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Effect of Subclinical Mastitis Detected in the First Month of Lactation on the Reproductive Performance of Dairy Cows in Western Algeria

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ABSTRACT

Background: The current study aimed to detect in the first month of lactation the prevalence and risk factors of subclinical mastitis (SCM) and its effects on reproductive performance in dairy cows in western Algeria.

Methods: 530 cows from 18 herds were examined in the study. California Mastitis Test (CMT) was used to screen subclinical mastitis for individual cows*and* a score of two or more for any quarter without any clinical symptoms and abnormalities in milk was considered as positive for SCM. Milk samples positive for subclinical mastitis were subjected to bacteriological analysis.

Result: The present study revealed a prevalence of subclinical mastitis of 34.90%, (185 of 530). The most common pathogen identified from CMT-positive milk samples was *Staphylococcus aureus* with 35.62%. Logistic regression analysis showed that parity, farming system and milking method affect significantly the prevalence of SCM. Subclinical mastitis during the first month of lactation affected significantly the reproductive performance by increasing calving to first service interval (CFSI), calving-to-conception interval (CCI)*and* number of services per conception (NSPC). The results of this study highlight the importance of mastitis control programs in dairy farms by showing that a high frequency of subclinical mastitis caused by *Staphylococcus aureus* may have a negative impact on the reproductive performance of dairy cows.

Key words: Dairy cow, Risk factor, Subclinical mastitis.

INTRODUCTION

Reproductive efficiency is one of the most important factors associated with dairy cows profitability and could be negatively affected by diseases such as mastitis. This latter is a serious challenge to global milk production (Ruegg, 2017; Hogeveen *et al.,* 2019; Safak *et al.,* 2023). Mastitis is defined as the inflammation of the mammary gland usually following an intramammary infection. Bovine mastitis is classified either as clinical mastitis (CM) or sub-clinical mastitis (SCM), based on the severity of the disease. Subclinical mastitis (SCM) is difficult to assess without diagnostic tests such as the California Mastitis Test (CMT) or somatic cell counts (Reza *et al.,* 2011). SCM is characterized by physical, chemical, bacteriological and cytological changes in milk*and* pathological changes in the mammary gland (Ruegg and Erskine, 2014). It's not easily detected, it can remain untreated for long periods, leading to chronic losses in milk production and fertility and becoming a major cause of economic losses in conventional dairy farms (Ndahetuye *et al.,* 2019).

The deleterious effect of SCM on fertility in conventional dairies is well known (Ruegg, 2017). Fertility is decreased in cows with intramammary infection, possibly due to hormonal changes and their effects on follicular growth (Fuenzalida *et al.,* 2015; Dolecheck *et al.,* 2019). Cows with mastitis have delayed estrus, decreased pregnancy rate at first service and augmented risk of abortion (Fuenzalida *et al.,* 2015; Wang *et al.,* 2021). Likewise, cows with mastitis showed higher number of services per conception and longer interval from calving-to-first-service interval (CFSI) or calving-to-conception interval (CCI) than that of healthy cows (Dolecheck *et al.,* 2019). In cows, mastitis causes an increase in inter estrus interval and a decrease in the luteal phase which impairs the establishment and maintenance of pregnancy and impedes embryonic development (Edelhoff *et al.,* 2020).

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Mastitis is usually caused by a variety of pathogens classified into contagious and environmental mastitis (Shaheen *et al.,* 2016), while the causative agents of SCM vary between countries and studies, the commonly isolated organisms in several studies are *Staphylococcus aureus, Streptococcus spp*., *Klebsiella spp*. and *Escherichia coli* (Rahularaj *et al.,* 2019). The reported effect of specific pathogens on reproductive performance has been inconsistent. Several studies have reported similar reductions in reproductive performance for mastitis caused by gram-positive and gram-negative pathogens (Santos *et al.,* 2004).

