

Estimation of Brucella species antibodies among Butchers in Dongola city, Northern state (2025)

Wafa Mohammed Hussein Abuelgasim¹, Fawzia Ahmed Salih Fadl Allah², Ahmed Mohamed Zain³, Ayman Anwar Mohammed osman⁴, Muslih Haroun Elhussein Gamea^{5*}

¹National Ribat university, medical laboratory Sciences, Assistant professor of medical microbiology.

²Dongola university, assistant Professor of biology, Sudan

³Dongola university, Professor of Microbiolgy, Sudan

⁴Institute of Forensic Sciences, National ribat university, Sudan

⁵Sudan International University, Register of the National Council for Medical and Health Professions, Sudan

Received: 27.10.2025 Revised: 22.11.2025

Accepted: 11.12.2025

Published: 26.12.2025

*Corresponding Author: Muslih Haroun Elhussein Gamea

Email: muslihlab0900@gmail.com

Abstract: This study was carried out in Dongola. Northern state, during January 2024– February 2025. The study aimed to describe the trend and determine the prevalence of brucellosis among butchers in Dongola city butchers. A total of 92 sera samples were collected from butchers and were tested by Rose Bengal test, Widal test, and ELISA test. The study showed that there was significance difference among different age groups to get infection with Brucellosis. The study revealed that joint pain, back pain, and weakness were most common representing symptoms among butchers. The prevalence of brucellosis was obtained by each test was as follow: The prevalence by Rose Bengal test was found (4.3%), and by using Widal test for *B.abortus* was found (22.8%), and by using Widal test for *B.melitensis* was found (21.7%), and was found (13%) by ELISA IgG test, and was found (2.2%) by using ELISA IgM test. The percents of total positive results by different serological tests was 7% by Rose Bengal test, and was 36% by Widal test for *B.abortus*, and was 34% by Widal test for *B. melitensis*, and was 20% by ELISA IgG test, and was 3% by ELISA IgM test. This study reflects the clear difference between the results obtained by the Rose Bengal test, Widal test, and ELISA test in the detection of the infection status as past infection (chronic infection), or present now (acute infection) with Brucellosis.

Keywords: Brucellosis, Butchers, Seroprevalence, Rose Bengal Test, ELISA, Dongola.

Citation: Wafa Mohammed Hussein Abuelgasim *et al.* Estimation of Brucella species antibodies among Butchers in Dongola city, Northern state (2025). e Jnl Med Phrm Sci, 2025 Oct-Dec 1(1): 7-13.

INTRODUCTION

Brucellosis is one of the world's major zoonotic problems. Though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand [1], but it remains an uncontrolled problem in regions of high endemicity such as the Africa, Mediterranean, Middle East, parts of Asia and Latin America [2].

Brucellosis is caused by members of genus *Brucella*. These are small, non-motile, aerobic, facultative intracellular, Gram-negative coccobacilli. The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity [3]. The species of *Brucella* and their major hosts are *B.abortus* (cattle), *B.melitensis* (goats), *B.suis* (swine) and *Br.ovis* (sheep). *Br.abortus* also causes infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen [4]. From public health view point, brucellosis is considered to be an occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians.

Brucellosis is spread to humans by direct contact with living or dead infected animals and their carcasses or secretions (including their tissues, blood, urine, vaginal discharges, aborted fetuses, and especially, placentas) Infection is transmitted by inoculation through non-intact skin or through direct contact with mucosal surfaces, and through ingestion of raw milk and dairy products (e.g., unpasteurized cheese) from infected animals.

There are so many factors that can affect the prevalence of brucellosis in various species of livestock. Prevalence of brucellosis can vary according to climatic conditions, geography, species, sex, age and diagnostic tests applied.

The first state-federal cooperative efforts towards eradication of brucellosis caused by *Brucella abortus* in the U.S. began in 1934.

