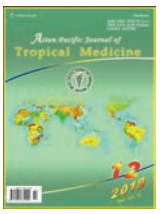

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# Seroprevalence of brucellosis among exposed agro−pastoral communities in southern Saudi Arabia

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ABSTRACT

**Objective:** To investigate the prevalence and risk factors of brucellosis in human and animal's communities in southern Saudi Arabia.

**Methods:** A cross-sectional sero-epidemiological study was conducted in Aseer and Jazan, Saudi Arabia (October 2017-October 2018). Human serum samples ($n$=339) were initially screened for *Brucella* antibodies and positive samples were further titrated for *Brucella* antibodies by immunocapture assay (titer of ⩾1:320 as positive). Animal samples ($n$=828) were screened using the Rose Bengal test. Relationship status was dichotomized to measure and predict independent contributions to variations in human using univariate and multivariate stepwise binary logistic regression model.

**Results:** The rate of brucellosis among the 339 human samples in the two regions was 33.9%, and the rate of acute brucellosis was 12.4%. The rate of brucellosis in animals was 4.7%. Human brucellosis among the target groups was higher in northwestern Aseer (53.3%) compared to Southeastern Aseer (25.9%) and Jazan region (20.6%). The disease was more prevalent among non-Saudi nationals (35.2%) compared to Saudis (30.5%). The rate of brucellosis among butchers and shepherds was 37.5% and 37.2%, respectively. The rate of brucellosis was 37.8% in people over 30 years of age. Our univariate analysis showed that residing in Aseer region (*OR*: 2.60, 95% *CI*: 1.50-4.40), especially residing in northwestern Aseer region (*OR*: 4.40, 95% *CI*: 2.40-7.90), frequent consumption of raw meat (*OR*: 2.90, 95% *CI*: 1.50-5.50), shepherds (*OR*: 2.10, 95% *CI*: 0.80-5.30), owning sheep (*OR*: 2.20, 95% *CI*: 1.10-4.40), daily contact with animals (*OR*: 2.10, 95% *CI*: 0.75-5.80), and those > 30-year-old (*OR*: 1.50, 95% *CI*: 1.00-2.40) were significantly associated with increased risks of brucellosis. Our multivariate analysis further showed that residing in northwestern Aseer (*OR*: 9.16, 95% *CI*: 3.39-24.76) and having sheep (*OR*: 1.16, 95% *CI*: 1.00-1.35) were significant and independent risks of brucellosis while residing in agricultural region (*OR*: 0.28, 95% *CI*: 0.10-0.78) was a significant and independent protector against brucellosis.

**Conclusions:** The study concluded that residing in northwestern Aseer area and having animals (sheep) are associated with significantly increased risks of brucellosis.

## 1. Introduction

Brucellosis (undulant fever, Malta fever, or Mediterranean fever) is a major zoonotic disease that causes considerable economic losses in addition to significant human morbidity, especially in endemic areas. This highly communicable disease is caused by *Brucella*

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spp., small, facultative intracellular gram-negative coccobacilli[1,2]. The disease remains a neglected zoonotic disease among agro-pastoral communities where unprocessed milk and meat products are incautiously consumed[3-5]. Its clinical manifestations differ from an asymptomatic infection to chronic illness associated with recurrence of symptoms[6,7] which may lead to serious morbidity in humans and remains to be the main health setback. The majority of brucellosis cases are found in people who engage in the agro-pastoral sectors, namely, those involved in farming, abattoirs, and processing of animal products. The source of human brucellosis is usually direct or indirect exposure to infected animals or their products[8]. Prevention of brucellosis is based on the elimination of such contact or better elimination of the disease from animals, which may be beyond the financial and human resources in many developing countries. Attempts to minimize the impact of the disease and to reduce the risk of infection by personal hygiene, adoption of safe working practices, protection of the environment and food hygiene seem more tangible. Prophylaxis has an insignificant role in the prevention of human disease owing to the fact that vaccines are lacking or unsafe[8,9].

