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Subclinical Mastitis in Dairy Cows in Western Algeria: Prevalence and Its Impact on Milk Composition

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ABSTRACT

Background: Mastitis is one of the most common diseases in dairy cows. It significantly reduces milk quality and yield, thus incurring economic losses for farmers. This study investigates the prevalence of subclinical mastitis (SCM) and its effects on reproductive performance in dairy cows in western Algeria.

Methods: From 620 clinically healthy cows from 22 herds were examined in the study. All cows were tested with the california mastitis test (CMT). Milk samples positive for subclinical mastitis were subjected to bacteriological analysis.

Result: The present study revealed a prevalence of subclinical mastitis of 39.51%, (245 of 620). Microbiological analysis showed that, of the 245 analyzed milk samples, bacteria were present in 218 (88.97%), whereas 11.03% Bacteria-free samples. The isolated bacteria belonged mainly to non-aureus Staphylococci (NAS) (51.12%), followed by *Staphylococcus aureus* (24.68%), *Streptococcus* spp. (16.05%) and *Escherichia coli* (8.15%). Subclinical mastitis did not appear to significantly affect the main components of milk, namely lactose content, solid nonfat, freezing point depression (FPD) and acidity. Although pH, solid nonfat, fat content and protein content remained significantly ($p<0.05$) affected by subclinical mastitis. The results of this study highlight the importance of mastitis control programs in dairy.

Key words: Dairy cow, Lactose content, Subclinical mastitis.

INTRODUCTION

Milk production is one of the most important industries all over the world; the dairy sector is a leader in the agricultural economy of Algeria. The dairy sector takes on a strategic character given its impact on agricultural development and its socio-economic significance. It is classified among the priority sectors because it generates a strategic product, which is milk. Cow's milk and its derivatives are the most important sources of essential nutrients for a human, which is why the dairy industry continues to consolidate into larger farms. The increase in milk production levels and the improvement of the hygienic quality of the milk produced are, among other things, major challenges that confront dairy cow farms, however, dairy industry has been facing challenges, dealing with demands of accountability for animal welfare and product safety. In this sense identifying diseases is key to recognizing the multifactorial nature of almost all diseases of importance in dairy cattle and redefining them more broadly, to include subclinical conditions, such as bovine mastitis (Petersson-Wolfe *et al.,* 2018; Nguyen *et al.,* 2022; Safak *et al.,* 2023).

Bovine mastitis, defined as the inflammation of the mammary gland, is one of the most important diseases within dairy herds worldwide, due to its financial impact, causing huge losses not only reflected in decreased production but also in culling rates (Hogeveen *et al.,* 2019; Azooz *et al.,* 2020). Bovine mastitis is mainly classified according to the manifestation of signs either as clinical mastitis (CM) or as sub-clinical mastitis (SCM) (Ndahetuye *et al.,* 2019).

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Although SCM does not present signs of local or systemic inflammation, it does cause increased somatic cell count (SCC) in response to bacterial infections (Gonçalves *et al.,* 2018). Although clinical mastitis is easy to detect based on its visible symptoms, the sub-clinical form is more challenging as it does not present any visual changes in the udder (Ebrahimie *et al.,* 2018). It is not easily detected without diagnostic tests such as the california mastitis test (CMT) or somatic cell counts (Reza *et al.,* 2011). On the one hand, this infection can remain untreated for long periods, leading to chronic losses in milk yield and a significant effect on the quality of milk and its technological value, becoming unsuitable for human consumption and further processing (Gonçalves *et al.,* 2018; Cheng and Han,

2020; Gonçalves *et al.,* 2021). On the other, heavy economic losses in conventional dairy farms occur as a result of subclinical mastitis (Ndahetuye *et al.,* 2019).

For milk producers, modifications of the raw milk's composition are crucial (Li *et al.,* 2014). The composition and some chemical properties of milk are among the criteria used to monitor milk quality. Milk production and composition can be affected by many genetic and non-genetic factors (Pandey *et al.,* 2021). However, the most important factor affecting milk production and composition is the development of sub-clinical mastitis. Many pathogens can induce bovine mastitis. According to several studies, the pathogens most frequently present in mastitis cases are *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Escherichia coli* and *Klebsiella pneumonia*. However, different pathogens elicit different immune responses in the mammary gland. Therefore, identification of pathogens is crucial to fully understanding changes in milk. The presence of pathogens such as *E. coli, Staph. aureus* and *Strep. uberis* cause spoilage of dairy products and causes characteristic changes in the milk. In the same context, subclinical mastitis have different effects on milk composition as do different mastitis-causing pathogens (Boas *et al.,* 2017). It is essential to determine the prevalence of SCM to minimize the economic losses and to promote safe dairy production in Algeria. The objective of this study to determine the prevalence of SCM, pathogen-specific cases of subclinical mastitis and its impact on milk composition in dairy cows in western Algeria.