In Sudan many surveys were conducted to determine the incidence of human brucellosis. In 1978 it was found that 14% of persons contacted with dairy animals gave positive reaction, and some studies were taken brucellosis in animal as Musa [5] cited prevalence of the disease in man, cattles, camels, sheep, goats and equines, while *Brucellamelitensis* was isolated from goat milk among British resident in the Gezira area [6]. The organism was also isolated from camels in Butana area [7].

In north Sudan Abdullah [8] was study brucellosis prevalence rates in villager's cattle. In Darfur states, Musa [9] cited prevalence rate of brucellosis in cattle.

Brucellosis is an important but neglected disease in Sudan. And only a few recent studies have addressed the prevalence and importance of brucellosis as a human disease problem in Sudan, although it affects the population life and export of livestock.

The disease may be overlooked and misdiagnosed because of the difficult diagnosis and the absence and lack of facilities in laboratory fields. Alertness of medical staff is needed to recognize and diagnose the disease in many studies revealed that the highest prevalence rate was in the intensive farm system and under nomadic condition. [9]. Human cases remain unrecognized and treated symptomatically as other diseases or as "fever of unknown origin", because the disease expresses many non specific symptoms which similar other febrile conditions, because of that reason this study was undertaken to evaluate the prevalence of brucellosis among butchers in Khartoum State.

MATERIALS AND METHODS

A cross sectional survey study was conducted in Northern state. Dongola city between January 2024 and February 2025. Were estimated 97 butcher's sera to detect *Brucella* species antibodies by serological tests. Rose Bengal test with use of 1 drop of Rose Bengal *Brucella* stained suspension reagent and 40 ul of serum sample in serological card circle. If agglutination occur it's a positive and if not, it's a negative result.

Widal test for *B.abortus* and Widal test for *B.melitensis* were done by the agglutination way of serial diluted sera with constant volume of *Brucella* antigen suspension reagent.

Elisa IgG test and Elisa IgM test were performed after multi steps of sera dilution and add conjugate and substrate, stop solution and reading in Elisa reader at 450 nm, calculation and interpretation the results.

RESULTS

A total 92 butcher were included in this study, with age range is (18 - 80), and mean (38.8), all of them were males, their sera screened to detect the presence of *Brucella* antibodies .

The butchers healthy status and time of exposure and also age were analyzed statistically to detect any significance relationship between them and the presence of *Brucella* species antibodies in their sera .

4.1.Age distribution of objects

According to the butcher's age groups, we have seven age groups distributed as follow:

The age group 1 (18 - 26 year old) represented 15.2%, age group 2 (27 - 35 year old) represented 37%, age group 3 (36 - 44 year old) represented 12%, age group 4 (45 - 53 year old) represented 20.7%, age group 5 (54 - 62 year old) represented 10.9%, age group 6 (63 - 71 year old) represented 3.3%, age group 7 (72 - 80 year old) represented 1.1%.(Figure-1)



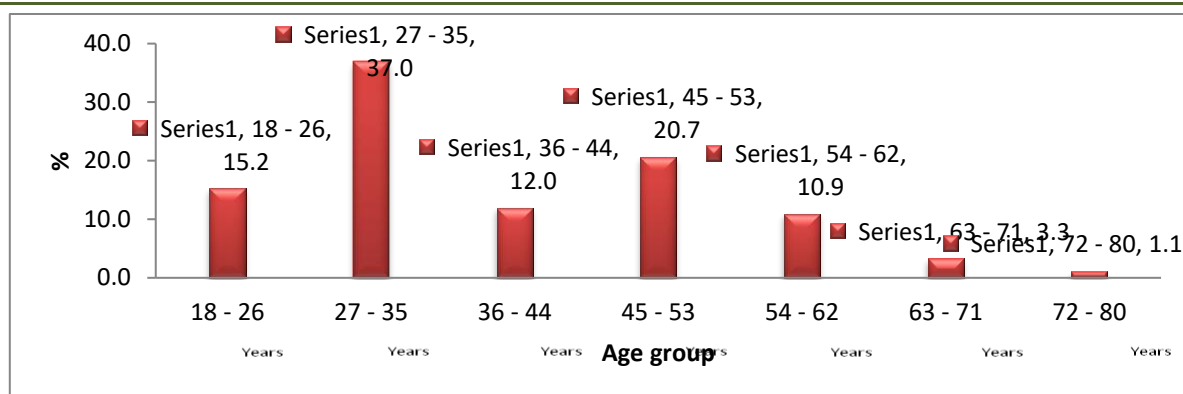


Figure-1: Percentages of age groups distribution.

