



## Systematic review and meta-analysis on brucellosis seroprevalence in ruminants (1970 – 2020) of India

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### ABSTRACT

Brucellosis is one of the most common contagious and neglected bacterial zoonotic diseases. The disease has been reported in ruminants in almost all Indian states. But there is a lack of comprehensive information on the seroprevalence of brucellosis in the Indian subcontinent. A systematic review and meta-analysis on the seroprevalence of brucellosis among ruminants of India was conducted from the published articles (January 1970 - June 2020) by including 172 studies screened from 567 publications. The estimated brucellosis seroprevalence of cattle, buffalo, sheep, goat, yak and mithun was 14% (95% CI: 12% - 16%), 8% (95% CI: 6% - 9%), 8% (95% CI: 7% - 10%), 8% (95% CI: 7% - 9%), 16% (95% CI: 7% - 28%) and 26% (95% CI: 12% - 42%), respectively. Sub-group analysis was performed based on diagnostic tests, regions, publication year, and sample size. The estimated seroprevalence of brucellosis in cattle and goats was found to be higher in the central region compared to other regions. Similarly, the western region showed a higher seroprevalence for brucellosis in buffalo and sheep. Given the estimated animal population of 2021, the meta-analysis estimated that the total number of seropositive animals would be 26.95 million cattle (95% CI: 23.09–30.78), 8.78 million buffaloes (95% CI: 6.59–9.89), 5.94 million sheep (95% CI: 5.20–7.43), 11.91 million goats (95% CI: 10.42–13.40), 9.6 thousand yaks (95% CI: 0.0042–0.0168), and 100 thousand mithun (95% CI: 0.05–0.16). Further, the comprehensive picture of the brucellosis seroprevalence may help the decision-making authorities in formulating better prevention and control strategies for brucellosis in India.

**Keywords:** *Brucella*, India, Meta-analysis, Ruminants, Seroprevalence, Systematic review

Brucellosis is one of the most contagious re-emerging bacterial diseases in the world (Schaeffer *et al.* 2021). It was first identified in India in 1942 and causes abortion in pregnant animals and reduced fertility (Khan and Zahoor 2018, Renukaradhya *et al.* 2002). Globally, it is one of the most prevalent zoonotic diseases with a substantial social and economic burden (Dean *et al.* 2012). Brucellosis in animals is an important source of human infection (Li *et al.* 2013). The two routes of *Brucella* infection are direct (contact with infected animal or secretions) and indirect contact (consumption of infected under pasteurized milk or raw milk and dairy products). Brucellosis transmission to humans through meat consumption is very uncommon (Olsen and Palmer 2014, Migisha *et al.* 2018). Meta-analysis may give rise to the development of new research procedures, and address the policymakers to find better prevention and control strategies for various infectious diseases (Fagard *et al.* 1996). It is the gold standard for evidence-based research with the highest clinical relevance. It has been conducted successfully in finding the prevalence

of other infectious diseases like bluetongue (Rupner *et al.* 2020), classical swine fever (Patil *et al.* 2018, Malik *et al.* 2020) and Peste des petits ruminants (Barman *et al.* 2020) in India. Meta-analysis studies on the prevalence of brucellosis have been carried out in Tanzania (Alonso *et al.* 2016), China (Ran *et al.* 2018, Ran *et al.* 2019), Middle East region (Abedi *et al.* 2020), for analyzing the treatments in human brucellosis (del Pozo *et al.* 2012), to identify the seasonal pattern of the disease (Moosazadeh *et al.* 2016), and to identify the susceptibility of *Brucella* meta to antibiotics (Khademi *et al.* 2018). In India, Barman *et al.* (2020) performed a meta-analysis to determine the prevalence of brucellosis in the North-East region among the different species of livestock. Several seroprevalence studies in India were carried out either on non-random samples or on suspected samples, making the results biased to determine the incidence and prevalence in the general population. Hence, a meta-analysis on brucellosis seroprevalence was undertaken to estimate the pooled seroprevalence of brucellosis in the random population of ruminants in India.

