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Updated Seroprevalence and Risk Factors of *Brucella* spp. infection in Sheep Flocks

Maab I. Al-Farwachi[1]*, W.A. Alobaidii[2], Hanin B. Al-Malachi[1]

[1]Department of Internal and Preventive Medicine, University of Mosul, Mosul, Iraq; [2]Department of Microbiology College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Abstract | Brucellosis in farm animals is a zoonotic contagious illness that has significant global financial impact. From January 2024 to January 2025, sera were collected from 400(320 ewes and 80 rams) unvaccinated adult sheep(1.5 to 7 years) from 25 flocks. All sera were tested for the presence of serum antibodies against *Brucella abortus*, *Brucella melitiensis*, and *Brucella suis* All sera were initially screened using the Rose Bengal test (RBT). Additionally, they were tested with a competitive ELISA (C-ELISA; Svanovir®, Sweden) for antibodies against *Brucella abortus*, *B. melitensis*, and *B. suis*, and with an indirect ELISA (I-ELISA; IDEXX *B. ovis* Ab Test kit) for anti-*B. ovis* antibodies. C-ELISA and I-ELISA detected antibodies in 24.3% and 15.5% of samples, respectively, with 8.3% of animals positive in both assays. Overall seroprevalence was 37.8% with a 95% confidence interval of 31.8 to 44.3. Rose Bengal test demonstrated a lower positive rate of 20.8%. Significant risk factors for general *Brucella.* spp. included a history of abortion, the presence of stray dogs, a younger age (1.5-3 years), the 3-5 year age group, and mixed-species management with cattle. For animals that tested seropositive for *Br. ovis*, significant risk factors included mixed-species management with goats, the presence of orchitis, and the animals' ages, which ranged from 1.5 to 5 years. In conclusion, brucellosis is still a prevalent disease in sheep flocks, indicating the continued risk of transmission in mixed-species livestock systems. Variable detection rates were observed, with minimal overlap between the results obtained from the C-ELISA and I-ELISA kits. Ewe abortion history, the presence of stray dogs, co-management with goats or cattle, younger age, and ram orchitis are key risk factors, highlighting areas that require targeted preventive measures.

Keywords | Prevalence, Sheep, Abortion, Survey, Iraq

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***Correspondence** | Maab Ibrahim Al-Farwachi, Department of Internal and Preventive Medicine, University of Mosul, Mosul, Iraq; **Email**: maabalfrwche@yahoo.com
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INTRODUCTION

Ovine brucellosis is primarily caused by two bacterial species: *Brucella melitensis* and *B. ovis*, despite small domestic ruminants being susceptible to other *B. species*. *B. melitensis* is predominantly recognised as a reproductive disorder in ewes, which is characterized by abortion and stillbirth in ewes and occasionally by epididymo-orchitis in rams (Rossetti *et al.*, 2022). In contrast, another type of ovine brucellosis results from infection by *B. ovis*; it predominantly manifests as epididymitis in rams, occasionally resulting in abortions and neonatal mortality (Elderbrook *et al.*, 2019).

Generally, antibody detection tests (serology) remain the most cost-effective method for the screening and detection of infected herds, especially under resource-limited settings (Legesse *et al.*, 2023; Islam *et al.*, 2025).

In many epidemiological investigations, indirect ELISA is the method of choice because of its high specificity and sensitivity (Xu *et al*., 2020; Bulashev and Eskendirova, 2023; Legesse *et al*., 2023). Major variations exist in the occurrence of ovine brucellosis between countries and between parts of the world. According to a meta-analysis and systematic review, the overall seroprevalence of sheep in America, Africa, and Asia was 6.2%; the Americas had the lowest prevalence at 1.9%, while Africa had the highest at 8.5% (Wang *et al*., 2023). Several studies have established the issue of ovine brucellosis in various Iraqi provinces (Al-Farwachi *et al*., 2010; Al-Khafaji and Al-Farwachi, 2012; Al-Tae and Al-Samarrae, 2013; Al-Farwachi *et al*., 2019; Abdulhameed *et al*., 2020).

