



Epidemiology of Brucellosis in Humans and Livestock: A Systematic Review and Meta-Analysis (2000–2025) from Pakistan

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Abstract

This is a systematic review and meta-analysis based on the synthesis of epidemiological evidence on brucellosis within the Pakistani population in the period 2000 to 2025. Based on 87 included studies, the national prevalence of brucellosis was 8.7% (95% CI: 6.910.4) by pooling studies, and livestock had a higher prevalence of brucellosis seroprevalence (9.4%) than in humans (6.2%). It showed that there is a lot of heterogeneity in terms of regions with Punjab (10.8%) and Sindh (8.9%) being major hotspots because of intensive farming of livestock. The major reservoirs of animals were small ruminants and especially goats (12.3%). The human occupational risk was greatest among veterinarians and abattoir workers (9.1%), and farmers (7.6%). Key routes of transmission were eating unpasteurized dairy products and direct contact with infected animals and their tissues. Although the decrease in prevalence was rather modest after 2010, the disease remains a major endemic issue. It is mainly perpetuated by its lack of a national policy on its eradication, scattered control measures, inconsistent diagnostic protocols and poor surveillance. High statistical heterogeneity ($I^2 = 93.4\%$) highlights the inconsistency in the methods of study. The evidence elucidates the urgent necessity of integrated One Health approach, including standardized diagnostics, animal-human surveillance, mass livestock vaccination and focused public health education that will help decrease the high socioeconomic cost of this overlooked zoonosis in Pakistan.

Keywords: Epidemiology of Brucellosis, Human and Livestock, Risk Factors, Public Health, Systematic Review and Meta-Analysis, Endemic.



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Introduction

Brucellosis is a worldwide big-neglected zoonotic disease that is caused by gram-negative, facultative intracellular bacteria of *Brucella* genus and is a serious public health and veterinary issue (Ali *et al.*, 2018). According to World Health Organization (WHO), the disease is among the top neglected zoonosis in the world, especially in South Asia, where the disease is perpetuated by lack of awareness among the population, lack of diagnostic centers and close contact between humans and livestock in the rural economies (Gul *et al.*, 2015). Pakistan has endemic disease spread in all the provinces among domestic ruminants like cattle, buffaloes, sheep, goats, and camels and the human population. The *Brucella abortus*, mostly related to the bovines, and *Brucella melitensis* species, which are commonly found in small ruminants and the most virulent in humans, are the most common species in the country (Awais *et al.*, 2024).

Infection in humans in Pakistan takes place via numerous routes, the most common ones being consumption of unpasteurized milk and dairy goods or direct contact with infected animal body tissues and fluids like placenta, aborted fetus and vaginal discharges (Jamil *et al.*, 2021). The livestock farmers, veterinarians, butchers, and abattoir workers are the high-risk groups because they often get in touch with infectious aerosols and contaminated biological materials during their daily occupational practice (Shehzad *et al.*, 2021). Brucellosis has a tremendous economic impact on the economy; in animals, the disease causes reproductive losses in the form of abortions, stillbirth, and infertility; considerable reduction in milk production and weight gain. The disease in the human population occurs in the form of a wavy febrile disease that without treatment, may develop debilitating chronic complications, such as endocarditis, neuroborreliosis, and osteoarticular disease, which further reduces the productivity of households and adds to the cost of health care (Gul & Khan, 2007).

Irrelevant of these harsh consequences, brucellosis is not well controlled in Pakistan because there is no strong policy of national eradication, the free flow of livestock and the system of monitoring it is not strong (Ullah *et al.*, 2020). Punjab province has been discovered to be a disease hot spot, mostly because of the irrigated land and great concentration of cattle and buffalo animals in the province (Saeed *et al.*, 2019). Although a variety of studies are carried out in the region, the amount of available information is still scattered and disjointed, with the most significant gap in the existing knowledge about the epidemiological situation in the country in the past 20 years. The urgent need is thus a national-level synthesis and meta-analysis to give an overall evaluation of the disease rates and trends from the year 2000 to 2025 (Hussain *et al.*, 2008).

The main research questions of this review will be to find the combined seroprevalence of brucellosis in both people and animals in Pakistan, the most important risk factors of the infection, and the effectiveness of current control measures. Through the response to these questions, the study will be able to give evidence-based recommendations on a One Health approach to reduce the burden of this zoonosis on the national health and the economy.

