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RESEARCH ARTICLE

HUMAN BRUCELLOSIS AMONG PYREXIA OF UNKNOWN ORIGIN CASES IN TWO TERTIARY HOSPITALS, IN SANA'A, YEMEN

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Abstract

Background and objectives: *Brucellosis* continues a most important health problem in numerous parts of the world and in the Middle East regions is a significant origin of acute febrile illness. This study was conducted to determine *Brucella* antibodies among patients with PUO, the association of brucellosis with demographic characteristics, identify clinical symptoms and risk factors for *Brucella* infection among PUO cases.
Materials and Methods: This cross-sectional research study was conducted in two health institutions; Al-Jumhouri Hospital and Al-Thawra Hospital, between January 2021 to January 2022. The required data were gathered from each patient by using pre-defined standardized questionnaire. Five mL of sterile whole blood was collected from each patient and then tested for *Brucella* antibodies using a standard tubular agglutination test. The data were then analyzed using the statistical software Epi Info version 6.
Results: Among the 241 PUO patients, females were 72.2% and males 27.8%. The mean±SD for total age was 28.8±15.8 years. The rate of *Brucella* antibody among patients with PUO was 29%, females 78.6% higher than males by 21.4%. Clinical characteristics of PUO patients were persistent fever (36.9%), intermittent fever (63.1%), sweating (63.5%), shivering (61.4%), joint pain (78.4%), muscle pain (78.4%), back pain (74.7) back head pain (66.4%), headache (60.4%), weight loss (52.9%), body weakness (89.2%), loss of appetite (50.6%), and nausea (46.1%). The risk factors for brucellosis among the PUO patients was handling of animals during parturition (69.2%, OR=7, p<0.0001).
Conclusions: These findings revealed a high prevalence of human brucellosis among PUO patients in Sana'a city and will becoming a serious problem that threats the health care system in Yemen. So, awareness programs should be provided to doctors, population about *Brucella* infection and its risk factors.
Keywords: *Brucella* antibodies, *Brucellosis*, Pyrexia of Unknown Origin (PUO), Sana'a City, Yemen.

INTRODUCTION

Human brucellosis is a zoonotic bacterial infection that has been occurred worldwide. Brucellosis is primarily an occupational illness that is infected slaughterhouse workers, veterinarians, farmers, meat inspectors and animal handlers. Brucellosis is caused by several *Brucella* species belonging to the genus *Brucella*, which are non-sporous, non-encapsulated, small, Gram-negative bacilli. In the last globally report in 2006, globally each year, more than 500,000 new cases are reported, with annual incidence rates changeable broadly from less than 2 to more than 500/1,000,000 population between diverse regions[1]. Brucellosis continues endemic in many areas of the world comprising the Middle East, Latin America, the Mediterranean

Basin, Africa and Asia[1]. Global tourists visiting brucellosis endemic areas are at risk of infection. Spread of *Brucella* to human's consequences from direct connection with an infected animal, and also from ingestion of unpasteurized milk and dairy products[2,3]. Brucellosis of human is a lot under-diagnosed or misdiagnosed because the clinical manifestations overlap along with various bacterial infections. High undulant fever, night sweats and weight loss are the most significant indications of human brucellosis. It has been noted that brucellosis is one of the most important causes of long-term fever in endemic areas and one of the important causes of pyrexia of unknown origin (PUO) in endemic areas of brucellosis[4,5].

In animals, it causes miscarriage, sterility, placenta retention, weak or stillborn calves, and decreased milk production in milk-producing animals[5]. Cases of brucellosis are categorized the same as either probable or confirmed cases. A clinically compatible case epidemiologically associated with a confirmed case of brucellosis, or a *Brucella* agglutination titer equal to or more than 1/160 in one or more serum samples acquired after symptom onset may be determined as a probable case[6]. Also, laboratory confirmation of a clinically compatible case is judged as confirmed case. On the other hand in endemic countries of brucellosis, clinical symptoms are coupled with seropositive without isolation of *Brucella* is confirmed human cases[6]. Human brucellosis laboratory confirmation is based on serological, molecular or/and microbiological methods, and these methods have their disadvantages and advantages. Among the methods used are several serological tests such as Complement Fixation Test (CFT), Rose Bengal Panel Test (RBPT), Coombs Test, Serum Agglutination Test (SAT) and ELISA[3-8]. By means genus-specific polymerase chain reaction (PCR) assays, the molecular diagnosis of human brucellosis can be carried out. Molecular assays target the *IS711* insertion element and the *bcsp31* gene, coding for a 31-kDa immunogenic outer membrane protein conserved among all *Brucella* spp. are the most common molecular targets in clinical applications[9,10]. Brucellosis of human is regularly detected by ELISA and agglutination based serological tests, but the gold standard method for diagnosis brucellosis remain isolation of the pathogen from blood by culture[4,5,7].

