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REVIEW

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Seroprevalence of Bovine Brucellosis in Ethiopia: Systematic Review and Meta-Analysis

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Abstract: Brucellosis is an infectious and widespread zoonotic disease caused by bacteria of the genus *Brucella* and can induce considerable human suffering and huge economic losses in animals. Thus, the aim of this study was to conduct a systematic review and meta-analysis on the seroprevalence of bovine brucellosis in Ethiopia. PubMed, Science Direct, African Journals Online, and Google Scholar were used to search the articles. All references were screened and articles, which reported seroprevalence of bovine brucellosis in Ethiopia was made to calculate the pooled seroprevalence of brucellosis. This review included 15 papers. The estimated pooled seroprevalence of brucellosis was found to be 3.0% (95% CI: 2.0, 4.0). The subgroup analysis showed that there was a statistically significant association between the disease and geographical location, setting, laboratory technique employed and study years. Also, there was some evidence of publication bias (Egger's test, p = 0.0003) on studies reporting the prevalence of brucellosis in Ethiopia. This review proves a high seroprevalence of brucellosis in the country and appropriate control strategies are recommended. Moreover, further study on the risk factors is also required to develop cost-effective preventive strategies.

Keywords: brucellosis, cattle, Ethiopia, seroprevalence, meta-analysis

Background

Brucellosis, which is an infectious disease caused by gram-negative bacteria of the genus *Brucella*, is a widespread zoonosis disease that can induce considerable human suffering and huge economic losses in animals.^{1–3} Bovine brucellosis is usually caused by *B. abortus*.^{4,5} However, it has occasionally been caused by *B. melitensis* and *B. suis* in some instances where mixed farming is practiced.⁵

Even though brucellosis in livestock has been eradicated in industrialized countries, it is one of the most prevalent zoonotic diseases worldwide⁶ with approximately 500,000 new cases of human infection every year,³ especially, in low-income tropical countries where agriculture is the mainstay. In this part of the world, the disease is endemic and neglected, with huge health and livelihood burdens. This is due to lack of effective control and proper disease surveillance.^{7,8} In many developing countries like Ethiopia, brucellosis remains endemic and continues to be a major public and animal health problem.⁹ Brucellosis has been noted as one of the important livestock diseases in the country as it has been depicted the first report of brucellosis in the 1970s in Ethiopia¹⁰ and its reported seroprevalence is ranging from 1.5% to 22.6% were reported in the country.^{1,11} The disease impairs socio-economic development for livestock owners

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by causing significant loss of productivity through abortion, prolonged calving, kidding, or lambing interval, low herd fertility, and comparatively low milk production in farm animals.

Even though, most reports have made either limited geographic coverage or are relatively confined to a single agro-ecology, these stated evidences strongly suggest that bovine brucellosis might be a widespread problem in Ethiopia.^{7,11–14} Compiling the results of different studies fragmented by area and time is very important to show the disease burden at the country level within the specified period of time. Moreover, a comprehensive understanding of the disease occurrence in cattle across the country should be identified as this helps to shape possible future intervention programmes. Thus, the objective of this systematic review and meta-analysis was to estimate the seroprevalence of bovine brucellosis for evidence-based disease control in Ethiopia.

Methods

Study Protocols

The study was performed according to the Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) guideline (<u>Supplementary Figure 1</u>).^{1,15} 1 The checklist was used to make sure the inclusion of relevant information in the analysis. The outcome of interest was the proportion and odds ratio of seropositive for brucellosis. The study protocol had been registered on PROSPERO with reference number ID: CRD42020171243 and Available from: <u>https://www.crd.york.ac.uk/prospero/dis play_record.php</u>? ID=CRD42020171243.

