Original Research

Journal of Advanced Veterinary Research
(2025) Volume 15, Issue 3, 370-374

Prevalence and zoonotic implication of animal brucellosis in Libya: A systematic meta-analysis

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ARTICLE INFO

Recieved: 02 June 2025

Accepted: 30 June 2025

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Keywords:

Brucella, Cattle, Human, MENA region, Sheep.

ABSTRACT

Brucellosis, a significant zoonotic bacterial infection, is transmitted from animals to humans through various *Brucella* species. The present report conducted a meta-analysis and focused on the prevalence of brucellosis and its zoonotic implication in Libya, adhering to the PRISMA guidelines throughout the systematic review process. The following databases were searched: Web of Science, Ovid, EBESCO, Sage, Scopus, and Google Scholar. A comprehensive search yielded eligible articles for data extraction and analysis. Employing a random-effects model, the analysis was executed using a specialized meta-analysis software, producing results that included effect size, confidence intervals (CI), heterogeneity metrics, and publication bias. In total, 16,980 animals were examined across 11 studies regarding *Brucella* infection, with 1255 yielding positive results, resulting in a prevalence rate of 7.39 %. The peak prevalence rates (28.34%) occurred in the years 2010. From the random-effects analysis, the pooled effect size was 0.12, the Z-value calculated was -6.35 (p = 0.00) indicated statistical significance, while the heterogeneity metrics revealed a Q-value of 1512,16, I-squared of 99.33, and a p-value of 0.000. Furthermore, the results of Egger's linear regression test suggested publication bias, yielding an intercept of -14.46 and a 95% CI ranging from – 35.76 to 6.75. the results of Egger's linear regression test suggested publication bias, yielding an intercept of -14.46 and a 95% CI ranging from – 35.76 to 6.75. Overall, this meta-analysis underscores a significant prevalence of *Brucella* infections in Libya, particularly highlighting zoonotic value. Consequently, a greater emphasis on disease prevention and control measures is warranted.

Introduction

Brucellosis, a significant zoonotic bacterial infection, is transmitted from animals to humans through various *Brucella* species (Qureshi *et al*., 2023). It is caused by members of the genus *Brucella*, intracellular Gram-negative pathogens with a range of host species preferences (Bonilla-Aldana *et al*., 2023).

It mostly impacts warm-blooded animals such as cattle, goats, camels, pigs, and dogs, all of which have a high susceptibility to the disease (Meletis *et al*., 2024). In animals, brucellosis can cause significant economic loss due to abortion, premature birth, reduced fertility, and decline in milk production (Kiiza *et al*., 2023). Infected animals exhibit distinct clinical symptoms, such as miscarriages in pregnant females and infertility in males (Selim *et al*., 2019).

Brucellosis presents a considerable public health challenge globally, with a pronounced prevalence in regions like Africa (Sibhat *et al*., 2022; Legesse *et al*., 2023). Human infection occurs primarily through close contact with infected animals or their by-products (Njeru *et al*., 2016; Franc *et al*., 2018; Modise-Tlotleng *et al*., 2024). The species most frequently associated with human disease are *Brucella melitensis*, *Brucella suis*, *Brucella abortus*, and, to a lesser extent, *Brucella canis*. *B. melitensis* is the most frequently isolated strains in small ruminants in Mediterranean and Middle East countries (Yumuk and O'Callaghan, 2012; Bagheri Nejad *et al*., 2020; Khairullah *et al*., 2024). Humans acquire the disease through direct contact with livestock and consumption of their products, mostly raw milk and dairy products made from unpasteurized milk (Tuon *et al*., 2017).

Several studies have been conducted on the prevalence of brucellosis in Libya (Alshekh *et al*., 2024) most of these studies were conducted on small ruminants. Other studies were conducted on cattle. Moreover, zoonotic significance of the disease has also been highlighted (Alshekh *et al*., 2024). However, in Libya, human cases are often underreported due to limited surveillance and diagnostic challenges.

A meta-analysis serves as an epidemiological approach aimed at systematically evaluating findings from prior research to draw conclusions regarding a specific research inquiry (Haidich, 2010).This method involves an aggregated and quantitative examination of a substantial and often intricate body of literature, which may also show apparent discrepancies (Moher *et al*., 2010b). Furthermore, the outcomes derived from a meta-analysis can yield a more precise estimation of the impact of risk factors or treatments on particular diseases or other results than can be achieved by any single study included in the combined analysis (Moher *et al*., 2009). However, case reports are not suitable to be included the meta-analysis (El-Khodery *et al*., 2008b). Worldwide systematic review and meta-analysis on brucellosis has been conducted (Khoshnood *et al*., 2022; Freire *et al*., 2024). However, this type of research has not been performed in Libya. Consequently, the objective of the present meta-analysis was to get a conclusion about the disease in Libya with special emphasis on its zoonotic importance.