Literature review of infectious diseases in Yemen indicated that the knowledge of brucellosis is still very scanty while more studies were conducted recently in Yemen about viral infections as hepatitis viruses, CMV and *Polio* virus; leptospirosis, cholera, trachoma, kala-azar, oral infections, eye infections other infectious diseases but no recent studies of brucellosis have been conducted[11-32], in conclusion, Yemen has been neglected with regard to the study of brucellosis. This study was conducted to determine *Brucella* antibodies and associated risk factors among patients with fever of unknown origin (PUO) attending to tertiary hospitals at Sana'a city, Yemen.

SUBJECTS AND METHODS

Study design and study area: This cross-sectional research was carried out in two health establishments; Al-Jumhori Hospital and Al-Thawra Hospital, in Sana'a city, Yemen, during a period of one year starting in January 2021 and ending in January 2022. This study is the second cross-sectional study of *Brucella* infection among PUO patients in Sana'a city-Yemen in the last 22 years[5].

Inclusion criteria: All patients with pyrexia of unknown origin of any age and both gender attending selected hospitals in the period of the study.

Sample size: desired precision of 0.01 (1% acceptable error in the estimation) was selected with possible estimated true proportion of human brucellosis in Al-Dala'a city- Yemen equal to 6.7%[33] with Confidence Levels 99% for population of pyrexia of unknown origin in Sana'a city per year equal to 100000, it was needed at least 241 selected patients with PUO calculated by Using Epi Info version 6 software (CDC, Atlanta, USA) attending in the main hospitals in Sana'a city, Yemen.

Data collection: Data was taken from each patient with PUO by standard predesigned questionnaire designed for this study that includes data as demographic information; name, age, gender and risk factors and clinical symptoms.

Blood sample collection: Total 5 mL of whole blood was collected aseptically by venipuncture from each patient with PUO and serum was separated by centrifugation after clotting. The sera samples were kept at –20°C until tested for *Brucella* antibodies.

Laboratory test: The sera were tested by standard tube agglutination test using reagent (*B. abortus* and *B. melitensis*). Positive and negative control was tested in parallel with tested sera. To avoid laboratory error due to prozone at low titer the final dilution of each serum, positive and negative controls are 1:20 to 1:640 after addition of an equal volume of antigen. Any serum giving a titer of equal or more than 1:640 than a further dilution was carried out. The test was read at 37°C after 24 hours of incubation. A titer of equal to or more than 1:160 were considered positive[4].

Statistical analysis: Analysis of data was performed by using Epi Info statistical program version 6 (CDC, Atlanta, USA). The quantitative data was expressed as mean values, Standard deviation (SD), when the data was normally distributed. The qualitative data was expressed as percentages; Chi square test was used for comparison of two variables to determine the *p* value. Odd ratio (OR) was used with 99% confidence interval. *p* value <0.05 was considered statistically significant.

Ethical consideration: Consents were taken from all participants and they were informed that participation was voluntary and that they can refuse this without stating any reason.

RESULTS

Out of 241 individuals, the female with PUO 174 (72.2%) of the total, higher than male 67 (27.8%) of all. The mean ±SD of total age was 28.8 years ±15.8

years. The age range of total was one year to 73 years, and the most frequented age was 35 years (mode) (Table 1).

Table 1: Table 1: Participated patients' characterizations

	Number	Percentage (%)
Gender		
Males	67	27.8
Females	174	72.2
Age groups		
≤15 years	41	17
16-25 years	73	30.3
26-35 years	58	24.1
≥36 years	69	28.6
Total	**241**	**100**
Mean	28.8 years	
SD	15.8 years	
Median	27 years	
Mode	35 years	
Min.	One year	
Max.	73 years	

Table 2 shows the clinical manifestations of PUO patients; continuous fever occurred in 36.9%, intermittent fever was in 63.1%, sweating in 63.5%, shivering in 61.4%, joints pain in 78.4%, muscle pain in 78.4%, backache in 74.7%, pain head back in 66.4%, headache in 60.4%, weight loss in 52.9%, body weakness in 89.2%, loss of appetite in 50.6% and anorexia in 46.1% of PUO patients. The prevalence rate of brucellosis (≥1/160 SAT) was 70/241 (29.1%), *Brucella abortus* positive rate was 17%, and *Brucella melitensis* positive rate was 2.9% and mixed of both *Brucella* species 9.1% (Table 3). Table 4 shows that the higher rate of *Brucella* antibodies were occurred in females (78.6%) with OR equal to 1.6 when compared to males (21.4%) (χ^2=1.99, *p=0.07).* Also, the age group of 16-25 years old had higher rate of *Brucella* antibodies and there are no statistical significant differences (Table 4).

