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RESEARCH ARTICLE

Epidemiological diversity and diagnostic accuracy of cow-side test for subclinical mastitis in cows of Braj region of India

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Abstract: The aim of this study was to determine the prevalence of subclinical mastitis in native breeds of cows in the Brij region of Mathura and to assess the accuracy of the cow-side test. Milk samples were taken from selected population and screened through California mastitis test. Positive and ambiguous findings were further tested for Somatic cell count. The overall clinical mastitis prevalence was 55.83%. Organized farms had a lower prevalence of 44.12%, while unorganized farms exhibited a significantly ($p<0.05$) higher prevalence of 71.15%, indicating epidemiological diversity. In the context of two commonly reared breeds, Sahiwal cows showed a higher prevalence at 61%, in contrast to Haryana cows with a prevalence of 49%. Group with a paroty of 3-5 demonstrated the highest prevalence at 63.7%. Diagnostic tests, including the California mastitis test (CMT) and Somatic cell count (SCC), unveiled variations in prevalence rates.

Keywords: SCC, Udder health, milk and fat, sensitivity, accuracy

Introduction

Subclinical mastitis is the most frequently occurring form of mastitis in dairy cattle where there are no visible signs of abnormalities in the milk or udder (Radostits et al. 2007). It is detected through silent evidences of some inflammatory indicators such as a high somatic cell count level above to 200,000 cells/ ml (Satu ,2003) , increased serum proteins due to cellular damage in the udder tissue (Hussein et al. 201) and alterations in milk pH due to efflux of bicarbonates of serum (Qayyum et al. 2016 and Sani , 2021) . Diagnosing and managing of subclinical mastitis in dairy cattle still remains a significant challenge, because of its elusive nature also posing a potential source of infection for healthier animals (Abebe et al. 2016 and Ruegg 2017). Apart from these, the milk from antibiotic-treated animals with subclinical mastitis poses a dual risk, carrying both a potential zoonotic threat and the presence of antibiotic residue. In the One Health concept, it is necessary to map subclinical mastitis with a holistic approach because it directly impacts animal health, human health, and the economy of farmers as well as the country. Several contributing factors involves such as animal-related aspects like host age, breed, lactation level and parity, along with environmental factors such as temperature, humidity, human interactions and hygiene (Almaw et al. 2008; Doherr et al. 2007; Karimuribo et al. 2008; Madut et al. 2009). The most commonly reported contagious pathogen is *Staphylococcus aureus* and the environmental pathogen *Escherichia coli* (Cheng et al. 2020) Considering these factors, this study aims to assess the holistic diversity and accuracy of commonly used diagnostic tests for subclinical mastitis in lactating cows in the Brij region of India. By investigating epidemiological aspects, we strive to enhance understanding diversity of subclinical mastitis in dairy animas and insights on its diagnosis tools. These findings might be helpful to develop suitable preventive measure in this specific geographical area.

Materials and Methods

Location and geography of study area

Mathura, is an ancient district in the north-central part of Uttar Pradesh state, India, situated between Lat. 27°14' and 27°58' N and Long. 77°17' and 78°12' E, hold a renowned legacy for cow rearing associated with Lord Krishna. The region is currently overpopulated with indigenous cattle. The climatic conditions in the area exhibit variable temperature and humidity, averaging 30.6°C and 86.7%, respectively, throughout the year.

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Epidemiological study:

Population and sample collection

The selected population comprised 57 cows of Haryana and 63 cows of Sahiwal breeds from various age groups, all with multiple parities and over 30 days of lactation. Sixty-eight cows were sourced from the organized farm LFC, and 52 were sourced from Gaushalas in Mathura District. A total of 480-quarter milk samples were aseptically collected from 120 lactating cows and tested for the presence of subclinical mastitis using California Mastitis Test (CMT and somatic cells count. After discarding the first few milk squirts, 15 ml milk sample was collected in a sterile plastic bottle in a clean environment from each individual cow's quarters and leveled properly from randomly selected population.

Assessment of subclinical mastitis

Cow-side assessment

All the 120 samples examined at cow-side by used of California Mastitis Test (CMT) with modified Scandinavian scoring system 0 to 4. In the scoring system, 0 is negative (no gel formation), 1 is trace (possible infection), and 2 or 3 indicates a positive result and 4 has the thickest gel formation. A sample was considered as positive to subclinical mastitis when one or more quarters with more than 2 score were detected a Schukken, et al. 2003.

