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Comprehensive Protocol for Assessing Risk Factors of Subclinical Mastitis in Albanian Dairy Farms: A Practical Approach

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Abstract

Subclinical mastitis (SCM) presents a significant challenge in Albanian dairy farming due to its asymptomatic nature, which impedes early detection and prevention. Environmental factors such as nutrition, hygiene, milking and housing remain insufficiently studied, and despite high prevalence, standardized detection and prevention protocols are lacking. This study adapted and evaluated a practical protocol for assessing SCM risk factors across nine Holstein dairy farms in Albania. The protocol included: (i) collection of farm and husbandry data; (ii) assessment of feeding status; (iii) establishment of a testing group of cows; (iv) administration of the California Mastitis Test (CMT); (v) evaluation of udder hygiene and mastitis management; (vi) milk sampling for pathogen and quality analyses; and (vii) data analysis with formulation of practical recommendations. The adapted protocol was evaluated under real production conditions on nine Holstein dairy farms, aiming to identify biotic and abiotic risk factors for SCM. On each farm, 6 to 11 cows in lactation years 1 to 5 were randomly selected. Herd sizes ranged from 21 to 140 animals, with daily milk yields between 12 and 30 kg. The average prevalence of SCM was 43.2% (range 6–83%). Significant associations were found between SCM prevalence and mineral and vitamin intake—particularly calcium, phosphorus, zinc, selenium, and vitamins E, A, and D. Additionally, udder hygiene, teat infection status, and post-milking udder disinfection were significantly correlated with SCM prevalence ($p = 0.01$). SCM prevalence was also significantly influenced by housing and milking systems ($p = 0.001$). Significant regression was observed between lactose content, conductivity, SNF content, protein, and milk density to SCM prevalence (R^2 = 0.26 - 0.41).These findings demonstrate that the adapted protocol enables comprehensive identification of both biotic and abiotic risk factors and is appropriate for practical application on Albanian dairy farms, provided farmers receive basic training.

Keywords: subclinical mastitis; dairy cows; biotic and abiotic factors; working protocol

1. Introduction

Mastitis remains a major health and economic burden in Albanian dairy farming, with subclinical mastitis (SCM) being particularly problematic due to its asymptomatic nature. The absence of overt clinical signs often delays detection, enabling disease progression and facilitating pathogen transmission within herds. The etiology of mastitis is multifactorial, shaped by interactions among environmental factors, microbial agents, and the immune status of the host (Mecaj et al., 2023; Algharib SA et al., 2024).

Research in Albania is more concentrated on contagious pathogens, while environmental contributors—such as feeding and housing conditions, and hygiene practices— have been neglected, despite their critical role in mastitis occurrence. Improper antibiotic use, often uninformed by diagnostics such as SCC or antibiograms, further exacerbates antimicrobial resistance—an issue also observed regionally, such as in Kosovo, where most Staphylococcus isolates showed multidrug resistance (Mehmeti et al., 2016; Puvača et al., 2021).

Despite the high prevalence of SCM in dairy farms, Albania still lacks standardized and comprehensive

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protocols for the detection, monitoring, and prevention of mastitis—particularly cases of environmentally induced SCM (Mecaj et al., 2023). In contrast, other countries implement structured mastitis management programs involving regular herd assessments, housing, as well as milking hygiene evaluation and hygiene management (EFSA, 2009; Bouchoucha et al., 2024; Kamphues et al., 2024; LKV Kur(h)ier, 2023). The National Mastitis Council (2025) and EFSA (2009) offer guidelines emphasizing early detection, prevention, hygiene, and proper milking techniques to maintain udder health.

In Albanian dairy farm practice, interventions are typically reactive, caused usually by visible symptoms or reduced milk production. Farm-level documentation of animal health, feeding, and milk quality is frequently inadequate or absent, thereby obliging veterinarians and animal production advisers to rely on unreliable observations or insufficiently verified data. Due to a weak national milk quality assurance system, monitoring of somatic cell count (SCC) is infrequent and remains unlinked to milk pricing, which fails to encourage the use of routine test such as the California Mastitis Test or pathogen-specific analyses. Consequently, antibiotic treatments are largely empirical, increasing costs and resistance risks.

