ISSN No:-2456-2165

Performance of Serum Tube Agglutination Test and Enzyme-Linked Immunosorbent Assay IgG and IgM Tests for Diagnosis of Human Brucellosis among Employees of Widam Slaughter Establishments

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Abstract:-

➢ ***Objectives***

Brucellosis is reported as important food borne zoonotic infection worldwide. There are number of techniques utilize to detect the presence of *Brucella* species in human sera and these techniques have different levels of specificity and sensitivity and the purpose of this study is to assess the Diagnostic Performance of Serum Tube Agglutination Test and Enzyme-Linked Immunosorbent Assay IgG and IgM Tests for Diagnosis of Human Brucellosis among the study group at Widam slaughter establishments.

➢ ***Methods***

A total of 141 human sera samples were collected from large animals' slaughter establishments in Qatar, namely, Abu Hamour slaughter establishment, Alshamal Slaughter establishment, Muaither slaughter establishment, Abu Hamour slaughterhouse for the Public, Um Salal slaughterhouse, AlMazroua slaughterhouse, Al Rayan slaughterhouse, Al shahaniya slaughterhouse from August 2017 to September 2017. The samples were tested by serological tests, serum tube agglutination test (STAT), and enzyme-linked immunosorbent assay (ELISA) (immunoglobulin M [IgM] and immunoglobulin G [IgG]).

➢ ***Results***

130 participants were divided into two age groups (18 - 45 and ≥ 46), in which, 82.3% were aged 18 – 45 years and 17.7% were aged ≥ 46 yrs. The age range across the samples was 18-68 years with a mean age of 38.35 Years (SD = 8.62). Additionally, the majority of the participants were male (99.2%, n=129) and 77.7% (n= 101) were married. Overall, 73.1% (n=95) were from South East Asian Countries, followed by 26.1% (n=34) from Arab African Countries. ELISA IgG and IgM has sensitivity (83.4% and 16.67%) respectively and specificity (93.3% and 99.13%).

➢ ***Conclusion***

Conclusion that ELISA IgM tests should not be used to confirm brucellosis.

Keywords:- *Brucellosis, STAT, ELISA, IgG and IgM, Qatar.*

I. INTRODUCTION

Brucellosis is a non-endemic disease in Qatar and has a small incidence compared to other EMR nations. Between 2004 and 2012, the prevalence of brucellosis has decreased where the peak prevalence occurred in 2006 (4.2 cases per 100,000 residents). The transmission route was found to be consumption of raw milk and milk products at a rate of 41.7% of the study group get infected by consumption of raw milk or milk products, and 12.5% of the study group acquire the infection via contact with animals or animals' parts infected with brucella. An outbreak of human brucellosis due to consumption of raw camel milk, was reported in Qatar in 14 members of a family live in the rural area of Qatar (Garcell, 2016).

Human brucellosis can be diagnosed by serologic tests and by blood cultures or by using both testing techniques. Patients should be placed on treatment if bacteria brucella genes were identified (Al-Nassir, 2016).

Serological tests such as serum tube agglutination test (STAT), Rose Bengal plate test (RBPT), enzyme-linked immunosorbent assay (ELISA), are among the laboratory testing techniques used for diagnosis of human brucellosis. Among these also is the molecular test technique like polymerase chain reaction (PCR) and blood culture technique to isolate brucella organism.

There is a lack of serological test technique that can solely confirm the diagnosis of brucellosis. However, PCR is considered as an excellent choice to test for human brucellosis. PCR is used as sign to foresee the development of the disease (Prakash, 2012; Mangalgi, 2012). Most of the serological tests used at laboratories are not particularly useful in delivering an appropriate sensitivity and specificity for brucella organism. For instance, Enzyme-linked immunosorbent assay (ELISA) technique which detect immunoglobulin G (IgG) has high sensitivity (Vakili, 2010) however, has low specificity (Amirzargar, 2009)

This study was conducted to compare STAT with ELISA IgG and IgM tests for diagnosis of human brucellosis among in Widam slaughter establishments.

II. METHODS

- *Study Design*

Cross sectional study was conducted at Widam slaughter establishments, to assess Diagnostic Performance of Serum Tube Agglutination Test and Enzyme-Linked Immunosorbent Assay IgG and IgM Tests for the Diagnosis of Human Brucellosis among the study focus group from Widam slaughter establishments.

