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The prevalence of environmental and contagious bovine mastitis, risk factors associated with feed supplements, farm and milking hygiene in Albania dairy cattle

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Abstract
Despite the existence of traditional mastitis control programs, subclinical mastitis remains the most prevalent infectious disease affecting dairy cattle. In developed countries, national mastitis councils are typically established to address udder health and implement control strategies. In contrast, Albania lacks both a formal mastitis control program and the organizational framework needed to establish such a council, despite the recognized burden of subclinical mastitis in dairy herds.

This study aimed to (i) assess the prevalence of subclinical mastitis, (ii) identify the causative bacterial pathogens, (iii) determine the antimicrobial susceptibility of major isolates, and (iv) evaluate key risk factors associated with disease occurrence. A cross-sectional survey was conducted across 35 small to medium-sized dairy farms using the California Mastitis Test (CMT) for screening. Milk and feed samples, along with farm-level metadata, were collected and analyzed for bacteriological profiles, antimicrobial resistance patterns, and mineral content.

The overall prevalence of subclinical mastitis at the cow level was 59.5% (95% CI: 52.8–65.9%), while herd-level prevalence reached 97.1% (95% CI: 85.5–99.5%), with 34 out of 35 farms testing positive. Bacteriological analysis revealed both contagious and environmental pathogens, with Staphylococcus aureus being the most frequently isolated, followed by Pseudomonas aeruginosa, Escherichia coli, and Enterobacter spp. Antimicrobial susceptibility testing showed widespread multidrug resistance among isolates; notably, enrofloxacin was the only antibiotic effective against more than half (59.6%) of the isolates. Significant associations were found between subclinical mastitis and poor udder hygiene ($P = 0.001$), as well as deficiencies in dietary supplementation. Positive correlations were observed with zinc ($R^2 = 0.136–0.197$; $P = 0.041$), selenium ($R^2 = 0.33$; $P = 0.001$), vitamin A ($R^2 = 0.35$; $P = 0.001$), and vitamin E ($R^2 = 0.31$; $P = 0.001$) intake.

The high prevalence of subclinical mastitis and the emergence of antimicrobial resistance highlight the need for urgent action. The lack of awareness among farmers and the absence of a structured control program suggest that national strategies, including education, coordinated interventions, and possibly the establishment of a mastitis council, are essential to improve udder health in Albanian dairy farms.

Keywords — Udder health, bovine contagious subclinical mastitis, Zinc and Selen suplement, vitamin A and E, Staphylococcus aureus, antibiotic resistance

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I. INTRODUCTION

Bovine subclinical mastitis is a serious disease that affects dairy cattle all over the world. It is characterized by inflammation of the mammary gland with no obvious clinical indications. Unlike clinical mastitis, which is characterized by abnormal milk, swelling, and redness, subclinical mastitis is undetectable without laboratory testing, making it a silent but economically significant disease (Animal Health Ireland, 2022, Quinn *et al*, 2011).

Bovine subclinical mastitis is caused largely by bacterial invasion of the udder through the teat canal. Understanding and managing subclinical mastitis is critical for dairy herd health, milk quality, and profitability in the dairy business. Despite the fact that around 150 pathogens are implicated in bovine mastitis, several of them are more commonly isolated. Pathogens associated in bovine mastitis are classed as contagious (directly transmitted from cow to cow) or environmental (found in bedding, soil, and water). The most common contagious pathogens are *Staphylococcus aureus* (which plays an important role, forms biofilms, and is resistant to antibiotics), *Streptococcus agalactiae* (which colonises the udder and spreads during milking), and *Mycoplasma* spp. Environmental pathogens include *Escherichia coli* (which creates endotoxins that cause inflammation), *Streptococcus uberis* (which lives in organic materials and invades mammary tissues), and *Klebsiella* spp. Once infected, pathogens adhere to the mammary epithelium, replicate, and trigger an immunological response, resulting in inflammation and an increase in somatic cell count; however, in subclinical cases, the infection remains hidden without visible signs, resulting in decreased milk quality and yield (Mohammed & Bakr, 2019, De Vliegher *et al,* 2012, Quinn *et al*, 2011, Radostits *et al*, 2007).

Although subclinical mastitis has no obvious symptoms, it is identified by laboratory tests. Subclinical mastitis can be detected using both direct and indirect techniques. Indirect testing include the California Mastitis Test (CMT), somatic cell count (SCC) analysis, and electrical conductivity test, followed by direct tests include milk culture and polymerase chain reaction (PCR) Antibiotic susceptibility tests are required to determine the efficacy of antibiotics chose.

