**Reviewer’s Comments**

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# HUMAN BRUCELLOSIS AMONG PYREXIA OF UNKNOWN ORIGIN CASES IN TWO TERTIARYHOSPITALS, IN SANA'A, YEMEN

**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. However, consistent with the World Health Organization, *brucellosis* is schedule as one of seven neglected zoonotic diseases. *Brucellosis* is an important cause of PUO in endemic areas. Living near animals is a major risk factor for disease. Estimates of the prevalence of brucellosis among PUO patients are not available for many countries of the world including Yemen.This study was conducted to determine *Brucella* antibodies among patients with fever of unknown origin, and to determine the association of brucellosis with demographic characteristics, including age, sex, residence, educational level and occupation. Also, identify clinical symptoms and risk factors for *Brucella*infection among PUO cases.

**Materials and Methods**: This cross-sectional research study was conducted intwo health institutions; Al-Jumhouri Hospital and Al-Thawra Hospital, between January 2021 to January 2022. The required data were gatheredfrom each patient byusing pre-defined standardized questionnaire that included data as demographic; Name, age, sex, risk factors and clinical symptoms. Five mLof 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 pain (66.4%), headache (60.4%), weight loss (52.9%), body weakness (89.2%), loss of appetite (50.6%), nausea (46.1%). The risk factors for brucellosis among the PUO patients were animal husbandry at home and handling of animals during parturition (69.2%, OR = 7, P < 0.0001).

**Conclusion:**High incidence of *Brucella*antibodies among PUO patients (29%). The rate of brucellosis antibodies among females was much higher than that of males. There was no significant variation of brucellosis among the different age groups, as the distribution was almost between all ages, and there was a significant correlation between brucellosis and animal husbandry at home, as well as dealing with animals during childbirth.

 **Keywords**: *Brucella* antibodies, *Brucellosis*, Pyrexia of Unknown Origin (PUO), Sana'a City, Yemen.

**INTRODUCTION**

Human *brucellosis* is a zoonotic bacterial disease that has been reported worldwide. It is primarily an occupational disease that is reported by veterinarians, slaughterhouse workers, farmers, meat inspectors and animal handlers. Brucellosis is caused by several *Brucella*species belonging to the genus *Brucella*, which are small, Gram-negative, non-sporous, non-encapsulated bacilli. Globally each year, more than 500,000 new cases are reported, with annual incidence rates changeable broadly from <2 to >500 per 1,000,000 population between different regions1.Brucellosis continues endemic in many areas of the world including the Middle East, Latin America, the Mediterranean Basin, Africa and Asia1. International tourists visiting brucellosis endemic areas are at risk of infection.

Spread of Brucellato humans consequences from direct contact with an infected animal, and also from consumption of unpasteurized milk and dairy products2,3. Brucellosis of human is a lot under-diagnosed or misdiagnosed because the clinical manifestations overlap with many bacterial infections. High undulant fever, night sweats and weight loss are the most important symptoms 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 brucellosis4,5. 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 greater than or equal to 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. Laboratory confirmation of human brucellosis 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 ELISA3-8.The molecular diagnosis of human brucellosis can be carried out by means of genus-specific polymerase chain reaction (PCR) assays. 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 agglutination based serological tests and ELISA, but isolation of the pathogen from blood culture remains the gold standard4,5,7.

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 infectionsother infectious diseases but no recent studies of brucellosis have been conducted11-32, in conclusion, Yemen has been neglected with regard to the study of brucellosis.This study was conducted to estimate *Brucella* antibodies among patients with pyrexia of unknown origin, determine the association of brucellosis with demographic characteristics, including age, gender, residence, educational level, and occupation. Also, determine the clinical symptoms and risk factors of *Brucella* infection among the PUO cases.

**MATERIALS AND METHODS**

**Study design andstudy 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.

**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:** We selected desired precision of 0.01 (1% acceptable error in the estimation) with possible estimated true proportion of human brucellosis in Al- Dala’a city- Yemen equal to 6.7% 33with Confidence Levels 99% for population of pyrexia of unknown origin in Sana'a city per year equal to 100000 , we need at least241 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 patients with PUO by standard predesigned questionnaire designed for this study that include data as demographic information; name, age, gender and risk factors and clinical symptoms.

**Blood sample collection:** Five mLofwhole blood was collected aseptically by venipuncture from each patients with PUO andserum was separated by centrifugation after clotting. The sera samples were kept at – 20°C until tested for *Brucella* antibodies.

