



## Full Length Article

## Sero-diagnosis of brucellosis in sheep and humans in Assiut and El-Minya governorates, Egypt

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## ABSTRACT

Egypt is an endemic area with brucellosis, so wherever herd problem associated abortion is present, brucellosis should be suspected, and its sero-diagnosis is needed. The present study aimed to estimate the seroprevalence of brucellosis in sheep and their contact humans in Assiut and El-Minya Governorates where a history of abortion in sheep was the chief complaint of the farmers; besides; the appearance of signs of undulant fever among some contact humans. Also, to identify the risk factors for brucellosis seropositivity at human and animal level. Serum samples were collected from 189 sheep and 53 in contact humans in Assiut and El-Minya Governorates, Egypt, during 2017. Antibodies against brucellosis were measured in the serum samples using Rose Bengal Plate test (RBPT) and were further confirmed using Serum Tube Agglutination Test (STAT). The overall seroprevalence of brucellosis using RBPT was 15.87% in sheep and that in humans was 9.44% (5 of 53), respectively. STAT confirmed that 40% of the RBPT-positive reactor sheep were infected by *Brucella abortus*, 16.67% were containing *Brucella melitensis* and 20% were experiencing a mixed infection of both *Br. abortus* and *Br. melitensis*. Additionally, 80% of the RBPT-positive reactor humans were infected with *Br. abortus* only and 20% were having both species of *Brucella*. Among different analyzed variables in this study, the age and farming system of the examined sheep were statistically significant. There was no significant effect in relation to gender, abortion history and pregnancy status of the examined animals, however, higher exposure rates were found among males, abortive animals, and recently calved animals. Gender of the tested humans and presence of seropositive sheep in contact differed significantly in acquiring the infection. Nevertheless, other factors, including age, education, and profession were statistically insignificant on getting human brucellosis. These results indicate the high seroprevalence of brucellosis in humans and sheep in the study areas and generally in Egypt, and therefore, control programs should be implemented.

## 1. Introduction

Brucellosis is a common neglected zoonosis with a global geographical distribution, and as such jeopardizes human health and animal production [1]. The etiological agents causing brucellosis belong to genus *Brucella*, and the classical disease in humans is mainly caused by *Br. abortus* (from cattle), *Br. melitensis* (from goats and sheep), and *Br. suis* (from pigs) [2]. Human can acquire brucellosis via contact with infected animal secretions and carcasses or consumption of their products, mostly unpasteurized milk and milk products [3].

In sheep, brucellosis causes late abortion, stillbirth, reduced fertility and decreased milk production resulting in significant economic losses

while in humans, it has a variety of clinical manifestations such as undulant fever, malaise, insomnia, arthralgia, sexual impotence, nervousness and depression [4]. Human brucellosis is also known for multiple organ involvement causing encephalitis, meningitis, endocarditis, arthritis, orchitis, and prostatitis [4]. Additionally, brucellosis can induce spontaneous abortions in pregnant women [5].

In Egypt, brucellosis is still endemic and the true incidence of human brucellosis is underestimated [6]. In a hospital based survey conducted over two-years in two large hospitals in Cairo, 5% of the admitted patients were positive for brucellosis [7]. Between 1999 and 2003, out of 10,130 Acute febrile illness (AFI) patients from 13 infectious disease hospitals in different regions of Egypt, 3% and 11%

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were positive for brucellosis by culture and serology, respectively [8]. In 2003, seroprevalence within a village in Gharbia Governorate was 1.7% [9]. In another study, a population-based survey of AFI patients in Fayoum Governorate reported 7% seropositivity for human brucellosis [10]. Additionally, the seroprevalence of brucellosis in humans in contact with animals was 5% to 8% [11]. A prospective study based on Ain Shams hospitals found that brucellosis was the most common infectious cause of fever of unknown origin among Egyptian adults [12]. Recently, seroprevalence of brucellosis in human participants was reported to be 21% at Sharkia Governorate [13].