The age group 1 (18 - 26) there gave 2 positive results with Widal test for *B.abortus*, and 4 positive results were represented with Widal test for *B.melitensis*, and 3 positive results were represented with ELISA IgG test (Table – 1). The age group 2 (27 - 35) there gave 3 positive results with Rose Bengal test, and 9 positive results were represented by Widal test for *B.abortus*, and 4 positive result were represented with Widal test for *B.melitensis*, and 2 results were represented with ELISA IgG test, 1 positive result was represented with ELISA IgM test (Table – 1).

Table-1: Age groups distribution in comparison with the results of serological tests

Age groups	Rose Bengal			Widal test				ELISA			
				<i>B.abortus</i>		<i>B.melitensis</i>		IgG		IgM	
	+ve	-ve		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
18 – 26	0	14		2	12	4	10	3	11	0	14
27 – 35	3	31		9	25	4	30	2	32	1	33
36 – 44	0	11		2	9	3	8	0	11	1	10
45 – 53	1	18		4	15	7	12	5	14	0	19
54 – 62	0	10		4	6	2	8	2	8	0	10
63 – 71	0	3		0	3	0	3	0	3	0	3
72 – 80	0	1		0	1	0	1	0	1	0	1
Total	4	88		21	71	20	72	12	80	2	90

The age group 3 (36 - 44) there gave 2 positive results with Widal test for *B.abortus*, and 3 positive results were represented by Widal test for *B.melitensis*, and 1 positive result was represented by ELISA IgM test (Table – 1).

The age group 4 (45 - 53) there gave 1 positive results by Rose Bengal test, and 4 positive results were represented with Widal test for *B.abortus*, and 7 positive result were represented with Widal test for *B.melitensis*, and 5 positive results were represented with ELISA IgG test (Table – 1).

The age group 5 (54 - 62) there gave 4 positive results with Widal test for *B.abortus*, and 2 positive results were represented by Widal test for *B.melitensis*, and 2 positive results were represented by ELISA IgG test (Table – 1).

The age group 6 (63 - 71) and group 7 (72 – 80) shown that no positive results were represented by each of three serological tests. That means the high prevalence of brucellosis were increased in young age group (27 – 35), and were decreased in the older age group (72 – 80) (Table – 1).

The results were obtained that supported the association between the ages and increased of infection rate by *Brucella* species.

4.2. Health status of objects

Seven butchers 7(7.6%) represented a fever sign (Figure -2), that percent represented 2 positive results with Rose Bengal test, 5 negative results were represented with all serological tests.