Laboratory assessment

The somatic cell count of milk samples were done using an automatic somatic cell counter (Lacto scan SSC, Milkotronic Ltd.; Nova Zagora, Bulgaria) in accordance with the manufacturer's manual and method in the literature (Salvador et al. 2014). The SCC is quantified as the number of cells per ml of milk. All the collected samples having CMT score 0 and 1,2, 3 and 4 were tested of individual cows were categorized having SCC of 100,000 or less indicates an 'uninfected' cow and cattle having SCC of 200,000 would determine whether a cow is infected with mastitis. Cows with a result of greater than 200,000 are highly likely to be infected on at least one quarter (Salvador et al. 2014)

Assessment of diagnostic accuracy

Data of both the test statistically tabulated as true positive, false positive, false negative (Table-4). The values of sensitivity, specificity, positive prediction, negative prediction and accuracy were calculated by using following formula:

a) Sensitivity = True positive/True positive + False negative

b) Specificity = True negative/True negative + False positive.

c) Positive predictive value = True positive/True positive + False positive.

d) Negative predictive value = True negative/True negative + False negative.

e) Accuracy = True negative/Total value

Results and Discussion

The overall prevalence of subclinical mastitis in indigenous cows of Brij region, Mathura district, was 45% based on cow-side California mastitis test, while laboratory analysis showed 55.83% positivity in somatic cell count (Fig. 1).Similarly, prevalence of subclinical mastitis also have been reported by Birhanu et al. (2017), Reddy et al. (2015), and Badiuzzaman et al. (2015) .

Grading of bovine sub-clinical mastitis

Based on cow side test, out of 120 lactating cows 66 cows were found CMT negative, 10 were identified as weakly positive (+),14 were distinctly positive (++) and 30 were diagnosed as strongly positive (+++) for sub-clinical mastitis (Table). However, laboratory analysis of somatic cell counts revealed that 67 milk samples found positive and having more than 2 lakhs SCC per ml of milk. Among these, 26 samples showed more than 10 lakhs/ml, 30 samples were ranges in 5-10 lakhs/ml and 11 samples were range lower range of somatic cell count 2-5 lakhs/ml (Table 2)

Epidemiological diversity

Based on somatic cell count epidemiological diversity of SCM in unorganized farm was significantly ($p<0.05$) higher as compared to organized farm as 71.15% and 44.12% respectively (Table -1). The higher infection rate in unorganized farms potentially influenced by various detrimental factor such as nutritional imbalance, poor hygiene, lack of awareness and improper ways to mange others farm practices. Cheng et al. (2020),Bangar et al. (2015), Krishnamoorthy et al. (2021), Khan et al. (2021) and Mbindyo et al. (2020).

Among, two most commonly indigenous breed of cows in brij region, subclinical mastitis was detected higher in Sahiwal breed compared to the Haryana breed as recorded with prevalence of 61% and 49% respectively but it was non-significant (Table-2). It

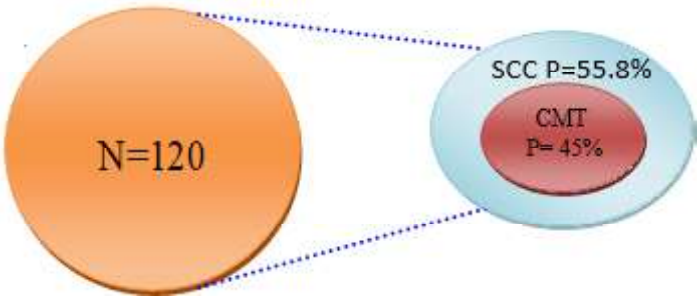

Fig1 -: Overall Status of sub-clinical mastitis in lactating cow in brij region of Mathura

might be due to the phenotypic characteristic of udder that mostly having pendulous and large udder and synthesis more milk as

Table: 1 Overall Status of sub-clinical mastitis in indigenous cattle

S.N.	Overall Status of Subclinical mastitis in indigenous dairy cattle	
1	Total Number of cows (N)	120
2	Total Positive of cows (P/%)	67/ 55.83%

Table-2 Status of subclinical mastitis based on Farming

S.N	Type of Breed	Total cow	Positive cow (N)	Positive cow (%)	(p>0.05)
1	Sahiwal	63	39	61.90	$X^{2=}$1.738
2	Haryana	57	28	49.12	*P* =0.18
	Total	120	67	55.83	