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### MATERIALS AND METHODS

To estimate the brucellosis seroprevalence a literature

search was performed for the systematic review and meta-analysis covering the published literature of the past 51 years (1<sup>st</sup> January, 1970 to 30<sup>th</sup> June, 2020) (Supplementary Table 1). The data was gathered using a computer literature search of electronic databases through Google Scholar, Science Direct, Springer, Krishikosh, PubMed, ICAR-CeRA, and non-electronic material search of the thesis, journals, symposium, abstracts, and ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) annual reports. The keywords used for search on the electronic media were brucellosis, *Brucella*, animals, cattle, buffalo, sheep, goat, yak, mithun, epidemiology, prevalence, and seroprevalence.

The schematic representation for the PRISMA procedure followed in the literature selection in this systematic review and meta-analysis of brucellosis seroprevalence in India is represented in Fig. 1. The quality assessment was carried out using the Joanna Briggs Institute (JBI) critical appraisal checklist for studies reporting seroprevalence data. Inclusion and exclusion criteria are described in Table 1. Following the set criteria, two independent investigators (MVL and DKS) screened these studies manually and a third investigator (VOR) resolved any disagreement between the two investigators.

The extraction and coding of the study details such as the author, state, publication year, type of test, the total

number of samples tested, number of positive samples, and geographical region were done in Microsoft excel spreadsheets. For each species, studies were further divided into various sub-groups based on diagnostic tests, region, publication year, and sample size. The states which reported the seroprevalence of brucellosis were categorized into the Northern region (Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Delhi, Uttarakhand, and Uttar Pradesh), Southern Region (Andhra Pradesh, Telangana, Karnataka, Kerala, and Tamil Nadu), Western region (Rajasthan, Gujarat, Maharashtra and Goa), Central region (Chhattisgarh and Madhya Pradesh), Eastern region (Bihar, Jharkhand, West Bengal, and Odisha), Northeastern region (Sikkim, Assam, Meghalaya, Arunachal Pradesh, Nagaland, Manipur, Mizoram, and Tripura), Andaman & Nicobar Islands, and all India. The sub-groups based on the publication year were done for three publication periods (1970-1986, 1987-2003 and 2004-2020) and based on the sample size were completed for two groups (sample sizes of 1- 200 and >200).

Publication bias was assessed by visualizing the symmetry of the funnel plot, rank correlation and Eggers test. Brucellosis seroprevalence was estimated using 92 studies in cattle (sample size= 1,23,273), 52 studies in buffalo (sample size = 40,336), 50 studies in sheep (sample size = 51,911), 61 studies in goat (sample size= 53,837), 4 studies