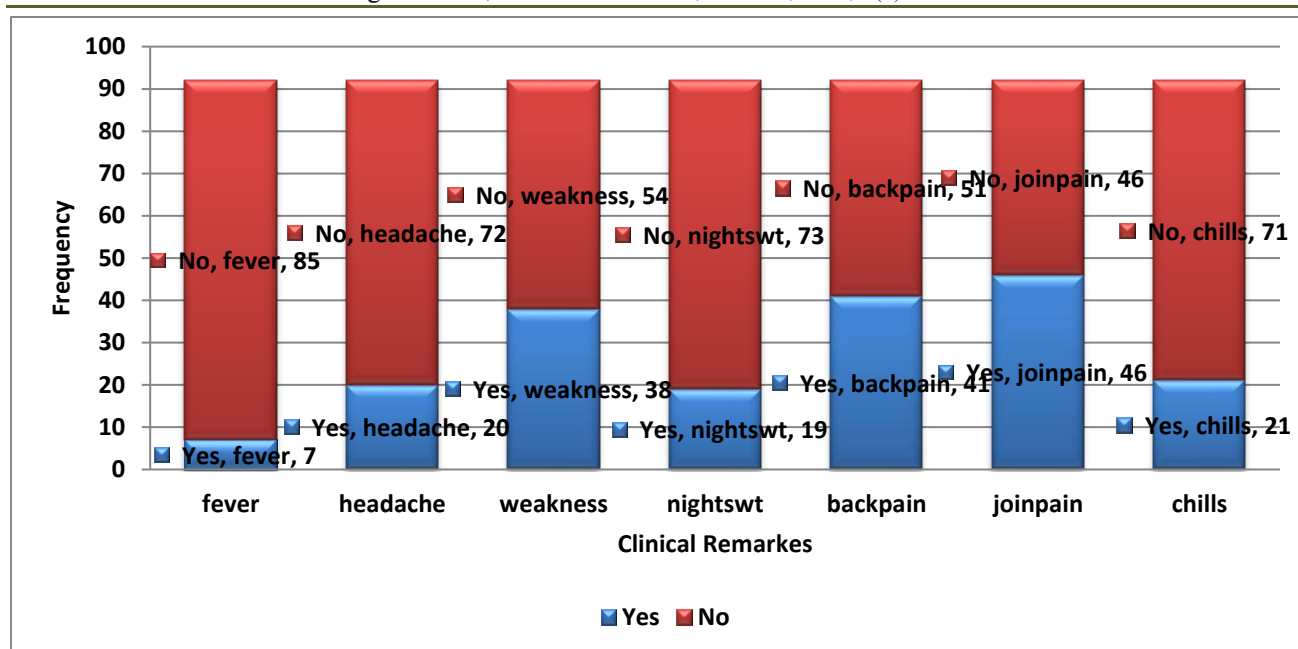


Figure-2: Health status of objects

(%21.7)20 were represented a headache sign (Figure -2), That percent represented 2 positive results with Widal test for *B.abortus*, and 4 positive results were represented with Widal test for *B.melitensis*, and 2 positive results were represented with IgG test, and 12 negative results were represented negative results with all serological tests.

(%44.6)41 were represented a back pain sign (Figure -2), That percent represented 2 positive results with Rose Bengal test, and 9 positive results were represented with Widal test for *B.abortus*, and 9 positive results were represented with Widal test for *B.melitensis*, 5 positive results were represented with ELISA IgG test, 19 negative results were represented with all serological tests.

(%50)46 were represented a joint pain sign (Figure -2), That percent represented 2 positive results with Rose Bengal test, and 9 positive results were represented with Widal test for *B.abortus*, and 7 positive results were represented with Widal test for *B.melitensis*, 5 positive results were represented with ELISA IgG test, 27 negative results were represented with all serological tests.

38 (%41.3) were represented a weakness sign (Figure -2), That percent represented 2 positive results with Rose Bengal test, and 9 positive results were represented with Widal test for *B.abortus*, and 9 positive results were represented with Widal test for *B.melitensis*, 5 positive results were represented with ELISA IgG test, 16 negative results were represented with all serological tests.

%20.7)19 were represented a night sweating sign (Figure -2), That percent represented 1 positive results with Rose Bengal test, and 3 positive results were represented with Widal test for *B.abortus*, and 1 positive result was represented with Widal test for *B.melitensis*, 5 positive results were represented with ELISA IgG test, 13 negative results were represented with all serological tests.

(22.8)21 were represented a chills sign (Figure -2), That percent represented 4 positive results with Widal test for *B.abortus*, and 4 positive results were represented with Widal test for *B.melitensis*, 1 positive result was represented with ELISA IgG test, 14 negative results were represented with all serological tests.