It is essential to determine the prevalence and potential risk factors for SCM to minimize the economic losses and to promote safe dairy production in Algeria. Further understanding of the extent of the performance reduction caused by SCM is crucial for raising awareness among dairy farmers, leading to the development and implementation of strategies to prevent and mitigate the burden of SCM in Algerians herds. Therefore, the current study was conducted with the aim of detecting in the first month of lactation the prevalence and risk factors of SCM and its effects on reproductive performance in dairy cows in western Algeria.

MATERIALS AND METHODS

Reproductive parameters

The current study was performed from October 2019 to October 2022 in18 dairy herds located in north western of Algeria. Cows were surveyed after calving to record their reproductive parameters. Herd- and management-related information was collected by interviewing the farm managers through a pretested, structured questionnaire (Table 1). Follow-up visits were conducted to complete the recording of data and to confirm the reproductive status of cows. The collected records were used to calculate the reproductive indices.

California mastitis test

Cows with clinical mastitis were initially identified and excluded from the study and the California Mastitis Test (CMT) was used 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.

Bacteriological examination of milk samples

Quarters with CMT≥2 were sampled for bacteriological analyses for culturing and identification of SCM causative agents according to the National Mastitis Council (NMC, 2017). The CMT positive samples were further cultured for the isolation and identification of bacteria. Bacteriological examination of the composite milks samples was carried out at the laboratory of the Department of biology, Faculty of Sciences and Technology, University of Ain Témouchent, Algeria. The goal of the study was to isolate and identify the three primary genera of mastitis-causing bacteria that have been found in Algeria: *E. coli,Streptococcus* spp. and *Staphylococcus spp.* All milk samples were cultured inoculated on to blood agar enriched with 5% of bovine blood. Then, inoculated plates were aerobically incubated at 37°C for 24 to 48 h to detect growth of pathogens and hemolytic characters of the colonies.

For the primary isolation and identification of mastitis causing pathogens colony size, shape, colour, pigmentation, haemolytic characteristic, Grams staining, Oxidase test were performed. To obtain a pure culture, these colonies were then subcultured on several media, including MacConkey agar, Manitol salt agar, Eosin methylene blue medium (EMB) *etc*. In addition, the secondary biochemical tests such as, coagulase test, urease test; indole, citrate tests, sugar tests, *etc*. were done for bacterial species identification. Results of Gram staining, colony morphology*and* biochemical tests were used to arrive at a final bacteriological diagnosis.

Data analysis

The prevalence of SCM on cow level was calculated as the number of mastitis-positive cows (with one or more

Table 1: Frequency distribution of explanatory variables.

Variable	Level	Frequency (%)
Parity	Primiparous	213 (40.18%)
	Multiparous	317 (59.81%)
Cow breed	Holstein	307 (57.92%)
	Montbéliarde	223 (42.05%)
Farming system	Semi-intensive	193 (36.14%)
	Intensive	337 (63.85%)
Milking method	Machine milking	224 (42.96%)
	Hand milking	306 (57.73%)
Daily milk production	<10 L	317 (59.81%)
	>10 L	213 (40.18%)

quarters with SCM) divided by the total number of cows tested. Cows with a history of reproductive problems such as retained placenta, repeat breeding, or any other systemic disease at the time of examination were excluded from the study. Microsoft Excel® 2007 (Microsoft Corporation®, Redmond, USA) was used for some of the descriptive analyses. Data obtained were summarized and entered into Microsoft excel 2007 with the result for each test recorded. Statistical analysis was conducted using SPSS 21 for Windows software (IBM, USA), with cow as the experiment unit. In all analysis a confidence level with 95% and values of $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Prevalence of SCM and associated pathogens

Initial screening using the CMT identified 34.9% (185/530) of the cows as positive for SCM (CMT score ≥2). Milk sample from each cow with CMT-positive was subjected to microbiological analysis. Microbiological analysis showed that 83.78% (155/185) of all CMT-positive cows exhibited a bacterial infection, whereas 11.9% were culture-negative and 4.32% were contaminated. Based on the number of mastitis pathogens isolations, the most frequent pathogens was *Staphylococcus aureus* (35.62%), followed by non-aureus staphylococci (NAS) (25.4%), *Streptococcus spp*. (13.85%) and *Escherichia coli* (9.52%).