Literature Search Strategy

This systematic review and meta-analysis were aimed to determine the weighted seroprevalence of brucellosis. We retrieved published articles from various English electronic databases, including: PubMed/PubMed Central, Science Direct, Scopus, African Journals Online, and Google scholar until February 29, 2020. Article search was made following the medical subject heading (MeSH) terms. The key search terms were: "Brucellosis" OR "Brucella" AND "Seroprevalence" OR "Prevalence" OR "Seroepidemiology" AND "Risk factors" OR "Potential factors" AND "Cattle" OR "Bovine" AND "Ethiopia". We used "OR" and "AND" Boolean operators to identify studies with any of the keywords in their titles, abstracts and full texts. Moreover, unpublished thesis manuscripts were also accessed from various Ethiopian Universities and research centers.

Inclusion and Exclusion Criteria

We used the following inclusion criteria to confirm the eligibility of the searched papers: 1) original peerreviewed research articles and thesis conducted in Ethiopia; 2) cross-sectional studies that reported the seroprevalence of brucellosis; 3) studies with full texts; 4) targeted study population included bovine within any of the management system (intensive or extensive); in this context intensively managed bovine are those cattle which are kept indoor for whole day or that only be out of the house for only few hour in a day for recreation whereas extensively managed bovine are cattle that are kept on the grazing pasture and get their feed by grazing with or without supplementation in the early morning and late afternoon; 5) studies were performed using serological diagnostic tests RBPT for screening and CFT or ELISA for confirmation; 6) studies provided the total sample size and the outcome of interest (number of positive samples); 7) studies published in English language; and 8) studies published online between 2009 up to February 29, 2020. Papers which did not meet the above-mentioned criteria were excluded. Besides, the references of the selected papers were checked manually to find relevant papers that were not retrieved in the database search.

Study Selection and Data Extraction

Records identified from various electronic databases, indexing services and directories were exported to MENDELEY reference manager software version 1.19.5 (Mendeley Ltd.) with compatible formats. Duplicate records were identified, documented and removed with Mendeley. Some duplicates were addressed manually due to variation in reference styles across sources. Thereafter, two authors (HD and AT) independently screened the title and abstracts with predefined inclusion criteria. Two authors (HD and AT) also independently collected fulltexts and evaluated the eligibility of them for final inclusion. In each case, the rest authors played a critical role in solving discrepancies arose between two authors to come up to consensus.

Similarly, data extraction format was prepared based on first author, publication year, study year, geographical location (region and zone), study design, sampling method, sample size, diagnostic test, setting, number of positive samples, and their corresponding negative cases

among the study groups (Data Supplement). Seroprevalence of brucellosis was calculated by dividing the number of positive cases by the total number of individuals used for the study in a given population at a given period. The study effect size and their corresponding confidence intervals were calculated from the extracted data. Study searching strategies and exclusion criteria are presented in detail in Supplementary Figure 1. Mendeley version 1.19.5 was used to catalogue the initial literature search results and to manage citations. Microsoft Excel datasheet was used to code and manage all extracted information from all relevant studies.

Study Quality Assessment

Two independent researchers were evaluated the quality of the included papers using a quality assessment checklist (standard strengthening the Reporting of Observational Studies in Epidemiology checklist (STROBE)) (Supplementary Check List 1). This quality assessment checklist includes 22 items constituting various sections of the articles such as title, abstract, introduction, methods, results, and discussion. The checklist included items assessing objectives, different components of the methods (eg, study design, sample size, study population, bias, statistical methods), results, limitations, and funding of the studies. The assigned scores were determined from 0 to 44. Following the checklist (STROBE), searched papers were classified into 3 groups: low quality score (<15.5), moderate quality (15.5–29.5), and high quality: (30.0–44.0).^{16,17} The quality of the included studies was indicated in Supplementary Table 1.

