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REVIEW ARTICLE

Seroprevalence of brucellosis in livestock in Iran: a meta-analysis

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Abstract
The main target of the current investigation was to estimate the overall seroprevalence rate of brucellosis in Iranian livestock (sheep, goats, and cattle) using a meta-analysis of available documents. In this review, different electronic databases were searched for the relevant studies published until January 2022, about the seroprevalence of brucellosis in animals in Iran. A chi-squared test and also a random effect model (REM) were used to determine the heterogeneity of studies and to estimate the pooled seroprevalence among subgroups, respectively. The overall seroprevalence of brucellosis was 3%, 4%, and 5% in cattle, sheep, and goats, respectively (Pooled seroprevalence = 3%). The highest pooled seroprevalence was reported 75% in West Azerbaijan, northwest of Iran, while the lowest rate was 1% in Charmahal and Bakhtiyari and Khuzestan, southwest regions of Iran. This was a review study on brucellosis in animals in Iran. There was no comprehensive data on animal brucellosis from some locations in Iran; which was the main limitation of our work. Further studies of the brucellosis seroprevalence rate in animals, especially in endemic regions of Iran, as well as associated risk factors, are highly recommended. This is essential for developing a launch control strategy for eliminating the disease. Also, healthy measures such as increasing livestock vaccination and farmers' education must be improved in the areas with higher seroprevalence rates.

Keywords Brucellosis · Seroprevalence · Animal · Meta-analysis · Iran

Introduction

Brucellosis, a bacterial zoonotic disease that can affect both humans and animals, is of particular importance, and has significant economic implications worldwide. This disease has been identified as having a higher incidence rate among human and animal populations in Asia, the Middle East, and neighboring tropical countries (Pappas 2010). Brucellosis has caused irreversible economic losses in the animal husbandry industry due to reduced milk production and increased rates of abortions. In the USA alone, the estimated annual disease cost is around US $600 million (Angara et al. 2016). *Brucella abortus* is the main cause of bovine brucellosis, while *Brucella melitensis* is the dominant species in sheep and humans (Rubach et al. 2013; Golshani and Buozari 2017). *Brucella* tends to infect the genital organs of domestic animals, leading to complications such as abortion, stillbirth, metritis, reduced milk production, and infertility (Zowghi and Ebadi 1989; Pal et al. 2020). Moreover, infected animals' aborted materials and genital discharges may contaminate the environment and infect other hosts. Milking from infected cows can also lead to the infection of newborn calves. It is not advisable to treat brucellosis in animals. Instead, infected animals should be immediately culled upon detection to control *Brucella* infection among animals and humans (ZareBidaki et al. 2022).

In Iran, brucellosis is an endemic disease that is considered a major risk to public health. The country's veterinary services have included it in its control programs, making it one of the strategic diseases. Comprehensive information is needed to conduct an effective control program on brucel-

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losis in animals. Animal vaccination against brucellosis is the most effective tool in the endemic areas. Additionally, it is recommended to test and cull infected animals as a second step (Gharekhani et al. 2016).

The transmission of brucellosis in humans primarily occurs via direct contact with secretory materials of sick livestock and/or the consumption of unpasteurized dairy products, especially raw milk and traditional cheeses. Therefore, the occurrence of the disease in humans heavily relies on the infection rate among domestic animals. Although several countries have made significant progress in controlling brucellosis, the risk of infection remains high in endemic regions (Dadar et al. 2019). Regarding the implementation of Iranian Veterinary Organization (IVO) controlling programs, animal brucellosis, especially in dairy cattle farms, has decreased in recent years. These control measures have reduced the prevalence of bovine brucellosis in intensive industrial breeding to zero in some regions (Dadar et al. 2021).

