

# Herd Practices and Their Association with Subclinical Mastitis Prevalence in Dairy Cows in Semiarid Regions of Northeastern Algeria

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## Abstract

In order to complete the national picture of livestock disease caused by bovine mastitis, a cross-sectional study design was conducted in a large dairy farm in the northeastern region of Algeria. Between November 2019 and September 2020, a total of 154 lactating cows were sampled from 11 randomly chosen dairy farms. Milk samples were examined for subclinical mastitis using the California Mastitis Test (CMT: Teepol<sup>®</sup> vial, opaque blister packs), and binary logistic regression was performed to test the influence of several risk factors (management type, herd size, breed, age of animal, and stage of lactation) on cow mastitis status (0 = negative and 1 = positive). Our examination revealed that 90.91% (almost all) of the herds observed had at least one cow suffering from subclinical mastitis. At cow level, the frequency of mastitis in the present study was 12.9%. This prevalence value is the lowest in the country, since it is positioned under the range of cow level mastitis prevalence (26–55.16%) recorded by the most

recently published studies. The presence of mastitis was significantly influenced by the lactation stage and its connection with the animal's age. The odds of finding a cow with a positive CMT result was 15.9-fold more in the late stage of lactation than in the first stage. Moreover, cows in the mid-lactation stage were significantly more likely to have mastitis than cows in the early lactation stage. The Hosmer–Lemeshow goodness-of-fit test suggested that the model fit the data ( $\chi^2 = 3.62$ ;  $p = 0.92$ ), and explained 23.5% of the deviance in the mastitis occurrence and 20.5% of the variance. Thus, we can say that poor hygiene practices, the age of cows, and the lactation stage are the greatest risk factors and are the focal issues for which preventive veterinary programs and control measures could be developed and implemented.

**Keywords:** Farm hygiene, livestock welfare, logistic regression, milk yield, odds ratio, subclinical mastitis

## Introduction

Subclinical mastitis (SCM) is one of the most detrimental diseases facing dairy farms and affecting milk yield worldwide (Ismail, 2017; Nielsen et al., 2010). This problem in the dairy farming industry has had a devastating effect in several domains such as social, economic, and human welfare. In addition to the decline in the production of milk and its derivatives, bovine mastitis directly affects livestock productivity, due to the premature culling (Ahmed & El Zubeir, 2013; Ismail, 2017), the cost of disease control actions and veterinary care, and last but not the least, the general health, chronically infected cows, occasional deaths, and the poor reproductive performance of

animals (Dobson et al., 2008; Seegers et al., 2003). Since the indiscriminate use of antibiotics in farm animals is also associated with a number of concerns (increasing antimicrobial resistance) (Van et al., 2020), dairy farm management and good hygiene practices are the main alternatives to antibiotic applications. Epidemiological data and etiological studies are fundamental to understand the magnitude of the problem, and to determine the key parameters that ensure management quality and hygienic credibility of any dairy farm. By identifying some of the factors that are likely to be of greatest risk, prevention and control measures can be developed and implemented to focus on the ones that are most likely to create problems. Many studies worldwide have been concentrated on

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the investigation of the prevalence and risk factors for mastitis. At the cow level, parity, stage of lactation, and genetic structure are the main characteristics that influence susceptibility to mastitis (Busato et al., 2000; Zadoks et al., 2001). At the herd/farm level, management and hygiene practices such as maintaining cleanliness of the animals and farms, washing and drying udders, and maintaining cleanliness of hands, contact surfaces, and the equipment used to milk the cows were found to be significantly associated with low numbers of somatic cells in milk, thereby decreasing the risk of SCM (Kelly et al., 2009; Vasilev et al., 2007).

Dairy cow production in Algeria is concentrated mainly in three zones; Zone I (60% of dairy cows) is a coastal and a sublittoral area with a humid and subhumid climate, Zone II (26% of dairy cows) is comprised of agropastoral and pastoral regions with a semiarid and arid climate, and Zone III (14%) is located in the Saharan region with a desert climate (Ait-Kaki et al., 2019). Due to the fact that Algeria is an oil-producing country and does not depend so much on such farming activities, focused studies on contagious diseases are lacking and little or no effort has been made to assess management and hygiene practices and their association with the prevalence of bovine mastitis; this applies mainly to Zone II in which this study was done. Hence, with the current study, we aim to complete the national clinical picture of this animal epidemic and provide evidence for the significant association of several management and hygiene practices at both the herd level and the cow level that may affect mastitis occurrence, and could serve as key parameters for selection to reduce mastitis in dairy cattle. We believe that such studies are of paramount importance for designing feasible prevention and control strategies.

