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MASTITIS DISEASES TEST IN DAIRY COW

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Abstract: The Dairy Industry is one of the world's industries with the highest rate of growth. With an increase in global milk output, there is also a rise in the number of cattle. The health of cattle is one of the greatest obstacles faced by the Agriculture and Dairy industries. Dairy cannot be alone responsible for monitoring the health of varied animals; consequently, farmers must help by taking care of their livestock. Mastitis is recognized as a major disease affecting the dairy industry. The somatic cell of the milk is one of the features, which cross the threshold value in the case of an infected cow. Elevated somatic cell count is an important indicator for mastitis in milk. Different test methodology of the mastitis detection has been in this work, test output as score present the Infection is positive or negative. Change in electrical conductivity detected subclinical mastitis; its reliability further increases when used together with the other diagnostic methods.

Keywords: Somatic cell count; Mastitis; Sensitivity; Specificity; Electrical Conductivity.

Introduction:
Agriculture is the backbone of the developing country's economy. Agri-business like dairy farming is for long-term milk production processed at a farm or dairy plant. The commercial dairy farm is one species enterprise; it puts together mammal can produce milk; it consists of high producing dairy cows. There are some other species, including goats, sheep, and camels. A dairy cow produces milk at the peak level 40-60 days after calving; the cow is dried off for about 60 days after calving again. The milking period is around 10 months in a year. There are the major 4 reasons when dairy cows fail to produce milk

A. Infertility- Cow are failing to conceive milk for 60-80 days after calving
B. Mastitis – fatal Infection in the mammary gland leads to high somatic cell counts ultimately loss of production.
C. Lameness – An infection in the foot of a cow causing infertility and loss of cow production
D. Production - Some cow fails to produce an adequate amount of milk.

Mastitis is the most common disease in the cow where no gross change in milk production but significant enhancement in somatic (inflammatory) cells. With Infection, the mammary gland starts appearing red and painful. It generally occurs as an immune response to the bacterial invasion of the teat canal by the different categories of the bacterial source present in the dairy. It's a multifactor disease closely related to the milk production system. Mastitis disease in the cow resulting due to the presence of an inflammatory reaction into the mammary gland. The most common indication of mastitis is swelling in the udder, redness, heat, pain, hardness; other symptoms of the disease are fever, off-bread and may lead to death in most severe cases. The presence of mastitis significantly declines the quality of milk and its production. The appearance of the milk also changes to watery flakes or the presence of pus. The curtailment in the milk gives in, shoots up the body temperature, lack of hunger, and reduction in mobility are other symptoms founds. Mastic-infected cow milk declines potassium, casein (protein of milk).and enhances the lactoferrin. Milk from mastic-infected cows has higher somatic cell counts, which lowers the milk quality. Mastitis is classified into two categories (i) Contagious mastitis during the milking process, the transmission of contagious bacteria from one cow to another cow, (ii) Environmental infections bacteria in the environment infect the cow.

Existing Research:

Early detection of mastitis disease saves the animal from going into pain and coves the dairy farm's economic condition. There is no standard delimitation of mastitis detection; however, clinical and subclinical measures are mostly used for detection; (i) Clinical Mastitis (CM) is characterized by heat, pain, and swelling that may occur with or without these signs. The udder secretion is usually abnormal affects milk yield and quality. The severity of the effects leads toa susceptibility of the cow and the extent of udder damage. Another one, (ii) Sub-clinical Mastitis (SCM), is difficult to detect clinically. A feature of the yield milk processed to detect, as an increase in the cell content, counts more than 500,000 cells per ml is regarded as indicative of subclinical mastitis, which farmers frequently do not notice. Indirect tests such as California Mastitis Test (Rapid Mastitis Test in Australia), cell counts, or white side tests are required to make the diagnosis.

