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Meta-analysis of Prevalence of Subclinical and Clinical Mastitis, Major Mastitis Pathogens in Dairy Cattle in India

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A B S T R A C T

In the present study, pooled prevalence of subclinical mastitis [SCM], clinical mastitis [CM] and major bacterial pathogens *viz*., *Staphylococcus species*, *Streptococcus species* and *Escherichia coli* in dairy animals was studied by using meta-analysis. Based on systematic review of studies on mastitis from 2005-2016 using online and offline databases, meta-analysis was done. Meta-analysis of 43 and 22 studies on SCM and CM, respectively was carried out with random effects model using Metaprop package in R-Software. The pooled prevalence of SCM and CM were 41% [33-49%] and 27% [19-37%], obtained by using 25,455 and 6,978 dairy animals. Pooled prevalence estimate of *Staphylococcus sp.*, *Streptococcus sp.* and *Escherichia coli* were 45%, 13% and 14%, respectively. Period-wise analysis revealed prevalence of SCM, *Staphylococcus sp.*, increased and CM, *E. coli* prevalence decreased indicating the importance of SCM and *Staphylococcus species* in mastitis. Zone-wise estimates for SCM and CM were high in South and North zone, respectively. The prevalence of subclinical and *Staphylococcus species* mastitis was high, which may cause low productivity in dairy cattle and economic loss to dairy farmers. Hence, there is a need to reduce the prevalence of subclinical and clinical mastitis by adopting scientific management, feeding and therapeutic measures for better economy of the dairy farmers.

Introduction

Mastitis is an economically important disease of dairy animals and causes economic loss to dairy farmers in India. It is the most expensive disease of dairy cattle resulting in the reduction in milk production, losses in milk quality and quantity, losses due to discarded milk, premature culling, treatment costs and labour cost (Hogeveen *et al*., 2011). The severity of the inflammation can be classified into sub-clinical, clinical and chronic forms, and its degree is dependent on the nature of the causative pathogen and on the age, breed, immunological health and lactation state of the animal. Sub-clinical mastitis is difficult to be detected due to the absence of any visible indications, and it has major cost implications. Clinical mastitis is a condition in which swelling of the udder and flakes in milk are common signs. Chronic mastitis is a rare form of the disease but results in persistent inflammation of the mammary gland. In India, the total annual economic losses due to mastitis was calculated to be 7165.51 crore rupees (Bansal and Gupta, 2009).

Previous studies on prevalence of subclinical and clinical mastitis conducted in different states of India have reported to be high is some studies and low in another. The prevalence of mastitis in dairy animals was found to be variable from study to study due to the variations in the period and location of study, breed of animal, stage of lactation, number of samples tested, sampling strategies and management practices of the dairy farms. Previous literature has reported the meta-analysis of prevalence of subclinical mastitis in dairy cows for the period 1995-2014 (Bangar *et al*., 2015), however, the meta-analysis of prevalence of clinical mastitis and major mastitis pathogens were not reported so far. Among the bacteria isolated in bovine mastitis, *Staphylococcus* species occupies an important place. Till date, more than 50 Staphylococcal species and subspecies have been characterized. The prevalence of the major mastitis pathogens in India like *Staphylococcus sp.*, *Streptococcus sp.* and *Escherichia coli* are also variable in milk of dairy animals. It is pertinent to collect the information on the prevalence of subclinical and clinical mastitis and major mastitis pathogens across different states of India, which may act as baseline information for devising mastitis control strategies and will be useful to reduce the economic losses due to mastitis in dairy sector. Hence, the present study was undertaken to do meta-analysis of available literatures on prevalence of mastitis in India to obtain the pooled and efficient estimates for subclinical and clinical mastitis, and major mastitis pathogen prevalence among dairy animals in India during the period 2005-2016.

Materials and Methods

Search strategy

Systematic research was performed on the literature about prevalence of subclinical and clinical mastitis in dairy animals in India. The databases included were Pubmed, Springer, Elsevier, Indian journals.com, Consortium of e-Resources in Agriculture (CeRA) under Indian Council of Agricultural Research, conference, seminar and symposium abstracts, etc. The studies addressing the SCM, CM and major mastitis pathogens viz., *Staphylococcus sp.*, *Streptococcus sp.*, and *E. coli* were collected for the meta-analysis. More than 100 articles were searched and results were subjected to meta-analysis to determine the prevalence rate with respect to various periods and states in India. The articles from study area based on the states in India was divided into five zones viz., North, East, West, South and Central zones. The literature search from different publications was undertaken the period from 2005 to 2016 for subclinical, clinical mastitis and major mastitis pathogen prevalence. Given a vast quantity of literature, the type of items that were collected includes the characteristics of the publications (author, year, country, state, no of samples tested and prevalence rate in different livestock species such as cattle and buffalo). The retrieval dates were limited to the period from January 2005 to August 2016 and the retrieval language was limited to English. Finally, the original articles, review articles and references cited from the retrieved articles were searched.

Data entry and filtration

Two reviewers independently extracted the characteristics of each included study onto predesigned Excel forms. These included publication year, authors, study participant eligibility criteria, study period, numbers of animals positive for mastitis, the total number of animals tested and the test used. The test used for diagnosis of prevalence of SCM and CM was California mastitis test and for major mastitis pathogens was isolation and identification by molecular methods by PCR.

Results from the previous research found in the literature must be entered in a database. The coding of the database was done with numerous variables identifying the experimental objectives of all the experiments selected. Hence, numerous columns are potentially added to code each objective found in all the sources of experimental data. This coding is necessary so as to avoid the improper aggregation of results across the studies with very different objectives. During the coding, we have chosen to transform the continuous variable to discrete variable with n levels coded in a single column with levels of discrete variables as entries. Different criteria can guide the selection of classes, such as equidistant classes, classes with equal frequencies or probability of occurrence. The important point was that sum of these descriptive columns must entirely characterize the objective of all studies used. There were at least three steps necessary to effective data filtering. First, the analyst must ensure that the study under consideration was coherent with the objectives of the meta-analysis. The meta-analysis objectives dictate that some traits must be measured and reported. The second step consisted of a thorough and critical review of each publication under consideration, focusing on the detection of errors in the reporting of the quantitative results. Verification of data entries was essential component to this process. In the third step, it was important to ensure that a selected publication does not appear to be an outlier with respect to the characteristics and relations under considerations.