Mastitis is neither covered by Albania's official veterinary surveillance nor monitoring plan. Control efforts focus on treating acute cases, while preventive measures and pathogen-specific diagnostics are seldom applied. The national veterinary service is also deficient in a formalized legal framework governing udder health management. Although mastitis is not designated as a notifiable disease under the EU Animal Health Regulation (Regulation 2016/429), the regulation mandates national surveillance measures (Articles 24–25), biosecurity protocols (Articles 10–13), and responsible antibiotic usage (Regulation (EU) 2019/6), thereby providing a regulatory framework that Albania has yet to implement.

The aim of the present study is to adapt a practice-relevant methodological model as an working protocol for professionals and trained farmers, enabling an integrated assessment of the biotic and abiotic factors contributing to the occurrence of subclinical mastitis in dairy farms in Albania.

2. Material and Methods

This model quasi-protocol integrates a range of practical tools, including questionnaires for both quantitative and qualitative data collection, direct testing on dairy farms and animals, and rapid analytical methods for milk and feed samples. Additionally, professional evaluations from veterinarians or animal production experts are conducted, with findings communicated to farmers in a simple practical format.

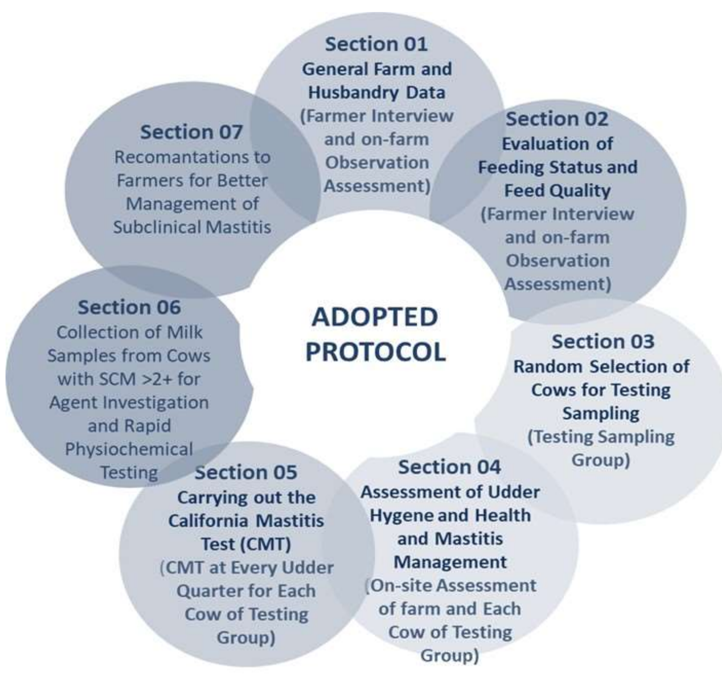

Figure 1: The conceptual structure or key sections of the adopted protocol

Section 1) Collection of general farm data and husbandry data, by an interview of the farmer as well as on-farm observation/assessment carried out by the researcher/expert.
Section 2) On-farm evaluation of feeding status and feed quality were conducted through a farmer interview and on-site observation/assessment by the researcher/expert. Moreover, feed samples from the farm were analyzed using the NIRS (Near-Infrared Reflectance Spectroscopy) method.
Section 3) For each farm, a testing/sampling group was established through random selection. In dairy farms with up to 100 cows, at least six cows (2–3 cows per lactation stage) were randomly selected for testing.
Section 4) Assessment of udder hygiene, udder health, and mastitis management were conducted through an on-site evaluation of the farm and each cow included in the Testing Group.
Section 5) The California Mastitis Test were performed on all four udder quarters of each cow included in the Testing Group.
Section 6) Milk samples were collected from cows with subclinical mastitis scores greater than 2+ for pathogen identification and rapid physicochemical testing.
Section 7) Analysis of the results and provision of recommendations to the farmers. Targeted guidance was offered to improve the management of subclinical mastitis.

3. Results and Discussion-

Implementation of the adopted Protocol

As the study focuses on the adaptation of a protocol, the results fundamentally involves its implementation or testing under the usual conditions prevailing in dairy cattle farms across the country.

3.1. Farms

The adopted protocol was evaluated through a study conducted on nine Holstein dairy farms in Albania, representing three regions (Durrës, Shkodra, and Korça). Data collection and testing occurred over an 8-month lactation period, extending through to the onset of the dry period. On each farm, 6 to 11 cows from lactations 1 to 3 were randomly selected for the administration of the California Mastitis Test (CMT) and milk sampling. The following average productivity parameters were recorded: herd sizes ranged from 21 to 140 cows, daily milk production varied between 12 and 30 kg, and the average days in lactation were: 32–83 d for the first testing, 146–213 d for the second, and 241–300 d for the third testing. No modifications were made to the housing, hygiene, or feeding practices of the investigated farms, although these variables were documented.