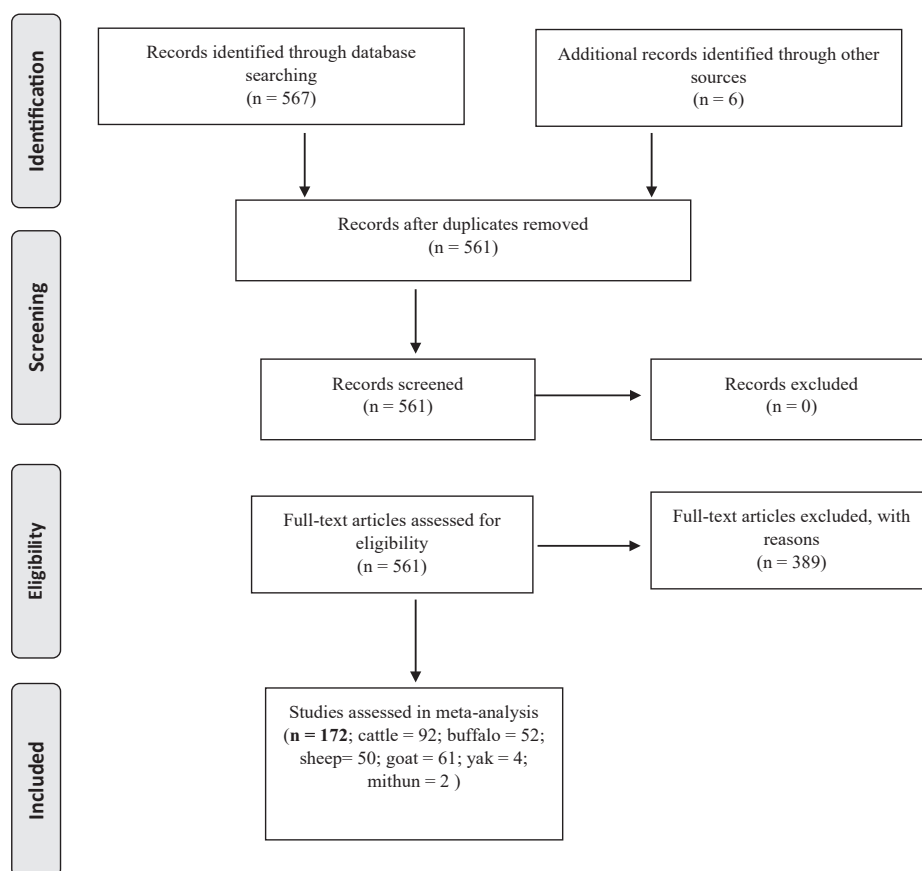


Fig. 1. PRISMA schematic diagram showing the brucellosis seroprevalence studies (January 1970- June 2020) included for meta-analysis.

Table 1. Inclusion and exclusion criteria used for systematic review and meta-analysis on brucellosis seroprevalence in ruminants (1970 – 2020) of India

Inclusion criteria	Exclusion criteria
Studies related to brucellosis with accessible full text.	Studies not related to brucellosis
Studies on brucellosis status in India.	Studies on brucellosis status in countries other than India
Random sampling and sample size mentioned	Purposive sampling and sample size or species were not mentioned
Publication year of the studies (1970 - June, 2020).	Publication year of the studies before 1970 and after June, 2020
Studies that include ruminants only.	Studies which does not include ruminants
Serological diagnosis*	Non- serological diagnosis
Studies specifying the test used for diagnosis, excluding the tests 2-ME and MRT (for bulk milk samples)	Studies not specifying the test used for diagnosis as well as the tests 2-ME and MRT (for bulk milk samples)
Seroprevalence studies about brucellosis	Studies that deal with isolation, serotyping, differentiation of biovars, and review articles that are not related to prevalence of brucellosis

\* Studies with single test results and results >1 tests in parallel and sequential testing were included in the meta-analysis and the test giving higher sensitivity was used for estimation of the overall seroprevalence. For diagnostic test-based sub-group analysis, results of the same diagnostic methods employed in each study were combined to estimate the diagnostic test-wise seroprevalence.

in yak (sample size= 760) and 2 studies in mithun (sample size= 170). Baujat plot (Baujat *et al.* 2002) (Supplementary Fig. 1), Cochran's Q and Higgin's I<sup>2</sup> methods were used to assess the study heterogeneity. The meta-analysis was performed using the inverse-variance model (DerSimonian and Laird 1986) and Freeman-Tukey double arcsine transformation (Harris *et al.* 2008, Nyaga *et al.* 2014). The pooled estimate was reported as seroprevalence with 95% confidence intervals (CI), and prediction intervals (PI). Using the forest plots (Supplementary Fig. 4), the seroprevalence in each study along with the combined estimated seroprevalence was visualized. To identify the influential studies, a set of case deletion diagnostics such as covariance ratio (COVRATIO), studentized residuals, Cook's distances, the difference in fits values (DFFITS), and leave-one-out estimates, were used (Viechtbauer and Cheung 2010). Sub-group analyses were performed to identify the stratified seroprevalence of brucellosis by different diagnostic tests, regions, publication year, and sample size. However, the sub-group analyses were not conducted for yak and mithun because of fewer studies in each stratum.

The statistical analyses were carried out using the R statistical platform (R Foundation for Statistical Computing, Vienna, Austria version 3.5.1) with "metafor" package (version 2.0-0) and "meta" package (version 4.9-2).