Materials and methods

Ethical approval

According to PRISMA guidelines, this meta-analysis was directed. Thus, it is not necessary to get approval from the ethical committee for animal use in scientific research.

Reference cases

This meta-analysis involved all articles focused on the prevalence of brucellosis in Libya using different techniques. All animals were sampled to detect *Brucella* antigen by serological tests or by using molecular techniques. However, the sample size of this study was not limited.

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Inclusion criteria

Only acceptable papers those are in English language. All publications on brucellosis in Libya. Cross sectional and case-control and studies. papers on the seroprevalence of brucellosis were included. Articles mentioned the prevalence of brucellosis with any diagnostic technique.

Exclusion criteria

Articles on brucellosis other than prevalence. Articles in languages other than English. Case reports, review articles and preprint.

Study selection

The objective of this study was to search all publications that had been written about the prevalence of brucellosis in Libya and its zoonotic implication. We searched the PubMed, Web of Science, Sage, BESCO, Ovid, CABI, Scopus, database with a combination of the following search terms ("Libya" "brucellosis") (title/ abstract) ("*Brucella* spp.") (title/ abstract) and ("Libya") (title / abstract) and ("Prevalence" "Incidence") (title/ abstract) OR ("Libya") (title / abstract). Preliminary screening of the articles was based on the title and abstract from the earliest data available by May 2025. This procedure was supplemented by manual searching, Google Scholar searching, expert recommendations, and citation reviews. Database outputs were integrated using EndNote software. The standard identification, selection, and eligibility criteria of the selected studies are described (Figure 1).

Data extraction

The extracted information encompassed the study region, authors, publication year, diagnostic technique, sample size, and positive cases (Table 1).

Quality control

According to PRISMA (Moher *et al*., 2010a), the current meta-analysis was carried out. All available published publications on the assessment of the prevalence of brucellosis in Libya based on blood sample collection from infected animals were included to reduce publication bias.

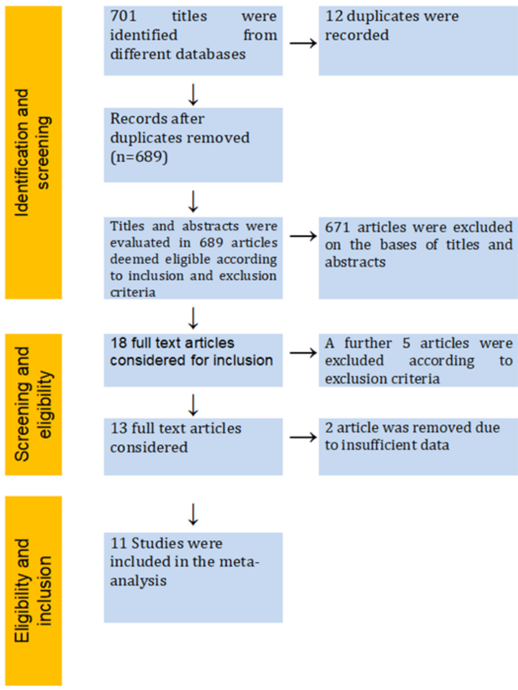

Figure 1. Results of the literature search and inclusion regarding prevalence of brucellosis in Libya.

Data analysis

Data Analysis: The prevalence of brucellosis was first estimated, followed by the application of the Chi-square test to evaluate significant variations among studies (GraphPad Prism for Windows version 9, USA). The commercial software for meta-analysis (Comprehensive Meta-Analysis software version 2, Biostat, Englewood, NJ, USA) was utilized to analyze the data. The primary tests conducted using a random effects model included effect size, 95% confidence intervals, variance, heterogeneity, relative weight, and publication bias.

Table 1. Descriptive data regarding animal brucellosis in Libya.

