Human Brucellosis: Methods of Diagnosis and Risk Factors among Egyptian Patients at Assiut Fever Hospital

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Original Article

ABSTRACT

Background: Human brucellosis, a common zoonotic disease, is major public health problem in many countries worldwide including Egypt.

Objectives: To define brucellosis patients' risk-factors and to assess diagnostic lab methods of brucellosis at Assiut Fever Hospital.

Patients and Methods: The study recruited 98 patients with brucellosis and an equal number of controls. All participants were subjected to interview, clinical examination, and lab investigations.

Results: Older age, males, rural residence, low socioeconomic status were significant risk-factors (OR=3.76, 2.04, 2.86, 2.72; respectively). Occupations had animals' contact were significant risk-factor (OR=4.7); the most risky were butchers/slaughter workers (OR=8.0) and farmers/dairy workers (OR=3.59). Longer occupational exposure was risk-factor (OR=15.57). The main significant presenting symptoms were fever and musucloskeletal affections. The main significant signs were high temperature and hepato- and spleno-megaly. Standard agglutination test (SAT) titer 1/320 was the cut-off point for diagnosis and significantly lies in area under the ROC curve, sensitivity=96.4% and specificity=100.0%. Blood culture was positive in 58.2% of cases with no significant differences between SAT titer and blood culture positivity. ELISA IgM and IgG results were positive in 69.4% and 65.3% of the cases with no significant differences between SAT titer and IgM and IgG results.

Conclusions: Human brucellosis has many preventable risk-factors; its diagnosis depends mainly on presence of risk-factors, clinically suspected, and SAT titer ≥1/320.

Key Words: Brucellosis, clinical, diagnosis, risk-factors, sociodemographic.

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INTRODUCTION

Human brucellosis is a common neglected, re-emerging, zoonotic disease with worldwide distribution; it jeopardizes human health and animal production[1]. It's caused by bacteria of genus Brucella; human pathogens are B. abortus, melitensis, suis, etc. [2]. The disease is infectious; transmitted to humans by contact with fluids of infected animals or derived food products[3]. Over than 500.000 cases are reported yearly in many countries[4]. Brucellosis prevalence had increased in many developing areas[5]; up-to 17.0%[6]. However, its epidemiology had drastically changed over the past decade because of socioeconomic, sanitary, global travel development, and political reasons[7]. As an effect of farm animal screening and vaccination programs, and pasteurization of dairy-products, the overall incidence of brucellosis become lower[8].

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In Egypt, brucellosis still endemic; its true incidence is underestimated[9]. In rural Gharbia, brucellosis seroprevalence was 1.7%[10]. While, brucellosis seroprevalence among exposed workers in Sharkia was 21.0%[11]. A hospital-based study in Ain-Shams University Hospitals showed brucellosis was the commonest infectious disease, in adults, causes fever of unknown origin (FUO)[12]. Also, 3.0% and 11.0% of 10, 130 acute febrile illness (AFI) patients, from 13 Egyptian Fever Hospitals, were positive for brucella using culture and serology, respectively[13].

Brucellosis is systemic infection; any body organ can be involved[2,14 - 14]. It has high morbidity for humans and animals; it's an important cause of public health problem and economic loss in many developing countries[15]. It's included in the differential diagnosis of FUO/AFI in endemic areas. It's a disease of protean manifestations; however fever is fixed. Examination is non-specific; but lymphadenopathy, hepatomegaly, and/or splenomegaly are often present[16]. Acute illness is characterized by high swinging fever, rigors, sweating, lethargy, headache, and joint/muscle-pains[17].

Development of definitive diagnostic test for brucellosis is an elusive target[16]. Various serological tests have been deployed for brucellosis screening in humans[18]. Definitive and dependable method for brucella diagnosis depends on its isolation from blood or other tissues[19]. Because Brucella is difficult to culture, diagnosis usually depends on positive Brucella agglutination or enzyme linked immunosorbent assay (ELISA) test results with high titers of antibody (Ab)[14]. Serological methods have proven useful in the study of brucellosis in developing countries because they are simple, cost effective, robust and reproducible[20].

OBJECTIVES OF THE STUDY

They are to determine the sociodemographic, lifestyle, and risk-factors of brucellosis patients; to define duration of antibiotic use and relapse rate; to evaluate diagnostic lab methods (standard agglutination test (SAT), blood culture, ELISA)) of brucellosis; and to assess SAT as a significant, standard diagnostic lab method for brucellosis.

PATIENTS AND METHODS

I. Study design, setting, and time: A hospital-based, case-control, follow-up study design was chosen to perform this research at Assiut Fever Hospital, from February 2018 to January 2019.

II. Administrative design: Approvals to conduct the study were obtained.

III. Study population: Patients with clinical and epidemiological features suspected of brucellosis, admitted to the hospital to verify the diagnosis, were the target population.

IV. Patients and controls: Patients were checked by SAT to prove the diagnosis, titer ≥1320/ was considered positive (case). Equal number of apparently healthy subjects (other out-patients without abnormal findings) was enrolled as controls.