Literature Review

The Brucellosis is endemic in all the administrative units of Pakistan, as well as the provinces of Punjab, Sindh, Balochistan, Khyber Pakhtunkhwa (KPK), Gilgit-Baltistan, and the Azar Jammu and Kashmir territory (Akram *et al.*, 2021). The disease has been widely mapped in Punjab, where the greatest density of livestock in the country is found, and recent surveys have shown a pooled seroprevalence of 13.13% in the general population and as large as 15% in some urban dairy clusters such as Chak Shahzad (Abubakar *et al.*, 2012). Seroprevalence rates in sheep and goats were recently reported to be 13.5 and 12.5 percent in the Khyber Pakhtunkhwa province, in the districts of Mohmand and Charsadda the first reported case of *Brucella melitensis* in these regions. Studies in Balochistan have discovered the introduction of disease in organized dairy farms in Quetta and in Sindh seroprevalence in camels, which demonstrates the national distribution of the pathogen in host species of various types (Saeed *et al.*, 2020). Gilgit-Baltistan is also found to be an endemic area with a region of concern and research has been done on the risk at the interface between livestock and human handlers (Ullah *et al.*, 2024). The countrywide disease mapping since 2000 indicates that although there are certain areas that have kept the prevalence rates below 3-6.5, there are still prominent disease hotspots such as Southern Punjab with 16.5% and 13.6% seropositivity of cattle and buffaloes respectively (Awais *et al.*, 2024).

Cattle, buffaloes, sheep, goats and camels constitute the major hosts of livestock brucellosis in Pakistan with *Brucella abortus* and *Brucella melitensis* as the commonest species detected using molecular methods (Khan *et al.*, 2017). Cattle are particularly prone to the highest seroprevalence than buffaloes and small ruminants, although the two species are commonly co-carried in mixed farming (Abbas *et al.*, 2025). Household husbandry methods are very much involved in the prevalence of the disease as they include application of common grazing grounds and the nomadic mobility of herds between the neighbors, which encourages quick transmission of the infection across district and provincial boundaries (Jamil *et al.*, 2020). Biosecurity in smallholder and peri-urban areas is also of extreme low quality, with introduction of new animals to the farm conducted without any quarantine measures or of unknown origin being a main source of herd-level infection (Nisar *et al.*, 2025). Natural breeding rather than artificial insemination promoted by the management facilitates the likelihood of spreading the disease greatly, and low educational level and insufficient awareness among the farmers about the use of biological materials contribute also to the further development of the disease (Hasni *et al.*, 2025).

The occupational exposure to brucellosis is closely associated with the human burden of this infectious disease, and livestock owners, veterinarians, butchers, and those working in an abattoir are in the greatest risk of being infected (Ali *et al.*, 2025). Among workers in Pakistani slaughterhouses, prevalence has been noted to be about 21.7% usually due to direct contacts with infected tissues or the inhalation of infectious aerosols in the daily meat processing activities with these workers (Nawaz *et al.*, 2021). The rural pregnant women in Pakistan constitute a highly susceptible population with a seroprevalence percentage of 5.8% that has been linked to the past occurrence of spontaneous abortions and intrauterine fetal death. General Pakistani population Clinical manifestations are often undulant fever, night sweats, arthralgia and persistent fatigue but the disease is often diagnosed wrongly because of the limitation of diagnosis in rural health care (Ali *et al.*, 2016). Majority of clinic practices in rural areas do not have the ability to conduct complex confirmatory tests such as ELISA or PCR, but use simple screening tests, which might not be able to identify chronic or subclinical cases (Ullah, 2013). This is compounded by the lack of coordination in One Health where absence of communication between veterinary sectors and medical professionals does not allow tracking human infections to animal reservoirs.

Table 1:
Reported Seroprevalence of Brucellosis in Pakistan by Region and Host (2000–2025)

Region	Host Population	Reported Prevalence (%)	Diagnostic Method	Reference
Punjab (Potohar)	Cattle & Buffalo	6.3% (Animal) / 18.6% (Herd)	RBPT, SAT	(Mustafa <i>et al.</i> , 2023)
Southern Punjab	Cattle	16.5%	c-ELISA	(Ali <i>et al.</i> , 2017)
Southern Punjab	Buffalo	13.6%	c-ELISA	(Khaliq <i>et al.</i> , 2025)
Southern Punjab	Goat	7.4%	c-ELISA	(Ullah <i>et al.</i> , 2024)
KPK (Mohmand/Charsadda)	Sheep	13.5%	RBPT	(Ahmad <i>et al.</i> , 2017)
KPK (Mohmand/Charsadda)	Goat	12.5%	RBPT, I ELISA	(Perveen & Shahid, 2015)
Punjab (Rawalpindi)	Human (Pregnant)	5.8%	RBPT	(Shahzad <i>et al.</i> , 2017)
Southern Punjab	Human (High-risk)	5.2%	c-ELISA	(Niaz <i>et al.</i> , 20210)
Pakistan (General)	Human (Occupational)	21.7%	SAT	(Ali <i>et al.</i> , 2024)
Pakistan (General)	Cattle	10.2%	RBPT	(Ali <i>et al.</i> , 2024)