Effective prevention and control of subclinical mastitis involve proper milking techniques (including washing, pre and post milking disinfection and milking machine maintenances), environment hygiene control, selective and rational antibiotic therapy, herd health monitoring (monthly SCC and culling chronic cases). It is important to provide balanced feed enriched in vitamins and microelements to ensure good udder health condition. Economic losses due to subclinical mastitis stem from reduced milk production, increased culling rates, and the costs of treatment and prevention measures (Costa *et l,* 2024, Hogeveen *et al,* 2011). Additionally, the presence of pathogens in milk poses a risk to public health, emphasizing the importance of early detection and control strategies in dairy herd management (Khan *et* al, 2024, Mohammed & Bakr, 2019, Quinn *et al*, 2011, Radostits *et al*, 2007).

Aim of this study was to assess the prevalence of subclinical mastitis in Albania dairy herds, as well as the significance of zoo-technical criteria such as nutrition and sanitary conditions in frequency of subclinical mastitis. Furthermore, identification of bacteria involved and their antibiotic susceptibility were performed.

MATERIAL AND METHODS

Dairy farms and animals

The study included 35 small dairy herds from 13 different districts in Albania. The average farm size of milking cow was 28.5 animals (range from 8 to 121 milking caws). Holstein-Friesian is the most frequent breed among the sample animals, followed by Simental, other crossbreeds such as Jersey, native, and Taranteze. Six milking cows from each farm were assigned at random from each farm and tested using the California Mastitis Test (CMT).

The study's animal participants had to meet two requirements: the farm had to have more than ten animals, and cows had to be chosen at random from among animals lactating for between 10 and 140 days.

A visual inspection and statistics on age, milk production, time from calving to successful insemination, and day of lactation were recorded for each animal that was recruited. The amount of DNA in milk secretions is estimated qualitatively by the CMT. A quick test to identify cow mastitis at the cow's side in the early stages. Elevated somatic cell counts (SCC) are quickly detected by the test as a sign of a mastitis infection. It is feasible to identify specific quarters with higher SCC and detect mastitis in its early stages by using CMT at regular intervals and documenting the results. The CMT was run in the farm and data for each quarter was recorded in hard copy, than where transfer into the excel document. Briefly, after the milk has been properly collected in the CMT paddle, the reagent is added in the same amount while being swirled horizontally for ten to fifteen seconds. The CMT kit provide sodium alkyl aryl sulfonate, which reduces surface tension, changes the structure and conductivity of the cell membrane and nucleus, disturbs the osmotic balance, inhibits oxidation, stimulates proteolytic enzymes, and enhances the viscosity of milk. Table 1 displays the assessing criteria for presence of bovine subclinical mastitis.

Score	Likely somatic cell count (SCC) range	Animal health status	Comment
0	0 - 200.000	Healthy quarter	Mixture of milk and test fluid remain unchanged when swirled
T	200.000 – 400.000	At border line	Mixture of milk and test fluid becomes very slightly mucoid
1	400.000 – 1.200.000	Weakly positive	Mixture of milk and test fluid becomes slightly mucoid
2	1.2 – 5 million	Positive	Mixture become mucoid, but still tip out of small volume of liquid
3	> 5.000.000	Strong positive	Mixture become mucoid, and jelly-like, with no excess fluid to tip out

Table 1 – *Criteria used to assess the subclinical health status of tested cows* (Animal Health Ireland, 2022, Mohammed & Bakr, 2019, Dereje *et al,* 2012, Quinn *et al*, 2011, Radostic *et al,* 2007).

Udder cleanliness was visually assessed and categorized into four levels: Very Clean – No visible dirt or debris; Lightly Soiled – Small amounts of dirt, indicating moderate cleanliness; Moderately Dirty – Noticeable dirt accumulation; Very Dirty – Heavy contamination, suggesting poor hygiene. These hygiene scores were then compared to the prevalence of subclinical mastitis.

Milking hygiene practices, including pre- and post-milking teat disinfection, were documented. The prevalence of mastitis was compared between farms that performed post-milking teat dipping and those that did not; Dietary Supplementation and Udder Health

Based on data on feed composition and the currently used feed rations, the amounts of energy, organic matter, minerals, and vitamins provided by the actual feed rations of each farm were calculated. These values were then compared with the nutritional requirements according to GfE (2023). On this basis, the extent to which nutrient requirements were met for each dairy farm was assessed.

The influence of mineral-vitamin supplementation on subclinical mastitis prevalence was examined. Nutrients analyzed included: Zinc (Zn) – Evaluated for its role in immune function and epithelial integrity; Selenium (Se) – Studied for its impact on inflammation reduction; Vitamin A – Assessed for its role in maintaining epithelial tissue integrity;Vitamin D3 – Investigated for its immune-modulating effects and Vitamin E – Examined for its influence on immune cell function.

Farm records, when available, were used to estimate daily intake levels. Correlation analysis was conducted to determine the statistical significance of dietary supplementation in reducing mastitis prevalence.