Laboratory methods based on microbiology, serology, and molecular biology are used to diagnose brucellosis in livestock (Priyanka et al. 2018a, b, 2019a). Serology techniques are user-friendly and cheap for rapid diagnosis of the disease. The Rose Bengal rapid test, Wright and 2-Mercaptoethanol, and enzyme-linked immunosorbent assay (ELISA) are the most common serologic tools that are used in Iran (Priyanka et al. 2017; Adabi et al. 2022). Nowadays, ELISA with 100% sensitivity and 99.2% specificity has been used as a suitable alternative to culturing techniques. SAT accounts for aggregated quantities of IgM and IgG, while IgG for *Brucella* infection is calculated using the treatment of sera samples. IgG tracing is important for determining the active stage of brucellosis (Al Dahouk and Nöckler 2011).

Currently, there is no comprehensive summarized data on the brucellosis seroprevalence in Iranian animals. The main target of the current investigation was to determine the overall rate of brucellosis seroprevalence in cattle, sheep, and goats in Iran using a meta-analysis of available documents.

Materials and methods

Search strategy

A systematic search was conducted to retrieve the published articles on seroprevalence of brucellosis in cattle, sheep, and goats in Iran. For this reason, electronic databases such as PubMed, Scopus, Web of Science, and Google Scholar were applied in the search process until January 2022. The start date for the search was not restricted. After analyzing the dominant Medical Subject Headings (MeSH) terms, the following Keywords were applied: "Brucellosis" OR "Animal Brucellosis") AND (cows OR cattle OR bovine OR sheep OR goat OR Livestock) AND (epidemiology_ OR seroprevalence_ OR incident_ OR surve_) AND (Iran). We removed duplicate reports of identical studies. In addition, we checked the reference lists of all accessed published articles to achieve a complete document.

Study selection and inclusion criteria

Both the importance of study questions and the consistency of methodology were applied for the inclusion and exclusion of literature. In our work, all reports on seroprevalence of brucellosis in Iranian livestock (cattle, sheep, and goats) were included without consideration of the language (English and/or Persian), publication date, and study locations. All seroprevalence studies with the primary target of control strategies on brucellosis were excluded. When the research results were found in different articles, only the most recent and comprehensive report was included in the analysis. Also, we conducted a manual search for articles with relevant titles. We adhered to the PRISMA guidelines to extract the pertinent articles (Moher et al. 2010). The criteria for inclusion were determined using the CoCoPop mnemonic (Condition, Context, and Population) recommended by the Joanna Briggs Institute.

(a) Population: Livestock with brucellosis in Iran without location/province consideration.
(b) Condition: "Animal brucellosis" is a zoonotic bacterial disease caused by various *Brucella* species, which mainly occurs as a chronic infection in domestic animals such as cattle, sheep, and goats. The main variable of interest was the "prevalence of brucellosis." "Point prevalence of brucellosis" was defined as number of livestock with brucellosis/the total number of examined livestock at a particular point of time × 100. "Period prevalence of brucellosis" was defined as number of cattle with brucellosis/number of cattle during a particular period × 100.
(c) Context: Studies developed in Iran, regardless of the province, language, and period.

Screening process and extracting information

We used the EndNote X8 software to collect and analyze all publications that may be relevant. The data were extracted for detecting the eligible works. Following the final assessment, the essential data was extracted and saved: author name, study date, study locations (province), the number of animals (sample size) in each study, study design, and the relative frequency of brucellosis in the investigations. If necessary, we contacted the authors to achieve complementary information. So, qualified studies were selected for primary screening. The last report on the frequency of brucellosis considered the type of animals and their location.