V. Ethical consideration: Study protocol was approved by local Ethical Committee of Al-Azhar Faculty of Medicine, Assiut. Study aims were explained to the participants; accordingly informed consents were taken from them.

VI. Study tools:

1. Interviewing form: A specially designed, comprehensive interviewing form was used. Socioeconomic level was determined according to El-Gilany *et al.*[21] with modification.

2. Clinical examination: The participants were subjected to full clinical examinations.

3. Investigations: The needed investigations (e.g. pelvic-abdominal sonography, CT-abdomen, etc.) were done for the cases.

4. Laboratory tools and methods:

4.1. Routine laboratory tools: The participants (patients and controls) were subjected to complete blood count (CBC), erythrocyte sedimentation rate (ESR), liver- [alanine amino-transferase (ALT), aspartate amino-transferase (AST), total serum bilirubin] and renal-functions (urea and creatinine).

4.2. Specific laboratory tools and methods: Whole blood samples were collected in 5ml plain Vacutainer tubes and transported directly to the laboratory where they left to clot, then centrifuged for 15minute at speed of 1500g, finally sera were separated and preserved at −20°C until tested.

4.2.1. SAT: Serum samples, from the participants, were analyzed using suspension of B. abortus and melitensis (Wellcome Laboratories, UK). The procedure was according to Salata[22]. Agglutinins detected in serum are usually IgM or IgG. In some sera, a blocking factor may interfere with agglutination at low serum dilution; may be due to presence of IgA or other non-agglutinating Ab. Positive results, available after 24hrs, were defined as any sample showing visible agglutination with naked eye after gentle agitation of the mixture. Any positive subject of the controls was excluded.

4.2.2. ELISA (for the patients only): ELISA is based on reaction of Abs in the sample tested with Ag adsorbed on a polystyrene surface. Unbound immune globulin is washed-off and an enzyme labeled with anti-human globulin binds the Ag-Ab complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution (tetramethylbenzidine) to render a blue

colored soluble product that turns into yellow after adding acid stopping solution[23].

Results interpretation: Ab-index=Sample optical density (OD)/cut-off serum mean OD x 10; Ab-index <9: -ve, 911-: equivocal, and >11: +ve. Samples with equivocal results must be retested and/or a new sample obtained for confirmation. Samples with the Ab-index <9 were considered as not having IgG specific Abs against Brucella. Samples with Ab-index >11 were considered as having IgG specific Abs against Brucella[23].

4.3. Blood culture (for the patients only): The most conclusive mean of proving the diagnosis of brucellosis is a positive culture[4]. Blood samples were inoculated aseptically into blood culture bottles containing serum dextrose broth agar and subculture done every three days. The medium pH was adjusted in between 6.6 and 7.4; sterilized using autoclave at 121°C for 20minutes with 1% glucose and 5% inactivated serum-horse before dispensing into Petri dish or tubes for slants.

5. Follow-up: Patients were followed-up for 3months to monitor duration of treatment and relapse by SAT, ELISA, and/or blood culture as required.

VII- Statistical analysis

Data analysis was done using statistical package for the social sciences version20. Data were presented as mean± standard deviation (SD) for quantitative variables and frequency and percentage for qualitative variables. Groups' comparison was done using independent sample t-test for quantitative data and Yates chi-square ($\chi 2$) or Fischer's exact (FE) tests, as appropriate, for qualitative variables. To determine risk-factors, odds ratio (OR) was used. Receiver operated characteristic (ROC) curve was constructed with area under curve (AUC). It provides a useful way to evaluate the sensitivity and specificity for quantitative diagnostic tool that categorize cases into one of two groups. Analysis was done to detect the cut-off point of SAT titer to detect patients with brucellosis. *P-value* <0.05 was considered statistically significant difference for t-, $\chi 2$, and FE tests. While, 95% confidence interval (CI) or exact confidence limits (ECL) were used, as appropriate, for OR.

RESULTS

Among 150 cases suspected clinically to have brucellosis, diagnosis was proved in 98 (65.3%) cases by SAT; B. abortus (38.8%), melitensis (18.4%), and mixed (42.8%). The M±SD of hospital stay, antibiotics' courses duration, and time for relapse occurrence were 13.0457.68±29.94 ,8.15±26.67 ,4.52±day; respectively (Table1).

Table 1: Frequency distribution of the studied cases according to clinical characteristics

Variables	Number=98	Percent
Cases suspected to have brucellosis (n=150):		
Cases proved by laboratory diagnosis to haven't brucellosis	52/150	34.7
Cases proved by laboratory diagnosis to have brucellosis	98/150	65.3
Brucella abortus	38	38.8
Brucella melitensis	18	18.4
Mixed infection	42	42.8
Seasonal variation:		
Winter (December-January- February)	12	12.2
Spring (Marsh-April-May)	20	20.4
Summer (June-July-August)	38	38.8
Autumn (September- October-November)	28	28.6
Hospital stay (Mean± SD* day)	13.04±4.52	
Duration of complete symptoms disappearance (Mean± SD day)	7.15±2.31	
Total time of antibiotic use in- and out-hospital (Mean± SD day)	26.67±8.15	
Time of relapse occurring (Mean± SD day)	29.94±57.68	
Relapse rate:	26	26.5

*SD: Standard deviation

Age group 4563-year, males, and rural residence are significant risk-factors for brucellosis (OR=3.76, 2.04, 2.86; respectively). Unskilled labor [OR=2.17] and low socioeconomic level (OR=2.72) are significant risk-factors. Drinking un-pasteurized, raw-milk, and slaughtering animals 1month before disease are significant risk-factors (OR=2.63, 3.64, 4.42; respectively) (Table2).