3.2. Description of the implementation of different sections of adopted protocol.

3.2.1. Section 1. Collection of general farm data and husbandry data

In this section of the protocol, data were collected through structured interviews with the farmer, along with on-farm observations and assessments carried out by the researcher. The data include the following parameters:

a) Location, region and district; type of farm (e.g., dairy, mixed-species), animal species, and farm size (measured by the number of animals).

b) Zootechnical characteristics: animal breed, lactation year and stage, production performance, reproductive performance.

c) Economic and cost-related data: production costs, revenue and profitability data.

d) Husbandry system: type of management system (intensive, extensive, or semi-intensive).

e) Housing and hygiene Conditions: housing type (e.g., tethered or free), floor type and bedding material, hygiene status of housing facilities.

f) Milking techniques and equipment: milking techniques aplied, status and maintenance of milking equipment and facilities.

g) Herd health status: frequency of main animal diseases, types of common disorders affecting the herd.

The objective to maintain mastitis at a manageable level in dairy cattle requires a limited exposure of the animals to the pathogens causing the infection (Meçaj et al., 2023). Location of the farm, climatic conditions, and season (summer mastitis) are directly related to mastitis susceptibility in cattle (Hogan & Smith, 2012; Ishiyama et al., 2017). Intensity of management, hygienic conditions of the stable, floor type, and bedding (composted recycled manure, fresh recycled manure, etc.) are related to mastitis occurrence (Cole and Hogan, 2016). Milking technique (mechanical or manual), maintenance of milking facilities/equipments, regular cleaning, sanitizing, and cleaning the hands of milking staff are crucial to reduce mastitis prevalence (Zigo et al., 2021).

The present findings illustrate the influence of housing conditions and milking systems on the prevalence of SCM in dairy herds. A statistically significant effect of the housing type was observed, with free-stall systems demonstrating clear advantages over tie-stall housing in terms of reducing SCM prevalence. Similarly, the choice of milking techniques showed a significant impact: herds milked in milking station showed a lower prevalence of SCM compared to those milked using mobile milking equipment. Overall, subclinical mastitis, along with lameness, emerged as one of the most prevalent health disorders within the studied herds, underscoring the need for improved housing and milking management practices.

3.2.2. Section 2: On-farm evaluation of feeding status and feed quality

The on-farm evaluation of feeding status and feed quality was conducted through farmer interviews and on-site observations/assessments by the researcher/expert. Additionally, feed samples from the farm were analyzed using the Near-Infrared Reflectance Spectroscopy (NIRS) method. Specifically, the information in this section includes data on the following parameters:

a) On-site organoleptic assessment of feed quality according DLG (2004).

b) Analysis of daily ration components on the testing day and classification of feed rations throughout the year.

c) On-site evaluation of feeding parameters, including structural fiber content (Penn State Particle Separator Test) according Kononoff et al. (2003) and Body Condition Score (BCS) assessment according Edmonson et al. (1989).

d) Collection and laboratory analysis of feed samples using Near-Infrared Reflectance Spectroscopy (NIRS).

e) Assessment of nutrient supply and ration optimization according GfE (2023) and NRC (2001).

f) Examination of feeding strategies during the lactation and dry periods.

g) Evaluation of feeding processing methods and techniques.

Nutrition, particularly the provision of essential minerals and vitamins, is pivotal in the immune defense against mastitis (Smith et al., 1984). This influence is exercised both directly, through specific nutrient deficiencies, and indirectly, via the dietary disbalances and resulting metabolic disorders—for example, subacute rumen acidosis due to a lack of structural fiber or forage particles (Kononoff et al., 2003) that stimulate rumination, or severe deviations in body condition score (BCS) (Edmonson et al., 1989) caused by suboptimal energy balance during early lactation stage and the transition period (GfE, 2023)—which impair immune function. Key immune mechanisms affected by nutrition include the rapid elimination of pathogens by phagocytes, the secretion of anti-inflammatory cytokines, and the migration of polymorphonuclear neutrophils (PMNs) to the infected udder quarter (Erskine, 1993).