Statistical analysis

The estimate of animals' brucellosis was based on the total number of animals who were examined for brucellosis (denominator) and the total number of animals with confirmed brucellosis (numerator). The meta-analytic integration of the seroprevalence estimate was carried out utilizing Stata software version 14 (StataCorp, College Station, TX, USA) and its "metaprop" commands. The "metaprop" command was developed specifically for meta-analysis of proportions and is based on the Freeman-Tukey double arcsine transformation for stabilizing variances. The level of heterogeneity in a meta-analysis mainly determines the effort to reach general conclusions. The I^2 statistic is commonly used to assess the degree of heterogeneity among the studies included. If $I^2 < 50$, it signifies least heterogeneity, $I^2 > 50\%$ characterizes least-moderate heterogeneity, and $I^2 > 95\%$ indicates high heterogeneity. To identify moderator variables that account for seroprevalence variance, we conducted a meta-regression analysis using a random-effects model. To generate the pooled estimates, the random-effects model was utilized. The pooled associations with 95% confidence intervals (CI) were demonstrated separately for different types of animals and study locations. Also, inverse of variance method was used for variance estimation. The "metaprop" command uses the numerator, and the denominator carries out the Freeman-Tukey double arcsine transformation and uses inverse variance weighting to model fixed and/or random effects. The data from the numerator and denominator were utilized to estimate the seroprevalence. The data were transformed into the Freeman-Tukey double arcsine equivalent with standard errors using Excel, then they were used to generate the Galbraith plots. Additionally, the Begg and Egger tests were applied to examine the possibility of publication bias. We used subgroup analysis by types of cattle and province to explore reasons for heterogeneity.

Results

Explanation of reports

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) literature search flowchart is demonstrated in Fig. 1. After reviewing and screening 138 published articles, 18 articles were selected that could be included in this systematic review and meta-analysis. Exclusion reasons consisted of reports that were duplicated on the same population ($n = 5$), wrong design for measuring seroprevalence ($n = 3$), narrative qualitative study ($n = 2$), lack of relevancy ($n = 7$), and single case report ($n = 3$). From 216,678 investigated domestic animals (cattle, sheep, and

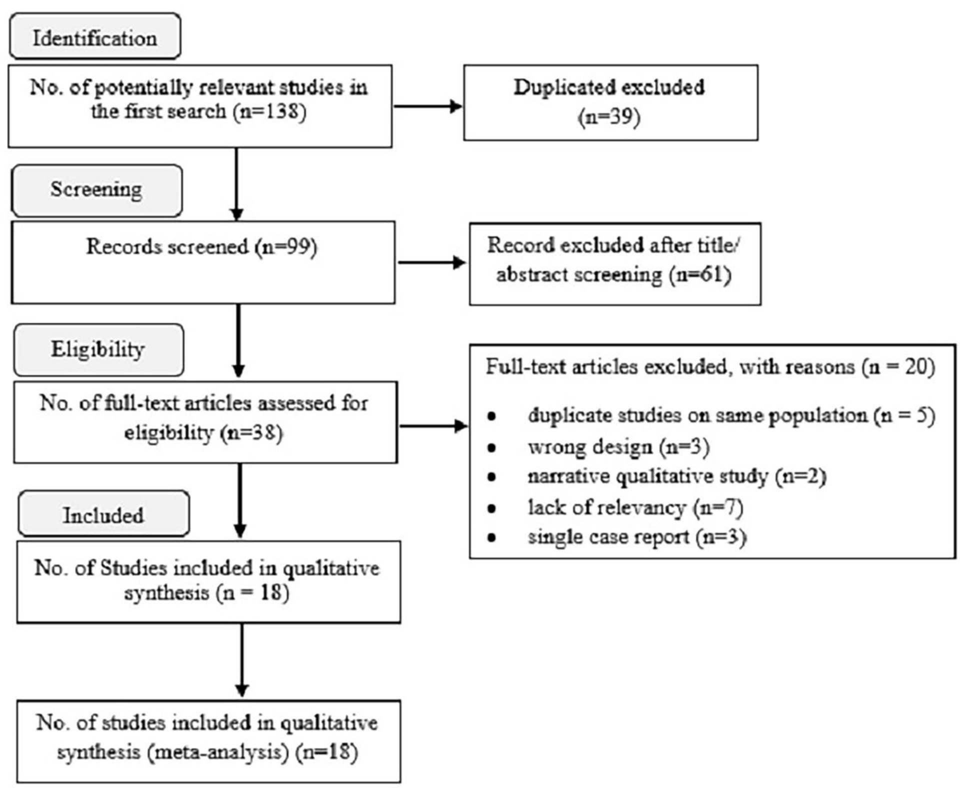

Fig. 1 Flow diagram of the articles selection process for the meta-analysis study on seroprevalence of brucellosis in animals from Iran

goats), 9335 animals tested positive for brucellosis. The studies were published from 12 locations/provinces in Iran.

