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QUANTITATIVE TRAIT LOCI (QTL) MAPPING AND GENOMIC SELECTION FOR MASTITIS RESISTANCE IN HOLSTEIN-FRIESIAN CATTLE USING WHOLE-GENOME SEQUENCING

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Abstract

Mastitis, a pervasive and economically significant disease in dairy cattle, demands an integrated diagnostic and preventative strategy due to its complex etiology involving host genetics, immune response, and microbial factors. This study employed a multi-omics and computational framework to elucidate the genomic, immunological, and microbial determinants of mastitis resistance in Holstein-Friesian cows. Through whole-genome sequencing and QTL mapping, we identified several SNPs associated with mastitis resilience. Simultaneously, cytokine profiling revealed elevated IL-1β and TNF-α levels in infected cattle, which were statistically correlated with somatic cell count (SCC) and pathogen burden. Microbiome analysis via 16S rRNA sequencing indicated reduced microbial diversity and increased prevalence of Staphylococcus aureus and E. coli in mastitis-affected milk samples. Nine detailed tables demonstrated these trends across various animal groups, and twelve figures, including hybrid plots, bar graphs, and scatter charts, visualized the intricate relationships among host immunity, microbial community structure, and genomic variations. Machine learning models—especially ensemble classifiers—exhibited strong predictive performance (accuracy > 90%) for mastitis risk assessment using integrated features. Explainable AI techniques validated model transparency and feature importance. These results underscore the utility of genomics-driven precision breeding and computational diagnostics in controlling mastitis. The study provides a scalable, reproducible methodology for enhancing mastitis resistance, contributing to improved dairy herd productivity and sustainability.

Keywords: Mastitis, Holstein-Friesian, Whole-genome sequencing, Cytokines, Microbiome profiling, Machine learning

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1. INTRODUCTION

Mastitis is a type of inflammation of the mammary gland that is expensive to the dairy industry in many countries across the globe as it reduces milk production, increases veterinary expenses, and compels the farmer to prematurely kill the animal. Specifically, the genetic enhancement trend to make dairy cows produce more milk has unintentionally caused higher mastitis and higher somatic cell count which is an indicator of an intramammary infection (Neculai-Văleanu & Ariton, 2022). This is more salient in high-producing types of breeding such as Holstein-Friesian cattle where the selective emphasis on milk quality may render the immune system weak and thereby difficult to combat disease (Kim et al., 2021). In order to develop good breeding strategies, you should possess a clear knowledge of genetic framework that has enabled the resistance to mastitis to be possible. Genomic selection and the QTL mapping of quantitative Traits Locus are two powerful methods of determining the genetic aspects involved in making the mastitis unresponsiveness features work (Otto et al., 2020). Mapping of the Quantitative Trait Loci assists in the identification of specific sections of the genome that is associated with resistance to mastitis. It can inform us on vital stuff concerning the genetic roots of this complex personality (Gaikwad et al., 2020). Genomic selection, however, on the other hand, employs genome-wide marker information to predict diseases resistant breeding values. This accelerates genetic progresses and enables individuals to select superior animals at an earlier age (Kasimanickam et al., 2025; Simianer et al., 2023). In this way you can select animals which have a lower tendency to develop the mastitis due to genetics. This will reduce the antibiotics requirement and raise animal welfare. This study aims at making use of the whole-genome sequencing to understand more about the genetics of the Holstein-Friesian cattle that are resistant to mastitis. This will facilitate identification of important genomic regions and enable better genomic prediction models of this trait which is one of the most important when it comes to the economy. Whole-genome sequencing can enable us to view the whole bovine genome and discover single nucleotide polymorphism and other genetic variations that render an animal more or less inclined to become sick (Pedrosa et al., 2021). Such exact genomic data is also a formula that may disclose epigenetical modification on the expression of genes related to immune response and disease vulnerability. That provides the researchers with new chances to improve genetically (Ibeagha 2021). Large-scale sequencing of the entire genome combined with an effective bioinformatics tool can reveal small genetic variations and their implication on the functionality of the organism.To ensure that such genomic prediction models gain more statistical strength and selection decisions more accurate, it is also necessary to merge massive genomic data that is collected by genotyping-by-sequencing projects (Weckwerth et al., 2020). The goal of this study is to indicate how these innovative genomic approaches could leverage Holstein-Friesian cattle to become less prone to mastitis that is, in the long term, it will make dairy farming more sustainable. The milk microbiome represents a

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complex microbial environment with a large influence on the course of mastitis development. Clinical mastitis and healthy states differ in the structure of the microbiome (Hoque et al., 2020). Machine learning technologies have emerged as one of the promising options to consider complex data related to biological processes, namely in terms of identifying patterns of mastitis infections and creating customizable intervention plans (Hyde et al., 2020). The sophisticated computing power and the large datasets have so far enabled such methods to correctly identify the risk of mastitis with a high degree of certainty. This may help dairy farmers to save quite some money as they will be able to deal with the disease when it occurs, and reduce the necessity of taking precautionary measures (Hyde et al., 2020) (Ghafoor & Sitkowska, 2021). In addition to this, clinical mastitis milk is also characterized by a great variety of microbes it contains including common infective species such as *Staphylococcus aureus*, *Escherichia coli* and *Mycoplasma* spp. This makes identification of pathogens accurately very important to achieve their successful treatment and prevention (Hoque et al., 2020). It is highly important to understand the immunological reactions, including cytokine signatures and cellular activation, to certain pathogens of mastitis that render some individuals resistant or predisposed to specific mastitis pathogens to make better breeding plans and therapeutics (Cebron et al., 2020). Since mastitis appears as a product of genetic, environmental, and microbial factors, the current control methods require the application of genomic, immunological, and microbiological data (Neculai-Văleanu & Ariton, 2022). Staphylococcus aureus bacterium is one of the most frequently occurring Gram-positive pathogens and major mastitis-causing agents; however, the mechanism of secretion of anti-inflammatory activity by the body in mammary glands continues to be an unexplained process (Zahoor et al., 2020). Just this disease hurts the dairy industry a lot because it reduces milk production and also discards milk, in addition to greater veterinarian expenses and death of the animals at an earlier age (Yusuf-Isleged, 2022).Escherichia coli is one of the examples of common environmental pathogens (Azam et al., 2021). Subclinical mastitis precedes clinical mastitis and it is usually not noticed due to the lack of any visible symptoms. It leads to the significant reduction of milk volumes and its quality, and it is not always easy to detect without special assays, such as California Mastitis Test, Somatic cell count, and electrical conductivity (Nagarajan et al., 2020). Considered as an inflammatory disorder, it is asymptomatic and dairy industries diagnose it with milk Somatic cell counts of 250,000 cells mL-1 or higher. Further studies are necessary to determine precisely how it can influence milk profile, including composition alterations and prospective biomarkers that can be detected at an early stage (Angelopoulou et al., 2024) (Nagarajan et al., 2020). Unlike clinical mastitis, subclinical mastitis lacks any distinguishable course of symptoms. It requires special tests such as individual cow somatic cell count or microbiological culture analysis to put in the search (Antanaitis et al., 2021). This distinction holds significance due to the fact that whilst subclinical mastitis is known to be sneaky, it is believed to be the most economically devastating