- *Blood Samples*

A total of 141 human sera samples were collected from different Qatar slaughters, namely, Abu Hamour slaughter establishment, Alshamal Slaughter establishment, Muaither slaughter establishment, Abu Hamour slaughterhouse for the Public, Um Salal slaughterhouse, AlMazroua slaughterhouse, Al Rayan slaughterhouse, Al shahaniya slaughterhouse. Animals awaiting slaughter are kept in pens located outside of the main slaughter building. Veterinary care is provided to these animals for the entire period they spent in pens. Livestock caretakers are assigned to feed and take care of these animals until they are sent to the slaughter est. for slaughter.

III. SEROLOGICAL TESTS

- *STAT*

STAT was performed utilizing a business pack [Tulip Diagnostics (p) Ltd. Goa, India]. The unit contains prepared to utilize institutionalized explicit antigen suspensions of Brucella having explicit reactivity toward antibodies to B. abortus and B. melitensis. The test was performed by the organization rules. From the start, 8 test cylinders were taken and numbered as 1-8. The test cylinder marked with 8 was taken for negative control. The primary test cylinder was topped off with 1.9 ml phenol saline, and other 7 test cylinders were topped off with 1 ml phenol saline. Next, 0.1 ml test serum was taken to the primary test cylinder to get 1/20 weakening. After that from first test tube, 1 ml weakened serum test was moved to the second test cylinder, and this procedure was rehashed from the second to seventh test cylinders to get 1/40, 1/80, 1/160, 1/320, 1/640, and 1/1280 weakening. Next, 1 drop (50 µL) of antigen was added to each test tube alongside negative control and blended. From that point forward, all the test cylinders were kept in hatchery at 37°C for 24 hrs. An immune response titer of 1:160 or more was viewed as positive for brucellosis.

- *ELISA*

The Brucella ELISA test was performed utilizing a business pack (Immunolab immunoglobulin M [IgM] and immunoglobulin G [IgG], Germany), and the method of the test was trailed by the unit guidance. From the start microtiter, wells (which are covered with Brucella antigen (B. abortus strain, w99) were topped off with 100 µL each of the diluted (1:101) samples together with ready to use standards and controls except one well which was used for the substrate blank. This was followed by placing the plate at room temperature for 1 hour. Plates were then washed with wash buffer and 100 µL of enzyme conjugate was pipetted into each well. Plates were then covered with foil and incubated at room temperature for 30 minutes. Wells were washed with diluted wash buffer. 100 µL of substrate solution was pipetted into each well. Plates were placed in full darkness and incubated at room temperature for 20 minutes. Finally, 100 µL of stop solution was pipetted to each of the wells to halt reaction. Optical density was then measured using a photometer at 450 mm.

- *Statistical Analysis*

The sensitivity, specificity and the kappa value of the test were calculated using MedCal (16) software at 95% confidence interval. A p-value <0.05 was considered as statistically significant.

IV. RESULTS

130 participants were divided into two age groups (18 - 45 and ≥ 46), in which, 82.3% were aged 18 – 45 years and 17.7% were aged ≥ 46 yrs. The age range across the samples was 18-68 years with a mean age of 38.35 Years (SD = 8.62). Additionally, the majority of the participants were male (99.2%, n=129) and 77.7% (n= 101) were married. Overall, 73.1% (n=95) were from South East Asian Countries, followed by 26.1% (n=34) from Arab African Countries table (1).

The levels of IgG & IgM antibodies were assessed by commercial ELISA kits in the sera of 130 participants working at Widam slaughter establishments in Qatar. The

seroprevalence of Brucellosis is shown in table (2). The overall *Brucella* IgG and IgM was 9.3% (n=12) and 3.84% (5), respectively.