Meta-analysis

In the most general terms, meta-analysis was one method of research synthesis. It employs specialized techniques for data gathering and analysis developed specifically for the purposes of research synthesis (Koricheva *et al*., 2013). Using a rigorous systematic protocol, systematic reviews was used to synthesize findings from available research reports is the gold standard for measuring the effectiveness of preventive and therapeutic interventions for the specified conditions. The overall effect calculated from a group of sensibly combined and representative condition or criteria provides an essentially unbiased estimate of the effect, with an increase in the precision of this estimate.

Test of significance of homogeneity

The test for equality of k variances was carried used in this study. The k study-specific summary statistics shared a common mean θ. A statistical test for the homogeneity of study means was equivalent to testing,

H_0: $\theta = \theta_1 = \theta_2 = \ldots\ldots \theta_k$ against

H_1: At least one θ_i is different. Under H_0, for large sample sizes,

$Q_w = \sum_1^k w_i (y_i - \hat{\theta}_{MLE})$ Follows Chi-square with (k-1) degrees of freedom

Where, $\hat{\theta}_{MLE} = \frac{\sum w_i y_i}{\sum w_i}$ and $w_i = \frac{1}{s_i^2}$

After testing for the significance of heterogeneity of studies based on chi-square test and Tau^2, depending upon the Tau^2 (heterogeneity coefficient), fixed effect and random effect models were selected for integrating the results. When the Tau^2 value is zero, the Inverse variance method was used as fixed effect model, otherwise a random effect model by Der Simonian and Laird Method (Koricheva *et al*., 2013). The random effect model allows the study variation to some extent while integrating the results.

Inverse-Variance Method (Fixed Effect model): The Inverse-Variance Method (IV method) was used for pooling either binary, continuous or correlation data. This approach was used, which has wide applicability since it can be used to combine any estimate that has standard error available. The effect size or mean are combined to give a pooled estimate (denoted by θ) by calculating weighted average of the treatment effects from the individual studies as follows.

$$\theta_{IV} = \frac{\sum w_i \theta_i}{\sum w_i}$$

Where the weights w_i are calculated as, $w_i = \frac{1}{SE(\theta_i)^2}$

That is, the weight for the i^{th} study is equal to its precision of the estimate.

The standard error of θ_{IV} is given by $SE(\theta_{IV}) = \frac{1}{\sqrt{\sum w_i}}$

The heterogeneity statistic (denoted by Q_w) is given by,

$$Q_w = \sum w_i (\theta_i - \theta_{IV})^2$$

The Q_w follows chi-square distribution with (k-1) degrees of freedom, where k is the number of studies included in the meta-analysis.

DerSimonian and Laird Method (Random Effect Model): The DerSimonian and Laird method (DL method) of meta-analysis is based on the random effects model. Under the random effects model, the assumption of common effect is relaxed, and the effect size or mean θ_i are assumed to have a normal distribution with mean θ and variance τ^2. The usual DL estimate for τ^2 is given by,

$$\tau^2 = \frac{Q_w - (k-1)}{\sum w_i - \frac{\sum w_i^2}{\sum w_i}}$$

Where Q_w is the heterogeneity statistic, and the weights w_i are calculated as in the IV Method, and k is the number of studies. The τ^2 is set to zero if Q_w < (k-1). In this approach, the weights for each study effect size w_i' are as given below.

$$w_i' = \frac{1}{SE(\theta_i)^2 + \tau^2}$$

The pooled estimate is given by,

$$\theta_{DL} = \frac{\sum w_i' \theta_i}{\sum w_i'}$$

With standard error,

$$SE(\theta_{DL}) = \frac{1}{\sqrt{\sum w_i'}}$$

The heterogeneity statistic and its test of significance are as given in the IV method.

Forest plots

Graphical representation of meta-analysis was done using forest plots. The forest plots are known as confidence interval plots, which displays effect estimates and their confidence intervals for each study and odds ratios (or log odds ratios), standardized mean differences (effect sizes), correlations (transformations), proportions (transformations), risk ratios (or log risk ratios) and differences in means. It includes the result of the overall effect from a meta-analysis, normally at the bottom of the graph, and often using a diamond to distinguish it from the individual studies (Higgins *et al*., 2013). Meta-analysis was done by using "Metaprop" package of R 3.1.0 software (Comprehensive R Archive Network). The Q statistics were also calculated using the formula as given below.

$$Q = \frac{(1-K)}{(I^2-1)}$$

Where, K-Number of studies, I^2- I squared value.

The Forest plots were prepared for the subclinical and clinical mastitis, and major mastitis pathogens based on total studies, year wise [2005-10 and 2011-16] and five zones separately. The prevalence estimates for subclinical, clinical and major pathogens in mastitis was expressed as percentage and also with confidence interval [CI] at 95 % level.

Results and Discussion

Details of prevalence studies

The present study was carried out based on systematic review of prevalence of subclinical, clinical, *Staphylococcus sp.*, *Streptococcus sp.* and *E. coli* mastitis in dairy animals of India for the period 2005-2016. The details of the studies on mastitis with location, zones and prevalence studied are presented in table 1. The forest plot analysis was carried out to show the meta-analysis results for better understanding and to determine the heterogeneity between the various studies. The prevalence studies included in this study were from nineteen states in India which includes Andhra Pradesh, Arunachal Pradesh, Bihar, Chhattisgarh, Gujarat, Haryana, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Sikkim, Tamil Nadu, Uttarakhand and Uttar Pradesh. The highest number of studies on mastitis prevalence was reported from Karnataka [10] followed by other states in India. The number of studies across different zones reported was 27, 7, 12, 24 and 2 from North, East, West, South and Central zones, respectively. There were fewer studies on mastitis from East and Central zones compared to other zones.

Meta-analysis of subclinical and clinical mastitis

The detailed results of meta-analysis of prevalence studies of subclinical and clinical mastitis in India are presented in table 1. The Forest plot for subclinical and clinical mastitis is depicted in figures 1 and 2. The number of studies on subclinical and clinical mastitis were 43 and 22, respectively was studied with a total of 25,455 and 6,978 samples from dairy animals for testing for subclinical and clinical mastitis, respectively. The pooled estimates for prevalence of subclinical and clinical mastitis were 41% (95% CI 33-49%) and 27% (95% CI 19-37%), respectively.