Despite, Sheep and other ruminants infected with *Brucella spp.* are considered the primary source of human infection in Egypt, limited data are available on the prevalence of brucellosis in sheep in different localities of Egypt [14]. It was estimated that the proportion of seropositive sheep decreased during the period between 1999 and 2011, except in 2001 and 2009 in which a significant increase was observed [6]. In addition, the seroprevalence of brucellosis in sheep in the Kafrelsheikh district of Egypt was estimated at 15% [15], 12.2% [16] and 20% [17]. Furthermore, the antibodies against *Brucella spp.* were detected in 4% of slaughtered sheep at Elmuneeb abattoir in central Egypt [18]. In the period between 2001 and 2003, the prevalence of brucellosis was reported to be 7.8% in sheep in El-Minya Governorate [19]. Indeed, the epidemiological situation of brucellosis based on reliable data needs more clarification. Thus, effective control programs are urgently needed and brucellosis surveillance is highly recommended. In response to the appearance of undulant fever in some shepherds and a veterinarian managing a private sheep farm in Assiut Governorate where late abortion among pregnant ewes were noticed coupled with the presence of abortion in some individual farms in the nearby governorate, El Minya, we have been motivated to investigate some sheep private farms and people in contact in such Governorates for the presence of brucellosis. Also, to identify the risk factors for brucellosis seropositivity at human and animal level.

## 2. Materials and methods

### 2.1. Ethical approval

All the participants were informed about the objectives of the study, methods, voluntary participation and the individual information will be concealed. Written consent was not possible because neither literate nor illiterate participants agree to sign a written consent based on the Egyptian cultural settings especially in field studies. Therefore, oral consent was obtained from all participants and their rights were clearly explained to each one of them. Also, animal samples and data were obtained after agreement of the owners and the animals were handled following the Assiut University regulatory rules for animal research. The present study, oral consent of participants; besides; both animal and human work were approved by the Assiut University Ethics Committee.

### 2.2. Study area, study period and sample size

The study was carried out during 2017, in two governorates (Assiut and El-Minya) where a simultaneous abortion was observed in private sheep farms and signs of fever appeared in the contact humans. Thus, a total of 189 sheep and 53 humans in contact were included in the study. Sheep samples were collected from one private farm at Assiut Governorate (number = 40), and 3 private farms at El Minya Governorate (first farm = 51, second farm = 46 and third farm = 52).

### 2.3. Sampling

For the human participants, once oral consent was obtained, their information were collected including gender, age, education, profession, and keeping seropositive sheep. All information were anonymized.

Sheep information were also recorded including gender, pregnancy, farming system, history of abortion and age.

Human or sheep whole blood samples were collected from the cephalic or the jugular vein, respectively, in 5 mL plain Vacutainer tubes and transported directly to the laboratory where the samples were centrifuged, for 15 min at a speed of 1500g [20], and sera were then separated and preserved at  $-20^{\circ}\text{C}$  until tested.

### 2.4. Serology

Serum samples were initially screened using Rose Bengal Plate test (RBPT) and RBPT positive samples were further confirmed using Serum Tube Agglutination Test (STAT) [21]. Using STAT, significant titers were those determined to be  $\geq 1/160$  in humans and  $\geq 1/80$  in sheep [22]. Seropositivity was considered only when sera were reacted with both tests, while those gave negative results to either RBPT or STAT were considered seronegative. Rose Bengal brucella antigen (ID.vet innovative diagnostics, Grabels, France) was used for RBPT, on the other hand, *Br. abortus* and *Br. melitensis* antigens (Cromatest, Linear Chemicals, Spain) were used for the STAT. All techniques were following the manufactures' instructions.

### 2.5. Statistical analyses

To measure the impact of each factor individually on the occurrence of the disease in animals (e.g. Age, abortion, pregnancy and farming system) and humans (e.g. Gender, age, education, profession and keeping seropositive sheep), Fisher's exact test and odds ratios (OR) with 95% confidence interval (95% CI) in the GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) were used. Values of  $P < 0.05$  were considered significant.

## 3. Results

Upon initial screening of sera by RBPT, 15.87% (30 of 189) and 9.43% (5 of 53) of sheep and humans were positive for brucellosis, respectively. STAT further confirmed that 40% (12 of 30) of the RBPT-positive reactor sheep were infected by *Br. abortus* only with agglutination titers ranged between 1:80 and 1:1280, 16.67% (5 of 30) were containing *Br. melitensis* only with agglutination titers ranged between 1:80 and 1:640 and 20% (6 of 30) were having mixed infection of both *Br. abortus* and *Br. melitensis*. Additionally, 80% (4 of 5) of the RBPT-positive reactor humans were infected by *Br. abortus* only with agglutination titers ranged between 1:160 and 1:1280 and the other patient was having both species of *Brucella* with agglutination titers of 1:640 and 1:320 for *Br. abortus* and *Br. melitensis*, respectively. Statistically, no significant difference was found between brucellosis infection rate in sheep compared to that in humans (Table 1).