Human brucellosis seroprevalence was recorded high (17%) in Kiboga district, Uganda[5]. The estimated herd-level prevalence of brucellosis in Ouagadougou, Burkina Faso was 3.6%[10], 5.6%[11] and up to 11.4% in Maranhao State, Brazil[12]. The animal disease factor remains the main risk factor for human infection. There have been indications that human brucellosis is widely distributed in Saudi Arabi[13,14]. Reports have revealed insufficient and patchy indications of the disease in its epidemiological setting from Aseer region. One report from Al-Qassim, Aseer, and Hail indicated higher rates reaching 25%. Young, male Saudi citizens living in highly endemic areas were at greatest risk of acquiring brucellosis[15]. The rate is, however, significantly decreased from 22.9% in 2004 [95% confidence interval (*CI*)=22.3, 23.5] to 12.5% in 2012 (95% *CI*=12.1, 13.0)[15].

Zoonotic diseases such as brucellosis are difficult to control mainly because of their animal reservoirs. Also, the estimate of their prevalence would be insignificant and lacks accuracy if no appropriate knowledge of the sampling method is applied[16]. The wide variance in the results among regions may be due to sampling size, time and area of investigations[17]. Large multicenter studies are needed to determine prevalence and risk factors to establish appropriate control measures. Endemic sites of the disease are needed to be determined, and efficient educational and health control measures are also needed to be enforced in such sites.

This study aimed to investigate the prevalence of brucellosis in rural and suburban communities in two regions in southern Saudi Arabia and to identify some potential risk factors.

## 2. Materials and methods

### 2.1. Study design

This was a cross-sectional sero-epidemiological study which was conducted in two regions (Aseer and Jazan) of southwestern Saudi Arabia between October 2017 and October 2018 covering nine different sites. Participants were included conveniently after explaining the purpose of the study. Informed consent was obtained from all individuals who agreed to participate in the study ($n$=339). All procedures performed involving human and animal participants were in accordance with the ethical standards of the institution. Ethical approval was obtained from the College of Medicine, King Khalid University (REC#2017-05-21).

### 2.2. Collection of samples

Blood samples from humans ($n$=339) and animals 828 (629 sheep and 199 camels) were collected from communities in nine areas in two study regions. The sampling strategy from humans and animals has targeted the agro-pastoral communities in the two regions between October 2017 and October 2018 following standard methods. At the time of sampling, a questionnaire to collect data regarding risk factors for brucellosis was completed. Data on the questionnaire covered socio-demographic characteristics and human brucellosis related risk factors. The questionnaire was previously constructed by the researchers after an intensive literature review and expert consultation.

Briefly, blood samples were collected from volunteers at random at scheduled visits to each of the nine sampling locations. A total of 5-10 mL blood was collected aseptically using sterile disposable syringes and vacutainer tubes. Tubes were labeled and allowed to stand at room temperature for at least 30 min to permit a solid clot to form and retract. The tubes were then centrifuged at 3 500 rpm for 5 min; the serum was removed, placed in another clean tube and kept in the refrigerator before transportation. Tubes were transported to the laboratory in a cool box.

### 2.3. Screening procedure for Brucella antibodies in human sera

Samples were initially assayed for *Brucella* antibodies by single-step immunocapture agglutination assay using *Brucella abortus (B. abortus)* antigen through commercially available kits (VIRCELL, Granada, Spain). Briefly, reagents were brought to room temperature and samples were assayed in a precoated microtiter plate to capture immunoglobulins in serum. Two wells were used for each patient where 100 μL of serum diluent was added to the first well and

50 μL to the second well, then 5 μL of serum was added to the first well and mixed well. After that 50 μL was transferred from the first well to the second well followed by addition of 50 μL of bacterial suspension to each well. Dilution in the first well was 1:40 and in the second well was 1:80. Plates were sealed with adherent tape and incubated for 24 h at 37 ℃. Positive wells showing agglutination at 1:40 dilution was further titrated to get the exact titer for *Brucella* antibodies.