The pooled seroprevalence of brucellosis in animals

The overall seroprevalence of brucellosis was 3% in cattle (95% CI, 0.02 to 0.04; I^2, 99.70), 4% in sheep (95% CI, 0.03 to 0.05; I^2, 99.79%), and 5% in goats (95% CI, 0.03 to 0.06; I^2, 97.87%). The pooled seroprevalence of brucellosis in animals was estimated at 3% (95% CI, 0.02 to 0.04; I^2, 99.58%) (Table 1 and Fig. 2).

The pooled seroprevalence of brucellosis in different locations

Regarding random effect model analysis, the highest pooled seroprevalence of brucellosis was reported 75% (95% CI, 53–89%; I^2, 0.00%) in West Azerbaijan, northwest of Iran, while the lowest rate belonged to Charmahal and Bakhtiyari (1%, 95% CI, 0–2%; I^2, 99.51%) and Khuzestan (1%, 95% CI, 0.9–2%; I^2, 0.00%), southwest regions of Iran (Table 2 and Fig. 2).

Table 1 Data on all selected articles for the meta-analysis study of seroprevalence of brucellosis regarding animals in Iran

Animals	Study (year)	Seroprevalence	Standard error (SE)	Weight	References
Cattle	2011	0.023256	0.016252	1.17	(Akbarmehr and Ghiyamirad 2011)
	2013	0.506093	0.013386	1.66	(Bahonar et al. 2013)
	2006	0.000083	0.000083	15.06	(Bokaei et al. 2009)
	2018	0.002541	0.000179	13.16	(Hajkazemi et al. 2020)
	2016	0.006382	0.000501	14.89	(Kaboli Boroujeni et al. 2020)
	2013	0.015674	0.000735	14.71	(Mahzounieh et al. 2015)
	2010	0.750001	0.096825	0.04	(Morshedi et al. 2010)
	2014	0.033670	0.006043	5.70	(Semironi et al. 2018)
	2013	0.000393	0.000077	15.06	(Shahbazi et al. 2016)
	2016	0.146572	0.008598	3.49	(Soleimanzadeh et al. 2017)
	1990	0.163473	0.004596	7.73	(Zowghi et al. 1990)
	1984	0.034115	0.004839	7.34	(Zowghi et al. 1984)
Random pooled effect size = 0.03 (0.02, 0.04); I^2, 99.70%					
Sheep	2011	0.041892	0.007365	9.15	(Akbarmehr and Ghiyamirad 2011)
	2019	0.035088	0.024372	4.73	(Amouei et al. 2019)
	2015	0.030196	0.003389	9.89	(Gharekhani et al. 2016)
	2018	0.045149	0.003218	9.91	(Hajkazemi et al. 2020)
	2005	0.099773	0.014271	7.27	(Javadi et al. 2007)
	2016	0.000057	0.000040	10.10	(Kaboli Boroujeni et al. 2020)
	2012	0.030104	0.000495	10.10	(Mombeni et al. 2014)
	2014	0.038863	0.003493	9.87	(Semironi et al. 2018)
	2013	0.111204	0.005262	9.59	(Shahbazi et al. 2016)
	2012	0.054895	0.005419	9.57	(Sharifi et al. 2015)
	1987	0.009434	0.003833	9.83	(Zowghi et al. 1984)
Random pooled effect size = 0.04 (0.03, 0.05); I^2, 99.79%					
Goats	2011	0.050001	0.017230	11.16	(Akbarmehr and Ghiyamirad 2011)
	2015	0.045714	0.007894	14.67	(Gharekhani et al. 2016)
	2018	0.050553	0.008708	14.41	(Hajkazemi et al. 2020)
	2005	0.120001	0.018762	10.57	(Javadi et al. 2007)
	2016	0.001538	0.001087	15.98	(Kaboli Boroujeni et al. 2020)
	2014	0.030075	0.002015	15.91	(Semironi et al. 2018)
	2013	0.269231	0.061511	2.45	(Shahbazi et al. 2016)
	2011	0.070559	0.007293	14.85	(Sharifi et al. 2015)
Random pooled effect size = 0.05 (0.03, 0.06); I^2, 97.87%					
Total seroprevalence = 0.03; I^2, 99.58%					