Nutritional imbalances, such as excess or deficiency in energy, acidosis, alkalosis, and a lack of antioxidants, have been shown to increase the susceptibility to mastitis (Erskine, 1993). Additionally, feeding-related disorders, such as diarrhea-induced bacterial contamination, exacerbate the risk of infection, particularly from *Escherichia coli*. Metabolic diseases, including ketosis and fatty liver syndrome, compromise immune defense during the dry period and peri-calving, significantly raising the likelihood of mastitis (O'Rourke, 2009). Deficiencies in minerals diminish immune cell activity and predispose to the onset of mastitis (Libera et al., 2021). Deficiencies in vitamin E and selenium are associated with reduced PMN activity, whereas supplementation with these micronutrients prior to calving has been shown to improve bacterial clearance (Weiss et al., 1997). Selenium also exerts anti-inflammatory effects through the modulation of inflammatory mediators (Wang et al., 2018). The concurrent supplementation of vitamin E and selenium in cows undergoing antibiotic treatment is linked to improved immune responses, reflected in a reduction of somatic cell counts (Mukherjee, 2008). Furthermore, deficiencies in vitamin A and calcium have been correlated with heightened mastitis susceptibility (Ganda et al., 2016).

The research on the impact of vitamins and trace elements on immune function, particularly in relation to mastitis, gained momentum in the 1980s, establishing vitamins A and E as essential for immune efficacy (Chew et al., 1982; Smith et al., 1984). These findings prompted significant revisions in nutritional guidelines, which influenced subsequent NRC recommendations (NRC, 2001; NRC 2005) regarding vitamins and trace elements (Hogan et al., 1992; Weiss et al., 1997). In addition, the GfE (2023) recently reassessed the nutritional requirements for trace elements and vitamins in dairy cows, leading to an increase in the recommended levels.

The evaluation of feed quality across the studied farms included the use of dried alfalfa and/or hay as roughage of moderate quality, with varying botanical compositions and nutrient content determined by NIRS. Maize silage represented the main roughage feed, except during short periods of insufficient supplies in two of studied farms. The maize silage used in the dairy farms examined in this study was of moderate quality, characterized by a dry matter content of 30–33% and frequently a suboptimal grain fraction, influenced by the timing of harvest and the effectiveness of the ensiling process. Energy-rich concentrates included mainly maiz corn, with wheat and barley comprising smaller portions, mostly purchased commercially, though a few farms produced their own grain. Soybean meal, the primary protein concentrate, was used on all farms, also sourced commercially. Nine from 27 rations analysed contained alaso a performance-enhancing dairy concentrate, also commercially procured.

The assessment of feeding levels showed that farms use an average of 40% concentrates in the ration, ranging from 22% to 50%, particularly on farms with a milk yield of 25–30 kg/day. Of the 27 rations analyzed, 21 included a mineral-vitamin premix from Schaumann Austria Premix, with an average intake of 140 g/day, a and a maximum of 200 g/day. The analysis confirmed that the premix is the primary source of trace elements and vitamins, while roughage and concentrates meet macroelement needs. Mineral and vitamin balancing showed the greatest variability. Despite this, the rations generally meet energy and protein requirements, with over half exceeding the needs for milk production up to 25 kg/day, linked to the high proportion of concentrates. Variance analysis showed a significant ($p \leq 0.05$) impact of mineral intake levels (Ca, Zn, Se, Vitamins E, A, and D per kg DM) on the prevalence of subclinical mastitis in this study.

3.2.3 Section 3: Establishment of the Testing Cow Group

For each farm include in the study, a representative sampling cohort is established through randomized selection. Within farms housing up to 100 lactating cows enrolled in this study, a minimum of six animals—generally two to three from each lactation stage—were randomly selected for sampling and subsequent analysis, or in a total of six to eleven cows per farm.

For each cow included in the testing group, production-related parameters were recorded, including breed, lactation number, daily milk yield, number of days in lactation, and the interval between calving and successful insemination.

The recorded data involved the following characteristics: lactation year ranged from one to five (mean 2,18 lactations); days in lactation were between 32 and 83 d at the first testing, 146 to 213 d at the second, and 241 to 300 d at the third one. Daily milk yield during the investigation period varied between 12 and 30 kilograms.

