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Nationwide cross-sectional study on brucellosis sero-prevalence in buffaloes using competitive ELISA

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ABSTRACT

Brucellosis is a bacterial disease caused by various *Brucella* species, which mainly infect bovines (cattle, buffalo), small ruminants (sheep, goat), swine, and dogs and humans. Earlier studies have reported brucellosis sero-prevalence only in cattle or both in cattle and buffaloes (bovine brucellosis) and actual disease burden in buffaloes was not available. The current study aimed to record countrywide brucellosis sero-prevalence in Indian buffaloes using competitive ELISA (cELISA). For the study, 1086 female buffalo (*Bubalus bubalis*) serum samples were collected from the top 10 states of India having the highest buffalo population. Overall, samples were drawn from 62 districts, 104 blocks and 242 epiunits from five regions of the country ({Punjab-100, Haryana-109, Uttar Pradesh-101, Rajasthan-100, Gujarat-156, Andhra Pradesh-100, Madhya Pradesh-110, Maharashtra-108, Karnataka-102, Andhra Pradesh-100 and Tamil Nadu-100)}. Samples were tested for anti-brucella antibodies using in-house developed monoclonal-based competitive ELISA (cELISA). Overall, apparent prevalence (AP) of 15.38% (CI-95%;13.35-17.64) and true prevalence (TP) of 15.85% (CI-95%; 13.77-18.19) were recorded. Sero-prevalence was highest in Punjab state (AP:57%; 47.22-66.27) in the Northern region and lowest in Madhya Pradesh (AP:0.90; 0.16-4.97) state of Central region with some of the districts displaying up to 90% and 70%, seropositivity in the Punjab state. Low brucellosis prevalence was noted in young (2.1 to 5 years) and older age groups of animals (11.1 to 13 years) and significantly high in the age group between 8 to 11 years (32.23%). The highest brucellosis seropositivity was observed in buffalo breeds such as Nagpuri and Murrah (28.79% and 20.67%), respectively and significant association was noted among four breeds. In conclusion, India has the highest buffalo population with very high-yielding buffalo breeds (109.85 million). Periodical surveillance is essential to detect and control brucellosis in buffaloes.-the pride species of the country.

Keywords: *Bubalus bubalis*, Brucellosis, cELISA, India, Sero-prevalence

Brucellosis is a zoonotic disease with important epidemiological, economic, and global health implications, particularly for a developing-country, for both human and animal populations, that rely on intense farming and agricultural methods (Moreno 2014). The pathogen's potential to seamlessly and swiftly adapt to the contemporary environment has been proved by evidence of shifting ecology and re-emergence of brucellosis in recent years, demanding continuous epidemiological analysis and intervention design/s (Pappas 2010). In buffaloes (*Bubalus bubalis*), brucellosis is caused mainly by *Brucella abortus* which is transmitted by consumption of feed contaminated with tissues or bodily fluids, contact with mucosal membranes, direct injection, and fomites. The most common indications of infection are late-term abortion and pre-term delivery with retained placenta. India has the world's highest buffalo population (109.85 million or 36.35%) as per the Livestock Census, DAHD, GoI (2019-2020) and disease surveys and control programs are very important to identify economically important diseases like brucellosis in buffaloes.

Brucellosis causes prolonged infection due to its ability to evade innate and adaptive immunity and there is currently no vaccine available for human use to prevent the disease (Jonsson 2013). Also, controlling the disease in animals can help prevent brucellosis in humans. As a result, comprehensive surveillance, control, and eradication activities in bovines and other animal populations are required to reduce brucellosis transmission (Jindal *et al.* 2017). From 2019, brucellosis control program was implemented in the country by immunizing cattle and buffalo calves between the ages of 4 to 8 months with *B. abortus* S19. Another component of the

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control program is brucellosis sero-prevalence reporting, which is based on the RBPT and indirect enzyme linked immunosorbent assay (iELISA). Both RBPT and iELISA tests are affiliated with FPSR (false positive serological reactions) with vaccinated animals. Other assays, such as FPA (Fluorescence polarization assay) and cELISA, were investigated in infected farms and FPA sensitivity was nearly equal to RBPT and in-house cELISA sensitivity was greater than RBPT (Kalleshamurthy *et al.* 2020). Based on these findings, cELISA was taken up for screening buffaloes with unknown vaccination/disease status.

