

Sero-prevalence and Perception of Brucellosis among High-Risk Groups, A cross-sectional Study

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ABSTRACT

OBJECTIVE: To determine the seroprevalence of Brucellosis and assess the disease's perception among high-risk groups.

METHODOLOGY: This descriptive cross-sectional, community-based study was conducted among 125 high-risk group individuals vulnerable to infection with Brucellosis and compared to 50 individuals of non-risk groups. Obtained sera were tested for the presence of antibodies to Brucella using the Rose Bengal plate test and standard tube agglutination test. A questionnaire was designed to assess the risk behavior and previous knowledge about the disease.

RESULTS: The males were 78%, and the females were 22%. The seropositivity of Brucellosis was 3.2% among people at high risk and nil for the non-risk groups. The disease had a high prevalence in dairy farmers, which was more prevalent in dairy farmers (2.4%) and slaughterhouse workers (0.8%). All infected persons were males; the disease is most common in the age group 15-25 years.

CONCLUSION: There was a statistically significant correlation between education level and the prevalence of Brucellosis. The two laboratory methods used to diagnose Brucellosis gave the same results. Routine checkups and education of at-risk individuals are recommended to help control the disease.

KEYWORDS: Brucellosis, High risk groups, Perception, Sero-prevalence, Sudan, Weil Flix Test Method, Widal Method

INTRODUCTION

Brucellosis is an infectious disease previously known as Rock, Cyprus, Undulated, Gibraltar, Malta, and Mediterranean¹. Bacteria of the genus *Brucella* are major zoonotic pathogens responsible for considerable human morbidity in areas where they are endemic in livestock. Either direct contact with infected animals or their infected products infects humans². People who are working in jobs requiring frequent contact with animals or meat such as slaughterhouse workers, farmers, veterinarians, and dairy workers are at high risk¹. Brucellosis has been documented worldwide, over a number of years in a great variety of wildlife. Recently Brucellosis has been reported in a wide variety of marine mammals. A very important consideration in regard to Brucellosis in wildlife is to distinguish between spillover infection from domestic animal management and environmental factors. *Brucella* organisms are shed in milk, urine, and vaginal discharges and they contaminate the environment. The infection occurs through the ingestion of non-boiled milk of infected animals, contact with vaginal discharge, urine, stools, and the blood of infected animals, through breached skin and the mucous membrane of the conjunctiva, and also by inhalation³. In this study, we aimed to determine the seroprevalence of Brucellosis among the people in the high-risk group in Atbra, River Nile State, Sudan, and to assess the knowledge of the study population about the disease and to compare the two laboratory methods that are commonly used in the diagnosis of Brucellosis (Rose Bengal Plate (RBP) method and Standard tube agglutination (STAT) Method).

METHODOLOGY

This was a descriptive cross-sectional community-based study conducted among people with a high risk for acquiring Brucellosis compared to non-risk groups. The study was conducted at the veterinarian laboratory research and special lab in Atbara city, Sudan. The investigation for Brucellosis was conducted by two methods Rose Bengal Plate (RBP) method and the Standard tube agglutination (STAT) Method. The Study area was Atbara city which is located in River Nile State in Sudan is about 310 km north of Khartoum and lies on the junction of River Nile and Atbara River with a total population of 134568. The participants were recruited from the security forces' agricultural project, the slaughterhouse, the veterinary hospital, Um-Altur, and the Atbara market, Sudan. The study was conducted in June 2011. The sample size of 175 individuals including 125 from at-risk groups and 50 recruited from non-risk groups. The high-risk group was as follows: veterinarian, dairy worker, butcher, farmer worker, and slaughterhouse. We selected 25 participants per group (A total of 125 participants). The control group was recruited from Nile Valley University Employees and housewives reside in the same area. The number of the control group was 50 individuals. During the period of study, the samples were taken by simple random technique. We included a healthy population who agree to participate in the study and exclude the very elder population and anyone who refused to participate in the study. The data were collected by a pre-tested questionnaire.

Methods used for detection of Brucellosis:

Method 1 Rose Bengal Plate (RBP): Acid antigen stained with Rose Bengal plate being highly and sensitive and rapid for human diagnosis, the test is simple to spot agglutination and buffered to low PH 3.65 + 0.05. (O.I.E).