Although previous studies have assessed prevalence of brucellosis in Mosul city , this study is the first to simultaneously compare multiple serological tests (C-ELISA and I-ELISA) and evaluate the seroprevalence of antibodies against both *B. ovis* and other *Brucella* species in the same sheep flocks. Additionally, it identifies and compares the associated risk factors for each *Brucella* species, providing a comprehensive and novel understanding of ovine brucellosis in the region, despite the implementation of vaccination programs.

MATERIALS AND METHODS

Study area

The study was conducted in Mosul, a major city in northern Iraq situated 400 km north of Baghdad on the Tigris River. The metropolitan area covers approximately 32,308 square kilometers. Sheep are extensively raised in the Mosul region due to its temperate environment and the availability of natural pastures. Also, the city is a transit point for animals imported from countries neighboring Iraq.

This increases the risk of disease introduction into small ruminant population. Informal animal trade poses a significant risk for disease introduction. Additionally, the internal movement of sheep and goats between neighboring regions poses a risk of disease transmission. The study was carried out in several regions of the Mosul city , including Bazwaia, Kokjali, Bab Shamash, Veterinary Teaching Hospital, University of Mosul, between January 2024 to January 2025.

Twenty-five flocks of sheep were used in a cross-sectional study in Mosul, Iraq. The number of farms selected was proportional to the livestock population in the area of study, and the sample size within each farm and for different age groups within each farm was determined proportionally. Approximately 10% of animals from each flock were randomly sampled, with statistical analyses accounting for differences in flock size. To obtain an estimate with 5% precision and 95% confidence, and an estimated illness prevalence of 25 %. The sample size was calculated as:

$$n = Z^2P(1-P)/d^2$$ (Charan and Biswas, 2021)

Z= Value of the normal distribution for a 95% confidence level; P= Expected prevalence; d= Absolute error; N= 289.

To improve the precision of prevalence estimates, accommodate potential sample attrition, and allow for accurate subgroup analysis across variables such as age, sex, and management techniques. A total of 400 serum samples were collected from non-vaccinated adult sheep (1.5-7 years old), comprising 320 ewes and 80 rams. The sample information, such as age, sex, origin and abortion history was documented.

Animal sampling

Five milliliters of venous blood was collected from each animal in a sterile, plain red-top tube and allowed to clot. The samples were transported on ice to the diagnostic laboratory of the College of Veterinary Medicine, University of Mosul. Serum was harvested after centrifugation (at 3000 rpm for 10 minutes) and stored at -20˚C until tested.

Serological tests

All sera were tested by the following serological tests. The Rose Bengal test RBT (Institut Pourquier, Montpellier, France) was performed as described by Alton *et al*. (1988) and competitive ELISA (C-ELISA) kits (Svanovir®, Sweden). *B. suis, B. melitensis*, and *B. abortus* antibodies were detected by C-ELISA based on the smooth lipopolysaccharide (s-LPS) antigen and indirect ELISA (I-ELISA; IDEXX *B. ovis* Ab Test kit; Idexx Montpellier, France). After setting and incubating (Memmert®, Germany), the tests were washed (Biochek, USA). The optical density at 450 nm was was measured using an ELISA microplate reader. The S/P (sample-to-positive) ratio for each specimen was calculated according to the manufacturer's instructions.

Statistical analysis

The data were analyzed by the Chi-square test and odds ratios (OR) with 95% confidence interval (95% CI) in SPSS V25 at a significance level of P≤ 0.05.

RESULTS AND DISCUSSION

In this investigation, 97 sera (24.3%) tested positive for the C-ELISA, which detects antibodies against *Br. suis*, *Br. melitensis*, and *Br. abortus*, and 62 sera (15.5%) tested positive for the I-ELISA, which detects antibodies against *Br. ovis* as shown in Table 1. However, 33/400 sera (8.3%) tested positive for both ELISA kits, as shown in Figure 1.

Overall seroprevalence was 37.8% (151/400) with a 95% confidence interval of 31.8 to 44.3. In contrast, the Rose Bengal test identified antibodies in 83 sera (20.8%), with a 95% CI of 16.5 to 25.7 as shown in Figure 1.

Table 1: Prevalence of *Brucella* spp. antibodies among sheep in Mosul City, Iraq (N= 400).