Brucellosis epidemiology is always changing and continual disease reporting is helpful for prioritising vaccination efforts in highly endemic regions to prevent and control disease spread. Countrywide species-specific brucellosis updates based on sero-surveillance supports the identification of priorities and inform the researchers and policymakers about the disease burden in the region/s.

MATERIALS AND METHODS

Ethics statement: The study was approved by the Institutional Animal Ethics Committee, Indian Council of Agricultural Research-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru, India and written consents and permission were also obtained from farm owners to publish the data (project code:OXX5174).

Study plan and sample design: The cross-sectional study was undertaken during 2021-2022 to decipher brucellosis seropositivity in buffaloe. For the study, top 10 states of India having highest buffalo population were chosen across five regions. In the selected states, two stage random sampling methodology was adapted, in the first strata, districts within the state, clusters within the district and epiunits within the cluster were selected by means of a simple random approach and animals were selected based on probability proportional to buffalo population. In the second strata, the households (HH) were randomly selected to collect the designated buffalo samples using survey tool box (Sargent *et al*. 2018). Because the small scale diary farmers in rural India follow almost same farming practices at epiunits (village) level with respect to breeding, feeding and management practices and hence sampling at HH in the epiunits were proportionate to buffalo population.

A questionnaire was designed for the study with animal (sex and age) and demographic details such as the name of the village/epi unit, block/ cluster, district and state.

Serum samples: The veterinary officers were instructed to collect approximately 5-7 mL of blood aseptically by jugular vein using vacutainers without anticoagulant (Becton Dickson, Oxford, UK). Separated serum samples from blood clots were transported to ICAR-NIVEDI, Bengaluru, India by maintaining cold chain. Serum samples received in the institute were centrifuged at 2500 rpm for 3-5 min and separated clear sera was stored at -20°C until tested.

Serological tests: Serum samples were analyzed by in-house developed and standardized monoclonal antibody (mAb) based competitive ELISA (cELISA) (Kalleshamurthy *et al.* 2020), results were interpreted based on per cent inhibition (PI) values with reference to conjugate control. The samples with PI values >30 and <30 were considered positive and negative, respectively as per the standerdized protocol. The test has determined sensitivity (Se) of 97.5% and specificity (Sp) of 100%, respectively. The experiments including the culture work, *B. abortus* S-99 smooth lipopolysaccharide (sLPS) antigen extractions, mAb purification and serum sample processing were carried out in Biosafety Laboratory level-II plus laboratory facility.

Statistical analysis: Information from the questionnaire were digitized into a Microsoft excel spreadsheet (Microsoft Corporation) and serological results were interpreted as seronegative=0 or seropositive=1. Apparent prevalence (AP) and true prevalence (TP) were calculated using online software at 95% confidence interval (CI) in which assay sensitivity of 97.50% and specificity of 100% were taken into consideration (https://epitools.ausvet.com.au). The chi-square value to find the significant differences between the age and breed was carried out using Graph Pad Prism software 9 (https://www.graphpad.com/quickcalcs/chisquared1/) with the significant p-value ($p<0.01$) to assess the differences. All the maps were created using QGIS (Geographic Information System) software version 3.22 (Fig. 2).

RESULTS AND DISCUSSION

Brucellosis is under-appreciated zoonotic disease of livestock (Smits and Kadri 2005). Despite gains in control elsewhere, brucellosis remains a major public health threat (Mehra *et al.* 2000) causing massive economic losses due to abortions, infertility, and decreased milk production. First brucellosis was reported in buffaloes at Indian Veterinary Research Institute, Mukteshwar, India (Annual Report, 1917–1918) and *Brucella abortus* isolation during 1942 (Polding 1942). Later, several serological studies have indicated 3% brucellosis in buffaloes (Renukaradhya *et al*. 2002) and followed by reports from few states of India (Aulakh *et al*. 2008, Jagapur *et al*. 2013, Shome *et al*. 2019). India has diverse livestock species inhabiting different geographical regions and many reports highlight region-specific brucellosis prevalence in buffaloes. Brucellosis needs effective control measures such as vaccination of the young heifers, countrywide periodical surveillance and elimination or quarantining of diseased herds.

