Original Article

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 was prevalent 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 Mediterranean[1]. 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 infect humans[2]. People working in jobs requiring frequent contact with animals or meat, such as slaughterhouse workers, farmers, veterinarians, and dairy workers, are at high risk[1]. Brucellosis has been documented worldwide over several years in various wildlife. Recently Brucellosis has been reported in a wide variety of marine mammals. A significant consideration regarding Brucellosis in wildlife is distinguishing between spillover infection from domestic animal management and environmental factors. Brucella organisms are shed in milk, urine, and vaginal discharges, contaminating the environment. The infection occurs through ingesting the non-boiled milk of infected animals, contact with vaginal discharge, urine, stools, and the blood of infected animals, breached skin and the mucous membrane of the conjunctiva, and inhalation[3].

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 descriptive cross-sectional community-based study was 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, located in River Nile State in Sudan 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-Altuor, 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.

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doi: 10.22442/jlumhs.2023.01005
Received: 19-12-2022 Revised: 07-03-2023
Accepted: 16-03-2023 Published Online: 18-04-2023

We selected 25 participants per group (A total of 125 participants). The control group was recruited from Nile Valley University Employees and housewives in the same area. The number of the control group was 50 individuals. During the study period, the samples were taken by simple random technique. We included a healthy population who agreed to participate in the study and excluded the very elder population and anyone who refused to participate. 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 susceptible 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 appears as visual agglutination. Agglutination means a positive result; if there is 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 stood till the reagents became room temperature. Then we took 25µl of serum volume by automatic pipette into the white plastic plate and gently shacked the reagents, 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 positive. 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 killed and stained to enhance the reading of the agglutination reaction. There were two bottles, one containing Brucella abortus, and the other was Brucella melitenisis and control rabbit sera. Principle: The Ag/Abs reaction was based and gave 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 serum dilutions 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 shacked well, and we added one 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 incubation, 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. We used negative and positive control to ensure the reagent worked well. This procedure used for two antigen Brucella melitenisis and Brucella abortus.

Statistical analysis

We enter data, clean, and analyze 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 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 signs 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%)

Table II: The association between the Rose Bengal method and the 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.%	25.%	0.%	0.%	25.%	0.%	100.%
Negative	Count	35	54	45	25	7	5	171
	% within Rose Bengal	21.%	32.%	26.%	15.%	4.%	3.%	100.%
Total	Count	37	55	45	25	8	5	175
	% within Rose Bengal	21.%	31.%	26.%	14.%	5.%	3.%	100.%

P-value < 0.05

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

Well Flexreaction Method		Sex		Total
		Male	Female	
Positive	Count	5	0	5
	% within well Flex reaction	100.%	0.%	100.%
Negative	Count	132	38	170
	% within well Flex reaction	78.%	22.%	100.%
Total	Count	137	38	175
	% within well Flex reaction	78.%	22.%	100.%

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.%	3.%	16.%	74.%	100.%
Basic	Count	6	0	3	24	33
	% within education	18.%	0.%	9.%	73.%	100.%
Secondary	Count	15	3	14	22	54
	% within education	28.%	6.%	26.%	41.%	100.%
University	Count	20	3	22	12	57
	% within education	35.%	5.%	39.%	21.%	100.%
Total	Count	43	7	44	81	175
	% within education	25.%	4.%	25.%	46.%	100.%

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%. This result is less than the fact result because the standard tube agglutination method gives a false negative in chronic Brucellosis. So, it must be confirmed by other common techniques such as the 2ME method or the (PCR) method. This result is lower than that reported by Ismail A 2007 (11.4%) [4], similar to the study 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 2007[4], 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 2005[6] in western Anatolia. The study documented 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 no difference between the two methods 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 significant reasons leading to this increase in the prevalence of Brucellosis, such as educational status, prior knowledge, and being at occupational exposure, such as dairy and slaughterhouse workers. These risk factors should be addressed to control and prevent the disease. The health authorities need to highlight the health education of the population, especially 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 treating infected animals and humans promptly, following up with the livestock, and advising 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 specific communities. Moreover, the vaccination of animals is crucial to eradicating the disease.

Ethical permission: Behri University, Sudan, ERC letter No. 11204/006/018.

Conflict of Interest: No conflict of interest.

Financial Disclosure / Grant Approval: No funding agency was involved in this research.

Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTIONS

Bushara MOE: All research was done by the principal author

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