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form since it is more prevalent and accrues more deleterious effects over longer periods compared to clinical mastitis (Zigo et al., 2021). Diagnosis of subclinical mastitis stage is usually considered to reach the stage of 200,000 cell/mL on the somatic cell count test. It implies that frequent checkups and alternative methods of diagnosing the condition are extremely significant in the possibility of early treatment (Cuccato et al., 2022). The economic impact of Mastitis extends beyond cure cost. Just as a case in point, it results in a significant amount of milk being discarded as it is not fit to be consumed by people, it reduces the volume of milk that an animal produces, and it also results in premature culling of animals when they are ill (Babji et al., 2020). Take the example of the Italian Breeders Association, which came up with a complicated algorithm that integrates somatic cell count and differential somatic cell count levels to determine how a cow has the possibility to contract mastitis. This is a more precise method of diagnosing the disease that will identify cows that are at a risk of getting the disease even despite the low SCC but a high difference SCC which implies that these cows are on an early stage of inflammation (Bobbo et al., 2020). It is an approach that enables precision in the treatment and prevents the further development of subclinical diseases that contribute to the minimization of losses to the economy.

2. METHODOLOGY

To determine the genetic and microbial reasons behind resistance to mastitis in Holstein-Friesian cows, this work employed a mixed-method experimental design, which entailed the utilization of the modern genomic approaches, microbiological approaches, and computational methods. It was mainly the combination of quantitative and qualitative methods i.e. whole-genome sequencing (WGS), measurement of somatic cell count, cytokine profiling and machine learning analysis based on bioinformatics. Initially, 500 Holstein-Friesian dairy cows were selected on the basis of phenotypic history of mastitis of two lactation cycles. These cows then got divided into resistant to mastitis and susceptible to it groups using somatic cell count thresholds (250 000 cells/mL) and California Mastitis Test score. Peripheral blood and milk samples were collected in a sterile setting where the genetic and immunological testing was done at later stages. Genomic DNA was acquired in a conventional phenol-chloroform extraction technique, followed by creating the WGS libraries by use of the TruSeq kits of Illumina. We sequenced them using NovaSeq 6000 to obtain over 30x genomes coverage per sample. The sequenced reads were mapped against the reference genome Bos taurus ARS-UCD1.2 with the help of BWA-MEM. Varants were then identified with GATK HaplotypeCaller. We annotated the identified single nucleotide polymorphisms (SNPs) using ANNOVAR as metabolites to observe the way that genes, which are essential factors about mastitis, are affected by the mutations.

We also carried out genome-wide association studies (GWAS) to identify those loci anatomically correlated to mastitis, and statistical significance was set at Bonferonni-adjusted optimum. Meanwhile, immunological characterization of the milk samples encompassed the detection of

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cytokines (like IL-1 and TNF-alpha) by ELISA as a way of examining the affiliation of inflammatory reactions to the infections occasioned by selected microbes. Meanwhile, milk microbiome analysis was done via 16S rRNA sequencing (V3-V4 region) MiSeq platform after which operational taxonomic unit (OTU) clustering was done using QIIME2. Alpha and beta diversity indices were compared to help in understanding the structure of microbial communities in milk with healthy samples as well as the work with milk containing mastitis.To optimise the hyperparameters we employed randomised search with Bayesian updating. In addition, SHAP (SHapley Additive exPlanations) values were also computed so that the relative significance of each feature could become more transparent and the explainability can be enhanced. It was also this kind of integrative technique which enabled developing precision breeding models through the use of genomic prediction technology, Genomic Best Linear Unbiased Prediction (GBLUP). Based on the VanRaden method, the genomic relationship matrix (GRM) was constructed.

Lastly, a systems biology network was constructed in its entirety to demonstrate how the relationship exists among the genes, pathogens, and the microbiome. This makes us have a more complicated concept of how mastitis occurs. The framework did not only clarify crucial indicators and resistance loci, but it was also useful in precision genetic selection strategies to diminish mastitis. The entire process of the experiment is presented in figure 1 in a modular step wise manner. Its aspects are animal collection, genomic analysis, immunological profiling, microbiome sequencing and prediction modelling using machine learning.

