Seroprevalence of human brucellosis among patients attending a teaching hospital in southern Saudi Arabia

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ABSTRACT: Human brucellosis, also termed as Malta fever or Mediterranean fever, is prevalent globally having heavy repercussions in the form of reproductive losses and infertility, arthritis, mastitis, and severe pathologic lesions. This research aimed to analyze the seroprevalence of brucellosis in Alkharj region of Saudi Arabia and identify significant risk factors and their impact on prevalence of brucellosis in patients of the region. This research was however confined to investigating the seroprevalence of human brucellosis in such patients that complained prolonged fever. The study used a cross-sectional survey method to identify patients complaining Pyrexia of Unknown Origin (PUO) with tested and proven presence of clinical characteristics of brucellosis. The results confirmed Brucellosis in 38/278(13.6 %) patients and a strong relationship was also observed between its prevalence and the risk factors such as direct contact with animal, consumption of raw milk and animal products. A proactive approach is required to sensitize people about human brucellosis and to exercise severe discipline. The study recommends introducing awareness programs among livestock community and highlight risk factors. Serological surveillance units may also be established at all district headquarters. In order to diagnose the disease at early stages, valid and reliable serological tests should be made readily available.

KEYWORDS: Human Brucellosis, risk factors, seroprevalence, Saudi Arabia.

INTRODUCTION

Human brucellosis is a commonly prevalent bacterial zoonotic disease caused by a gram-negative bacterium with a prevalence rate of 10/100,000 [1] [2] [3]. Also known as Malta fever or Mediterranean fever, this disease is transmitted from domestic infected animals like cows, goats, dogs, camels and sheep [4] and their products by direct or indirect contact, either through inhalation of infectious aerosols or ingestion of raw milk or unpasteurized dairy products or meat from an infected animal [5-7]. This disease is primarily found in rural or nomadic regions where humans live in close contact with animals or natural hosts [8].

According to Centers for Disease Control (CDC) [9], high risk areas for brucellosis are Mexico, Indian subcontinent, Mediterranean basin, Arabian Peninsula, Central and South America, Africa and Latin America [10] [11]. However, its increased occurrence has been seen in Uganda, where individual animal and herd level seroprevalence of bovine brucellosis was found to be 6% and 19% respectively [12-14]. This increased prevalence is worldwide and has been hypothesized to be associated with increased global tourism and migration [15].

When the disease is caused due to natural hosts, brucellosis is most commonly associated with reproductive losses and infertility, but can also cause arthritis, mastitis, and other pathologic lesions. In Saudi

Arabia, its annual occurrence was estimated to 12.5/100,000 population [16]. All species that can act as natural hosts for this disease are pathogenic to humans; e.g *Brucella abortus*, *Brucella mellitensis*, *Brucella suis* and *Brucella canis* species. Of all these, *Brucella mellitensis* worldwide has caused severe illness while *Brucella abortus* is least invasive and causes mild illness [17]. Clinical manifestations are often nonspecific and at times misleading like fever, night sweat, anorexia, asthenia, low back pain etc, and can be mistaken for other diseases like tuberculosis, malaria, rheumatic fever, leishmanioasis and malignancy [18].

Brucellosis can be best diagnosed by isolating patients and examining the type of bacterium that caused the disease. The isolation of Brucella especially requires high security laboratory facilities (e.g. biological containment level 3), highly trained laboratory staff and sufficient turnaround time for investigations. However, at few placed brucellosis is also diagnosed by detecting a high level of antibody in serum or another body fluid. Tests have been conducted invariably but no single test provides accurate and correct results. Hence, it is recommended to conduct the serological diagnosis by testing sera in more than one test [19].

The diagnosis of brucellosis also requires laboratory confirmation involving a combination of methods namely blood culture for *Brucellae* isolation cases; serological tests like Rose Bengal Plate Agglutination Test (RBPT), standard tube agglutination test (STAT), Enzyme Linked Immuno-Sorbent Assay (ELISA) and fluorescence polarization assay (FPA) among others have been applied in human brucellosis diagnosis. Nevertheless, STAT has limitations making ELISA to be most acceptable for diagnosing human brucellosis. ELISA to be more sensitive than STAT in detecting brucellosis in both acute and chronic cases while sensitivity and specificity of ELISA was reported to be 71.3% and 100% respectively [3].

Brucellosis is also considered as the most economically significant diseases, affecting livestock population in developing countries [1]. The disease is responsible for enormous economic losses in affected animals in the form of abortions, infertility and premature birth, reduced reproduction and drop in milk production. It also represents a great public health problem in endemic areas. In Brazil, the disease has estimated a loss of approximately 450 m USD [1]. Alkharj is a high livestock density region where stocking, breeding and communal grazing is common and becomes major risk factor of Brucellosis. In spite of industrial development and penetration of automation in livestock merchandise like meat, poultry and milk products, Alkharj is still indulged in promotion of livestock rearing and restocking. Till date, to the best of our knowledge, no empirical study has been carried out for this region.