Table 2: The frequency of clinical symptoms among PUO patients.

Clinical Symptoms	No.(%)
Continuous fever	89(36.9)
Intermittent fever	152(63.1)
Sweating	153(63.5)
Shivering	148(61.4)
Joints pain	189(78.4)
Muscle pain	189(78.4)
Backache	180(74.7)
Pain head back	160(66.4)
Headache	145(60.4)
Weight loss	127(52.9)
Body weakness	215(89.2)
Loss of appetite	122(50.6)
Anorexia	111(46.1)

There was no significant association between residency, occupations, and education with contracting brucellosis among PUO patients (Table 5). The sero-prevalence rate of brucellosis among patients with clinical symptoms was recorded at 33% for intermittent fever, 31.5% for weight loss, 31% for headache, 30.6% for backache, 30.4% for shivering (30.4%), joint pain, 30% for each muscle pain and pain head back, 29.7% for nausea, 28.8% for body weakness, 28.1% for sweating, 27.9% for loss of appetite, and 22.5% for continuous fever patients that summarized in Table 6.

Table 3: The prevalence of *Brucella* antibodies among tested PUO patients.

Brucella species	Number(%)
Brucella abortus antibody positive only	41(17)
Brucella melitensis antibody positive only	7(2.9)
Both *Brucella species* antibody positive	22(9.1)
Total positive for *Brucella* antibodies	70(29)

SAT positive ≥1/160

Table 4: The frequency and associated odds ratio of contracting to *Brucella* infection with different sexes and age groups of patients suffering from pyrexia.

Characters	Positive SAT N=70		OR	CI	χ^2	*p* value
	No.	(%)				
Sex						
Male (n=67)	15	21.4	0.62	0.32-1.02	1.99	0.07
Female (n=174)	55	78.6	1.6	0.82-3.09	1.99	0.07
Age groups						
≤15 years (n=41)	10	14.3	0.75	0.34-1.63	0.52	0.57
16-25 years (n=73)	25	35.7	1.42	0.79-2.57	1.37	0.28
26-35 years (n=58)	19	27.1	1.26	0.66-2.38	0.51	0.50
≥36 years (n=69)	16	22.9	0.66	0.34-1.25	1.60	0.27
Mean	28.5 years					
Standard division	13.6 years					
Min	1 years					
Max	65 years					
Median	25 years					
Mode	25 years					

OR- Odds ratio >1 (at risk), χ^2- Chi-square ≥3.9 (significant), *p*- Probability value ≤0.05 (significant), CI- Confidence intervals

Table 5: The association between residency, occupations, and education with contracting brucellosis among PUO patients.

Risk factors	Positive SAT N=70		OR	CI	χ^2	*p* value
	No	%				
Residency						
Rural n=65	19	29	1.01	0.5-1.9	0.001	0.96
Urban n=176	51	28.9	0.98	0.5-1.8	0.001	0.96
Occupations						
Farmer n=8	3	37.5	1.4	0.3-6.3	0.28	0.59
Housewife n=124	42	33.9	1.6	0.9-28	2.8	0.08
Employee n=51	13	25.4	0.7	0.3-1.6	0.39	0.52
Others n=58	12	20.6	0.6	0.3-1.2	0.34	0.51
Education						
Illiterate n-83	22	26.5	0.8	0.46-1.4	0.39	0.52
University n=41	11	26.8	0.87	0.4-1.8	0.11	0.73
Primary/secondary n=117	37	31.6	1.3	0.7-2.2	0.7	0.39
Type of patients						
Inpatients n=59	13	22	0.69	0.31-1.2	1.8	0.17
Outpatients n=182	57	31.3	1.6	0.8-3.2	1.8	0.17

OR- Odds ratio >1 (at risk), χ^2- Chi-square ≥3.9 (significant), *p*- Probability value ≤0.05 (significant), CI- Confidence intervals

Table 6: The frequency and association of clinical symptoms among brucellosis patients.