The subclinical mastitis showed increased prevalence from 29 to 45% during 2011-16 period compared to the clinical mastitis. This endorses the importance of subclinical mastitis than the clinical mastitis over the years. The zone wise analysis revealed increased prevalence of subclinical mastitis in East zone (50%) and clinical mastitis in North zone (35%). The clinical mastitis studies were limited or no studies from East and Central zones in India. The Q statistics for SCM and CM showed highly significant ($P<0.0001$) difference between the studies and are depicted in table 2. This indicated there was significant heterogeneity between the various studies on SCM and CM prevalence reports from various states in India.

Meta-analysis of major mastitis pathogens

The results of meta-analysis of prevalence studies of major mastitis pathogens *viz., Staphylococcus sp., Streptococcus sp.* and *Escherichia coli* are presented in table 1 and 2. The Forest plots for major mastitis pathogens are depicted in figures 3, 4 and 5. The number of studies on prevalence of *Staphylococcus sp., Streptococcus sp.* and *Escherichia coli* were 45, 40 and 39 with the overall samples studied were 9856, 13058 and 13315, respectively. Pooled estimate of prevalence of *Staphylococcus sp., Streptococcus sp.* and *Escherichia coli* were 45, 13 and 14%, respectively. The period-wise analysis showed increased prevalence for *Staphylococcus sp.*, whereas it showed decreasing trend for *E. coli* during 2011-16. However, there was more or less same trend over the years for prevalence of *Streptococcus species* from dairy animals. The zone wise analysis showed high prevalence of *Staphylococcus sp., Streptococcus sp.* in North zone and *Escherichia coli* in West zones, respectively. The Q statistics revealed highly significant difference, indicating the heterogeneity between the studies for major mastitis pathogen prevalence in dairy cows. However, there was no significant difference for *Streptococcus sp.* prevalence during 2005-10, and *Staphylococcus species*, *Streptococcus species* and *E. coli* in Central zone, which indicated homogeneity between the prevalence studies.

The results of meta-analysis provides a quantitative level of mastitis prevalence by considering differences in the results and approaches followed by various researchers in different studies from various states of India. A substantial benefit of meta-analysis is the potential to investigate the new hypothesis using existing data, both through the development of a priori hypothesis and by examination of heterogeneity. The dairy animals are affected by mastitis and causes great economic loss to dairy farmers despite adopting many preventive measures for SCM and CM in dairy cows. The present study revealed the pattern of prevalence of subclinical, clinical and major mastitis pathogens in mastitis in dairy animals from various organized and unorganized farms over the period of twelve years from different parts of the India. This study showed that the SCM was increasing and CM prevalence was decreasing over the past six year period [2011-16]. Further, there was high heterogeneity was observed between various studies for the estimates of mastitis prevalence and it might be due to various factors like parity of cattle, lactation stage and genetic makeup of animals, agroclimatic conditions and farm managemental practices (Joshi and Gokhale, 2006). More number of studies on SCM (32) and CM (19) prevalence are being reported during 2011-16 indicating the importance of mastitis in dairy cows in India during recent years.

In the present study, prevalence of subclinical mastitis at cow level was 41% and was slightly less when compared to previous meta-analysis study on subclinical mastitis was 46.35% in cows (Bangar *et al*., 2015). However, the past study reported the prevalence of subclinical mastitis ranges from 15 to 75 % (Cynthia, 2005) and concurred with the present study. The estimated prevalence observed in the present study was in agreement with results reported earlier for Bangladesh (Rahman *et al*., 2010) and Sri Lanka (Samarakoon *et al*., 2014). However, less prevalence was reported as 29.5% (Islam *et al*., 2011) and 20.2 % (Sarker *et al*., 2013) in Bangladesh for SCM. The pooled estimates of CM prevalence were 27%, which was less when compared to the SCM, indicating the importance of SCM in India.

Table.1 Details of the mastitis studies included in systematic review and meta-analysis

No.	Study [Author and year]	Study locations in India	Zones	Prevalence [SCM = 1, CM=2, MMP = 3]
1.	Sudhan *et al.*, 2005	Jammu and Kashmir	North	1, 3
2.	Bulla *et al.*, 2006	Haryana	North	1, 3
3.	Sharma N *et al.*, 2007	Chhattisgarh and Madhya Pradesh	Central	1, 3
4.	Sharma A *et al.*, 2007	Haryana	North	1, 2, 3
5.	Sumathi *et al.*, 2008	Karnataka	South	3
6.	De and Reena, 2009	Uttar Pradesh	North	1, 2, 3
7.	Kavitha *et al.*, 2009	Andhra Pradesh	South	1
8.	Sindhu *et al.*, 2009	Haryana	North	1
9.	Sudhakar *et al.*, 2009	Maharashtra	West	3
10.	Bhikane *et al.*, 2010	Maharashtra	West	3
11.	Dubal *et al.*, 2010	Sikkim	East	1, 2, 3
12.	Elango *et al.*, 2010	Tamil Nadu	South	1, 3
13.	Sharma *et al.*, 2010	Rajasthan	West	1, 3
14.	Stanikzai *et al.*, 2011	Karnataka	South	1
15.	Bhatt *et al.*, 2011	Gujarat	West	1, 2
16.	Guha *et al.*, 2011	Haryana	North	1, 3
17.	Harini and Sumathi, 2011	Karnataka	South	1,3
18.	Ranjan *et al.*, 2011	Jharkhand	East	3
19.	Bhandari and Garg, 2012	Punjab	North	1
20.	Bhanot *et al.*, 2012	Haryana	North	3
21.	Mubarack *et al.*, 2012	Tamil Nadu	South	1, 2, 3
22.	Patel *et al.*, 2012	Gujarat	West	1, 3
23.	Sadashiv and Kaliwal, 2012	Karnataka	South	3
24.	Sharma *et al.*, 2012	Punjab	North	1
25.	Sharma A *et al.*, 2012	Jammu and Kashmir	North	1
26.	Sukumar and James, 2012	Kerala	South	2
27.	Sundareshan *et al.*, 2012	Hyderabad, Andhra Pradesh	South	3
28.	Tufani *et al.*, 2012	Jammu and Kashmir	North	2, 3
29.	Visvanath *et al.*, 2012	Tamil Nadu	South	1, 2, 3
30.	Ayyappadas *et al.*, 2013	Tamil Nadu	South	3
31.	Bajjaragi *et al.*, 2013	Karnataka	South	3
32.	Hegde *et al.*, 2013	Karnataka	South	1,3
33.	Hussain *et al.*, 2013	Jammu and Kashmir	North	2, 3
34.	Jeyakumar *et al.*, 2013	Tamil Nadu	South	3
35.	Karabasanavar *et al.*, 2013	Uttarakhand	North	3
36.	Mohanty *et al.*, 2013	Odisha	East	3
37.	Pachauri *et al.*, 2013	Uttar Pradesh	North	1, 2
38.	Prakash *et al.*, 2013	Uttar Pradesh	North	1, 2