Meta-Analysis

Data on the seroprevalence and corresponding 95% confidence intervals (CIs) of the disease were calculated for each study. The pooled prevalence estimate was computed using the formula given by Barendregt et al.¹⁸ Forest plot diagram was employed to present the variations among studies, outcomes of meta-analysis that display estimates of the seroprevalence, and their corresponding CIs of all included studies together with the pooled effect size. Similarly, subgroup analyses for the primary outcome (seroprevalence of brucellosis) will be done by study region, laboratory technique employed (CFT or ELISA) and setting. Cochran's Q-statistics and inverse variance index (I^2) were computed to determine the heterogeneity and inconsistency (true variation) among studies, respectively. Similarly, we considered the I^2 values of 25, 50, and

as low, medium and high heterogeneity, 75% respectively.¹⁹ The *tau* statistics (τ^2) was used to assess the variance of the effect size estimates across the population of the study. Galbraith plot diagram was also constructed to assess the heterogeneities of study level estimates. Based on the heterogeneity assessment result, we used Der Simonian and Laird's random-effects method (if the *p*-value of the O test was <0.05 and I^2 was >50%) or Mantel-Haenszel's fixed-effects method to pool the estimations.²⁰ Small study effects and publication bias presence were then visualized using funnel plot diagrams and, Egger's and Begg's asymmetry tests.²¹ A funnel plot was computed using the logarithm of effect size and its corresponding standard error of the effect size. STATA software version 16 (Stata Corp., College Station, TX, USA) was used to do the meta-analysis.

Result

Search Result

A total of 134 potentially relevant studies were identified from several sources including PubMed/PubMed Central, Science Direct, Scopus, African Journals Online, and Google scholar. From these, 20 duplicated articles were removed with the help of MENDELEY and manual tracing. The remaining 114 records were screened using their titles and abstracts and 85 of them were excluded. Full texts of 29 records were then evaluated for eligibility. From these, 14 articles were excluded due to the outcome of interest was found missing, insufficient and/or ambiguous. Finally, a total of 15 articles fulfilled the eligibility criteria and quality assessment and thus included for systematic review and meta-analysis (Supplementary Figure 1).

Study Characteristics

The 15 eligible studies which were considered for determining the seroprevalence of brucellosis in cattle are summarized in <u>Supplementary Table 1.</u>^{4,7,11–14,22–30} The studies were published in the year between 2009 and 2019. All the selected studies were cross-sectional study design in nature (Supplementary Table 1).

Publication Bias and Small Study Effect Assessment

We assessed bias and small study effects by funnel plot observation and Egger's test for small study effects. The result of effect estimates against its standard error showed that there was some evidence of publication bias on studies reporting the seroprevalence of brucellosis in Ethiopia (Egger's test, p = 0.0003) (Supplementary Figure 2).

Meta-Analysis

Due to the expected variation between studies, randomeffects meta-analyses were carried out using the total sample size and number of positives (effect size and standard error of the effect size). The meta-analysis indicated that between-study variability was high ($T^2 = 0.00$; $I^2 =$ 96.3%, Q-test = 377.8, df = 14 and P < 0.001). Individual study prevalence estimates ranged from 0.0% to 11% with the overall random pooled prevalence of 3% (95% CI: 2.0, 4.0). Studies weighted approximately equal with weights on individual studies ranging from 1.7% to 8.0% due to high heterogeneity between studies. <u>Supplementary Figure</u> <u>2</u> presents the forest plot derived from the meta-analysis (<u>Supplementary Figure 3</u>).