To have a deeper understanding [29], of the condition, delve deeply into the causative factors, established paradigms, and the application of innovative technology for mastitis diagnosis, treatment, and prevention. Mastitis and other diseases and introducing the use of omics sciences, particularly metabolomics and related analytical tools. All types of mastitis are followed and diagnosed following childbirth, when the incidence is known to be highest. The pathobiology of mastitis has been illuminated by a number of longitudinal investigations and current advancements in the biomarker field for periparturient illnesses. In this study, we discuss the relationship between mastitis and other disorders that occur around calving, as well as the potential for using metabolomics to identify predictive biomarkers. Several laboratories have identified postpartum or prepartum biomarkers in blood, urine, and breast milk. The repercussions of mastitis have a significant influence on the dairy business; consequently, prepartum diagnosis and monitoring of mastitis are of the utmost significance. Validation and manufacture of predictive biomarkers on a larger scale are required.

According to [31] Bovine mastitis is one of the most significant diseases in the dairy business, and a greater knowledge of the role biofilm plays in the disease is crucial for developing more effective therapies. Human medicine recognizes chronic biofilm infections as dangerous and difficult-to-treat conditions. The majority of research on biofilm and bovine mastitis has thus far concentrated on in vitro investigations; however, direct approaches are required to detect the presence of biofilm in the udders of dairy cows with mastitis. Some of the approaches employed in the diagnosis and study of biofilms in human illnesses may be applicable to the study of biofilm in bovine mastitis. There is a need for in vivo research investigating the presence and distribution of biofilms directly in the udders of dairy cows with mastitis and correlating these findings with those in milk samples. Antibiotic resistance is one of the greatest hazards to human and animal health, and the continued failure of antibiotic therapy for possible biofilm mastitis infections can raise the likelihood of antibiotic resistance. The role of biofilm infections in bovine mastitis appears to be the key to unlocking the knowledge required to develop novel diagnostic approaches and to treat persistent and chronic instances of bovine mastitis.

The bovine [28] population in India is affected by mastitis, and the bovine population in the state of Madhya Pradesh is also at risk. Madhya Pradesh ranks fifth in India for milk output. Numerous state residents earn their living through milk production and distribution. Cases of clinical and subclinical mastitis in bovines, caused by a variety of infections, are recorded from the state and result in direct and indirect economic losses for dairy animal owners. The institutions in the state routinely administer a variety of mastitis diagnostic tests for early and timely detection of mastitis. It is assisting the authorities involved in livestock health to treat mastitis more effectively. National Mastitis Council-recommended measures for the prevention and control of mastitis in bovines are currently being promoted through farmer awareness programs; however, more effort is required to disseminate this information to farmers.

A different factor in computing risk presented in [1] is (a) somatic cell counts (SCC) (more than 2×105/ml of milk), (b) breed hierarchy (genetic vulnerability), (c) Stress (stress in the cattle due to inadequate diets, deficiency of amino acid, minerals, etc.). However, SCC values are variable threshold values shown in [4] 3,000, 00 cells/ML presents milk appears healthy. A unique detection methodology presented in [2] is based on a milk flow rate to detect infection susceptibility. Hand-based milking is more immune to spread Infection. An analytic tool, the Biosensor presented in [7], converts the presence of biological compounds into an electric signal. Electrical conductively observed its counts in lymphocytes; monocytes and granulocyte counts investigate the milk qualities. Enhancing granulocyte counts using flow cytometer and electrical conductivity is the reliable technique for detecting mastitis explored in [8]. A deterministic model presented in [3] indicates whether a cow has a risk of having mastitis diseases or not overall risk, chronic risk, and days to next sample collected; based as on real-time information about milk. The mathematical equation efficiently computes, and early detection of mastitis provides useful decisions with sensitivity rather than 80% and specificity of more than 90%. A farmer makes decisions about diseases based on experience and observation. At the same time, a farm management information system (FMIS) in [5] enables farmers to make decisions based on a machine-learning algorithm to have a prediction model. An experiment conducted in [6] based on the sample collected from 1000 farms over three months block over 18 periods, the random forest-based model predicts with higher accuracy with 98% true prediction value and 86% negative. Sensor-based data collection and proceeds through computing engines like Raspberry Pie is the most attractive methodology; Difference sensors like temperature, sound, motion, and rumination collect the basic information from the farm. An artificial neural network (ANN) based classifier compares the collected samples with a threshold value of healthy cow intimidates farmers through the internet of things (IoT) [9]. The presence of mastitis presented in [15] in milk was indicated by variable SCC, which indicated a change in electrical conductivity. Support vector machine (SVM) classifiers classify the infected milk with 89% sensitivity and 92% specificity, and 50% error in detection.
A low-cost kit for the detection of subclinical mastitis (SCM) was presented in [16], following criteria from a sample of milk, cow considered as positive for mastitis when CMT and BMT score was ≥1+ and SCC value was $\geq 2 \times 10^5$/ ml of milk (threshold value). CMT and BMT tests characterized the milk SCC as the gold standard