Among different analyzed variables in this study, the age and farming system of the examined sheep were statistically significant. Although there was no significant effect in relation to gender, abortion history and pregnancy status of the examined animals, employing OR revealed higher exposure rates among males (OR = 7.50, 95% CI, 0.45–124.3), abortive animals (OR = 3.56, 95% CI, 0.85–14.95) and recently calved animals (OR = 2.86, 95% CI, 0.84–9.72) (Table 2).

In this study, the impact of some sociodemographic factors on brucellosis seroprevalence in humans was evaluated. Gender of the tested humans and presence of seropositive sheep in contact differed significantly in acquiring the infection. Nevertheless, other factors, including age, education, and profession were statistically insignificant on getting brucellosis (Table 3).

## 4. Discussion

Brucellosis is still an uncontrolled endemic public health problem in Egypt [11]. The control measures were unsuccessful due to the

**Table 1**  
Brucellosis infection rate in sheep and humans.

Species	Rose Bengal Test (Screening)			Standard Tube Agglutination Test (Confirmation)				
	#tested	<i>Brucella</i> spp. No. (%)	Odds ratio (95% CI)	#tested	<i>Br. abortus</i> only No. (%)	<i>Br. melitensis</i> only No. (%)	Mixed infection No. (%)	Negative No. (%)
Sheep	189	30 (15.87)	1.81 (0.66–4.93)	30	12 (40)	5 (16.67)	6 (20)	7 (23.33)
Human	53	5 (9.43)	0.55 (0.20–1.50)	5	4 (80)	0 (0.00)	1 (20)	0 (0.00)
Total	242	35 (14.46)		35	16 (45.71)	5 (14.28)	7 (20)	7 (20)

economic implications [15,16]. The annual incidence of human infection in Egypt increased from 0.5/100.000 in 1994 to 70/100.000 population in 2003 [10,14]. Sheep and other ruminants are the primary source of brucellosis. Therefore, our study was designed to investigate some sheep private farms and people in contact in Assiut and El Minya Governorates, Egypt for the presence of brucellosis after a history of abortion in late months of pregnancy, stillbirth in sheep and signs of fever in the contact individuals. Also, to identify the risk factors for brucellosis seropositivity at human and animal level.

Although RBPT is a highly sensitive screening test for diagnosis of animal and human brucellosis, it should be followed by a quantitative test for further confirmation [23]. Accordingly, we employed RBPT for brucellosis screening and then confirmed the results by STAT; this combination was used to minimize measurement errors of false positives [24,25].

The seroprevalence of brucellosis was 12.17% in the examined sheep. This result is typically similar to a 12.2% reported previously by Hegazy et al. [16], lower than that recorded by Hegazy et al. [15] and is much higher than those cited by Sedeek [26] and Abdel-Hafeez et al. [27]. The high seroprevalence reported in the present study could be explained by the inclusion of high hazard sheep groups in the study. This may be as a result of distribution of the disease from infected animals to healthy ones, particularly during free grazing and movement of sheep [28].

Our findings documented the presence of antibodies against both *Br. abortus* and *Br. melitensis* in the examined sheep and humans, demonstrating that one host can be infected with two different species of *Brucella* at the same time. Close contact of different animal species, the existence of mixed animal shelters and uncontrolled animal movements

**Table 3**  
Influence of sociodemographic factors on brucellosis seroprevalence in human.

Factor	No. tested	Positive no. (%)	Negative no. (%)	Odds ratio (95% CI)
Gender				
Male	29	5 (17.24)	24 (82.76)	11 (0.58–210.1) 0.091 (0.005–1.736) P = 0.0414
Female	24	0 (0.00)	24 (100)	
Total	53	5 (9.43)	48 (90.57)	
Age (Year)				
12–34 Y	24	1 (4.16)	23 (95.83)	0.27 (0. 0.028–2.61) 3.7 (0.38–35.40)
35–63 Y	29	4 (13.79)	25 (86.21)	
Total	53	5 (9.43)	48 (90.57)	
Education				
Yes	24	1 (4.16)	23 (95.83)	0.27 (0. 0.028–2.61) 3.7 (0.38–35.40)
No	29	4 (13.79)	25 (86.21)	
Total	53	5 (9.43)	48 (90.57)	
Profession				
Housewives	23	0.00(0.00)	23 (100)	Reference value 9.97 (0.48–206.4) 12.37 (0.54–282.7)
Farmers	19	3 (15.79)	16 (84.21)	
Others	11	2 (18.18)	9 (81.82)	
Total	53	5 (9.43)	48 (90.57)	
Keeping seropositive sheep				
Yes	14	4 (28.57)	10 (71.43)	15.20 (1.52–151.6) 0.066 (0.007–0.66) P = 0.0143
No	39	1 (2.56)	38 (97.44)	
Total	53	5 (9.43)	48 (90.57)	