39.	Singh *et al.*, 2013	Uttar Pradesh	North	1, 2
40.	Srinivasan *et al.*, 2013	Tamil Nadu	South	1, 3
41.	Thakor *et al.*, 2013	Gujarat	West	1
42.	Biswas *et al.*, 2014	Arunachal Pradesh	East	3
43.	Chougule *et al.*, 2014	Maharashtra	West	1, 2, 3
44.	Dar *et al.*, 2014	Jammu and Kashmir	North	1
45.	Kathiriya *et al.*, 2014	Gujarat	West	1
46.	Krishnaveni *et al.*, 2014	Karnataka	South	1, 2, 3
47.	Kurjogi *et al.*, 2014	Karnataka	South	1, 2
48.	Langer *et al.*, 2014	Gujarat	West	1, 3
49.	Mir *et al.*, 2014	Punjab	North	1, 3
50.	Patnaik *et al.*, 2014	Jharkhand	East	1, 3
51.	Sahu *et al.*, 2014	Uttar Pradesh	North	3
52.	Sarkar *et al.*, 2014	Haryana	North	3
53.	Ali *et al.*, 2015	Uttar Pradesh	North	3
54.	Bhagat *et al.*, 2015	Gujarat	West	3
55.	Bharathy *et al.*, 2015	Tamil Nadu	South	3
56.	Chandrasekaran *et al.*, 2015	Tamil Nadu	South	3
57.	Ganai *et al.*, 2015	Jammu and Kashmir	North	2
58.	Gupta *et al.*, 2015	Rajasthan	West	2, 3
59.	Jena *et al.*, 2015	Rajasthan	West	1, 3
60.	Kaur A *et al.*, 2015	Punjab	North	1, 3
61.	Kaur M *et al.*, 2015	Punjab	North	3
62.	Kumar *et al.*, 2015	Jharkhand	East	3
63.	Kutar *et al.*, 2015	Uttar Pradesh	North	1, 2, 3
64.	Prabhu *et al.*, 2015	Karnataka	South	1, 2, 3
65.	Sharma *et al.*, 2015	Uttar Pradesh	North	3
66.	Sudhanthiramani *et al.*, 2015	Andhra Pradesh	South	3
67.	Hardenberg *et al.*, 2016	Bihar	East	1, 2, 3
68.	Jagadeesh *et al.*, 2016	Karnataka	South	1
69.	Laxmi *et al.*, 2016	Kerala	South	3
70.	Monica *et al.*, 2016	Tamil Nadu	South	3
71.	Panchal *et al.*, 2016	Haryana	North	1, 2
72.	Singh *et al.*, 2016	Madhya Pradesh	Central	2, 3

Note: SCM-Subclinical mastitis; CM-Clinical mastitis; MMP-Major mastitis pathogens

Table.2 Meta-analysis of prevalence studies on subclinical mastitis (SCM), clinical mastitis (CM), *Staphylococcus* sp., *Streptococcus* sp., and *Escherichia coli* mastitis in India

No.	Parameters	Period	Number of studies	Total events	Pooled Prevalence Estimates [%] (Confidence interval at 95% level)	I- squared value (%)	Tau-squared value	Degrees of Freedom	Q statistics
1	SCM	2005-16	43	25,455	41 (33-49)	98.0	1.127	42	2100.0***
2	CM	2005-16	22	6,978	27 (19-37)	98.5	1.159	21	1400.0***
3	*Staphylococcus Species*	2005-16	45	9,856	45 (39-50)	94.1	0.59	44	745.8***
4	*Streptococcus Species*	2005-16	40	13,058	13 (11-16)	92.4	0.497	39	513.1***
5	*Escherichia coli*	2005-16	39	13,315	14 (11-17)	95.3	0.7167	38	808.5***
Period wise									
6	SCM	2005-10	11	7,581	29 (20-41)	98.3	0.6917	10	588.2***
7	SCM	2011-16	32	17,874	45 (36-55)	97.6	1.176	31	1291.7***
8	CM	2005-10	3	3,409	31 (9-66)	99.3	1.667	2	285.7***
9	CM	2011-16	19	3,569	26 (18-37)	97.2	1.161	18	642.8***
10	*Staphylococcus Sp.*	2005-10	9	788	39 (30-50)	86.6	0.3589	8	59.7***
11	*Staphylococcus Sp.*	2011-16	36	9,068	46 (39-53)	94.9	0.6522	35	686.2***
12	*Streptococcus Sp.*	2005-10	8	4,185	12 (11-13)	30.3	0.0001	7	10.0^{ns}
13	*Streptococcus Sp.*	2011-16	32	8,873	13 (10-17)	91.4	0.598	31	360.4***
14	*Escherichia coli*	2005-10	9	4,279	15 (9-25)	96.5	0.7772	8	228.5***
15	*Escherichia coli*	2011-16	30	9,036	13 (10-17)	93.7	0.7183	29	460.3***
Zone wise									
16	SCM - North zone	2005-16	20	7,621	39 (30-49)	97.0	0.8141	19	633.3***
17	SCM - East zone	2005-16	3	384	50 (15-85)	97.0	2.25	2	66.7***
18	SCM- West zone	2005-16	7	12,263	37 (16-64)	98.8	2.224	6	500.0***
19	SCM - South zone	2005-16	12	3,187	47 (33-61)	96.8	1.004	11	343.7***
20	SCM - Central zone	2005-16	1	-	-	-	-	-	-
21	CM - North zone	2005-16	11	4,988	35 (23-50)	98.6	0.9795	10	714.2***
22	CM - East zone	2005-16	1	-	-	-	-	-	-
23	CM- West zone	2005-16	3	480	19 (4-56)	97.1	2.118	3	68.9***