4.3. Time of exposure as risk factor to get Brucellosis

According to time of exposure to meat or total time of job as butcher, we have five groups distributed as follow:

Total time of exposure group 1 (1 - 10 year), group 2 (11 - 20 year), group 3 (21 - 30 year), group 4 (31 - 40 year), group 5 (41 - 50 year.)

The total time of exposure group 1(1 – 10 years) represented 2.2% positive results with Rose Bengal test, and were represented 10.9% positive results with Widal *B.abortus* test, and 7.6% positive results were represented with Widal



B.melitensis test, and 3.3% positive results were represented with ELISA IgG test, and 1.1% positive result was represented with ELISA IgM test. (Table – 2.)

Table-2: Total time of exposure groups in comparison with the results of serological tests

Time of exposure groups	Rose Bengal			Widal test						ELISA					
				B.abortus			<i>B.melitensis</i>			IgG			IgM		
	+ve	-ve	P-value	+ve	-ve	P-value	+ve	-ve	P-value	+ve	-ve	P-value	+ve	-ve	P-value
1 – 10	2.2 %	33.7 %	0.878	10.9 %	25%	0.316	7.6 %	28.3 %	0.05	3.3%	32.6 %	0.725	1.1 %	34.8 %	0.602
11 – 20	1.1 %	33.7 %		4.3 %	30.4 %		4.3 %	30.4 %		5.4%	28.3 %		0%	34.8 %	
21 – 30	1.1 %	14.1 %		3.3 %	12%		3.3 %	12%		1.1%	14.1 %		1.1 %	14.1 %	
31 – 40	0%	12%		4.3 %	7.6%		6.5 %	5.4%		3.3%	8.7 %		0%	12 %	
41 – 50	0%	2.2%		0%	2.2%		0%	2.2%		0%	2.2 %		0%	2.2 %	

The significant P-value is less than 0.05

The total time of exposure group 2(11 – 20 years) represented 1.1% positive results with Rose Bengal test, and were represented 4.3% positive results with Widal B.abortus test, and 4.3% positive results were represented with Widal *B.melitensis* test, and 5.4% positive results were represented with ELISA IgG test (Table – 2.)

The total time of exposure group 3(21 – 30 years) represented 1.1% positive results with Rose Bengal test, and were represented 3.3% positive results with Widal B.abortus test, and 3.3% positive results were represented with Widal *B.melitensis* test, and 1.1% positive results were represented with ELISA IgG test, and 1.1% positive result was represented with ELISA IgM test. (Table – 2.)

The total time of exposure group 4(31 – 40 years) represented 4.3% positive results with Widal B.abortus test, and 6.5% positive results were represented with Widal *B.melitensis* test, and 3.3% positive results were represented with ELISA IgG test. (Table - 2)

The total time of exposure group5 (41 – 50 years) represented negative results with all serological tests.

In this results showed insignificant association between time of exposure and that results were obtained by Rose Bengal test(P-value = 0.878), and found insignificant association between time of exposure and that results were obtained by Widal B.abortus test (P-value = 0.316), and found significant association between time of exposure and that results were obtained by Widal *B.melitensis* test (P-value=0.05), and the insignificant association was found between time of exposure and that results were obtained by ELISA IgG test (P-value = 0.725), and also found insignificant association between time of exposure and that results were obtained by ELISA IgM test (P-value = 0.602).(Table – 2)

4.4. Different serological test results

This study showed (4.3%) serologically positive cases, and (95.7%) serologically negative cases by Rose Bengal test, and found that (22.8%) serologically positive cases and (77.2%) serologically negative cases by Widal test for *Brucellaabortus*, and (21.7%) serologically positive cases, and (78.3%) serologically negative cases by Widal test for *Brucellamelitensis*.



It studies also showed that (13%) positive cases, and found (87%) negative cases by ELISA IgG test, and (2.2%) positive cases, and (97.8%) negative cases by ELISA IgM test.

