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Spatial seroprevalence of bovine brucellosis in India—A large random sampling survey



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ABSTRACT

Brucellosis caused by *Brucella* spp. is an important zoonosis and constitutes a serious public health hazard. In India, the disease is increasingly prevalent among bovine population with high zoonotic potential and negative impact on national economy. The investigation was conducted to study seroprevalence of brucellosis through random sample survey using survey tool box software. A total of 12,054 [cattle-9236, buffaloes-2818] bovine serum samples sourced from 15 states of India were tested by protein G indirect ELISA. The true prevalences of brucellosis observed in cattle and buffaloes were 8.3% and 3.6%, respectively. The highest prevalence of brucellosis was observed in the state of Punjab in both cattle and buffaloes (23.51 and 10.2%). Comparatively higher prevalence was recorded in cattle than the buffaloes in all the states except Manipur. The true prevalence greater than 5% was recorded in 8 and 3 states for cattle and buffaloes, respectively [(cattle- Punjab, Maharashtra, Rajasthan, Karnataka, Madhya Pradesh, Tamil Nadu, Gujarat and Kerala) and (buffaloes-Punjab, Gujarat and Manipur)] indicating wider prevalence of brucellosis. This study conclusively highlighted the seroprevalence of bovine brucellosis at state level which might be useful for prioritizing regions for vaccination, designing control strategies and improvisation of clinical surveillance system.

1. Introduction

Brucellosis in humans and animals is second most important zoonoses after rabies and most challenging issues for health and the economy in number of developing countries including India [1]. Despite several surveillance and control efforts, the disease is increasingly prevalent among bovine population and humans causing huge impact on national economy [2,3]. The economic losses in animals due to brucellosis can be reduced through restrictions in commerce, slaughter, replacement of seropositive animals from farms and vaccination [4]. Conversely, it is difficult to measure economic losses due to human brucellosis such as medical costs, morbidity and reduced work efficiency [5]. Brucellosis causes persistent infection by its capacity to escape from innate and adaptive immunity and till date there is no vaccine for human use for prevention of brucellosis [6]. Hence, prevention of brucellosis in humans can be achieved by controlling the disease in animals. Thus, control of transmission of brucellosis infection demands effective surveillance, control and eradication efforts in bovines and other livestock population [7].

India has the largest buffalo population (105.34 million- 57.3%) and

second largest cattle population in the world (199.08 million- 14.7%). Brucellosis is a significant and increasing veterinary and public health problem in India as the 80% of the population livelihood depends on livestock and they live in close contact with domestic/ wild animal population owing to their occupation. Hence, human population stands at a greater risk of acquiring zoonotic diseases including brucellosis [7]. Brucellosis has been reported in organised, unorganised dairy sector and through national surveys [8–13]. Most of these reports are based on non-random or purposive sampling approaches or samples collected for screening other diseases. To determine the status of brucellosis in the state of Punjab, India, two stage random sampling approach using survey tool box has been reported [8]. Brucellosis epidemiology is ever changing in the country and continuous reporting of the disease is useful for prioritizing vaccination strategies in highly endemic brucellosis states to prevent and control the disease spread. The objective of the study was to record seroprevalence of brucellosis through two stage random sample survey using survey tool box software in a few selected states of India.

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2. Materials and methods

2.1. Random sampling

In India, farmers follow similar husbandry practices at the village level and animals have equal probability to acquire infection. Thus for the study, all the bovines in a village were considered to belong to one herd and samples were collected randomly by two stage sampling method of survey tool box software [8]. In the first stage sampling, villages were selected by means of simple random approach and animals were selected via probability proportional to population size in second stage. Approximately 5–7 mL of blood was collected from the jugular vein of each animal using vacutainers without EDTA (Becton Dickson, UK). Samples were labelled using codes describing state names and species. The clotted blood in the tubes was centrifuged at 1000 g for 10 min to obtain clear serum and stored at -20 °C until tested. A total of 12,054 samples [cattle -9236, buffaloes -2818] sourced from 15 and 11 states for cattle and buffaloes from India were tested to estimate the disease prevalence.

2.2. Serodiagnosis by iELISA

Serum samples were analyzed by in-house developed and validated protein G based indirect enzyme linked immunosorbent assay (iELISA). For the assay, smooth lipopolysaccharide (sLPS) antigen from *Brucella abortus* 99 strain was extracted [2] and cut-off values were established after thorough screening and validation [14]. Any sample of percent positivity (PP) value below 55% was taken negative; between 55–65% as moderate positive, more than 65% as strong positive and sample with only 55% PP were recommended for re-sampling after 45 days. The test has determined sensitivity (Se) of 98 and 92.0% and specificity (Sp) of 95 and 98%, respectively for cattle and buffaloes (Indian patent application No. 5031/CHE/2013).