Table3: Status of subclinical mastitis based on breed of population

S.N	Parity	Total cow	Positive cow (N)	Positive cow (%)	(p<0.05)
1	3-5	80	51	63.75	$X^{2=}$6.099*
2	More than 5	40	16	40	*P*=.013
	Total	120	67	55.83	

Table: 4 Status of subclinical mastitis based on Parity

S.N	Parity	Total cow	Positive cow (N)	Positive cow (%)	(p<0.05)
1	3-5	80	51	63.75	$X^{2=}$6.099*
2	More than 5	40	16	40	*P*=.013
	Total	120	67	55.83	

Table-5: Comparative analysis CMT and SCC against subclinical mastitis

Comparative study of CMT and SCC test				
Test	Scoring system	Positive cow (N)	Positive cow (%)	Total (N/%)
CMT	+	10	18.52	54/ 45%
	++	14	25.93	
	+++	30	55.56	
SCC	* (2- 5 lakhs)	11	16.42	67/ 55.83%
	**(5- 10 lakhs)	30	44.78	
	***(< 10 lakhs)	26	38.81	

Accuracy of diagnostic test			
True positive	False positive	False negative	True negative
48	6	13	53

Comparative Diagnostic accuracy of CMT		(95% CI)
Sensitivity	78.69%	66.32% to 88.14%
Specificity	89.83%	79.17% to 96.18%
Positive Likelihood Ratio	7.74	3.58 to 16.70
Negative Likelihood Ratio	0.24	0.15 to 0.39
Disease prevalence (*)	55.83%	
Positive Predictive Value (*)	90.72%	81.92% to 95.48%
Negative Predictive Value (*)	76.93%	67.14% to 84.48%
Accuracy (*)	83.61%	75.75% to 89.74%

Table6: Status of subclinical mastitis based on affected udder and teats of cow

Udder Morphology		Status of SCM			
Shape of udder	Total	Cow negative	Cow positive (N)	Cow positive (%)	(p>0.05)
Pendulous	50	16	34	68.00	
Bowl	33	18	15	45.45	
Round	37	19	18	48.65	
Total	120	53	67	55.83	$X^{2=}5.217$ p=.073
Teat Morphology			Status of SCM		
Teat Shape	Total	Cow negative	Cow positive (N)	Cow positive (%)	(p>0.05)
Bottle	90	40	50	55.5	$X^{2=}85.27$ P=.001
Cylindrical	250	80	170	68	
Funnel	140	65	75	53.5	
Total	480	185	295	61.4	

well also lead physiological stress, it allowed to easily contacting to environmental opportunistic.

Among all the three parity groups, 3-5 was one of significantly ($p<0.05$) affected parity group with the highest 63.7% prevalence rate (Table-3). Lactating animals under this range, having fully grown udder that optimized our peak milk production. However, within the same age group, animals experienced more udder stress and possibility to breakdown the teats canal barriers and udder parenchyma tissue with progressive ageing the body's immune system undergoes a transition, adapting to shifts in various patho-physiological pathways from adulthood to old age. Similarly, the results and facts behind it agreed with (Cheng et al. (2020);Lakew et al. (2009) and Badiuzzaman et al.(2015).

Statistical validation of diagnostic test against subclinical mastitis

Statistical validation of the tests showed that the sensitivity, specificity, and diagnostic accuracy of the CMT test against SCC were 78.69%, 89.83%, and 83.61%, respectively. (Table - 4).These values are consistent with the findings of Kandiwa et al. (2017); Badiuzzaman et al. (2015) and Sumon et al. (2020), who reported sensitivity of CMT as 80.08% and specificity as 69.40%, with diagnostic accuracy ranging from 87.1% to 97.4%.

Conclusion

In the present study, overall prevalence rate of subclinical mastitis was found slightly higher, in indigenous cows of the Brij region in Mathura district. Unorganized farms exhibited significantly higher SCM prevalence compared to organized farms likely influenced by factors such as nutritional imbalance and poor hygiene. Sahiwal breed showed a higher subclinical mastitis rate compared to Haryana breed possibly due to phenotypic characteristics. Animals in the 3-5 parity group had the highest prevalence reflecting the challenges of udder stress and tissue breakdown with aging .This study also concluded that CMT test, demonstrated low sensitivity of 78.69%, specificity of 89.83%, and diagnostic accuracy of 83.61% against SCC. It indicates that screening criteria should be based on laboratory analysis rather than cow side and furthermore develop advance cow side test based on modern technology such as dye, markers or sensor with higher accuracy.

