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Seroprevalence of Brucellosis from the city Mosul Iraq

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Abstract---Background *Brucellosis is a common neglected zoonotic disease transmitted from an animal reservoir to humans. Both, wildlife and domestic animals, contribute to the spreading of disease and it is endemic in most of Middle eastern countries including Iraq. Thus, brucellosis has negatively impacted the economy as well as livestock and health sectors.*

Aim: *The aim of this study was to provide data on the seroprevalence of brucellosis and investigate the status and prevalence among gender, age groups, and to evaluate and compare the level of immunoglobulin classes IgG and IgM among the patients with brucellosis symptoms.*

Methods:*A prospective cohort study was conducted among patients with brucellosis symptoms from July 2017 to June 2019 at city of Mosul in northern Iraq. Blood samples were collected randomly and aseptically from patients with brucellosis symptoms at Mosul General Hospital. Rose Bengal test (RBT) was used to measure the level of immunoglobulin classes IgG and IgM, as well as enzyme linked immunosorbent assay (ELISA) for sensitivity and specificity against brucellosis.*

Results:*A total of 385 sera individuals suffering with clinical features suggestive of Brucellosis who had positive for RBT test appear 115 (29.9%) showed positive reaction in RBT. The study recorded the highest seroprevalence 28 (38.9%) in females while16 (23.1%) in male in 2017. But the highest 35 (34.6%) in female and 19(26%) in male in 2018. The highest seroprevalence 7(16.2%) in female, and 3(11.2%) in male in 2019.The study showed a higher seroprevalence 35 (34.3%) was observed among the age group of (31- 40) year, then 34 (29.1%) , 28(28.2%) and 15(26.8%) among the age > 40 ,(21-30) and (11-20) year respectively. The study record from 30 serum positive by RBT were appear 17(56.6%) positive by ELISA, estimated sensitivity and specificity of the ELISA, RBT 69% and 59% respectively.*

Conclusion: *In conclusion, the frequency of brucella complications varied between differentgenders and age groups in the city of Mosul, Iraq. Hence, systemic monitoring as well aseducating of population are necessary to prevent the (re)emergence of brucellosis and itscomplications.*

Keywords---*Brucellosis, seroprevalence, prospective cohort study, clinical symptoms, Mosul, Iraq.*

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I. Introduction

Brucellosis is a major bacterial zoonotic infectious disease. Brucellosis is one of the neglected diseases in the developing countries (Franc et al., 2018). It is distributed worldwide, however, it is rare in most industrialized countries and more common in in low-income and middle-income countries (Franc et al., 2018). Brucellosis is endemic in Iraq (Shareef, 2006) and Middle East has the highest reported prevalence of brucellosis in the world (Pappas et al., 2006).

Brucella abortus mostly causes infections in cattle. Though, *Brucellamelitensis* and *Brucella. suis* known for their high virulence factor affects can be very dangerous for human(Corbel, 2006).Brucella species are intracellular,facultative, non-encapsulated, small non-motile gram-negative coccobacilli bacteria(Percin, 2013). Human can acquire brucellosis via contact with infected animal secretions and carcasses or consumption of their products, mostly unpasteurized milk and milk products(Köse et al., 2014). In human it mostly effect bones and joints, largely reported in all ages and sexes in high-risk areas (Adetunji et al., 2019). It has a variety of clinical manifestations such as undulant fever, malaise, insomnia, arthralgia, sexual impotence, nervousness, asthenia (Doganay & Aygen, 2003), depression(Jafari et al., 2015) and sexual impotence (Khan & Zahoor, 2018). Human brucellosis is also known for multiple organ involvement causing encephalitis, meningitis, endocarditis, arthritis, orchitis, and prostatitis(Samaha et al., 2008).