3.2.4 Section 4: Assessment of Udder Hygiene, Health, and Mastitis Management

Udder hygiene, udder health status, and mastitis control practices were evaluated through systematic on-site assessments. This evaluation was conducted at each studied farm and involved both the farm conditions and individual animals included in the testing group.

This section provides a detailed overview of the following items:

a) Mastitis history, prevention, and management practices applied, including previous prevalence of mastitis; frequency of mastitis control, and standard mastitis treatment protocols.

b) Milking hygiene, assessed based on cleanliness of the milking station and animals prior to milking, techniques used for udder cleaning before milking, disinfection practices employed before and after milking, and condition and maintenance of milking equipment and machines.

c) Udder cleanliness, categorized as: *clean* - no visible contamination, *slightly contaminated* - 2-10% of the udder (external) surface is unclean; *moderately contaminated* -10–30% of udder surface is unclean, and *highly contaminated* - more than 30% of udder surface is unclean (LKV Kur(h)ier, 2023).

d) Teat health condition, evaluated through: formation of rings and signs of hyperkeratosis or edema (≤ 20% of teats), hemorrhagic lesions (< 1%

of teats), redness, bruising, or swelling around the teats, palpable or visible hardening around the teat canal, and chronic hyperkeratosis indicating prolonged stress or irritation (LKV Kur(h)ier, 2023).

Udder hygiene is crucial for mastitis control; to achieve it from the moment the animal enters the milking parlor, a workflow must be applied in each step. Starting with washing and drying the teat with an individual paper towel, fore-stripping the first streams, applying pre-dipping to the teats, and post-dipping them with antiseptic solution (Bradley et al., 2018; Zigo et al., 2021). Several key findings emerged in relation to factors influencing the prevalence of SCM. Udder cleanliness and teat health status were significantly associated with the occurrence of SCM ($p = 0.01$ and $p = 0.001$, respectively), indicating that hygiene-related variables play a crucial role in mastitis dynamics. Furthermore, the implementation of post-milking teat disinfection was shown a highly significant effect in reducing SCM prevalence ($p = 0.001$), underscoring its importance as a preventative management practice.

3.2.5 Section 5: Administration of the California Mastitis Test (CMT)

The early identification of subclinically infected cows is crucial for effective mastitis control. Subclinical mastitis, which lacks visible signs or abnormal milk, leads to increased somatic cell counts. CMT is carrying out according José et al. (2018) and is a cost-effective, simple, and rapid method for detecting such infections without the need for laboratory analysis.

Equipment: Milk samples were collected hygienically in a clean CMT dish, with four shallow cups labeled A, B, C, and D for each quarter of the udder.

Procedure: Approximately 2 ml of milk was taken from each quarter, and an equal amount of CMT reagent was added. The plate was rotated for up to 10 seconds to mix the contents. The results were read within 20 seconds, as the reaction dissipates over time. The formation of gel was visually assessed: greater gel formation corresponds to a higher result.

Following table (Table 1) presents the evaluation criteria for the presence of subclinical mastitis in dairy cattle.

Table 1. Criteria used to assess the subclinical health status of tested cows

Score	Likely somatic cell count (SCC) range	Animal health status	Description
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

CMT has some advantages and disadvantages. As advantages can be mentioned: high sensitivity, low cost, and ease of use; requires minimal equipment and maintenance. And as disadvantages can be mentioned: results may vary based on technique; provides estimates, not exact counts; and may yield false positives in fresh cows or those nearing dry-off. Rare cases of acute mastitis may not test positive if somatic cells are destroyed.

Generally, the CMT is a reliable tool for detecting subclinical mastitis, although proper technique and timing are crucial for accurate results. However, in dairy farm practice in Albania, this simple test is still not used routinely. Unfortunately, even practicing veterinarians who support or advise dairy farms rarely use the CMT test.

The prevalence of subclinical mastitis was assessed based on the proportion of cows exhibiting a CMT score of ≥1+, relative to the total number of animals examined (9 cow farms; n = 178; 54-63 cows or 216 – 252 udder quarters were tested in each lactation period; a total of 712 udder quarter's CMT-s carried out). Across the study population, the mean prevalence of SCM was 43.2%. However, the distribution of values revealed substantial variability, with prevalence rates ranging from a minimum of 6% to a maximum of 83.3%. The interquartile range further highlighted this variation, with the first quartile (Q1) at 21.1%, the median (Q2) at 33.3%, and the third quartile (Q3) at 66.7%.