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References

Abebe R, Hatiya H, Abera M, Megersa B, Asmare K (2016) Bovine mastitis: prevalence, risk factors and isolation of Staphylococcus aureus in dairy herds at Hawassa milk shed South Ethiopia. BMC Vet Res 121: 1-11

Almaw G, Zerihun A, Asfaw Y (2008) Bovine mastitis and its association with selected risk factors in small holder dairy farms in and around Bahir Dar, Ethiopia. Anim Health Prod 40: 427-432

Badiuzzaman M, Samad, M A, Siddiki S, Islam MT, Saha S (2015) Subclinical mastitis in lactating cows: Comparison of four screening tests and effect of animal factors on its occurrence. Bangladesh J Vet Med 13(2): 41-50.

Birhanu M, Leta S, Mamo G, Tesfaye S(2017) Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu Town, Ethiopia. BMC Resh Notes 10(1): 1-6

Cheng WN, Han SG (2020) Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments-A review. Asian-Australas J Anim Sci 33(11): 1699-1713

Doherr MG, Roesch M, Schaeren W, Schallibaum M, Blum JW (2007)Risk factors associated with subclinical mastitis in dairy cows on Swiss organic and conventional production system farms. Veterinarni Medicina-praha 52(11): 487

Kandiwa E, Iraguha B, Mushonga B, Hamudikuwanda H, Mpatswenumugabo J P (2017) Comparison of cow-side diagnostic tests for subclinical mastitis of dairy cows in Musanze district, Rwanda. J South African Vet Association 88(1): 1-6

Kathiriya J, Kabaria B, Saradava A, Sanepara Y (2014) Pervalence of subclinical mastitis in dairy cows in Rajkot district of Gujarat. Int J Sci Nature 5: 35-43

Khan I, Javaid A (2021) Identification of biologically important compounds in neem leaves through GC-MS analysis. Jordan J Pharmaceutical Sci 14(3)

Krishnamoorthy P, Goudar AL, Suresh KP, Roy P (2021) Global and countrywide prevalence of subclinical and clinical mastitis in dairy cattle and buffaloes by systematic review and meta-analysis. Res Vet Sci 136: 561-586

Lakew M, Tolasa T, Tigre W (2009) Prevalence and major bacterial causes of bovine mastitis in Asella,South Eastern Ethiopia. Tropical Anim Health Prod 41: 1525-1530

Madut NA, Godir AEA,El-Jalil I M (2009) Host determinants of bovine mastitis in semi-intensive production system of Kharfoum State, Sudan. J Cell Animal Biol 3: 71-77

Mbindyo CM, Gitao GC, Mulei CM (2020) Prevalence, etiology, and risk factors of mastitis in dairy cattle in Embu and Kajiado Counties, Kenya. Vet Med Int (1)

Pyorala S(2003)Indicators of inflammation in the diagnosis of mastitis. Vet Res 34 (5): 565-578

Radostitis O, Gay C, Hinchcliff K and Constable P (2007) Veterinary medicine. A text book of the diseases of cattle, horses, sheep, pigs and goats. 10th Edn. Saunders Elsevier

Reddy BS, Shobhamani B, Sreedevi B, Kumari KN, Reddy YR (2015)Diagnosis of subclinical mastitis in cross bred cattle. Res Rev J Vet Sci Technol 4: 39-43

Salvador RT, Soliven RL, Balagan EJY, Abes NS, Gutierrez CA, Mingala CN(2014) Evaluation of a portable somatic cell counter in the diagnosis of bubaline subclinical mastitis. Thai J Agric Sci 47(4): 205-209

Sumon SM, Parvin MS, Ehsan MA, Islam MT(2020) Relationship between somatic cell counts and subclinical mastitis in lactating dairy cows. Vet World 13(8):170

Sani N, (2021) Alteration of milk ph, somatic cell count (scc), lactate dehydrogenase (ldh) and alkaline phosphatase (alp) activities in buffalo milk related to udder health status Buffalo Bull 40 (3): 443-450

Hussein HA, Abd El-Razik KA, Gomaa AM, Elbayoumy MK, Abdelrahman KA, Hosein HI (2018) Milk amyloid A as a biomarker for diagnosis of subclinical mastitis in cattle, Vet World 11(1): 34-41