The relationship between udder hygiene, dietary supplementation, and mastitis prevalence was assessed using correlation coefficients (R^2) and p-values. The significance threshold was set at $P \leq 0.05$, with a stronger emphasis on results with $P \leq 0.01$, indicating a highly significant relationship.

RESULTS

Out of the 996 cows in total, 210 lactating cows were tested, and 125 of them tested positive for subclinical mastitis. The farms involved in the study are show in Table 2. There is a wide range of the prevalence. The district, number of the farms, tested animals, positive animals, prevalence of subclinical mastitis, lower and upper confidence intervals are shown in Table 2 and Figure 1.

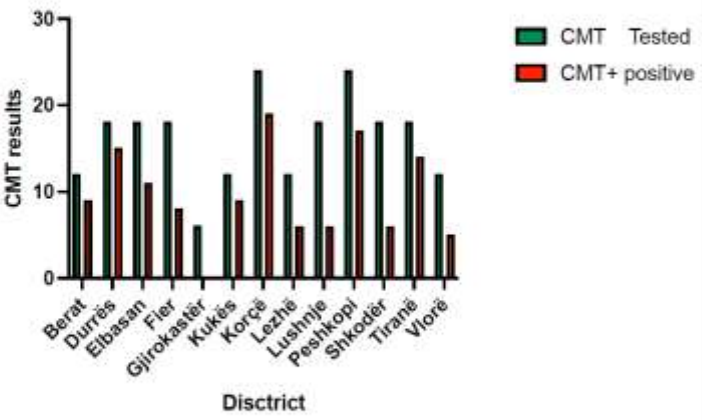
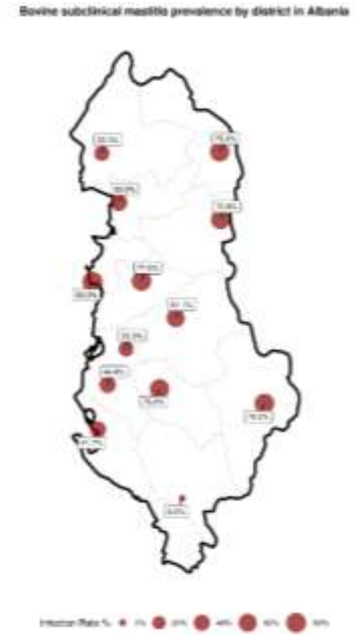


Figure 1 *– California Mastitis Test results by regions. This bar graph displays California Mastitis Test (CMT)* ***results*** *across different districts. The x-axis represents various districts, while the y-axis indicates the number of CMT tests performed.*

Nr	District	Farm number	CMT Tested	CMT+ positive	Percentage	Lower CI 95%	Upper CI 95%
1	Berat	1	6	4	66.7	30	93
2		2	6	5	83.3	43.7	97
Subtotal		2	12	9	75.0	46.8	91.1
3	Durrës	1	6	4	66.7	30	90.3
4		2	6	6	100.0	61	100
5		3	6	5	83.3	43.7	97
Subtotal		3	18	15	83.3	60.8	94.2
6	Elbasan	1	6	4	66.7	30	93
7		2	6	3	50.0	18.8	81.2
8		3	6	4	66.7	30	93
Subtotal		3	18	11	61.1	38.6	79.7
9	Fier	1	6	3	50.0	18.8	81.2
10		2	6	2	33.3	9.7	70
11		3	6	3	50.0	18.8	81.2
Subtotal		3	18	8	44.4	24.6	66.3
12	Gjirokastër	1	6	0	0.0	0.0	0.0
Subtotal		1	6	0	0.0	0.0	0.0
13	Kukës	1	6	4	66.7	30	93
14		2	6	5	83.3	43.7	97
Subtotal		2	12	9	75.0	46.8	91.1
15	Korçë	1	6	4	66.7	30	93
16		2	6	5	83.3	43.7	97
17		3	6	4	66.7	30	93
18		4	6	6	100.0	61	100
Subtotal		4	24	19	79.2	59.5	90.8
19	Lezhë	1	6	3	50.0	18.8	81.2
20		2	6	3	50.0	18.8	81.2
Subtotal		2	12	6	50.0	25.4	74.6

21	Lushnje	1	6	3	50.0	18.8	81.2
22		2	6	1	16.7	3	56.4
23		3	6	2	33.3	9.7	70
Subtotal		3	18	6	33.3	16.3	56.3
24	Peshkopi	1	6	5	83.3	43.7	97
25		2	6	6	100.0	61	100
26		3	6	5	83.3	43.7	97
27		4	6	1	16.7	3	56.4
Subtotal		4	24	17	70.8	50.8	85.1
28	Shkodër	1	6	2	33.3	9.7	70
29		2	6	2	33.3	9.7	70
30		3	6	2	33.3	9.7	70
Subtotal		3	18	6	33.3	16.3	56.3
31	Tiranë	1	6	6	100.0	61	100
32		2	6	5	83.3	43.7	97
33		3	6	3	50.0	18.8	81.2
Subtotal		3	18	14	77.8	54.4	91
34	Vlorë	1	6	4	66.7	30	93
35		2	6	1	16.7	3	56.4
Subtotal		2	12	5	41.7	19.3	68
Total		35	210	125	59.5	52.8	65.9