Table 2: Distribution of the studied cases with brucellosis and controls according to their demographic, socioeconomic, lifestyle, and clinical risk-factors

Variables	Cases (n=98)		Controls (n=98)		OR(95% CI)* OR(95% ECL)**
	No.	%	No.	%	
Demographic risk-factors					
Age (years):					
3-18	14	14.3	18	18.4	0.74(0.32-1.69)*
19-44	62	63.3	73	73.5	0.59(0.31-1.14)*
45-63	22	22.4	7	7.1	3.76(1.43-10.3)*
Gender:					
Male	64	65.3	46	46.9	2.04(1.11-3.77)*
Female	34	34.7	52	53.1	0.49(0.27-0.9)*
Residence:					
Rural	65	66.3	40	40.8	2.86(1.53-5.33)*
Urban	33	33.7	58	59.2	0.35(0.19-0.65)*
Socioecono¬mic risk-factors					
Educational status:					
Illiterate	57	58.2	39	39.8	2.1(1.14-3.88)*
Elementary	17	17.3	25	25.5	0.61(0.29-1.29)*
Secondary	14	14.3	15	15.3	0.92(0.39-2.17)*
University	10	10.2	19	19.4	0.47(0.19-1.15)*
Occupational status:					
House wife	30	30.6	37	37.8	0.73(0.38-1.37)*
Unskilled labor	43	43.9	26	26.5	2.17(1.14-4.13)*
Skilled labor	17	17.3	23	23.5	068(0.32-1.46)*
Professional	8	8.2	12	12.2	0.64(0.22-1.78)*
Socioeconomic level:					
Low	63	64.3	39	39.8	2.72(1.47-5.07)*
Middle	27	27.6	38	38.8	0.6(0.31-1.14)*
High	8	8.2	21	21.4	0.33(0.12-0.83)*
Lifestyle risk-factors					
Eating cottage-cheese (unprocessed)	56	57.1	38	38.8	2.11(1.14-3.88)*
Drinking un-pasteurized milk	49	50.0	27	27.6	2.63(1.39-4.98)*
Drinking raw-milk	33	33.7	12	12.2	3.64(1.65-8.12)*
Eating ice-cream from street vendor	21	21.4	8	8.2	3.07(1.2-8.04)*
Breeding animals at home	27	27.6	11	11.2	3.01(1.32-6.98)*
Slaughtering animals 1 month before disease onset	12	12.2	3	3.1	4.42(1.13-25.04)**
Follow preventive measures at dealing with risk	6	6.1	11	11.2	0.52(0.15-1.61)**
Clinical risk-factors					
Past history of similar attack	43	43.9	6	6.1	11.99(4.62-36.28)**
Family history of similar attack	17	17.3	4	4.1	4.93(1.51-20.81)**
History of diseases (e.g. DM, liver & renal disease, etc)	49	50.0	38	38.8	1.58(0.86-2.9)*

*CI: Confidence interval **CI: Exact confidence limits

Occupation that has animal contact is significant risk-factor for brucellosis (OR=4.7). The significant occupations risk-factors are butchers/slaughtering (OR=8.0) and farmers/dairy workers (OR=3.59). Exposure ≥20year has the highest significant risk (OR=15.57) (Table 3).

All symptoms and signs are significantly common among cases than controls except jaundice, hypertension/heart disease, and tender spine (Table 4). Mean Hb level and RBCs count are significantly lower among cases. Meanwhile, means of 1st and 2nd hours ESR and liver function are significantly higher among cases (Table 5).

SAT titers of cases are ≥1320/ Vs ≤1160/ of controls. The differences are significant (P<0.05 for each titer except ≥12560/). Meanwhile, the differences between SAT titers of B. abortus and melitensis are insignificant (Table 6).

Cut-off point of SAT titer 1320/ discriminates between cases and controls. Cases lie significantly in AUC with high sensitivity (96.4%) and specificity (100.0%) (Table 7).

Positive Brucella cultures represent 58.2% of the cases. There are insignificant differences between SAT titers and blood culture positivity among the cases. Positive and negative IgM results are 69.4% and 30.6% of the cases, respectively with statistically insignificant differences except for titer 1640/ (P=0.03). Positive and –ve IgG results are 65.3% and 34.7% of cases, respectively with statistically insignificant differences at all titers. There is insignificant difference in SAT titers as a whole neither between IgM +ve and –ve groups nor between IgG +ve and –ve groups. This indicates there isn't association between SAT titers [as one entity] and ELISA results (Table 8).