The present study revealed that the prevalence of SCM at cow level was 34.9% in the study area. The current finding was lower than 45% previously reported in Tizi-Ouzou, central province of Algeria (Bentayeb *et al.,* 2023) and the 37.66% (Zaatout *et al.,* 2019) in Eastern Algeria but higher than the 29.44% in eastern regions of Algeria (Mamache *et al.,* 2014).

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. However, the average cow level SCM prevalence in the present study was lower than the SCM prevalence of 62% previously reported in dairy herds linked to milk collection centers in Rwanda (Ndahetuye *et al.,* 2020) or the 76.2% in peri-urban areas of Kigali in Rwanda (Ndahetuye *et al.,* 2019) and 49.0% in Sri Lanka (Rahularaj *et al.,* 2019). 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 deficiency*and* causative factors.

The results of bacteriological analysis of the milk samples from cows showed that *S. aureus* and NAS were the most common isolated bacterial pathogen in the CMT positive milk samples. These findings were similar to the results reported by Abebe *et al*. (2016), Ndahetuye *et al* (2020) and Rahularaj *et al*. (2019). Staphylococcal mastitis frequency was around 18% for *S. aureus* and 50% for NAS in previous study from Algeria (Bentayeb *et al.,* 2023).

The isolation rate of NAS (25.4%) was much lower than the findings of Zaatout *et al*. (2019, 61.94%) and Ndahetuye *et al*. (2019, 40.2%). According to the microbiological finding of the study, *S. aureus* was the most common pathogen identified from CMT positive milk samples (35.62%), 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%). *Escherichia coli* was isolated with 9.52%. Naidu *et al*. (2022) reported a higher 24.79% incidence of *E. coli* from cases of mastitis.

The cause of the high prevalence of *S. aureus* reported in this study could be multifactorial. Beside unhygienic hand milking and lack of a mastitis-control plan mentioned earlier, contagious spread of *S. aureus* in the studied herds could also have been facilitated by not milking mastitis-infected cows last. As the main source of infection between uninfected and infected udder quarters, *S. aureus* and other infectious bacteria are typically discovered on the udder or teat surface of infected cows, generally during milking. The etiological prevalence of SCM caused by *S. aureus*, (27%), *Streptococcus* spp. (15%) and *E. coli* (6.5%) respectively in study of Solanki *et al*. (2023) in Southern Rajasthan.

Effect of SCM on reproductive performance

Table 2 showed that SCM in the first month of lactation had statistically significant effect on CFSI, CCI, NSPC and CRFS ($P\geq0.05$). The results of the current study showed that SCM has a negative impact on calving interval. The mean

Table 2: Effect of subclinical mastitis (SCM) on reproductive parameters of dairy cows.

Reproductive parameters	SCM negative	SCM positive	P-value
No. of cows (%)	345 (65.1)	185 (34.9)	-
CFSI (Mean±SE days)	116.38±2.10	128.28±4.67	0.008
CCI (Mean±SE days)	133.15±2.56	156.30±5.42	0.000
NSPC	1.66±0.81	1.88±0.78	0.004
CRFS (%)	55.07	37.83	0.000

CFSI: Calving to first service interval; CCI: Calving-to-Conception interval.

CRFS: Conception rate at first service; NSPC: Number of services per conception.

SE: Standard error.

duration of CFSI were significantly (P = 0.008) longer in cows with SCM (128.28±4.57 days) compared to healthy ones(116.38±2.10 days).

Calving to conception interval for the SCM-positive and healthy cows were 156.30±5.42 and 133.15±2.56, respectively and significantly different between the two groups (P= 0.000). The overall mean for the number of services per conception (NSPC) in healthy cows was 1.66±0.81 while positive cows had 1.88±0.78 (P= 0.004). The occurrence of SCM during the first month of lactation was associated with reduced CRFS compared with cows without mastitis. Conception rate at first service (CRFS) was reduced in cows with SCM (37.83%) compared to healthy cows (55.07%) (P= 0.000).

