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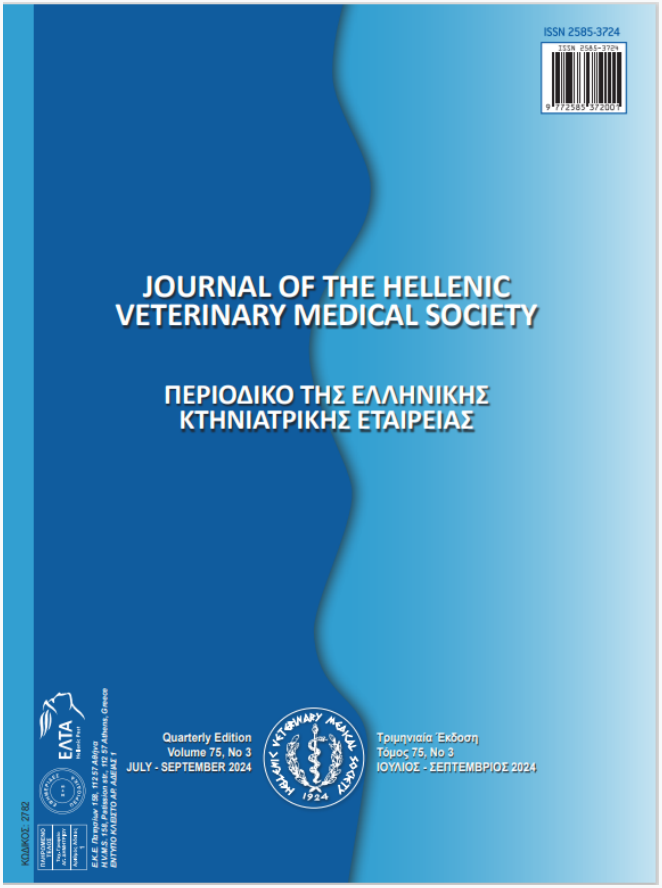


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# Seroprevalence of brucellosis among cows and sheep in Assiut province: A pilot study

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**ABSTRACT:** Brucellosis is a common neglected zoonotic disease with a global geographic distribution that endangers human health and animal production. However, there is a scarcity of studies focusing on brucellosis in neglected regions such as Assiut governorate in Egypt. Therefore, the objective of this study was to investigate the prevalence of antibodies reactive to *Brucella* sp. in sheep and cows, as well as the associated risk factors, in Assiut governorate. A total of 184 sheep and 166 cows were included in the study, and their serum samples were subjected to screening using the Rose-Bengal plate test, followed by confirmation through the serum tube agglutination test. The overall seroprevalence of *Brucella* antibodies in sheep was 8.2% (15/184), while in cows, the seroprevalence was 1.2% (2/166). Among sheep, four cases (2.2%/184) tested positive for *Brucella abortus*, seven cases (3.8%/184) were seropositive for *Brucella melitensis*, and four cases (2.2%/184) had a mixed infection with both *Brucella abortus* and *Brucella melitensis*. In cows, one cow displayed seropositivity for *Brucella abortus*, while the other exhibited mixed infection with both *Brucella abortus* and *Brucella melitensis*. Moreover, animals originating from villages bordering Sohag governorate demonstrated a higher risk of contracting brucellosis while other risk factors did not impact the occurrence of brucellosis. In conclusion, this study provides compelling evidence of the endemic nature of brucellosis in the investigated areas, with a particularly high prevalence observed in sheep. Both cows and sheep are susceptible to infection by either *Brucella abortus* or *Brucella melitensis*. To gain a comprehensive understanding of the epidemiological factors associated with infection, it is imperative to conduct large-scale surveys that incorporate molecular isolation of various *Brucella* species to facilitate the development of appropriate prevention strategies.