In the present study, a total of 1086 female buffalo serum samples were drawn from 62 districts of the total 741 Indian districts (8.36%) and 242 epiunits of the total 6,64,369 villages (0.03%) from five regions of India. Out of 1086 buffaloes tested, 167 samples were positive by cELISA with overall AP of 15.38% (CI- 95%;13.35-17.64) and TP of 15.85% (CI-95%; 13.77-18.19). The AP ranged

from 0 to 57% (Table 1 and Fig. 1) with highest being in Punjab (57%) followed by Andhra Pradesh (28%), Tamil Nadu (19%), Haryana (15.60%), Maharashtra (13.89%), Uttar Pradesh (12.87%) and lowest in states of Karnataka

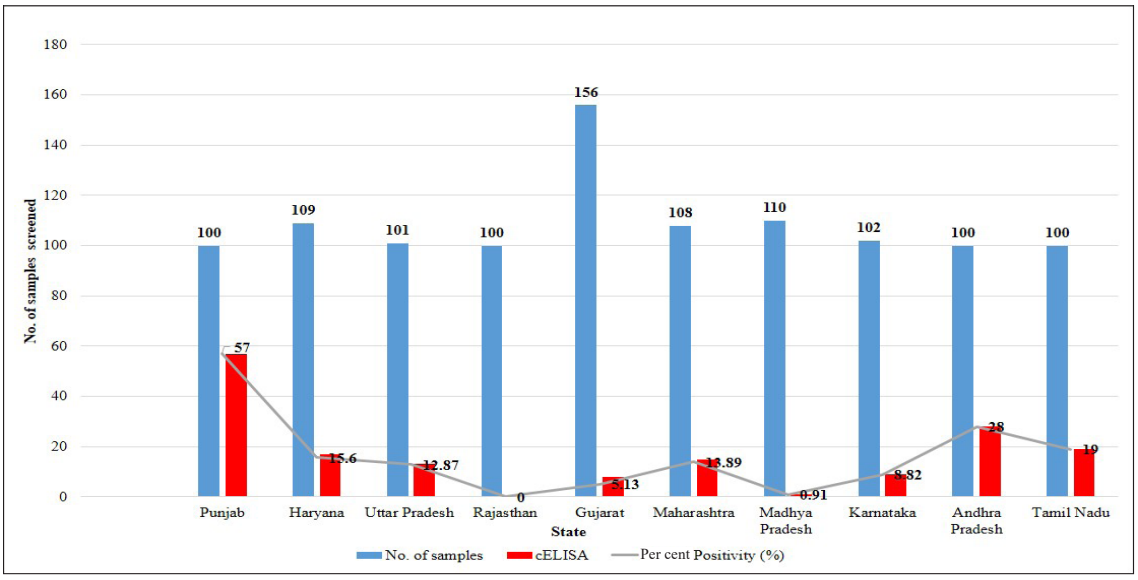

Fig.1. State wise sero-prevalence of brucellosis in buffaloes.

Table 1. Reporing of brucellosis sero-prevalence in buffaloes in 10 states of India using cELISA