3.2.6 Section 6: The collection of milk samples for the identification of causes and the quality of the milk

Milk sampling for pathogen identification: In cows presenting CMT scores of ≥2+, 5–10 ml of milk was aseptically collected from each affected quarter into sterile containers. These samples were submitted to the Albanian Institute of Food Safety and Veterinary laboratory for microbiological analysis and pathogen identification.

Milk sampling for physico-chemical analysis: After filtration and thorough mixing in the farm's bulk milk cooling tank, two sterile plastic bottles containing 100–150 ml of milk were collected. These samples were subjected to physico-chemical analysis using a rapid testing method (milk analyzer – Lactosan, Milkotronic Ltd., Bulgaria), in accordance with standard practices used by dairy processing facilities.

Based on bacteriological findings, both contagious and environmental pathogens were identified as agents of SCM in dairy cows. The predominant pathogen isolated was *Staphylococcus aureus*, a major causative agent of bovine contagious mastitis. *Staphylococcus aureus* was detected in 48.6% of farms, while the majority of other bacterial isolates were of environmental origin (43.8%).

Using data from tests of all udder quarters (712 udder quarter tests), the significance and correlation of SCM on changes in the physicochemical properties of milk were evaluated through Robust Compound Regression (RCR). A higher prevalence of SCM was associated with a significant increase in milk conductivity, as well as notable decreases in milk lactose, protein, solid non-fat (SNF) content, and density. The prevalence of both mild and severe SCM exhibited seasonal and lactational stage-dependent variations ($p \leq 0.05$). Significant regression was observed between lactation year, lactose content, conductivity, SNF content, protein, and milk density to SCM prevalence ($R^2 = 0.28$; $R^2 = 0.41$; $R^2 = 0.26$; $R^2 = 0.36$; $R^2 = 0.39$). These findings suggest that physicochemical milk parameters, routinely measured in Albanian milk companies/dairies using rapid methods, can serve as reliable early-warning indicators for dairy farmers to detect potential cases of SCM.

3.2.7 Section 7: Analysis of the results and provision of recommendations to the farmers

The results obtained from all sections of the protocol were analyzed using the standard Excel software package and interpreted by veterinary and zootechnical professionals. Based on the analysis of these results, the veterinary or animal production professionals provide the farmer with a comprehensive and clear explanation of the status of SCM on the farm, identify potential risk factors, and offer recommendations for improving farm management practices. These recommendations aim to prevent further spread of SCM in cow herd, facilitate the recovery of infected cows, and halt its progression to clinical mastitis.

In light of the identified risk factors and the predominance of specific mastitis-causing pathogens, a comprehensive set of recommendations was developed and carried to farms included in this study with the aim of improving the control and management of SCM. These recommendations included several critical domains: optimizing nutritional management during both lactation and the dry period; improving environmental hygiene and housing conditions; ensuring adherence to proper milking hygiene and technique, including the regular servicing and sanitation of milking equipment/facilities; and implementing appropriate veterinary interventions, encompassing both preventive measures against SCM and therapeutic protocols for clinical mastitis case

4. Conclusions and Implications

Subclinical mastitis represents a substantial economic burden on dairy farming operations in Albania, posing challenges to both milk production and overall herd health. This study aimed to rigorously validate a practical model specifically developed to assess the multifactorial etiology of SCM, acknowledging the complex interactions between various risk factors. The adopted model offers an integrated approach that considers both biotic factors, such as pathogen prevalence and microbial resistance patterns, and abiotic factors, including environmental conditions, farm management and feeding practices.

The methodology adopted in this study emphasizes a holistic view of SCM, enabling a better understanding of the interactions between these diverse factors related to SCM. Results from the testing process indicate that the model is not only feasible for implementation but also highly relevant and adaptable to the specific conditions of Albanian dairy farms.

Furthermore, the protocol has been shown to be practically applicable under the usual farm conditions, providing a valuable management tool that can be utilized effectively by veterinarians, zootechnicians, and trained farmers. This model facilitates a more targeted approach to SCM management, potentially leading to improved herd health and milk quality of Albanian dairy farms.

Given the current absence of a comprehensive and legally recognized protocol for subclinical mastitis management in Albania, the protocol adapted in this study may contribute to the development of a standardized guideline for subclinical mastitis management. Experience from the study clearly demonstrates that successful implementation requires thorough training of dairy farmers.

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