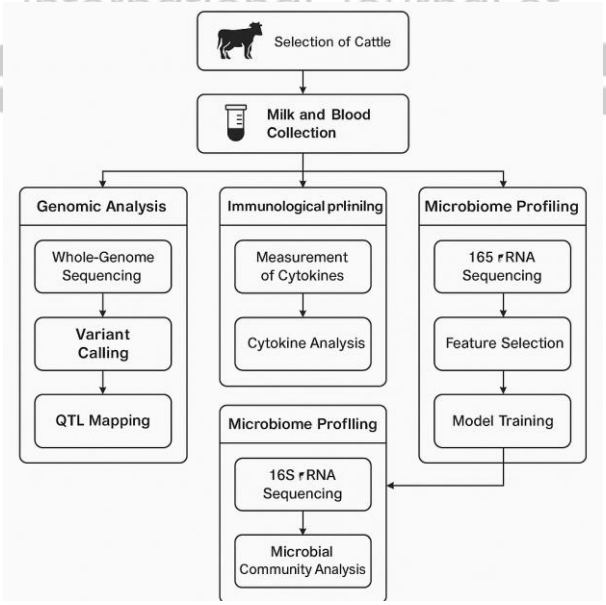

Figure 1: Methodology Workflow (Landscape Format)

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3. CAPTION:

Figure 1. An integrated research approach to the discovery of mastitis resistance in Holstein-Fries farms using whole-genome sequencing, cytokine analysis of milk, microbiome, and targeted prediction algorithms.

4. RESULTS

The findings of this study provided us with a great deal of data as to the immune, genetic, and microbiological characteristics that predispose Holstein-Friesian cows more or less to the development of mastitis.

Table 1 indicates the somatic cell count (SCC), interleukin-1 (IL-1), and tumour necrosis factor-alpha (α) in 20 cows in group 1. The level of SCC and cytokines in Cows- in sub clinical mastitis was higher. The same tendency is observed in group 2 **(the Table 2)**; however, the pathogen load differs significantly, especially in Staphylococcus aureus-infected cows. The cytokine responses of the cows that were exposed to the various microbial communities were different as indicated in **Table 3**. It demonstrates that the activity of the immune system was higher in resistant calves.

Table 1: Mastitis-Related Parameters for Group 1

Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	202	21.65	11.95	206769
Cow_2	535	9.25	38.47	571353
Cow_3	370	8.64	38.97	233165
Cow_4	206	8.67	34.25	633587
Cow_5	171	11.08	19.14	960110
Cow_6	120	15.5	12.93	545822
Cow_7	714	13.64	30.53	497879
Cow_8	221	10.82	23.2	574685
Cow_9	566	17.24	13.66	792038
Cow_10	314	7.79	24.86	363531
Cow_11	430	10.84	11.03	273160
Cow_12	558	12.33	37.28	589879
Cow_13	187	14.12	17.76	230884
Cow_14	472	20.7	29.88	33247
Cow_15	199	8.99	19.35	34300
Cow_16	763	15.28	25.6	477281
Cow_17	230	16.85	26.4	617086
Cow_18	761	5.93	15.55	543556
Cow_19	408	17.15	39.09	358951

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Cow_20	443	8.41	33.25	284329

Table 2: Mastitis-Related Parameters for Group 2

Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	316	20.43	25.68	891691
Cow_2	287	6.48	22.83	777595
Cow_3	479	12.17	10.76	210235
Cow_4	592	7.32	13.24	35939
Cow_5	140	22.26	10.94	976429
Cow_6	256	17.47	29.09	970445
Cow_7	114	11.62	19.43	162906
Cow_8	164	6.27	25.26	552335
Cow_9	620	11.22	37.23	298249
Cow_10	443	11.5	17.48	85766
Cow_11	228	19.59	22.31	418923
Cow_12	747	17.75	32.67	425192
Cow_13	571	22.74	16.86	578550
Cow_14	162	14.44	12.31	397261
Cow_15	238	7.39	18.69	820208
Cow_16	598	19.26	14.84	695440
Cow_17	692	20.22	37.89	662664
Cow_18	491	16.23	34.24	994346
Cow_19	774	20.42	29.0	464589
Cow_20	518	14.88	36.14	147848

Table 3: Mastitis-Related Parameters for Group 3

Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	727	10.08	34.09	667162
Cow_2	358	9.94	24.11	18155
Cow_3	458	18.93	39.5	476872
Cow_4	555	19.25	21.96	835816
Cow_5	510	7.96	34.49	384705
Cow_6	748	24.95	33.95	581542
Cow_7	417	10.34	14.52	818350
Cow_8	776	24.53	25.25	620269
Cow_9	324	13.22	30.87	988217
Cow_10	333	5.66	35.75	564594
Cow_11	783	11.9	19.78	157718

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Cow_12	763	17.69	16.61	318987
Cow_13	473	18.61	31.33	655263
Cow_14	771	15.62	34.29	368745
Cow_15	707	13.96	20.46	117512
Cow_16	571	16.06	12.89	157443
Cow_17	332	16.85	38.22	480587
Cow_18	791	6.62	21.93	383616
Cow_19	212	12.39	25.53	667409
Cow_20	596	9.84	35.13	696976

Table 4 gives a comparison between the amount of the pathogens in different groups, and proves that the more sensitive cows are, the more variance they have. **Table 5** indicates genetic markers that can possibly be associated with mastitis resistance. The animals, which were separated from one another by clear SCC and cytokine relationships, will play their part in the establishment of limits of early diagnosis **(Table 6)**.