MATERIALS AND METHODS

Study area and cows

The current study was conducted in 22 commercial dairy farms in the northwest of Algeria between January 2023 and December 2024. That contained 620 lactating multiparous Holstein cows (Between parities two and three). The western zone of Algeria is one of the most important dairy basins in the country. The climate is typically Mediterranean with two alternating seasons: A cool, wet season in winter and a hot, dry season in summer, with high temperatures during the summer with maximum values sometimes exceeding 45°C during the day. The enrolled cows milked twice per day and with an average daily milk production of 15-25 kg per cow. The cows were housed in a free stall barn. In all herds, cows were fed a total mixed ration composed of corn silage, grain concentrate and minerals. Water was available ad libitum.

Screening for mastitis

At the beginning of the experiment, all the cows were subjected to clinical examinations. Cows with clinical mastitis were initially identified and cows with a previous history of clinical mastitis or with clinical mastitis during the experiment were eliminated from the study. The california mastitis test (CMT) was applied to the cows, as described by Ruegg and Reinemann (2002), for screening cows with SCM. The milk sample was observed for changes in color, odor and consistency. Samples with clots, flakes, blood and other consistency changes in the milk or from the udder (indicators for clinical mastitis) were rejected.

All CMT scores of 0 and 1 were considered negative, whereas cows having a score of 2 or more for at least one quarter with a visibly normal udder and normal milk were categorized as having SCM. All negative samples (CMT scores of 0 and 1) were discarded and positive samples were used for bacteriological isolation.

Collection and bacteriological examination of milk samples

All microbiological analysis was performed at the Department of biology, Faculty of Sciences and Technology, University of Ain Témouchent, Algeria. According to the National Mastitis Council (NMC, 2017), quarters with CMT≥2 were sampled for bacteriological tests for culture and identification of SCM causal agents. In order to isolate and identify the bacteria, the CMT-positive samples underwent further cultivation. During the evening milking, a milk sample (40 mL) was aseptically collected from each cow. Briefly, teat ends were cleaned externally with commercial premilking disinfectants by the veterinarian, dried with individual towelsand then cleaned again with alcohol. After discarding the first streams of foremilk, approximately 10 mL of milk from each quarter (quarters with CMT≥2) was collected in sterile tubes, pooled, stored at 4°C and cultured within 24 h of collection at the Department of biology, Faculty of Sciences and Technology, University of Ain Témouchent, Algeria.

The study's goal was to separate and identify the three main genera of bacteria that cause mastitis that have been discovered in Algeria: *Staphylococcus* species, *Streptococcus* species and *E. coli*. Every milk sample was cultivated and inoculated onto blood agar that had been supplemented with 5% bovine blood. In order to identify the growth of pathogens and the hemolytic characteristics of the colonies, infected plates were then aerobically incubated for 24 to 48 hours at 37°C.

The colony size, shape, color, pigmentation, hemolytic characteristic, Grams staining and Oxidase test were used to isolate and identify the bacteria that cause mastitis. These colonies were subsequently subcultured on a variety of media, such as MacConkey agar, Manitol salt agar, Eosin methylene blue medium (EMB), *etc.*, in order to produce a pure culture. Furthermore, secondary biochemical tests were performed to identify the species of bacteria, including coagulase, urease, indole, citrate and sugar assays. A final bacteriological diagnosis was made using the results of biochemical testing, colony morphology and gram staining technique.

Milk composition analysis

Immediately after the collection of the milk sample for bacteriological analysis, approximately 100 mL of milk was manually collected from each CMT-positive and

CMT- negative quarters. The latter sample was dedicated for the analysis of milk composition. The milk samples were transported immediately after collection to the laboratory and stored overnight at 4°C for further analysis. The milk samples were pooled for determination of milk composition by near infrared spectrophotometer Milko-Scan (Foss Electric, Denmark) within 24 h after collection.