Tests	Number of positive sera (%)	95% confidence interval (95%CI)	Chi-squared	P value
C-ELISA	97 (24.3)	19.7 to 29.6	9.704	P < 0.001
I-ELISA	62 (15.5)	11.9 to 19.9		

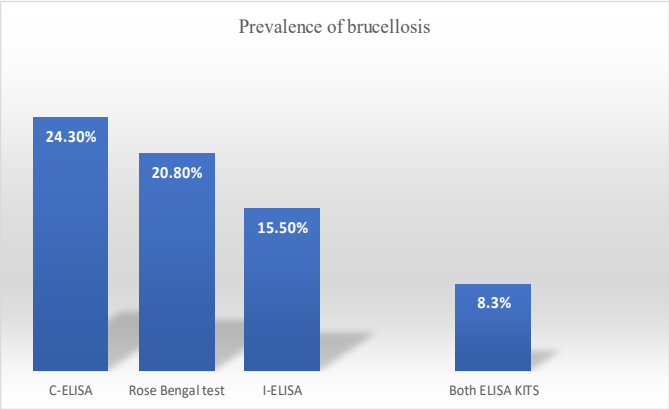

Figure 1: Percentage of *Brucella* spp. seropositive sheep using C-ELISA, I-ELISA and Rose Bengal tests.

Ovine brucellosis is widely recognised as a considerable global challenge for sheep producers, particularly in areas that utilise extensive management practices for livestock rearing. Surveillance and vaccination, together with controlling animal movement, are the main measures to control brucellosis in livestock. The co-circulation of multiple *B.* species noticed in this study reflects Iraq's complex brucellosis epidemiology. Risk factors such as unrestricted animal movement, ineffective immunisation programs, traditional husbandry practices, and a lack of awareness all contribute to the persistence of illness in livestock. Additionally, efforts to control the disease are further restricted by diagnostic difficulties such as serological cross-reactivity and inadequate disease reporting.

Compared to previously reported rates in Iraq, the seroprevalence of brucellosis in sheep in the current study was 37.8%, which is considered high. Lower prevalence has been observed in a number of previous investigations. Alshwany and Robertson (2018) reported a prevalence of 6.9% in Kirkuk province, but Ilyas *et al.* (2024) recorded a prevalence of 10.6% in Baghdad. According to various research, the prevalence in northern Iraq (Kurdistan Region), especially in Duhok, is moderately high, ranging from 31.7% to 35.4% (Al-Hamada *et al.*, 2021; Ameen *et al.*, 2023). Another study found that the Diyala Governorate had a higher prevalence rate of 59.5% (Al-Busultan *et al.*, 2018).

The higher prevalence identified in this study could be attributable to factors such as poor vaccination coverage, inadequate biosecurity measures, and traditional husbandry practices. Iraq currently lacks a program to vaccinate cattle and buffaloes against brucellosis, despite the fact that the Rav1 vaccine is widely used to vaccinate sheep. Furthermore, the prevalence of stray dogs in sheep flocks plays a substantial role in disease transmission (Gharekhani *et al.*, 2019; Mahmmodi *et al.*, 2025).

This is supported by our findings, which identified contact with cattle and stray dogs as significant risk factors. Furthermore, unrestricted animal movement, including the importation of sheep from various countries, contributes to disease spread.

The current study demonstrated a significant prevalence of brucellosis among the animals under study in Iraq using various serological assays. In particular, 62 (15.5%) serum samples tested positive by I-ELISA, which targets *B. ovis*, while 97 (24.3%) and 83(20.8%) samples tested positive by C-ELISA and Rose Bengal test respectively, which detect antibodies against *B. melitensis*, *B. abortus*, and *B. suis*. This difference can be explained by the characteristics of the tests. C-ELISA is generally more sensitive and specific, detecting lower antibody levels and providing better differentiation between vaccinated and naturally infected animals. In contrast, RBT is a rapid screening test that may miss low-titer infections or early-stage seroconversion, leading to slightly lower prevalence estimates (Trangadia *et al.*, 2015).