Table 3: Distribution of the studied cases of brucellosis and controls according to their Occupational-risk factors

Variables	Cases (n=98)		Controls (n=98)		OR* (95% CI)** OR(95% ECL)***
	No.	%	No.	%	
Occupational exposure:					
Contact with animals:	41	41.8	13	13.3	4.7(2.2-10.19)*
Butcher and slaughtering workers	14	14.3	2	2.0	8.0(1.74-73.91)**
Farmers and dairy workers	13	13.3	5	5.1	3.59(1.05-15.62)**
Veterinarians	4	4.1	1	1.0	4.13(0.4-205.33)**
Meat transporters and driver	10	10.2	5	5.1	2.11(0.63-8.17)**
No contact with animals:	57	58.2	85	86.7	0.21(0.1-0.45)*
House wife	30	30.6	37	37.8	0.73(0.38-1.37)*
Student	12	12.2	19	19.4	0.58(0.25-1.35)*
Clerical work	9	9.2	19	19.4	0.42(0.16-1.05)*
Others e.g. manual and skilled worker	6	6.1	10	10.2	0.57(0.16-1.83)**
Duration of occupational exposure (years):					
<5	10	10.2	7	7.1	1.48(0.49-4.53)*
5-9	11	11.2	4	4.1	2.97(0.84-13.21)**
10-19	13	13.3	3	3.1	4.84(1.26-27.19)**
≥20	24	24.5	2	2.0	15.57(3.63-138.58)**

*OR: Odds ratio, **CI: Confidence interval, ***CI: Exact confidence limits

Table 4: Distribution of the studied cases of brucellosis and controls according to their symptoms and signs

Variables	The studied groups				Yates χ^2	*P-value*
	Cases (n=98)		Controls (n=98)			
	No.	%	No.	%		
Clinical symptoms						
Fever, rigor, and/or sweating	89	90.8	9	9.2	127.37	0.0000
Muscloskeletal: Joint affection, body-aches, and/or back-pains	86	87.8	21	21.4	84.3	0.0000
Headache	85	86.7	39	39.8	[4]4.46	0.0000
Anorexia, nausea and/or vomiting	69	70.4	21	21.4	45.38	0.00000
Abdominal pains and/or constipation	58	59.2	13	13.3	42.76	0.0000
Cough/dyspnea/chest pain	46	46.9	12	12.2	26.67	0.0000
Genitourinary symptoms	38	38.8	11	11.2	18.39	0.00001
Clinical signs						
High temperature	87	88.8	4	4.1	137.93	0.000
Lymph node enlargement (peripheral)	25	25.5	9	9.2	8.01	0.004
Pallor	31	31.6	13	13.3	8.47	0.003
Jaundice	14	14.3	6	6.1	2.73	0.098
Abdomen:						
Hepatomegaly and/or tender liver	42	42.4	9	9.2	27.14	0.0000
Splenomegaly and/or tender spleen	34	34.7	12	12.2	12.53	0.0004
Chest affection	32	32.7	12	12.2	10.58	0.001
Hypertension and/or heart diseases	24	24.5	19	19.4	0.48	0.489
Tender and/or swollen joints	11	11.2	5	5.1	1.7	0.19
Tender spine	9	9.2	4	4.1	1.32	0.25
Swollen and/or tender testes	21	21.4	6	6.1	8.42	0.003

Table 5: Distribution of the studied cases of brucellosis and controls according to the results of routine lab tests

Variables	The studied groups		*t-value*	*P-value*
	Cases (n=98)	Controls (n=98)		
	Mean±SD	Mean±SD		
Routine lab tests (Mean± SD)				
CBC (Mean± SD):				
Hb (mg/dl)	12.9±1.6	13.6±1.7	-2.968	0.001
RBC (millions/cmm)	5.2±0.9	5.6±0.8	-3.288	0.0005
WBC (thousands/cmm)	5.9±2.7	5.7±2.6	-0.528	0.298
ESR (Mean± SD):				
1st hour	27.2±14.3	21.4±11.6	-3.118	0.001
2nd hour	41.2±17.8	34.3±16.7	-2.799	0.002
Liver function tests (Mean± SD)				
T. Serum bilirubin (mg/dl, Mean± SD)	1.2±0.1	1.0±0.1	-14.0	0.000000
ALT (U/L, Mean± SD)	50.1±15.4	32.5±9.2	-9.713	0.00000
AST (U/L, Mean± SD)	51.2±13.1	35.6±8.3	-9.958	0.00000
ALP (U/L, Mean± SD)	109.6±35.3	76.9±21.4	-7.842	0.00000
Kidney function tests (Mean± SD)				
Urea (mg/dl, Mean± SD)	30.4±8.3	28.6±8.2	-1.527	0.06
Creatinine (mg/dL, Mean± SD)	0.9±0.8	0.8±0.1	-1.228	0.111