***Keywords*:** Brucellosis;Risk factors; Cows;Sheep; Seroprevalence

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## INTRODUCTION

Brucellosis is a common neglected zoonotic disease with a global geographic distribution that endangers human health and animal production (Seleem et al. 2010). The etiological agents of brucellosis are members of the genus *Brucella*, with *Brucella melitensis* (from goats and sheep), *Brucella abortus* (from cattle), and *Brucella suis* (from pigs), being the most common (Corbel 2006). In cattle and sheep, brucellosis causes late abortion, stillbirth, lower fertility, and reduced milk yield resulting in significant economic losses, whereas in humans, the disease is presented commonly with undulant fever and spontaneous abortions in pregnant women (Pappas et al. 2006).

The initial record of brucellosis in Egypt was reported in 1939. Subsequently, the disease has been identified in most of Egyptian provinces, indicating its endemic nature (Refai 2002). This situation is further exacerbated by the common practice of mixed breeding of ruminant animals, uncontrolled animal movement between different governorates, and the lack of effective control programs (Wareth et al. 2014).

In the Delta region (Kafrelsheikh governorate), the prevalence rates of brucellosis in cattle milk tanks and sheep were remarkably high as 15% (Hegazy et al. 2009), 12.2% (Hegazy et al. 2011), and 20% (Hegazy et al. 2016). On the other hand, in the upper Egypt, a high prevalence of brucellosis among cows (7.77%) and sheep (7.91%) was reported in Beni-Suef, based on a multicenter study (Samaha et al. 2008). Sheep and goats had a higher prevalence than other ruminants and *Brucella melitensis*is the most dominant isolate in Egypt (Wareth et al. 2014). In Assiut governorate, a few surveillance studies have been performed, showing a prevalence rate of brucellosis in cows as 1.34% (Koriem et al. 2013) and that in sheep as 11.6% (Oraby et al. 2007), and1.6% (Abdel-Razek et al. 2006).

In the past decade, a single report of brucellosis was published in response to the occurrence of abortion in sheep and cases of fever in humans in Assiut (Abdelbaset et al., 2018). However, the epidemiological situation of brucellosis in cows and sheep remains unclear and necessitates additional investigation. Consequently, the present study sought to assess the seroprevalence of antibodies reactive to *Brucella* sp. in sheep and cows, as well as investigate the associated risk factors in Assiut governorate.

## MATERIALS AND METHODS

### Animals and study area

This study was conducted in the villages of Assiut province, spanning from September 2020 to October 2021. Assiut is located approximately 389 kilometers south of Cairo, the capital of Egypt. Sheep were reared on a small scale, either individually or as part of a herd, with a range of 2 to 100 animals. A total of 184 sheep and 166 cows were included in the study. Sheep samples were randomly selected, consisting of 172 females and 12 males and were obtained from three sheep farms. The average age of the chosen animals was 3.28 ± 1.19 years. The female sheep were categorized into three groups based on their pregnancy status: pregnant, non-pregnant, and aborted. Cows' samples were collected from cases admitted to the infectious diseases' clinic of the veterinary teaching hospital at the Faculty of Veterinary Medicine, Assiut University, with an average age of 4.95 ± 2.02 years. The female cows were classified as pregnant or non-pregnant based on their pregnancy status, and as having a history of abortion or not based on their abortion record.

### Sampling

Whole blood samples from sheep and cows were collected in plain vacutainer tubes (5 mL) via the Jugular venipuncture. These samples underwent centrifugation for 15 minutes at a speed of 1500g. Following centrifugation, the sera were carefully separated and stored at -20 °C until they were ready to be tested.

### Serology

Serum samples were initially subjected to screening using the Rose-Bengal plate test (RBPT). Samples that tested positive on RBPT were further confirmed using the serum tube Agglutination Test (STAT) (Abdelbaset et al. 2018). In the STAT, significant titers were defined as ≥1/40 in cows and ≥1/80 in sheep. Seropositive results were only considered when sera reacted positively in both RBPT and STAT tests. Samples that yielded negative results in either RBPT or STAT were classified as seronegative. The RBPT utilized the Rose Bengal *Brucella* antigen (ID. vet innovative diagnostics, Grabels, France), while the STAT employed *Brucella abortus* and *Brucella melitensis* antigens (Cromatest, Linear Chemicals, Spain). All techniques were performed following the instructions provided by the respective manufacturers.