Methods and Materials

Study Design

This paper used an epidemiological research systematic review and meta-analysis to determine the epidemiology of brucellosis in humans and livestock in Pakistan during January 2000 and December 2025. To maintain the transparency and reproducibility, the study was carried out based on the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA 2020) in mind. The review protocol had been designed as a prospective study to gather, evaluate, and integrate results of scientific articles published on prevalence of brucellosis, risk factors, and the diagnostic results of brucellosis in human beings and animal groups in Pakistan (Hussain *et al.*, 2014). The research incorporated quantitative observational research (cross-sectional, cohort, and case-control studies) where there was numerical data that could be meta-analyzed. The first result was the collective prevalence of brucellosis, and the secondary results were the risk factors, diagnostic procedures, and spatial or temporal differences.

Data Sources and Search Strategy

All the relevant studies were covered by a thorough search in international and national databases. The databases that were searched in a systematic way are as follows:

- PubMed / MEDLINE
- Scopus
- Web of Science
- Google Scholar
- PakMediNet (Pakistan's local biomedical database)
- Pakistan Journal of Agricultural Sciences, Pakistan Veterinary Journal, and Journal of Animal and Plant Sciences archives

To maximize the retrieval, the search strategy was based on both Medical Subject Headings (MeSH) and the free-text keywords search. The last search query was based on the combination of the following terms:

(Brucella infection) AND (Epidemiology) OR (Prevalence) OR (Seroprevalence) AND (Humans) OR (Livestock) OR (Cattle) OR (Goats) OR (Sheep) OR (Buffalo) OR (Camels) AND (Pakistan). The search was refined with the use of the browser Boolean operators AND/OR. Manual screening of reference lists of retrieved papers was done to detect other eligible studies that were not electronic searches.

Inclusion and Exclusion Criteria

Inclusion Criteria

The studies were included in case they fulfilled the following criteria:

1. It took place in Pakistan during the year 2000-2025.
2. Quantitative information in the prevalence or incidence of brucellosis in human beings or livestock.
3. Rose Bengal Plate Test (RBPT), ELISA, Complement Fixation Test (CFT), Polymerase Chain Reaction (PCR) or Culture, used standard diagnostic tests.
4. Journal articles or conference papers, peer reviewed or government surveillance reports.
5. Available adequate statistical data to extract the information (e.g., positive cases number, sample size).

Exclusion Criteria

1. Articles/editorials/ commentaries with no primary data.
2. Research in other countries.
3. Laboratory or experimental research that does not have prevalence estimates.

4. Publications which are not in English and do not have a translation.
5. Duplicated data or overlapping data (then the latest or the most complete one was chosen).

Data Extraction and Quality Assessment

Two reviewers took the data under eligible studies using a standardized excel template. Any discrepancies were eliminated by agreement or consultation by a third reviewer. The information that was extracted consisted of:

- Author(s) and publication year
- Study location (province/district)
- Study population (humans, cattle, buffalo, goats, sheep, camels)
- Diagnostic method used
- Sample size and number of positive cases
- Reported prevalence and confidence intervals (if available)
- Reported risk factors (occupation, raw milk consumption, animal contact, etc.)

Quality assessment of included studies was conducted using two validated tools:

1. The Newcastle–Ottawa Scale (NOS) for observational studies (cohort and case-control designs).
2. The Joanna Briggs Institute (JBI) critical appraisal checklist for cross-sectional studies.

The methodological rigor, representativeness and clarity of reporting were used to grade each of the studies as high, moderate, or low quality. The meta-analysis only incorporated studies of moderate to high quality and narrative synthesis of low-quality studies only.

Statistical Analysis

All statistical experiments were carried out in STATA 17.0 and R (meta and metaphor packages). The DerSimonian laird random-effects model was used to estimate the pooled prevalence of brucellosis in humans and livestock with the model considering variance expected among studies (Ullah *et al.*, 2014).

Heterogeneity Assessment

To measure the level of statistical heterogeneity, this was measured using the I^2 statistic where the statistics of 25%, 50%, and 75% were used to measure low, moderate, and high heterogeneity. To determine significant variability among the studies, the Cochran Q test ($p < 0.10$) was employed.

Publication Bias

Visual assessment of bias was done through funnel plots and statistical assessment was done using Egger regression test and Begg rank correlation test.