CBC: Complete blood count, Hb: Hemoglobin, RBC: Red blood corpuscle ESR: Erythrocyte sedimentation rate, WBC: White blood cells
ALT: Alanine amino-transferase, AST: Aspartate amino-transferase ALP: Alkaline Phosphatase

Table 6: Distribution of the studied cases of brucellosis and controls according to results of standard agglutination test (SAT) titer

SAT* titer	The studied groups				χ2 FE**	*P-Value*
	Cases (n=98)		Controls (n=98)			
	No.	%	No.	%		
≤1/80	0	0.0	86	87.8	149.69	0.000
1/160	0	0.0	12	12.2	10.74	0.001
1/320	12	12.2	0	0.0	10.74	0.001
1/640	36	36.7	0	0.0	41.68	0.000
1/1280	46	46.9	0	0.0	57.52	0.000
≥1/2560	4	4.1	0	0.0	FE	0.121
SAT titer	Discovered brucella species (n=140***)				χ2	*P-value*
	Abortus (n=80=81.6%)		Melitensis (n=60=61.2%)			
	No.	%	No.	%		
1/320	11	13.8	6	10.0	0.17	0.681
1/640	29	36.2	24	40.0	0.08	0.782
1/1280	36	45.0	28	46.7	0.0	0.98
≥1/2560	4	5.0	2	3.3	FE	0.7

*SAT: Standard agglutination test, **FE: Fisher exact test, ***42 mixed infection cases, B. abortus and melitensis

Table 7: SAT predictive ability to discriminate Brucella cases using receiver operated characteristic (ROC) curve

SAT predictive ability to discriminate Brucella cases from controls					
AUC*	95% CI**	*P-value*	Titer cut-off point	Sensitivity	Specificity
0.98	0.96-0.99	0.0001	1/320	96.4%	100.0%

*AUC: Area under the ROC curve **CI: Confidence interval

Table 8: Distribution of the results of blood cultures, and immunoglobulin (Ig) M and G among the studied cases of brucellosis according to standard agglutination test (SAT)

SAT* titer	Blood culture (N=98)				χ2 FE**	*P-Value*
	Positive (N=57=58.2%)		Negative (N=41=41.8%)			
	No.	%	No.	%		
1/320	4	7.0	5	12.2	FE	0.484
1/640	22	38.6	10	24.4	1.59	0.207
1/1280	26	45.6	25	61.0	1.68	0.194
≥1/5120	5	8.8	1	2.4	FE	0.395
SAT titer	IgM results (n=98)				χ2	*P-value*
	Positive (n=68=69.4%)		Negative (n=30=30.6%)			
	No.	%	No.	%		
1/320	12	17.6	5	16.7	0.03	0.863
1/640	28	41.2	5	16.7	4.56	0.032
1/1280	22	32.4	14	46.7	1.27	0.259
≥1/2560	3	4.4	2	13.3	FE	0.195
≥1/5120	3	4.4	1	6.6	FE	0.64
			χ2= 7.41	*p-value*= 0.115		
SAT titer	IgG (N=98)				χ2	*P-value*
	Positive (N=64=65.3%)		Negative (N=34=34.7%)			
	No.	%	No.	%		
1/320	10	15.6	7	20.6	0.11	0.735
1/640	23	35.9	10	29.4	0.18	0.67
1/1280	21	32.8	13	38.2	0.1	0.753
≥1/2560	6	9.4	3	8.8	FE	1.0
≥1/5120	4	6.3	1	2.9	FE	0.655

*SAT: Standard agglutination test, **FE: Fisher exact test

DISCUSSION

This study showed 65.3% of suspected patients proved, using SAT, to have brucellosis, which is the commonest infection causes FUO[12]. Prakash *et al.*[24] found 25.7% seropositivity of Brucella Abs in FUO patients. Our figure is much higher; our patients were clinically and epidemiologically potential cases. Basyony *et al.*[25] cleared 82.3% of patients were seropositive. We found B. abortus the commonest pathogen, 38.8%; Pappas *et al.*[16] cleared majority of the cases worldwide were B. melitensis. Our result might be explained, animal hosts of B. abortus are cows& buffalos that common in Egypt. Also, Abdelbaset *et al.*[26] found 80.0% of the positive reactors had B. Abortus only and 20.0% had mixed infection. While, El-Hamshary *et al.*[27] reported infection with B. melitensis and Abortus were 49.4% and 30.4%, respectively in Banha Fever Hospital. Further, Elbeltagy[28] showed 13.9%, 44.5%, and 40.9% of their patients had B. abortus, melitensis, and mixed, respectively. Most (38.8%) of our cases were presented in the summer months. This result is consistent with Fouad *et al.*[29] and Abd-Elall[30]. We reported mean total time of antibiotics' use was 26.7457.68±day; Yang[2] cleared sufficient period of drug therapy, 6weeks-6months, has significant role in cure achievement. Our short antimicrobial time use could be explained; high cost and socio-cultural factors. We showed relapse occurred in 26.5% of cases. Gotuzzo[4] cleared after antimicrobial therapy, 10.0% of the patients experienced relapse. Our high relapse rate could be explained; therapy discontinuation (short period and/or intermittent use) and continuous exposure to infection in high risk groups. Further, in tuberculosis-endemic populations as Egypt, community-acquired rifampin resistance should be taken into account in brucellosis treatment.