**Statistical analyses**

To assess the individual impact of various factors on the seroprevalence of brucellosis in the examined sheep (including age, sex, pregnancy status, abortion status, and farm) and cows (including age, pregnancy status, and abortion status), relative risk and chi-square tests were performed using IPM SPSS Statistics software (IBM Corp, USA, Version 26). Additionally, odds ratios and corresponding 95% confidence intervals were estimated. Statistical significance was determined based on a probability value (*P*-value) of $P<0.05$.

## RESULTS

By using the Rose-Bengal test, the overall seroprevalence of *Brucella* antibodies in sheep was 8.2% (15 out of 184). Among sheep, four cases (2.2%/184) tested positive for *Brucella abortus*, seven cases (3.8%/184) were seropositive for *Brucella melitensis*, and four cases (2.2%/184) had a mixed infection with both *Brucella abortus* and *Brucella melitensis*. In cows, one cow displayed seropositivity for *Brucella abortus*, while the other exhibited mixed infection with both *Brucella abortus* and *Brucella melitensis*. The majority of seropositive animals were older than 2 years, and there was no statistically significant difference in age across the groups. Both genders were equally seropositive. In the tested animals, there was no statistically significant correlation between abortion and *Brucella* infection; none of the positive cases had experienced abortion. In comparison to non-pregnant and abortive ewes (5.8%), pregnant ewes showed a non-significantly higher seroprevalence of *Brucella* antibodies (10.5%). Farm 3 differed statistically significantly from the other farms in the study in that it had a higher seroprevalence of *Brucella* infection (16.1%), which was particularly relevant for *Brucella melitensis* (Table1).

Among the examined cows, two out of 166 (1.2%) tested positive for brucellosis. Only one cow was seropositive for *Brucella abortus* antibodies compared to the other one that were seropositive for both *Brucella abortus* and *Brucella melitensis* antibodies. Age, abortion, or pregnancy had no statistically significant impact on the seroprevalence of *Brucella* infection in the examined cows (Table2).

## DISCUSSION

As an intracellular bacterium impacts humans and animals worldwide, particularly in developing countries, our knowledge regarding the status of *brucellosis* in animals needs to be expanded. Given the scarcity of studies focusing on brucellosis in the last decade in neglected areas in Egypt such as Assiut Governorate, there is a need for more research to evaluate the status of this abortifacient disease.