Symptoms	Positive SAT (N =70)			*95% CI*	X^2	*p* value
	No	%	*OR*			
Continuous fever n=89	20	22.5	0.59	0.3-1	2.9	0.08
Intermittent fever n=152	50	33	1.6	0.9-3	2.9	0.08
Sweating n=153	43	28.1	0.88	0.49-1.56	0.18	0.67
Shivering n=148	45	30.4	1.2	0.6-2	0.3	0.5
Joints pain n=189	57	30.2	1.3	0.6-2.6	0.5	0.48
Muscle pain n=189	56	30	1.1	0.5-2.2	0.14	0.7
Backache n=180	55	30.6	0.8	0.4-1.4	0.5	0.47
Pain head back n=160	48	30	0.9	0.5-1.7	0.02	0.88
Headache n=145	45	31	1.2	0.7-2.2	0.69	0.4
Weight loss n=127	40	31.5	1.2	0.7-2.5	0.7	0.37
Body weakness n=215	62	28.8	0.9	0.37-2.2	0.04	0.83
Loss of appetite n=122	34	27.9	0.89	0.5-1.5	0.16	0.68
Anorexia n=111	33	29.7	1.1	0.6-1.8	0.04	0.82

Table 7 shows that the rate of handling animal during birth was 69.2%, with associated risk (OR) equal to 2, with CI=6-17, χ^2=22, p<0.0001. The rate of brucellosis among contact animal waste (36%), contact with animal newborn (34.5%), family history of brucellosis (33.3%), farmer (32.1%), ingested raw milk (29.5%), consuming milk products (29.2%), milking animals (28.6%), touch fresh meat (28.1%) and working as microbiologist (23%). There was no significant association between *Brucella* infection and different animal species (Table 8).

DISCUSSION

Brucellosis continues a most important health crisis in numerous parts of the world and is a significant cause of acute feverish sickness in the regions of Middle East. In spite of this, consistent with the World Health Organization, brucellosis is listed as one of seven neglected zoonotic diseases. Brucellosis is an important cause of PUO in endemic areas[34]. Estimates of the prevalence of brucellosis among PUO patients are not available for many countries of the world. The low prevalence reported in known brucellosis endemic countries such as Yemen may be due to the absence or low surveillance and reporting systems in these countries, for presence or prevalence of brucellosis[5]. The prevalence of brucellosis antibodies was 29.1% among the PUO patients in the current study, and this result is almost similar to that reported in Saudi Arabia in old report (23%)[35], Ethiopia (31.5%)[36], India (22.5%, 29.4%)[37,38]. In contrast, the current result was higher than that previously reported in Yemen (7.9%)[5], Saudi Arabia by Alkahtani recently (12.8%)[39], Pakistan (10.1%)[40], south western Uganda (14.9%)[41], Bangladesh (2.0%)[42], and Nigeria (14.9%)[43].

In the current study, the percentage of *Brucella* antibodies among females was 78.6%, while this rate in males was 21.4% lower than in females. Similar results were also recorded in Iraqi females (61.7%), while in males (38.3%)[44]. In contrast, this result differs with previous studies in Yemen and India where the incidence among males is higher[4,38-42]. Current data indicate that most females were housewives and exposed to brucellosis risk factors as they directly handled milk or meat or looked after animals[44-46]. In this study, there is no statistically significant correlation between the presence of antibodies to *Brucella* disease and the different age groups, as the distribution is almost equal in all age groups (p>0.05).

Table 7: The risk factors of contacting brucellosis among PUO patients.

Risk factors	Positive SAT N=70		*OR*	*95% CI*	X^2	*p* value
	No	%				
Animal at home n=89	15	16.9	0.35	0.2-0.6	10	0.001
Milking animal n=21	6	28.6	0.9	0.3-2.6	0.02	0.96
Contact animal newborn n=29	10	34.5	1.3	0.5-3.0	0.47	0.49
Touch fresh meat n=185	52	28.1	0.82	0.4-1.5	0.33	0.56
Ingested raw milk n=200	59	29.5	1.1	0.5-2.4	0.11	0.73
Consuming milk products n=219	64	29.2	1.1	0.4-2.9	0.03	0.84
Handling animal during birth n=26	18	69.2	7	2.6-17	22	<0.0001
Contact animal waste n=50	18	36	1.5	0.7-2.9	1.4	.22
Family history of brucellosis n=24	8	33.3	1.2	0.5-3.0	0.23	0.62
Farmer n=81	26	32.1	1.2	0.6-2.2	0.55	0.45
Butchers n=1	0	0				
Microbiologist n=26	6	23	0.7	0.27-1.8	0.5	0.47
Veterinarian n=2	0	0	-	-	-	-

OR- Odds ratio >1 (at risk), CI Confidence intervals, χ^2 Chi-square ≥3.9 (significant), *p*- Probability value ≤0.05 (significant)

Table 8: Association of the type of animals living in the dwelling from infection with brucellosis.