Higher seroprevalence detected by C-ELISA implies the presence of smooth *Brucella* species (*Br. abortus*, *Br. melitensis*, and *Br. suis*) in the examined population. This is particularly concerning in rural areas where close animal-human contact is common and consumption of raw dairy products is widespread. Previous Iraqi investigations have found human brucellosis to be prevalent, with *Br. melitensis* identified as the principal cause of human infections (Hou *et al.*, 2013). The average yearly incidence of human brucellosis in Iraq was 41.88 cases per 100,000 individuals, with higher rates in Kurdistan (Hou *et al.*, 2013). Research in Duhok discovered a 7% prevalence of brucellosis among children, highlighting the need for focused public health interventions (Alamian and Dadar, 2020).

In the present study, the seroprevalence of anti-*Brucella ovis* antibodies was lower than that seen for other *Brucella* species in this investigation. This finding may be explained by several factors. First, *B. ovis* has a restricted host specificity, with transmission occurring almost exclusively

among rams and through venereal routes, whereas other *Brucella* species can spread more efficiently through horizontal and vertical pathways (Elderbrook *et al*., 2019).

Al-Farwachi *et al*. (2019) documented a *Br. ovis* seroprevalence of 4.8% among rams with epididymo-orchitis in Mosul city, northern Iraq. Similarly, in Karbala province, a study utilizing PCR on epididymal tissues from slaughtered rams found an overall *B. ovis* prevalence of 5%, with a significantly higher detection rate of 18% among animals (Wedajo *et al*., 2015). The higher seroprevalence of *B. ovis* observed in our study (15.5%) compared to the 4.8% reported by Al-Farwachi *et al*. (2019) may be attributed to several factors, including differences in sample size, animal selection criteria, variations in flock management and biosecurity measures.

Generally, younger animals, their mixing with other species, the presence of stray dogs among sheep flocks, abortion, and orchitis showed a statistically significant association with *Br*. spp. seropositivity compared to the other factors included in this study.

A statistical analysis of risk factors for *Brucella* spp. seropositivity in sheep demonstrated that ewes with a history of abortion were six times more likely to be seropositive for *Brucella* spp. than those without such a history (P < 0.001) as shown in Table 2. Ewes with a history of abortion had a prevalence of 43.0% (*P* < 0.001), compared to 11.0% in those without abortion history. This is consistent with the known pathogenesis of *Brucella* spp., which are often implicated in reproductive disorders. The pathogenicity of *Brucella* is closely linked to its ability to localize and multiply in the reproductive organs, especially the uterus and placenta. A key factor contributing to this is the presence of erythritol, a sugar alcohol highly concentrated in the placenta of ruminants, which promotes the growth of *Br.* Species. *Brucella* induce placentitis, necrosis of placental tissue, and restriction of foetal development, ultimately leading to abortion (Narouei *et al*., 2022).

In the present study, rams with orchitis had a significantly higher rate of anti-*Brucella ovis* antibodies (28.9%) compared to those without lesions (7.1%), with an odds ratio of 5.3 (95% CI: 1.3–20.8, *P* = 0.01) as shown in Table 2. This finding indicates that testicular inflammation is an important clinical marker of *Brucella ovis* infection. The organism has a well-documented tropism for the male genital tract, where it causes epididymitis and orchitis, resulting in reduced semen quality and impaired fertility (Hou *et al*., 2013; Elderbrook *et al*., 2019; Rossetti *et al*., 2022). Further supporting the role of reproductive tract infections in disease dynamics. Both orchitis and abortion serve not only as disease indicators but may also promote pathogen transmission within flocks, especially in systems lacking proper reproductive management (Rossetti *et al*., 2022).

The current investigation showed a significant association between the presence of stray dogs and increased seropositivity in sheep flocks. Specifically, flocks exposed to stray dogs were 3.4 times more likely to be seropositive for *Brucella* spp. than those not exposed (P < 0.001), as

Table 2: Animal factors-wise and the seroprevalence of *Brucella* spp. and *B. ovis* antibodies among sheep at Mosul city governorate using ELISA kits.

