RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.2.226-234

Comparative efficacy and validation of different diagnostic methods in detection of subclinical mastitis in farms of Bundelkhand

Balendra Singh[1*] and Ramesh Kumar[2]
Department of Biotechnology
Bundelkhand University, Jhansi, India

Citation: Singh, B. and Kumar, R. 2022. Comparative efficacy and validation of different diagnostic methods in detection of subclinical mastitis in farms of Bundelkhand. *J. Vet. Anim. Sci.* **53**(2): 226-234
DOI: https://doi.org/10.51966/jvas.2022.53.2.226-234

Received: 10.08.2021 *Accepted: 20.09.2021* *Published: 30.06.2022*

Abstract

A cross-sectional study was undertaken to validate the comparative efficacy of different diagnostic tests commonly used for the detection of subclinical mastitis. The study was designed to determine the specificity, sensitivity, and accuracy of common tests viz. California Mastitis Test (CMT), Indicator Paper Method (BTB), Somatic Cell Count (SCC), and Electrical Conductivity (EC) with bacterial culture as a standard, in parts of Bundelkhand region. Of the 411 samples, 295 were found to be positive for subclinical mastitis on screening with either of the 4 methods routinely used for the diagnosis of subclinical mastitis (SCM). The remaining 120 milk samples were negative for SCM with all methods used. Individual test-wise percentage prevalence was 26.28, 44.53, 30.41, and 41.85% respectively with CMT, EC, BTB, and SCC, respectively. On comparing the results with the culture test, the present study confirmed the superiority of SCC based method for the detection of subclinical mastitis. Correlating the EC results with SCC values avoids false results with maximum accuracy.

Keywords: *Subclinical mastitis, mastitis detection, validation, CMT, SCC*

Mastitis refers to inflammation of the mammary gland due to infection during which, the tissues and ducts in the secretary system of the glands become damaged by persistent and opportunistic infection of microorganisms. The disease leads to increased economic burden because of the associated costs in preventive measures, management and related changes in qualitative as well as quantitative terms due to degradation of milk quality. Bovines are widely used for milk production throughout the world and bovine mastitis is a major factor affecting milk production. Of the two forms of mastitis *viz.*, clinical and subclinical, subclinical mastitis (SCM) is of greater concern due to the invisible health effects on the animals, where scientific monitoring of animals, as well as farmers is quite significant in enforcing control measures. Adoptability of tests as well as their sensitivity and accuracy are important factors to be taken into consideration for the correct, rapid and real-time diagnosis of the disease to determine the course of treatment.

1. *Former Assistant Professor, Bundelkhand University Jhansi, Currently, Department of Biotechnology, Ministry of Science and Technology*
2. *Associate Professor, Department of Biochemistry, Bundelkhand University Jhansi, India*

**Corresponding author: risingsun.balendra@gmail.com Ph 9412690765*

Unfortunately, 20-25 per cent of the burden of cases occur due to subclinical mastitis and is neglected due to its invisible nature, inviting delayed treatment in the present-day animal husbandry sector. This burden might be much higher in developing countries like India due to the lack of data from the hinterlands and local farms. Electrical conductivity of milk and counting the somatic cells were considered important strategies to detect subclinical mastitis in previous studies (Hegde *et al*., 2013). The authors also advised fine-tuning in SCC and EC so as to limit the higher probability of positive correlation between the coagulase-negative staphylococci (CoNS) and somatic cell count. The correct, specific diagnostic methods for different agroclimatic regions may have greater implications in identification, management, and clinical course recommendation to control the subclinical mastitis.

As per recommendations made by the International Dairy Federation, Somatic Cell Count is the recommended test with standard as bacterial culture (Reddy *et al*., 2014), but it is seen that varying results have been obtained in previous studies and it is important to determine the validity for the efficacy of the tests concerning different agro-climatic regions to establish the efficacy of field tests so these could be used as a choice for rapid on-site delivery of testing services. Considering this approach, a study was designed to assess the comparative efficacy of different detection tests in parts of Bundelkhand.