**Laboratory test:** The sera was 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 positive4.

**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* <0.05was 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). The prevalence rate of brucellosis (≥1/160 SAT) was 70/241 (29.1%), *Brucellaabortus* positive rate was 17%, and *Brucellamelitensis* positive rate was 2.9% and mixed of both *Brucella*species 9.1% (Table 2). Table 3 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.Table 4 shows that the higher rate of *Brucella* antibodies were occurred in females (78.6%) with OR equal to 1.6 when compared tomales (21.4%) (χ 2 =1.99*, P=0.07).*  Also, the age group of 16-25 years odl had ahiher rate of *Brucella* antibodies and there are no statistical significant differnces (Table 4).There was no significant association between residency, occupations, and education with contracting brucellosis among PUO patients (Table 5). The seroprevalence 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 patientsthat summarized in Table.Table 7 shows the risk factors of contacting brucellosis among PUO patients.When we considered the handling animal during birth, it was (69.2%), handling animal during birth (CI=2, CI=6-17, χ 2 =22,  *P<0.0001).* The rate of brucellosis among contact animal waste (36%),contact animal newborn (34.5%),family history of brucellosis (33.3%), farmer (32.1%), ingested raw milk (29.5%), consuming milk products (29.2%), milking (28.6%), touch fresh meat (28.1%) and microbiologist (23%).Table 8 shows association of the type of animals living in the dwelling from infection with *brucellosis*. There was no significance association with the different animals.

**DISCUSSION**

*Brucellosis* remains a major health problem in many parts of the world and is an important cause of acute febrile illness in the Middle East regions. However, according to the World Health Organization, *brucellosis* is listed as one of seven neglected zoonotic diseases. Brucellosis is an important cause of PUO in endemic areas34. 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 the presence or prevalence of brucellosis. For example, this study is the second cross-sectional study of *Brucella* infection among PUO patients in Sana'a city-Yemen in the last 22 years5.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, southwestern 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. Our data indicate that most females were housewives and exposed to brucellosis risk factors as they directly handled milk or meat or looked after animals44-46. 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). 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. Our 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 Yemen3-5,49, Iran50 and Pakistan40. 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 Pakistan52,53, and in Egypt recently47 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, χ2 = 22, P<0.0001). These findings are similar to previous studies conducted in Yemen5,33, in northern Uganda54, in Pakistan40 and in Nigeria43, where they found that handling animals during childbirth is a risk factor for brucellosis.

**CONCLUSIONS AND RECOMMENDATIONS**

High incidence of *Brucella*antibodies among PUO patients (29%). The rate of brucellosis antibodies among females was much higher than that of males. There was no significant variation of brucellosis among the different age groups, as the distribution was almost between all ages, and there was a significant correlation between brucellosis and handling animal during birth. These findings revealed a high prevalence of human brucellosis among PUO patients in Sana'a city and will becoming a serious problem that threat 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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**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

**AUTHOR CONTRIBUTIONS**

This research is part of a master's degree in the Department of Medical Microbiology, first author Ahlam Ali Ahmed Maher, who conducted field work in hospitals, and who did laboratory work, and other authors contributed to data analysis, drafting and review of the paper, and gave final approval to the research.

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**Results**

|  |  |  |
| --- | --- | --- |
|  | Number (No.) | 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 1**:**The age and sex distribution of patients with PUO who were tested for,*Brucella* antibodies in Sana'a city, Yemen, 2022.

Table 2: The prevalence of *Brucella*antibodies among tested PUO patients.

|  |  |  |
| --- | --- | --- |
| *Brucella species* |  Accept Frequency(%) | ~~percentage~~ |
| *Brucellaabortus*antibody positive only | 41(17) | ~~17~~ |
| *Brucellamelitensis*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 3:**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~~ |

**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 No. (%)  | OR | CI | χ 2 | *P* value |
| 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 SATN=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)**, **CI** Confidence intervals, **χ 2** Chi-square **≥3.9 (significant***)*

 ***P*** Probability value **≤0.05 (significant)**

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Symptoms | Positive SAT (N =70) | *95% CI* | *X2* | *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 |

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

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Risk factors | Positive SATN=70 | *OR* | *95% CI* | *X2* | *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 SATN=70 | OR | 95% CI | X2 | 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 |