Authors	Total samples	Positive (%)	Technique	Species	Location	Zoonotic study
(El Sanousi and Omer, 1985)	3753	11	Rose Bengal Plate Test (RBPT)	cattle	Benghazi	-
(Aboudaya, 1986)	8607	125	Rose Bengal Plate Complement Fixation test "CFT"	Cattle	Central	
(Gameel *et al*., 1993)	967	40	Rose Bengal Plate Test (RBPT) Complement Fixation test "CFT"	Camels	Central	-
Abd El-Aal and Salem (2007)	720	139	Rose Bengal Plate Complement Fixation test "CFT"	Sheep and goat	Western Libya	No
(Ahmed *et al*., 2010)	561	159 (28.34%)	IgM IgG	Sheep, goats, cattle, camels	Western mountains	Yes 546 (221, 40% positive)
(Abo Rokia *et al*., 2013)	2230	90	Rose Bengal Plate	Sheep	Western, Middle and Southern areas	No
(Al-Griw *et al*., 2017)	1612	342	Rose Bengal Plate Complement Fixation test "CFT"	All ruminants	North west region of Libya	
(Eissa *et al*., 2017)	400	152	Rose Bengal Plate	Sheep and goat	Al- Jabal Al- Akhdar area, Libya	No
(Younis *et al*., 2018)	600	49	Rose Bengal Plate ELISA PCR	sheep	Al Jabel Al Akhdar	No
(Alshekh *et al*., 2024)	555	15	Rose Bengal Plate Test (RBP Indirect ELISA	Sheep (235) 9 and goat (320) 6	South Libya (Al Jufrah)	-
(Altalhy and Shukri, 2024)	728	133	Rose Bengal Plate, ELISA IgG, IgM, PCR	Sheep and goat	around Al Bayda, Libya	No

Results

A comprehensive database search yielded a total of 701 items. Following the application of specific exclusion criteria, 11 qualifying studies were included in this meta-analysis, as detailed in Table 1 and illustrated in Figure 1. The combined dataset comprised 16980 animals examined for *Brucella* infections. Among these, 1255 animals tested positive, resulting in an overall prevalence of 7.39 %.

From the random-effects analysis, the pooled effect size was 0.12, the Z-value calculated was -6.35 (p = 0.00) indicated statistical significance, while the heterogeneity metrics revealed a Q-value of 1512.16, I-squared of 99.33, and a p-value of 0.000 (Figures 2-3). The analysis of publication bias is illustrated in Figures 4 and 5, where the funnel plot appeared asymmetric, indicating non-significant publication bias. Furthermore, the results of Egger's linear regression test suggested publication bias, yielding an intercept of -14.46 and a 95% CI ranging from – 35.76 to 6.75 and degrees of freedom set at 9. The 1-tailed p-value stood at 0.07, while the 2-tailed p-value was recorded at 0.15. Additionally, Kendall's tau, with continuity correction, yielded a value of – 21.0, accompanied by a 1-tailed p-value of 0.05 and a 2-tailed p-value of 0.11. The classic fail-safe N indicated that approximately 1679 additional studies would need to be included to ensure the results of this meta-analysis are significant. Furthermore, Orwin's fail-safe N suggested an event rate of 0.12 in observed studies versus a mean event rate of 0.5 in the studies deemed missing.

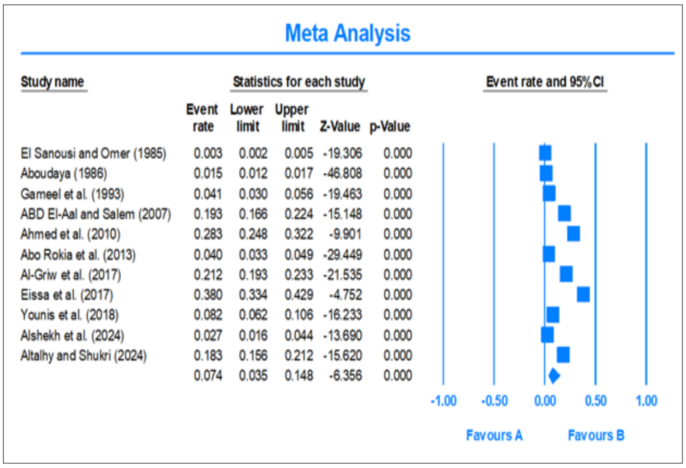

Figure 2. Forest Plot on the prevalence of brucellosis in Libya shows the event rate, 95% confidence interval, Z- value, P- value on random effect model.