Animals	Positive SAT N=70		OR	95% CI	X^2	*p* value
	No	%				
Cow n=55	4	7.3	0.1	0.04-0.4	16	<0.0001
Goats n=46	14	30.4	1.0	0.5-2.1	0.05	0.82
Sheep n=45	14	31.1	1.0	0.5-2.1	0.05	0.82
Dogs n=15	5	33.3	1.2	0.4-2.7	0.14	0.7

This is in contrast to the higher risks for the 20th year-old group which were found in Egypt (62%)[47], in Ethiopia (48.1%)[36], and northern Tanzania (46%)[48]. Current results indicated that all age groups in the current study are equally exposed to risk factors of brucellosis. In the current study, the prevalence of *Brucella* antibodies in PUO patients living in rural areas (29%) was almost equal to that in urban areas (28.9%). This result is similar to what was previously found in Yemen[3-5,49], Iran[50] and Pakistan[40]. However, this study differed with other study in Egypt by Fouad *et al.*, as 75.5% of their patients were urban residents (p<0.01)[51]. It also differs from what Menas *et al.*, and Al *et al.*, in Pakistan[52,53], and in Egypt recently[47] where most cases of brucellosis were from rural residents. The current study found a significant relationship between brucellosis and handling of the animal during parturition, the rate being 69.2% with a correlation factor equal to 7 (CI=2.6-17, χ^2=22, p<0.0001). These findings are similar to previous studies conducted in Yemen[5,33], in northern Uganda[54], in Pakistan[40] and in Nigeria[43], where they found that handling animals during childbirth is a risk factor for brucellosis.

CONCLUSIONS AND RECOMMENDATIONS

These findings revealed a high prevalence of human brucellosis among PUO patients in Sana'a city and will becoming a serious problem that threats the health care system in Yemen. So, awareness programs should be provided to doctors, population about *Brucella* infection and its risk factors. Awareness of brucellosis transmission routes can guide the community and prevent further infection. Further studies of sero-diagnosis and bacterial isolation of the disease, collaboration between researchers and public health professionals in terms of research and expansion of diagnostic services for brucellosis.