We cleared the older age was significant risk-factor. Our result agrees with Al-Sekait[31], he showed age ≥45year was significant risk associated with seropositivity. Hussein *et al.*[32] found brucellosis increased among patients aged 41 - 50 year. Further, Tumwine *et al.*[6] cleared 22.2% of patients were >60 year. On contrary, Al-Tawfiq and Abukhamsin[33] found patients aged 2040-years had the highest rate. Also, Fallatah *et al.*[34] observed 60.3% of the patients were 1340-year. Meanwhile, 14.3% of our patients aged up-to 18year. Gotuzzo[4] cleared brucellosis in school-aged children, worldwide, accounts for up-to 10.0%. But, it's up-to 20.0%-25.0% in endemic areas. However, Abdelbaset *et al.*[26] found insignificant risk of age on contracting brucellosis; individuals in age group 35–63years had increased risk of exposure compared to younger age group.

We found male gender was significant risk-factor. Worldwide, males have higher prevalence of brucellosis, that is constant epidemiological feature[6,26,33,34,35]. Our result agrees with this feature, which could be explained; types of males' occupations and the differences in the practice and habits. Fouad *et al.*[29] found 70.0% of the patients were males. On contrary, Hussein *et al.*[32] showed brucellosis prevalence was significantly higher in females. Also, Abdelbaset *et al.*[26] found insignificant risk of the male gender in acquiring brucellosis.

We showed rural residence was significant risk-factor (OR=2.86). Our result agrees with Al-Sekait[31] and Tumwine *et al.*[6]; they reported significant risk-factors (OR=2.8 and 3.16, respectively). On contrary, Fouad *et al.*[29] observed 75.5% of their patients were urban residents (p<0.01). Minas *et al.*[35] showed urban population isn't at great risk to acquire brucellosis; commercial dairy-products were manufactured from pasteurized milk.

We noticed illiteracy, unskilled labor, and low socioeconomic level were significant risk-factors. The most affected population are the poorly educated[18]. Al-Sekait[31] found unskilled labor was significant risk-factor (OR=3.8). While, Elbeltagy[28] cleared 54.0% and 44.5% of patients had no- and moderate-education, respectively. Tumwine *et al.*[6] showed most of the cases had no- or primary-education. On contrary, Abdelbaset *et al.*[26] showed illiterates were insignificant risk-factor to catch brucellosis. Cetinkaya *et al.*[36] found brucellosis wasn't related to educational level. These results lightened the need for health-education program for such risky group.

Regarding lifestyle risks; eating cottage-cheese, drinking raw- and/or un-pasteurized milk, eating polluted ice-cream, breeding animals at home, and slaughtering animals were significant risk-factors. The organisms may survive in un-pasteurized goat cheese for up-to 8weeks. Freezing dairy-products or meat doesn't destroys the organisms that are killed by pasteurization and boiling[37]. Consumption of raw-milk and milk-products were the most prevalent risk-factors[29]; Al-Sekait[31] reported 5.5 significant risk for drinking raw-milk. Further, Saleh[38] cleared 54.1% of patients had history of raw-milk ingestion. Tumwine *et al.*[6] elicited consuming milk-products and locally processed milk-products were significant risk-factors (OR=2.36, 2.54; respectively). On contrary, Minas *et al.*[35] showed 8.5% of their cases infection was attributed to consumption of dairy-products. Also, Meky *et al.*[39] found drinking raw-milk and eating cottage-cheese were insignificant risk-factors, while eating polluted ice-cream and breeding animals at home were significant risk-factors (OR=1.8, 2.3; respectively). On contrary, Tumwine *et al.*[6] showed breeding animals at home was insignificant risk-factor. We found family history of similar attack was significant risk-factor. Household members of patients may have been exposed to the pathogen and became infected/ill[40]. More than 1 / 3 (37.6%) of the patients

had positive family history[37].