Sl. no	Region	State	No. of samples	cELISA PositiveS	Per cent positivity (%)	Apparent prevalence*	True Prevalence*
1		Punjab	100	57	57	57 (47.22-66.27)	58.76 (48.68-68.32)
2	Northern States	Haryana	109	17	15.60	15.60 (9.97-23.56)	16 (10.28-24.29)
3		Uttar Pradesh	101	13	12.87	12.87 (7.68-20.78)	12.27 (7.92-21.43)
Total			310	87	28.06	28.06 (23.35-33.31)	28.93 (24.08-34.34)
4		Rajasthan	100	0	0	0 (0-3.7)	0 (0-3.81)
5	Western states	Gujarat	156	8	5.13	5.13 (2.62-9.79)	5.29 (2.7-10.09)
6		Maharashtra	108	15	13.89	13.89 (8.6-21.66)	14.32 (8.87-22.33)
Total			364	23	6.31	6.32 (4.25-9.3)	6.51 (4.38-9.59)
7	Central states	Madhya Pradesh	110	1	0.91	0.90 (0.16-4.97)	0.94 (0.05-5.12)
Total			110	1	0.91	0.90 (0.16-4.97)	0.94 (0.05-5.12)
8		Karnataka	102	9	8.82	8.82 (4.71-15.92)	9.1 (4.86-16.42)
9	Southern states	Andhra Pradesh	100	28	28	28.00 (20.14-37.49)	28.87 (20.76-38.65)
10		Tamil Nadu	100	19	19	19.00 (12.51-27.78)	20.22 (12.94-30.09)
Total			302	56	18.54	18.54 (14.56-23.31)	19.12 (15.02-24.03)
Grand total			1086	167	15.38	15.38 (13.35-17.64)	15.85 (13.77-18.19)

*CL, 95%; diagnostic sensitivity (Dse), 97.5% and diagnostic specificity (Dsp), 100%.

(8.82%), Gujarat (5.13%) and Madhya Pradesh (0.91%).

When region-wise brucellosis was reviewed among three Northern states, highest apparent prevalence (57%) was recorded in Punjab while the other studies have reported comparatively low prevalence rates of 13.4% and 15.12% in buffalo farms (Dhand *et al.* 2005, Malik *et al.* 2018) and 10.2% in stratified random sampling survey (Shome *et al.* 2019). However, all the reported studies have clearly showed >10% brucellosis prevalence in buffaloes similar to that of cattle in Punjab (Holt *et al.* 2021). Similarly, in neighbouring state Haryana, overall 15.60% apparent prevalence of brucellosis was noted which is in agreement with reported study of 11.71% (Khurana *et al*. 2012). In last 10 years, disease has showed increasing trend and it has to be noted for implementation and compliance of National Animal Disease Control for brucellosis in the state. Yet another biggest state of the country-Uttar Pradesh too recorded high sero-prevalence (12.87%) whereas, its noteworthy that few studies have reported 36.34% in buffalo farms (Jagapur *et al.* 2013) and 46.6% in aborted buffaloes (Jain *et al.* 2013) from the state. Overall 28.06% brucellosis sero-prevalence has been recorded in three Northern states having sizeable buffalo population.

Among three states in the Western region of the country, Maharashtra showed highest brucellosis seropositivity in buffaloes (13.89%) and almost same percentage (14.3%) was recorded in buffalo farms with history of abortions (Das *et al.* 1990). Maharashtra has highest population of Nagpuri breed of buffalo and disease detection and control is very essential as both the recent and old reports have revealed highest brucellosis sero-prevalence in buffaloes. In the adjoining state Gujarat, sero-prevalence was 5.13%. On the contrary, very high prevalence was recorded in purposively tested samples from organized buffalo herds with a history of abortion (72%) and 13.04% in animals without history of abortion (Trangadia and Patel 2016). Whereas in another Western state-Rajasthan, disease prevalence was negligible in buffaloes as per current study whereas earlier studies have reported ~12% (Priyanka *et al.* 2018). This variation in the prevalence reporting is because of sampling methodology and sensitivity of the tests employed for the brucellosis diagnosis. Among three states investigated from Western region of the country, overall 6.3% brucellosis prevalence in buffaloes was noted.