Principle: The test is based on antigen-antibody reaction. If the antibody in the serum corresponding to the antigen, the reaction appeared as visual agglutination. Agglutination means a positive result if no agglutination it means a negative result. The Procedure was done according to the manufacturer's instructions.

Blood sampling: we took 3 ml of venous blood drawn from each participant in a dry glass tube by standard technique and biosafety. We prepared the serum by centrifugation of the blood. After that, we took the reagents from the refrigerator and stand till the temperature of the reagents became room temperature. Then we took 25µl of serum volume by automatic pipette into the white plastic plate and shacked the reagents well gently and an equal volume of antigen was placed in the serum spot. Immediately, after that mix suspension using a glass plastic rod for each test to produce an oval or circular zone. The mixer was rotated gently for 4 minutes. After 4 minutes the agglutination was read any visible reaction was considered a positive result. Comparing with positive control in the patch.

	T	C
R	25 µl	25 µl
S	25 µl	-
C	-	25 µl

R: Rose Bengal reagent, S: Serum, C: Positive control.

Method 2 Standard tube agglutination method (STAT): These stained bacteria antigen suspensions were bacteria killed and stained to enhance the reading of the agglutination reaction. There were two bottles one of them containing Brucella abortus, and the other was Brucella melitensis and control sera of rabbit. Principle: The Ag/Abs reaction was based and gives agglutination. We required: 8 small plastic test tubes for each sample, dispensing pipette 0.005 ml – 1 ml, 0.85% normal saline, and a water bath. Procedure: we placed eight test tubes in a rack and labeled 1 – 7 and control, we put 1.9 ml of 0.85 ml of normal saline

into the first tube (tube 1) and 1 ml saline in the remaining 7 tubes. Then, we added 0.1 ml of undiluted serum into the first tube and mixed them well. Then, we transferred 1 ml from the first tube into the second tube and mixed it well. This process as double dilutions of serum were continued and 1 ml was discarded from the seventh tube. The eighth tube was the control and contained 1 ml of saline only. The tubes were shaken well, and we added 1 drop of the undiluted antigen suspension to all the tubes including the control, and mixed them well. The tubes were incubated in a water bath at 37°C for 24 hours. After the incubation period, we carefully removed the rack, and agglutination was observed. The titer was taken as the last tube showing agglutination and then considered a positive result. While, if no agglutination occurred then it was considered a negative result. Using negative and positive control to ensure that the reagent working well. This procedure used for two antigen *Brucella melitensis* and *Brucella abortus*.

Statistical analysis

We enter data, clean, and analyzed using the statistical package for social sciences (SPSS) Version 26 (IBM Corp., Armonk, NY, USA). In the descriptive analysis, we analyzed the socio-demographic variables and presented them as frequencies, and in inferential statistics, Chi-square and t-tests were applied. We considered statistically significant for all two-sided p-values was ≤ 0.05 and 95% confidence interval (CI).

RESULTS

In this study, we identified that headache and joint pain were the most common symptoms of Brucellosis among the patients 48 (27.4%) and 42 (24%) respectively, while the lymph node was the least sign 3 (1.7%) (Table I). The most common age group of positive results was 15-25 years occurred in 2 patients. (Table II). Most of the participants were males 137 (78%) and the female were 38 (22%) (Table III). We identified that the most educational level was university level 57 (32.6%). The study reported that there was a significant correlation between education level and the prevalence of Brucellosis (Table IV).