24	CM - South zone	2005-16	6	1,090	20 (6-34)	97.3	1.285	5	185.1^{***}
25	CM - Central zone	2005-16	No data	-	-	-	-	-	-
26	*Staphylococcus Sp.*-North zone	2005-16	16	6,422	47 (35-52)	96.5	0.7539	15	428.5^{***}
27	*Staphylococcus Sp.*-East zone	2005-16	6	651	38 (27-50)	87.3	0.3486	5	39.3^{***}
28	*Staphylococcus Sp.*-West zone	2005-16	6	577	43 (22-67)	88.4	1.382	5	43.1^{***}
29	*Staphylococcus Sp.*-South zone	2005-16	15	2,072	45 (36-54)	93.3	0.4812	14	208.9^{***}
30	*Staphylococcus Sp.*-Central zone	2005-16	2	134	52 (43-60)	10.5	0.0123	1	1.1^{ns}
31	*Streptococcus Sp.*-North zone	2005-16	14	9,914	15 (10-20)	95.9	0.4258	13	317.1^{***}
32	*Streptococcus Sp.*-East zone	2005-16	7	796	8 (4-17)	91.5	1.018	6	70.5^{***}
33	*Streptococcus Sp.*-West zone	2005-16	5	505	13 (7-22)	69.5	0.3707	4	13.1^{*}
34	*Streptococcus Sp.*-South zone	2005-16	12	1,709	13 (9-19)	89.9	0.4782	11	108.9^{***}
35	*Streptococcus Sp.*-Central zone	2005-16	2	134	20 (10-36)	58.9	0.1941	1	2.4^{ns}
36	*Escherichia coli*-North zone	2005-16	10	9,276	8 (5-13)	97.7	0.6697	9	391.3^{***}
37	*Escherichia coli*-East zone	2005-16	6	651	15 (10-21)	68.3	0.1761	5	15.7^{**}
38	*Escherichia coli*-West zone	2005-16	7	707	19 (9-34)	89.9	1.074	6	59.4^{***}
39	*Escherichia coli*-South zone	2005-16	14	2,547	15 (10-23)	93.3	0.7098	13	194.0^{***}
40	*Escherichia coli*-Central zone	2005-16	2	134	13 (9-20)	0.0	0.0	1	1.0^{ns}

Note: ns- Non Significant; *- Significant at P<0.05; ** - Significant at P<0.01; ***Significant at P<0.001

Fig.1 Forest plot showing the prevalence of subclinical mastitis reported by various studies and events indicates number of mastitis affected bovines

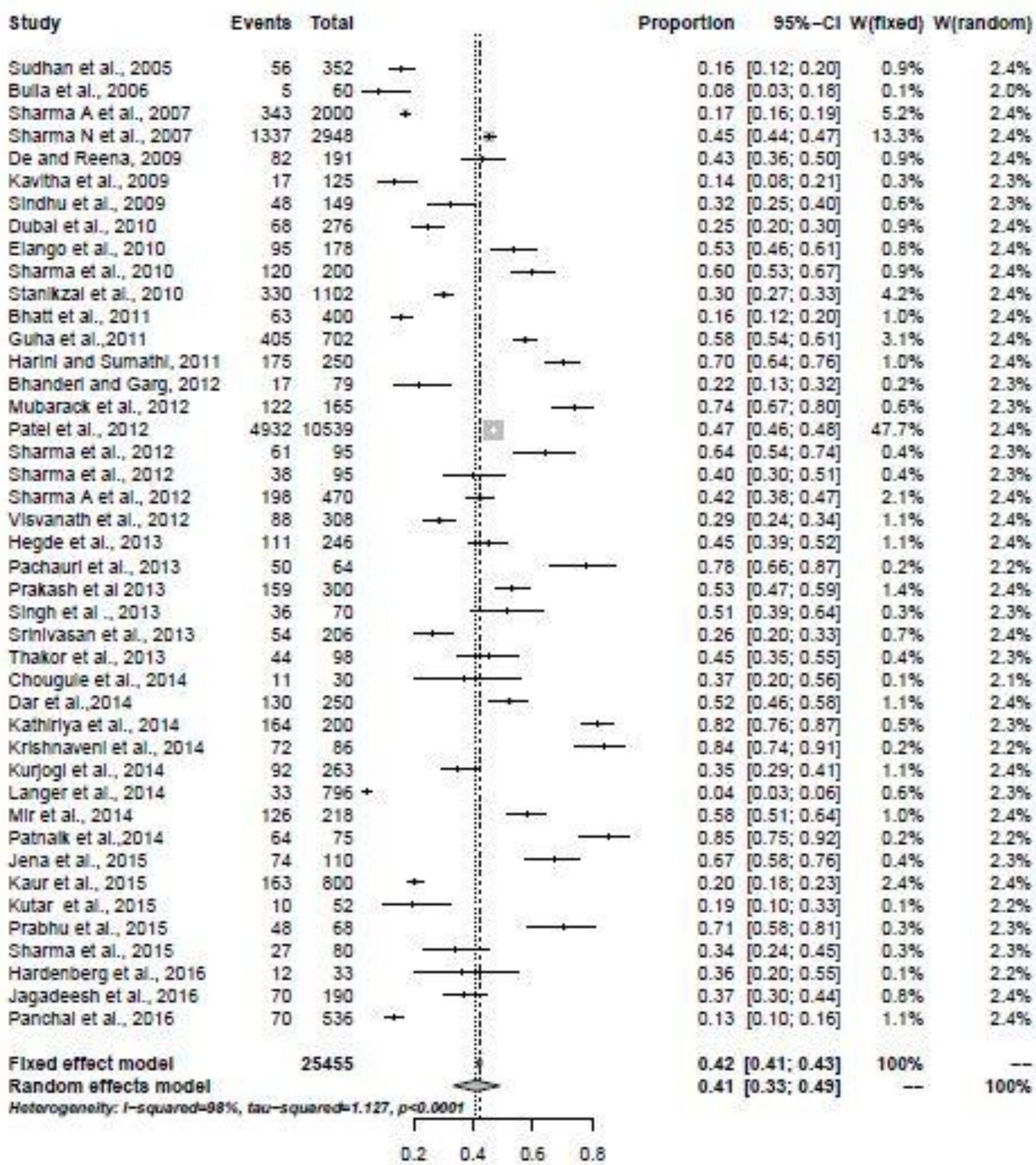


Fig.2 Forest plot showing the prevalence of clinical mastitis reported by various studies and events indicates number of mastitis affected bovines