Accuracy = TP + TN/TP + FP + TN + FN × 100

Sensitivity = TP/TP + FN × 100

Specificity = TN/TN + FP × 100, PPV = TP/TP + FP × 100, NPV = TN/TN + FN × 100 Where:

TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative.

Comparatively, BMT isa rapid and inexpensive test kit to access the SCM of animals [18]. CMT effective test kit for assessing SCM [17] However, only 20.2% and 25% found the prevalence of SCM in dairy cows in [19] and [17], respectively. 50.4% and 58% prevalence of SCM were reported by [20] and [21], respectively.

Mastitis Test Principle:

Early mastitis detection in the cow improves the health level of dairy animals and brings up the economic level of the farmers. Sample of milk collected at different intervals of time and applied to reaction reagent. The infected cell has a variable amount of somatic cell count; upon reaction, the postreaction product indicates the presence of mastitis in the milk with careful observation. This work aimed to estimate the sensitivities and specificities of three mastitis screening tests, i.e., California Mastitis Test (CMT), White Side Test (WST), and Surf Field Mastitis Test (SFMT).

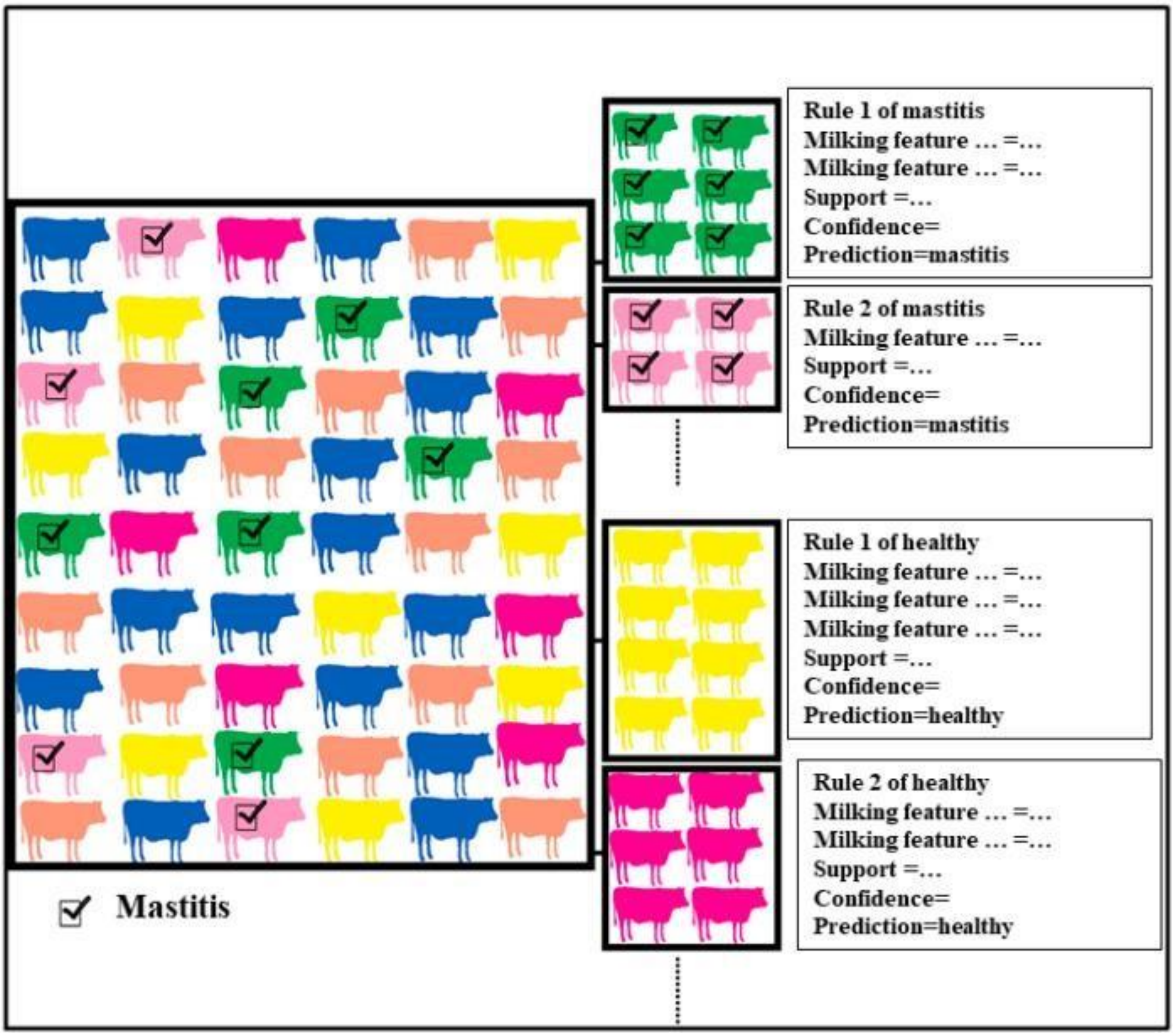


Fig: Detecting Mastitis in Cow
Source: (Ebrahimie et al., 2021)

California Mastitis Test (CMT) is a simple and widely used diagnostic tool for subclinical mastitis detection, indicate the quality of milk with a CMT (1-5) score based on reaction. CMT score 1 indicates a completely negative reaction and 2-5 increasing degree if of an inflammatory reaction of the udder. The reactions 2-5, 3-5, or 4-5 may indicate subclinical mastitis and may produce false either positive or false-negative results—the variation in SCC access the sensitivity and specificity of the CMT compared to SCC. About 2 ml of milk drawn from