## Method

### Sample Size and Study Design

A cross-sectional study design was followed to address the objective of the study. The study was conducted in a large dairy farm in the northeastern region of Algeria, rearing up to 10,000 primiparous or multiparous dairy cows. Between November 2019 and September 2020, a total of 154 lactating cows were sampled from 11 dairy farms of the Khenchela District. According to the estimated size of the cow population in the region, our sample size would allow the study to determine the prevalence of subclinical mastitis with a confidence interval of  $\pm 7.5\%$ , which is a very acceptable margin of uncertainty (Conroy, 2015). From the sampled cows, 1848 milk samples were collected during the routine morning milking. Dirty cows and those presenting any symptom of illness, abnormal udder (lesions, redness, heat, etc.), and sample milk that presented any change in color and consistency were excluded from the study. Before starting our collection of milk samples, several standard aseptic conditions were applied. The cow's udder and the milker's hands were washed with clean water and the teats were swabbed with cotton soaked in 70% alcohol. After discarding the first strip, approximately 40 mL of milk of each

teat was collected into sterile bottles and the samples were then transported immediately to storage at 4°C in the laboratory where they were examined for SCM using the California Mastitis Test (CMT: Teepol® vial, opaque blister packs). The test was carried out according to the procedure described by Quinn et al. (1999). All CMT scores of 0 and traces were considered as negative, while CMT scores of 1, 2, and 3 were considered as positive, and an individual cow having at least one quarter with a CMT score of 1+ was considered a positive cow (Abebe et al., 2016). Finally, a herd was considered as positive for mastitis if a minimum of a single animal was detected with a CMT-positive result.

### Data Analysis

The management and hygiene practices employed by farmers in the dairy farms that were examined in the Khenchela District were determined by a questionnaire and direct interview with the farms' owners. We considered farms with good hygiene as those who applied a good design and management of the housing (e.g., all resting areas should be of sufficient size and should be kept clean and dry, the passageways and access routes should be free from accumulations of dung and slurry), with provision for good animal cleanliness (manual removal of dirt and encouraging grooming with cow brushes) (Regulations, 2006).

The prevalence odds ratio of mastitis was calculated as the ratio of mastitis-positive cows against the total number of animals investigated, based on the transformation  $p = \text{Odds}/(1 + \text{Odds})$ , where  $p$  is the probability of being mastitis-positive (Bruce & Bruce, 2017). We used Generalized Linear Models (GLM) with binomial distribution (logistic regression) to test the influence of risk factors (independent variables) on cow mastitis status (dependent variable), that is, 0 = negative and 1 = positive. For the cow level model, we considered the Farms as a random factor. The independent variables evaluated were management types (semi-intensive and intensive), herd size ( $\leq 10$  heads and  $> 10$  heads), breed (Fleckvieh, Holstein and Friesian), cow's age ( $\leq 3$  years and  $> 3$  years) and stage of lactation (early, mid and late). The intensively managed cows were kept indoors and regularly nurtured with a supplementary customized cattle feed in addition to hay and crop residues (such as corn stalks, wheat/barley straw and other leftovers from grain threshing). On the other hand, the semi-intensively managed cows foraged freely on pasture but received supplementary feeds in the morning and evening when they were milked.

For each set of variables, we developed an all-inclusive design (all possible combination models) by using multimodal inference. Models were then ordered by increasing Akaike Information Criterion with correction (AICc) (Burnham & Anderson, 2002). Finally, the model was assessed for goodness-of-fit using the Hosmer–Lemeshow test (Hosmer, 2000). We used the package "MASS" to apply Generalized Linear Models (GLM) with the binomial distribution, the package "MuMIn" to calculate AICc (Bartoń, 2015), and the package "Resource

Selection” to test the best model performance. Statistical analysis was carried out in R-3.0.2 software (R Development Core Team, 2013), and statistical significance was set at  $p < .05$ .