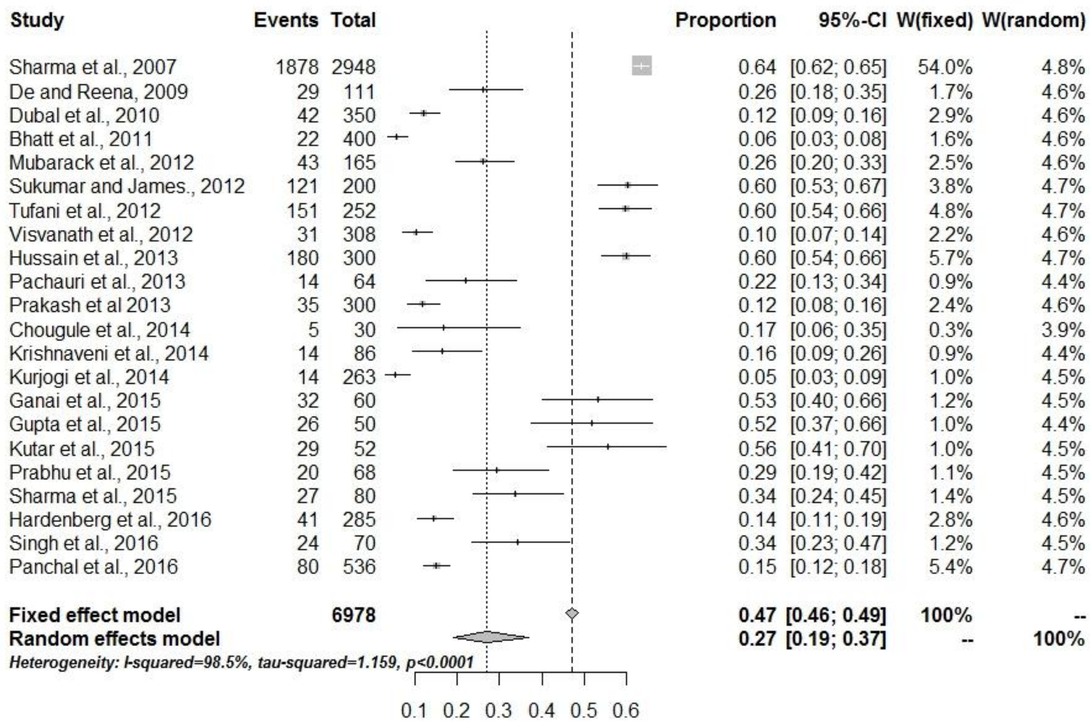


Fig.3 Forest plot showing the prevalence of *Staphylococcus species* mastitis reported by various studies and events indicates number of mastitis affected bovines