tube to CMT reagent, reaction developed immediately with highly concentrated SCC milk within 10-20 seconds. The DNA in the cell membrane reacts with the test reagent starts forming a gel. The CMT reaction indicates a score for each udder half depending on the thickness of gel formed as per table1

Table1: California Mastitis Test score

CMT Score	Reaction	Mean value of leukocytes /ml
0	No reaction	≤ 268000
1+	Distinct slime without gel	80000
2+	Immediate gel formation	2560000
3+	El developed a convex sifrace	≤10000000

White Side Test (WST): The WST was performed with a slight modification of the procedure described by Coles (1986). Briefly, milk samples were thoroughly mixed to ensure an even distribution of solids components of milk but with caution to avoid violent shaking. Then 50 µl of milk were placed on a glass slide with a dark back-ground by micropipette. Subsequently, 20 µl of WST reagent (4% NaOH) were added to the milk sample, and the mixture was stirred rapidly with a toothpick for 20–25 seconds. A breaking up of milk in flakes, shreds and viscid mass was indicative of positive reaction. On the other hand, milky and opaque and entirely free of precipitant was indicative for a negative reaction. Whiteside test (WST) is a modified test process of test methodology described by [22], milk sample mixes to ensure solid component of milk evenly distributed. About 50 micro ml of milk was placed on a glass slide (dark background) by micropipette, and 20 micro ml of WST reagent (4% NaOH) added to the milk sample; a mixture of stirring with a toothpick for 20-25 seconds, a positive reaction indicated by breaking of milk in flakes, shred and viscid mass. While opaque and entirely free of the participant, indicate a negative reaction. I am reading o milk sample presented in table 2.