## Results

### Management and Herd Practices

From a total of 11 dairy farms, various data on the rural and hygiene practices implemented by farmers were recorded during the course of the survey period (Table 1). The study animals included 154 cows belonging to three cattle breeds, the most common one being the Holstein Friesian that represented 70.3% of the studied animals (Table 1). Dairy cows were managed under two husbandry systems (intensive 63.2%, and semi-intensive 36.8%).

On the other hand, following our field surveys, it was noticed that hygiene practices and milking procedures were satisfactory in the study area, and there was no antecedent of subclinical mastitis in all sampled cows (Table 1).

### Prevalence of Mastitis

Based on the CMT results, 20 cows were found to have SCM, with a prevalence of (12.9%) (Table 2). At herd level, almost all (90.1%) surviving dairy farms have at least one SCM case, and at the quarter level, all cows have mastitis in just one quarter

**Table 1**

*Management and Rural Practices Employed by Farmers in the Dairy Farms Examined in Khenchela District, Northeastern Algeria*

Variable	Category	Number of Herds	Frequency (%)
Herd size	≤ 10	54	34.8
	> 10	101	65.2
Management type	Semi-Intensive	57	36.8
	Intensive	98	63.2
Lactation stage	Early	76	49.0
	Mid	56	36.1
	Late	23	14.8
Age	≤ 3	63	40.6
	> 3	92	59.4
Cattle breeds	Fleckvieh	20	12.9
	Holstein	26	16.8
	Friesian	109	70.3
Dry cow therapy	Yes		
	No	154	100
Previously Mastitic	Yes		
	No	154	100
Hygiene of the farm	Good	154	100
	Poor		

**Table 2**

*Overall Prevalence of Subclinical Mastitis in Dairy Cows at Khenchela District, North Algeria*

Observation	Subclinical Mastitis		
	Not Examined	No. Positive	Prevalence (%)
Herd level	11	10	90.91
Cow level	155	20	12.9
Quarter level	620	11 (Hind)	7.1
		9 (Fore)	5.8

of the udder (i.e., all cows do not have mastitis in both fore and hind quarters), and the hind udders were more infected than the fore quarters (Table 2).

### Mastitis Risk Factors

The prevalence of mastitis related to specific risk factors was determined as the proportion of affected cows out of the total cows examined, based on the concept of logistic regression. Several aforementioned factors were retained in the multivariable binary logistic regression analysis for the prevalence of mastitis. Among those factors, the stage of lactation was the unique variable found to be significantly related ( $p < .05$ ) to the occurrence of SCM (Table 3, Figure 1). If the odds ratio (OR) is greater than 1, then lactation stage and mastitis are considered to be associated (correlated), in the sense that the progress of lactation stage raises the odds of mastitis occurrence. Based on the transformation  $p = Odds/(1+Odds)$ , we notice that the probability of being mastitis-positive is 82.9% and 94.08% respectively at mid and late stages, compared to the early stage. We can also say that the likelihood of a cow being SCM positive was 15.9-fold or 4.85-fold at the late or mid-lactation stages, more than at an early stage. The Hosmer–Lemeshow goodness-of-fit test suggested that the model fit the data ( $\chi^2 = 3.62; p = .92$ ), and explained 23.5% of the deviance in mastitis occurrence and 20.5% of the variance.

The effect of the interaction of risk factors on the prevalence of SCM in dairy cows at Khenchela District was found to be significant only when applied to the relationship between the lactation stage and age of the cows, when old cows at the late stage of lactation were more affected than younger cows at the early stage of lactation (Table 4, Figure 1).

## Discussion, Conclusion, and Recommendations

In this cross-sectional study, we tried to assess the effectiveness of hygiene management in reducing the prevalence of SCM at dairy farms of Khenchela District, a region belonging to the Algerian high lands (high plateau) which experiences a Mediterranean semiarid sub-climate and is poorly documented. For this purpose, only cows whose farmers follow a strict hygiene procedure were included in this prevalence study.