Variables	Criteria	Number of samples	Brucella spp. Seropostive (%)	Odds ratio (95% CI)	P value	*Brucella ovis* Seropostive(%)	Odds ratio (95% CI)	P value
Age(years)	1.5-3	120	38(31.7)	2.4(1.4 to 4.2)	0.001	26(21.7)	2.5(1.3 to 4.9)	0.004
	3- 5	120	33(27.5)	1.95(1.0 to 3.5)	0.01	20(16.7)	1.8(1.0 to 3.6)	0.05
	> 5	160	26(16.3)	Reference		16(10.0)	Reference	
Sex	Ewes	320	72(22.5)	0.7(0.429 to 1.2)	0. 1	48(15.0)	Reference	
	Rams	80	25(31.3)	Reference		14(17.5)	0.7(0.3 to1.3)	0.11
Manage-ments	Sheep and cattle	120	41(34.2)	2.3 (1.4 to 3.9)	0.001	5(4.1)	Reference	
	Sheep and goats	100	23(23.0)	1.3(0.7 , 2.4)	0.2	18(18.0)	5.0(1.8 to14.1)	0.001
	Sheep only	180	33(18.3)	Reference		39(21.7)	3.4(2.4 to 16.7)	0.00008
Stray dogs	Yes	166	62(37.3)	3.4(2.1 to 5.5)	0.0	30(18.0)	1.5(0.9 to 2.5)	0.07
	No	234	35(15.0)	Reference		32(13.7)	Reference	
Abortion	Yes	130	56(43.0)	6.0(3.4 to 10.8)	0.0	22(16.9)	0.8(0.4,1.4)	0.2
	No	190	21(11.0)	Reference		26(13.7)	Reference	
Orchitis	Yes	38	11(28.9)	0.81 (0.31to 2.1)	0.67	11(28.9)	5.3 (1.3to 20.8)	0.01
	No	42	14(33.3)	Reference		3(7.1)	Reference	

(95% CI= Confidence Interval).

shown in Table 2, indicating a very significant association. Infected dogs can transmit *Br. abortus* and *Br. melitensis* to specific hosts, humans, and other animal species. Furthermore, infected dogs can play a vital role in the persistence of brucellosis in ruminants (Gharekhani *et al*., 2019; Mahmmodi *et al*., 2025).

In farms, the role of stray dogs in the epidemiology of brucellosis can be twofold. They may act as mechanical carriers by contacting aborted materials, placenta, or contaminated environments. Without being infected themselves, they can subsequently spread *Brucella* organisms to healthy flocks. This increases infection rates and compromises biosecurity measures. On the other hand, dogs can also become biologically infected by *Brucella canis* and occasionally *B. melitensis*, indicating their potential role as true reservoirs .Effective control strategies must include systematic screening of dogs associated with herds and implementing vaccination protocols for both sheep and dogs is essential for managing brucellosis outbreaks (Hou *et al*., 2013; Alamian and Dadar, 2020).

The highest seroprevalence for *Brucella* spp. was observed in the 1.5–3 years age group (31.7%), with an odds ratio (OR) of 2.4 (95% CI: 1.4–4.2, P< 0.001). The 3–5 years old group also showed increased risk (27.5%; OR = 1.95, 95% CI: 1.094–3.49, (P < 0.01), compared to animals older than 5 years (16.3%), which were used as the reference group, as showed in Table 2.

Young, sexually mature animals are more susceptible, possibly due to increased exposure or immunological naïveté. while older animals appear more resistant, probably due to previous exposure conferring some immunity**.** Similar findings have been reported in previous epidemiological studies of ovine brucellosis (Wedajo *et al*., 2015; Narouei *et al*., 2022).

Mohammed *et al*. (2017) and Sorsa *et al*. (2022) reported higher seroprevalence in older age groups than in younger animals, even though the difference was not statistically significant. In contrast, Hussen *et al*. (2023) reported a higher seroprevalence in the old age group than in the medium and young age groups.

Among sheep aged 1–3 years, 26 animals (21.7%) tested seropositive for *Brucella ovis* with an odds ratio of 2.5(95% CI: 1.3–4.9; *P* < 0.004), indicating a significantly higher likelihood of seropositivity in this age group as showed in Table 2. Indicates that that *Brucella ovis* seroprevalence correlates with sexual activity. This increased seropositivity in young adults may be attributed to several factors. First, sexually mature animals are more likely to be exposed to infection during mating . Second, animals in this age group may have relatively naïve immune systems, lacking previous exposure that could confer partial immunity. These results align with other studies reporting higher seroprevalence in younger, sexually active sheep compared to older animals, suggesting that age and sexual maturity are key determinants in the epidemiology of ovine brucellosis (Al-Farwachi *et al*., 2019; Elderbrook *et al*., 2019).