In the Southern region, highest brucellosis sero-prevalence was recorded in Andhra Pradesh state (28%) and it is the second highest state in seropositivity after Punjab. Various studies have reported 8-10% prevalence in buffaloes (Pushpa and Kumari 2005) and 5.98% in government dairy farms (Kumar and Gupta 2018) and all these are location/farm specific reports. The disease endemicity in other livestock species is very well-documented indicating high burden of brucellosis in livestock as such in the state (Shome *et al*. 2021). Yet another state (Tamil Nadu) showed high prevalence of brucellosis in buffaloes (19%) and there are no reports of brucellosis prevalence in buffaloes of Tamil Nadu. However, 6.70% was reported in bovines from 11 districts of the state (Naveenkumar *et al.* 2017). Karnataka state in Southern region, reported comparatively low sero-prevalence (8.82%). Other two studies have also reported lower prevalance (6%) in non-randomized samples from buffalo farms and still lower prevalence in stratified random sampling (1.22%) (Shome *et al.* 2014, 2019). Karnataka is adopting periodical milk testing, zoning and vaccinations in the highly endemic regions of the state which could be the probable reason for lower sero-prevalence among the three Southern states of India. Overall, 18.54% was recorded in Southern region of the country which is quite high in terms of disease burden on livestock and public health complications.

In Central region of Indian state-Madhya Pradesh, sero-prevalence was very negligible (0.90%) which has similarly been cited in two other studies from different regions of Madhya Pradesh (Verma *et al.* 2019) and stratified random surveillance (Shome *et al.* 2019) except a report of Mehra *et al.* (2000) who recorded 11.4% and 9.4% among buffaloes in organized and unorganized farms, respectively.

Disease distribution was found highly skewed at district level as it was observed in Patiala and Amritsar districts of Punjab displaying 90% and 70% prevalence, respectively as frequency of occurrence of brucellosis was also highest in this state (Fig. 2). Similarly, very high sero-prevalence of 71.43% was recorded in Nuh district of Haryana compared to other two sampled districts (Ambala and Kurukshetra) with each having 33.33% prevalence rate. Also, among four districts investigated in Andhra Pradesh, Krishna district reported highest 47.37% prevalence rate and similarly, two districts of Tamil Nadu (Tiruchirapalli and Virudhunagar) revealed 40% and 28.57% prevalence, respectively. Pune district of Maharashtra showed the highest brucellosis seropositivity (21.74%) among four district samples analyzed and even Belagavi district of Karnataka showed prevalence rate of 26.67% among seven other different districts surveyed (Fig. 2 and Table 2).

Buffalo rearing in unorganized sector follow natural breeding and detection and removal of infected male from the herds is prerequisite for the control of the disease in the regions. In the infected herds, direct or close contact is an important disease transmission mode and high disease prevalence observed in few regions is attributed to the circulation of bacteria within the herds/ regions (Seventer and Hochberg 2017). In the current study, isolation/ molecular detection was not performed due to large number of samples, hence it is unlikely to conclude that, the positivity is due to *B.abortus* or any other *Brucella* species.

Large number of buffalo samples were from 5.1 to 8 years age group (34.07%) which constitutes about one third of all the age groups in the study. Only, 12.52% (136) samples belonged to 11.1-13 years and the age information