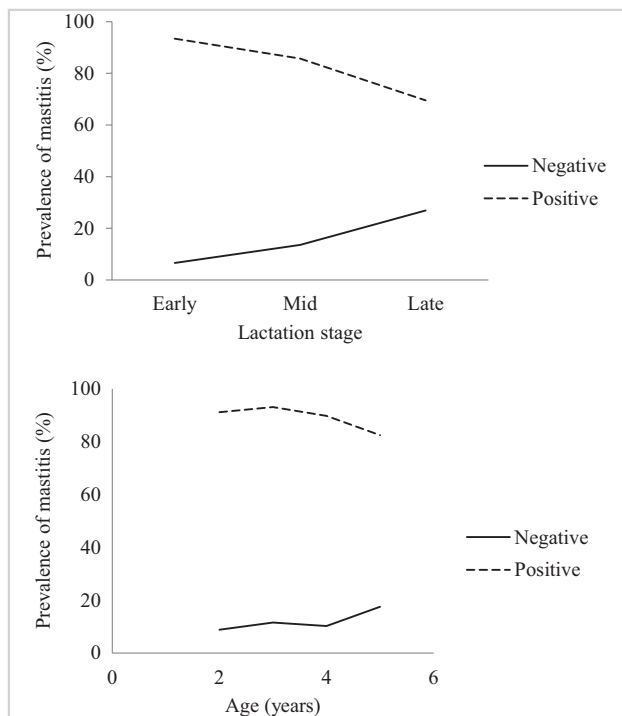
**Table 3**

Binary Logistic Regression Analysis (Multivariable) of the Association Between Subclinical Mastitis in Dairy Cows and Multiple Risk Factors

Variable	Category	OR	SE	Z	p	95% CI for OR	AIC
Herd Size	≤10	Ref					
	>10	1.83	.63	.93	.34	.53 6.31	11.99
Age	≤3	Ref					
	>3	.82	.57	.12	.73	.27 2.50	11.88
Lactation stage	Early	Ref					
	Mid	4.85	.73	4.63	<b>.03</b>	1.15 20.45	16.64
	Late	15.9	.96	8.33	<b>.00</b>	2.43 104.01	
Breed	Fleckvieh	Ref					13.56
	Holstein	.00	8615.6	.00	1.00	.00	
	Friesian	1.23	.70	.09	.77	.31 4.79	
Management type	Intensive	Ref					11.95
	Semi-Intensive	1.03	.02	1.76	.18	.99 1.08	
Constant				13.62	<.001		

Bold character: Significance at .05 level.

Note. OR = odds ratio; SE = standard error; Z = z-score; p = p-value of significance; 95% CI OR = the 95% confidence interval for the odds ratio; AICc = Akaike Information Criterion with correction.



**Figure 1**  
Effect of Lactation Stage and Animal's Age on the Prevalence Ratio of Subclinical Mastitis.

Our examination revealed that 90.91% (almost all) of herds observed had at least one cow suffering from SCM. It was difficult to compare our herd level results with other mastitis studies since investigations at similar regions (high plateau) are scarce. However, the prevalence (at herd level) is in line with many similar studies across the other parts of the country; it is at 100% in the Oran region of West Algeria (Benhamed et al., 2011), and every examined herd at the El-Taref district of East Algeria had at least one infection (Fartas et al., 2017). Finally, in the central part of the country, all the investigated herds were mastitis-positive (Saidi et al., 2013). At the cow level, the frequency of mastitis in the present study (12.9%) was the lowest one in the country, positioned under the range of cow level mastitis prevalence (26–55.16%) recorded by the most recently published studies (Ait-Kaki et al., 2019; Benhamed et al., 2011; Bouzid et al., 2011; Fartas et al., 2017; Saidi et al., 2013). Moreover, our mastitis prevalence was lower than that of other African countries whose climate and dairy management are relatively similar to ours; for instance, the prevalence rate was 42.9% in Egypt (Elbably et al., 2013), and 38.89% in Sudan (Hussein, 2012). This fact is logical, since we have selected only those cows whose farmers follow a strict hygiene procedure in terms of animal cleanliness, washing and drying udders, and maintaining cleanliness of hands, contact surfaces, and the equipment used to milk the cows. These practices and other milking procedures such as the use of teat disinfection post

**Table 4**

*Effect of Risk-Factor Interaction on the Prevalence of Subclinical Mastitis in Dairy Cows at Khenchela District, North Algeria (We Present Only the Top Significant Model)*

Model	OR	B	SE	Z	p	AICc	GOF (p)
Age*lactation stage	13.4	.13	.26	11.22	.04	104.95	.96
...							
Null model	1.13	.12	.26	22.96	0	105.14	.99

Reference odds is young cattle\*early stage.