Given that none of the examined sheep and cows

**Table 1.** Effect of different risk factors on Brucellosis seroprevalence in sheep.

| Factor | No. tested | Rose-Bengal test | | Odds ratio | 95% CI | P-value | STAT *B. abortus* | | Odds ratio | 95% CI | P-value | STAT *B. melitensis* | | Odds ratio | 95% CI | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive n (%) | Negative n (%) | | | | Positive n (%) | Negative n (%) | | | | Positive n (%) | Negative n (%) | | | |
| Age | | | | | | | | | | | | | | | | |
| **1-2 years** | 55 | 3 (5.5) | 52 (94.5) | Reference | | | 2 (3.6) | 53 (96.4) | 2.340 | 0.206 - 26.530 | | 1 (1.8) | 54 (98.2) | Reference | | |
| **> 2-3 years** | 63 | 6 (9.5) | 57 (90.5) | 0.548 | 0.130 - 2.304 | 0.500 | 1 (1.6) | 62 (98.4) | Reference | | 0.453 | 4 (6.3) | 59 (93.7) | 0.273 | 0.030 - 2.520 | 0.125 |
| **>3 years** | 66 | 6 (9.1) | 60 (90.9) | 0.577 | 0.137 - 2.422 | | 5 (7.6) | 61 (92.4) | 0.460 | 0.086 - 2.472 | | 6 (9.1) | 60 (90.9) | 0.185 | 0.022 - 1.588 | |
| **Total** | 184 | 15 (8.2) | 169 (91.8) | - | - | - | 8 (4.3) | 176 (95.7) | - | - | - | 11 (6) | 173 (94) | - | - | - |
| **Sex** | | | | | | | | | | | | | | | | |
| **Female** | 172 | 14 (8.1) | 158 (91.9) | 0.975 | 0.117 - 8.111 | 1.000 | 8 (4.7) | 164 (95.3) | 1.049 | 1.015 - 1.084 | 1.000 | 10 (5.8) | 162 (94.2) | 0.679 | 0.080 - 5.797 | 0.534 |
| **Male** | 12 | 1 (8.3) | 11 (91.7) | 1.026 | 0.123 - 8.537 | | 0 | 12 (100) | 0.953 | 0.923 - 0.985 | | 1 (8.3) | 11 (91.7) | 1.473 | 0.173 - 12.573 | |
| **Total** | 184 | 15 (8.2) | 169 (91.8) | - | - | - | 8 (4.3) | 176 (95.7) | - | - | - | 11 (6) | 173 (94) | - | - | - |
| **Pregnancy** | | | | | | | | | | | | | | | | |
| **Pregnant** | 86 | 9 (10.5) | 77 (89.5) | 1.894 | 0.607 - 5.902 | 0.404 | 4 (4.7) | 82 (95.3) | 1.000 | 0.242 - 4.134 | 1.000 | 5 (5.8) | 81 (94.2) | 1.000 | 0.279 - 3.587 | 1.000 |
| **Non-pregnant** | 86 | 5 (5.8) | 81 (94.2) | 0.528 | 0.169 - 1.646 | | 4 (4.7) | 82 (95.3) | 1.000 | 0.242 - 4.134 | | 5 (5.8) | 81 (94.2) | 1.000 | 0.279 - 3.587 | |
| **Total** | 172 | 14 (8.1) | 158 (91.9) | - | - | - | 8 (4.7) | 164 (95.3) | - | - | - | 10 (5.8) | 162 (94.2) | - | - | - |
| **Abortion** | | | | | | | | | | | | | | | | |
| **Yes** | 16 | 0 | 16 (100) | 0.910 | 0.866 - 0.956 | 0.368 | 0 | 16 (100) | 0.949 | 0.915 - 0.984 | 0.861 | 0 | 16 (100) | 0.936 | 0.898 - 0.975 | 0.601 |
| **No** | 156 | 14 (9) | 142 (91) | 1.099 | 1.046 - 1.154 | | 8 (5.1) | 148 (94.9) | 1.054 | 1.016 - 1.093 | | 10 (6.4) | 146 (93.6) | 1.068 | 1.026 - 1.113 | |
| **Total** | 172 | 14 (8.1) | 158 (91.9) | - | - | - | 8 (4.7) | 164 (95.3) | - | - | - | 10 (5.8) | 162 (94.2) | - | - | - |
| **Farm** | | | | | | | | | | | | | | | | |
| **Farm1** | 41 | 1 (2.4) | 40 (97.6) | Reference | | | 1 (2.4) | 40 (97.6) | Reference | | | 1 (2.4) | 40 (97.6) | 0.500 | 0.030 - 8.203 | |
| **Farm2** | 81 | 4 (4.9) | 77 (95.1) | 0.481 | 0.052 - 4.450 | **0.046** | 2 (2.5) | 79 (97.5) | 0.988 | 0.087 - 11.221 | 0.398 | 1 (1.2) | 80 (98.8) | Reference | | **0.002** |
| **Farm3** | 62 | 10 (16.1) | 52 (83.9) | 0.130 | 0.016 - 1.058 | | 5 (8.1) | 57 (91.9) | 0.285 | 0.032 - 2.533 | | 9 (14.5) | 53 (85.5) | 0.074 | 0.009 - 0.598 | |
| **Total** | 184 | 15 (8.2) | 169 (91.8) | - | - | - | 8 (4.3) | 176 (95.7) | - | - | - | 11 (6) | 173 (94) | - | - | - |