## RESULTS AND DISCUSSION

The proportions for brucellosis seroprevalence were estimated with the 172 included studies and the quantitative analyses provided a sample size of 123,273 in cattle, 40,336 in buffaloes, 51,911 in sheep, 53,837 in goats, 760 in yak and 170 in mithun. Three studies in cattle (Chakraborty *et al.* 2000, Kaushik *et al.* 2010 and Kushwaha *et al.* 2015), one in buffalo (Kant *et al.* 2015), three studies in sheep (Hussain *et al.* 2017, Padher *et al.* 2017, Sonekar *et al.* 2018), and one study in goat (Padher *et al.* 2017) contributed to the heterogeneity. Except, for the study of

Sonekar *et al.* (2018) on sheep other heterogeneity studies of cattle (3 studies), buffalo (one study), sheep (2 studies), and goat (one study) were identified as influential studies (Supplementary Figs. 2 and 3).

The pooled estimate of brucellosis seroprevalence by random effect (RE) model in cattle was 14% (95% CI: 12% - 16%), and in buffalo was 8% (95% CI: 6% - 9%). The seroprevalence estimate in sheep and goat was 8% (95% CI: 7% - 10%) and 8% (95% CI: 7% - 9%) respectively. The pooled seroprevalence estimate of yak and mithun was 16% (95% CI: 7% - 28%) and 26% (95% CI: 12% - 42%) respectively.

For cattle brucellosis, the estimated region-wise seroprevalence was found to be the highest in the Central region (16%), followed by the Western region (14%) and Andaman & Nicobar Islands (14%). Whereas in buffaloes, the highest seroprevalence was noticed in the Western region (12%), followed by the North-eastern region (9%). Suresh *et al.* (2022) conducted a meta-analysis for the period between 2000 -2020 and estimated brucellosis pooled prevalence of the Central region (19%), Western region (15%), Southern region (12%) and Northern region (11%). The present study meta-analysis between 1970-2020 and the study of Suresh *et al.* (2020) between 2000-2020 indicates the endemicity of brucellosis in different regions on India.

The national survey done by Isloor *et al.* (1998) found that the seroprevalence of brucellosis in cattle and buffalo was 1.9% and 1.8%, respectively which is much lower when compared to our study. The low level of seroprevalence could be attributed to the use of STAT alone in the estimation of prevalence. By using large random samples, Shome and coworkers (2019) estimated the seroprevalence of 9% in cattle and 5% in buffalo in India. A long-term study indicated the brucellosis seroprevalence of 5% in cattle and 3% in buffaloes (Renukaradhya 2002). Studies reported wide variations in seroprevalence of brucellosis, such as 6.6% in the Central state of Madhya