Subclinical mastitis in the first month of lactation was associated with calving interval. The CFSI and the CC interval were longer in cows with SCM compared to healthy ones and also had a higher number of services per conception and low conception rate at first service. Studies conducted in warm temperate zones such as: Argentina (Gómez-Cifuentes *et al.,* 2014) and Chile (Pinedo *et al.,* 2009) have shown an impairment of reproductive performance in cows with SCM. The main findings in cows with SCM were an increased CFSI and CCI and cows with SCM needed a higher number of services per conception and low conception rate at first service.

Results of this study confirm the negative effects of SCM on dairy cows' fertility. Cows with SCM showed an increased NSPC compared with healthy ones. These results agree with the work of Rahularaj *et al*. (2019) in which cows with SCM in major milk-producing regions of Sri Lanka, showed increased services per conception before the first service compared with healthy cows. It has been also reported that SCM significantly increased the number of services per conception in a pasture-based managed system (Gómez-Cifuentes *et al.,* 2014). In the study of Villa-Arcila *et al*. (2017), cows having SCM had an average 1.83 more services per conception than healthy cows in a rotational grazing system.

In the present study, CFSI in cows with SCM was 12 days longer compared with those recorded in healthy ones. These results agree with the study of Ranasinghe *et al*. (2021) in which days to first insemination was delayed by 15 days in infected cows compared with animals that were uninfected. Siatka *et al*. (2019) and Pinedo *et al*. (2009) have reported similar outcomes. In contrast, Villa-Arcila *et al*. (2017) stated that interval from calving to first service did not differ significantly between the cows with SCM and without SCM. In the current study, CCI was significantly different between healthy and SCM affected cows. Similar results have been reported in Chilean dairy cattle with high somatic cells count (Pinedo *et al.,* 2009). Previous reports showed that CCI was not significantly different between SCM-positive and healthy cows (Ranasinghe *et al.,* 2021).

A reduction in conception rate at first service has been demonstrated in cows with SCM. The results of this study revealed that cows with SCM were less likely to conceive at first service than non-affected cows. In the present study, pregnancy rate at first service (PRFS) decreased by SCM. Previous studies have also shown the presence of a relationship between mastitis and reduced pregnancy rates. According to Lavon *et al*. (2016), cows affected with SCM had a longer estrus-to-ovulation interval (56 h) than uninfected cows (28 h) in an experiment that used a hormonal method to fix the timing of ovulation. Lavon *et al*. (2010) observed about 30% of the cows affected with SCM has delayed ovulation. Delayed ovulation is associated with low levels of circulating estradiol concentrations and delayed pre-ovulatory LH surges (Lavon *et al.,* 2010).

Risk factors associated with SCM

Our findings showed that the risk of SCM occurrence in lactating cows was strongly influenced by parity (P<0.0001), farming system (P<0.0001) and milking method (P<0.0001) (Table 3).

The likelihood of SCM occurrence was 3.53 times higher in multiparous cows than in primiparous cows. Similar results were reported previously in many studies,

Table 3: Logistic regression analysis of the risk factors with subclinical mastitis (SCM) in dairy cows in western of Algeria.

Risk factor	Class	N positive/ N tested	Per cent	OR	95% CI	P-value
Parity	Primiparous	41/213	19.24	Ref	Ref	Ref
	Multiparous	144/317	45.25	3.53	2.35-5.30	<0.0001
Cow breed	Holstein	112/307	36.48	Ref	Ref	Ref
	Montbéliarde	73/223	32.73	1.18	0.82-0.69	0.371
Farming system	Semi-intensive	108/193	55.95	Ref	Ref	Ref
	Intensive	77/337	22.84	2.18	1.48-3.19	<0.0001
Milking method	Machine milking	37/224	16.51	Ref	Ref	Ref
	Hand milking	148/306	48.36	3.37	2.22-5.13	<0.0001
Daily milk production	<10 L	105/317	33.12%	Ref	Ref	Ref
	>10 L	80/213	37.55	0.83	0.57- 1.18	0.293