**Table 2**  
Effect of different risk factors on Brucellosis seroprevalence in sheep.

Factor	No. tested	Positive no. (%)	Negative no. (%)	Odds ratio (95% CI)
Gender				
Male	2	1 (50)	1 (50)	7.50 (0.45–124.3) 0.13 (0.008–2.21)
Female	187	22 (11.76)	165 (88.24)	
Total	189	23 (12.17)	166 (87.83)	
Age (Year)				
6–≤ 24 months	51	5 (9.80)	46 (90.20)	Reference value 11.59 (2.53–52.72) 0.26 (0.09–1.39) P = 0.0004
> 24–≤ 48 months	65	16 (24.62)	49 (75.38)	
> 48 months	73	2 (2.74)	71 (97.26)	
Total	189	23 (12.17)	166 (87.83)	
Pregnancy				
Pregnant	95	8 (8.42)	87 (91.58)	Reference value 2.86 (0.84–9.72) 1.66 (0.61–4.55)
Recently calved	24	5 (20.83)	19 (79.17)	
None pregnant	68	9 (13.24)	59 (86.76)	
Total	187	22 (11.76)	165 (88.24)	
Abortion history				
Yes	10	3(30)	7 (70)	3.56 (0.85–14.95) 0.28 (0.067–1.18)
No	177	19 (10.73)	158 (98.27)	
Total	187	22 (11.76)	165 (88.24)	
Farming system				
Separate (only sheep)	155	5 (3.23)	150 (96.77)	0.03 (0.01–0.09) 33.75 (11.04–103.1) P < 0.0001
Mixed with other livestock	34	18 (52.94)	16 (47.06)	
Total	189	23 (12.17)	166 (87.83)	

in Egypt increase the risk of a pathogen to cross the species barrier [29,30]. So, infection of sheep with *Br. abortus* can be acquired via natural exposure to infected materials from another species or indirectly through contact with soil contaminated with abortion and birth fluids [31]. *B. abortus* infections have been also reported in sheep in the USA [32], in Nigeria [33,34] and in Iran [35].

Although a greater exposure rate was found among the male than female sheep in this study, the comparison remained invalid due to high variation of numbers among examined animals from both genders.

Regarding the age, the present work detected antibodies to *Brucella* in the three studied age groups of sheep which poses a serious threat to the welfare of human beings residing in the study areas. The age group > 24–48 months had a higher exposure rate (OR = 11.59, 95% CI, 2.53–52.72) compared to the age group 6–≤ 24 months. Our result was in accordance with that reported elsewhere [36]. This may be attributed to the fact sex hormones and erythritol that stimulate multiplication of *Brucella* organisms tend to increase in concentration with age and sexual maturity of the animal [37]. Surprisingly, the age group above 48 months old had a lower exposure rate (OR = 0.26, 95% CI, 0.09–1.39) when compared to both younger groups. Convincing explanation in this case remains difficult; nevertheless, it could be related to the uncontrolled replacements in Egyptian sheep flocks.

As seen in our report, pregnancy status did not differ significantly between seronegative and seropositive animals. Additionally, high exposure rate of brucellosis was found among the examined recently calved (OR = 2.86, 95% CI, 0.84–9.72) compared to pregnant ewes. Generally, shepherds in Egypt usually introduce new animals to their herds without testing or even caring to the previous history of the purchased animals. Furthermore, the seropositive recently calved animals diagnosed in this study may have suffered previously from abortion since infected sheep can only abort once and carry the organism lifelong [38]. Sheep with history of abortions were at greater risk (OR = 3.56, 95% CI, 0.85–14.95) to brucellosis compared to those without, which confirms that abortions in the examined farms were likely due to infection with brucellosis.