### 2.4. Titration procedure for Brucella antibodies

Samples were initially screened for *Brucella* antibodies by single step immunocapture agglutination assay using *B. abortus* antigen[18] taking into consideration titer of ⩾1:320 as positive for *Brucella* according to manufacturer recommendations (VIRCELL, Granada, Spain). Similar to previous method, all reagents were brought to room temperature before use and samples were screened in a microtiter plate where 50 μL of serum diluent was added into well A; then 50 μL of serum diluent was added into all wells from A to H. Some 5 μL of each serum, positive and negative controls were added into well A. Doubling dilutions with 50 μL was made for each well from A to H. Some 50 μL of the bacterial suspension, previously homogenized by vigorous shaking, was added into all wells. Plate wells were sealed with adherent tape and incubated for 24 h at 37 ℃, in a humid chamber protected from light exposure. Results were read taking into account that titers will be: 1:40 for row A, 1:80 for row B, 1:160 for row C, 1:320 for row D, 1:640 for row E, 1:1 280 for row F, 1:2 560 for row G and 1:5 120 for row H.

### 2.5. Rose Bengal test for animal samples

Rose Bengal test, a rapid slide agglutination antigen, was used for the detection of anti-*Brucella* antibodies in sheep and camels sera from the two regions as described by the OIE[19] using commercially available Brucelloslide-Test kit (bioMerieux, 69280 Marcy l'Etoile France). Microtitration droppers were used to deliver 1 drop (0.03 mL) of stained *Brucella* antigen suspension to an equal volume of the animal serum sample. The mixtures were then mixed with individual wooden sticks, and slides were rocked gently for 4 min on an orbital shaker. Samples that tested positive by the Rose Bengal test are indicated by the presence of agglutination visible by the naked eye.

### 2.6. Statistical analysis

After data were extracted, it was revised, coded and fed to statistical software IBM SPSS version 22. The analysis was done using two-tailed tests and an alpha error of 0.05. A *P*-value less than or equal to 0.05 was considered statistically significant. Descriptive analysis including frequency and percent distribution was done for all variables including the prevalence of brucellosis among participants and also participants' bio-demographic data and risk factors. Univariate analysis was done to assess the crude relation between different studied risk factors and brucellosis using crude odds ratio estimates with 95% confidence limits. The multivariate stepwise binary logistic regression model was applied to assess the most significant predictors for brucellosis among the participants based on the adjusted odds ratio estimate with its 95% confidence limits.

## 3. Results

Among the 339 human samples investigated in southern Saudi Arabia, 115 (33.9%) were positive for brucellosis detected by anti-*B. abortus* antigen. Forty-two of the positive cases (12.4%) had acute infections (titer ⩾1:320) whereas 73 (21.5%) were chronic (titer<1:320).

The infection rate in animals (sheep and camels) in the two regions was 4.7% (39/828). The total number of camels was 199 with 7 positive cases (3.5%). All the 199 camel samples were collected from Khamis city in Aseer region. Whereas the total number of sheep was 629 with 32 positive cases presenting a prevalence of 5.1%. Among these, 399 sheep with 24 positive cases (6.0%) were from Aseer region. The samples were collected from Abha city (*n*=129); Khamis city (*n*=202) and Wadi Ibn Hashbal area (*n*=68). The remaining 230 samples were from Jazan region with 8 positive cases (3.5%).