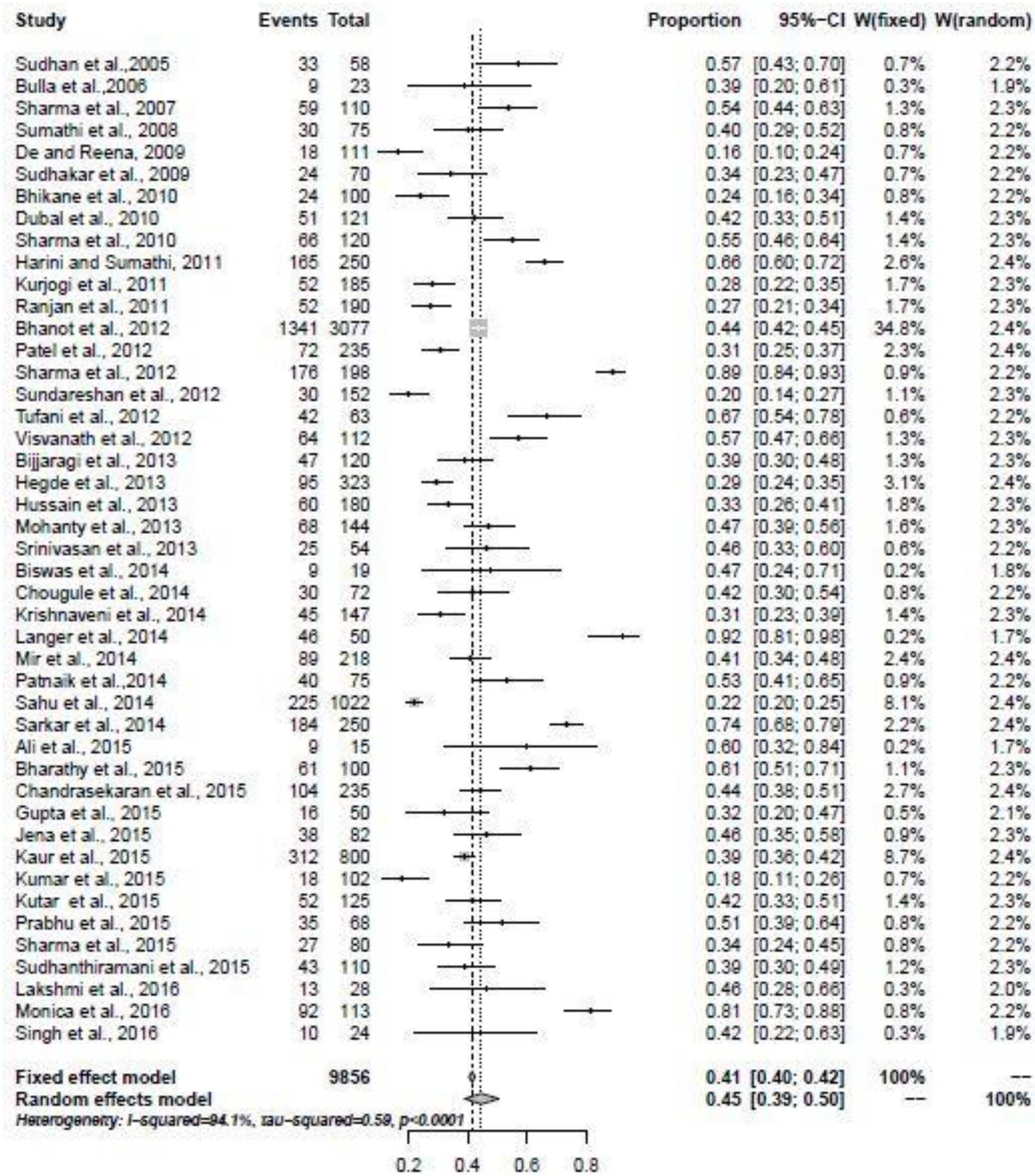


Fig.4 Forest plot showing the prevalence of *Streptococcus species* mastitis reported by various studies and events indicates number of mastitis affected bovines

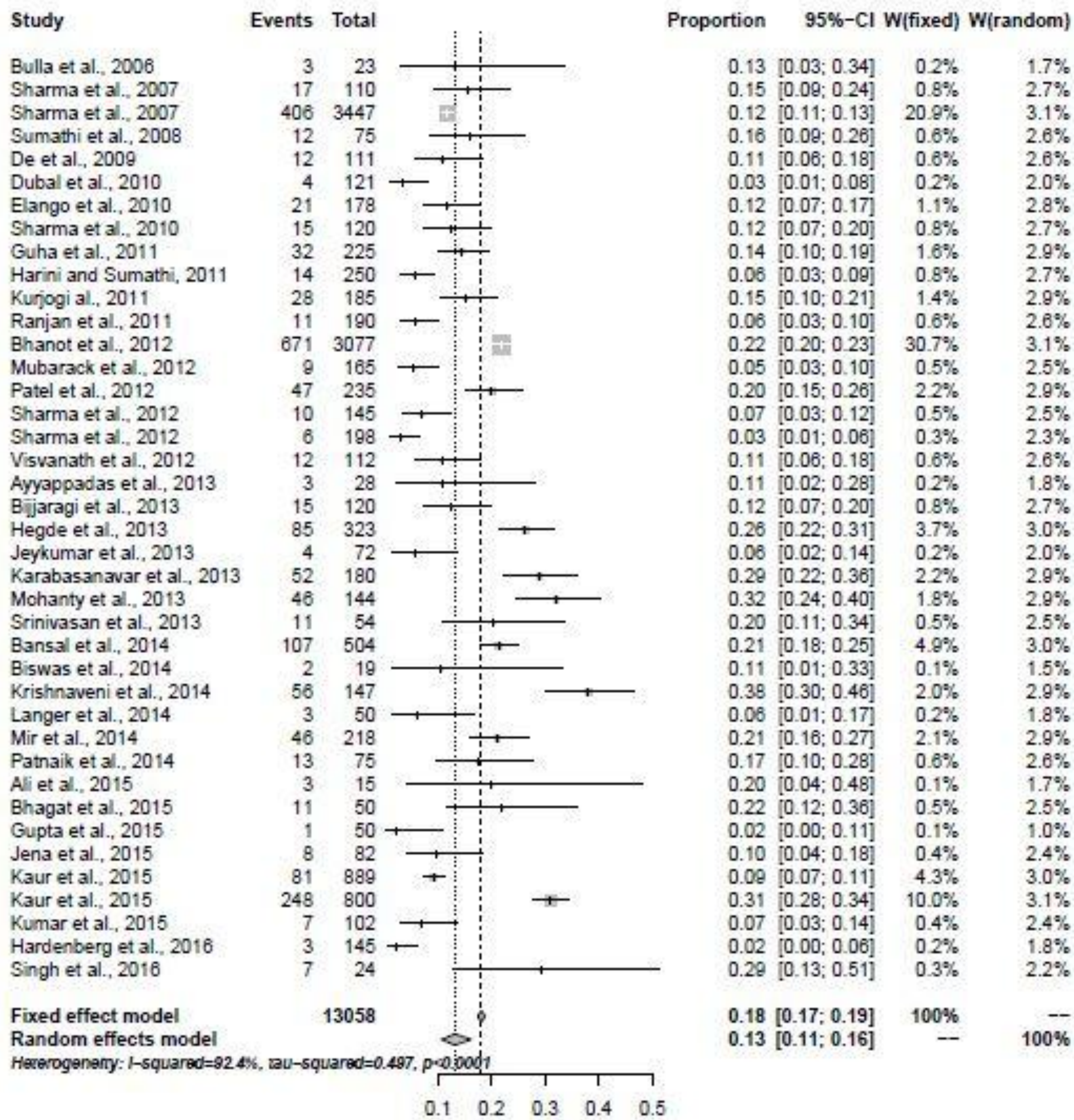
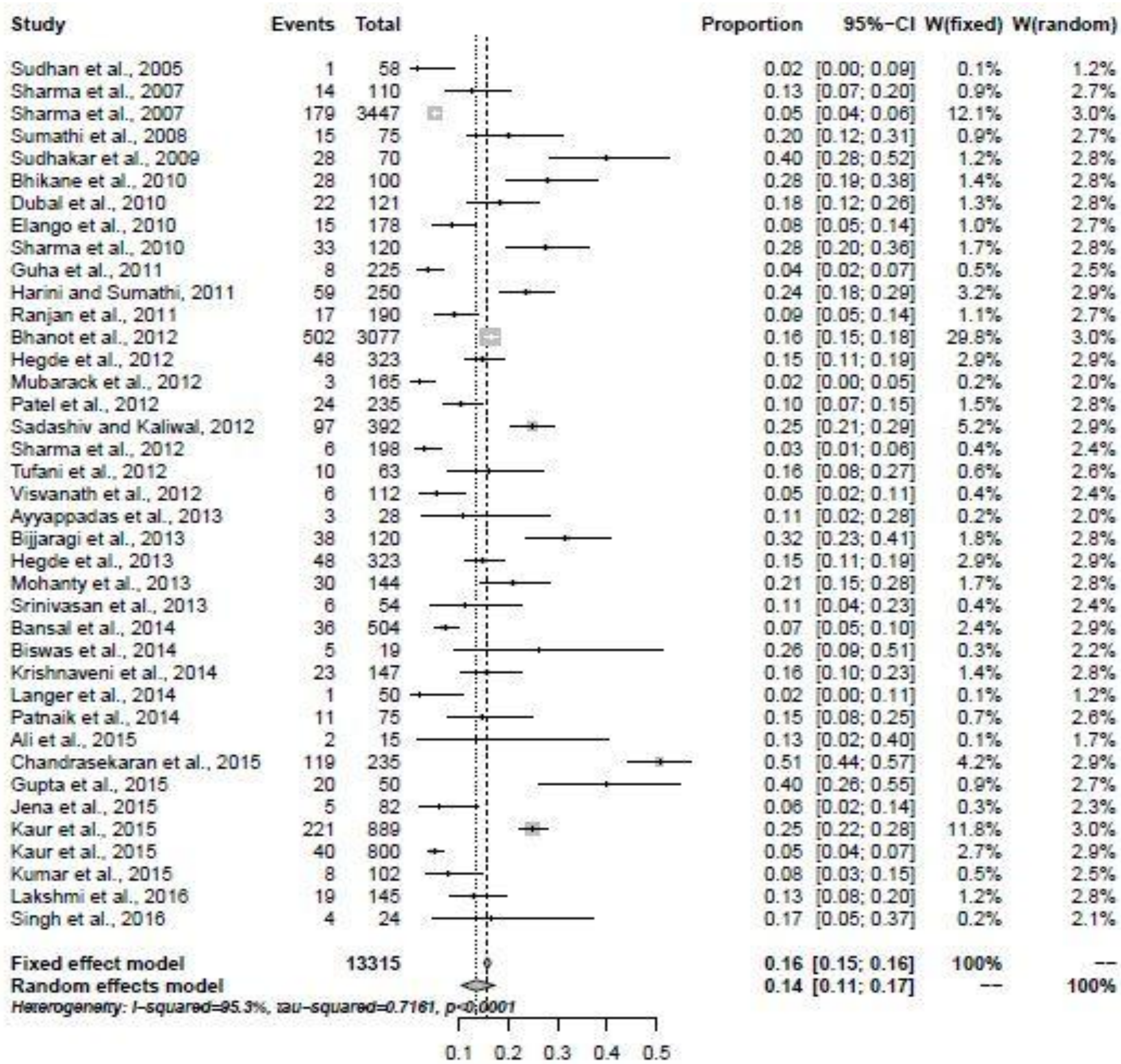