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

| Factor | No. tested | Rose-Bengal test | | | | | STAT *B. abortus* | | | | | STAT *B. melitensis* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive n (%) | Negative n (%) | Odds ratio | 95% CI | P-value | Positive n (%) | Negative n (%) | Odds ratio | 95% CI | P-value | Positive n (%) | Negative n (%) | Odds ratio | 95% CI | P-value |
| **Age** | | | | | | | | | | | | | | | | |
| **1-3 years** | 36 | 0 | 36 (100) | Reference | | | 0 | 36 (100) | Reference | | | 0 | 36 (100) | Reference | | |
| **> 3-5 years** | 76 | 1 (1.3) | 75 (98.7) | 0.987 | 0.962 - 1.013 | 1.000 | 1 (1.3) | 75 (98.7) | 0.987 | 0.962 - 1.013 | 1.000 | 1 (1.3) | 75 (98.7) | 0.987 | 0.962 - 1.013 | 1.000 |
| **>5 years** | 54 | 1 (1.9) | 53 (98.1) | 0.981 | 0.946 - 1.018 | | 1 (1.9) | 53 (98.1) | 0.972 | 0.920 - 1.027 | | 0 | 54 (100) | - | - | |
| **Total** | 166 | 2 (1.2) | 164 (98.8) | - | - | - | 2 (1.2) | 164 (98.8) | - | - | - | 1 (0.6) | 165 (99.4) | - | - | - |
| **Pregnancy** | | | | | | | | | | | | | | | | |
| **Pregnant** | 79 | 2 (2.5) | 77 (97.5) | 1.026 | 0.990 - 1.063 | 0.225 | 2 (2.5) | 77 (97.5) | 1.026 | 0.990 - 1.063 | 0.225 | 1 (1.3) | 78 (98.7) | 1.013 | 0.988 - 1.038 | 0.476 |
| **Non-pregnant** | 87 | 0 | 87 (100) | 0.975 | 0.941 - 1.010 | | 0 | 87 (100) | 0.975 | 0.941 - 1.010 | | 0 | 87 (100) | 0.987 | 0.963 - 1.012 | |
| **Total** | 166 | 2 (1.2) | 164 (98.8) | - | - | - | 2 (1.2) | 164 (98.8) | - | - | - | 1 (0.6) | 165 (99.4) | - | - | - |
| **Abortion** | | | | | | | | | | | | | | | | |
| **Yes** | 19 | 0 | 19 (100) | 0.986 | 0.968 - 1.005 | 1.000 | 0 | 19 (100) | 0.986 | 0.968 - 1.005 | 1.000 | 0 | 19 (100) | 0.993 | 0.980 - 1.007 | 1.000 |
| **No** | 147 | 2 (1.4) | 145 (98.6) | 1.014 | 0.995 - 1.033 | | 2 (1.4) | 145 (98.6) | 1.014 | 0.995 - 1.033 | | 1 (0.7) | 146 (99.3) | 1.007 | 0.993 - 1.020 | |
| **Total** | 166 | 2 (1.2) | 164 (98.8) | - | - | - | 2 (1.2) | 164 (98.8) | - | - | - | 1 (0.6) | 165 (99.4) | - | - | - |

under study had been administered the *Brucella* vaccine, the antibodies that were detected can be attributed to the natural occurrence of brucellosis infection. The sheep seroprevalence of brucellosis in the current study was 8.2% which is consistent with other investigators who recorded prevalence rates of 7.8% (El-Diasty et al. 2021), and 7.91% (Samaha et al. 2008) for sheep in Egypt. In addition, a higher seropositivity rates (12.2%, and 15.87%) were reported in Egypt (Hegazy et al. 2011; Abdelbaset et al. 2018). On the contrary, cows seroprevalence (1.2%) was lower than those reported in Nile Delta, Egypt (12.2%) (Hegazy et al. 2011), Ethiopia (8%) (Megersa et al. 2012), and in Western Algeria (15.7%) (Aggad and Boukraa 2006). These variations in prevalence rates can be attributable to the differences in animal susceptibility to disease, animal population, hygienic measures applied, and diagnostic tests used.