***Table 2** – Tested farms results and prevalence of subclinical mastitis at each farm, each district and total animal level*



***Map 1** – Prevalence of Subclinical Mastitis in Cows in Albania.*

The map shows the distribution of subclinical mastitis across districts in Albania. The highest prevalence is observed in Vlorë (83.3%), Korçë (79.2%), and Elbasan (77.8). These data help identify high-risk areas and support the planning of preventive measures.

Table 2 presents data on California Mastitis Test (CMT) results across various districts, assessing the percentage of CMT-positive samples in different farms. A total of 210 samples were tested across 35 farms in different districts. Out of these, 125 tested positive, resulting in an overall CMT+ positivity rate of 59.5% with a 95% confidence interval (CI) of 52.8% to 65.9%. Durrës had the highest CMT Positivity Rate at 83.3% (CI: 60.8% - 94.2%), followed by Tiranë at 77.8% (CI: 54.4% - 91%), Korçë at 79.2% (CI: 59.5% - 90.8%), and Peshkopi at 70.8% (CI: 50.8% - 85.1%). Gjirokastër had the lowest CMT Positivity Rates at 0.0% (CI: 0.0% - 0.0%), followed by Shkodër and Lushne. The 95% Confidence Intervals (CIs) represent the uncertainty in the percentage estimations. Durrës had narrower CIs (60.8% - 94.2%), while Lushnje had wider CIs (16.3% - 56.3%). This suggests increased variability, which could be attributable to a smaller sample size or inconsistencies in infection rates.

Bacteria species	Number	Percentage	Comments
Bacillus cereus	1	1.8	Environmental pathogen
Candida albicans	1	1.8	Mycotic pathogen
Corynobacterium bovis	1	1.8	*Contagious mastitis*. Primary reservoirs are infected udder and teat ducts
Staphylococcus hemolyticus	1	1.8	*Contagious mastitis* Coagulase Negative staphylococcus, originates from the teat skin
Escherichia coli	5	8.8	Environmental pathogen
Enterobacter spp.	7	12.3	Environmental pathogen
Pseudomonas aeruginosa	10	17.5	Environmental pathogen
Staphylococcus epidermidis (albus)	7	12.3	*Contagious mastitis* Coagulase Negative staphylococcus, originates from the teat skin
Staphylococcus aureus	24	42.1	*Contagious mastitis* Coagulase positive staphylococcus, main contagious pathogen

Table 3.0 *– Bacterial species isolated from subclinical mastitis cases, their frequency, and relative percentage, along with their classification as environmental or contagious pathogens.*

Bacteria	Number	Percentage	Comments
Staphylococcus aureus	24	42.1%	Coagulase-positive staphylococcus, major contagious pathogen.
Staphylococcus epidermidis	7	12.3%	Coagulase-negative staphylococcus (CNS), originates from the teat skin.
Staphylococcus hemolyticus	1	1.8%	Coagulase-negative staphylococcus (CNS), originates from the teat skin.
Corynebacterium bovis	1	1.8%	Found in the udder and teat ducts, associated with contagious mastitis.

Table 3.1 *– Contagious pathogens isolated from milk samples of CMT positive quarter of mammary gland. These bacteria are primarily transmitted during milking through contaminated equipment, hands, or bedding.*

Bacteria	Number	Percentage	Comments
Pseudomonas aeruginosa	10	17.5%	Found in water, soil, and bedding. Difficult to treat.
Enterobacter spp.	7	12.3%	Soil and manure contaminants. Can cause clinical mastitis.

Bacteria	**Number**	**Percentage**	**Comments**
Escherichia coli	5	8.8%	Opportunistic pathogen from manure-contaminated bedding.
Bacillus cereus	1	1.8%	Environmental spore-forming bacterium.
Candida albicans	1	1.8%	Fungal pathogen, sometimes associated with prolonged antibiotic use.

Table 3.2 *– Contagious pathogens isolated from milk samples of CMT positive quarter*

A total of 57 bacterial isolates were found in mastitis patients. The bacteria were classed as contagious or environmental pathogens, reflecting distinct sources and modes of transmission. The most prevalent pathogen was *Staphylococcus aureus* (42.1%), which is a primary cause of infectious mastitis. *Pseudomonas aeruginosa* (17.5%), an environmental pathogen, was the second most commonly found species (Table 3.0 and 3.1 and 3.2 at Annex 1).