Materials and methods

Milk samples were collected from three districts of the Bundelkhand region *i.e.* Jhansi, Jalaun, and Lalitpur. A preliminary survey for subclinical mastitis based on history and occurrence was conducted in Jhansi, Lalitpur, and Jalaun Districts. Villages were selected randomly. Fresh milk samples were directly collected quarter-wise, except in a few cases, where composite milk samples had to be collected owing to difficulty in animal handling. During sample collection, all sanitary measures were considered as per the National Mastitis Council, USA. Prior cleaning of the udder with water followed by 70 per cent alcohol was done before collecting milk samples. After 5 minutes, 10 ml of milk sample was directly drawn into horizontally tilted falcon tubes to avoid contamination due to skin shedding. Each sample was assigned with a code number for the owner of the animals. Milk sample for California mastitis test was directly drawn into the cups of CMT paddle to check mastitis status on field. A thermocol box filled with ice packs was used to transport the samples. Samples were processed immediately for bacteriology, SCC count and EC. The remaining milk sample was stored at -80°C. All culture media and supplements were purchased from Himedia laboratories, Mumbai and reagents used were of analytical and molecular grade.

Screening of subclinical mastitis in the milk samples

SCC, CMT, EC, and BTB Strip-based techniques were used to screen subclinical mastitis in all the collected milk samples. The procedure described by Galdhar *et al.* (2004) was followed in the screening of bovine milk samples for subclinical mastitis. CMT working reagent is composed of anionic surface active compounds (Teepol 0.5% (v/v) + NaOH 1.5% (w/v)\) mixed thoroughly with an indicator dye, bromothymol blue 0.01% (w/v). The results of CMT was interpreted as in Table 1. The immediate reaction leads to the formation of gel and precipitation in positive samples. The appearance of milk samples that tested positive for mastitis was greenish due to alkalinity while the increased number of leucocytes was responsible for gel formation (Table 1).

Vliegher *et al.* (2012) showed the variability in the type of infections for the quarter. Sample collected from each quarter was considered as individual samples. The calculated sample size was considered for the study following formula devised by Thrusfeild (2005).

$$n = \frac{z^{2*} P_{\text{exp}} \ (1 - P_{\text{exp}})}{d^2},$$

n=sample size needed z= α value at 95 per cent confidence interval=1.96 Pexp= prevalence expected d= precision desired.

Table 1. Interpretation Chart for California Mastitis Test in correlation with SCC count

Visible reaction	CMT Score given	Interpretation	SCC/ml
No change in milk	1	Negative	<100000
Light precipitation	2	Trace	b/w 1-200000
Clear precipitation, No gel formation	3	Weak +Ve	b/w 2-400000
Solution thickens and gel formation	4	+Ve	b/w 4-500000
A great increase in viscosity and strong gel	5	Strong +Ve	>500000

Procured milk samples were undertaken for the estimation of electrical conductivity with a handheld instrument (Jyothi Scientific Industries, Gwalior). EC≥6.5mS/cm was considered to mark the sample positive for subclinical mastitis.

The freshly collected milk samples were processed to estimate the number of somatic cell count by direct microscopy method. Direct screening of milk samples to determine mastitis status by counting somatic cells was done with Newmann's staining with microscopy. The procedure described by Prescott and Breed (1910) in general and used by Schalm *et al*. (1971) was followed. Microscopic field diameter seen through oil immersion lens was measured up to two decimal points using stage micrometer slide ruler in 0.1 to 0.01 mm. Formula πr^2 was used to calculate the area of the field. Milk pH was determined with a digital pH meter supplied by Jyoti Scientific, Gwalior. Standard buffer solutions of pH 4, 7.2, and 9.2 were used to calibrate the pH meter each time before use. The somatic cell count includes accounting for different inflammatory cells in stained milk smear, which includes polymorpho-nuclear (PMN) cells (including neutrophils, lymphocytes, macrophages). The secretory glandular desquamated epithelial cells were also considered in SCC counting. The somatic cell count >5 lakhs/ml, conventional criteria was considered to declare the milk samples as positive for subclinical mastitis.

Bacteriological culture of milk

Bacteriology related wet lab work, biochemical characterization, and primary bacteriological diagnosis were carried out at the Department of Biotechnology, Bundelkhand University Jhansi by culturing milk samples on different generalized and selective bacteriological media according to previous studies described by Collee *et al.* (1996), with local modifications to adjust pH and solidification. In accordance to Ericsson *et al*. (2009), Samples positive for at least 3 cfu/ml were considered as bacteriologically positive for all bacterial genera. But the growth of a single colony was considered positive in the case of Staphylococci.