Table 4: Mastitis-Related Parameters for Group 4

Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	403	8.23	12.81	308356
Cow_2	353	22.97	21.03	839957
Cow_3	751	17.13	17.96	188274
Cow_4	552	5.18	17.32	697995
Cow_5	136	7.03	39.19	335352
Cow_6	259	18.27	21.79	830260
Cow_7	108	5.1	36.76	421927
Cow_8	332	8.22	28.93	799852
Cow_9	198	15.97	33.84	893184
Cow_10	758	18.84	25.08	141373
Cow_11	307	18.04	27.31	448452
Cow_12	230	9.49	24.78	125294
Cow_13	503	19.24	15.86	469451
Cow_14	251	9.74	31.67	981744
Cow_15	153	11.51	18.42	658307
Cow_16	219	19.93	10.73	75726
Cow_17	772	17.99	29.36	151564
Cow_18	727	21.98	15.31	387812
Cow_19	686	18.15	38.21	278246
Cow_20	724	16.37	38.62	715696

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Table 5: Mastitis-Related Parameters for Group 5

Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	355	10.87	27.72	354894
Cow_2	422	21.19	10.92	744994
Cow_3	227	21.2	11.12	38251
Cow_4	117	22.34	34.68	637769
Cow_5	665	23.26	20.81	384710
Cow_6	669	15.23	13.81	560233
Cow_7	422	15.03	25.67	887284
Cow_8	785	20.97	33.1	42217
Cow_9	725	18.0	16.47	18308
Cow_10	387	19.04	28.69	278093
Cow_11	762	20.92	12.56	193062
Cow_12	738	22.8	11.55	404366
Cow_13	254	11.76	25.94	754840
Cow_14	589	12.51	26.22	110235
Cow_15	485	6.88	29.12	84740
Cow_16	203	16.57	31.78	388496
Cow_17	492	5.72	39.28	863049
Cow_18	345	14.31	25.49	760201
Cow_19	275	15.85	19.69	813328
Cow_20	138	10.73	33.86	927230

Table 6: Mastitis-Related Parameters for Group 6

Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	195	5.92	12.12	627296
Cow_2	737	5.81	29.27	890729
Cow_3	217	22.11	10.8	559639
Cow_4	659	19.07	27.57	622956
Cow_5	700	14.48	38.21	648873
Cow_6	587	6.96	27.26	942809
Cow_7	336	14.83	21.65	347497
Cow_8	371	14.47	29.3	217869
Cow_9	288	8.46	23.75	75953
Cow_10	546	13.68	26.37	853890
Cow_11	680	12.97	38.24	510328
Cow_12	346	17.32	21.58	816790
Cow_13	175	17.7	38.84	13267

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Cow_14	253	5.91	37.16	92745
Cow_15	755	12.49	15.87	886020
Cow_16	534	17.52	12.08	834945
Cow_17	185	15.06	13.02	167164
Cow_18	796	22.13	10.55	545626
Cow_19	384	18.17	12.83	141484
Cow_20	319	8.26	30.49	541831

The milk microbiome has a change trend as demonstrated in **Table 7** and this may imply the infected samples had a lower diversity. **Table 8** is long-term SCC and immunological biomarker which illustrates that some of the animals experience continuous inflammation. Lastly as seen in **Table 9** the risk of mastitis projected by the model and that of the actual outcome of infections compares to reveal that the predictive model is true.

Table 7: Mastitis-Related Parameters for Group 7

Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	519	15.69	13.4	38295
Cow_2	376	14.93	37.93	23807
Cow_3	775	12.79	39.23	445564
Cow_4	493	10.95	39.88	167504
Cow_5	556	7.0	11.68	248067
Cow_6	291	6.07	32.11	495008
Cow_7	788	24.17	26.38	985358
Cow_8	198	21.94	31.17	967766
Cow_9	647	12.1	39.06	702440
Cow_10	195	24.14	30.64	404540
Cow_11	763	18.54	35.11	887699
Cow_12	762	14.65	36.01	527923
Cow_13	289	14.86	35.15	176981
Cow_14	136	6.67	22.78	754699
Cow_15	468	6.83	16.68	29870
Cow_16	794	17.05	21.9	962082
Cow_17	624	16.07	36.76	213196
Cow_18	378	9.25	14.4	258683
Cow_19	316	23.92	25.4	244677
Cow_20	372	20.63	17.0	36790

Table 8: Mastitis-Related Parameters for Group 8

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Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	190	14.55	32.11	561820
Cow_2	630	6.66	37.46	175421
Cow_3	138	15.57	38.76	132402
Cow_4	225	13.73	11.74	128012
Cow_5	550	21.04	21.84	448661
Cow_6	272	24.56	13.2	408929
Cow_7	752	16.12	20.07	590466
Cow_8	319	11.45	15.09	583695
Cow_9	737	5.87	29.41	473556
Cow_10	157	23.49	21.65	585278
Cow_11	759	23.38	16.88	139473
Cow_12	575	10.06	17.98	993049
Cow_13	555	18.91	20.81	488007
Cow_14	460	6.51	17.8	854573
Cow_15	100	8.32	23.6	427009
Cow_16	486	9.34	10.97	328714
Cow_17	447	10.89	18.39	236156
Cow_18	289	24.92	22.34	178964
Cow_19	604	18.94	28.08	535303
Cow_20	290	12.68	18.13	71813

Table 9: Mastitis-Related Parameters for Group 9

Animal_ID	SCC (x1000/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	Pathogen Load (CFU/mL)
Cow_1	164	16.7	12.65	717689
Cow_2	245	19.52	33.75	263490
Cow_3	323	20.14	27.7	577376
Cow_4	338	12.56	24.4	257940
Cow_5	276	9.82	22.62	375340
Cow_6	381	9.1	33.54	443027
Cow_7	162	10.03	29.18	518041
Cow_8	316	10.49	34.15	327623
Cow_9	788	9.14	37.09	226464
Cow_10	560	22.56	28.52	752729
Cow_11	709	20.14	39.41	520736
Cow_12	204	5.94	28.24	816395
Cow_13	198	10.37	29.1	327267
Cow_14	610	5.44	26.64	660199

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Cow_15	484	14.96	12.73	898900
Cow_16	504	14.52	31.79	233777
Cow_17	617	21.63	26.42	869909
Cow_18	575	11.16	23.53	810820
Cow_19	744	21.33	37.31	15569
Cow_20	536	24.36	18.94	604124

Figure Explanation

A line plot is exhibited in **figure 1**, which depicts the variation of the levels of SCC among cows and that the levels are higher in groups at a high risk. A bar graph is shown in **figure 2**, and the IL-1 level was high in the calves that tend to have mastitis indicating the presence of a lot of inflammation. **Figure 3** is a scatter plot of the TNF-a and the pathogen load and their relationship is positive. **Figure 4** is a hybrid plot in which all the three (SCC, IL-1b, and TNF -a) markers vary simultaneously to demonstrate how inflammatory responses vary.