According to age groups, the prevalence of IgG (8.7%, n = 11) and IgM (3.1%, n = 4) was highest in the age group <45 years, however, did not differ significantly according to patient age (*P*= 0.472 and 0.819, respectively) (Table 3). Furthermore, the seroprevalence of *Brucella*-IgG (3.6%, n = 9), and IgM (3.8%, n=5) was highest among married workers (5.4%, n = 5) and lowest among single, but the difference was not statistically significant (*P* = 0.112 and 0.318, respectively). The *Brucella*-IgG seropositivity was higher among workers with primary (3.1%, n=4) and secondary school (3.8%, n=5) education and slaughtering & processing occupation (6.15%, n=8), but had no significant association. However, IgM positivity was slightly higher but had no significant association among workers with illiterate & primary education and livestock & slaughtering & processing occupation. Additionally, the level of IgG and IgM according to the duration of the job and different establishments or collection sites, was not significantly different. The prevalence of *Brucella*-IgG and IgM among workers from South East Asian countries was higher (5.38%, n = 7) than workers from Arab and African countries 3.84% (n = 5). Among the South East Asian and Arab & African workers, *Brucella*-IgG antibodies were detected higher in workers originating from India (4.61%, n = 6) and Egypt (3.1%, n=4). Additionally, IgM positivity was equal in both Indian and Nepalese workers (1.53%, n=2 in each) (table 2).

Furthermore, based on IgG/IgM ELISA and tube agglutination test, we extended our analysis to evaluate and compare the test sensitivity and specificity using receiving operating characteristic. and found higher AUC performance in ELISA IgG (0.886) and (0.902) when compared to *B. abortus* and *B. melitensis (table 3 and 4)*. Whereas in case of IgM, a low performance was noted which was equal to (0.507) and (0.508) when compared to *B. abortus* and *B. melitensis* (Table 5 and 6; Figure 1 and 2)

	Frequency	**Percentage**
Male	129	99.2
Female	1	0.8
18 – 45 Years	107	82.3
> 45 Years	23	17.7
South East Asian Countries	95	73.1
Arab African Countries	35	26.9
Married	101	77.7
Single	29	22.3

Table 1:- Demographic data of 130 workers of Widam Slaughter Establishments in Qatar

	IGG			**P. Value**	**IGM**			**P. Value**
	Equivocal	**Positive**	**Negative**		**Equivocal**	**Positive**	**Negative**	
Positive Titer	0	5	1	0.000	1	1	4	0.002
Negative Titer	9	7	108		1	4	119	
Total	9	12	109		2	5	123	

Table 2:- Brucella Abortus and ELISA IgG and IgM

	IGG			**P. Value**	**IGM**			**P. Value**
	Equivocal	**Positive**	**Negative**		**Equivocal**	**Positive**	**Negative**	
Positive Titer	0	6	1	0.000	1	1	5	0.006
Negative Titer	9	6	108		1	4	118	
Total	9	12	109		2	5	123	

Table 3:- Brucella Melitensis and ELISA IgG and IgM

	IgG	IgM
Sensitivity	83.33	16.67
Specificity	93.31	99.13
AUC	0.886	0.507
Weighted Kappa[a]	0.524	0.138

Table 5:- Sensitivity and specificity of IgG and IgM according to *Brucella abortus* ([a] **Linear weight)**

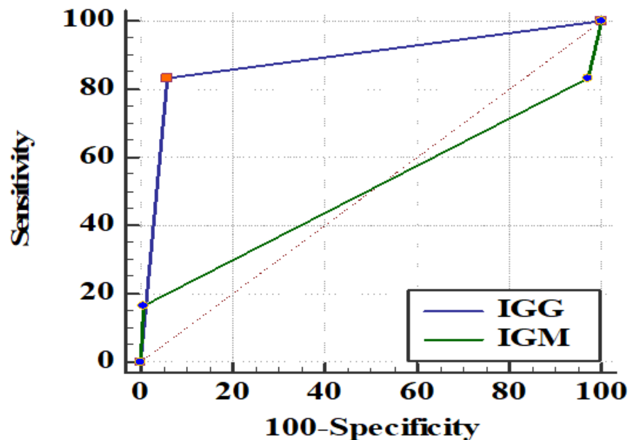

Fig 1:- Sensitivity and specificity of IgG and IgM according to *Brucella abortus*

	IgG	IgM
Sensitivity	85.71	14.29
Specificity	94.74	99.12
AUC	0.902	0.508
Weighted Kappa[a]	0.603	0.123

Table 6:- Sensitivity and specificity of IgG and IgM according to *Brucella melitensis* ([a] Linear weight)