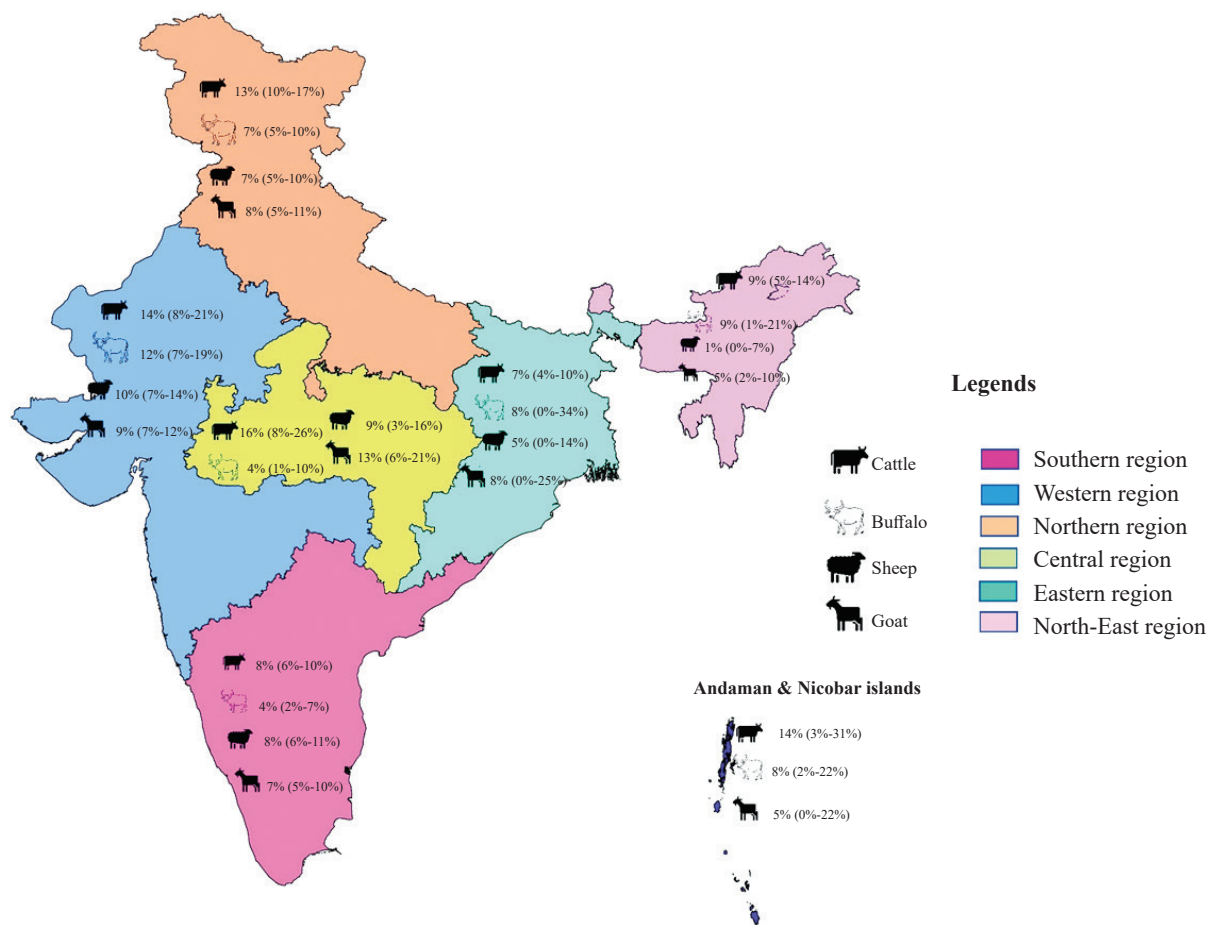


Fig. 2. Region-wise seroprevalence (in percentage with 95% confidence interval) map of brucellosis in cattle, buffalo, goats and sheep.

Pradesh (Mehra *et al.* 2000), and 60% in the north-eastern state of Assam (Chakraborty *et al.* 2000).

The region-wise seroprevalence for all the species is depicted in Fig. 2. Period-wise analysis depicted the highest seroprevalence was noticed during 2004-2020 in cattle, buffalo, sheep, and goat. In sheep, the highest seroprevalence was observed in the Western region (10%). While in goats, the highest seroprevalence was noticed in the Central region (13%), followed by the Western region (9%). The meta-analytic seroprevalence estimate in sheep and goat was 8% (95% CI: 7%-10%) and 8% (95% CI: 7%-9%), respectively. The annual report by NIVEDI (2019) showed the prevalence of brucellosis in goats and sheep nationwide as 5% and 11%, respectively. The present study was in agreement with Shome *et al.* (2015) who studied the seroprevalence of brucellosis in small ruminants considering all the different Indian regions and found it to be 9% and 6% in sheep and goats, respectively, in agreement with our study. A recent countrywide study by Shome *et al.* (2021) revealed higher seropositivity in sheep (11.55%) compared to goats (5.37%).

Several diagnostic tests were used in the identification of brucellosis in animals. Variation of brucellosis seroprevalence was noticed in diagnostic test-based subgroup analysis. However, the sample size-wise subgroup

analysis observed fewer variations in the brucellosis seroprevalence of ruminants in India.

Hitherto, given the estimated animal population of 2021, the meta-analysis estimated that the total number of seropositive animals would be 26.95 million cattle (95% CI: 23.09–30.78), 8.78 million buffaloes (95% CI: 6.59–9.89), 5.94 million sheep (95% CI: 5.20–7.43), 11.91 million goats (95% CI: 10.42–13.40), 9.6 thousand yaks (95% CI: 0.0042–0.0168), and 100 thousand mithun (95% CI: 0.05–0.16).