Subgroup Meta-Analysis

Subgroup analyses were done for study locations (central, eastern, northern, western, southern and selected areas of Ethiopia) (Supplementary Table 2), setting (farm and at field) (Supplementary Table 3), laboratory technique employed (CFT and ELISA) (Supplementary Table 4) and year of study (study year between 2009-2011, 2012–2015 and 2016–2018) (Supplementary Table 5). Thus, high seroprevalence was observed in Southern Ethiopia 8% (95% CI: 4.0, 12.0) followed by Northern Ethiopia, 3% (95% CI: 1.0, 7.0), whereas the least prevalence was observed in Central Ethiopia 1% (95% CI: 0.0, 3.0) and Eastern Ethiopia 1% (95% CI: 1.0, 3.0%) (Supplementary Table 2). Similarly, the pooled seroprevalence of bovine brucellosis from the different settings: at fields and farms was 4% (95% CI: 3.0, 6.0) and 1% (95% CI: 0.0, 2.0), respectively (Supplementary Table 3). Also, the prevalence of bovine brucellosis from the different laboratory technique employed: CFT and ELISA was 3% (95% CI: 2.0, 4.0) and 1% (95% CI: 1.0, 3.0), respectively (Supplementary Table 4). Between the different study years, the seroprevalence of bovine brucellosis was 4% (95% CI: 3.0, 5.0), 2% (95% CI: 1.0, 3.0) and 2% (95% CI: 0.0, 4.0) in studies conducted between 2009-2011, 2012-2015 and 206-2018, respectively (Supplementary Table 5).

Meta-Regression

Meta-regression analysis was done for each variable included in the study separately. The variables included

were setting, study year as categorical variable, study location and laboratory technique employed. Those variables with p-values <0.1 were used in the multivariable meta-regression analysis. Study location and setting had significant value and retained in the final multivariable analysis. Results of final multivariable meta-regression are summarized in Supplementary Table 6.

Discussion

Brucellosis is a widespread zoonotic disease that can induce considerable human suffering and huge economic losses in animals.¹⁻³ It has a significant public health implication for a pastoral community in consequence of lifestyles, feeding habits, close contact with animals, low awareness, and poor hygienic conditions which favors infection.^{1,11} Also, it can generally cause significant loss of productivity through abortion, prolonged calving, kidding, or lambing interval, low herd fertility, and comparatively low milk production in farm animals. The disease impairs socio-economic development for livestock owners, which represents a vulnerable sector in rural populations in general and pastoral communities in particular. Even though, most reports have made either limited geographic coverage or are relatively confined to a single agroecology, these stated evidences strongly suggest that brucellosis might be a widespread problem in Ethiopia.^{7,11–14}

Out of 15 original studies with 15,562 serum samples included in this study, the pooled seroprevalence of bovine brucellosis was 3.0%. This result is in concordance with the previous meta-analysis report from dairy cattle in Ethiopia where the pooled prevalence was 3.3%. Similarly, the current finding was similar with the reports of⁵ who reported prevalence of 3.4% from cattle selected in different areas in Cameron. However, the current finding was higher than the reports of³¹ and³² who reported a pooled prevalence of 1.9% from dairy cattle in China and 0.001% in Iran, respectively. Also, the current finding was higher when compared with the report in Uganda.³³ Meanwhile, the current finding was lower than the reports in Zimbabwe, Ghana, Pakistan, Jordan, Cameroon, Tanzania and India.5,34-39 Moreover, among the different countries, China, India and Iran are practiced different control strategies of bovine brucellosis^{3,31,32} however, the other countries did not practice control strategies.^{33–39} The difference in the seroprevalence of bovine brucellosis in the different studies could be due to differences in the geographical location and farming system between the different studies. Therefore, information on the actual seroprevalence of the disease in the country

In the present study, the subgroup analysis showed that there was a statistically significant association between the disease and geographical location, setting, laboratory technique employed and study years. Also, there was evidence of publication bias and small study effects (Egger's test, p = 0.0003) on studies reporting the seroprevalence of bovine brucellosis in Ethiopia.

Conclusion and Recommendations

This systematic and meta-analysis has certain limitations, including: limited and/or lack of studies in many regions of the country and the findings were heterogeneous. In conclusion, the results of this meta-analysis show that the pooled prevalence estimate of the disease in the country is 3.0%. Thus, by considering the significance of bovine brucellosis in the national economy, strategies to reduce the prevalence and burden of brucellosis should be prioritized and offered adequate funding.

Data Sharing Statement

All data generated during this study are included as supplementary information.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests.

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