengal Test (RBT). The test was carried out according to [24]. The following steps was followed: -

A. Test serum (0.03 ml) is mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.

B. The mixture is agitated gently for four minutes at ambient temperature, and then observed for agglutination.

C. Any visible reaction is considered to be positive.

2.4.**Isolation and identification of Brucella species from Human Blood**

The isolation of Brucella from human blood was done under sterile conditions according to [23 and 25]. The identification of *Brucella abortus* and *Brucella melitensis* were confirmed by Biochemical analysis, and the tests performed illustrated in Table (A).

2.5. Statistical Analysis

Data were analyzed using Chi-Square test and SPSS software version 15.

Table (A): Phenotypic characteristics of Brucella species isolated from human blood

Biochemical tests	*Br.abortus*	*Br.melitensis*
Growth on MacConkey agar	-	-
Blood haemolysis	-	-
Simmon's citrate	-	-
Indole test	-	-
Urease activity	+ive, hydrolyzing urea within 1 - 2 hours	Variable
Catalase test	+	+
Oxidase test	+	+
Triple Sugar Iron	-	-
Nitrate reduction	+	+

H2S production	+	-
Agglutinationwithmonospecific sera A	+	-
Agglutination with monospecific sera M	-	+
Thionin	+	-
CO2 requirement	+	-

3. RESULTS AND DISCUSSION

3.1. Prevalence of Human Brucellosis in Erbil Governorate According to Sex

Of the 280 Blood samples evaluated, 24 (8.6%) had appositive result for RBT. The result shows that the female was more exposed13 (9.7 %) to infection with Brucella, compared with male infection rate 11 (7.5 %) (Table 1).

Table (1): Prevalence of Human Brucellosis in Erbil Governorate According to Sex.

Sex	No of Samples examined	Positive		Negative		Chi Square	P Value
		No	%	No	%		
Male	146	7.5	11	92.5	135	0.42	0.52
Female	134	9.7	13	90.3	121		
Total	280	8.6	24	91.4	256		

3.2. Results of RBT Among Male Blood According to Age

This study showed that the prevalence rate of Brucella antibody among male was high in age group between 31- 40 years (10.3 %), followed by the group with age between 21–30 years (10.0%) Table 2.

Table (2): Results of RBT Among Male Blood According to Age.

Age Group (Years)	No of Samples Examined	Positive		Negative		Chi-Square	P Value
		No	%	No	%		
11 – 20	30	3.3	1	96.6	28	1.24	0.87
21 – 30	30	10.0	3	90	27		
31 – 40	29	10.3	3	90	27		
41 – 50	28	7.1	2	92.9	26		
51 – 60	29	6.9	2	93.1	27		
Total	146	7.5	11	92.5	135		

3.3. Results of RBT Among Female Blood According to Age

Table (3) show that the prevalence rate of Brucella infection in females were high (19.2%) in the age group between 21- 30 years, followed by the group with age between 31- 40 years (11.5%).

Table (3): Results of RBT Among Female Blood According to Age.

Age Group (Years)	No Examined	Positive		Negative		Chi-Square	P Value
		No	%	No	%		
11 – 20	27	3.7	1	96.3	26	4.28	0.37
21 – 30	26	19.2	5	80.8	21		
31 – 40	26	11.5	3	88.5	23		
41 – 50	27	7.4	2	92.6	25		
51 – 60	28	7.1	2	92.9	26		
Total	134	9.7	13	90.3	121		

3.4. Isolation of Brucella species from Human Blood According to Sex

Depending on Phenotypic characteristics of *Brucella abortus* and *Brucella melitensis* isolated from human blood, we achieved that Brucella isolates were found in 16 (5.7 %) among 280 samples. This result indicated that the isolation of *Brucella abortus* was 4 / 7 (57.1%) and 6 / 9 (66.7 %), while 3/7 (42.9%) and 3 / 9 (33.3 %) were *Brucella melitensis* from male and female respectively (Table 4).

Table (4): Isolation of Brucella species from Human Blood According to Sex

Sex	No. examined	Positive		*Br. abortus*		*Br. melitensis*		Chi Square	P value
		No	%	No	%	No.	%		
Male	146	7	4.8	4	57.1	3	42.9	0.15	0.70
Female	134	9	6.7	6	66.7	3	33.3		
Total	280	16	5.7	10	62.5	6	37.5		

3.5. The Relation between Result of RBT and Isolation of Brucella species from Human Blood

When we study the relation between result of RBT and isolation of *Brucella* species from human blood, we found that 11/146 (7.5 %) and 13/134 (9.7%) samples from male and female were positive according to RBT, compared with 7 (4.8 %) and 9 (6.7 %) samples gave isolates of *Brucella* species respectively (Table 5).

Table (5): The Relation between Result of RBT and Isolation of Brucella species from Human Blood.