2.3. Statistical analysis

From apparent prevalence (AP), true prevalence (TP) was calculated at 95% confidence interval (CI) using true prevalence program of the survey tool box in which sensitivity and specificity of the diagnostic test used and sample size were taken into consideration [15]. The data were analysed using IBM SPSS software Version 22 (India). The significance of difference was determined by chi square test and of $p = 0.05$ was considered statistically significant.

3. Results

Out of 12,054 bovines tested, 1024 samples were positive by iELISA with overall prevalence of 8.4% (95% CI 8.0–9.0) and true prevalence of 7.2% (95% CI 6.6–7.8). Among 9236 cattle and 2818 buffalo samples screened, 871 (8.3%) and 153 (3.6%) were seropositive, respectively and higher seropositivity was observed among cattle than buffaloes ($p = < 0.001$) (Table 1).

State wise true seroprevalence in cattle ranged from 0 to 23.51% across the states (Table 2 and Fig. 1) with highest in Punjab (23.51%) followed by Maharashtra (13.93%) and Rajasthan (13.60%) and lowest

Table 1
Species wise seroprevalence of bovine brucellosis in India.

Species	No. of samples tested	No. of positives	Apparent prevalence	95% CI*	True prevalence	95% CI*	Chi square	P- value
Cattle	9236	871	9.43	8.9-10.0	8.3	7.6-9.0	44.46	< 0.0018*
Buffalo	2818	153	5.43	4.7 - 6.3	3.6	2.6 - 4.5		
Total	12054	1024	8.4%					

* P- value < 0.05 is considered significant; * CI -95%; Sensitivity (Se) and Specificity (Sp) of iELISA for cattle are 98 and 95 respectively; Se and Sp of iELISA for Buffalo are 92 and 98, respectively.

in Odisha (1.95%), Andhra Pradesh (2.84%) and Manipur (3.05%) states. True seroprevalence greater than 10% from 3 states and zero prevalence in 2 states has been reported.

In buffaloes, overall true prevalence of 3.81% was recorded. Similar to the cattle seroprevalence, highest prevalence in buffaloes was recorded in Punjab (10.2%) followed by Gujarat (9.50%) and Manipur states (6.91%). True prevalence ranging from 5.0 to 10.0% was recorded from 3 states and the disease prevalence was very negligible in buffaloes of Madhya Pradesh, Karnataka, Odisha, and Jammu and Kashmir states (Table 3 and Fig. 1). Overall, the highest seroprevalence in both cattle and buffalo populations was recorded (23.51% and 10.2%) in Punjab state and seroprevalence was higher in buffaloes compared to cattle in the state of Manipur.

4. Discussion

Brucellosis is an important but neglected zoonotic disease of live-stock in India [16]. Despite the progress achieved in its control elsewhere, brucellosis still remains a major public health threat [4] and causing huge economic losses due to abortions, infertility and reduced milk production. Surveillance and vaccination are the two effective approaches for controlling the disease in endemic countries. To implement disease control strategies, detailed information about the spatial prevalence is essential for designing suitable approaches for confining the disease within acceptable limits.

In India, since the first report of brucellosis during 1918, several studies have reported the regional prevalence of the infection throughout the country. First national report comprising screening from 23 states of the country indicated seroprevalence of 1.9% and 1.8%, in 23,284 cattle and 7,153 buffaloes, by RBPT and SAT tests [10]. In another study from 25 states of India indicated 5% and 3% in 38139 cattle and in 9456 buffaloes by AB-ELISA, respectively [12]. The present study is a continued effort to record brucellosis during the period 2011–2015. Significant seroprevalence difference was observed between 9236 cattle and 2818 buffaloes with higher seropositivity in cattle (8.30%) compared to buffaloes (3.60%). Serological evidence of brucellosis in buffaloes of two states (Jammu and Kashmir and Odisha) could not be established similar to the previous reports [10,12].

Worldwide, a number of studies has reported seroprevalence of brucellosis in animals. In Bangladesh, the estimated animal level true prevalence in cattle was reported to be 9.70% [17] and in Libya, 42% of cattle were seropositive [18]. In India, prevalence as low as 0.13% [7] to as high as 50% have been reported [14]. Many of the high prevalence reports are based on investigations from the farms with a history of abortions and reduced productivity. The differences in seroprevalence reporting were observed even between rearing systems, wherein high seropositivity in organized farms (4.1%) compared to cattle owned by individual farmers 0.7% [10]. Similar reports of marginally higher prevalence in organized farms (6.5%) compared to unorganized farms 5.1% [15] have been documented. The variations in seroprevalence reporting are even attributed to laboratory tests employed. In most of the previous surveys, RBPT and in few studies AB-ELISA was used [8]. In the present study, protein G based iELISA test with overall sensitivity of 87.5% and specificity of 97.6%, was used.