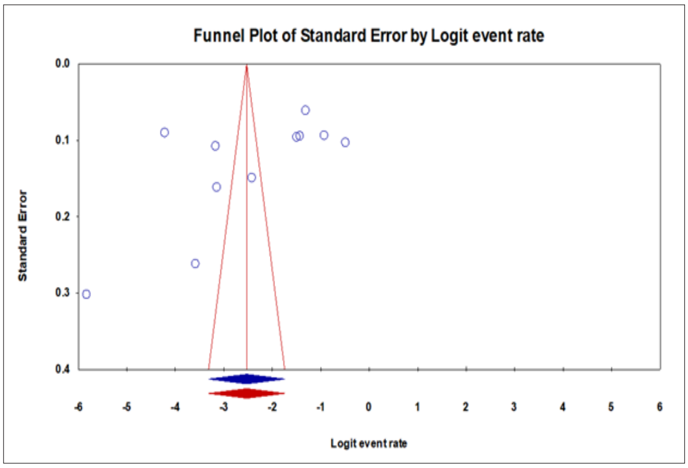

Figure 4. Funnel plot of the prevalence of brucellosis in Libya infection using the random effect model of 11 observed and imputed studies.

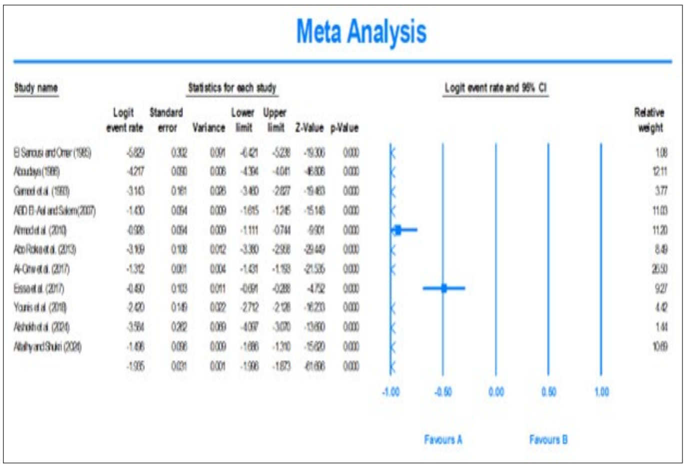

Figure 3. Forest Plot of the prevalence of brucellosis in Libya shows the logit event rate, 95% C, standard error, and variance on the random effect model of 11 observed studies.

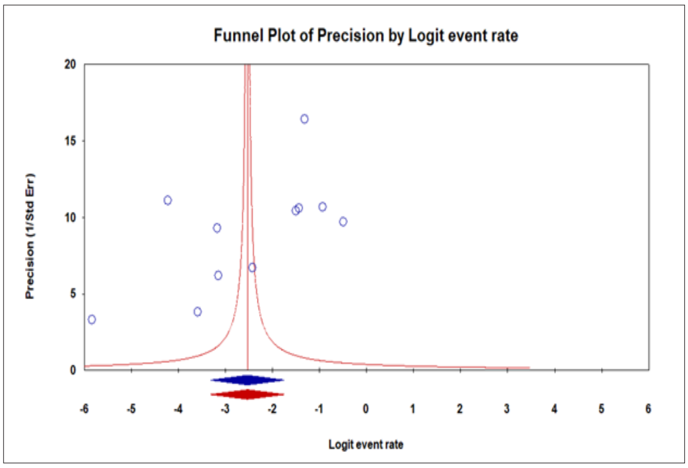

Figure 5. Funnel plot of the prevalence of brucellosis in Libya shows precision by logit event rate on the random effect model of 11 observed and imputed studies.

Discussion

Bovine brucellosis is an economically important zoonotic disease in many developing countries, including Libya. As usually known, diseases should be evaluated as it economic importance (El-Khodery *et al*., 2008a). Several studies at different localities of Libya have been conducted to estimate the prevalence of this disease in livestock, especially small ruminants.

In the present meta-analysis, eleven studies fulfilled the selection criteria. A total of 16980 animals were tested for *Brucella* infection with prevalence of 7.39 %. The prevalence of brucellosis varied among selected studies. This finding may be attributed to the used diagnostic test, animal species, age and gender of examined animals. Higher prevalence was recorded in the adjacent countries (Eltholth *et al*., 2024; Menshawy *et al*., 2025). The present meta-analysis included 11 studies, of which only one study used molecular technique was used for the detection of brucellosis. However, the remaining studies used traditional screening tests. This finding may reflect the potential of studies using this technique. The PCR is a convenient and rapid tool for investigating the clinical relevance of brucellosis (Khan and Zahoor, 2018; Khoshnood *et al*., 2022).

Regarding the zoonotic implication of the brucellosis, there was only one study which indicated an infection rate of 40 % in human (Ahmed *et al*., 2010). This indicated underestimation of the disease in this country. This opinion is supported by limited studies on this disease in human in Libya (Miller *et al*., 2023).