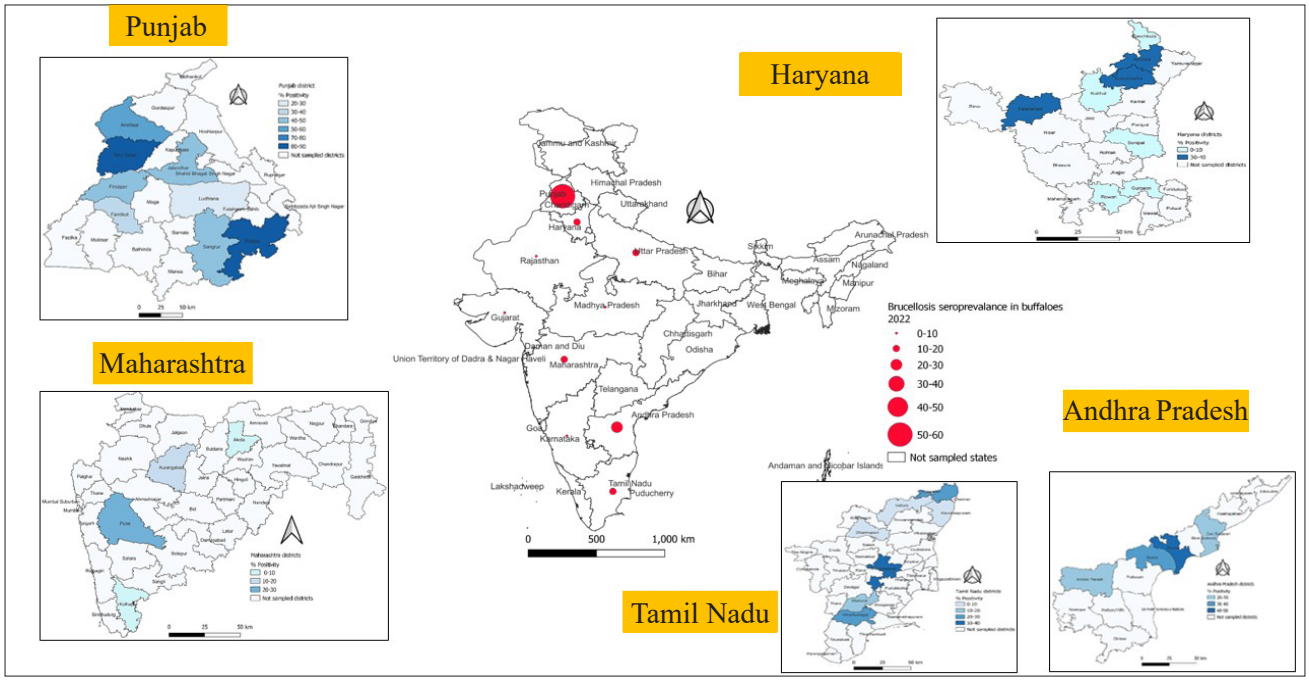

Fig. 2. Mapping of brucellosis in buffaloes from 10 representative states by DIVA test (cELISA).

Table 2. District-wise brucellosis sero-prevalence in buffaloes

Sl. no		State	District-wise	No. of samples	cELISA positives	Apparent prevalence*	True prevalence*
1	Northern States	Punjab	Amritsar	20	14	70 (48.10-85.45)	72.16 (49.59-88.10)
			Faridkot	10	4	40 (16.82-68.73)	41.24 (17.34-70.86)
			Ferozepur	10	5	50 (23.66-76.34)	51.55 (24.39-78.70)
			Jalandhar	10	5	50 (23.66-76.34)	51.55 (24.39-78.70)
			Ludhiana	15	4	26.67 (10.90-51.95)	27.49 (11.23-53.56)
			Patiala	10	9	90 (59.58-98.21)	92 (61.43-102)
			Sangrur	15	7	46.67 (24.81-69.88)	48.11 (25.58-0.72.04)
			Tarn Taran	10	9	90 (59.58-98.21)	92 (61.43-102)
			Total	100	57	57.00 (47.22-66.27)	58.76 (48.68-68.32)
2		Haryana	Ambala	12	4	33.33 (13.81-60.94)	34.36 (14.24-62.82)
			Gurugram	10	0	0 (0-27.75)	0 (0-28.61)
			Kaithal	19	2	0.1053 (0.0294-0.3139)	10.85 (03.03-32.37)
			Panchkula	11	0	0 (0-25.88)	0 (0-26.68)
			Kurukshetra	15	5	33.33 (15.18-58.29)	34.36 (15.65-60.09)
			Nuh	15	5	71.43 (35.89-91.78)	73.64 (37-94.62)
			Rewari	10	0	0 (0-27.75)	0 (0-28.61)
			Sonipat	17	1	5.88 (1.05-26.98)	6.06 (0.31-27.82)
			Total	109	17	15.60 (9.97-23.56)	16.00 (10.28-24.29)

(*Table 2 continued ...*)