Sex	No. examined	Result of RBT		Isolation of Br.spp		Chi-Square	P Value
		No.	%	No.	%		
Male	146	11	7.5	7	4.8	0.017	0.90
Female	134	13	9.7	9	6.7		
Total	280	24	8.6	16	5.7		

3.6. Prevalence of Human Brucellosis in Erbil Governorate According to Habitation

According to the habitation of the participants, the high prevalence of Brucella infection rate 15/ 155 (9.7%) according to RBT among rural area, whereas 9/ 125 (7.2%) among urban area (Table 6).

Table (6): Prevalence of Human Brucellosis in Erbil Governorate According to Habitation.

Source of samples	No examined	Positive		Negative		Chi Square	P Value
		No	%	No	%		
Rural Area	155	9.7	15	90.3	140	0.54	0.46
Urban Area	125	7.2	9	92.8	116		
Total	280	8.6	24	91.4	256		

3.7. Relationship between Months and Prevalence of Human Brucellosis during the period of study.

Table 7 illustrate that the relationship between months and prevalence of brucella antibodies in human blood during the period of study. From this table we noticed that the highest rate of prevalence of brucella antibodies according to RBT was found in September 6/50 (12.0%), then in August 5/45 (11.1%), while the lowest rate was found in July and December 3/ 45 (6.7 %) and 2/45 (4.4%) respectively.

Table (7): Relationship between Months and Prevalence of Human Brucellosis during the period of study.

Month	No of samples examined	RBT		Chi Square	P Value
		No	%		
July	45	3	6.7	3.28	0.46
August	45	5	11.1		
September	50	6	12.0		
October	50	4	8.0		
November	45	4	8,9		
December	45	2	4.4		
Total	280	24	8.6		

DISCUSSION

Human brucellosis represents a high public health hazard and poses a major threat to human health. Brucellosis remains an important sickness, and according to the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the Office International des Epizootics (OIE), it continues to have a significant impact on social and economic progress worldwide especially indeveloping countries[26- 28].Serological tests are more commonly used, and it is the most practical method available to confirmdiagnosis. Rose Bengal test (RBT) is used to screen human sera in some

countries. The RBT is an affordable, simple, sensitive and easiest methods to apply and the most globally used for recognizing antibodies of human brucellosis [29,30] In the work at hand, two hundred and eighty 280 (146 males and 134 female) blood samples were collected among Erbil Governorate, during the period from July 2017 to December 2017. The overall prevalence of brucella antibodies in blood from all collected human samples were 24 / 280 (8.6%), as shown in (Table 1), which is approach or slightly lower percentage with [31] in Pakistan whom found that the prevalence of Brucella infection was (9.33%) according to RBT, while [32] in Egypt reported that the prevalence of Brucella infection was (11.1%) according to RBT. In the study designed by [33] in Karnataka, India noticed that the prevalence of brucellosis by Rose Bengal plate test (RBPT) was 6.38 %. In Angola, namibe province, [22] mentioned that the general weighted prevalence of brucellosis was 15.6% from a population of 131 workers of butchers, slaughter rooms, slaughterhouse, and 192 breeders sampled randomly.Also,table1shows that the female are more exposed to infection with Brucella, the rate of infection in female was 13/ 134 (9.7%), compared with the rate of infection in males were 11/146 (7.5%).This result was compatible with [34] in Pakistan, who indicated that the prevalence of brucellosis in females (25.58%), while in males (10.18%). In another hand, [32] noticed that the prevalence of brucellosis in males (11.6%). higher than females (8.9%).Tumwine et al. [35] in Uganda reported that the seroprevalence of human brucellosis varied among males (20.5 %, *n*=78) and females (15.3 %, *n*=157). Statistical analysis using Chi-Square showed no significant difference at the level of 0.05 for the brucellosis existing in the human where the value of Chi-Square was (0.42) with the level of significance 0.52 ($p > 0.05$).According to age groups, the prevalence rate of brucella antibodies among male was high in age group between 31- 40 years (10.3 %), followed by the group with age between 21–30 years (10.0%), then from 41 to 50 years were (7.1%),after that from 51 to 60 years were (6.9%), finally the age group from 11 to 20 years were (3.3 %) (Table2).The obtained results indicated that there were no significant differences between the RBT among male blood according to age ($p>0.05$). In Yemen [25] noticed that the prevalence rate of Brucella infection among male was high (17.4%) in the age group between 31- 40 years according to RBT. Also, table 3, show that the prevalence of Brucella infection in female blood according to age groups. The results indicated that the higher rate (19.2 %) were in the age group between 21- 30 years, followed by the group with age between 31- 40 years (11.5%). Abdul-Razag [25] reported that the higher prevalence of brucella antibody in the group between 51- 60 years (33.3%), followed by the female with age between 21- 30 (20.6%) according to RBT. There were no significant differences between the RBT among female blood according to age ($p>0.05$).Anyhow, our results were compatible with [36] in India who found that the prevalence of brucellosis was higher in >35-50 years age group (middle-aged) (16.13%), compared to >20-35 years (young adults) (9.59%) and >50-65 years (elderly adults) (0.00%). While this result non-agreement with study conducted in Uganda by [35] where the seroprevalence of human brucellosis was higher in participants above