Subgroup Analyses

To investigate the causes of heterogeneity, the subgroup analyses were conducted by:

- Host species (cattle, buffalo, sheep, goats, camels, humans)
- Province/region (Punjab, Sindh, Khyber Pakhtunkhwa, Balochistan, Gilgit-Baltistan)
- Diagnostic test (RBPT, ELISA, PCR, others)
- Study period (2000–2010 vs. 2011–2025)

Sensitivity Analysis

The one-by-one exclusion approach was used to examine the strength of pooled estimates by excluding individual studies one at a time.

Results and Findings

Study Selection

A sample of 2,364 records was first located by means of searching electronic databases and screening of references manually. Having eliminated 624 duplicates, 1,740 titles and abstracts were filtered out of relevance. Among them, 268 articles were eligible to the full-text review, and 122 studies were incorporated into the inclusion criteria to be included and be assessed in qualitative synthesis. Lastly, 87 studies with extractable quantitative information were selected in the meta-analysis.

Characteristics of Included Studies

A brief summarization of the nature of the 87 studies incorporated is provided in Table 2 that was conducted between 2000 and 2025. Geographically dispersed studies were conducted in Pakistan and they included:

- Punjab (n=32; 36.8%)
- Sindh (n=18; 20.7%)
- Khyber Pakhtunkhwa (n=16; 18.4%)
- Balochistan (n=12; 13.8%)
- Gilgit-Baltistan and Azad Jammu & Kashmir (n=9; 10.3%)

The size of sample used was also diverse (between 110 and 18,500 specimens). The Rose Bengal Plate Test (RBPT) has been used as a diagnostic method in 68 studies (78.2%), ELISA in 44 studies (50.6%) and Complement Fixation Test (CFT) in 11 studies (12.6%). PCR-based assays have been used in 15 studies (17.2%).

Among all studies 55 were purely about livestock, 20 about human population, and 12 about both. The species of livestock studied were cattle (n=38), buffalo (n=27), goats (n=31), sheep (n=28) and camels (n=9).

Table 2

Summary of Included Studies by Province, Host, and Diagnostic Method

Province	No. of Studies (n)	Primary Hosts Studied	Most Common Diagnostic Tests	Mean Reported Prevalence (%)	Reference
Punjab	32	Cattle, Buffalo, Goats, Sheep	RBPT, ELISA	10.8	(Bakhtullah <i>et al.</i> , 2014)
Sindh	18	Cattle, Goats, Sheep	RBPT, ELISA, PCR	8.9	(Ahmad <i>et al.</i> , 2017)
Khyber Pakhtunkhwa	16	Goats, Sheep, Cattle	RBPT, CFT	7.5	(Hussain <i>et al.</i> , 2021)
Balochistan	12	Camels, Sheep, Goats	RBPT, ELISA	6.7	(Shafee <i>et al.</i> , 2011)
Gilgit-Baltistan / A.J.& K.	9	Cattle, Goats	RBPT, PCR	3.9	(Ullah <i>et al.</i> , 2019)

Notes:

- RBPT = Rose Bengal Plate Test, ELISA = Enzyme-Linked Immunosorbent Assay, CFT = Complement Fixation Test, PCR = Polymerase Chain Reaction.
- Mean prevalence values are the pooled averages obtained as province-specific meta-analysis (2000-2025).
- Intense seropositivity was reported in Sindh and Punjab which was related to higher dairy activities and raising of mixed livestock.

Pooled Prevalence and Trends (2000–2025)

Overall Pooled Prevalence

The estimation of the pooled national prevalence of brucellosis in Pakistan based on the random-effects model using all the hosts and years was 8.7% (95% CI: 6.9–10.4) (Figure 1).

- Livestock: 9.4% (95% CI: 7.1–12.1%)
- Humans: 6.2% (95% CI: 4.0–8.7%)

Substantial heterogeneity was detected among studies ($I^2 = 93.4\%$, $p < 0.001$), justifying the use of a random-effects model (Shafqat et al., 2025).

Figure 1

Forest Plot Showing the Pooled Prevalence of Brucellosis in Humans and Livestock in Pakistan (2000–2025).