Table 2 Whiteside Test Score

CMT Score	Reaction
(Negative)	The Mixture is opaque, free of participant
1+ (Weak positive)	Background Less opaque, the larger particle of coagulated material
2+ (Distinct positive)	Background More watery and large coagulated material
3+ (Strong positive)	The background is a very watery large mass of coagulated material

Surf Filed Mastitis Test (SFMT) [23] based on commercially available reagent 3 g of detergent (surf excel) to 100 nm of distilled water. A shallow half black paddle having 4 cups used to rinsed, about 2 ml milk was drawn into the cup, and 2 ml of reagent was squirted for a few seconds. Reaction developed immediately with milk containing somatic cells. A score of the reaction read like a CMT score in the table.

Methodology:

The dataset used to train and construct the machine learning model for predicting the risk of mastitis in this study was collected from Ankitha's (2020) recent research. This dataset consists of 6600 entries (three entries per cattle) for cattle with 10 attributes; cow ID, date, breed, months since giving birth, previous occurrence of mastitis, front left udder inhale limit (IUFL) front left udder exhale limit (EUFL), front right udder inhale limit (IUFR), front right udder exhale limit (EUFR), rear left udder inhale limit (IURL) rear left udder exhale limit (EURL) (healthy or mastitis).

The tweaked model was then put to 10-fold cross validation, and its average mean accuracy, sensitivity, and specificity were calculated. In addition, the model's sensitivity and specificity were calculated using Equation (1) and Equation (2), respectively.

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}$$

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}$$

The true positives were the number of healthy cows that were correctly predicted, the false positives were the number of healthy cows that were predicted to be at risk of mastitis, the true negatives were the number of cows at risk of mastitis that were correctly predicted, and the false negatives were the number of cows at risk of mastitis that were predicted to be healthy (Abdul Ghafoor & Sitkowska, 2021).

Evaluation of test principle:

The electrical conductivity of the mastitis infected milk is higher than the normal milk due to tissue damage. There is a considerable increased ion Na+ and Cl- ion of the milk. Change in electrical conductivity measures is a useful tool for the early detection of mastitis in the milk. Herd-specific conductivity threshold levels have crossed the threshold level. The available handheld conductivity meter indicates immediate treatment for a particular animal, and further investigation is needed, like temperature and udder examination. Sensitivity and specificity are the predictive measures of the CMT score. [24][25]

Sensitivity of the CMT indicates the ability the presence of the Intramammary infections (IMI), calculated as the proportion of quarter has an IMI and positive CMT. Specificity of the CMT is the ability to detect a quarter that does not have IMI and calculated as a proportion of non-infected quarter and negative CMT. Generally, sensitivity and specificity are inversely related. The predictive value of the CMT is how the test results should interpret.

False-positive and false-negative reactions occur with CMT; higher values of sensitivity (no false-negative CMT reaction) detect the majority of the quarter that had an IMI. A higher value of specificity indicates a false-positive reaction. A false-positive reaction occurs when somatic cells present in the milk are not being isolated from bacteria. In contrast, a false-negative reaction occurs due to bacteria in the gland but somatic cells not found.

False positive→ low specificity→High sensitivity

False-negative→low sensitivity→High specificity

High sensitivity and low specificity indicate the probability of having the disease, and further investigation is required. Alternatively, the cow initially tested positive with high sensitivity and low specificity, and during the second test, low sensitivity and high specificity indicate as disease negative. Interpretation of screening tests is difficult because data collection from dairy is not similar. The test produces false positive and false negative, which produce an estimate of the apparent prevalence. Prevalence is the proportion of dairy cow gives positive test result based on sensitivity and specificity. True prevalence does not represent the true disease status of an individual cow; it refers to the proportion of a population infected.

$$True\ prevalence = \frac{apparent prevalence + (specificity - 1)}{specificity + (sensitivity - 1)}$$

The value of true prevalence ranges from 0-1.