The mixed farming system had a significant impact on getting brucellosis infection in sheep with a higher exposure rate (OR = 33.75, 95% CI, 11.04–103.1) among sheep reared in mixed herds with other livestock compared to those kept in separate flocks (OR = 0.03, 95% CI, 0.01–0.09). Such significant impact could also explain the detection of antibodies against both species of *Brucella* in a single host as discussed earlier. Moreover, in endemic countries like Egypt, sheep are usually raised in close contact with cattle in the same pasture, so *Brucella* spillover from maintenance reservoirs to incidental hosts may occur, which increases the incidence of brucellosis [31].

On the other hand, seroprevalence of brucellosis in humans was 9.43% which is close to the result reported from Egypt by Samaha et al. [11] and Refai [14] and accounted for 8% and 11%, respectively. Meanwhile, our result came lower than that of Fouad et al. [39] 26% but higher than that of El Sherbini et al. [9] 1.7%. The low sample size of human individuals in this study was due to the rejection of many individuals to provide their samples, which was a major limitation in this research.

Our results showed a significant effect of the human gender on brucellosis seroprevalence. Men were at increased risk (OR = 11, 95% CI, 0.58–210.1) to acquire *Brucella* infection than women (OR = 0.091, 95% CI, 0.005–1.736). As documented by several reports, brucellosis can attack both men and women, but in contrary to that fact, none of the examined women reacted positively to any of the used tests because males included in this study were in more contact with animals [25,40,41]. Workers, butchers, and veterinarians are usually males and often help in flock management, so acquiring the infection by direct contact with animals was most likely seen in men than women. Previous investigations conducted in Egypt found that male individuals represented 64% and 65% of brucellosis cases [42] and [8], respectively. Also, Jennings et al. [10] found that males formed 70% of cases

of brucellosis. Despite the nonsignificant influence of age on contracting human brucellosis in the present study, individuals fall in the age group 35–63 years old were found to have increased odds of exposure (OR = 3.7, 95% CI, 0.38–35.40) compared to those within the younger group. This may be attributed to the heavily participation of individuals in such age group in the breeding and reproduction practices in sheep farms compared to the younger participants.

Although occupation of the human participants did not differ significantly between seropositive and seronegative humans, the OR showed a high variability. Compared to housewives, veterinarians and other individuals assisting animals, and farmers showed higher odds of exposure accounted for (OR = 12.37, 95% CI, 0.54–282.7) and (OR = 9.97, 95% CI, 0.48–206.4), respectively, thus the latter two occupational groups were at greater risk of acquiring brucellosis. This may be due to the handling of fetal membranes and aborted fetuses during handling abortion and parturition of infected animals [43] and the high concentration of *Brucella* organisms in the placenta of infected animals [44] as well as the high contamination of the pasture areas with miscarriages, fecal wastes, and animal secretions [13].

The association between keeping seropositive sheep and human brucellosis was a significant risk factor on getting brucellosis and people kept seropositive sheep during the sampling period were 10 times more seroreactive to brucellosis (OR = 15.20, 95% CI, 1.52–151.6) compared to who were in contact with seronegative ones. Similar findings were found previously [9,25]. In Egypt, due to > 70% of the total sheep population are owned by small farmers, they usually keep their sheep either close to or in their house, resulting in a higher exposure rate of humans to infected animals [9,10,45]. Exposure to aborted animals and assisting animal parturition [46,47] and/or sharing of water source with animals are important risk factors associated with occurrence of human brucellosis [48].

The relationship between the level of education and infection with *Brucella* was investigated and our results showed that non-educated individuals were at a higher risk (OR = 3.7, 95% CI, 0.38–35.40) to acquire *Brucella* infection than educated ones that underscores the urgent need for initiating a prompt health education program for such risky group of humans. Similar findings were reported by Al-Shamahy et al. [49], Kozukeev et al. [47] and Assenga et al. [44], meanwhile, opposed findings were reported by Cetinkaya et al. [50] who found that seropositivity was not related to educational level. Poor knowledge about the mode of transmission of brucellosis among individuals from rural areas appeared to be a risk factor that facilitates infection in human [47,51].

## 5. Conclusions

The present study confirmed the presence of *Brucella* specific antibodies in private sheep flocks and the human contacts which presents a serious public health and economic concern. So, public awareness of sheep farmers in rural areas about brucellosis and its method of transmission is a must. Cooperation between veterinary services and public health authorities in Egypt is necessary to control the spread of the disease. Measures should be taken to control sheep brucellosis especially in the mobile flocks, as it's the major source of *Brucella* in Egypt. Periodic examination of sheep flocks or newly purchased animals and vaccination of animals are recommended.

## Competing interests

The authors declare that they have no conflict of interest.

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