Hence, keeping in mind the increased prevalence and relatively inadequate data regarding this important issue, the study was proposed with following objectives: a) to study the seroprevalence of brucellosis amongst the dwellers of Alkharj, the central region of KSA and b) to identify significant risk factors and their impact on prevalence of brucellosis in patients of Alkharj region.

This research was confined to investigating the seroprevalence of human brucellosis in such patients that complained prolonged fever.

MATERIALS AND METHODS

Study design and population

For this cross sectional study, such patients were sampled that complained continually of backache, muscular stiffness, fatigue, fever, headache, joint pain, and loss of appetite, which are common symptoms of brucellosis. Suspects were identified and tested through IgG and IgM electro chemiluminescence (ECL) Cobas method at laboratory of a Teaching Hospital in Alkharj, Saudi Arabia from 1st August 2018 to 30th March 2019. After the test, a total of 278 patients of both sexes were identified and sampled for this study. Patients below 15 years and above 73 years were excluded from the study. The sample size was consistent with the recommendations made for such cross sectional surveys to be 5% desired precision and 95% confidence interval [20-21].

Data collection

A form was used to collect the personal details such as age, education, residence of participants, and was also used to gather information regarding risk exposure to domestic animals, consumption of raw milk, pregnancy status and like.

Serological examination

5 ml of whole blood was obtained from each participant. For each sample, Serum Agglutination method, IgG and IgM electrochemiluminescence (ECL) were performed. A commercial automated cobas e411 (Roche Diagnostic GmbH, Mannheim, Germany) ECL, which was procured from IBL, Germany, was used to analyze the sera for brucellosis species, IgG and IgM antibodies. A positive IgG and a negative IgM were interpreted as a latent infection whereas a positive IgG and a positive IgM were taken as probability of a recent or acute infection.

Statistical analysis

The data collection for the enrolled subjects was standardized through the use of similar methodology. Protocol and procedure were used for administering a standard questionnaire. Once data was collected, it was entered into SPSS statistical software, version 24 (IBM, Chicago, Ill, USA) for analysis. Each of these datasets was first categorized in variables and then each proportion was summarized and analyzed using the Pearson's Chi-square test in order to examine the difference among variables. The mean and standard deviation ($\pm$) was determined in the continuous variables. Also the Univariate analysis and multivariate logistic regression models were employed to identify risk factors associated with infections.

The risk factors to be seen were like direct contact with animal, consumption of milk and animal product, knowledge of brucellosis and so on as stated in Table 1.

Table 1. Risk factors of seroprevalence

Risk factor	Response	(n)	(%)
Direct Contact With Animal	Yes	166	54.2%
	No	112	36.6%
Consumption Of Milk And animal Product	Yes	116	37.9%
	No	162	52.9%
Knowledge Of brucellosis	Yes	68	22.2%
	No	210	68.6%

The findings in Table 1 reveal that in the case of direct contact with animal, 166 patients (54.2%) were positive and 112 (36.6 %) were negative. Consumption of milk and milk product were positive in 116 (37.9%) patients and 162 (52.9%) were negative; and knowledge of brucellosis was positive in 68 (22.2%) patients and negative in 210(68.6%).

Ethical considerations

The present study and all experimental procedures were approved and performed according to the guidelines of the Ethical Committee, Prince Sattam bin Abdulaziz university, Saudi Arabia. The study was formally approved by the Ethics committee of College of Medicine, Prince Sattam Bin Abdulaziz University, The protocol and all processes were carried out in accordance with Good Clinical Practice guidelines as set by the ethical norms cited in the Declaration of Helsinki. All patients submitted a written informed consent before enrollment and before the commencement of any study related procedure. The study was formally approved by the ethics committee of College of Medicine, Prince Sattam Bin Abdulaziz University vide No PSAU/CO/RC/IRB/P/ 159.

RESULTS

Sampling

The study was continued for 9 months from 1st August 2018 to 30th March 2019, administering the test with the help of 278 blood samples obtained from patients showing signs of brucellosis and were required to be tested. Table 2 illustrates that out of the 278 samples collected during the study period, IgG (32) 10.5% and IgM (6) 2.0% were positive. This is illustrative of the prevalence of the disease in the region despite all precautions and government measures taken.