Table 2. *Continued ...*

Sl. no		State	District-wise	No. of samples	cELISA positives	Apparent prevalence*	True prevalence*
3		Uttar Pradesh	Bareilly	101	13	12.87 (7.68-20.78)	13.27 (7.92-21.43)
			Total	101	13	12.87 (7.68-20.78)	12.27 (7.92-21.43)
4		Rajasthan	Alwar	20	0	0 (0-16.11)	0 (0-16.61)
			Jaipur	20	0	0 (0-16.11)	0 (0-16.61)
			Dausa	20	0	0 (0-16.11)	0 (0-16.61)
			Jhunjhunu	20	0	0 (0-16.11)	0 (0-16.61)
			Sikar	20	0	0 (0-16.11)	0 (0-16.61)
			Total	100	0	0 (0-3.7)	0 (0-3.81)
5	Western states	Gujarat	Gandhinagar	156	8	5.13 (2.62-9.79)	5.29 (2.7-10.09)
			Total	156	8	5.13 (2.62-9.79)	5.29 (2.7-10.09)
6		Maharashtra	Akola	22	2	9.09 (2.53-2.78)	9.37 (2.61-2.86)
			Aurangabad	20	3	15 (5.24-36.04)	15.46 (5.40-37.16)
			Kolhapur	20	0	0 (0-16.11)	0 (0-16.11)
			Pune	46	10	21.74 (12.26-35.57)	22.41 (12.64-36.67)
			Total	108	15	13.89 (8.6-21.66)	14.32 (8.87-22.33)
7	Central states	Madhya Pradesh	Bhopal	35	1	2.86 (0.51-14.53)	2.95 (0.15-14.98)
			Indore	75	0	0 (0-4.87)	0 (0-5.02)
			Total	110	1	0.09 (0.16-4.97)	0.09 (0.05-5.12)
8	Southern states	Karnataka	Ballari	20	3	15 (5.24-36.04)	15.46 (5.40-37.16)
			Belagavi	15	4	26.67 (10.90-51.95)	27.49 (11.23-53.56)
			Bidar	17	0	0 (0-18.43)	0 (0-19)
			Kalaburagi	10	0	0 (0-27.75)	0 (0-28.61)
			Kolar	10	1	10 (1.79-40.42)	10.31 (0.53-41.66)
			Mysore	20	1	5 (0.89-23.61)	5.15 (0.26-24.34)
			Tumkur	10	0	0 (0-27.75)	0 (0-28.61)
			Total	102	9	8.82 (4.71-15.92)	9.1 (4.86-16.42)
9		Andhra Pradesh	East Godavari	43	9	20.93 (11.42-35.21)	21.78 (11.78-36.29)
			Guntur	15	5	33.33 (15.18-58.29)	34.36 (15.65-60.09)
			Krishna	21	9	47.37 (27.33-68.29)	48.83 (281.18-70.40)
			Kurnool	21	5	0 (0-43.45)	0 (0-44.79)
			Total	100	28	28.00 (20.14-37.49)	28.87 (20.76-38.65)

(*Table 2 continued ...*)

Table 2. *Concluded*

Sl. no	State	District-wise	No. of samples	cELISA positives	Apparent prevalence*	True prevalence*
10	Southern states	Tamil Nadu	Dharmapuri	10	1	10 (1.79-40.42)	10.31 (0.53-41.66)
			Kanchipuram	16	1	6.25 (1.11-28.33)	6.44 (0.33-29.20)
			Madurai	18	2	11.11 (3.10-32.80)	11.45 (3.20-33.81)
			Tiruchirapalli	15	6	40 (19.82-64.25)	41.24 (20.44-66.24)
			Tiruvallur	15	4	26.67 (10.90-51.95)	27.49 (11.23-53.56)
			Vellore	12	1	8.33 (01.49-35.39)	8.59 (0.44-36.48)
			Virudhunagar	14	4	28.57 (11.72-54.65)	29.46 (12.08-56.34)
			Total	100	19	19.00 (12.51-27.78)	20.22 (12.94-30.09)
			Sub Total	1086	167	15.38 (13.35-17.64)	15.85 (13.77-18.19)

* CL, 95%.

was not available for 154 buffalo samples. Low brucellosis prevalence in young (2.1 to 5 years) and older age group of animals (11.1 to 13 years) was observed compared to 5 to 8 years and 8 to 11 years (Table 3). The susceptibility to disease increases with age and is more commonly associated with sexual maturity than age (Radostits *et al.* 2000). Few seropositives detected in the age group of 2.1 to 5 year animals may be due to exposure to brucellosis infected animals in the farms. The younger animals are more resistant to primary infection and frequently clear infections, although latent infection do occur (Walker *et al.* 1999). Also higher brucellosis prevalence in adult animals observed in the current study is also linked to prolonged contact with infected animals in the farm environment and this potential risk may be significant in herds where positive animals are not removed (Megersa *et al.* 2011). Brucellosis within the age groups disclosed significantly high prevalence ($p<0.004$) and it has been described for brucellosis that some of the infected animals do not become seropositive until pregnant.