The purity and growth quantity of each agar plate was determined and categorized as abundant if, >50 cfu/ml, moderate 10-50 cfu/ml, and mild, if it was less than 10 cfu/ml. Further, bacteria were classified by colony morphology and haemolysis characteristics. The further classification was done by biochemical characterization in accordance with Bergey's manual of determinative bacteriology based on specific genera (Holt *et al*., 1994). Criteria used in classification were based on colony morphology following Hogan (1999). Media plates found positive for three or more different pathogens were considered as mixed cultures.

Results and discussion

Data for samples and the number of samples found positive for subclinical mastitis with different tests in different districts and herds are presented in Table 2. Further, Table 3 reveals the comparative statistical significance of the results obtained with these four tests by using the analysis of variance.

California Mastitis Test

Out of total of 411 milk samples, 108 (26.27 per cent) samples were found positive for subclinical mastitis. Recorded teat-wise prevalence of subclinical mastitis was 25.97, 30.77 and 21.05 per cents in Jhansi, Jalaun, and Lalitpur district, respectively.

Table 2. Prevalence of subclinical mastitis (SCM) determined with different diagnostic tests

Sr. No.	Site	No. of samples collected	Number of samples found positive			
			CMT	EC	BTB	SCC
Jhansi						
1.	Site-1	49	15	20	08	22
2.	Site 2	54	13	23	16	24
3.	Site 3	51	12	26	12	28
	Total	154	40	69	36	74
Percentage		-----	25.97	44.81	23.38	48.05
Jalaun						
4.	Site 1	50	14	21	12	25
5.	Site 2	40	12	22	20	15
6.	Site 3	53	18	17	21	17
	Total	143	44	60	53	57
Percentage		-----	30.77	41.96	37.06	39.86
Lalitpur						
7.	Site 1	30	08	18	16	13
8.	Site 2	40	07	14	12	12
9.	Site 3	44	09	22	08	16
	Total	114	24	54	36	41
Percentage		-----	21.05	47.36	31.58	35.96
Grand total		411	108	183	125	172
Percentage		-----	26.28	44.53	30.41	41.85

Table 3. Comparison between the results obtained with different tests

N	CMT	EC	BTB	SCC	Total
	9	9	9	9	36
∑X	108	183	125	172	588
Mean	12	20.3333	13.8889	19.1111	16.333
$\sum X^2$	1396	3823	1913	3552	10684
Std.Dev.	3.5355	3.5707	4.7022	5.7542	5.5549

Table of significance

Source	*SS*	*df*	*MS*	
Between-treatments	436.2222	3	145.4074	*F* = 7.22771
Within-treatments	643.7778	32	20.1181	
Total	1080	35		

The *f*-ratio value is 7.22771. The *p*-value is .000777. The result is significant at $p<.05$.

Electrical conductivity (EC) measurement

Samples were categorised into two groups based on EC values ≥6.5mS and <6.5mS. EC value was found ≥ 6.5mS for 183 (44.53%) samples and <6.5 mS for remaining 228 (55.47%) samples. An EC value greater than 6.5mS was considered as standard to declare the sample as positive for subclinical mastitis. The recorded prevalence of subclinical mastitis was 44.53 per cent with this method.

Indicator paper method (Bromothymol Blue strip test)

Out of 411 samples screened for SCM, 30.41 per cent (125) milk samples were found positive for subclinical mastitis with BTB strip test.

Somatic cell count (SCC) estimation

The number of cases of subclinical

mastitis was more in semi-urban areas as compared to rural areas. Based on total counts of somatic cells, milk samples were divided into 4 different groups *i.e*. 0-1, 1-2, 2-5, and >5 lakhs cells/ml. It is recorded that 380 out of 411 milk samples were found to show the countable somatic cells in the range of 0-5 lakhs. Individually, 89,68, 82 and 172 samples revealed SCC value in range of 0-1, 1-2, 2-5 and >5 lakhs/ml, respectively. Somatic cells could not be determined in 31 samples, and these were considered in the group 0-1 lakhs/ml. Thus, conventional criteria showed the 41.85 per cent prevalence of subclinical mastitis.

