Seasonal, physiological and bacteriological risk factors for subclinical mastitis in dairy cows maintained under different farming conditions

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SUPPLEMENTARY FILE

Supplementary Tables

Table S1: Summary statistics of SCM prevalence rate (%), SCC in healthy and SCM affected cows, BMSCC and herd average SCC in dairy farm and field conditions

	SCM prevalence rate (%)			SCC in healthy cows ($\times 10^3$ cells/ml)			SCC in SCM cows ($\times 10^3$ cells/ml)			BMSCC ($\times 10^3$ cells/ml)			Herd average SCC ($\times 10^3$ cells/ml)		
	HF-farm	Deoni-Farm	HF-Field	HF-farm	Deoni-Farm	HF-Field	HF-farm	Deoni-Farm	HF-Field	HF-farm	Deoni-Farm	HF-Field	HF-farm	Deoni-Farm	HF-Field
Mean	65.08	31.11	55.29	88.60	60.64	89.83	895.40	715.82	671.67	632.90	292.09	494.17	618.40	272.82	411.83
SD	7.30	5.81	10.57	16.47	12.46	10.72	182.03	222.84	57.22	131.00	92.95	93.91	170.84	99.22	79.03
95% CI (±)	5.22	3.90	11.09	11.78	8.37	11.25	130.21	149.70	60.05	93.71	62.44	98.55	122.21	66.66	82.93
Min	52.00	22.73	42.11	62.00	42.00	82.00	710.00	348.00	593.00	414.00	177.00	347.00	399.00	124.00	331.00
Max	73.91	40.00	70.00	128.00	82.00	108.00	1369.00	1085.00	745.00	868.00	436.00	602.00	1035.00	434.00	554.00

SCM: subclinical mastitis, SCC: somatic cell count, BMSCC: bulk milk somatic cell count, SD: standard deviation, CI: confidence interval, Min: minimum, Max: maximum

Table S2: Bivariate analysis of risk factors for SCM in Deoni cows in organized farm

S. No	Variables	Udder health status		Chi square values	P values
		Healthy	SCM		
1	Age (years)			14.370	0.001
	1 (3-5)	70 (82.4)	15 (17.6)		
	2 (5.1-9)	43 (69.4)	19 (30.6)		
	3 (≥9.1)	49 (55.7)	39 (44.3)		
2	Parity			9.436	0.024
	1 (1^{st})	47 (77)	14 (23)		
	2 (2-3)	41 (80.4)	10 (19.6)		
	3 (4-5)	37 (60.7)	24 (39.3)		
	4 (≥ 6)	37 (59.7)	25 (40.3)		
3	Stage of lactation (days in milk)			31.133	0.000
	Early (up to 60)	54 (84.4)	10 (15.6)		
	Mid (61-120)	45 (88.2)	6 (11.8)		
	Late (≥ 121)	63 (52.5)	57 (47.5)		
4	Seasons			0.249	0.883
	Rainy	90 (70.3)	38 (29.7)		
	Winter	31 (67.4)	15 (32.6)		
	Summer	41 (67.2)	20 (32.8)		
5	Mastitis treatment history in previous lactation				
	Yes	72 (68.6)	33 (31.4)	6.818	0.009
	No	108 (83)	22 (16.9)		
6	Mastitis treatment history in current lactation				
	Yes	38 (76)	12 (24)	0.013	0.911
	No	142 (77)	43 (23.2)		
7	Previous lactation milk yield (Kg)			13.841	0.001
	Low (≤ 684)	66 (84.6)	12 (15.4)		
	Medium (685-1154)	47 (58.8)	33 (41.2)		
	High (≥ 1155)	49 (63.6)	28 (36.4)		
8	Test day milk yield (Kg)			2.168	0.001
	Low (≤ 2.5)	57 (63.3)	33 (36.7)		
	Medium (2.6-3.7)	49 (73.1)	18 (26.9)		
	High (≥ 3.8)	56 (71.8)	22 (28.2)		

P value <0.2 is considered as statistically significant.