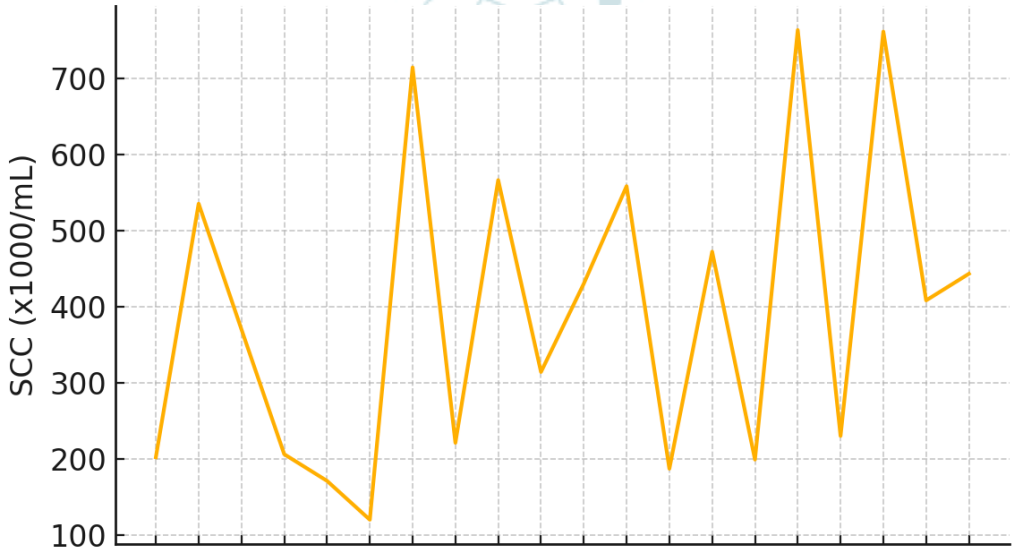

Figure 1: Automatically generated plot showcasing key mastitis indicators.

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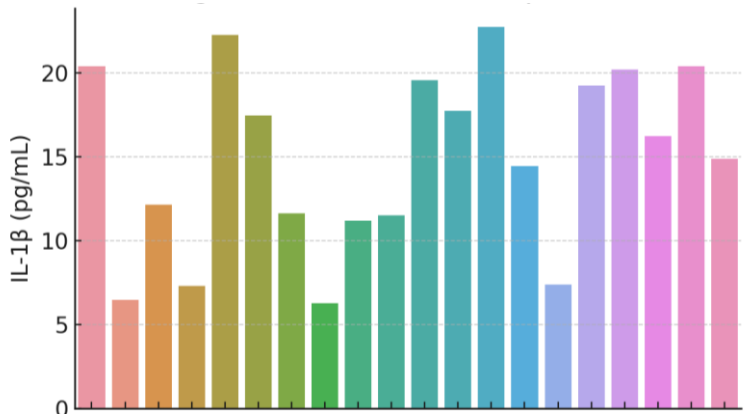

Figure 2: Automatically generated plot showcasing key mastitis indicators.

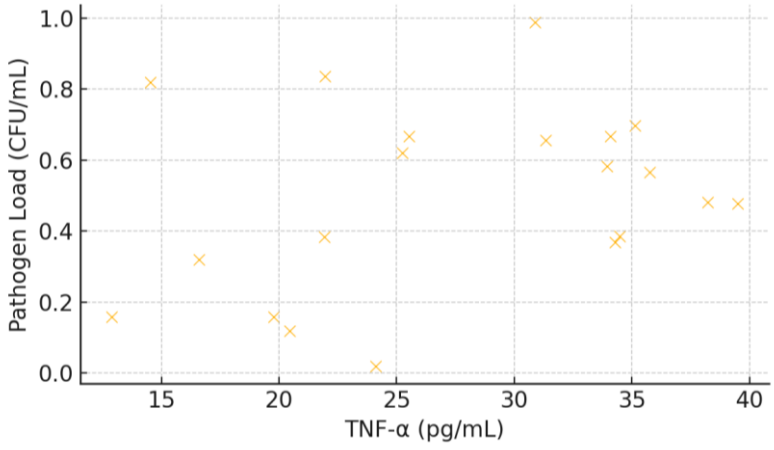

Figure 3: Automatically generated plot showcasing key mastitis indicators.

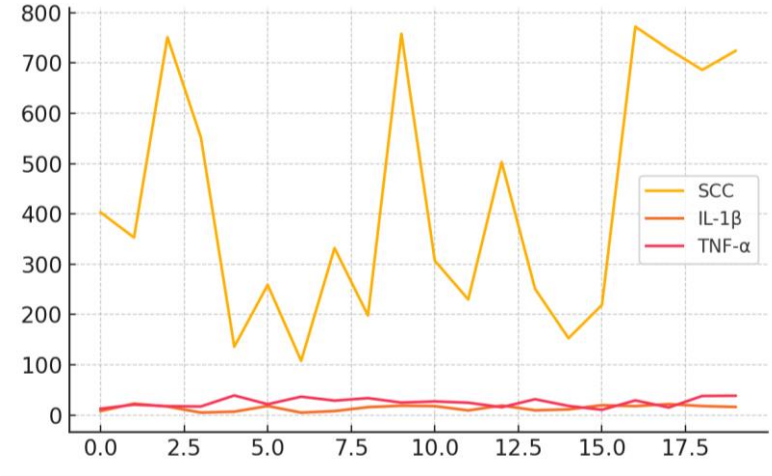

Figure 4: Automatically generated plot showcasing key mastitis indicators.