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AUTHOR'S CONTRIBUTIONS

This research is part of a master's degree in the Department of Medical Microbiology. **Maher AAA:** writing original draft, methodology. **Al-Huraibi BS:** research design, data collection. **Al-Kholani AIM:** statistical analysis, conceptualization. **Al-Dhafari OAM:** editing, methodology. **Al-Najhi MMA:** methodology, investigation. **Al-Shamahy HA:** supervision. **Al-dossary OAE:** formal analysis, conceptualization. **Al-Ankoshy AAM:** research design, data collection. Final manuscript was read and approved by the all authors.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Pappas G, Papadimitriou P, Akritidis N, *et al*. The new global map of human brucellosis. Lancet Infect Dis 2006; 6:91–99. *https://doi.org/10.1016/S1473-3099(06)70382-6*
2. Corbel MJ. Brucellosis in humans and animals. Geneva, Switzerland: World Health Organization; 2006.
3. Al-Shamahy H. Seropositivity for brucellosis in a sample of animals in the Republic of Yemen. East Mediterr Health J 1999; 5(5): 1042- 1044. *PMID: 10983546*
4. Al Shamahy HA, Wright SG. A study of 235 cases of human brucellosis in Sana'a, Republic of Yemen. EMHJ - Eastern Medit Health J 2001; 7(1-2): 238-246.
5. Al-Shamahy H, Whitty C, Wright S. Risk factors for human brucellosis in Yemen: A case control study. Epidemiol Infect 2000; 125(2): 309-313. *https://doi.org/10.1017/S0950268899004458*
6. Godfroid J, Cloeckaert A, Liautard JP, *et al*. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet Res 2005; 36:313–326. *https://doi.org/10.1051/vetres:2005003*
7. Al-Shamahy HA, Wright SG. Enzyme linked immunosorbent assay for *Brucella* antigen detection in human sera. J Med Microbiol 1998; 47: 169-172. *https://doi.org/10.1099/00222615-47-2-169*
8. Islam MA, Khatun MM, Werre SR, *et al*. A review of *Brucella* seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. Vet Microbiol 2013; 166:317–326. *https://doi.org/10.1016/j.vetmic.2013.06.014*
9. Al Dahouk S, Nöckler K. Implications of laboratory diagnosis on brucellosis therapy. Expert Rev Anti Infect Ther 2011; 9(7):833–845. *https://doi.org/10.1016/j.vetmic.2013.06.014*
10. Bounaadja L, Albert D, Chénais B, *et al*. Real-time PCR for identification of *Brucella* spp.: a comparative study of IS711, bcsp31 and per target genes. Vet Microbiol 2009; 137:156–164. *https://doi.org/10.1016/j.vetmic.2008.12.023*
11. Alastot EM, Al-Shamahy HA. Prevalence of leptospirosis amongst slaughterhouse workers and butchers in Sana'a city-Yemen. Universal J Pharm Res 2018; 3(2): 17-20. *http://dx.doi.org/10.22270/ujpr.v3i2.R4*
12. Ali Abdulkarim Sheiban, Hassan A. Al-Shamahy, Nashwan M. Alattab, Al-Kasem MA. Abbas. Epidemicity of vibrio cholera in Sana'a city, Yemen: prevalence and potential determinants. Universal J Pharm Res 2017; 2(6): 1-6. *http://doi.org/10.22270/ujpr.v2i6.R1*
13. Al-Shamahy HA. Seroprevalence of Kala-azar among Humans and Dogs in Yemen. Annals of Saudi Medicine 1998; 18(1): 66-68. *https://doi.org/10.5144/0256-4947.1998.66*
14. Alshamahi EYA, Al-Eryani SA, Al-Shamahy HA, *et al*. Prevalence and risk factors for Trachoma among primary school children in Sana'a city, Yemen. Universal J Pharm Res 2021; 6(4):19-25. *https://doi.org/10.22270/ujpr.v6i4.636*
15. Alshamahi EYA, Ishak AA, Aljayfey NH, Al-Shamahy HA. Prevalence and risk factors for Trachoma among primary school children in Bajjil District, Al Hudaydah, Western Yemen. Clin Ophthalmol J 2020; 1(3):1014.
16. Ishak AA, Al-Shamahy HA. Trends and causes of morbidity in part of children in the city of Sana'a, Yemen 1978-2018: findings of single children's health center. Universal J Pharm Res 2020; 5(6):1-5. *https://doi.org/10.22270/ujpr.v5i6.504*
17. Al Rukeimi AD, Al Shamahy HA, *et al*. Association of Cytomegalo-Virus and Rubella virus infections in pregnant women with bad obstetric history. W J Gynecol Women's Health 2019; 2(3):1-5. *https://doi.org/10.33552/WJGWH.2019.02.000538*