Figures in parenthesis under udder health status are percentage of samples

Table S3: Bivariate analysis of risk factors for SCM in HF crossbred cows in organized farm

S. No	Variables	Udder health status		Chi square values	P values
		Healthy	SCM		
1	Age (years)			4.551	0.103
	1 (2-4)	44 (42.3)	60 (57.7)		
	2 (4-7)	55 (34.4)	105 (65.6)		
	3 (>7)	15 (25.9)	43 (74.1)		
2	Parity			28.716	0.000
	1 (1st)	35 (39.8)	53 (60.2)		
	2 (2-3)	35 (30.2)	81 (69.8)		
	3 (4-5)	16 (20.8)	61 (79.2)		
	4 (≥ 6)	28 (68.3)	13 (31.7)		
3	Stage of lactation (days in milk)			7.793	0.020
	Early (≤100)	54 (41.9)	75 (58.1)		
	Mid (101-200)	37 (37.8)	61 (62.2)		
	Late (≥201)	23 (24.2)	72 (75.8)		
4	Seasons			0.980	0.613
	Rainy	47 (32.6)	94 (67.4)		
	Winter	27 (39.1)	42 (60.9)		
	Summer	40 (36.7)	69 (63.3)		
5	Mastitis treatment history in previous lactation				
	Yes	36 (38.7)	100 (43.7)	0.667	0.414
	No	57 (61.3)	129 (56.3)		
6	Mastitis treatment history in current lactation			17.242	0.000
	Yes	70 (45.8)	83 (83)		
	No	116 (68.6)	53 (31.4)		
7	Previous lactation milk yield (Kg)				
	Low (≤ 3214)	32 (31.4)	70 (68.6)	2.484	0.289
	Medium (3215-4974)	25 (27.8)	65 (72.2)		
	High (≥ 4975)	34 (38.6)	54 (61.4)		
8	Test day milk yield (Kg)			4.081	0.130
	Low (≤ 10)	39 (32.5)	81 (67.5)		
	Medium (10.1-13)	32 (31.1)	71 (68.9)		
	High (≥ 13.1)	43 (43.4)	56 (56.6)		

P value <0.2 is considered as statistically significant.

Figures in parenthesis under udder health status are percentage of samples

Supplementary materials and methods

Study area

The study was conducted in an organized research farm at the Livestock Research Centre, Southern Regional Station of the Indian Council of Agricultural Research (ICAR)-National Dairy Research Institute (NDRI), Bengaluru urban district and villages from Devanahalli taluk of Bengaluru rural district of Karnataka under non-organized dairy farming conditions. Devanahalli is one among the four taluks (Devanahalli, Doddaballapur, Hosakote, Nelamangala) in Bangalore rural district and located at an altitude of 900 meter above sea level in south eastern part of Karnataka on 13.23°N 24 latitude and 77.7°E longitudes. The climatic condition of the study area (both organized and non-organized) is subtropical in nature where the maximum temperature was 30 to 34ºC in summer and 16 to 19ºC during the winter season with average temperature of 24.37ºC during the study period. The average humidity (%), temperature humidity index (THI; ºF) and total rainfall (mm) during study period were 63, 77 and 1093, respectively (Srinivas, 2019). In general, Bengaluru urban region received the maximum rainfall in the month of June to October during southwest monsoon season followed by summer season and minimum rainfall during winter season. The average maximum and minimum temperature in the Bengaluru region were 36°C and 14°C respectively. The humidity ranged from 35-80% (Rajashekara, 2019).

In Devanahalli taluk, the majority of the dairy farmers reared crossbred cows (84%) than buffaloes (14%) or non-descript cows (2%). Most of the farmers allowed their animals for grazing about 4-6 hrs and maintained their animals in confined area attached with their own house with tie stall housing and stone slab flooring system. All the farmers followed Artificial Insemination (AI) for breeding their cows. Almost all the farmers had their own agriculture land where they mainly grown maize and ragi fodders, which was fed as green or dry roughage during lean season. Green fodder was fed in range of 5 to 10 kg per animal and maximum of 15-30 kg per animal/day. Most of the farmers procured commercially available concentrate feed from co-operative milk union (Bengaluru Milk Union Limited: BAMUL), under KMF (Karnataka Milk federation)

and fed @ 2-3 kg/day/animal during morning and evening milking times as equal portion. Hand milking was commonly practiced by all the farmers, twice daily as per their milk collection schedule of each Dairy Co-operative Societies (DCS).

Aseptic milk sampling

All the four quarters were washed and dried with clean cotton towel before sampling of milk in each cow. Before sampling, initial two to three strips of milk were discarded from each quarter. Total about 60 ml of milk from all four quarters and pooled milk of individual cows were collected aseptically and separately. A cotton soaked with 70% alcohol was used scrub the teat ends before collection of one to two ml of milk from each quarter for processing the composite milk samples for milk culturing purposes.