Our study revealed the existence of antibodies against *Brucella abortus* and *Brucella melitensis* in both cows and sheep. This result may be due to the intermixing of livestock and sharing animal shelters and pastures land, as well as uncontrolled animal movements to and from different localities and markets. Consequently, sheep can become infected with *Brucella abortus* through natural exposure to infected materials from cows or indirectly through contact with soil that is contaminated with fluids from abortion and birth processes (Wareth et al. 2014; Abdelbaset et al. 2018).

In relation to gender, our study denoted non-significant, higher seropositivity of brucellosis among female sheep than males. This finding is in the same line with recent studies (Wadood et al. 2009; Haggag et al. 2016; Rahman et al. 2018) which showed higher proportion of seropositivity in females than males. The female reproductive tract serves as a potential reservoir for *Brucella* to proliferate. This, coupled with the physiological stress experienced during gestation and lactation, likely contributes to the higher rates (Wadood et al. 2009; Haggag et al. 2016; Rahman et al. 2018). However, our results may be ascribed to sample bias rather than gender factor where the females were covering a large portion of the sampled animals.

Additionally, the current study showed that older sheep and cows had a non-significant, higher seroprevalence in comparison to younger ones. This result is consistent with a previous report in Assiut and El-Minya governorates (Abdelbaset et al. 2018). Erythritol and sex hormones, which stimulate the multiplication of *Brucella* organisms, tend to exhibit higher concentrations as the animal grows and reaches sexual maturity. Additionally, older animals have a high exposure rate to infection over time (Alhamada et al. 2017; Selim et al. 2019).

In our study, a slightly higher seroprevalence was noted in pregnant animals compared to non-pregnant ones, with both groups having relatively similar odds of exposure, which agrees with a recent report (Abdelbaset et al. 2018). This suggests that all animals were equally susceptible to acquiring the infection, and both pregnant and non-pregnant animals pose a potential zoonotic risk to humans living in the region as well as potential risk of spillover of bacterium.

Abortion serves as the primary means of transmitting infection within animal populations, as *Brucella* spp. are shed and can persist in the environment for

extended periods of time, posing a risk for reinfection of aborted animals as well as other animals in the same household (Samaha et al. 2008; Seleem et al. 2010). However, our study demonstrated that the history of abortion was not a significant factor associated with the occurrence of brucellosis infection among sampled animals, which is in line with a recent report from the same area (Abdelbaset et al. 2018).

Notably, a significant association was identified between the sheep seropositivity rate and the location of farm 3, which is situated on the border of Sohag governorate. Furthermore, one of the cows that tested positive for antibodies originated from the same area. The uncontrolled practice of smuggling and replacing animals across district boundaries could amplify the chances of interaction between local animals and those from neighboring villages, potentially leading to an increased risk of disease transmission among animals (Hegazy et al. 2009).

## CONCLUSIONS

This study provides evidence of the endemic nature of brucellosis in the areas under investigation, with a high prevalence observed in sheep. Both cows and sheep are susceptible to infection with either *B. abortus* or *B. melitensis*. Animals originating from villages bordering Sohag governorate exhibited a higher risk of contracting brucellosis. To gain a comprehensive understanding of the epidemiological factors associated with brucellosis, it is necessary to conduct large-scale surveys that incorporate molecular isolation of various *Brucella* species for the development of appropriate control strategies.

## CONFLICT OF INTEREST

The authors declare that no competing interest does exists.

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