Parameters	DM Intake, kg/d	Roughage, % /DM, %	Conc. in% DM	NEL, inMJ	NEL, MJ/kg DM	CP in DM, %	g CP /MJ ENL	StCF, % DM	ST_XZ % DM	Conc., ing/kg milk
Average	16,8	69,8	30,3	105,5	6,3	155,8	155,9	18,4	24,1	279,3
Min	9,5	52,1	0,0	64,2	5,4	123,4	123,2	13,7	5,2	0,0
Max	24,6	100,0	47,9	150,0	7,0	197,1	197,5	27,3	36,6	499,4
SD	3,6	11,7	11,7	24,2	0,5	21,2	21,2	4,0	8,0	110,8
CV, %	21,7	16,7	38,6	22,9	7,2	13,6	13,6	21,6	33,1	39,7

Table 4.0 *– The energy and nutrient contents and the balance ratios of the daily rations fed to the dairy farms (n=31).*

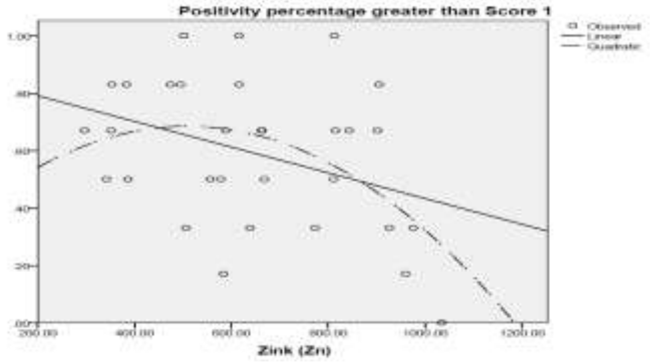
DM-Dry matter; CP-Crude Protein; StCF-structural crude fiber; ST_XZ- total sum of Starch and sugar; NEL-Neto Energy lactation.

Table 5 *– The mineral and vitamine contents of the daily rations fed to the dairy farms (n=31).*

Param.	Ca, g	P, g	Ca/P, g/g	Fe, mg	Cu, mg	Zn, mg	Se, mg	Vitamin A (1000 UI)	Vitamin D3 (1000 UI)	Vitamin E (mg)
Average	160,4	58,9	2,8	2553,5	192,5	645,6	4,2	110,5	13,8	250,5
Min	45,6	39,1	1,2	853,5	70,0	297,0	0,2	3,4	0,0	0,0
Max	335,5	110,1	5,1	3919,3	450,7	1034,4	16,3	407,6	56,3	988,0
SD	51,0	14,8	0,8	618,9	99,2	211,8	4,9	121,9	17,3	302,0
CV, %	31,8	25,2	29,3	24,2	51,5	32,8	116,1	110,3	125,7	120,6

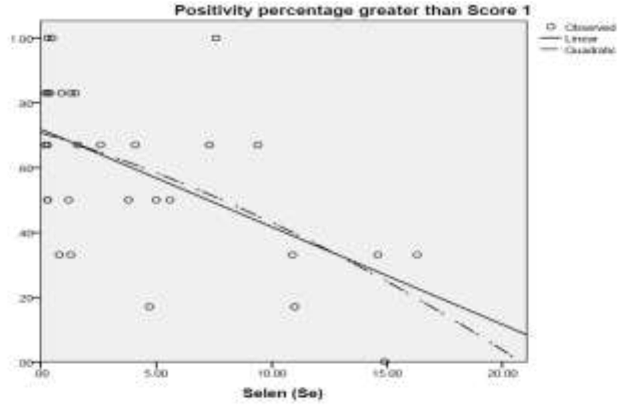
The data indicate that the average feed rations used in September 2024 in the studied farms align with normal values for daily milk production of 10–30 kg. However, some indicators exhibit high variability. Certain farms use rations with low NEL concentration (5.4 MJ NEL/kg DM), low protein content (12.3% CP in DM), and high fiber levels (27.3% structural CF in DM) mainly due to poor-quality roughage. Data on mineral and vitamin content in feed rations show high variation, largely due to nearly half of the farms omitting premixes. Deficiencies in trace elements (zinc, selenium, manganese, copper, iodine) and vitamins E and D are common. The new GfE (2023) recommendations set higher requirements for these nutrients, which are essential for immune function and mastitis prevention.

The majority of cows (53.1%) had lightly soiled udders, indicating moderate cleanliness. About 24.7% had very clean udders, while 19.6% were moderately dirty, and 2.6% were very dirty (data not shown). This suggests that hygiene conditions on most farms are not optimal, increasing the risk of mastitis.