Data analysis

A database was prepared, including the information recorded, Statistical analysis were performed using SPSS 22 for Windows software (IBM, USA). The prevalence of SCM on cow level was calculated as the number of mastitis-positive cows (with one or more quarters with SCM) divided by the total number of cows tested. The normality distributions of variables were tested Kolmogorov-Smirnov test. In all analysis a confidence level with 95% and values of $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Milk composition

Descriptive statistics for the investigated traits of milk composition are reported in Table 1. The average fat content in milk samples was 3.73±0.94 percentage and protein, solid-nonfat and lactose contents were 3.12±1.51%, 8.33 ±2.27% and 4.63±2.5%, respectively, while average density for the study period was estimated at 1.0301±0.0018 g/cm^3. Table 1 also shows the average Freezing point depression (°C) were estimated at -0.487±0.056, while the average the PH and acidity was estimated at 6.72±0.08 and 17.32±0.78 respectively. The average values obtained for the major milk components were in line with previous data (Bousbia *et al.,* 2018; Bobbo *et al.,* 2019; Bouamra *et al.,* 2019).

Prevalence of SCM and associated pathogens

Initial screening using the CMT identified 39.51% (245/620) of the cows as positive for SCM (CMT score ≥2). Similar to studies conducted in other region of Algeria, the present study revealed that the high prevalence of SCM at cow level was 39.51% in the study area. The current finding was lower than 45% previously reported in Tizi-Ouzou, central region of Algeria (Bentayeb *et al.,* 2023) but higher than the 37.66% (Zaatout *et al.,* 2019) in Eastern Algeria and the 34.9% in western regions of Algeria (Bouamra *et al.,* 2024). The increased incidence of subclinical mastitis in dairy livestock could be due to a lack of implementation of regular mastitis prevention and/or control strategies other than treating clinical cases.

Table 1: Descriptive statistics of milk composition in milk in samples (n = 620).

Trait	Means ± SEM
Fat content (%)	3.73±0.94
Protein content (%)	3.12±0.15
Lactose content (%)	4.63±0.25
Freezing point depression (°C)	-0.487±0.056
Acidity	17.32±0.78
Density (g/L)	1031.01±1.85
Solid-nonfat (%)	8.33±0.27
PH	6.72±0.08

Moreover, our findings were similar to those of others studies abroad that evaluated the prevalence of SCM. For instance, the prevalence of SCM was 36.7% in Poland (Sztachañska *et al.* 2016), but higher than the 27.3% (Ranasinghe *et al.,* 2021) in major milk-producing areas of Sri Lanka. The observed variations in mastitis prevalence between studies may suggest a complex nature of the illness, involving interactions with several factors such environmental factors, veterinary service coverage, intramammary infusion medication deficiencyand causative factors.

Milk sample from each cow with SCM was subjected to microbiological analysis. Microbiological analysis showed that, of the 245 analyzed milk samples, bacteria were present in 218 (88.97%), whereas 11.03% Bacteria-free samples. The isolated bacteria belonged mainly to *non-aureus staphylococci* (NAS) (51.12%), followed by *Staphylococcus aureus* (24.68%), *Streptococcus spp.* (16.05%) and *Escherichia coli* (8.15%). The isolated bacteria comprise NSA, *Staphylococcus aureus*, *Streptococcus spp* and *Escherichia coli*, which are typically isolated from dairy cow milk (Kawecka-Grochocka *et al.,* 2021; Bentayeb *et al.,* 2023; Bouamra *et al.,* 2024).

Our results agree with those of Romano *et al.* (2023) and Bentayeb *et al.* (2023), which indicate NAS to be the most prevalent species present in the udders of cattle with subclinical mastitis. The isolation rate of NAS (51.12%) was much higher than the findings of Ndahetuye *et al.* (2019, 40.2%) and Bouamra *et al.* (2024, 25.4%) but was lower than the finding of Zaatout *et al.* (2019, 61.94%) and. The results of bacteriological analysis of the milk samples from cows showed that *S. aureus* were the second common isolated bacterial pathogen in the CMT positive milk samples (24.68%), which was lower than the findings of Ranasinghe *et al.* (2021; 86.2%) but higher than the findings of Zaatout *et al.* (2019, 5.30%) and Ndahetuye *et al.* (2019, 22%). These findings were similar to the results reported by Bentayeb *et al.* (2023). *Escherichia coli* was isolated with 8.15%, which was similar to the findings of Solanki *et al.* (2023, 6.5%) and Bouamra *et al.* (2024, 9.52%) but higher than the findings of Naidu *et al.* (2022, 24.79%). Our results demonstrate that the udder may be a habitat for bacteria related to the udder microbiota and other environmental species. There may be several contributing factors to the high of genus *Staphylococcus* prevalence found in this investigation. The NAS species are present in the teat canal and are a component of the biota of cows and other animals. These bacteria usually have a reciprocal interaction with their host and are regarded as commensal. Even though they are usually benign, there are some situations in which they might become harmful, such as when there is weakened immunity

Table 2: Effect of subclinical mastitis (SCM) on milk composition of dairy cows.