Table 1 shows the univariate relation between participants' factors and brucellosis. A total of 40.1% of participants from Aseer region had positive *Brucella* compared to 20.6% of those at Jazan recording 2.6 times for likelihood for brucellosis (*OR*: 2.60, 95% *CI*: 1.50-4.40). About sub-region, participants in northwestern Aseer recorded 4.4 times more likely for brucellosis than Jazan region compared to 2.1 times for those who at southeastern Aseer (*OR*: 4.40, 95% *CI*: 2.40-7.90 and *OR*: 1.40, 95% *CI*: 0.71-2.50; respectively). Considering the nature of area, 36 brucellosis cases were detected in desert areas [66.7%; *OR*: 1.10, 95% *CI* (0.39-2.60)] and fewer risks were recorded in other natures of areas. Brucellosis affected more non-Saudi nationals [(35.2%) *OR*: 1.20, 95% *CI* (0.74-2.10)], compared to Saudis (30.5%). The majority of positive cases were males (34.4%) and have a higher risk, *OR*: 2.60, 95% *CI* (1.40-3.50). The prevalence rate of brucellosis in the two regions according to age groups is shown in Table 1. The largely affected age groups were those over 30-year-old [(37.8%) *OR*: 1.50, 95% *CI* (1.00-2.40)] compared to younger ages (28.7%). According to the occupation, butchers recorded 37.5%, followed by shepherds (37.2%), people with no permanent employment (Mutasabib) (27.6%).

The infection among those with frequent consumption of raw meat was found significant (*OR*: 2.90, 95% *CI*: 1.50-5.50) but not the consumption of milk (*OR*: 0.59, 95% *CI*: 0.29-1.20). According to the occupation: butchers (*OR*: 2.10, 95% *CI*: 0.34-11.40) and shepherds (*OR*: 2.10, 95% *CI*: 0.80-5.30) have high risks. Owning sheep and camels predisposed to brucellosis more than having other

**Table 1.** Univariate analysis for predictors of brucellosis among exposed agro-pastoral communities in southern Saudi Arabia.

| Risk factors | | *Brucella* | | $OR_u$ (95% *CI*) |
|---|---|---|---|---|
| | | Negative [*n*(%)] | Positive [*n*(%)] | |
| Region | Jazan | 85 (79.4) | 22 (20.6) | 1 |
| | Aseer | 139 (59.9) | 93 (40.1) | 2.60 (1.50-4.40)* |
| Sub-regions | Jazan Region | 85 (85.9) | 22 (20.6) | 1 |
| | Northwestern Aseer | 56 (43.8) | 64 (53.3) | 4.40 (2.40-7.90)* |
| | Southeastern Aseer | 83 (74.1) | 29 (25.9) | 1.40 (0.75-2.50)* |
| Nature of the area | Agricultural | 96 (80.0) | 24 (20.0) | 0.13 (0.05-0.30)* |
| | Desert | 18 (33.3) | 36 (66.7) | 1.10 (0.39-2.60) |
| | Mountainous | 100 (74.1) | 35 (25.9) | 0.18 (0.08-0.41)* |
| Nationality | Urban | 10 (33.3) | 20 (66.7) | 1 |
| | Non Saudi | 158 (64.8) | 86 (35.2) | 1.20 (0.74-2.10) |
| Gender | Saudi | 66 (69.5) | 29 (30.5) | 1 |
| | Female | 5 (100.0) | 0 (0.0) | 1 |
| Age in years[a] | Male | 219 (65.6) | 115 (34.4) | 2.60 (1.40-3.50)*# |
| | ⩽30 years | 102 (71.3) | 41 (28.7) | 1 |
| | > 30 years | 122 (62.2) | 74 (37.8) | 1.50 (1.00-2.40)* |
| Occupation | Farmer | 21 (77.8) | 6 (22.2) | 1 |
| | Butcher | 5 (62.5) | 3 (37.5) | 2.10 (0.34-11.40) |
| | Shepherd | 142 (62.8) | 84 (37.2) | 2.10 (0.80-5.30) |
| | Non-permanent job | 55 (72.4) | 21 (27.6) | 1.30 (0.47-3.80) |
| | Other | 1 (50.0) | 1 (50.0) | 3.50 (0.19-64.70) |
| Consumption of raw milk | Never | 89 (59.7) | 60 (40.3) | 1 |
| | Few times a week | 71 (65.7) | 37 (34.3) | 0.77 (0.46-1.30) |
| | Once a day | 29 (87.9) | 4 (12.1) | 0.21 (0.07-0.61) |
| | > once a day | 35 (71.4) | 14 (28.6) | 0.59 (0.29-1.20) |
| Consumption of raw meat and meat products | Never | 121 (70.8) | 50 (29.2) | 1 |
| | Few times a week | 70 (69.3) | 31 (30.7) | 1.10 (0.63-1.80) |
| | Once a day | 11 (57.9) | 8 (42.1) | 1.80 (0.67-4.60) |
| | > once a day | 22 (45.8) | 26 (54.2) | 2.90 (1.50-5.50)* |
| Contact with animals | Never | 34 (69.4) | 15 (30.6) | 1 |
| | Few times a week | 47 (63.5) | 27 (36.5) | 1.30 (0.60-2.80) |
| | Once a day | 12 (52.2) | 11 (47.8) | 2.10 (0.75-5.80) |
| | > once a day | 131 (67.9) | 62 (32.1) | 1.10 (0.54-2.10) |
| Animals species you have | None | 33 (68.8) | 15 (31.3) | 1 |
| | Camels | 23 (74.2) | 8 (25.8) | 0.76 (0.27-2.10) |
| | Cattle | 5 (50.0) | 5 (50.0) | 2.20 (0.55-8.70) |
| | Goats | 28 (82.4) | 6 (17.6) | 0.47 (0.16-1.40) |
| | Sheep | 63 (50.0) | 63 (50.0) | 2.20 (1.10-4.40)* |
| | Other | 72 (80.0) | 18 (20.0) | 0.55 (0.25-1.20) |
| Contact with aborted animals or retained placenta | No | 108 (67.1) | 53 (32.9) | 1 |
| | Not Sure | 34 (59.6) | 23 (40.4) | 1.40 (0.76-2.60) |
| | Yes | 82 (67.8) | 39 (32.2) | 0.98 (0.59-1.60) |