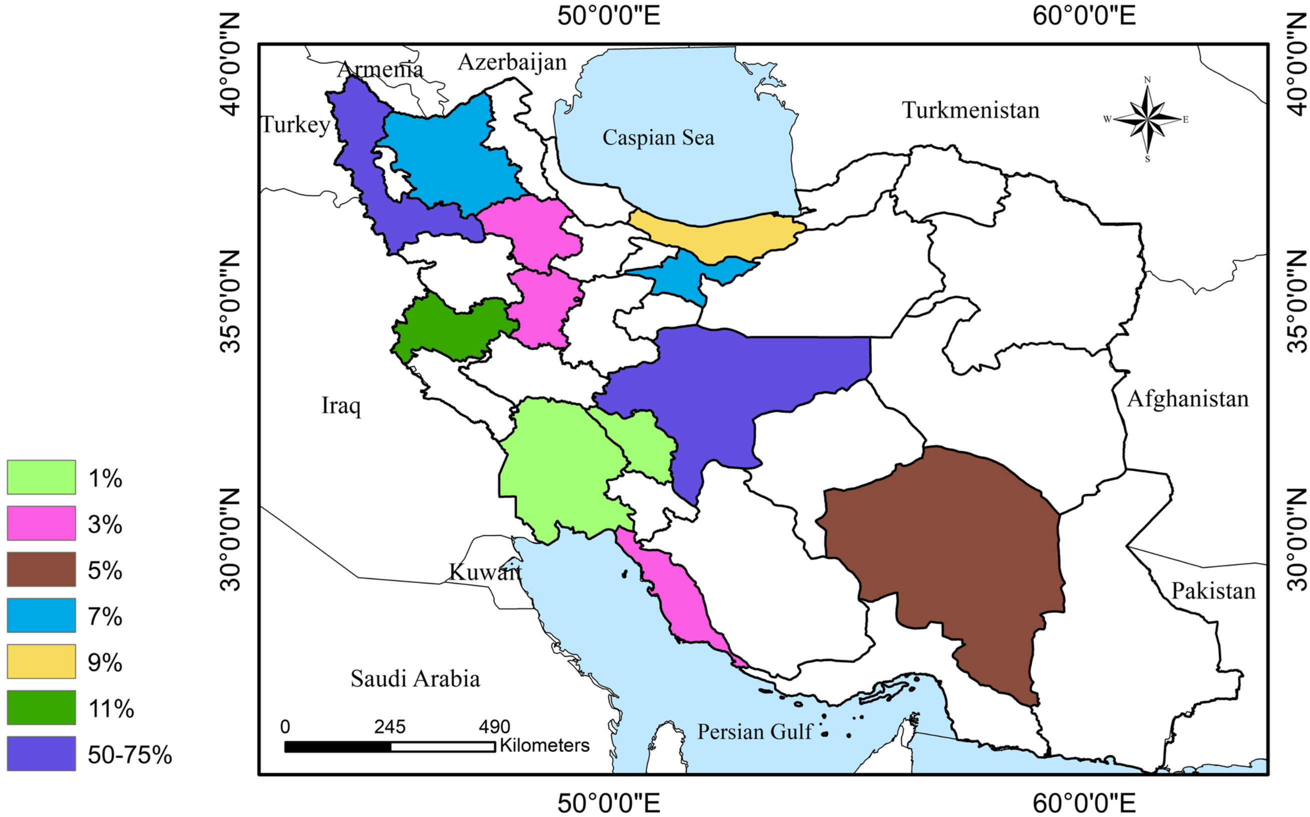

Fig. 2 Geographical locations with seroprevalence rate of animal brucellosis in Iran

Meta-regression assessment

The high heterogeneity detected in the pooled seroprevalence of brucellosis suggests the existence of investigation characteristics that influenced this variance. Therefore, we conducted a meta-regression analysis to evaluate whether publication year has a predictive impact, as well as province and type of livestock. All these variables led to a noticeable decrease in heterogeneity, although this decrease is not significant for all these variables (Table 3).