Brucella may transmitted to man through direct contact with infected animals or their secretions[14]. Brucellosis is usually related to occupational exposure[4]; some occupations were proved to be risk-factors as ranchers, veterinarians, and abattoir- and lab-workers[14]. Brucellosis is considered an important occupational disease[41]. We showed occupations with animal contact were significant risk-factors for acquiring brucellosis. Our result is compatible with Elbeltagy[28], Fouad *et al.*[29], Abd-Elall[30], Minas *et al.*[35], Saleh[38], Meky *et al.*[39], Farghaly *et al.*[41]; they elicited close contact with animals or their products was the commonest feature/significant risk-factor. Mishal *et al.*[42] showed almost all of the infected patients worked in cowshed, participated in calf deliveries, and had contact with cows' bloods and placentas. Meky *et al.*[39] and Fouad *et al.*[29] cleared farmers, butchers, and meat-transporter workers& vehicle-drivers were commonest occupations at risk. Saleh[38] reported direct contact with animals was found in 45.6% of the patients. Further, Prakash *et al.*[24] showed Brucella seropositivity was 37.1% in milkman and 26.7% in meat handlers/veterinarians. On contrary, Tumwine *et al.*[6] elicited contact with animals and slaughter animals were insignificant risk-factors. Further, we noticed occupations with no animal contact were significant protective factor. This result is expected and accepted as occupations that not exposing the subject to risk of infection might be decrease probability of infection. Also, we found the longer duration of occupational exposure the higher significant risk of disease. Again, this result is expected and accepted; there was a tendency towards increase infection rate with increase duration of exposure. Mahgoub[43] found 38.9% of seropositive workers exposed to risk of brucella infection for ≥5years. While, Refaat *et al.*[44] didn't find significant difference between veterinarians working more or less than 10 years.

We viewed most of the patients had many non-specific symptoms and signs. Brucellosis is a multisystem disease that can manifest with a broad spectrum of clinical features as fever, headache, back-pain, weakness, profuse sweating, chills, and joint-pain, etc. Fever is common symptom and sign; 72.0%-91.0%[2,45]. The most observed symptoms were fever (94.6%), fatigue (92.8%), body-ache (91.4%), sweating (87.4%), joint-pain (86.2%), back-pain (86.2%), chills (82.0%), headache (80.6%), loss of appetite (77.6%), weight-loss (65.2%), constipation (64.9%), abdominal-pain (45.0%), sleep-disturbances (37.0%), and cough (24.4%)[35]. While, the commonest signs were tender-spine (48.0%), arthritis (40.4%), lymphadenopathy (32.0%), splenomegaly (25.0%), pallor (22.0%), and epididymoorchitis (21.3%)[37]. Also, Fouad *et al.*[29] viewed the commonest symptoms and signs were fever (98.7%), weakness (80%), profuse sweating (74.7%), abdominal-pain (72%), and (34.9%). Further, Ruiz-Mesa *et al.*[46] found hepatomegaly and splenomegaly were 35.2% and 20.8%, respectively. Furthermore, El-Moselhy *et al.*[45] viewed most of symptoms were significantly more frequent among the patients than controls.

We showed mean Hb level and RBCs count were significantly lower among cases than controls, while mean ESR at 1st & 2nd hour, and liver functions were significantly higher among cases than controls. Meanwhile, means of WBCs count and kidney functions were insignificantly higher among cases. Young[14] cleared routine laboratory tests aren't particularly helpful. Anemia and leucopenia are common findings. The WBCs count is often normal or low and may not suggest an infectious process. The ESR is variable and of little diagnostic value. Our results regarding mild liver functions impairment during the course of brucellosis are agreed with LaSpada *et al.*[47]; 38.0% and 53.0% of patients had elevated baseline values of AST and ALT, respectively.

SAT could be considered a confirmatory test for other screening laboratory tests[48]. Brucellosis should be considered in individuals with unexplained chronic fever and non-specific complaints[2]. SAT titer ≥1160/ is considered diagnostic as long as the patient has signs and symptoms of disease. However, in endemic areas the diagnostic threshold value has to be 1320/ to provide sufficient high specificity[49]. We reported SAT titer ≥1320/ in all cases. Meanwhile, the entire control group SAT titer was ≤1160/. The cut-off point of SAT titer between cases and controls in current study was 1320/. This indicates that +ve SAT at titer ≤1160/ is common in healthy subjects because Brucella is endemic in Egypt leading to repeated exposure of the populations, particularly high risk groups, to infection. In endemic areas, titer ≥1320/ is recommended in the diagnosis of brucellosis[2]. Further, we noticed SAT titer 1320/ among the patients lies significantly in the AUC ROC with high sensitivity (96.4%) and specificity (100.0%). These results are similar to Abd-Elall[30] and Zaky *et al.*[50]. Also, Cakan, *et al.*[51] showed sensitivity and specificity of SAT is 95.6% and 100%, respectively, which are similar to our results.

We found 58.2% of blood cultures were positive. The sensitivity of blood culture varies depending on the quantity of bacteria in blood, specimen type, and the used methods; it varies from 15.0%-70.0%[2,52] up-to 90.0%[4]. The difference between our figure and these figures might be because our patients received many antibiotics therapy before diagnosis was confirmed. There were no statistically significant differences between SAT and blood culture positivity among the patients. Absolute diagnosis of brucellosis requires isolation of the bacterium from blood[14]. Also, Kiel& Khan[53] clarified although the cultures are not always positive; blood cultures have 50.0%-80.0%

sensitivity. So, diagnosis depends on serology, since cultures are not always positive. While, Ruiz-Mesa *et al.*[46] observed blood cultures were positive in 62.6% of the patients, while 37.4% of them were diagnosed according to clinical and serological criteria. On contrary, our result was higher than Abd-Elall[30]; he reported 37.0% positive cultures.