The prevalence greater than 10% in cattle of 3 states (Punjab,

Table 2
State wise seroprevalence of brucellosis in cattle.

State	No. of cattle samples tested	No. of positives	Apparent prevalence	95% CI [*]	True prevalence	95% CI [*]
Punjab	309	83	26.86	22.23-32.06	23.51	18.52-29.10
Maharashtra	557	100	17.95	14.99-21.36	13.93	10.74-17.59
Rajasthan	119	21	17.65	11.84-25.48	13.60	7.36-22.02
Karnataka	447	53	11.86	9.18-15.18	7.37	4.49-10.95
Madhya Pradesh	918	105	11.44	9.54-13.66	6.92	4.88-9.31
Tamil Nadu	152	17	11.18	7.10-17.18	6.65	2.26-13.10
Gujarat	593	65	10.96	8.69-13.73	6.41	3.97-9.39
Kerala	839	81	9.65	7.84 -11.84	5.00	3.05-7.36
Assam	198	19	9.60	6.23-14.50	4.94	1.32-10.22
Meghalaya	470	44	9.36	7.05-12.33	4.69	2.20-7.89
Manipur	523	41	7.84	5.83-10.46	3.05	0.89-5.87
Andhra Pradesh	785	60	7.64	5.98-9.72	2.84	1.06-5.07
Odisha	1072	73	6.81	5.45-8.48	1.95	0.48-3.74
Uttarakhand	140	07	5.00	2.44-9.66	0.00	0.0-5.33
Jammu & Kashmir	2114	102	4.82	3.99-5.82	0.00	0.0-0.89

* CI -95%.