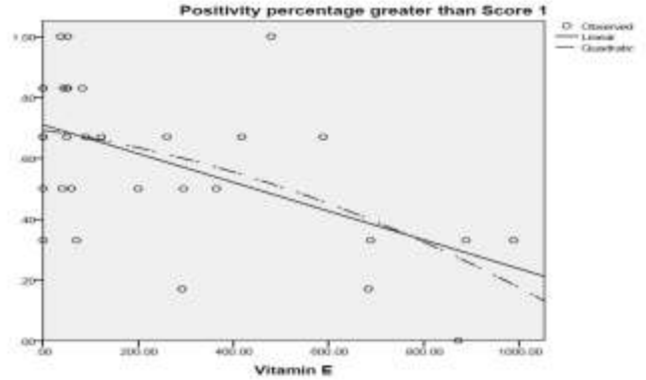
(R^2=0,136-0,197; P =0.041)

Figure 2 *– The impact of Zinc (Zn) levels (mg) in the diet on the prevalence of subclinical mastitis, expressed through CMT (California Mastitis Test) values of ≥ 1+ in dairy cows. The study found a significant correlation (P = 0.041, R^2 = 0.136-0.197) between dietary zinc levels and mastitis prevalence. Higher zinc intake was associated with lower California Mastitis Test (CMT) scores, suggesting that adequate zinc improves immune function and udder health.*



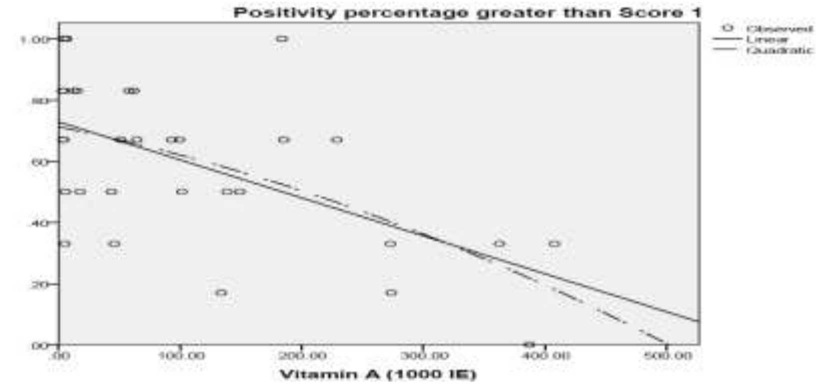
(R^2=0,33; P =0.001)

Figure 3 *– The impact of Selenium (Se) levels (mg) in the diet on the prevalence of subclinical mastitis, expressed through CMT (California Mastitis Test) values of ≥ 1+ in dairy cows. Selenium supplementation showed a strong inverse correlation (P = 0.001, R^2 = 0.33) with mastitis cases. Selenium is crucial for enhancing immune response and reducing inflammation in the udder.*



(R^2=0,31; P =0.001)

Figure 4 *– The impact of Vitamin E levels (mg) in the diet on the prevalence of subclinical mastitis, expressed through CMT (California Mastitis Test) values of ≥ 1+ in dairy cows. A similar correlation (P = 0.001, R^2 = 0.31) was observed between vitamin E levels and mastitis prevalence. Vitamin E boosts the activity of polymorphonuclear (PMN) cells, which are responsible for bacterial elimination in the udder.*



(R^2=0,35; P =0.001)

Figure 5 *– The impact of Vitamin A levels (1000 IU) in the diet on the prevalence of subclinical mastitis, expressed through CMT (California Mastitis Test) values of ≥ 1+ in dairy cows. Vitamin A supplementation was found to have a significant effect on reducing mastitis prevalence (P = 0.001, R^2 = 0.35). This vitamin supports epithelial tissue integrity and immune defense, making it essential for udder health.*

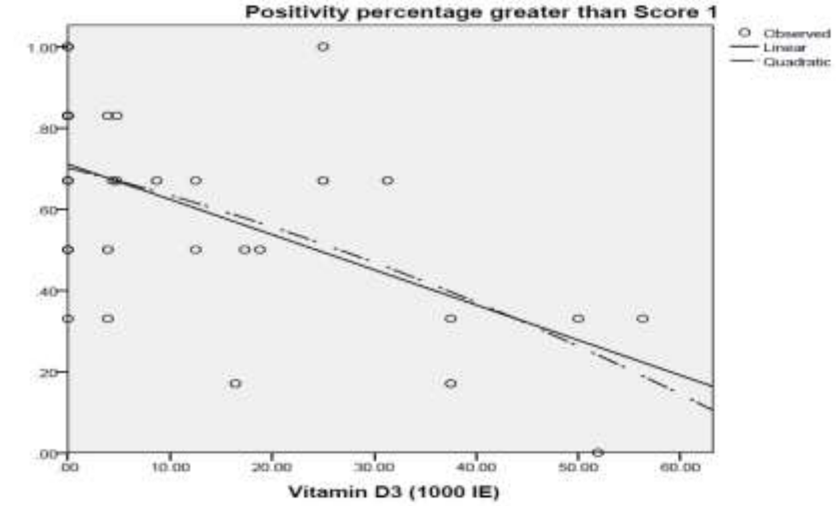


Figure 6 *– The impact of Vitamin D3 levels (1000 IU) in the diet on the prevalence of subclinical mastitis, expressed through CMT (California Mastitis Test) values of ≥ 1+ in dairy cows. Vitamin D3 also showed a strong inverse correlation with mastitis prevalence. Its role in modulating immune response and preventing bacterial adhesion makes it a key nutrient in mastitis control.*