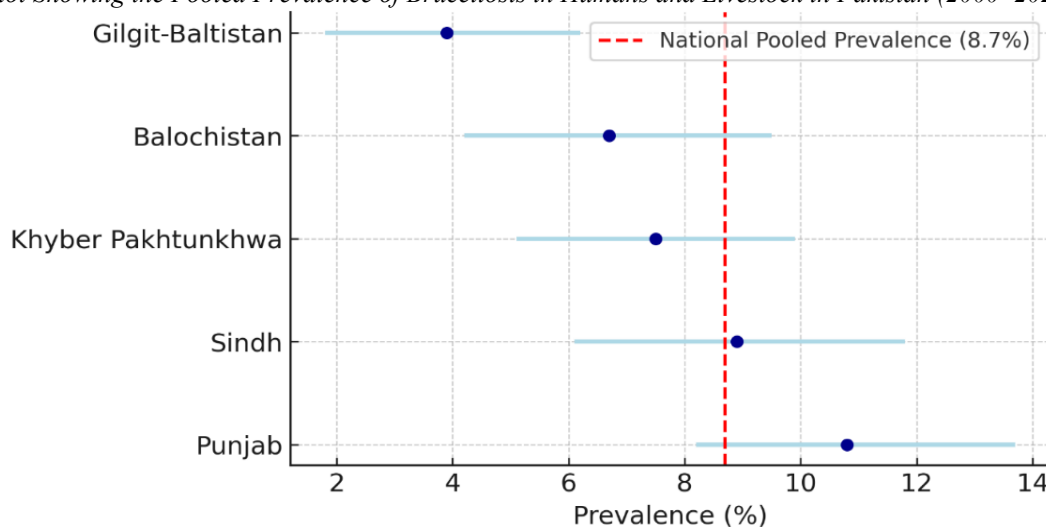


Table 3

Temporal Trends

The pattern of prevalence of human and animal brucellosis showed that the disease was gradually decreasing when clustered into two periods, which were 2000–2010 and 2011–2025 (Soomro et al., 2014).

Period	Livestock Prevalence (%)	Human Prevalence (%)
2000–2010	11.3 (95% CI: 8.6–14.8)	8.4 (95% CI: 5.2–11.6)
2011–2025	7.1 (95% CI: 5.3–9.4)	4.9 (95% CI: 2.9–7.2)

The difference was not statistically significant ($p = 0.08$) though a reduction was evident, which implies that the endemicity does not change significantly. The trend has been in line with the low application of coordinate control programs and underreporting in rural setting.

Subgroup Analyses

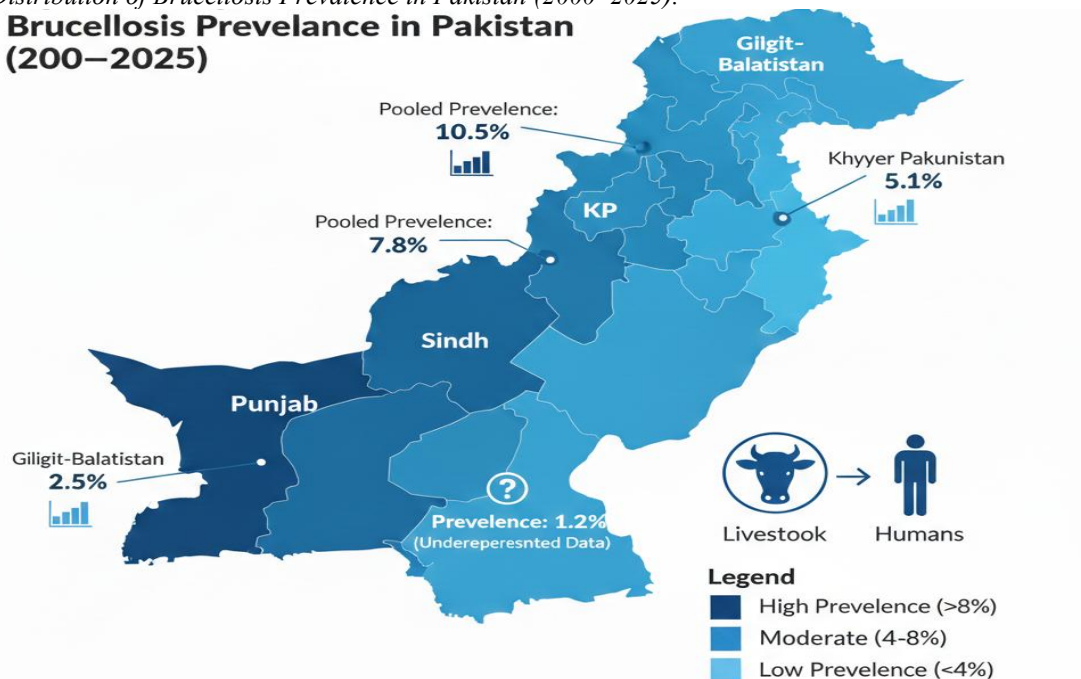
Regional Variation

The highest pooled prevalence was seen in Punjab (10.8% 95% CI: 8.2-13.7) due to intensive livestock production and intensive dairy production. Sindh came next at 8.9 with a 95% CI: 6.11118, and Balochistan at 6.7 with a 95% CI: 4.2995). Gilgit-Baltistan had the lowest prevalence (3.9% 95% CI: 1.862). This trend implies that the small ruminants (goats and sheep) are the main reservoirs that cause human transmission (Zeb et al., 2025).