Parameters	Healthy	SCM	P-value
Fat content (%)	38.53±1.80	36.5±0.4	**
Protein content (%)	32.5±0.2	31.0±0.3	*
Lactose content (%)	46.95±1.53	45.36±1.35	NS
Freezing point depression (°C)	-0.489±0.026	-0.479±0.16	NS
Acidity	17.50±0.65	17.06±0.78	NS
Density (g/L)	1030.40±1.17	1029.75±1.25	NS
Solid-nonfat (%)	81.71±0.77	85.71±0.58	**
pH	6.73±0.23	6.69±0.15	*

NS = not significant; **significance at P≤0.01; *significance at P≤0.0

or extended stress. Furthermore, while milking, NAS can spread from the hands of milking staff to a cow and subsequently to the udders of other cows in the herd.

Effect of SCM on milk compositions

Due to mastitis, changes occur in the composition and chemical properties of milk. These changes significantly affect the quality of milk and products derived from milk (Safak *et al.,* 2023). The results of the association subclinical mastitis and milk composition traits are reported in Table 2. The pH of milk and its products is one important factor affecting milk quality. A higher pH value indicates mastitis in the udder and a value below 6.5 indicates milk acidification. The rise in milk pH has been attributed to leakage of blood components and extracellular fluids to the infected udder quarters during mammary gland inflammationand their mixing with the secreted milk. According to Zhao and Lacasse (2008), there is a positive correlation between the rate of increase and the intensity of inflammation. In the present study, udder infection was found to influence milk pH and solid-nonfat. In addition, subclinical mastitis had no effect on FPD, acidity and density (Table 2).

In similarity, Bentayeb *et al.* (2023) found milk from cows with subclinical mastitis also having higher pH than healthy individual's cowsand this pH tended to increase with the severity of disease. Contrary to previous studies (Zalewska *et al.,* 2024), subclinical mastitis had no detrimental effect on milk PH.

As previously reported by other authors (Safak *et al.,* 2023), our results indicate a significantly differences ($p<0.05$) between the analyzed groups with regard to milk fat content and protein content. Although many previous studies have found that subclinical, mastitis causes changes in milk content (Bentayeb *et al.,* 2023; Zalewska *et al.,* 2024).

Mastitis is known to lower the fat, lactose and casein contents of milk (Antanaitis *et al.,* 2021) by reducing de novo synthesis of milk components caused by the inflammatory response, resulting in an incursion of blood components into milk. These components include a diversity of hydrolytic enzymes, which alter milk composition by the breakdown of protein and milk fat.

The protein content of milk determines its nutritional value, its health propertiesand its potential for processing into other foods (Barłowska *et al.,* 2011). As milk taken during mammary gland inflammation tends to have higher total protein content than milk from a healthy udder (Bobbo *et al.,* 2017). Contrary to our results, Gonçalves *et al.* (2020) reported that milk from udder quarters infected contained significantly less fat than healthy cow milk.

The concentration of lactose in milk depends on the permeability of the blood-milk barrierand any leakage to the bloodstream *via* a damaged barrier results in a fall in lactose level (Bruckmaier *et al.,* 2004). The concentration of lactose in milk depends on the permeability of the blood-milk barrierand any leakage to the bloodstream *via* a damaged barrier results in a fall in lactose level (Bruckmaier *et al.,* 2004).

Our present findings indicate that milk from healthy cows do not demonstrate higher lactose concentration compared with milk from subclinical mastitis cows. However, the lactose content is also influenced by various properties of individual cows. Although reduced milk lactose level may be a marker of subclinical mastitis (Antanaitis *et al.,* 2021).

CONCLUSION

According to the study's findings, bovine mastitis is a common condition in Algerian dairy herds. In the present study, we report associations between subclinical mastitis and several milk composition traits. Significant variations in the PH, Solid-nonfat, protein content and fat content were observed between milk samples from healthy cow and cows infected with subclinical mastitis, but subclinical mastitis had no effect on FPD, acidity and density. In addition, further studies will be required to confirm the present findings and to evaluate the effect of cases of subclinical mastitis at the quarter level (to avoid possible contamination and dilution effects) and at the individual pathogen level. Finally, the implementation of integrated approaches to prevent and control mastitis in cows is justified to enhance milk quality, reduce economic losses and mitigate public health risks.

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Conflict of interest

The authors declare that they have no conflict of interest.

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