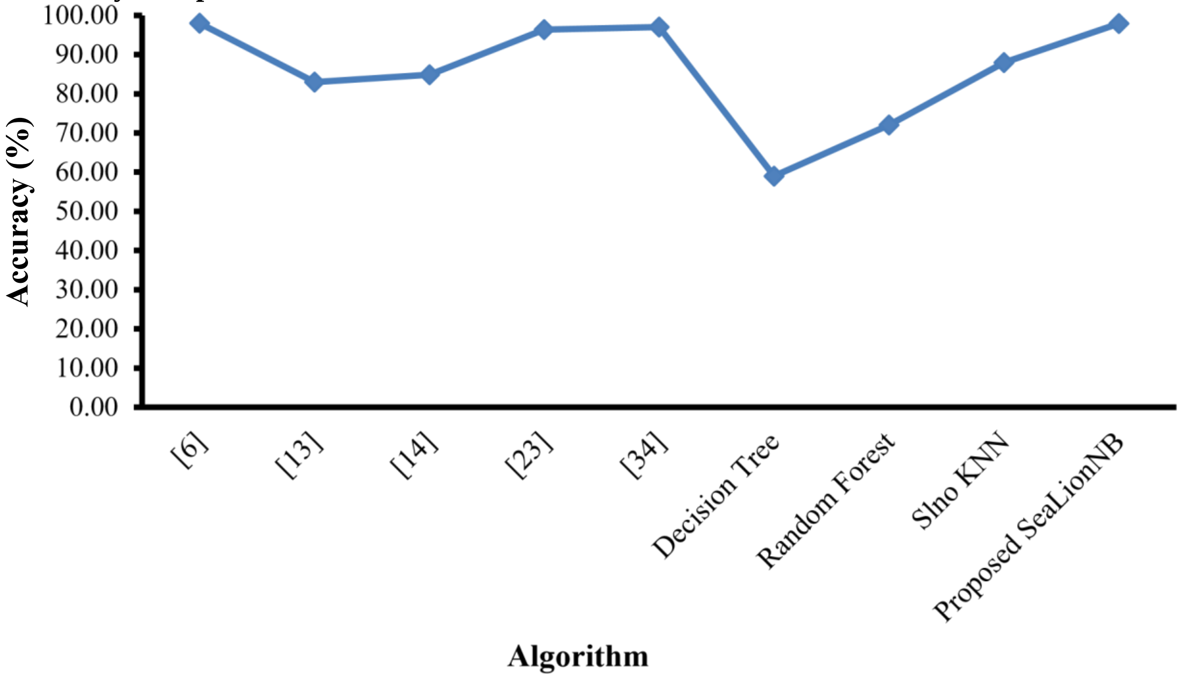
Table 1. Number of healthy and subclinical mastitis records, collected over 3 years during the study.

Year	Healthy Records	Subclinical Mastitis Records (Somatic Cell Count Cut-Off of ≥250,000 cells/mL)
Year 1	57,180	12,034
Year 2	118,126	24,218
Year 3	68,789	16,657

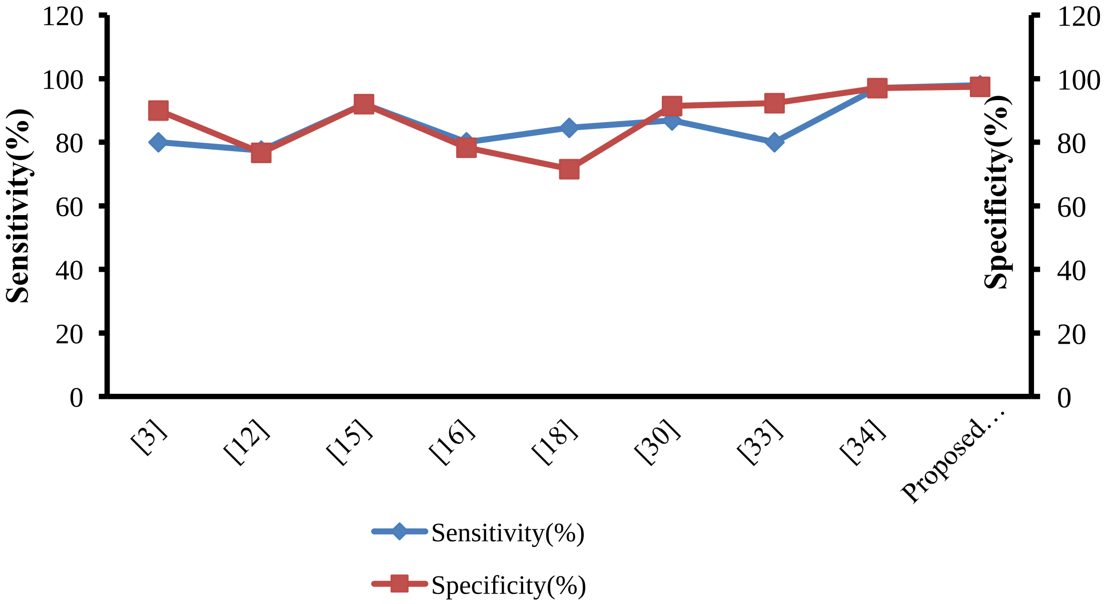
Source: (Ebrahimie et al., 2021)