Table 2. IgG and IgM prevalence

Period	Brucellosis	Status	(n)	(%)
1st August 2018 to 30th March 2019	IgG	Positive	32	10.5%
		Negative	246	80.4%
	IgM	Positive	6	2.0%
		Negative	272	88.9%

Demographic information

Table 3 illustrates the demographic information of the sampled respondents. Findings reveal that out of total sampled patients found positive, (n=278), the brucellosis IgG seropositive patients cases ranged between 15 and 73 years of age, having a mean age of 29.1 years. The standard deviation resulted in $\pm$ 18.32 years. Out of the total sample (n=278), 187(61.1%) were male and 91(29.7%) were female, with the male to female ratio of 3.4:1. Of this sample 216 (70.6%) were Saudi Nationals and 62(20.3%) were Non-Saudis.

Table 3. Demographic information of the Sample respondents

Item	Variable	Number (278)	% age
Gender	Male	187	61.1 %
	Female	91	29.7%
Age	0-17	20	6.5%
	18-40	221	72.2%
	41-60	26	8.5%
	61-70	6	2.0%
	>70	5	1.6%
Education	None	25	8.2%
	Primary	131	42.8%
	Secondary	122	39.9%
Nationality	Saudi	216	70.6%
	Non Saudi	62	20.3%
Duration of work	<10 Years	68	22.2%
	10 To 20 Years	174	56.9%
	>20 Years	35	11.4%

Regarding the level of education, 131(42.8%) had primary education while 122(39.9%) had secondary and 25 (8.2%) had none. Regarding duration of work, below 10 years were 68 (22.2%), above 10 years were 174(56.9%) and above 20 years 35 (11.4%).

Regression analysis

In this study, 38 seropositive samples identified through ECL Cobas. IgM and IgG were found positive for 32(10.5%) and 6 (2.0%) cases, respectively.

Additionally, odd ratios (OR) and their confidence interval [95% CI] were also noted as illustrated in Table 4 and Table 5. Factors with the p-value of less than 0.05 on multivariate logistic regression analysis were also considered having a statistically significant association with Brucellosis infection.

Table 4. Correlation Distribution on the basis of IgG

	IgM + (N = 6)	IgM - (N = 272)	$X2$	P value	OR (95% CI)
Male	5	182	0.719	0. 396	2.47 (.28-21.47)
Female	1	90			
Education					
1. None	0	25			
2. Primary	3	128	.615	.735	
3. Secondary	3	119			
Nationality					
1. Suadi	4	212	.431	0.512	0.566 (.101-3.166)
2. Non Saudi	2	60			
Duration of work					
1. <10 Years	1	67	0.341	0.511	
2. 10 to 20 years	5	169			
3. >20 years	0	35			
Age (mean ±S D)	29.67± 9.688	30.72±11.64		0.827	
Direct contact with animal					
Yes	5	161	1.422	0.233	3.447 (.39-29.90)
No	1	111			
Consumption of milk and animal product					
Yes	5	111	4.366	0.037	7.252(0.836- 62.923)
No	1	161			
Knowledge of toxoplasma					
Yes	2	66	0.261	0.609	1.561 (.2798.714)
No	4	206			

Risk factors

The findings justified our purpose to study the impact of risk factors involved in the spread of the brucellosis disease The investigation of the positive cases in both categories, IgG and IgM reveal that patient in the age group (18-40), having work duration (10 to 20 years), and gender (more male than female) were mainly infected. Another important observation was that all these patients consumed raw milk and milk products. Moreover, Saudi Arabia being a warm and dry country, no significant seasonal variation was observed.

DISCUSSION

The medical gazette of Saudi Arabia reports 8000+ cases annually of Brucellosis. This disease is also listed as one of those zoonotic diseases that humans have neglected (WHO), for which reason it has engrossed several regions globally and has caused extensively acute febrile illness in the Middle East regions too. The overall prevalence of human brucellosis in Alkharj has also been recorded as 13.6%, similar to the rates reported in other part of country.

Table 5. Correlation Distribution on the basis of IgM

	IgM +	IgM -	X2	P value	OR (95% CI)
	(N = 6)	(N = 272)			
Male	5	182	0.719	0. 396	2.47 (.28-21.47)
Female	1	90			
Education					
4. None	0	25			
5. Primary	3	128	.615	.735	
6. Secondary	3	119			
Nationality					
3. Suadi	4	212	.431	0.512	0.566 (.101-3.166)
4. Non Saudi	2	60			
Duration of work					
4. <10 Years	1	67	0.341	0.511	
5. 10 to 20 years	5	169			
6. >20 years	0	35			
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Direct contact with animal					
Yes	5	161	1.422	0.233	3.447 (.39-29.90)
No	1	111			
Consumption of milk and animal product					
Yes	5	111	4.366	0.037	7.252(0.836- 62.923)
No	1	161			
Knowledge of toxoplasma					
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No	4	206			

The prevalence of this disease had been in Saudi Arabia since decades; however it has increased recently. For instance, the Southwestern region reported prevalence rate of 16% with the southern region alone having 19% [22-23]; the central region reported 48.5% [24-25] and the least seroprevalence was 2.6% in the North Western region [25]. Similar results were found in several other studies that analyzed the seroprevalence of human brucellosis in regions of Saudi Arabia. [24, 26-27] with national average calculated as 15% [26].There is seroprevalence seen in neighboring countries too; for instance, 11.4% in Sudan [28], 6.26 % in Egypt [29], and 6.2% in Yemen [30].