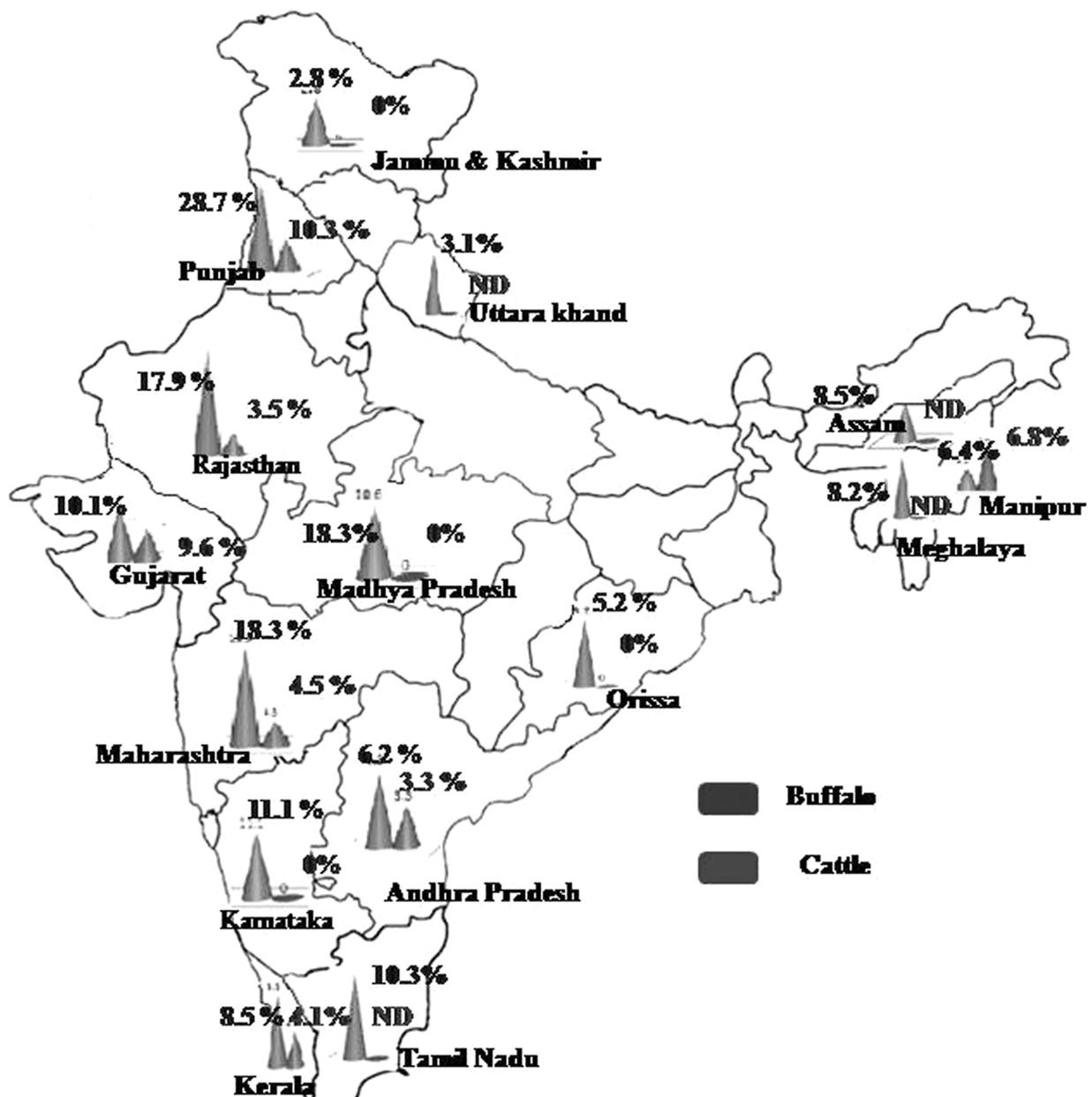


Fig. 1. State wise true seroprevalence in cattle in buffaloes in India.

Table 3
State wise seroprevalence of brucellosis in buffalo population.

State	No. of buffalo samples tested	No. of positives	Apparent prevalence	95% CI *	True prevalence	95% CI *
Punjab	170	19	11.18	7.27-16.8	10.2	5.86-16.44
Gujarat	455	48	10.55	8.05-13.71	9.50	6.72 -13.01
Manipur	146	12	8.22	4.76-13.82	6.91	3.07-13.13
Maharashtra	306	19	6.21	4.01-9.49	4.68	2.23-8.33
Kerala	68	04	5.88	2.31-14.17	4.31	0.35-13.52
Rajasthan	224	12	5.36	3.09-9.13	3.73	1.21-7.92
Andhra Pradesh	348	18	5.17	3.30-8.03	3.52	1.44-6.70
Madhya Pradesh	873	19	2.18	1.4-3.37	0.20	0-1.53
Karnataka	82	01	1.22	0.22-6.59	0	0-5.10
Odisha	129	01	0.78	0.14-4.26	0	0-2.51
Jammu & Kashmir	17	0	0	0-18.43	0	0-18.26
Total	2818	153	5.43	4.65 - 6.33	3.81	2.95 - 4.81

* CI -95%.

Maharashtra, Rajasthan) has been similarly documented earlier in 19 of 23 states surveyed [10]. Though the random sample frame was provided to all the 15 state units, buffalo sera samples were not received for screening from states of Assam, Tamil Nadu, Meghalaya, and Uttarakhnad. The disease is quite alarming in Punjab state which showed the highest seroprevalence in both cattle and buffaloes (23.51% and 10.2%). Earlier studies have reported high prevalence estimates ranging from 7.54% to 26.6% [13,19].

True prevalence greater than 5% in 8 and 3 states for cattle and buffaloes, respectively [(cattle- Punjab, Maharashtra, Rajasthan, Karnataka, Madhya Pradesh, Tamil Nadu, Gujarat and Kerala) and (buffaloes-Punjab, Gujarat and Manipur)] indicated endemicity of brucellosis in many states of the country [20–24]. However, overall results largely reflected prevalence of brucellosis in many regions and emphasise to institute immediate control measures such as restriction on movement of bovines to different regions, prior diagnosis before purchase and diagnosis of every productive and reproductive case in organized herds [13,20,21].

Department of Animal Husbandry, Dairying and Fisheries, Govt. of India has initiated the Brucellosis Control Program (Brucellosis-CP) during 2011 by providing grants-in-aid under the Livestock Health and Disease Control. As part of the program, surveillance and vaccination activities have been started in many states throughout the country. Serum based iELISA testing was suggested in-conjunction with milk ring test in states where milk procurement through dairy cooperatives is functional and good. Nationwide vaccination and strict surveillance are very difficult tasks due to limited availability of vaccines and laboratories as compared to huge bovine population of the country. Hence, the present study provides useful information in prioritizing the regions for carrying out vaccination and strengthening clinical surveillance system. Since, brucellosis is transmissible among different species, well-designed countrywide, evidence-based, and multidisciplinary studies of brucellosis at the human/livestock/wildlife interface are needed. This will help to generate potential impact estimates, to classify *Brucella* reservoirs, and to suggest control strategies of demonstrated efficacy.

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