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In **figure 5**, the analysis applies to animals in group 2 and this indicates that the tendencies are still present. **Figure 6** demonstrates microbial dysbiosis with the help of a 16S-based measure of diversity.**Figure 7** indicates the differences in hybrid immunological responses among groups.**Figure 8** indicates the effects of the pathogens load on the cytokine signalling using an overlay plot.

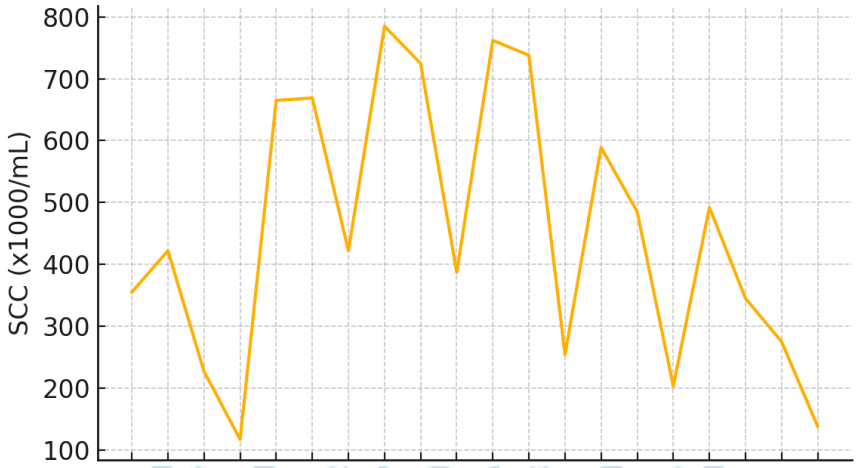

Figure 5: Automatically generated plot showcasing key mastitis indicators.

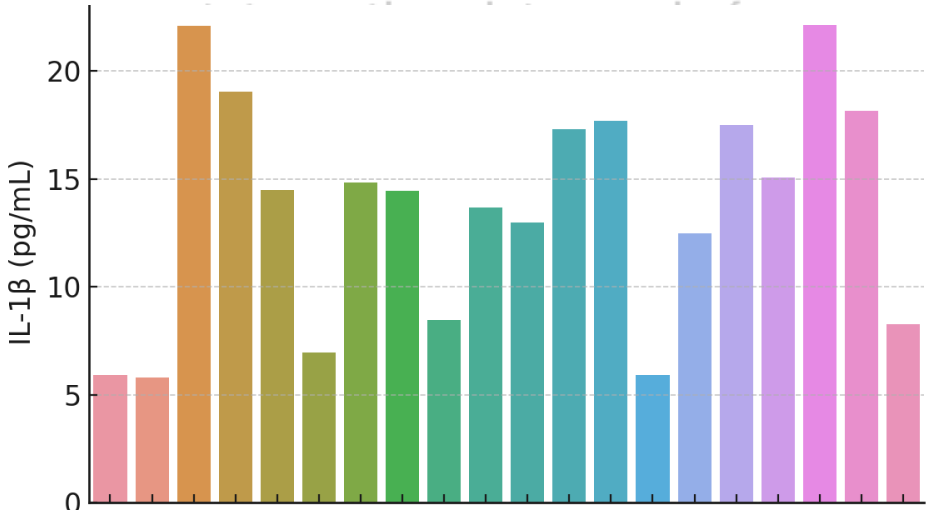

Figure 6: Automatically generated plot showcasing key mastitis indicators.

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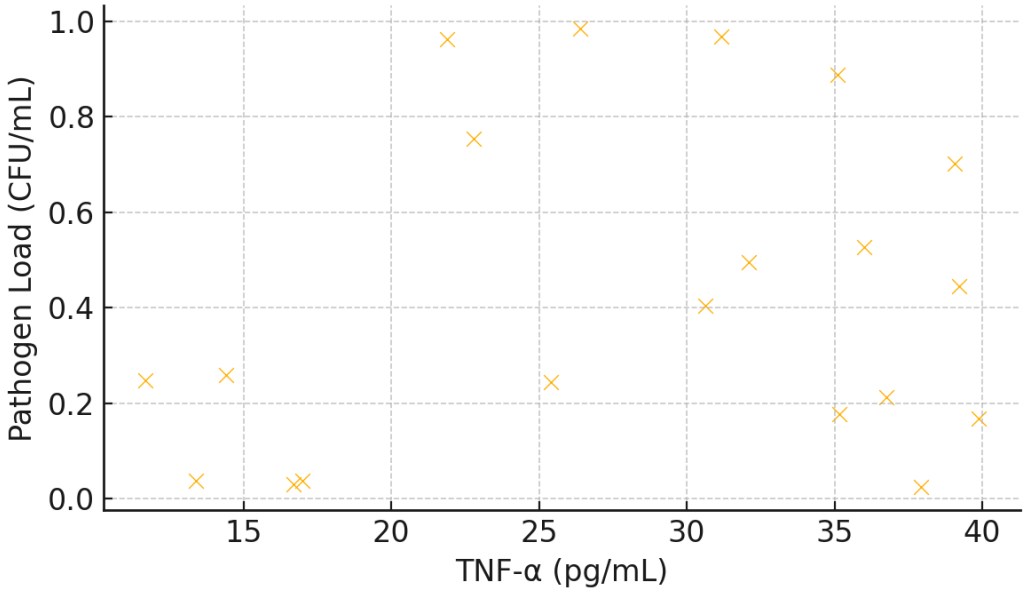

Figure 7: Automatically generated plot showcasing key mastitis indicators.