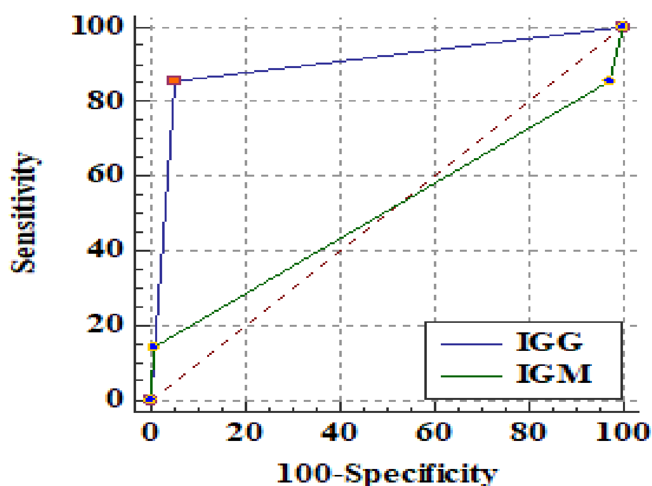

Fig 2:- Sensitivity and specificity of IgG and IgM according to *Brucella melitensis*

V. DISCUSSION

Brucellosis continues to be a disease of high risk to public health in developing nations (Khan, 2018). The disease imparks considerable effects and endemicity, as cause of acute febrile illness, in most Middle east countries among human and livestock productivity [McDermott, 2013; Dean, 2012]. The prevalence of disease varies greatly from country to country and across different regions with in a country (Acha and Szyfers, 2001). The present study aimed to compare STAT with ELISA IgG and IgM tests for diagnosis of human brucellosis among in Widam slaughter establishments, In this study, the overall seroprevalence of human brucellosis among slaughterhouse workers in Qatar is 9.3%. In earlier reports from neighboring countries, the seroprevalence rate shows a seroprevalence of 8.6% in Saudi Arabia, 11.4% in Sudan, 6.26 % in Egypt and 6.2% in Yemen (Rahamathulla, 2019; Tamador EA et al., 2014; Nawal, 2012; Al-Haddad, 2013). Worldwide, seroprevalence of human brucellosis is more common in males than in females [Al-Nassir, 2016]. In addition, young adult males predominate in most series of patients with brucellosis compiled in areas of endemic disease [Al-Nassir, 2016]. In this study, we observed brucellosis was more amongst male this is due to the fact that male is more involved in the risk of occupational exposure due to their direct contact with animals, meat, and milk products. Occupational exposure to animals likely plays an important role in the enhanced vulnerability of men to the development of brucellosis. It is unknown whether the increased risk manifested by males, is additionally influenced by aspects of personal hygiene, immunologic factors, or other circumstances. (Al-Nassir, 2016). Similar to our findings, other studies had shown a male predominance (Asad, 2012; Al-Tawfiq, 2009).

In this study, 4.61% of the individuals were Indian nationals and the highest percentage of IgG and IgM recorded (8.7% and 3.1%, respectively) was in the age group of <45 years. This may be due to the fact that people in this working age group are more in contact with domestic animals like cattle breeding, farming, butchering and consume raw milk and dairy products. In contrast to previous studies, these were not identified as major risk factors for brucellosis in this study (Memish, 2001; Al-Haddad, 2013). In addition, duration of work period also didn't show a significant role in getting the infection.

In this study, which employed Brucella SAT as the diagnostic reference standard, the ELISA IgG sensitivity were found to have (83.4%) compared to SAT, this result is lower than what has been reported in the literature (Osoba, 2001; Araj, 1986; De Klerk & Anderson, 1985; Araj & Kaufmann, 1989; Sippel, 1982). and higher than results obtained from (Welch and Litwin, 2010) and (Memish, 2002)

Contrary to previous studies ELISA IgM retrieves low sensitivity (16.67%) lower than what has been reported in the

literature (Osoba, 2001; Araj, 1986; De Klerk & Anderson, 1985; Araj & Kaufmann, 1989; Sippel, 1982; Welch and Litwin, 2010; Memish, 2002). ELISA IgG and IgM has higher specificity (93.3% and 93.1%) this finding in agreement with (Osoba, 2001; Araj, 1986; De Klerk & Anderson, 1985; Araj & Kaufmann, 1989; Sippel, 1982; Welch and Litwin,2010; Memish, 2002).

ACKNOWLEDGEMENTS

The author would like to express her gratitude to the managers of Widam Food Company and all individuals who volunteer to participate in this study.

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