Herd composition was another significant factor influencing the epidemiology of ovine brucellosis. Sheep raised in mixed flocks with cattle showed a significantly higher rate of anti-*Brucella* spp. antibodies(34.2%) compared to those in sheep-only flocks as showed in Table 2.

Also, *Br. ovis* seropositivity was found to be significantly influenced by management techniques. Sheep raised with goats had a significantly higher prevalence (18.0%) than those raised with cattle (4.1%) (OR = 5.0, 95% CI: 1.8-14.1, P < 0.001). Similarly, sheep raised in sheep-only systems showed a high seropositivity rate (21.7%) and were at higher risk (OR = 3.4, 95% CI: 2.428-16.66, P < 0.00008) as in Table 2. While young animals aged 1.5–3 years demonstrated the lowest odds at 2.5 as shown in Table 2.

These findings were consistent with previous studies demonstrating that multi-species farming systems facilitate the cross-species transmission of *Brucella* spp., particularly under extensive management or shared grazing conditions where biosecurity is often inadequate (Godfroid *et al*., 2013; Sibhat *et al*., 2024).

Mixed-species rearing is still frequent across Iraq due to traditional herding methods, which increases the possibility of interspecies transmission. Furthermore, shared water sources, the same grazing fields, and inadequate species separation during lambing and kidding seasons all increase the risk of transmission. As a result, effective control strategies in endemic areas should include species-specific interventions while also addressing the complex dynamics brought on by mixed-species livestock management (Martindah *et al*., 2025). In contrast, sheep-only herds presented a lower, yet still notable, odds ratio of 23%, indicating that while mixed herds increase risk, brucellosis remains prevalent even in single-species flocks, likely due to environmental contamination or animal movement.

CONCLUSIONS AND RECOMMENDATIONS

Brucellosis is still a prevalent disease in sheep flocks, highlighting the ongoing risk of transmission in mixed-species livestock systems. Variable detection rates were observed, with minimal overlap between the results obtained from the C-ELISA and I-ELISA kits. Ewe abortion history, the presence of stray dogs, co-management with

goats or cattle, younger age, and ram orchitis are key risk factors, highlighting areas that require targeted preventive measures.

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NOVELTY STATEMENT

This is the first study that provides updated epidemiological data on brucellosis in sheep flocks in Iraq using a mix of serological assays (RBT, C-ELISA, and I-ELISA). The research reveals the minimal overlap between different ELISA kits, demonstrating major disparities in diagnostic performance that have not been previously observed in the region. Furthermore, the identification of particular risk factors, such as ewe abortion history, ram orchitis, co-management with cattle or goats, and the presence of stray dogs, provides new insights into the transmission dynamics of *Brucella spp*. and *B. ovis* in mixed-species livestock systems. This study provides crucial data and provides significant guidance for focused control and preventative programs.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors. All required institutional ethical approvals were obtained before this study was conducted (Approval Date18/10/2024 and Number UM.VET.2024.101).

Generative AI and AI-assisted technology statement

All authors of this work declare that generative AI technologies including large language models (e.g., ChatGPT, Copilot) and text-to-image generators were not utilized in any capacity during the preparation, writing, or editing of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdulhameed, M.F., Sayhood, M.H., Aldeewan, A.B. 2020, Assessment of seroprevalence and the risk factors of sheep brucellosis in Basrah (Southern Iraq): A challenge to prospectively control brucellosis. Journal of Pure and Applied Microbiology, 14(4): 2543-2554. https://doi.org/10.22207/JPAM.14.4.30

Al Hamada, A., Habib, I., Bruce, M. 2021, Seroconversion to *Brucella* spp. and *Toxoplasma gondii* in sheep and Iraq and its association with pregnancy loss. Animals, 11(3): 1-836. https://doi.org/10.3390/ani11030836

Al-Khafaji, W.S., Al-Farwachi, M.I. 2012, Antioxidant status in pregnant ewes vaccinated with Rev 1 against brucellosis. Iraqi Journal of Veterinary Sciences, 26(1): 15-19. https://doi.org/10.33899/ijvs.2012.46890