Being an agrarian economy, India is home to an estimated 58% global buffalo population (Kumar *et al.* 2010) and has exquisite buffalo breeds such as Murrah, Nili ravi, Bhadawari, Jaffarabadi, Surti, Mehsana, Nagpuri (Or) Ellichpuri, Godavari breeds predominately found in Northern states and Toda breed of Southern region of India (Thiruvenkadan *et al.* 2013). Highest brucellosis seropositivity was observed in breeds such as Nagpuri, Murrah, and Mehsana (28.79%, 20.67% and 7.85%), respectively and least in Bhadawadi (6.70%). Significant association to brucellosis was observed among all the four breeds ($p<0.0019$) (Table 4). There are brucellosis reports in Indian Murrah buffaloes breeds where 50% of the animals were positive by both antigen and antibody detection tests (Shome *et al.* 2014) and 15.12% in Murrah buffalo farm (Malik *et al.* 2018). Buffalo breed

Table 3. Age-wise brucellosis sero-prevalence in buffaloes

Age in years	Number of samples	% of samples (in this interval)	No. of positives	Per cent positivity (%)	$\chi 2$ value	p-value
2.1 to 5	305	28.08	29	9.51	15.08	0.004538*
5.1 to 8	370	34.07	68	18.38		
8.1 to 11	121	11.14	39	32.23		
11.1 to 13	136	12.52	18	13.24		
Not mentioned	154	14.18	13	8.44		
Total	1086	100%	167	15.38		

*$p<0.05$, considered significant.

Table 4. Breed-wise brucellosis sero-positivity in buffaloes

Breed	No. of samples tested	No. of positives	Per cent positivity (%)	$\chi 2$ value	p-value
Nagpuri	132	38	28.79	14.80	0.0019*
Murrah	450	93	20.67		
Mehsana	191	15	7.85		
Bhadawari	313	21	6.70		
Total	1086	167	15.38		

*p-value<0.001, considered significant.

predisposition to brucellosis is not evinced much interest so far and since most important Indian breeds have showed high seropositivity, there is need to evaluate the same.

Another component of the brucellosis control program is brucellosis sero-prevalence reporting, which is based on the RBPT and iELISA, both of which are affiliated with FPSR in vaccinated (False Positive Serological Reactions). Other assays, such as FPA and cELISA, were investigated for sero-monitoring/surveillance proved to be sensitive and specific (Kalleshamurthy *et al.* 2020). This study was designed to determine the current status and prevalence of brucellosis in buffaloes using cELISA which has high sensitivity and specificity than RBPT.

The current work highlights baseline data of 15.38% of brucellosis sero-prevalevce in buffaloes. Buffalo keepers were totally unaware of the disease and the vaccine availability for the brucellosis (Kant *et al.* 2018). A recent report revealed high risk of brucellosis transmission in rural communities believing the purported medical benefits of raw buffalo milk consumption (Dadar *et al.* 2019). Because brucellosis can be transmitted between species, well-designed, evidence-based, multidisciplinary studies at the human/livestock/wildlife interface are required. The true epidemiological status of the disease in the country remains a concern owing to the absence of proper laboratory facilities, lack of awareness, under-reporting along with improper recording of the history of the disease. Further, cELISA could be used to test other species, having more sensitivity and specificity than RBPT and SAT makes the assay more robust and relevant for serological surveillance. Apart from testing, public awareness of brucellosis within rural populations is one of the inevitable factors in managing the risk of brucellosis in livestock as well as in humans.

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