DISCUSSION

Bovine subclinical mastitis is a silent yet economically significant disease that reduces milk quality and production. Since it lacks visible symptoms, early detection through SCC, CMT, and milk culture is essential. Cows that experienced multiple mastitis episodes had lower milk yield and lactose content compared to healthy cows (Costa *et al*, 2024). Monitoring SCC and lactose content can help detect subclinical mastitis early. Selection for cows with higher LC may contribute to better udder health, fertility, and longevity. Mastitis control strategies should focus on reducing cumulative infections to prevent irreversible damage (Animal health Ireland, 2022, De Vliegher, 2012, Quinn et al, 2011).

Infected cows produce less milk due to inflammation and damage to mammary tissue. Even mild infection can lead to production losses of 10-20% per affected quarter. Overall, subclinical mastitis can cost dairy farms $100–300 per cow per year, depending on severity and management practices. Increased somatic cell count (SCC) reduces milk quality, leading to lower milk prices, and eve may be rejected by processors. Mastitis increase treatment costs, antibiotics and veterinary expenses, costs associated with labor, diagnostics, and supportive care for controlling infections. Chronically infected cows may need to be culled, increasing herd turnover and replacement costs. Heifer rearing is expensive, impacting long-term profitability (Costa *et al,* 2024, Hogoveen, *et al,* 2011).

This study provides valuable insights into the prevalence, risk factors, and potential mitigation strategies for subclinical mastitis in lactating cows across multiple districts. The California Mastitis Test (CMT) results indicate significant regional differences in mastitis prevalence, emphasizing the importance of farm management, hygiene, genetics, and nutritional factors in disease control.

Out of 210 tested lactating cows, 125 (59.5%) were CMT-positive, indicating a high burden of subclinical mastitis. The prevalence varied widely across districts, with Durrës (83.3%), Korçë (79.2%), and Tiranë (77.8%) reporting the highest positivity rates, while Gjirokastër (0.0%) and Shkodër had the lowest. The confidence intervals (CIs) suggest that some districts experienced higher variability, possibly due to differences in farm sizes, management practices, and sample sizes. Our study results are quite similar with other studies (Bouchoucha *et al,* 2024 Ararsa et al, 2014, Bitew *et al,* 2010,).

The variation in CMT positivity highlights disparities in mastitis surveillance and control programs, suggesting that certain regions may require improved biosecurity measures, better milking hygiene, and enhanced farmer education (Animal Health Ireland, 2022, De Vliegher *et al,* 2012, Quinn *et al,* 2022).

A total of 57 bacterial isolates were identified, with *Staphylococcus aureus* (42.1%) being the dominant contagious mastitis pathogen, followed by *Pseudomonas aeruginosa* (17.5%), an environmental pathogen. Other pathogens included *Escherichia coli, Enterobacter* spp., and *Staphylococcus epidermidis*. The presence of both contagious and environmental pathogens indicates that both cow-to-cow transmission and environmental exposure contribute to the disease burden. The high prevalence of *Staphylococcus aureus* suggests poor milking hygiene, inadequate teat disinfection, and improper dry cow therapy as possible contributing factors. Additionally, the presence of environmental pathogens such as Pseudomonas aeruginosa and Enterobacter spp. suggests poor bedding management and inadequate sanitation (Katsande *et al,* 2013, Radostits *et al,* 2007)).

Kahn and co-authors explore the role of nutritional strategies in preventing periparturient mastitis, a condition affecting dairy cows due to **i**mmune suppression, oxidative stress, and metabolic disorders. The authors focus on how trace minerals, vitamins, and amino acids contribute to udder health and improve immune function during the periparturient period (Kahn *et al,* 2024, Radosits *et al*, 2007). Several factors were found to be significantly correlated with mastitis prevalence.

A strong correlation (P = 0.001) was found between poor udder hygiene and higher CMT positivity rates (Figure 5). 53.1% of cows had lightly soiled udders, while 19.6% were moderately dirty, indicating suboptimal hygiene practices in most farms. This underscores the need for improved cleaning protocols and farm sanitation to reduce bacterial load (Bitew *et al,* 2024, Khan *et al,* 2024, Mohammed *et al,* 2019).