We observed Brucella +ve IgM and IgG results were found among 69.4% and 65.3% of our cases. As IgM Abs appear earlier than IgG Abs, the detection of IgM in serum is the widely used approach for early serologic diagnosis of acute infection[14]; specific IgM Abs dominates during the acute phase of disease. ELISA discriminates between presence of specific IgM and IgG Abs and accesses illness stage[49]. ELISA has proved useful; many studies used it as confirmatory test for Brucella screening tests as Rose-Bengal Plate test[54]. Abd-Elall[30] found ELISA IgM and IgG were positive in 63.0% and 64.2% of cases, respectively. Also, Aranís *et al.*[55] cleared 80.0% and 50.0% of patients were ELISA IgG and IgM positive, respectively. Brucella ELISA test is considered to have higher sensitivity and specificity in determining Brucella specific Abs than other serological tests[56]. Also, ELISA had higher specificity and sensitivity compared with SAT[2]. However, Cakan *et al.*[51] showed ELISA test for brucellosis is more sensitive only when both IgG and IgM were used, though their titer alone didn't represent disease status. Awah-Ndukum *et al.*[57], Sanogo *et al.*[58], and Gatechew *et al.*[59] reported sensitivity and specificity of ELISA IgG were 95.6% &97.1%, 96.1% &95%, and 96.8% &96.3%; respectively.

CONCLUSIONS AND RECOMMENDATIONS

Brucellosis has many important sociodemographic, lifestyle, and clinical risk-factors. Diagnosis of brucellosis depends on presence of risk-factors, clinically suspected, and SAT titer ≥1320/. Titer ≥1320/ has high sensitivity and specificity. There are no significant relations neither between SAT titer and blood culture results nor IgM and IgG results. More studies are needed to define brucellosis seroprevalence in different areas and situations in Egypt and to understand the full epidemiology of this public health problem.

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CONFLICTS OF INTEREST

There are Conflicts of Interest

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الملخص العربى

داء البروسيلا البشرى: طرق التشخيص وعوامل الخطوره لدى المرضى المصريين فى مستشفى حميات أسيوط

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خلفية: داء البروسيلا البشرى، هو مرض شائع حيوانى المنشأ، ويمثل مشكلة صحية عامة رئيسية في العديد من البلدان في جميع أنحاء العالم بما في ذلك مصر.

الأهداف: تحديد عوامل الخطوره لمرضى داء البروسيلا وتقييم الطرق المختبريه لتشخيص مرض البروسيلا في مستشفى حميات أسيوط.

المرضى وطرق البحث: جندت الدراسة 98 مريضا يعانون من داء البروسيلات وعدد متساو من الاشخاص الأصحاء كمجموعة ضابطه. تم إخضاع جميع المشاركين للمقابلة، والفحص السريري، والفحوصات المخبرية.

النتائج: كان كبار السن، الذكور، والإقامة الريفية، والحالة الإجتماعية والإقتصادية المنخفضة عوامل خطوره مؤثرة (نسبة أودز=٣.٧٦ ، ٢.٠٤ ، ٢.٨٦ ، ٢.٧٢، على التوالي). و كانت المهن ذات الإتصال بالحيوانات لها عامل خطوره مؤثرة (نسبة أودز=٤.٧) ؛ و كان الأكثر خطورة هم الجزارين / عمال الذبح (نسبة أودز=٨.٠) والمزارعين / عمال الألبان (نسبة أودز=٣.٥٩). و كان التعرض المهني الأطول عامل خطوره مؤثر (نسبة أودز= ١٥.٥٧). وكانت أهم أعراض المرض عند التشخيص هى الحمى والإضطرابات العضلية الهيكلية. وكانت أهم العلامات الرئيسية هى إرتفاع درجة الحرارة وتضخم الكبد والطحال. وكان إختبار التراص القياسى (SAT) عيار ٣٢٠/١ هى نقطة الفصل للتشخيص ويقع بشكل مؤثر فى منطقة تحت منحنى ROC، بنسبة حساسية = ٩٦.٤٪ وخصوصية = ١٠٠٪. وكانت مزرعة الدم إيجابية في ٥٨.٢٪ من الحالات مع عدم وجود فروق ذات دلالة إحصائية بين إختبار التراص القياسى وإيجابية مزرعة الدم. وكانت الأجسام المضادة الموجبة من النوع م (IgM) و ج (IgG) والمكتشفه بواسطة إختبار الإليزا (ELISA) فى ٦٩.٤٪ و ٦٥.٣٪ من الحالات مع عدم وجود فروق ذات دلالة إحصائية بين نتائج إختبار التراص القياسى والأجسام المضادة الموجبة من النوع م (IgM) و ج (IgG).

الإستنتاجات : داء البروسيلا البشرى لديه العديد من عوامل الخطوره التي يمكن الوقاية منها و يعتمد تشخيصه بشكل أساسى على وجود عوامل الخطوره و الإشتباه سريريًا و إختبار التراص القياسى (SAT) عيار = أو أكبر من ٣٢٠/١.