OR: Odds ratio; P: Probability; CI: Confidence interval.

including those conducted in intensively managed herds (Taponen *et al.,* 2017; Ndahetuye *et al.,* 2019; Ranasinghe *et al.,* 2021). The increase in prevalence of SCM with parity could be related to many factors associated with impairment of leukocyte function, less effective defense mechanism and teat and udder conformation (Rainard and Riollet, 2006). The higher risk of the occurrence of SCM was associated with increased parity number. This may be related to the fact that multiparous cows have had cumulatively several exposures to mastitis pathogens from inadequate cleanliness during milking or from the environment (Ndahetuye *et al.,* 2019). It may also be due to their teat canals being more dilated, partially or permanently opened, possibly resulting from previous injuries, callosity of the teat and breakdown of the streak canal barriers and udder tissue with aging (Abrahmsén *et al.,* 2014). As a result, immunity is lowered, which raises the possibility that environmental and skin pathogens will enter the teat canal.

From farming system point of view, cows raised in an intensive system had a higher probability of developing SCM compared with those raised in a semi-intensive system (OR=2.18, 95%CI 1.48-3.19). Accordingly, a study conducted in different regions of Sri Lanka reported that cows reared under intensive management systems were 5.03 times more at risk of having SCM compared with cows managed under semi-intensive systems (Ranasinghe *et al.,* 2021). Unlike our results, Begum *et al*. (2015) reported that the cows kept under semi-intensive management systems had a greater prevalence of SCM (27.6%) than cows kept under intensive management systems (10.5%). We suggest that the high prevalence of SCM in intensive care units was related to the high sensitivity of Holsteins and Montbéliarde to unsanitary conditions which promote growth and spread of diseases, particularly during wet seasons. Variations in environmental conditions, milk hygiene procedures and milking settings that support the growth and transfer of pathogens could be the cause of the difference in the frequency of SCM between management systems.

Looking specifically to the milking methods, the results of the present study showed a significantly higher prevalence of SCM in cows milked manually (OR=3.37, 95% CI 2.22-3.13). Our result is in accordance with that reported by Rahularaj *et al*. (2019) but different to those reported in other studies. Indeed, no significant association was detected between the incidence of SCM and milking method by Wang *et al*. (2019) and Ranasinghe *et al*. (2021). Furthermore, Fadlelmoula *et al*. (2007) reported that the mechanic milking increased the likelihood for SCM because of the poor cleaning of the milking machine. Furthermore, machine milking alters the teat's appearance and tissue, which may have an impact on the teat's local defense systems and make it more vulnerable to udder infections inside.

Similar to the assertions of Ranasinghe *et al*. (2021), our study showed no effect of cows breed and milk production performance on the prevalence of SCM. Several reports from literature showed that high-yielding dairy cows are more susceptible to SCM (Zaatout *et al.,* 2019). Furthermore, a previous study from Algeria, reported that cows producing less than 10 L of milk had a lower risk of having SCM based on the low OR observed for milk production (OR= 0.56) (Zaatout *et al.,* 2019).

CONCLUSION

At the end of this study, one can assume that bovine mastitis is a prevalent disease in dairy herds in Algeria and *S. aureus* is the common pathogen identified from CMT-positive milk samples. Factors such as parity, farming system and milking methods were significantly associated with the prevalence of SCM. Their occurrence in the first month of lactation impairs significantly the reproductive performance by increasing calving to first service interval, calving-to-conception interval and the number of services per conception. Finally, implementation of integrated approaches to prevent and control mastitis in cows to improve the quality of milk, minimize the economic losses and limit the public health risks are fully justified.

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Conflict of interest

The authors declare that they have no conflict of interest.

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