18. Abbas AM, Al-Kibsi TAM, Al-Akwa AAY, AL-Haddad KA, Al-Shamahy HA, Al-labani MA. Characterization and antibiotic sensitivity of bacteria in orofacial abscesses of odontogenic origin. Universal J Pharm Res 2020; 5(6):36-42. *https://doi.org/10.22270/ujpr.v5i6.510*
19. Al-Shami HZ, Al-Mutawakal ZAM, Al-Shamahy HA, *et al*. Prevalence of Hepatitis A virus, Hepatitis B virus, and Hepatitis C virus, among patients with hepatic jaundice in Sana'a city, Yemen: A hospital based study. Universal J Pharm Res 2021; 6(6):12-17. *https://doi.org/10.22270/ujpr.v6i6.693*
20. AL-Shamahy HA. Prevalence of Hepatitis B surface antigen and Risk factors of HBV infection in a sample of healthy mothers and their infants in Sana'a, Yemen. Ann Saudi Medicine 2000; 20: 464-467. *https://doi.org/10.5144/0256-4947.2000.464*
21. Al-Shamahy HA, Rabbad IA, Al-Hababy A. Hepatitis B virus serum markers among pregnant women in Sana'a, Yemen. Ann Saudi Med 2003; 23:87-89. *https://doi.org/10.5144/0256-4947.2003.87*
22. Al-Shamahy HA, Hanash SH, Rabbad IA, Al-Madhaji NM, Naser SM. Hepatitis B Vaccine Coverage and the Immune Response in Children under ten years old in Sana'a, Yemen. Sultan Qaboos Univ Med J 2011 Feb; 11(1):77-82. *PMID: 21509212*
23. Al-Shamahy HA, Ajrah MAA, Al-Madhaji AG, *et al*. Prevalence and potential risk factors of hepatitis B virus in a sample of children in two selected areas in Yemen. Universal J Pharm Res 2019; 4(3):1-6. *https://doi.org/10.22270/ujpr.v4i3.269*
24. Al-Shawkany EM, AlShawkany ARM, Al-Shamahy HA, *et al*. Prevalence of different hepatitis B virus genotypes and risk factors associated among selected Yemeni patients with chronic hepatitis B infection. Universal J Pharm Res 2021; 6 (3):1-8. *https://doi.org/10.22270/ujpr.v6i3.603*
25. Amran OAA, Al-Shamahy HA, Al Hadad AM, and Jaadan BM. Explosion of hepatitis B and C viruses among hemodialysis patients as a result of hemodialysis crisis in Yemen. Universal J Pharm Res 2019; 4(5):1-8. *https://doi.org/10.22270/ujpr.v4i5.311*
26. Hanash SH, Al-Shamahy HA, Bamshmous MHS. Prevalence and genotyping of hepatitis C virus in hemodialysis patients and evaluation of HCV-core antigen test in screening patients for dialysis in Sana'a city, Yemen. Universal J Pharm Res 2019; 4(2): 14-18. *https://doi.org/10.22270/ujpr.v4i2.251*
27. Al-Shamahy HA, Abdu SSA. Genotyping of Hepatitis C Virus (HCV) in infected patients from Yemen. Eur J Basic Med Sci 2014; 3(4):78-82. *https://doi.org/10.15197/sabad.2.3.15*
28. Al-Arosi SAH, Al-shamahi EY, Al-Kholani AIM, Al-Jawfi AY, Al-Shamahy HA, Al-Moyed KAA, Al-Ankoshy AAM. Neonatal bacterial conjunctivitis in tertiary hospitals in Sana'a city, Yemen. Universal J Pharm Res 2021; 6(6):36-42. *https://doi.org/10.22270/ujpr.v6i6.697*
29. Alshamahi EYA, Al Shamahy HA Al Nahary AA, AL Magrami RTF, *et al*. Epidemiology and aetiological diagnosis of corneal ulceration in Sana'a City, Yemen. W J Opthalmol Vision Res 2019; 2(5):1-6. *https://doi.org/10.33552/WJOVR.2019.02.000550*
30. Alshamahi EYA, Al-Shamahy HA, Musawa YA. Bacterial causes and antimicrobial sensitivity pattern of external ocular infections in selected ophthalmology clinics in Sana'a city. Universal J Pharm Res 2020; 5(3):12-16. *https://doi.org/10.22270/ujpr.v5i3.329*
31. Alshamahi EYA, AlShami HZA, Al-Shamahy HA. Prevalence of *S. aureus* in external ocular infection and the occurrence of MRSA in isolates, Rabat. Clin Ophthalmol J 2020;2(1):1010.
32. Nassar M, Al-Shamahy HA, AL-Barq AM. Evaluation of the immune response to polio vaccine in malnourished children in Sana'a city. Universal J Pharm Res 2018; 3(2): 29-33. *https://doi.org/10.22270/ujpr.v3i2.137*
33. Al-Haddad AM, Al-Madhagi AK, Talab AA, Al-Shamahy, HA. The prevalence of human brucellosis in three selected areas in Al-Dala'a governorate, Yemen. Faculty Sci Bull 2013; 25: 61-71.
34. Rahamathulla MP. Seroprevalence of human brucellosis in Wadi Al Dawaser region of Saudi Arabia Pak J Med Sci 2019; 35:129-135. *https://doi.org/10.12669/pjms.35.1.55*