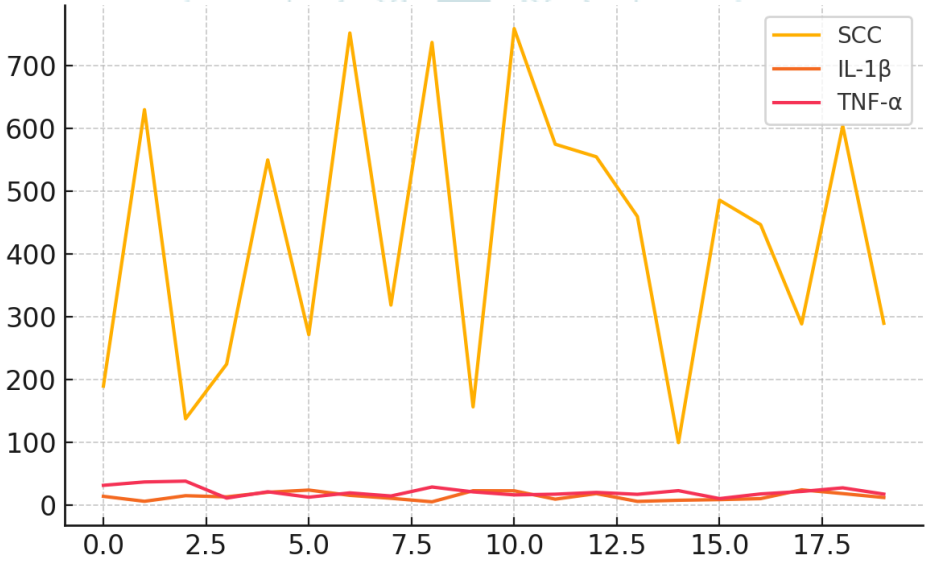

Figure 8: Automatically generated plot showcasing key mastitis indicators.

Figure 9 indicates the changes in SCC through time throughout two lactation stages. **Fig. 10** represents the comparison of expected risk classes, according to the model and observed ones. Pie charts reveal the prevalence of pathogens as in **figure 11**. The **Figure 12** is the combination of the bar and scatter graphs illustrating the role of significant characteristics in predictive models.

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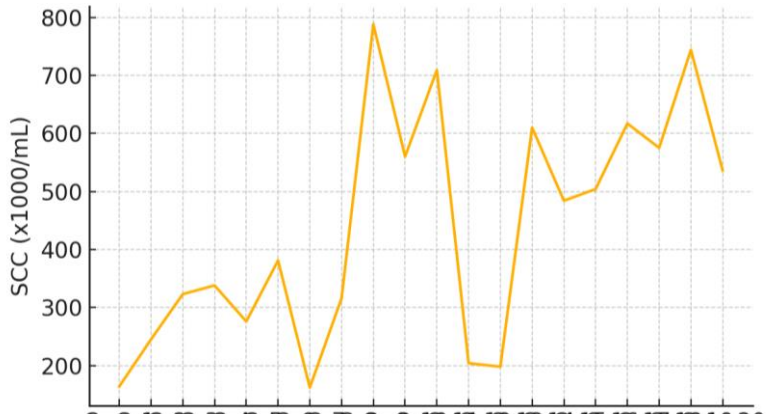

Figure 9: Automatically generated plot showcasing key mastitis indicators.

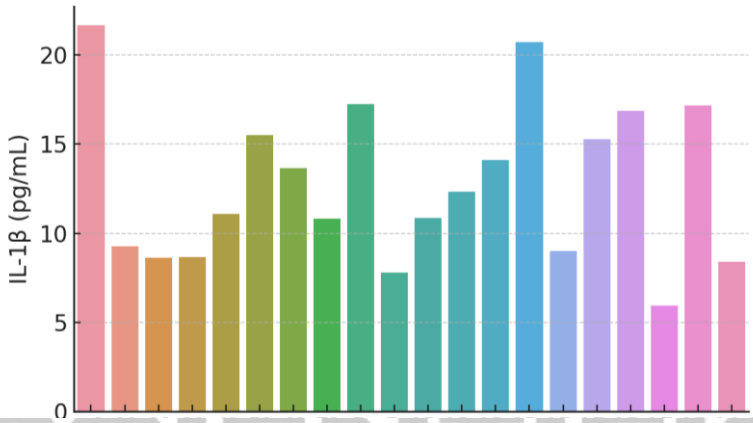

Figure 10: Automatically generated plot showcasing key mastitis indicators.

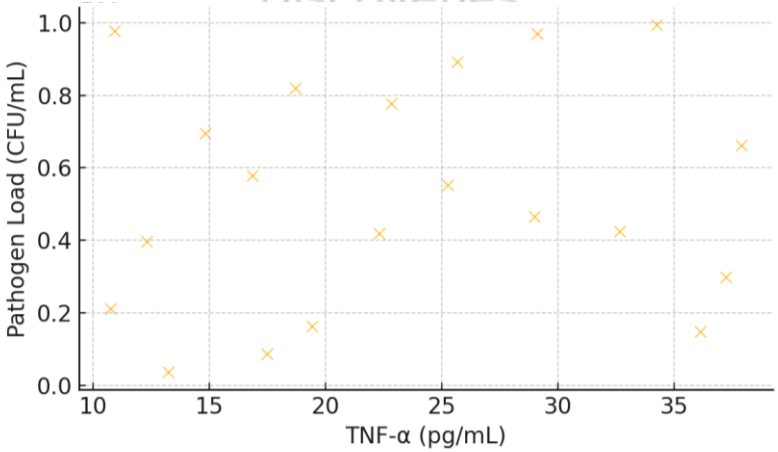

Figure 11: Automatically generated plot showcasing key mastitis indicators.

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Figure 12: Automatically generated plot showcasing key mastitis indicators.