Based on the results of meta-analysis, the study (Al-Griw *et al*., 2017) provided a relative weight of 26.5 %, whereas the small studies are given approximately 0.16 % and 0.15% of the relative weight, respectively (El

Sanousi and Omer, 1985; Alshekh *et al*., 2024). It is known that the common effect is well assessed by larger studies but not by small studies. Studies with small sample size had a negligible effect on the total value. Consequently, larger studies with smaller standard errors have greater weight than those of smaller studies with large standard errors. The present meta-analysis provided Z-values of -6.35 (p = 0.00). The Z-value here does not add to the results, as it is not the effect size, but only indicates the data distribution (Hak *et al*., 2016).

In terms of heterogeneity, the prevalence of brucellosis had an I2 of 99.33 and a Q-statistic of 1512.16 (p-value < 0.000). The null hypothesis for heterogeneity suggests that the studies assign a common effect size, whereas the Q-statistic incorporates the observed dispersion. The degrees of freedom are therefore taken to be equal to the Q-statistic (Thompson, 1994). However, I2 and tau-squared can offer an alternate, useful interpretation if the Q-statistic does not show effect size dispersion (Schulz *et al*., 1995).

According to the I2, there are clear variations in effect sizes across the studies, and random error can only predict 1% of the observed variation. The variance across studies, or tau-squared value, for *Brucella* infection was 1.71. This value is used to determine the weights. Although the eyeball test is a less formal way to quantify heterogeneity, it has been said that the I2 can be used to gauge the degree of heterogeneity in meta-analyses (Huedo-Medina *et al*., 2006). Typically, heterogeneity analysis demonstrates how the impact width differs between studies. According to this statistical test, the variation between studies can be attributed to either sampling mistakes or study differences (Hedges and Olkin, 1985; Borenstein *et al*., 2021). The conformance of the normal distribution of effect sizes is assessed using heterogeneity tests. The null hypothesis, which takes heterogeneity into account, states that both fixed and random influences will have no effect. The z-value, which is used to verify the null hypothesis, is often calculated using Hedges' g/standard error for the pertinent model (Higgins *et al*., 2019). Additionally, it has been said that the p-value is not a measure of the degree of heterogeneity because it is not an effect size. In this case, a low p-value indicates that there is probably some (unidentified) degree of heterogeneity (Duffield *et al*., 2008).

Concerning the publication bias, it is evident that publication bias is found in reports with small sample size (Joober *et al*., 2012). Therefore, detection of bias is needed because it has determinantal effect on the conclusion of systematic meta-analyses (Sutton *et al*., 2000). A common indication for determining the degree of bias is the funnel plot. The effect magnitude is typically shown versus precision or standard errors in this manner (Light and Pillemer, 1986). There was no sign of publication bias in this study, and the funnel plot was asymmetrical. Egger's linear regression test suggested publication bias, yielding an intercept of -14.46 and a 95% CI ranging from – 35.76 to 6.75. Zero level of regression slope indicates the absence of bias, according to the statistical meta-analysis (Rothstein and Borenstein, 2005). Publication bias is present if the Begg test shows a high correlation (Begg and Mazumdar, 1994).

The effect size and publication bias can also be assessed using Trim and fill test (Duval and Tweedie, 2000). Small studies near the extremes of the positive end of the funnel plot were excluded using a repeated method. Until the funnel plot was symmetric, the filling and trimming procedures were repeated (Duval, 2005).The trim-and-fill finding in this result is represented by closed dots for missing studies (no studies were trimmed) and open dots for observed studies (roughly 20 studies) that were imputed. This depends largely on the estimator (R0, L0, or Q0) that is chosen for imputing missing studies, and the adjusted correlation ranges from -0.19 to 0.034 (95% CI).

According to the fail-safe test, 980000 missing studies were needed to determine that the study's findings were significant (p = 0.000). Furthermore, a 0.47 event rate in observed trials and a 0.50 mean event rate in missing studies are recommended by Orwin's fail-safe N. Despite being typically employed in meta-analyses, these evaluations of publication bias may have low power and/or a type I error rate (Sterne *et al*., 2000; Terrin *et al*., 2003; Peters *et al*., 2006, 2007; Rücker *et al*., 2008).

Conclusion

The results of the present meta-analysis indicate a high prevalence of brucellosis in Libya and its zoonotic implication. More attention should be paid to prevention and control this disease.

Conflict of interest

The authors have no conflict of interest to declare.

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