In this result the prevalence of brucellosis obtained by Rose Bengal test was (4.3%), and by Widal test for *Brucella abortus* was (22.8%), and by Widal test for *Brucella melitensis* was (21.7%), and was (13%) by ELISA IgG test, and was (2.2%) by ELISA IgM test (Figure – 4.)

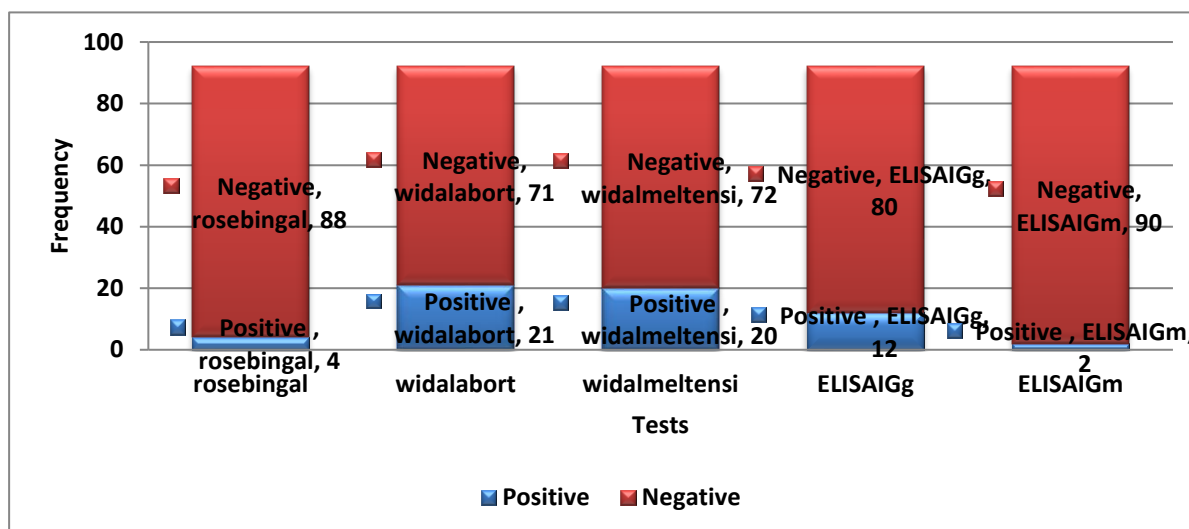


Figure-4: Prevalence of brucellosis by serological tests

The percent of total positive results represented by different serological tests was 7% by Rose Bengal test, and was 36% by Widal test for *Brucella abortus*, and was 34% by Widal test for *Brucella melitensis*, and was 20% by ELISA IgG test, and was 3% by ELISA IgM test (Figure-5).