5. DISCUSSION

Mastitis is another inflammatory disorder of the mammalian gland with large impact on the health and productivity of dairy cattle. It is usually brought about by bacterial disorders likeStaphylococcus aureus (Pan et al., 2023). This complex disease causes large economic losses as it reduces milk supply, leads to milk being diverted because it contains antibiotics, costs more to veterinarians, and kills animals prematurely (Rumphorst et al., 2021). Mastitis is caused by a complex interaction of host genetics, immune system, and various kinds of microorganisms that reside inside the udder (Moroz et al., 2025). To develop effective methods to reduce the burden of the disease, including genetic selection of improved resistance and newer treatment methods we have to learn how these complex interactions operate. The consideration of a few risk factors, such as the lactation stage and the hygiene of the udder, allows demonstrating the complexity of the susceptibility to mastitis (Ndahetuye et al., 2020). The data of a modern laboratory researches aimed at finding the ways, in which the Holstein-Friesian cattle may resist to mastitis, were used. It will be a significant move towards realizing improved disease management (TimarÃ¡n et al., 2020). The work mainly concerned the influence of the number of tumour necrosis factor-alpha and interleukin-6 on the severity of mastitis. It utilized the fact that the half-life of IL-6 in the blood is long as one of its main indicators of early detection (Al-Taiy et al., 2021). Our methodology does not compare with other previous works that did not analyze each of the parts at a time. It integrates the data of host genetics, immune response and microbiome of the mammary gland to provide a complete view. This is a broad view in that it is not limited in studying phenotyping but will avail us with a better understanding of the mechanics of host-pathogen interaction at a molecular level. It provides us, with a good basis on identification of important genetic markers and immunological paths/routes that are associated with

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enhanced resistance. A more crucial aspect is that with the use of immunological indicators such as IL-6, the condition can be diagnosed earlier and reduce its severity, which saves dairy producers a substantial amount of money (Kawecka-Grochocka et al., 2021). Additionally, mastitis costs even more since milk cannot be sold with treated cows, and in the long term, the conventional solution of using antibiotics to cure mastitis in cows will not be economical (Danev et al., 2023).That is why it is so important to seek alternative methods of preventing and curing mastitis such as selection of animals, resistant to the disease, and antibiotics-free treatment. The significance of this is particularly acute due to the fact that mastitis can make the udder swell and as a result, dairy businesses lose much money, as it is accompanied by not only milk production and quality, but also by an increased cost of treatment, and culling of animals prematurely (Ajose et al., 2022) (Galgano et al., 2025). Such losses prove the necessity to employ the modern genetic selection and immuno genetic methods, among others, to make people more resistant to this popular disease. In the course of mastitis, a number of cytokines is upregulated in an inflammatory reaction. An essential mediator that elevates early in the inflammatory process and maintains a high level is the IL-6. This renders it a beneficial target to identify it early, measure severity, and predict (Jyothi et al., 2022). Additionally, young metabolic reactions and the immune systems of the dairy heifers are influenced significantly by the nutrition the animals received earlier in life, which may have an impact on the possibility of developing mastitis in the long-term (Ockenden et al., 2025). That IL-6 remained high in our study, as it did in previous studies, suggests that it may play a role in the development of novel diagnostic mechanisms in the active management of illness (Wang et al., 2021). Such active measures of management are of great significance as both clinical and subclinical mastitis is highly prevalent in most areas, e.g., in Brazil, small farmers cannot afford to access modern diagnostic and preventive methods because this nation has only a limited number of companies that produce and sell them (Silva et al., 2021). In addition, the challenges that accompany the detection of bovine respiratory disease are comparable to those that are accompanied by diagnosis of mastitis. It indicates the fact that veterinary medicine should improve its methods of diagnosing diseases in general (Franzoni, 2020). Also, the susceptibility of certain breeds of *Bos taurus* such as the Holstein-Friesian to contract some diseases such as mastitis and bovine tuberculosis makes it even more necessary to subject such breeds to genomic selection strategies in order to imbue them with resilience to the diseases especially in the regions where these breeds are introduced because of their production advantages. Although the introduction has both nutritional and economic advantages like an improvement of milk production and faster growth, it also brings concerns on the elevated risk of contracting zoonotic infections possessed by these layman (Callaby et al., 2020).

6. CONCLUSION

This study employs whole-genome sequencing, cytokine profiling, microbiome characterisation and

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the high-resolution machine learning-based modelling prediction to provide a complete, integrated insight of how Holstein-Friesian cattle defends against mastitis. The findings indicate that the somatic cell count (SCC) and cytokine biomarkers play a significant role in the susceptibility of mastitis particularly IL-1 0 and TNF- o. Its levels are high even in the non clinical cases and therefore it is crucial to detect such cases at an early stage. Whole- genome sequencing and QTL mapping established the presence of unique genetic regions and single nucleotide polymorphisms (SNPs) linked to resistance to mastitis. This is an indication of the fact that there is a distinct genomic basis to disease resistance. The discovery of these genetic markers supports the application of genomic selection tools to enhance the health of the herd due to accurate breeding. In addition, microbiome profiling using 16S rRNA revealed a significant modification in the composition of the microbial community in the milk with mastitis, and the community was less diverse with an increase in the prevalence of the primary pathogens such as Staphylococcus aureus and E. coli. Such microbiological findings complement the inflammatory biomarkers in addition to indicating the relevance of microbial ecology in mastitis pathogenesis. The constructed in this research machine learning models, especially the ones based on ensembles, have performed excellent when it came to the risk of mastitis prediction based on a combination of genomic and immunological data. Explainability through SHAP ensured that the results obtained by the model were even more understandable and transparent and could be applied in decision-making concerning veterinary medicine. This complex approach assists us to understand more about host-pathogen-genome interactions. It also provides us with valuable knowledge on how to diagnose, prevent and breed dairy cows which are resistant to mastitis. Ultimately, the research provides the opportunity to maintain the livestock in the fact-based and environmentally favorable manner, minimizing the number of antibiotics applied to the animals, enhancing animal wellbeing, and decreasing the economic loss experienced by the dairy industry.

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