Alamian, S., Dadar, M. 2020, *Brucella melitensis* infection in dog: A critical issue in the control of brucellosis in ruminant farms. Comparative Immunology, Microbiology and Infectious Diseases, 73: 101554. https://doi.org/10.1016/j.cimid.2020.101554

Al-Busultan, A.S., Al-Bassam, L.S., Al-Shidid,i A.M. 2018, Cross sectional study on the seroprevalence of brucellosis in sheep, goat and man in Diyala Governorate. Mirror of Research in Veterinary Sciences and Animals, 7(1):1–19.

Al-Farwachi, M.I., Al-Badrani, B., Al-Nima, M. 2010, Detection of Brucella antigen in the aborted ovine fetal stomach contents using a modified ELISA test. Iraqi Journal of Veterinary Sciences, 24(1): 1-4. https://doi.org/10.33899/ijvs.2010.5577

Al-Farwachi, M.I., Al-Hankaw, O., Al-Iraqi, O. 2019, First Serodiagnosis of *Brucella ovis* among rams with orchid-epididymitis in Mosul city, Iraq. Egyptian Journal of Veterinary Sciences, 50(1): 13-16. https://doi.org/10.21608/ejvs.2019.6540.1055

Alshwany, E.A., Robertson, I.D. 2018, Epidemiology of Brucellosis in Sheep and Goats in the Iraqi Kurdistan Region. The Online Journal of Science and Technology, 8(4): 33–37.

Al-Tae, A.H., Al-Samarrae, E.A. 2013, Detection of *Brucella* antibodies of sheep in al-anbar province by using some serological tests. The Iraqi Journal of Veterinary Medicine, 37(1), 7-12. https://doi.org/10.30539/iraqijvm.v37i1.316

Alton, G.G., Jones, L.M., Angus, R.D., *et al*. 1988. Techniques for the Brucellosis Laboratory. Paris: INRA.

Ameen, A.M., Abdulaziz, N.S., Ghaffar, N.M. 2023, Molecular detection and associated risk factors of *Brucella melitensis* in aborted sheep and goats in Duhok Province, Iraq. Pathogens (Basel, Switzerland), 12(4): 544. https://doi.org/10.3390/pathogens12040544

Bulashev, A., Eskendirova,, S. 2023, Brucellosis detection and the role of *Brucella* spp. cell wall proteins. Veterinary World, 16(7): 1390–1399. https://doi.org/10.14202/vetworld.2023.1390-1399

Charan, J., Biswas, T. 2021, How to Calculate Sample Size for Different Study Designs in Medical Research? Indian Journal of Psychological Medicine, 35(2):121-126. https://doi.org/10.4103/0253-7176.116232

Elderbrook, M., Schumaker, B., Cornish, T., Peck, D., Sondgeroth, K. 2019, Seroprevalence and risk factors of Brucella ovis in domestic sheep in Wyoming, USA. BMC Veterinary Research, 15(1): 246. https://doi.org/10.1186/s12917-019-1995-5

Gharekhani, J., Sazmand, A. 2019, Detection of *Brucella* antibodies in dogs from rural regions of Hamedan, Iran. Avicenna Journal of Clinical Microbiology and Infection, 6(4): 122-126. https://doi.org/10.34172/ajcmi.2019.22

Godfroid, J., Al Dahouk, S., Pappas, G., *et al*. 2013, One Health: surveillance and control of brucellosis in developing countries: Moving away from improvisation. Comparative Immunology, Microbiology and Infectious Diseases, 36(3): 241-248. https://doi.org/10.1016/j.cimid.2012.09.001

Hou, Q., Sun, X., Zhang, J., Liu, Y., Wang, Y., Jin, Z. 2018, Modeling the transmission dynamics of sheep brucellosis in Inner Mongolia Autonomous Region, China. Mathematical Biosciences, 242 51–58. https://doi.org/10.1016/j.mbs.2012.11.012

Hussen, A.M., Alemu, F., Hasan, H.A. 2023, Herd and animal level seroprevalence and associated risk factors of small ruminant brucellosis in the Korahey zone, Somali regional state, eastern Ethiopia. Frontiers in Veterinary Science, 10: 1236494. https://doi.org/10.3389/fvets.2023.1236494