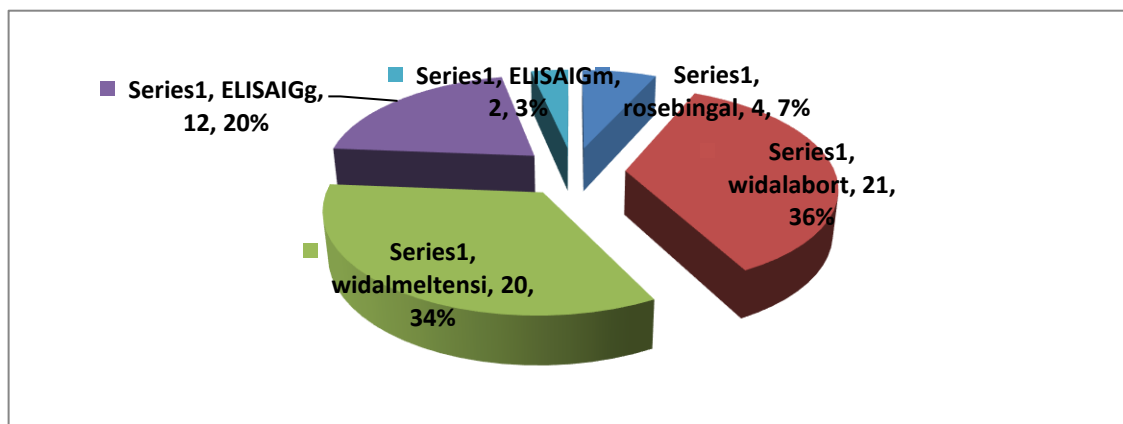


Figure-5: Percentages of total positive results by serological tests

4.5. Detection of *Brucella* species among objects

The main *Brucella* species that caused Brucellosis in butchers was *Brucella abortus* and *Brucella melitensis* species, and the prevalence detected among the target group by using of different serological tests were shown in (Figure – 4)

DISCUSSION

Brucellosis is diagnosed either by isolation of *Brucella* organism in culture or by a combination of serological tests and clinical findings consistent with brucellosis. Isolation of the *Brucella* organism is the definitive means of diagnosis but in practice it is difficult due to the early tissue localization, exacting culture requirements of the organism and also prolonged time required for isolation. In practice blood cultures are positive in 10-30% of brucellosis and the remainder is diagnosed serologically [10].

Our study findings correlated with the findings reported by [11] Dubray G who found the classical Rose Bengal test, Widal test, and ELISA tests have not shown similar results, the sensitivity of Widal test is higher than that of Rose Bengal test but the specificity can be disappointingly low. However, no single test provides 100% sensitivity and specificity, Rose Bengal test is often used as a rapid screening test. In Widal tests, the titers reported are in the range of 1:160 through 1:1280 IU.

The ELISA has the advantage of being highly sensitive, specific, but expensive and need special equipments to performed and need highly qualified technician. The ELISA used in this study can detect significantly the presence of brucellosis infection than Rose Bengal test and Widal test Reguera JM [12], sera showed the presence of IgG antibodies was 12 out of 92(13%), and the presence of IgM antibodies was 2 out of 92 (2.2%) butchers, these results supported that study reported by Reguera JM [12].

Cross-reactions and false-positive test results can occur in *Brucella* antibody tests. The primary immune determinant and virulence factor for *Brucella* species is the cell wall surface lipopolysaccharide, which is antigenically similar to the lipopolysaccharide of other gram-negative rods. False-positive *Brucella* antibody test results can be caused by cross-reactivity of antibodies to *Escherichia coli* O157, *Francisella tularensis*, *Moraxella phenylpyruvica*, *Yersinia enterocolitica*, and certain *Salmonella* species [13]. Most cross-reacting antibodies are IgM [14].

In this study the titers obtained in Rose Bengal test showed a low degree of correlation with those obtained by Widal test, and ELISA test, in contrast with other study reported by Najjar D *et al.* [15] which that found no significance difference between Widal test and Rose Bengal test in diagnosis of brucellosis.

According to other studies done found that no significance difference among different age groups to get infection with Brucellosis Najjar D *et al.* [15], in comparison with our study in which we found that the sensitive age group that affected by brucellosis were (27-35) and (36-44) that groups with high prevalence found, and the not affected groups were (63-71) and (72-80), because the older groups may be immunized due to repeated exposure to infection and the titer of antibodies was very low to be detected by conventional methods.

In this study the overall prevalence of brucellosis among butchers were obtained by Rose Bengal test was (4.3%), and by Widal test for *Brucella abortus* was (22.8%), and by Widal test for *Brucella melitensis* was (21.7%), and was (13%) by ELISA IgG test, and was (2.2%) by ELISA IgM test, compared with the study of Teoman *et al.*, 2007 which prevalence was found in sample size 1436 humans, and 3.2% (46) of the population was found to be positive by Rose Bengal test (RBT) and 3.0% (43) gave positive results with the standard tube agglutination test (STAT). Immunoglobulin class analysis of RBT-positive sera using the ELISA test gave positive results for 33 people (75%) with *Brucella* ELISA IgG and 11 people (25%) with *Brucella* ELISA IgM. The prevalence rate of our study was less than that obtained by Teoman *et al.*, 2007) that may be due to difference in sample size.