Table I: The frequency distribution of symptoms and signs of Brucellosis among the participants

Symptoms and sign of Brucellosis	Yes Frequency (%)	No Frequency (%)
Headache	48 (27.4%)	127 (72.6%)
Joint pain	42 (24%)	133 (76%)
Neck pain	33 (18.9%)	142 (81.1%)
Testes pain	38 (21.7%)	131 (74.9%)
Fever	29 (16.6%)	146 (83.4%)
Liver and spleen	5 (2.9%)	170 (97.1%)
Lymph node	3 (1.7%)	172 (98.3%)

TableII: The association between Rose Bengal method and age group of the participants

Rose Bengal		Age group						Total
		15-25	26-35	36-45	46-55	56-65	66-75	
Positive	Count	2	1	0	0	1	0	4
	% within Rose bengal	50.0%	25.0%	0.0%	0.0%	25.0%	0.0%	100.0%
Negative	Count	35	54	45	25	7	5	171
	% within Rose bengal	21.0%	32.0%	26.0%	15.0%	4.0%	3.0%	100.0%
Total	Count	37	55	45	25	8	5	175
	% within Rose bengal	21.0%	31.0%	26.0%	14.0%	5.0%	3.0%	100.0%

P-value < 0.05

Table III: the association between Weil Flex reaction method and sex of the participants

Well Flexreaction Method		Sex		Total
		Male	Female	
Positive	Count	5	0	5
	% within well Flex reaction	100.0%	0.0%	100.0%
Negative	Count	132	38	170
	% within well Flex reaction	78.0%	22.0%	100.0%
Total	Count	137	38	175
	% within well Flex reaction	78.0%	22.0%	100.0%

P-value < 0.05

Table IV: Relationship between education level and mode of transmission

Educational level		Transmission				Total
		Raw milk	Raw meat	Both	I don't know	
Illiterate	Count	2	1	5	23	31
	% within education	7.0%	3.0%	16.0%	74.0%	100.0%
Basic	Count	6	0	3	24	33
	% within education	18.0%	0.0%	9.0%	73.0%	100.0%
Secondary	Count	15	3	14	22	54
	% within education	28.0%	6.0%	26.0%	41.0%	100.0%
University	Count	20	3	22	12	57
	% within education	35.0%	5.0%	39.0%	21.0%	100.0%
Total	Count	43	7	44	81	175
	% within education	25.0%	4.0%	25.0%	46.0%	100.0%

DISCUSSION

This study was conducted in northern Sudan to determine the seroprevalence and perception of Brucellosis among risk groups. One hundred and seventy five individuals were selected randomly. One Hundred and twenty five individuals from at-risk groups and 50 from control groups. The Sero-prevalence of Brucellosis among the study group was 2.3% actually this result is less than the fact result because of standard tube agglutination method gives false negative in chronic Brucellosis so it needs to be confirmed by other standard methods such as the 2ME method or (P C R) method. This result is lower than that reported by Ismail A 2007 (11.4%)⁴, and is similar to that reported by Mohammed N et al (3.3%)⁵ in Bangladesh. The study revealed the highest prevalence of Brucellosis among dairy workers and slaughterhouse workers and this is similar to that reported by Ismail A 2007⁴, and Muhammad N et al.⁵. The study reported that there was a significant correlation between educational level and the prevalence of Brucellosis and these findings disagreed with Cetinkaya Z 2005⁶ in western Anatolia. The study documented that there was a statistically significant association between the prevalence of the disease and previous knowledge of transmission of the disease, knowledge of Brucellosis, and causative agent. However, the study showed that there was no difference between the two methods that were used in the detection of Brucellosis, as they gave the same results. This fact is in agreement with Muhammad N et al.⁵.

CONCLUSION

From our findings and relevant previous studies, it is clear that there are some major reasons leading to this increase in the prevalence of Brucellosis such as educational status, previous knowledge, and being at occupational exposure such as dairy workers and slaughterhouse workers, and these risk factors should be addressed in order to control and prevent the disease. The health authorities need to highlight on the health education of the population especially among those in contact with animals to raise their awareness about Zoonotic diseases, the method of transmission from animal to human, and how to treat the residents of animal and their products. The role of animal health and veterinary health care can be strengthened by early discovering and excluding the infected animals from the herd. Additionally, the health authority should start to treat the infected animals and humans promptly and follow up with the livestock, and advise the animal owners about the methods to be used in diagnosis and treatment. Furthermore, the population needs to be educated about avoiding drinking unpasteurized milk and eating raw meat, as this is a social habit present in certain communities. Moreover, the vaccination of animals is crucial to eradicating the disease

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AUTHOR CONTRIBUTIONS

Bushara MOE: All research was done by principle author

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