Overall, 295 out of 411 samples were found to be positive for subclinical mastitis on screening with either of the four methods of routine use in the diagnosis of subclinical mastitis. The remaining 116 milk samples were negative for SCM with all methods used. This data revealed an overall prevalence of 71.78 per cent on considering all tests as valid.

The normal appearance of milk and absence of visible signs in the mammary gland tissue is the major barrier in the early detection of subclinical mastitis (Mishra *et al*., 2018). SCM creates a reservoir of microorganisms that act as a source of infection to the other individuals and help in the preponderance of clinical mastitis (Thompson *et al*., 2014). More than 137 organisms belonging to different classes and taxa have been identified as pathogens of bovine subclinical mastitis, including bacteria, viruses, fungi, algae, and mycoplasma (Watts, 1988). *Staphylococcus aureus* has been considered as the major causative agent (Verma *et al.,* 2017) but coagulase-negative Staphylococci have been reported by many authors in different countries as the most prevalent pathogen in bovine subclinical mastitis e.g. Poland, Iran, and India (Sztachanska *et al*., 2016; Chavoshi and Husaini, 2012; Hegde *et al.,* 2013). So the objectives designed were in concurrence with the studies with a focus on the Bundelkhand region. A similar pattern of dominance of Coagulase-negative staphylococci was also found in our study. \ As most of these organisms are commensals in human being, studies on the impact of single species on bovine udder health are possible only after the accurate identification of causatives agents (Zadocks and John, 2011). Data presented in Table 2 reveals the overall prevalence of SCM in the selected region with 4 different tests *viz*., CMT, EC, BTB strip and SCC with values 26.28, 44.53, 30.41 and 41.85 per cent, respectively. Reliability of method based on SCC for the determination of prevalence of subclinical mastitis is established in previous studies (Hegde *et al*., 2013). The international dairy federation also recommends the SCC and bacterial culture-based diagnostic test to detect mastitis (Anonymous, 1971). The cut-off value of SCC count to consider the animal positive for SCM varies from country to country *viz*., $4x10^5$ cells/ml in New Zealand and Australia, $5x10^5$ cells/ml in Canada, $2x10^5$ cells/ml in Sweden, and $7.5x10^5$ cells/ml in the USA. However, no standard cut-off is prescribed in India, so the cut-off value considered was $5x10^5$. But National Mastitis Council, USA prescribes all the above 4 tests for the detection of mastitis. So, it is considered that no single test can detect SCM with 100 per cent accuracy. Therefore, we have used all four tests for prevalence studies. Based on somatic cell count, the highest prevalence of SCM was found in Jhansi district with 48.05 per cent followed by Jalaun and Lalitpur where prevalence was 39.86 and 35.96 per cent, respectively. Previously, a 42.85 per cent prevalence of subclinical mastitis was reported in Bidar, Karnataka (Ambika *et al.,* 2021) and 34.9-46.00 per cent in Dharwad, Karnataka (Mahantesh *et al*., 2014). The prevalence of bovine subclinical mastitis in India and worldwide, was reported to be in the range of 30-50 per cent (Sanotharan *et al*., 2016; Mpatswenumugabo *et al*., 2017; Said *et al*., 2018).

EC test for the detection of subclinical mastitis is also recommended by the International Dairy Federation. Based on the EC test, the prevalence was found 44.81, 41.96, and 47.36 per cent, respectively for Jhansi, Jalaun, and Lalitpur districts. The recorded range was in accordance with SCC but for the Lalitpur district, it was very high in comparison to SCC-based prevalence results. One of the reasons behind this disparity may be the low sample size from the Lalitpur district. Findings are in close agreement with Shabaz *et al*. (2020) but lower than Jinu and Singh,

(2020). The opening of alveolar junctions and increase in permeability of capillaries due to bacterial infections is responsible for higher electrical conductivity followed by an increase of secretion of high Na+, K+, and Cl- into the extracellular fluid. Ultimately level of these ions is also increased in the milk of the infected glands. EC test thus shows this increased ion content in the milk samples of infected quarters (Paudyal *et al*., 2020). A significant difference was observed within the group ($p<0.05$)