"..." refers to additional models examined, but not listed in detail to avoid an overlong table, as they were minimally informative.

Note. OR = odds ratio; B = unstandardized regression weight; SE = standard error; Z = z-score; p = p-value of significance; AICc = Akaike Information Criterion with correction; GOF (p) = The goodness of fit

milking were found to be significantly associated with low SCM, as reported by Kelly et al. (2009). Moreover, the high level of dirtiness of farms and animals is well known to be one of the most important predictors associated with the presence of the high number of somatic cells in milk and an increased risk of SCM (Vasilev et al., 2007). Thus, our results provide additional proof of the effectiveness of hygiene strategies in reducing SCM, especially at the cow level.

In this study, the presence of mastitis was significantly influenced by the stage of lactation and its relationship to the age of the animal. The odds of finding a cow with a positive CMT result was 15.9-fold greater in the late stage of lactation than in the first stage. Moreover, cows in the mid-lactation stage were significantly more likely to have mastitis than cows in the early lactation stage. This trend of increasing prevalence at the late stage complies with the reports of Fartas et al. (2017) at El-Taref district in East Algeria (this is the only previous study in the country that investigates the prevalence of bovine mastitis and the associated risk factors), and is also in line with other African countries (Belayneh et al., 2013). Correspondingly, the late stage of lactation in this study coincides with the summer, and because of the heat stress in this season, immunosuppression may have a significant influence in the higher occurrence rate of bovine mastitis during late lactation (Moosavi et al., 2014). Another factor that may be mentioned here is the accumulation of germs in the mammary gland as we progress through the lactation number, leading thereby to an increase in the likelihood of disease incidence during the later stage of lactation (Fartas et al., 2017; Morse et al., 1987; Sargeant et al., 1998). The late lactation is also a critical stage for older cows, and the likelihood of mastitis in the current study was 13.4-fold higher in older cows at a later stage compared with younger cattle at an early stage. The group of older animals faced the stress of calving and lactation together, with the reason that high-milking cows are more susceptible to udder infection (Fadlelmula et al., 2009). The older cows are multiparous (have more than one calving), and the effect of parity on the number of mastitis events has been documented in many studies which show

that higher parity cows have an increased risk of experiencing mastitis compared to primiparous (younger) cows (Jamali et al., 2018; Jong, 2005; Wolf et al., 2010). This is mainly due to the fact that increasing parity and age deteriorate the cow's intramammary and anatomical (e.g., teat sphincter patency) defense mechanisms (Zadoks et al., 2001). Additionally, we noticed that in our study, the oldest cow had a pendulous udder, placing the teats in a position of greater exposure to the ground (environmental factors), which could cause their injury and allow the adhesion of pathogens to the mammary tissue (Awale et al., 2012; Bind et al., 1980). On the other hand, several studies have found a significantly higher prevalence in the early and mid-stages of lactation (Elbably et al., 2013; Iraguha et al., 2015; Zeryehun et al., 2013). Since we have revealed in this study the negative interaction between age of cows and lactation number on the prevalence of bovine mastitis, we could suggest that the variations in the effect of lactation stage among these different studies were mainly related to differences in the age of the sampled animals.

The present study adds valuable information about the etiology and prevalence of bovine SCM at dairy farms in Algeria, mainly in semiarid regions that were poorly known (this is the first study in the region). We confirm here the importance of cleanliness and hygiene practices in mitigating the prevalence of this disease, as the overall mastitis rates obtained are the lowest against all the aforementioned studies. Additionally, when a regression model was used on the data, milk SCM was lower by 15-fold in younger animals and those in the early lactation stage than in older cows and at a late stage. Thus, we can say that hygiene practices, the age of cows, and the stage of lactation are the greatest risk factors that preventive veterinary programs and control measures could be developed and implemented to focus on.

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