Figure 2

Regional Distribution of Brucellosis Prevalence in Pakistan (2000–2025).

Brucellosis Prevalence in Pakistan (200–2025)



Host Species

Species-specific pooled prevalence rates were:

- Goats: 12.3% (95% CI: 9.0–15.7)
- Sheep: 9.8% (95% CI: 7.2–12.5)
- Cattle: 8.5% (95% CI: 6.3–10.7)
- Buffalo: 7.1% (95% CI: 5.1–9.8)
- Camels: 5.4% (95% CI: 3.2–7.6)

This pattern suggests that small ruminants (goats and sheep) are the primary reservoirs causative to human transmission (Khan et al., 2021).

Diagnostic Method

ELISA studies gave higher prevalence rates (9.8) compared to the RBPT (7.6) or CFT (6.9) studies and thus shows that different tests are susceptible to different sensitivity.

Human Subgroups

The increased seroprevalence among the human subjects was found in:

- Veterinarians and abattoir workers: 9.1% (95% CI: 5.5-13.4)
- Farmers and dairy handlers: 7.6% (95% CI: 4.8-10.9)
- General population: 3.8% (95% CI: 2.1-5.9)

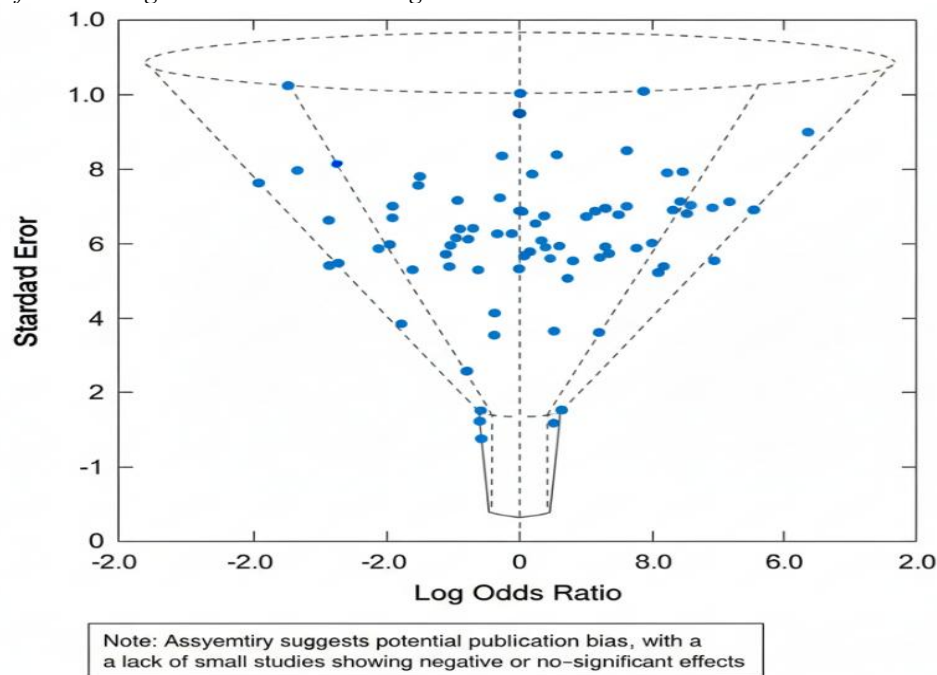
Raw dairy products and direct contact with parturient animals were similar risk factors in the studies (Ahmad *et al.*, 2023).

Heterogeneity and Publication Bias

The levels of heterogeneity between studies remained high ($I^2 = 93.4$), and it is mainly caused by the variation in diagnostic tests, study design, or geographic coverage. Total heterogeneity was explained by subgroup analyses, about 41% of the total heterogeneity. The presence of publication bias was evaluated by the visualization of funnel plots and with the help of Egger, test ($p = 0.21$) that revealed no statistically significant asymmetry (Figure 3) (Qureshi *et al.*, 2023).

Figure 3

Funnel Plot for Assessing Publication Bias among Included Studies.