$OR_u$: Crude Odds Ratio; *CI*: Confidence interval; #: Calculated based on Yates correction; *Significant relation ($P$<0.05). [a]30 years was the cutoff point which yielded a balanced groups for analysis.

animals (*OR*: 2.20, 95% *CI*: 1.10-4.40). Daily contact with animals had risks (*OR*: 2.10, 95% *CI*: 0.75-5.80) but not the contact with an aborted animal or a retained placenta (*OR*: 0.98, 95% *CI*: 0.59-1.60).

Multivariate stepwise logistic regression model for predicting brucellosis among exposed agro-pastoral communities in southern Saudi Arabia is shown in Table 2. Among all included risk factors, northwestern Aseer, Agricultural region, and having sheep, were the most important predictors keeping all other factors constant. Northwestern Aseer region showed the highest risk of infection compared to the other two sub-regions [$P$<0.01, *OR*: 9.16, 95% *CI* (3.39-24.76)]. Also, owning sheep represented a high risk of infection [$P$=0.04, *OR*: 1.16, 95% *CI* (1.00-1.35)]. Besides, agricultural regions showed a protective effect against having brucellosis [*OR*: 0.28, 95% *CI* (0.10-0.78)].

**Table 2.** Multivariate stepwise logistic regression model[a] for predictors of brucellosis among exposed agro-pastoral communities in southern Saudi Arabia.

| Predictors | B | SE | *P*-value | $OR_a$ | 95% *CI* for *OR* | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| Northwestern Aseer | 2.22 | 0.51 | <0.01 | 9.16 | 3.39 | 24.76 |
| Agricultural region | -1.26 | 0.52 | 0.02 | 0.28 | 0.10 | 0.78 |
| Have sheep | 0.15 | 0.08 | 0.04 | 1.16 | 1.00 | 1.35 |
| Constant | -1.88 | 0.81 | 0.02 | 0.15 | | |

Model Pseudo $R^2$; Significance, 0.28; 0.001*; Model accuracy, 77%; B: Regression coefficient; SE: Standard error; $OR_a$: Adjusted odds ratio; *CI*: Confidence interval; *Significant relation ($P$<0.05). #Stepwise logistic model reduced the included predictors to the most important which had direct relation with the outcome after adjusting all other factors unlike enter model which is explanatory not confirmatory.