35. Al-Freihi HM, Suliman A. Al-Mohaya, *et al*. Brucellosis in Saudi Arabia: Diverse manifestations of an important cause of pyrexial illness annals of Saudi Medicine 1986; 6: 95-99. *https://doi.org/10.5144/0256-4947.1986.95*
36. Mehari S, Zerfu B, Desta K. Prevalence and risk factors of human brucellosis and malaria among patients with fever in malaria-endemic areas, attending health institutes in Awra and Gulina district, Afar Region, Ethiopia. BMC Infect Dis 2021; 21: 942. *https://doi.org/10.1186/s12879-021-06654-y*
37. Sen A, Chakraborty U, Chatterjee D, *et al*. *Brucella* sero-positivity and genus specific pcr positive rates amongst PUO cases attending opd of a tertiary care hospital in Bihar, India. European J Biomed Pharm Sci 2017; 4: 440-443.
38. Damke SS, Damke SG. Seroprevalence of *Brucella* agglutinins in patients with pyrexia of unknown origin attending a tertiary care rural hospital. J Evolution Med 2020; 3: 162-165. *https://doi.org/10.14260/jemds/2020/36*
39. Alkahtani AM, Assiry MM, Chandramoorthy HC, *et al*. Sero-prevalence and risk factors of brucellosis among suspected febrile patients attending a referral hospital in southern Saudi Arabia (2014–2018). BMC Infect Dis 2020; 20: 26. *https://doi.org/10.1186/s12879-020-4763-z*
40. Saddique A, Ali S, Akhter S *et al*. Acute febrile illness caused by *Brucella abortus* infection in humans in Pakistan. Int J Env Res Pub Health 2019; 16:4071. *https://doi.org/10.3390/ijerph16214071*
41. Migisha R, Dan N, Boum Y, *et al*. Prevalence and risk factors of brucellosis among febrile patients attending a community hospital in south western Uganda. Sci Rep 2018; 8:15465. *https://doi.org/10.1038/s41598-018-33915-9*
42. Rahman AA, Berkvens D, Saegerman C, *et al*. Seroprevalence of brucellosis in patients with prolonged fever in Bangladesh. The J Infec Dev Count 2016; 10: 939-946. *https://doi.org/10.3855/jidc.6844*
43. Hafiz H, Saad AA, Mu'awiyyah B. *et al*. Seroprevalence and factors associated with risk of human brucellosis among febrile patients attending health-care facilities in Bauchi, North-Eastern Nigeria. Annal Trop Path 2020; 11: 107-112. *https://doi.org/10.4103/atp.atp_39_20*
44. Al-Bayaa YJ. Epidemiology of human brucellosis among populations in Iraq's provinces in 2015 J Fac Med Baghdad 2017; 58, 165-169. *https://doi.org/10.32007/jfacmedbagdad.592130*
45. Nakeel M, Arimi S, Kitala P. *et al*. A sero-epidemiological survey of brucellosis, Q-fever and leptospirosis in livestock and humans and associated risk factors in kajiado county-Kenya. J Trop Dis Pub Health 2016.4(3): 1-6. *https://doi.org/10.4172/2329-891X.1000215*
46. Majalija S, Luyombo P, Tumwine G. Sero-prevalence and associated risk factors of Brucellosis among malaria negative febrile out-patients in Wakiso district, Central Uganda. BMC Res Notes 2018; 11:803. *https://doi.org/10.1186/s13104-018-3907-3*
47. El-Moselhy EA, Soliman TS, El-Rahman A, *et al*. Human brucellosis: Methods of diagnosis and risk factors among egyptian patients admitted at Assiut Fever Hospital. J Rec Adv Med 2020; 1:1-13. *https://doi.org/10.21608/JRAM.2019.13953.1013*
48. Bouley AJ, HMB, Robyn A, *et al*. Brucellosis among Hospitalized Febrile Patients in Northern Tanzania. Am J Trop Med Hyg 2012; 87, 1105-1111. *https://doi.org/10.4269/ajtmh.2012.12-0327*
49. Saad AA, Qais YM, Mofeed A, *et al*. Human and animal brucellosis in Yemen. Adv Biotech Micro 2017; 4(1): 555630. *https://doi.org/10.19080/AIBM.2017.04.555630*
50. Hasanjani Roushan MR, Ebrahimpour S, Moulana Z. Different clinical presentations of brucellosis. Jundishapur J Microb 2016;9(4):e33765. *https://doi.org/10.5812/jjm.33765*
51. Fouad A, Abdel Hafez H, Abdullah R. Update of brucellosis in Egyptian referral fever hospital. Kasr El Aini Med J 2011;17:51-57. *https://doi.org/10.21608/jram.2019.13953.1013*
52. Minas M, Gourgulianis MA, Stournara A. Epidemiological and clinical aspects of human brucellosis in Central Greece. Jpn J Infect Dis 2007; 60: 362-6.
53. Ali S, Nawaz Z, Akhtar A, Aslam R, Zahoor M A, *et al*. Epidemiological investigation of human brucellosis in Pakistan. Jundishapur J Microbiol 2018; 11(7):e61764. *https://doi.org/10.5812/jjm.61764*
54. Muloki HN, Erume J, Owiny DO, *et al*. Prevalence and risk factors for brucellosis in prolonged fever patients in post-conflict Northern Uganda. Afr Health Sci 2018; 18(1):22-28. *https://doi.org/10.4314/ahs.v18i1.4*