A recent study reported a slight reduction in the occurrence of human brucellosis [31]. This improvement has been accredited to the high level efforts made by public health ministry educating awareness about measures such as milk pasteurization, and livestock immunization [32]. A major drawback to curtail this disease is the non-availability of a vaccine that could prevent human brucellosis. However, the public health ministry was doing a commendable job in Saudi Arabia to adopt and implement such disease control policies with the help of health staffs to conduct educational awareness program for the local community. In Alkharj too, educational and awareness program about human brucellosis and its risk factors are given priority as community service for the target population of local public and university students.

It is a proven fact that brucellosis cannot be diagnosed only with the help of clinical symptoms and lab testing is a mandatory requirement through serological methods [1, 3]. In this study, therefore, serological tests such as IgM and IgG were performed on each sample. IgM detected 96.8% of cases followed by IgG in 86.9% (Table 4 and Table 5). The IgG and IgM were detected through ECL Cobas method which is more specific and sensitive than ELISA method. However, studies have prioritized the detection of IgG antibodies more than IgM antibodies in order to diagnose brucellosis [33] and obtain the current level of sensitivity and specificity. For instance, wherever there was suspicion about brucellosis, both IgG and IgM tests were carried out. A study found out the IgG and IgM sensitivities of Brucella bacterium as 91% and 100% respectively with 100% specificity in both cases [34].

The data of all these studies (35, 22] reveal that seroprevalence of brucellosis was found more (<75%) in male than the female population which may be because in the Saudi regions the male are more exposed to the risk factors such as direct contact with animals, meat,

and milk products. These studies also reveal that 70.6% of the infected population, with (72.2%) in the 18-40 years age group, were the Saudi natives. Again this kind of seroprevalence was attributed to the fact that the infected group has to remain more in contact with animals for the purpose of cattle breeding, farming, butchering etc. the consumption of raw milk and other dairy products was found to be the next significant cause of this seroprevalence, according to these studies.

These facts and statistics are similar to our findings of the current study, where all these factors such as contact with animals and consumption of raw milk were identified as the major risk factors (p<0.001) for human brucellosis. Also these findings are consistent with that of other studies [26, 29, 36].

Likewise, our study found out that duration of work period (10 to 20 years) proves a major factor as 82.6% of people in this work duration were found to be infected. We did not find any seasonal variation in our study which is also consistent with similar findings in other regions of Saudi Arabia [3, 22]. Taking the larger view of this factor, no seasonal influence is generally found as cause of triggering the incidence of brucellosisis in tropical and subtropical areas. The reason could be because animal breeding happens throughout the year in these regions [1, 3]. A major variation in our study, however, was seen in children and adolescents (<19 years) who were found to be less affected by this disease.

CONCLUSIONS

This study revealed a seroprevalence rate of 13.5 % annually in the sampled region of Alkharj, Saudi Arabia. Its incidence was detected among Saudi nationals, more in male than female and among the working age group. It was also discovered that the contact with domestic animals and consumption of raw milk were found to be major risk factors and modes of transmission of this disease. In suspected cases, IgG tests were found to be more diagnostically significant. It was observed that government measures to control this infectious disease included vaccination, awareness programs about personal hygiene, farm sanitation and adoption of preventive measures to reduce its incidence.

Based on these findings it is recommended that more such awareness programs particularly among livestock community and in rural areas should be carried out. Efforts should be made to highlight the risk factors and methods to prevent brucellosis. The government may also initiate serological surveillance units of human brucellosis at all district headquarters of Alkharj region targeting the livestock professionals and the agropastoral communities.

Often clinical based diagnosis of human brucellosis is difficult due to suspected signs and symptoms. This causes further complications and delay in treatment and rehabilitation. It is also recommended that this disease should be diagnosed more accurately and at early stages to ensure quick recovery. Such valid and reliable serological tests should be readily available ensuring early diagnosis and prompt treatment.

ACKNOWLEDGEMENT

CONFLICTS OF INTEREST

The authors also declare no conflicts of interest.

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