## 4. Discussion

Brucellosis continues to be an important public health problem, especially in rural communities. It still remains neglected and its clinical or subclinical effects are largely unknown in many regions especially among marginalized sectors of the communities as animal workers. Prevalence cannot be accurately estimated unless a proper understanding and use of suitable sampling techniques are applied[16]. It has been suggested that differences in the results may be due to the time and area of investigation[17]. This study tried to determine the seroprevalence of *Brucella*-specific antibodies in rural and suburban communities in different regions of Aseer, South Saudi Arabia and to identify the potential risk factors in rural areas around Aseer region. We have targeted the high-risk group and two regions were compared. Our results revealed the occurrence of both human and animal brucellosis ranging from 33.9% in humans, 5.1% in sheep and 3.5% in camels. Brucellosis in animals in Saudi Arabia according to the national seroprevalence was estimated as 15.0%[20]. This is contrasting with our present results (4.7%) which is significantly lower than the national level.

A collection of studies in the Middle East concluded that brucellosis surveillances were heterogeneous in the number of samples and laboratory tests used[21]. The high percentage among humans is clearly due to the bias in sample selection giving the fact that the present survey targeted the potential groups at risk. A previous survey conducted in Najran[17], a neighboring region to the current two regions, found the prevalence of brucellosis was 7.3% in humans and 15.0% in diseased animals. Similarly, their study targeted sick humans and animals. In the present study, infection rate among butchers was 37.5% (3/8) compared to 4.0% in a previous study[22]. The overall prevalence of brucellosis in the above-mentioned study was 4.0% among abattoir workers. Infection was more common among butchers (8.9%), veterinarians and veterinary assistants (5.4%), and administrative personnel (1.1%)[22]. As expected, and from the findings of the present study, shepherds were the most affected sectors with an astonishingly high rate 37.2% (84/226), this means that more than three out of 10 shepherds is positive.

The difference in means and therefore the risk getting the infection among shepherds, butchers compared to other professions was considerable. The infection is higher among non-Saudi compared to Saudi national with the former being at a higher risk. Contact with an aborted animal or a retained placenta are some of the serious risks of brucellosi[3]. Our results showed more or less similar results with variation among the different responses which could be attributed to the inaccuracy of the questionnaire or its understanding. Daily consumption of milk has shown no increase in the risk of infection as did the consumption of raw meat products (*OR*: 2.90, 95% *CI*: 1.50-5.50). Although it is known that the *Brucella* organism is shed in milk more[23].

The high infection rate of brucellosis was noticed in the northwestern Aseer region which showed a significantly higher rate than other regions ($P<0.01$). The *OR* was 4.40 (95% *CI*: 2.40-7.90) in the univariate analysis and 9.16 (95% *CI*: 3.39-24.76) when using the multivariate stepwise logistic regression model. In this region and particularly in Wadi Ibn Hashbal in which 40 were positive out of the 55 examined. This was found due to the fact that the majority of the volunteers were shepherds and we have pointed out, as in preceding similar studies, that this sector is the most affected. Also, the teamwork has noticed frequent complaints of people having or suspecting brucellosis. It has been noticed that the area is crowded with domestic animals as well as the fact that a considerable number of them are imported from outside. Additionally, the regular veterinary inspection of animals and meat in this area is lacking. This phenomenon needs to be studied further. None of the previous reports had pointed to a particular area with an exceptionally higher rate of brucellosis. Apart from a previous laboratory analysis which disclosed that a significant proportion (19.2%) of the population in the southern region of Saudi Arabia had serological evidence of brucellosis[13,17].

There is an obvious variation of human brucellosis prevalence between the two regions: Southeastern Aseer region, 25.9%; northwestern Aseer region, 53.3% compared to Jazan region, 20.6%. This obviously suggests the low frequency of brucellosis in Jazan compared to Aseer.