Performance Comparison of Proposed work with existing

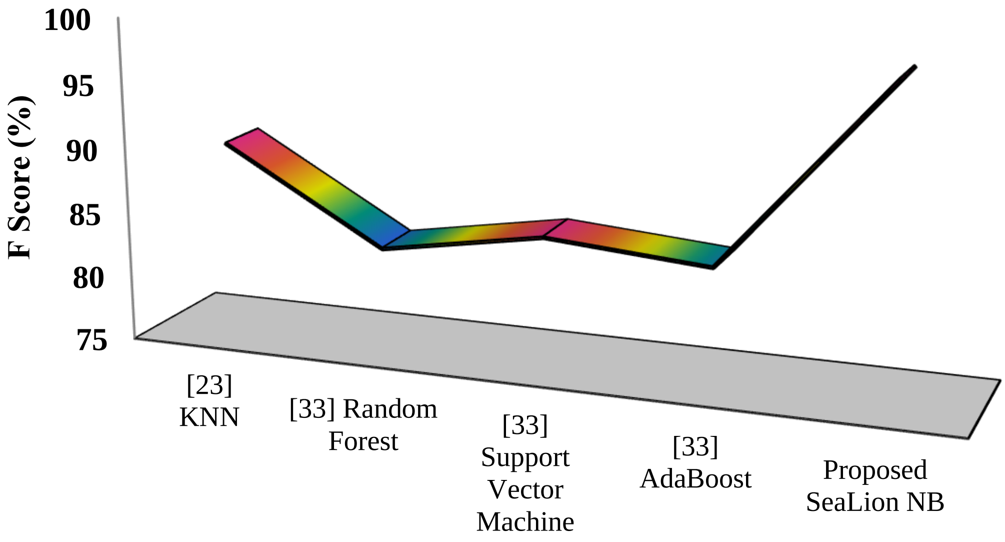
(A) Accuracy Comparison



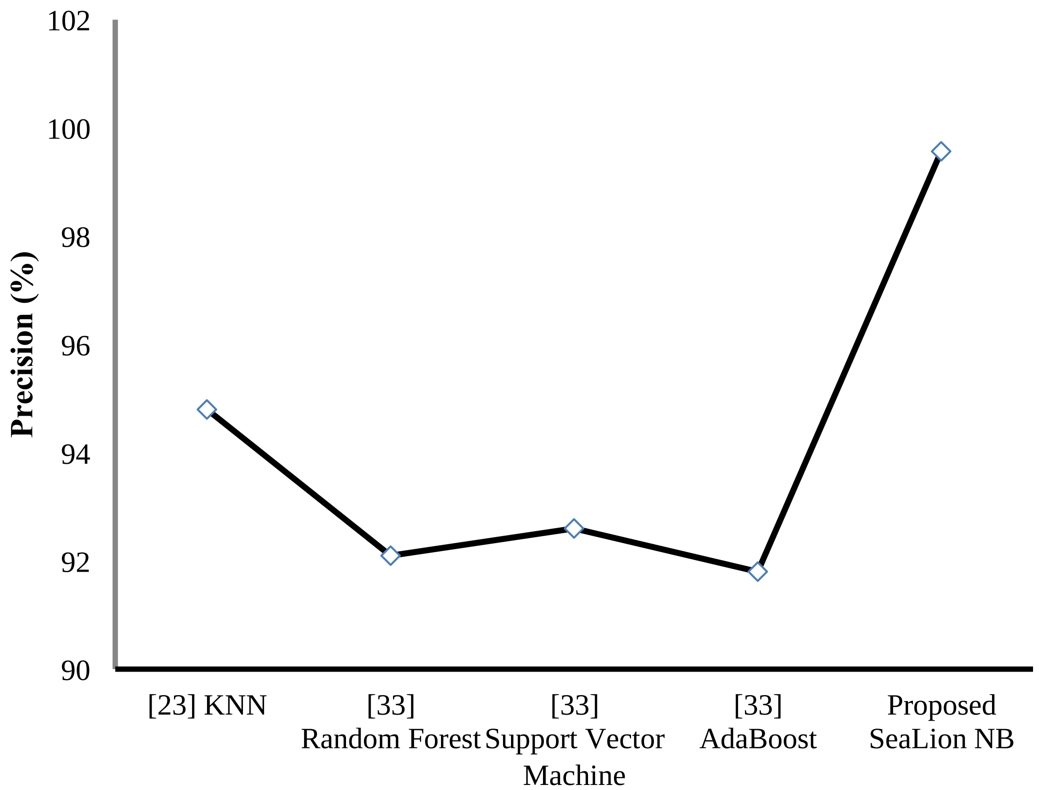
(B) Sensitivity-Specificity Comparison



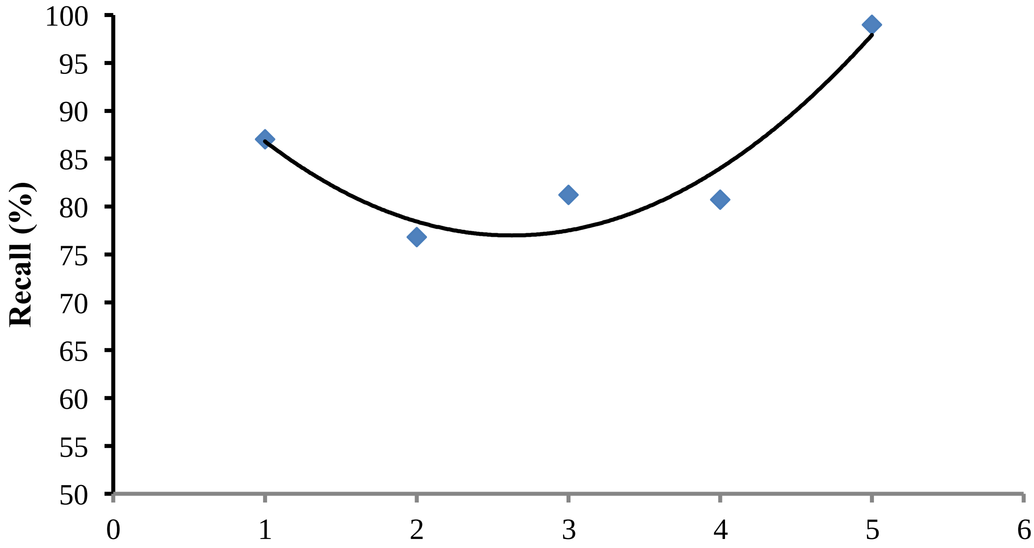
(C) F-Score Comparison



(D) Precision Comparison



(E) Recall Comparison



Conclusion:

In this work, detection of mastitis disease in the dairy cow was analyzed with the variation of somatic cell counts. The presence of mastitis varies the electrical conductivity. During testing principle, CMT, WST, and SFMT parameter sensitivity and specificity indicate the collected samples' tests are false positive or false negative with SCC into consideration. The core value of the test directs attention to individual udders, which are secreting milk of high somatic cell content. The higher value of true prevalence presents disease positive.

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