Therefore, demonstrating high or rising antibody titers in Brucella agglutination tests establishes the presumptive diagnosis of Brucellosis(Memish et al., 2002; Shemesh & Yagupsky, 2011). The Rose Bengal test (RBT) is a rapid plate agglutination test on pure serum using a stained antigen at pH 3.6(Díaz et al., 2011). RBT is economical, simple and rapid, however, low sensitivity (Chernysheva et al., 1980) particularly in chronic cases (Mizanbayeva et al., 2009), and relatively low specificity in endemic regions(Konstantinidis et al., 2007). Thus, enzyme linked immunosorbent assay (ELISA) is used as an alternative for qualitative determination and verification of specific antibody classes against Brucella in human plasma or serum (Thepsuriyanont et al., 2014).

Considering the above facts, the study was carried out to assess the seroprevalence of brucellosis among patients at Mosul General Hospital. RBT and ELISA were used to measure the level of IgG and IgM immunoglobulin classes.

II. Materials and Methods

Ethical approval and informed consent

A questionnaire was prepared to gather information related to patients who infected with the Brucellosis such as their drinking water quality and sources, toilets used and food. Agreement of the patient were gathered before they enrolled in the study. Before starting data and samples collection; a permission was obtained from all the enrolled patients after giving them explanations and clarifications about the purpose and components of the study. Each participant patients were reassured that the obtained information will remain secret and would be used only for the purpose of this study.

Study design and sampling

A total of (385) case from (169 males and 216 females) Ranging in age from 1 to up 60 years, from July 2017 to June 2019 in the Mosul city. Blood samples (≥5 mL) were collected randomly and aseptically at the level of the median and cephalic veins from patients suffering from symptoms of brucellosis who visited Mosul General Hospital

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during the study period. Patients were not subjected to discomfort. The biomarkers employed in the investigation included total IgG and IgM anti-brucella.

Rose Bengal Test (RBT)

RBT was performed as per suggested methods (Al et al., 2003). Samples were centrifuged for 15 min at a speed of 1500g. Sera were then separated and preserved at −20°C. All collected sera were screened using RBT, antigen manufactured by Standard Febrile Antigens Rose Bengal by slide agglutination tests (Biolabo SAS, France).Briefly, place 50µl of serum sample on 50µl of RBT antigen and were placed alongside on one well of glass slide and mixed thoroughly. The slide was gently rocked for 4 minutes and thereafter, any visible agglutination was considered as a presumptive positive (+). The absence of agglutination within 4 min was regarded as negative (−).

Enzyme Linked Immunosorbent Assays (ELISA)

Commercialised Ab C-ELISA kit (Svanova Biotech, Sweden) was used for detection of anti-brucella antibodies. The test was performed in accordance with the manufacturer's instructions.

Sensitivity and specificity of RBT and ELISA was evaluated using the following formulas (Rahman, 2015)

$$Sensitivity = \frac{Total\ positive}{Total\ positive + false\ negative} x\ 100$$

$$Specificity = \frac{Total\ negative}{Total\ negative + false\ negative} x\ 100$$

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III. Results and Discussion

Brucellosis are a matter of concern to economy and public health.Risk factors for human infections by zoonotic pathogens are related to local disease ecology, including the animal reservoirs. Wildlife can be the source of human disease directly transmitted or indirectly transmitted via domestic animals. Brucellosis is an endemic zoonotic disease in most Middle Eastern countries (Shareef, 2006), and Iraq in the past two decades has been reported for increase in prevalence of human brucellosis(Gwida et al., 2010; Zhao et al., 2019).

We determined brucellosis seroprevalence in human in the city of Mosul, Iraq.Brucellosis seroprevalence in total of 385 sera individuals suffering with clinical features suggestive of brucellosis showed to 115 (29.9%) positive in RBT. The study recorded the highest seroprevalence 28 (38.9%) in females while 16 (23.1%) in male in 2017 (Table 1). In 2018, the highest was recorded among female with 35 (34.6%) and 19 (26%) in male (Table 1). The highest seroprevalence in 2019 was also among female with 7(16.2%) in female, and 3(11.2%) in male (Table 1).