Some factors which could be deficiency factors in this study such as the bias in collection and the fact that samples from animals were mainly from slaughterhouses rather than community animals since owners tend to refuse to have their animals punctured for blood donation. Also, Jazan enjoys its own raised animals unlike areas in Aseer notably Wadi Ibn Hashbal where imported animals are large. Also, the number of camels is few in Jazan compared to Aseer.

In general, high incidence rates are clearly due to their indwelling contact with animals mainly their handling and helping animal parturition, notably the handling of aborted fetuses. This high-risk factor was noticed in this study as in previous ones[24]. Risk is recorded in people helping with animal parturition, but no significant risk associated with other direct (unspecified) animal contact[25]. Direct contact with animals (*OR*: 2.10, 95% *CI*: 0.75-5.80) and people dealing with aborted animals in this study showed no risk of infection (*OR*: 0.98, 95% *CI*: 0.59-1.60). Our results in this factor disagreed with previous findings[25-29].

It is known that hazards resulting from direct or indirect exposure[26] to infected animals or their products or secretions are the most important cause of brucellosis morbidity and mortality especially among shepherds, animal owners, and abattoir personnel. The estimated herd-level prevalence of brucellosis in some parts of

the world was as high as 17.0%[5] and 11.4% in some regions[12], but generally lower than rates at 3.61%[10] and 5.6%[11]. Animal factor remains the main risk for human infection. Human cases are seen in people occupationally or domestically exposed to livestock or practicing risky social-cultural activities such as consumption of raw meat, dairy products and slaughtering of animals within the homesteads[28].

The acute infections detected in the current results were 12.4% with titer ⩾1:320, which seems relatively high ratio when compared with previous findings which recorded a 1.1% prevalence in Najran, a neighboring region to Aseer in south Saudi Arabia[30]. Both acute and chronic brucellosis in Najran region were noticed as shown from IgM (3.3%) and IgG (7.3%) results[17].

In this study, the antibodies against *Brucella* spp. in humans was found relatively high (33.9%). National registry from 2004 to 2012 found that there were greater numbers of cases in the 15 to 44 year age group than in any other age group. This is accorded with the present findings which showed that 37.8% of the cases were >30 year old. The 2004 to 2012 survey noticed a significant decrease from 22.9% in 2004 to 12.5% in 2012. Males had a significantly greater incidence rate than females. Most cases were reported during the spring and summer seasons. In our study, non-Saudi were slightly more affected (35.2%) than Saudis (30.5%). This could be explained by the fact that non-Saudis, and as the labor market indicates, are more in contact with animals than Saudis.

In conclusion, the hypothesis that brucellosis is endemic in the investigated rural and urban areas of Aseer region in south Saudi Arabia was substantiated in the present study with significant variability among the three sub-regions examined. Seropositivity for *Brucella* spp. was 33.9% of the considered population. Although the collection strategy targeted the envisaged risk groups, the rate is relatively high (three out of 10 in these sectors were positive for brucellosis). The probability of infection is more likely to occur, and the risk, in the northwestern Aseer areas, older (>30 years), those having animals (sheep) with frequent contact or consuming their products represented the highest predictors.

The study recommends the introduction of routine isolation of *Brucella* spp. and direct PCR detection in clinical samples to increase the detection rate and those needing treatment for acute disease and to enforce control measures. As recommended earlier, vaccinating susceptible animals against brucellosis would greatly improve prevention.

## Conflict of interest statement

The authors declare that they have no competing interests.

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## Authors' contributions

M.E.H. and A.M. Al-Hakami conceived and designed experiments in the study; A.J.A., S.K.K., T.Y.G., A.F.B. and A.M. Almobty carried out community surveillance and collected data; R.A.M. and I.A. performed the serological tests; A.M. Alkahtani and I.A. participated in community surveillance and collected data; S.F.S. conducted the statistical analysis and interpretation; A.M. Al-Hakami led the project and participated in its coordination. M.E.H. and R.A.M. drafted and critically revised the manuscript; all authors read and approved the manuscript.

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