Table 2 The pooled seroprevalence estimates of brucellosis in animals regarding different locations in Iran

Location (provinces)	Pooled seroprevalence (95% CI)	Heterogeneity (I^2)	Weight (%)
Charmahal and Bakhtiyari	0.01 (0.00 to 0.02)	99.51	18.83
Khuzestan	0.01 (0.009 to 0.02)	0.00	9.50
Mazandaran	0.09 (0.06 to 0.12)	82.78	4.75
West Azerbaijan	0.75 (0.53 to 0.89)	0.00	0.02
East Azerbaijan	0.07 (0.03 to 0.10)	93.70	6.32
Booshehr	0.03 (0.02 to 0.04)	0.00	10.09
Esfahan	0.51 (0.48 to 0.53)	0.00	0.49
Hamadan	0.03 (0.02 to 0.04)	0.00	5.26
Kerman	0.05 (0.04 to 0.07)	95.40	18.36
Kermanshah	0.11 (0.01 to 0.20)	0.00	7.45
Tehran	0.07 (0.02 to 0.16)	0.00	9.12
Zanjan	0.03 (0.00 to 0.07)	0.00	9.51
Overall	0.03 (0.02 to 0.04)	99.58	100.0

Discussion

Our work has presented a picture of the seroepidemiology of brucellosis in Iranian livestock. Brucellosis is a significant zoonotic disease worldwide as well as a significant public health problem in some regions such as the Middle East (Lindahl et al. 2014; Priyanka et al. 2018a, b). Human

Table 3 Meta-regression analysis of the predictors of animal brucellosis

Variable	Coefficient	Standard error	*P*value	I^2(%)
Publication year	0.0020008	0.0048508	0.682	13.34
Livestock type	−0.0002638	0.0014698	0.858	13.81
Province	−0.0007772	0.0013616	0.571	14.12
Constant	1.572099	2.7471560	0.570	-

brucellosis has been reported to be 0.001% in Iran, with the highest incidence occurring in the western and northwest regions (Bahmani and Bahmani 2022). The eradication and control of brucellosis require sustainable budgets for long-term surveillance programs, which can be costly (Zhang et al. 2018). In this regard, epidemiological analysis plays a crucial role in identifying the main livestock reservoirs of brucellosis and implementing comprehensive preventive and control strategies. Numerous researches on brucellosis have been conducted in Iran. However, the seroprevalence of disease in wild animals, the main routes of infection transmission in the regions, *Brucella* diversity, disease management in animals and herds level, and controlling strategies are unclear (Dadar et al. 2021). In Dadar et al. (2019), molecular evaluation from Iranian isolates of *Brucella*, the infection in sheep was exclusively related to *B. melitensis*, while *B. abortus* and *B. melitensis* were common in cattle. The Rev1, vaccine strain of *B. melitensis* is detected in sheep and goats. *B. melitensis* biovar 1 and *B. abortus* biovar 3 are the dominant biovars that were reported from Iran. Identification of circulating *Brucella* species from animals, especially sheep and goats is so important because of the high activity of traditional small farms as well as mixed farming models in rural regions of Iran.

In our work, the pooled seroprevalence of brucellosis was calculated at 3%; this rate was 3%, 4%, and 5% in cattle, sheep, and goats, respectively. Zowghi et al. (2008) reported a high level of *Brucella* infection in animals in different regions of Iran (14.7% for small ruminants, and 17.6% for cattle). In a report by Suresh et al. (2022), the rate of brucellosis in livestock was 8% in the Asian and African continents. In the Middle East, animals' brucellosis is estimated to be 0.85–23.3% (Bahmani and Bahmani 2022). This rate in Iranian livestock was 10.18% (14.66% in cattle, 12.83% in sheep, and 4.34% in goats) (Dadar et al. 2021). In addition, bovine brucellosis was reported 1.8% in Argentina (Aznar et al. 2015), 1.9% in China (Ran et al. 2019), 2% in Tajikistan (Lindahl et al. 2014), 2.6% in Bangladesh (Rahman et al. 2011), 3% in Ethiopia (Tesfaye et al. 2021), 3.56% in Turkey (Yumuk and O'Callaghan 2012), 8.7% in Pakistan (Arif et al. 2019), and 17% in India (Barman et al. 2020). In another study from India, bovine brucellosis was reported at 13.9%, 15.3%, and 12.6% by using RBPT, i-ELISA, and molecular biology techniques, respectively (Priyanka et al. 2017, 2019b).