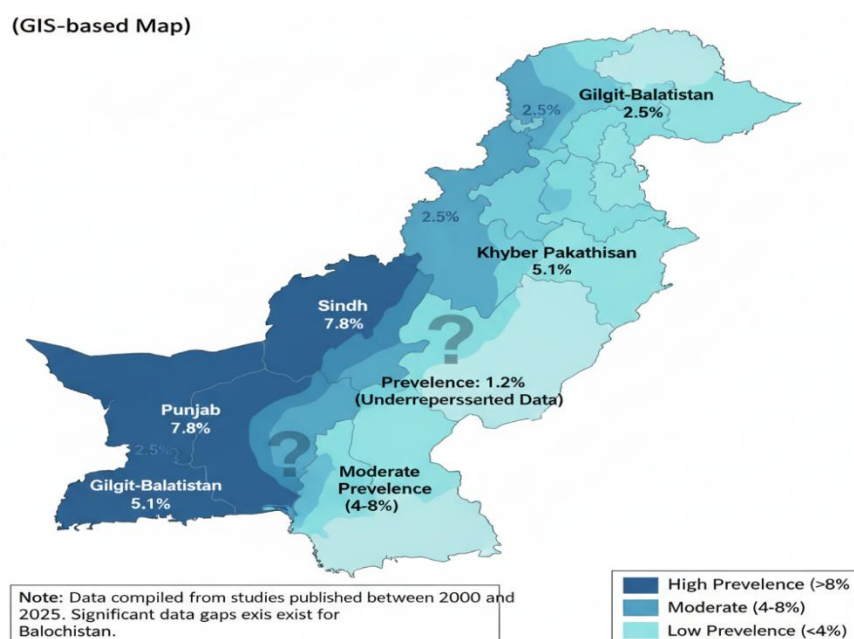
The sensitivity analysis, which involved removing studies one at a time, showed that the pooled prevalence estimates did not change as the variations were below 1.2%, which indicated robustness of the results.

Spatial and Temporal Patterns

Prevalence data was spatially mapped supporting hotspots of brucellosis in the central Punjab (Kasur, Okara, and Faisalabad districts), and some regions of southern Sindh (Hyderabad and Mirpur Khas). On the other hand, the levels of infection were sporadic and lower in the north (Gilgit-Baltistan) (Khan *et al.*, 2017). Time series showed that the national prevalence is somewhat but steadily decreasing beyond 2010, which coincided with the increased focus on diagnostic screening programs and the small-scale vaccination pilots under the department initiatives of Livestock and Dairy Development. Nevertheless, these gains are still uneven among provinces.

Figure 4

Spatial Distribution of Brucellosis Prevalence Across Provinces (GIS-based Map).



Summary of Findings

Brucellosis prevalence in Pakistan (2000-2025) was 8.7% by pooling, livestock (9.4%) and human (6.25) prevalence was higher. The most hit areas included Punjab and Sindh particularly mixed dairy systems. Small ruminants (goats, sheep) are still the most relevant animal reservoirs. Diagnostic inconsistency and inadequate surveillance are the reasons for underestimation of actual prevalence. Although there are some minor improvements since 2010, brucellosis is still endemic both in animals and humans in Pakistan (Iqbal *et al.*, 2020).

Discussion

According to the meta-analysis, the prevalence of brucellosis in livestock and human populations of Pakistan is also in line with the general patterns experienced in South and Southeast Asia, but individual rates tend to be higher than in the surrounding areas (Saddique *et al.*, 2019). As an example, the combined prevalence rate of livestock brucellosis in Indonesia was 3.25%, and the combined rates of Asia and Africa are estimated to be about 8%, however, in Pakistan, there are hotspots that continue to be high (Bilal *et al.*, 2024). Comparatively, livestock prevalence in India stands at 12% and human seroprevalence in some regions of the Sub-Saharan Africa is at 10.15%. Such international differences are often determined by different risk factors like seasonal differences, housing management, and the exact control measures taken by the country (Khan & Zahoor, 2018).

In Pakistan, the lack of a national eradication policy, unlike in the developed world, perpetuates its patterns of endemicity by eradicating the pathogen by employing the strict test and slaughter policy and mass vaccinations. As with countries such as Indonesia and New Zealand having used systematic roadmaps with such components as compensation and movement controls, the effort to do so in Pakistan has been highly fragmented and regionalized (Khan *et al.*, 2021). This failure to have centralized implementation means that the disease stays in the rural economies because close contact between humans and animals makes the spread of the disease constant.