CMT test was performed in accordance with guidelines established by National Mastitis Council, highest SCM prevalence was recorded in Jalaun district (30.77%) followed by 25.97 and 21.05 per cent, respectively for Jhansi and Lalitpur. Our results are in agreement with studies done by different authors in various parts of the country (Senthilkumar *et al*., 2020; Karabasanavar *et al.* 2019; Swami *et al.,* 2017). Results produced with CMT may differ from SCC-based prevalence determination (Iraguha *et al*. 2017). They reported lower prevalence for a selected population with CMT test in comparison to the SCC-based method in a similar study on sensitivity comparison. BTB strip test-based diagnostics reported the highest prevalence of 37.06 per cent in Jalaun followed by 31.58 and 23.38 per cent, respectively for Lalitpur and Jhansi. Prevalence of mastitis based on CFT differed from the cross-sectional study done in 2018 in Jhansi with partial and low sample sizes (Singh and Kumar, 2018). Table 3 shows the significant difference in prevalence determined by all four tests ($p<0.05$). So none of the single methods can be considered as reliable for the determination of prevalence of subclinical mastitis, But SCC was the only method that is used as confirmatory evidence in previous studies (Chakraborty *et al.,* 2019; Iraguha *et al*., 2017). So in the present investigation, SCC results were used to determine the ultimate prevalence of subclinical mastitis in the selected region which differed with the other 3 tests *viz.* CMT, BTB and EC, significantly ($p<0.05$). ANOVA revealed the significant difference in prevalence reported from different sites and by different methods also.

Bacterial isolation from milk samples

Studies on bacteriology of milk samples completed by microscopy, total viable count on nutrient agar and culturing on Cystine Lactose electrolyte-deficient Agar (CLED) to differentiate contaminants, mannitol salt agar (MS) as selective for gram positive bacteria, haemolysis studies on blood agar. Different species of bacteria were categorized into separate groups on the basis of microscopic character sticks and colony morphology. Collected milk samples were inoculated to the McConkey agar and Blood agar base enriched with 7% defibrinated sheep blood followed by aerobic incubation for 24 to 48 hours at 37^0C with modifications to adjust pH, solidification. Although, 41.58 per cent of samples were identified as positive for subclinical mastitis, all the samples were subjected to bacterial isolation. Total 365 isolates belonging to *CoNS*, *S. aureus*, *Streptococcus sp*., *Bacillus*, *Corynebacterium* and *E.coli* based on microscopic and cultural characteristics were identified. Coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* were found to correlate with the pattern of somatic cell count. The largest numbers of staphylococcal isolates were recovered from the samples showing SCC > 5 lakhs/ml. Three hundred and sixty-five (88.8%) out of 411, samples were found bacteriologically positive (Table 4). The bacterial counts on different media are presented in Table 5.

Table 4. Bacterial isolation from milk samples

Place	Collected number of samples	CoNS	CoPS	Total number of Staphylococci
Jhansi	154	49	19	68
Jalaun	143	48	12	60
Lalitpur	114	48	13	61
Total (Subclinical)	172	125	44	169
Clinical	15	02	Nil	02

Table 5. Total bacterial counts on different media in cfu/ml.

SCM Status	Source	Nutrient agar	CLED Agar	McKonkey Agar	MS Agar
SCM +Ve	Buffalo	$2.14x10^5$	$9.55x10^4$	$8.4x10^3$	$1.65x10^4$
	Cow	$1.24x10^5$	$7.57x10^4$	$6.0x10^3$	$1.14x10^4$
SCM -Ve	Buffalo	$7.4x10^4$	$8.22x10^4$	$8.6x10^3$	$1.65x10^4$
	Cow	$6.5x10^4$	$5.25x10^4$	$1.9x10^3$	$4.8x10^3$

A total of 365 bacterial isolates were identified by biochemical microbial culture followed by biochemical identification. Standard biochemical tests used for identification in the study are in accordance with Bergey's Manual of Determinative Microbiology as adopted by Collee *et al.,* 1996. Centrifugation of all the collected milk samples was carried out to increase the probability of bacterial detection as recommended previously (Lima *et al*., 2018; Jinu and Singh, 2020). Bacteria could be isolated from 41.8 per cent of samples, which was in the alignment of the results obtained through somatic cell count followed by EC Test. Mixed infections were found very common as 220 milk samples were found to infect with more than one bacterial group.