Gender	**Total**	**Brucella** - IgG, IgM Positive (%)	**Brucella** - IgG, IgM Negative (%)	**Years**
male	69	16 (23.1)	53 (76.8)	1/1 - 31/12 /2017
female	72	28 (38.9)	44 (61.01)	

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Table 1.

total	141	44 (31.2)	97 (68.7)	
male	73	19 (26)	54 (74)	
female	101	35 (34.6)	66 (65.3)	1/1 - 31/12 /2018
total	174	54 (31)	120 (69)	
male	27	3 (11.2)	24 (88.8)	
female	43	7 (16.2)	36 (83.7)	1/1 - 30/6 /2019
total	70	17 (24.2)	53 (75.8)	
All total	385	115 (29.9)	270 (70.1)	2017-2018-2019

Seroprevalence of Brucellosis positive & gender differences by Rose Bengal Test (2017 - 2019)

A higher seroprevalence 35 (34.3%) was observed among the age group of (31- 40) year, then 34 (29.1%), 28(28.2%) and 15(26.8%) among the age > 40, (21-30) and (11-20) years old respectively (Table 2). No femaleparticipant (n=334) tested positive for brucellosis even though these were in the majority compared to males (n=91). Brucella seroprevalence was recorded in three occupational categories out of the six that were considered in our study (Table 2).

Table 2: Age groups distribution of Seropositivity of Brucellosis (2017 - 2019)

Age	**No. of case**	**Brucella -** IgG, IgM Positive (%)	**Brucella** - IgG, IgM Negative (%)
1 - 10	17	3 (17.6)	14 (82.4)
11 - 20	56	15 (26.8)	41 (73.2)
21 - 30	99	28 (28.2)	71 (73.6)
31 - 40	96	35 (34.3)	61 (65.7)
> 40	117	34 (29.1)	83 (70.9)
All total	385	115 (29.9)	270 (70.1)

Rose Bengal plate test and ELISA used to determine the prevalence of brucellosis(Hussein et al., 2019). The study recorded from 30 serum positive by RBT showed 17(56.6%) positive by ELISA, estimated sensitivity and specificity of the ELISA. The sensitivity and specificity of the RBT test for the bacteria infected patients were 30 (100.0%) respectively, while that of the ELISA IgG & IgM positivity were 31 (43.3%) respectively (Table 3 & 4).

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Table 3: Distribution of human brucellosis using RBT & ELISA

Test	No. of samples	IgM, IgG Positive + ve (%)	IgM, IgG Negative - ve (%)
RBT 2ME	385	115 (29.9)	270 (70.1)
ELISA	30	17 (56.6)	13 (43.4)

Table 4: The compare positive and negative results of RBT and ELISA for sensitivity & specificity.

ELISA 2ME RBT	Positive Results No. sample (%)	Negative Results No. sample (%)
RBT	30 (100)	0 (0)
2ME	TP	FP
ELISA	31(43.3)	270 (70.1)
	FN	TP

Legend: TP: true positive; FN: false negative; TN: true negative; FP: false positive

Previous findings on serological reactions to *Brucella* sp. report on ELISA to have yielded higher sensitivity and specificity values compared with other conventional tests (Al Dahouk et al., 2005). However, another study reports that ELISA had higher sensitivity than RBT, but, RBT yielded higher specificity than ELISA (Matope et al., 2011). Thus, this suggests that using both RBT and ELISA in the diagnosis of Brucellosis among patients in Mosul, would yield better results.

IV. Conclusion

In Mosul, brucellosis is an emerging disease, however, the reported human cases may not reflect the actual prevalence as revealed in this study leading to a greater extend of the disease. There was considerable conditional dependence between RBT and ELISA implying that a combination of two of the serological tests may be a better choice for a better diagnosis. Our data highlights the need for further research, including the isolation and characterization of bacterial sp.

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