60 years(22.2 %), while the prevalence rate (16.7%) among all participants of age group below 13 years, age group from 13 to 19 years, and age group from 20 to 59 years. Recently, [37] in Iran, indicated that the most incidences of this disease were in patients who were over 50 years old (30%) and the least incidences were in the 31–40 ages group (12.3%).Nielsen and Gall [38] mentioned that the sensitivity and specificity of the RBT reportedly vary with pH of the antigen, ambient temperature of antigen and test serum. The principle of RBT depends on the detection of IgA, IgM, and IgG1, IgG2 present in the test serum. An acidic pH of the buffer is recorded to minimize the IgM activity. According to the Table 4, we noticed that 8 /10 (80%) and 2/3(66.7%) of isolates were *Brucella abortus*, while 2/10 (20%) and 1/3 (33.3%) were *Brucella melitensis* respectively. This observation indicate that *Brucella abortus* was the predominant species in cow milk. There are no significant differences between isolation of Brucella species from male and female blood according to sex where the significant value was more than 0.05 (0.70).The results obtained in table 4, illustrated that Brucella isolates were found in 16 (5.7 %) among 280 samples. This result indicated that the isolation of *Brucella abortus* were 4 / 7 (57.1%) and 6 / 9 (66.7 %), while 3 / 7 (42.9%) and 3 / 9 (33.3 %) were *Brucella melitensis* from male and female respectively. This observation indicate that the reason of high numbers of infection cases specially in developing countries may be due to poor sanitation, lack of health awareness, also may be due to the drinking of milk without of heat treatment, or consume dairy products made from it [39].When we study the relation between result of RBT and isolation of Brucella species from human blood, we found that 11 / 146 (7.5 %) and 13 / 134 (9.7%) samples from male and female were positive according to RBT, compared with 7 (4.8 %) and 9 (6.7 %) samples gave isolates of Brucella species respectively (Table 5). With ($p>0.05$) there is no significant difference between result of RBT and isolation of Brucella species from human blood. Our result was approach with result found by [40] in Greece, where they got the percent rate of isolation of Brucella species from human blood at (8.49%), also our result was compatible with [41] in Iran, whom found that the isolation rate of Brucella species from human blood was (6.0%). While this result were lower than result confirmed by [42] in Kenya whom reported that the isolation rate of Brucella species from human blood was (14.1%). Depending on the habitation of the participants, we noticed that the high prevalence of Brucella infection rate 15/155 (9.7%) according to RBT was recorded among rural area, whereas 9/125 (7.2%) among urban area (Table 6). Ebrahimpour et al. [43] in Iran, reported that 337 participants were found positive for brucellosis, 74.28% of these lives in rural areas, and 25.72% is in the city. In the study conducted by [44] in Uganda, mentioned that rural patients are likely to be either raising livestock or to come in contact with livestock, also the consumption of unboiled milk may play a significant mode of transmission. There is no significant differences between the prevalence of human brucellosis and habitation ($p>0.05$).Another point of this study include the relationship between months and prevalence of brucella antibodies among human samples during period of research in Erbil Governorate were followed up.

Results which described in table 7, explain that the highest rate of prevalence of brucella antibodies according to RBT were found in September 6/50(12.0%), then in August 5/45 (11.1%), while the lowest rate were found in July and December 3/45(6.7%) and 2/45(4.4%) respectively.With ($p<0.05$) there is no significant difference in the relationship between months and prevalence of human brucellosis during the period of study. The observed seasonality for Brucella infection may rotate to recent findings that Brucella species may be transmitted via different routes of infection especially through food such as milk and meat [23]. Minas et al.[40] in Greece reported that the occurrence of human brucellosis shows seasonality, with the majority of the cases diagnosed from December to May. Riabi et al. [37] in Iran indicated that from 176 patient cases, 94.8% of the people lived in rural areas and 5.2% lived in the urban. Most reported cases were in June and July and the lowest statistic was occurred in January. It seems that the disease process starts in the spring and in the summer reaches its peak and, then, begins to decline in autumn. Lai et al. [45] in China, whom studied the prevalence of brucellosis during the period from 1990 to 2014, a total of 513,034 brucellosis cases, were recorded, of which 99.3% were reported in northern China, and 69.1% (258, 462/374, 141) occurred during February–July.

CONCLUSION

Brucellosis is still one of a significant public health challenge for Iraqi Kurdistan. Based on the results, in order to prevent Brucellosis it's essential to applied Rose Bengal Test (RBT) for surveillance of this disease in human, because RBT is an affordable, simple, sensitive, and easiest method for the detection of human brucellosis, also dissemination of health awareness through the media (audio, visual media, and newspapers), highlighting the mode of transmission of these bacteria . In addition to educate the community, specifically at rural area, well to promote the drink milk after pasteurization and consume dairy products made from it. The eradication of Brucellosis as a major zoonotic trouble in food-producing animals is essential step to control this debilitating disease in human.

CONFLICT OF INTERESTS

The author has declared that he has no conflict of interest.

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