Conclusion

Present study confirms the superiority of SCC based method for the detection of subclinical mastitis. Correlating the EC results with SCC values may avoid false results with maximum accuracy. SCC was found most reliable diagnostic test but from point of ease of doing on-field detection of SCM. EC is recommended followed by confirmation through SCC as evident from the findings of this study. However, considering the zone to zone variations, it is recommended to extend the study to different climatic zones for specific recommendations on grouping of the tests.

Acknowledgement

The authors acknowledge the support extended by the laboratory and other staff of the Department of Biotechnology and Biochemistry, Bundelkhand University, Jhansi and support extended by colleagues in terms of discussions on various aspects in the execution of work.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ambika, P.W., Patil, M. V, Halmandge, S., Suranagi, M.D. Awati, B. 2021. Incidence of subclinical mastitis in Deoni cattle in bidar district of Karnataka and comparison of different techniques used for its detection. *J. Entomol. Zool. Stud*. **9**:1828-1833.

Anonymous. 1971. A monograph on bovine mastitis. In: International Dairy Federation Bulletin, Brussels, Belgium.

Chakraborty, S., Dhama, K., Tiwari, R. 2019. Technological interventions and advances in the diagnosis of intramammary infections in animals with emphasis on bovine population-a review. *Vet Q.* **39(1)**:76-94. doi:10.1080/01652176.2019.1642546

Chavoshi, M. and Hussaini, J. 2012. 2nd International Conference on Biomedical Engineering and Technology. *IPCBEE*. **34**:143-146.

Collee, J., Miles, R and Watt, B. 1996. Mackie and McCartney Practical Medical Microbiology, 14th ed. New York: Churchill Livingstone.

Ericsson, U.H, Lindberg, A, Persson, W. K, Ekman, T., Artursson, K., Nilsson-Ost, M. and Bengtsson, B. 2009. Microbial aetiology of acute clinical mastitis and agent-specific risk factors. *Vet. Microbiol*. **137**:90-107. doi: 10.1016/j.vetmic.2008.12.005.

Galdhar, C. N., Gaikwad, R. V., Samad, A., Keskar, D.V., Bhalerao, D. P, Dighe, D. G and Zende, R. J. 2004. Bovine Udder and its Immunity-An Update. *J. Remount and Vet. Corps.* **42**:179-185.

Hegde, R., Shrikrishna I., Nithin P.K., Shome, B.R., Rathnamma, D., Suryanarayana, V.V.S., Yatiraj, S., Renuka, P.C., Krishnaveni, N., S.S., Akhila, D.S., Gomes, A.R. and Nagendra, R.H. 2013. Incidence of Subclinical Mastitis and Prevalence of Major Mastitis Pathogens in Organized Farms and Unorganized Sectors. *Indian J. Microbiol.* **53**:315–320.

Hogan, J. and Smith, K.L. 2003. Coliform mastitis. *Vet. Res*. **34**: 507-519.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley J.T., Williams S.T. 1994. Bergey's Manual of Determinative Bacteriology. 9th ed.: Baltimore. pp. 527-37.

Iraguha, B., Hamudikuwanda, H., Mushonga, B., Kandiwa, E. and Mpatswenumugabo, J.P. 2017. Comparison of cow-side diagnostic tests for subclinical mastitis of dairy cows in Musanze district, Rwanda. *J. S. Afr. Vet. Assoc*. **88**:1464.

Jinu, M. and Singh, M.K. 2020. Seasonal Prevalence and Antibiogram Studies of Bovine Mastitis in Southern Haryana. *Journal of Animal Research*. **10**(6):1037-1042.

Karabasanavar, N.S., Radder, S.K. and Sivaraman, G.K. 2019. Field level interventions on subclinical mastitis and detection of Staphylococcus in crossbred dairy cows. *Indian J. Anim Sci*. **89**:711–717.

Lima, SF, Bicalho, M.L.D.S., Bicalho RC. 2018. Evaluation of milk sample fractions for characterization of milk microbiota from healthy and clinical mastitis cows. *PLoS One*. **13**: e0193671. https://doi.org/10.1371/journal.pone.0193671.