We found a large heterogeneity in the rate of brucellosis in animals due to the presence of various factors affecting the variance. Also, in the meta-regression evaluation, the variables of year of reports, location (province), and type of animals showed a notable decrease in heterogeneity with no significant statistical connections ($P > 0.05$). In the meta-analysis from African and Asian continents (Ran et al. 2019; Suresh et al. 2022), location and laboratory diagnostic methods had a significant ($P < 0.05$) impact on reducing the heterogeneity; this is agreement with the previous report in Iran (Dadar et al. 2021).

The mentioned work demonstrated different results compared to other studies, possibly due to differences in the time and location of the research. There was no comprehensive data on animal brucellosis from some locations in Iran, which was the main limitation of our work. Also, there were numerous reports on animal brucellosis without scientific support in designing the investigation such as sample size, sampling, and laboratory techniques, which were omitted in this study. Most of the presented articles in Iran focused on bovine brucellosis; 827,503 animals had been tested up to 2021. Improving management strategies and surveillance systems for brucellosis is necessary to reduce the infection transmission in animals at both individual and herd levels. Brucellosis in farms with intensive systems of breeding is higher than in extensive systems because of the close contact of the animals. Also, animals' susceptibility to infections differs depending on breed (Golshani and Buozari 2017). In some areas of Iran, the high density of animals in pastures, lack of sunlight especially in mountain regions, and unsanitary measures in the processing of dairy products caused the distribution of brucellosis. Additionally, in compared studies, the main reasons for the results differences are the different study design and protocol methods, sample size, diagnostic methods, herd size, animals' density, and farms' biosecurity (Dadar et al. 2021). Additionally, the rhythmic changes in immunological profiles have a high impact on implementing control measures for brucellosis in animals (Priyanka et al. 2021a). Cytokines play a significant role in the immunological responses and protection against *Brucella* infection, which may help establish quick diagnostic tools as well as develop vaccines against brucellosis (Priyanka et al. 2021b). According to the report by Bahmani and Bahmani (2022), the endemic regions face significant challenges due to the absence of efficient vaccines and difficulties in culling positive animals. It is recommended to keep various types of livestock, including cattle, sheep, goats, and genders separated to prevent the spread of non-specific *Brucella* spp. To prevent further transmission, each herd should be assigned specific pastures, and the cross-movement of animals between herds should be blocked. Using multiple laboratory methods simultaneously can improve sensitivity, specificity, and positive predictive value when conducting epidemiological investigations. This approach reduces the likelihood of misdiagnosis and increases the chances of detecting brucellosis antibodies (Al Dahouk and Nöckler 2011).

Conclusion

The study revealed a significant prevalence of brucellosis among livestock in Iran. However, estimating the rate of brucellosis in animals can be a challenging task due to limited data on animal populations and the mobility of most herds. To address this, further research on brucellosis in animals, particularly in regions with no reported cases in Iran, as well as associated risk factors is essential. According to the significant role of domestic and wild animals as potential sources of *Brucella* infection, a more comprehensive and reliable risk assessment is needed to develop effective strategies for disease control. This may involve measures such as increasing livestock vaccination and providing education to farmers in areas with higher prevalence rates.

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Compliance with ethical standards

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Conflict of interest The authors declare no competing interests.

Ethical approval For this type of study, formal consent is not required.

Informed consent For this type of study, informed consent is not required.

Consent for publication For this type of study, consent for publication is not required.

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