The region-wise analysis of different species of ruminants showed that brucellosis seroprevalence was higher in the Western region for buffalo (12%) and sheep (10%), meanwhile the Central region for cattle (16%) and goats (13%). Kanani *et al.* (2018) have also reported a high seroprevalence of 23.57% among sheep in Gujarat, the Western state of India. The increase in the number of samples covering a wide geographical area could be the reason for this high seropositivity. High seroprevalence of brucellosis (31-37.8%) was reported among buffalo in different Western states of India (Soni *et al.* 2014, Kala *et al.* 2018). The use of ELISA as the diagnostic test may be the result of this high seroprevalence. Jain *et al.* (2019), Shome *et al.* (2019), Namrata *et al.* (2016), and Maiti *et al.* (2012) have also reported a high prevalence of brucellosis among

cattle in the Central region of India at 15%, 11%, 25.8%, 31.2%, respectively. The prevalence reported by Jain *et al.* (2019) of 15% is similar to our study. Sai *et al.* (2018) have reported a prevalence (13.6%) similar to our study among goats in the central region of India. Bandyopadhyay *et al.* (2009) studied the seroprevalence of brucellosis in yak using RBPT, STAT and AB-ELISA and the seroprevalence ranged between 18.98% - 23.79% by these three tests. A study conducted by Rajkhowa *et al.* (2005) in mithuns maintained at the National Research Centre on Mithun, Nagaland, India revealed that the number of animals found positive for brucellosis in AB-ELISA, STAT, RBPT were 34, 20 and 11%, respectively.

Publication period-wise analysis showed that the seroprevalence of brucellosis was highest during 2004-2020 in cattle (15%), buffalo (11%), goats (9%), and sheep (9%). Overall, an increasing trend of brucellosis seroprevalence was observed since 1970. Gill *et al.* (2000) observed an increase in seroprevalence of brucellosis in Punjab state from 1990 to 1999 among ruminants. The absence of a vaccination program among the majority of the Indian farms may be the cause of this increasing trend. Shome *et al.* (2020) also identified the drastic increase in brucellosis seropositivity among small ruminants of Indian states between 2006-2018, similar to this study. This increase in brucellosis seroprevalence is believed to be highly attributed to the lack of vaccination among small ruminants (Pushpa 2005).

In this study, we have found that there is a high variation in the choice of diagnostic test used for brucellosis. This meta-analysis identified approximately 15 different kinds of diagnostic tests used for the estimation of brucellosis seroprevalence. This systematic review identified a few studies that used STAT or RBPT for seroprevalence, both the tests have lower specificity and sensitivity compared with the methods recommended (indirect-ELISA and Fluorescence Polarization Assay) by WHO (Corbel *et al.* 2006, Franco *et al.* 2007). A comparison study based on sensitivity and specificity analysis of various diagnostic tests for brucellosis showed the buffered plate agglutination test (BPAT) to be the best among the conventional tests (Gall and Nielsen 2004). Hence, the selection of a proper diagnostic test is important for brucellosis serosurveillance.

The Brucellosis Control Programme (B-CP), which started in 2010 focused only on selected districts of India, however, the recently implemented National Animal Disease Control Programme (NADCP) focuses on all the districts of India and targets vaccination bovine calves of 4-8 months. This systematic review and meta-analysis on brucellosis seroprevalence may give a comprehensive idea to the decision-making authorities for implementing an efficient brucellosis control programme.

A few limitations of the study are that the risk factors associated with brucellosis seroprevalence were not analyzed due to the scarcity of random sample studies in each species including the risk factors which was not sufficient to perform an effective meta-analysis. Most of

the studies mixed-up the risk factors and risk indicators. This systematic review and meta-analysis indicate that brucellosis is endemic across India, hence widespread surveillance is required for understanding the overall prevalence of brucellosis. Being zoonotic, brucellosis prevalence should be monitored more intensively to gather comprehensive information and to identify the high-risk areas to adopt better prevention and control measures.

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