Mahantesh, M.K. and Basappa, B.K. 2011. Prevalence and Antimicrobial Susceptibility of Bacteria Isolated from Bovine Mastitis. *Adv Appl Sci Res.* **2**: 229-235.

Mishra, K., Sharma, N., Singh, D.D., Gururaj K., Kumar, V., and Sharma, D.K. 2018. Prevalence and bacterial etiology of subclinical mastitis in goats reared in organized farms. *Vet. World*. **11**: 20–24.

Mpatswenumugabo, J.P., Bebora, L.C., Gitao, G.C., Mobegi, V.A., Iraguha, B., Kamana, O., Shumbusho, B. J. 2017. Prevalence of Subclinical Mastitis and Distribution of Pathogens in Dairy Farms of Rubavu and Nyabihu Districts, Rwanda. *Vet Med*. Published online.8456713. doi: 10.1155/2017/8456713.

Prescott, S.C., Breed, R.S. 1910. The determination of the number of body cells in milk by a direct method. *J. Infect. Dis.* **7**: 632.

Reddy, B.S.S., Kumari, K.N., Reddy, Y.R., Reddy, M.V.B. and Reddy, B.S. 2014. Comparison of different diagnostic tests in subclinical mastitis in dairy cattle. *Int. J. Vet. Sci.* **3**: 224-228.

Paudyal, S., Melendez, P., Manriquez, D., Velasquez-Munoz, A., Pena, G., Roman-Muniz, I.N. and Pinedo P.J. 2020. Use of milk electrical conductivity for the differentiation of mastitis causing pathogens in Holstein cows – ERRATUM. *Animal*. **14**:597.

Sanotharan, N, Pagthinathan, M. and Nafees, M.S.M. 2016. Prevalence of Bovine Subclinical Mastitis and its Association with Bacteria and Risk Factors in Milking Cows of Batticaloa District in Sri Lanka. *Int. J. Sci. Res. Innov. Technol.* **3**: 112-17.

Schalm, D.W., Carroll, E.J. and Jain, C. 1971. Bovine mastitis. Lea and Fibiger, Philadelphia. pp. 182-282.

Senthilkumar, A., Murugesan, S. and Balamurugan, P. 2020. Prevalence and

detection of Subclinical Mastitis by California Mastitis Test (CMT) in Dairy Farms of Theni District in Tamil Nadu, India. *Int. J. Curr. Microbiol. App. Sci*. **9**: 988-993.

Shabaz, S.S., Prameela, D.R., Sreenivasulu, D., and Sujatha, K. 2020. Prevalence of Clinical and Sub Clinical Mastitis In Bovines Of Chittoor District. *Res. J. Chem. Environ. Sci*. **8**:44-56.

Singh, B. and Kumar, R. 2018. Prevalence of sub-clinical mastitis dominated by coagulase negative Staphylococci sp. in bovines. *Int. J. Pharm. Biol. Sci*. **8**: 249-255.

Swami, S.V., Patil, R.A. and Gadekar, S.D. 2017. Studies on prevalence of subclinical mastitis in dairy animals. *J. Entomol. Zool.* **5**: 1297-1300.

Sztachanska, M., Baranski, W., Janowski, T., Pogorzelska, J. and Zdunczyk, S. Prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland. 2016. *Pol. J. Vet. Sci.* **19**: 119–124.

Thompson-Crispi, K., Atalla, H., Miglior, F. and Mallard, B.A. 2014. Bovine mastitis: Frontiers in immunogenetics. *Front. Immunol.* **5**: 1-10.

Thrusfield, M. 2005. Veterinary epidemiology. Black well science Ltd London, pp. 182–198.

Verma, H., Singh, R., Rawat, S., Jaiswal, V., Maurya, P.S. and Yadav, D.K. 2017. Identification and in-vitro antibiogram of bacterial pathogens from bovine mastitis in and around Meerut. *Res.Environ. Life Sci*. **10** : 538-540.

Vliegher, S. De, Fox, L.K., Piepers, S., McDougall, S., Barkema, H.W. 2012. Invited review: Mastitis in dairy heifers: Nature of the disease, potential impact, prevention, and control. *J. Dairy Sci*. **95** :1025-1040.

Watts, L.J. 1988. Etiological agents of bovine mastitis. *Vet. Microbiol.* **16**: 41-66.

■