Ilyas, M., Harpke, M., Wareth, G. 2024, Brucellosis in Iraq: A comprehensive overview of public health and agricultural. German Journal of Microbiology, 4(3): 10-20. https://doi.org/10.51585/gjm.2024.3.0039

Islam, M.S., Habib, M.A., Tonu, N.S., Haque, M.S, Rahman, M. 2025, Beyond serology: A meta-analysis of advancements in molecular detection of *Brucella* spp. in seronegative animals and biological samples. Veterinary Medicine and Science, 11(1):e70200. https://doi.org/10.1002/vms3.70200

Legesse, A., Mekuriaw, A., Gelaye, E. 2023, Comparative evaluation of RBPT, I-ELISA, and CFT for the diagnosis of brucellosis and PCR detection of Brucella species from Ethiopian sheep, goats, and cattle sera. BMC Microbiology, 23(1): 216. https://doi.org/10.1186/s12866-023-02962-2

Mahmmodi, Sh., Shayegh, J., Alamian, S. 2025, Brucellosis in dogs: A comparison of Brucella infection in herding and stray dogs in Iran (Tehran and East Azerbaijan). Journal of Zoonotic Diseases, 9(3): 945-952.

Martindah, E., Noor, S.M., Wahyuwardani, S., *et al*. 2025, Seroprevalence and risk factors of small ruminant brucellosis in Jabodetabek, Indonesia, Veterinary World, 18(4): 888-895. https://doi.org/10.14202/vetworld.2025.888-895

Mohammed, M., Mindaye, S., Hailemariam, Z., *et al*. 2017, Sero-prevalence of small ruminant brucellosis in three selected districts of Somali region, eastern Ethiopia. Journal of Veterinary Science and Animal Husbandry, 5(1): 105. https://doi.org/10.15744/2348-9790.5.105

Narouei, M., Saadati, D., Najimi, M., *et al*. 2022, Prevalence and risk factors of Brucella infection in sheep and goats in the Sistan region by PCR method. Journal of Zoonotic Diseases, 6(3): 129-137.

Rossetti, C.A., Maurizio, E., Rossi, U.A. 2022, Comparative review of *Brucellosis* in small domestic ruminants. Frontiers in Veterinary Science, 9: 887671. https://doi.org/10.3389/fvets.2022.887671

Sibhat, B., Adamu, H., Benti, T., *et al*. 2024, Brucella Seropositivity and Associated Risk Factors in Pastoral Livestock System in Northeastern Ethiopia. Veterinary Science, 11(12): 620. https://doi.org/10.3390/vetsci11120620

Sorsa, M., Mamo, G., Waktole, H., *et al*. 2022, Seroprevalence of caprine brucellosis and associated risk factors in south Omo zone of southern Ethiopia. Infection and Drug Resistance, 15: 387-398. https://doi.org/10.2147/IDR.S340866

Trangadia, B.J, Toft, N., Nagamani, K., Rana, S..K., Srinivasan, V.A. 2015, Evaluation of ELISA kits for brucellosis in naturally infected Indian cattle population by latent class analysis. Indian Journal of Animal Sciences, 85(1): 27–31. https://doi.org/10.56093/ijans.v85i1.46145

Wang, Q., Shi, J., Li, T. 2023, Seroprevalence and potential risk factors of brucellosis in sheep from America, Africa and Asia regions: A systematic review and meta-analysis. Research in Veterinary Science, 165: 105048. https://doi.org/10.1016/j.rvsc.2023.105048

Wedajo, M.T., Fekadu, R.G., Tefera, Y.M., *et al*. 2015, Seroprevalence of small ruminant brucellosis and its effect on reproduction at Tellalak District of Afar region, Ethiopia. Journal of Veterinary Medicine and Animal Health, 7(4), 111-116. https://doi.org/10.5897/JVMAH2014.0287

Xu, N., Dong, X., Yao, Y. 2020, Improved Early Detection of Focal Brucellosis Complications with Anti-Brucella IgG. Journal of Clinical Microbiology, 58(10): e00903-20. https://doi.org/10.1128/JCM.00903-20