In this study the prevalence rate when compared with [16] was less than found, which is that found the seroprevalence of brucellosis in Saudi Arabia among abattoir workers was (4.0%), infection was more common among butchers (8.9%).

Araj and Azzam [17] found higher prevalence in Lebanon, their overall seroprevalence based on standard agglutination test was 1.7%, and by ELISA IgG test was 57%, 61% by ELISA IgM test. In our studies we found the prevalence by Widal *B. abortus* test was (22.8%), and by Widal *B. melitensis* test was (21.7%) and by ELISA IgG (13%) and by ELISA IgM (2.2%), this might be due to use five tests in this study and who used three tests only (Figure – 4).

The present study showed that the brucellosis is still a hazard in the butchers and the cases of brucellosis may be easily misdiagnosed because of the deceptive nature of the clinical signs and symptoms. All the cases that showed the presence of antibodies to *Brucella abortus* had varied clinical manifestations of brucellosis. Clinicians usually miss many cases of brucellosis because it is not considered a common disease. The clinicians should keep in mind the possibility of an occupational or environmental exposure in cases of fever. It would also be worthwhile to create awareness about the disease in such butchers so that necessary precautions and periodic screening of such occupationally exposed people can be done. Elimination of the infection in animals by vaccination to produce *Brucella* free animals/animal products can prevent the infection in humans. The practice of universal precautions among high-risk population cannot be overemphasized.

CONCLUSION

The results of this study showed that butchers who contacted animal meat were likely to get infection of Brucellosis. In this study the results supported the importance of including the *Brucella* diagnosis as the one of the differential diagnosis when dealing with clinical symptoms with special consideration of the subject's history in contact with meat.



The control programme of brucellosis was insufficient, and the facilities of continuous health checking and the orientation of the disease is not enough.

The administration of the slaughterhouses in Northern state is needed to be more healthy and dual monitoring from the Ministry of Animal Resources.

Substantial progress has been achieved in understanding the molecular basis of the genetics of *Brucella* and the pathogenesis of the infection. However, further progress is needed, especially in relation to diagnostic procedures and therapy.

Recommendation

The control of the zoonotic disease as Brucellosis is difficult in the Sudan, because it is a big country and contains many farms which played a major role in spreading of the disease from animal to human in close contact areas.

A control programme of human brucellosis would depend on large extent on the public health education, and ensuring the maximum cooperation of community.

Active cooperation between health services and veterinary services should be controlled by a Ministry of Health and Ministry of Animal Resources.

Authors' contributions: All authors contributed equally

Declaration: All authors read and approved the final manuscript.

Funding:None

Ethical Approval: None

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Questionnaire: Brucellosis Among Butchers in Dongola (2025)

Section A: Demographic Data

1. Age:
2. Gender:
3. Education level:
4. Residence:

Section B: Occupational History

5. How long have you been working as a butcher?
6. Do you handle raw meat daily? (Yes/No)
7. Do you use protective equipment (gloves, masks)?
8. Do you have any cuts or wounds during work?

Section C: Exposure Risk

9. Do you handle animal blood or tissues directly?
10. Do you consume raw milk or unpasteurized dairy products?
11. Have you had contact with sick animals?

Section D: Clinical Symptoms

12. Do you experience fever?
13. Do you have joint pain?
14. Do you have back pain?
15. Do you feel weakness?
16. Do you experience night sweating?
17. Do you have chills?

Section E: Medical History

18. Have you ever been diagnosed with brucellosis?
19. Have you received any treatment for similar symptoms?
20. Do you regularly visit a healthcare facility?

Section F: Laboratory Investigation

21. Have you undergone blood tests for brucellosis?
22. If yes, which tests were performed? (Rose Bengal / Widal / ELISA)
23. Do you know your test result?