Fig.5 Forest plot showing the prevalence of *Escherichia coli* mastitis reported by various studies and events indicates number of mastitis affected bovines



However, the SCM was caused by many pathogens, without any clinical signs and unobservable nature of the infection, which is leading to major economic loss to the dairy farmers in India. The period-wise prevalence of CM was decreasing during 2011-16 when compared to 2005-11, which might be due to the awareness among the dairy farmers about the mastitis. The prevalence of clinical mastitis in Badulla district in Sri Lanka was 3.8% (Samarakoon *et al*., 2014), which was low when compared to the present study. Many studies on subclinical and clinical mastitis have been reported from North and South zones which may be due to the presence of more cases of mastitis and also more population of cattle and buffaloes in these regions. The pooled prevalence of *Staphylococcus sp.* was more (45%) when compared to *Streptococcus sp.* (13%) and *E. coli* (14%), which might be due to mastitis caused by both coagulase positive and negative *Staphylococcus species*. Further, more number of species from *Staphylococcus* group causing mastitis than other species and it is the predominant mastitis pathogen in India. The finding of *Staphylococcus sp.*, to be predominant bacteria involved in mastitis milk concurred with previous report (Bhanot *et al*., 2012). In a study in

North West of Iran, the Staphylococcal species associated with mastitis was found to be 71.5% (Hosseinzadeh and Saei, 2014) and was higher than the present study. Over the years, the pooled prevalence estimate revealed increase in *Staphylococcus* species mastitis when compared to other two major mastitis pathogens. This might be due to detection and identification of more number of *Staphylococcus* species by advanced molecular methods by species specific PCR, multiplex PCR and two tube PCR as well as by conventional methods.

Zone wise analysis revealed higher prevalence of *Staphylococcus* and *Streptococcus* species from North and Central zones and for *E. coli* was high in East and South zones. The number of prevalence studies on mastitis was increasing in recent years and might be due to advanced diagnostic methods and also due to the awareness among the dairy farmers about the quality of milk which will help them in getting more price for the milk produced.

The prevalence estimate in the present study should be viewed with certain limitations, as it does not provide the estimate for the association of disease incidence with risk factors. Further, there are many factors which causes variation between the studies are breed, lactation number, milk yield, management practices, climatic conditions, geographical conditions and period of study may affect the prevalence of SCM and CM in dairy cattle. In order to compare the results between different studies and diagnostic tests, a common test for SCM and CM were considered as diagnostic criteria, in case of major mastitis pathogens, isolation of organisms is considered as diagnostic criteria.

In conclusion, the present study estimated the pooled prevalence of subclinical, clinical mastitis and major mastitis pathogens in dairy cattle in India using the random effects model. There is high prevalence of SCM than CM indicating the importance of SCM and need for scientific management methods to be adopted to minimize the incidence of SCM in dairy cows in India. The high prevalence of subclinical mastitis in dairy cattle may cause low milk yield over the years and lead to greater economic loss to the dairy farmers. *Staphylococcus species* is more prevalent in mastitis than other pathogens in India. Hence, the study advocates, to overcome the occurrence of mastitis, there is need for scientific management practices and timely therapeutic interventions by field veterinarians, so as to improve the productivity of dairy animals in India. Further, more studies were reported from North and South zones, however, to assess the overall prevalence levels of mastitis in India and across the zones, there is a need for more studies on mastitis prevalence from Central and East zones of India.

Conflict of Interest

The authors declare no conflict of interest.

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