Holstein and Jersey cows had the highest mastitis prevalence, whereas Simmental cows were more resistant (Figure 2). This suggests that genetic selection for mastitis resistance could be an effective long-term strategy (Bitew *et al,* 2024, Khan *et al,* 2024, Mohammed *et al,* 2019).

Farms that practiced teat dipping after milking had lower mastitis prevalence, although the effect was not statistically significant (P = 0.44) (Figure 3). While the significance level was low, this finding reinforces the importance of post-milking hygiene in reducing bacterial transmission. In addition, number of farms that applied rigorosely post milking teatd dipping was very low, which may affect the significance of finding. Furthermore, this finding it point out that subclinical mastitis control can be achieved by employing a full mastitis control program (Bitew *et al,* 2024, Khan *et al,* 2024, Mohammed *et al,* 2019, Quinn *et al,* 2011, Radostits *et al,* 2007).

The results highlight the significant impact of trace elements (Zn, Se) and fat-soluble vitamins (E, A, D) on subclinical mastitis in cows, primarily linked to the use of premix feed (see previous section). These micronutrients influence mastitis by modulating immune responses. Nutrition affects mastitis susceptibility through two main pathways: direct impact: nutritional deficiencies impair immune function, reducing resistance to mastitis, and indirect impact: dietary imbalances cause metabolic disorders, weakening immune defenses. Deficiencies in vitamin E and selenium, especially during the dry period, reduce polymorphonuclear leukocyte (PMN) activity. Supplementation before calving enhances PMN function and bacterial clearance (Weiss et al., 1997). Recent studies (Ma et al., 2018; Wang et al.,

2018) also show selenium's anti-inflammatory effects. Combining vitamin E and selenium with antibiotic therapy reduces somatic cell counts (SCC) compared to antibiotics alone (Mukherjee, 2008). Vitamin A and calcium deficiencies also increase mastitis risk (Gillund & Mork, 1998; Ganda et al., 2016). Supplementing with vitamins A, D3, E, and H aids recovery by enhancing protective gene expression. Additionally, fatty acids with vitamin D3 regulate gene adhesion and bacterial internalization, potentially aiding in Staphylococcus aureus control (Frutis-Murillo et al., 2019). This survey underscores the need to raise farmer awareness regarding the importance of premix feed in dairy cow nutrition.

Mineral and vitamin supplementation showed significant associations with lower mastitis prevalence. Higher dietary zinc levels correlated with reduced CMT scores ($P = 0.041$, $R^2 = 0.136$-0.197) (Figure 6). Selenium (Se): Strong negative correlation with mastitis prevalence ($P = 0.001$, $R^2 = 0.33$) (Figure 7). The data indicates that Vitamin E, A, and D3 showed significant inverse correlations ($P = 0.001$, $R^2 > 0.3$) (Figures 8-10). These findings highlight the role of immune-boosting nutrients in reducing mastitis risk, suggesting that balanced nutrition is essential for udder health (Khan *et al,* 2024).

The study findings indicate that effective mastitis prevention should integrate based on following aspects: Improved milking hygiene and biosecurity measures: Routine CMT testing and early detection programs: Selective breeding for mastitis-resistant cows: Enhanced farm management, including bedding sanitation and proper ventilation: Nutritional interventions, particularly Zinc, Selenium, and Vitamin E supplementation.

Limitations

The study primarily examines the short-term effects of dietary supplementation. Long-term field trials are required to examine the long-term effects of mastitis prevention on dairy cow productivity. Housing conditions, hygiene, and milking practices vary amongst farms, which may influence findings. A more controlled study design could assist distinguish the impacts of diet from other management aspects. The study does not thoroughly investigate whether different dairy breeds react differently to nutritional interventions. Genetic factors can alter immune response and mastitis susceptibility. While the study emphasises the benefits of supplementing, it does not include the cost-effectiveness for dairy farmers. The financial viability of large-scale deployment is questionable.

CONCLUSION

This study highlights the high prevalence of subclinical mastitis in some districts and identifies key risk factors contributing to infection. The findings emphasize the need for region-specific intervention strategies, including hygiene improvements, genetic selection, and proper nutrition, to reduce the disease burden and improve milk quality.

The study highlights the importance of nutritional strategies in reducing per subclinical mastitis by improving immune function, reducing oxidative stress, and promoting udder health. The supplementation of trace minerals, vitamins, and amino acids is a promising approach for enhancing dairy cow productivity and preventing infections. Further research is needed to optimize dietary formulations for mastitis prevention. The findings emphasize a dual approach: improving milking hygiene and ensuring adequate mineral-vitamin nutrition. Proper post-milking disinfection, combined with targeted supplementation of Se, Zn, and Vitamins A, D, and E, is crucial for reducing subclinical mastitis and enhancing dairy herd health.

Further research is needed to evaluate the long-term impact of these interventions and explore additional risk factors affecting mastitis prevalence.

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