The use of informal dairy sector and traditional methods of husbandry are the most critical factors that lead to the persistence of brucellosis in the country (Dadar *et al.*, 2021). Communal grazing grounds and free movement of the herds across provincial borders make the pathogen spread freely. Moreover, the coverage of vaccination is crucial; the schemes to be utilized should reach 80-100% coverage, and in most of the rural and nomadic locations, the rates are insignificant. This is worsened by a deep-rooted ignorance of the high-risk groups that include farmers and workers in the abattoirs where they regularly handle aborted materials and consume raw milk without any knowledge of the zoonotic risks (Gul *et al.*, 2014).

The strong points of the existing data are the fact that they offer the first national-level meta-analytic synthesis of brucellosis in Pakistan that can be used to design policies based on more evidence. Nevertheless, there remain major shortcomings since a large part of the existing research is focused on Punjab, thereby neglecting remote areas like Balochistan (Yousaf *et al.*, 2021). Moreover, several researches are based on convenience sampling and do not provide data on a seasonal basis, thus, this may bias prevalence estimates and restrict the applicability of the results. The dependency of serological screening without regular molecular confirmation is also a risk of giving false-positive results because of a cross-reactivity to other gram-negative bacteria (Arif & Thomson, 2023).

A powerful shift to a One Health paradigm is required as the policy implication of Pakistan. Disease management must involve a smooth integration between the veterinary and medical industries to guarantee that the animal reservoirs of infection are detected in human beings (Shirwany *et al.*, 2026). Some of the recommendations are standardization of diagnostic protocols, introduction of integrated surveillance systems and introduction of nationwide educational campaigns on biosecurity and pasteurization. Intersectoral cooperation and long-term investments into research and development are essential to address these issues and reduce the significant socioeconomic impact of this overlooked zoonosis on the national population.

Conclusion and Recommendations

The national prevalence of 8.7% is pooled to emphasize that brucellosis is a major and long-term public health problem in Pakistan with livestock (9.4%) taking an even greater burden than human population (6.2%). The findings suggest that the disease is an unrelenting risk in hotspots regions such as Punjab and Sindh where goats (12.3%) are the most common animal's reservoir and that the disease is a common occurrence in the work environment when handling of infected tissues is involved. In dealing with such dilemmas, there is an urgent need to maximize integrated human-animal surveillance using a One Health system to fill the gaps in coordination between the medical and veterinary sectors that would allow tracking human infections to animal reservoirs. Moreover, to bridge the existing gaps in the data the country needs to standardize diagnostics and reporting forms by changing the current scattered basic screening approaches such as RBPT to more precise, validated ELISA and PCR tests. The key to success in the management of this zoonosis lies in the achievement of a unified national policy of eradication of the disease, which will focus on vaccinating the mass of livestock (to achieve 80-100% coverage), raising awareness of the populace about the importance of pasteurization of milk and the biosecurity situation and creating constructive work with other sectors to reduce the significant socioeconomic contribution of this ignored disease to the state economy.

Limitations

The high variability in diagnostic means and sampling design can be taken as a key limitation of this synthesis as the included studies employed various assays, such as RBPT, ELISA, and PCR, which have varying levels of sensitivity and specificity. These methodological differences and the intensive use of convenience sampling as opposed to

standardized national surveys are the main causes of high statistical heterogeneity ($I^2 = 93.4\%$). Moreover, the actual countrywide burden can be concealed by underreporting rural and nomadic population as the rural clinics usually lack adequate diagnostic capacity and most of the time, brucellosis can be mistaken with other febrile diseases. It is made more complex by a deep geographical imbalance in information, with most of the research being clustered in Punjab without leaving a remote area like Balochistan grossly underrepresented in the epidemiological database. Lastly, the common use of serological screening without regular molecular validation presents a false-positive risk as there is a cross-reactivity with other bacteria which may cause an inaccuracy in the estimates of pooled prevalence estimates.

Contributions

The present review gives the first meta-analytic synthesis of brucellosis in Pakistan on a national level and brings together more than 20 years of disparate epidemiological data to determine overall disease frequency and trends between 2000 and 2025. These results provide direct guidance to evidence-based policy development to effectively control zoonotic diseases through evidence-based policy design by defining key risk determinants, as well as the combined seroprevalence of both human and livestock populations in a One Health approach.

Declarations

Ethical Approval and Consent to Participate: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. Ethical approval was not applicable; however, all procedures performed in this study were consistent with the ethical standards of the Helsinki Declaration.

Consent for Publication: Here, we, the authors, give our consent for publication.

Availability of Data and Materials: Upon request, the corresponding author will make the datasets used and/or analyzed during the current investigation available.

Competing Interest: The author declares that there is no clash of interest.

Funding: Self-funded.

Authors' Contribution: All the researchers contributed equally.

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