# Analysis of the impact of risk factors on the occurrence of subclinical mastitis on dairy cattle farms in eastern Algeria

B. Bouchoucha<sup>\*</sup>, N. Zeghilet, R. Aimeur, N. Lakhdara and O. Bouaziz



### Abstract

In order to study the effect of various risk factors on the prevalence of subclinical mastitis, we studied the prevalence of subclinical mastitis in 104 dairy cows (416 udders) from 18 bovine herds in three wilayas in eastern of Algeria using the California mastitis test (CMT) combined with bacteriological analysis. The aim of this research was to determine the effect of 27 risk factors related to the animal, its environment and milking practices on prevalence, as well as the effects of breed, age, stage of lactation, housing and litter type, hygiene level, season, mil production, vacuum level and elimination of first jets on subclinical mastitis frequency. A survey was carried out to collect data on cow numbers, breeds, type of breeding, housing, litter and the level of hygiene in farms, the method of milking and milking practices. The CMT combined with bacteriological examination was performed to detect subclinical mastitis, the Chi 2 test was used to test for difference between the means. Prevalence values were: 24% by CMT vs 17% by bacteriology analysis. Concerning udders, we recorded a rate of 10% vs. 8.7% respectively. The results of the survey show that the Holstein breed is the most exploited and affected (P<0.05). The number of dairy cows varied

from 3 to 20 cows. In this study, 56% of cows were raised on farms built of cinder blocks, which had a lower rate compared to farms built of wood and reeds (P<0.001). Animal housing consisting of a concrete floor 33% vs. 36% raised with straw bedding and were more affected (P<0.001), litter is removed only once a day for 13% of cows, which is a factor that predisposes cows to this disease. The frequency is higher during early and late compared to mid-lactation phases (P<0.05); low udder conformation and the right side of udders also had an impact on this disease alongside animal hygiene (P<0.05), and cattle producing more than 12 litres per day were more susceptible (P<0.05). However, the frequency of mastitis was only slightly modified by the lactation number of cows. High and low vacuum level of milking machines and pulsation frequency predisposed cows more to this disease (P<0.05). In this study, cows milked manually were significantly more affected (P<0.05) particularly in summer and the winter, showing high rates (*P*<0.05).

Key words: dairy cattle; subclinical mastitis; investigation; CMT; bacteriological analysis; risk factors

Brahim BOUCHOUCHA\*, (Corresponding author, e-mail: brahim.bouchoucha@umc.edu.dz), Noureddine ZEGHILET, Rachida AIMEUR, Omar BOUAZIZ, GSPA Research Laboratory (Management of Animal Health and Productions), Institute of Veterinary Sciences of El-Khroub, Mentouri Brothers University of Constantine 1, Algeria; Nedjoua LAKHDARA, (Institute of Veterinary Sciences of El-Khroub, Mentouri Brothers University of Constantine 1, Algeria

## Introduction

As part of an epidemiological study on the effect of various risk factors related to the animal, its environment and milking practices on the occurrence of subclinical mastitis in small to medium-sized farms (average 6 cows) in three wilayas of eastern Algeria, the results obtained are described below. Herd risk factors seemed to have a strong influence on the occurrence of subclinical mastitis. Indeed, cows were exposed to unfavourable milking practices (hygiene of the milking machine, washing of udders, elimination of the first jets on the ground, soaking, draining and over-milking, etc.), housing of lactating cows (stall type, hygiene of sleeping areas, frequency of insufficient mulching, etc.), and of the animal and the health of cows' udders have already been implicated in cow mastitis (Bareille, 2000; Rakotozandrindrainy et al., 2007; Cvetnić et al., 2016; Hanzen, 2016; Cvetnić et al., 2022). The aim of this study was to determine the risk factors for subclinical mastitis in dairy farms in eastern Algeria. Also, using CMT and bacteriology, we tried to determine the effect of breed, number and stage of lactation of cows, the season, and milking practices on the frequency of subclinical mastitis.

## **Materials and methods**

#### Description of farms and animals

This study focused on a workforce of 104 lactating dairy cows, including 62 of the Holstein breed (60%) and 42 of the Montbéliarde breed (42%), on 18 farms located in Mila (n= 7), Constantina (n= 6) and Guelma (n=5). Herd sizes were small, with the number of cows per herd varying from two to twenty cows per farm, with an average of 5.77 dairy cows. The farming system is extensive in 14 farms and semi-intensive in 4 farms.

# Epidemiological investigation of risk factors

An epidemiological survey on the influence of risk factors on the occurrence of subclinical mastitis was carried out on all included farms, using an information sheet relating to the type of farm, management and hygiene (Lamari et al., 2021). The questionnaire was completed by questioning the staff in charge of the farms and by attending all stages of milking. This survey therefore aims to study the factors closely related to the onset of udder infections. It focused on characteristics related to the animal, characteristics relating to the conduct and hygiene of milking, and on characteristics relating to the environment (housing conditions). The assessment of the conformation of the mammary system and the state of cleanliness of cows and the barn were based on the standards given by Bareille (2000).

#### Occurrence of subclinical mastitis

In this study, the milk of 416 udders (104 cows) was exanimated using the California mastitis test (CMT), which is a test that uses a Teepol substance to highlight milk containing polynuclear cells. The CMT result was interpreted as negative (0+) or 1+ (trace), 2+ (gel) and 3+ (clumps) (Busato et al., 2000). Bacteriological tests were performed according to the guidelines of the International Dairy Federation (NMC, 1987). The cow is considered to have illness when it has one or more than udder with a positive CMT score (CMT≥2) and infected, or with negative CMT score (CMT≤1) but with positive bacteriological analysis.

#### Statistical analysis

In this study, we used the Chi-square test to test the differences between the mean rates of subclinical mastitis.

## **Results and discussion**

In this study, the California mastitis test (CMT) combined with bacteriological examination was performed to detect subclinical mastitis on 18 farms in 104 dairy cows (416 udders) in eastern Algeria. The prevalence rates detected were 24% by CMT vs 17% by bacteriology, while for udders we obtained unregistered rates of 10% vs. 8.7%, respectively by testing method. The Chi-square test is used to test for differences between the means of disease rates, and the results of the survey showed that this disease is impacted by several risk factors, as outlined below.

# Impact of animal-related factors on the occurrence of mastitis subclinical

Table 1 reports the results of animal-related risk factors for the occurrence of subclinical mastitis.

#### Breed

Holstein cows (19%) were more susceptible to subclinical mastitis than Montbéliarde cows (11%) (P<0.05), corroborating previous studies (Busato et al., 2000; Bouchoucha, 2007; Hamlaoui et al., 2019).

The average milk production observed in our study was more than 12 litres of milk per day and per cow for 61% of cows. This result is comparable to the data of  $14.3 \pm 4.77$  kg, 14 litres and 12.13

**Table 1.** Effect of several factors related to the animal and its hygiene on the prevalence of subclinical mastitis

Factors	Characteristics	Cows <i>N</i>	Cows with mastitis <i>N</i> (%)	Р
Breed	Montbéliarde	42	6 [14]	0.46*
	Holstein	62	12 (19)	
Age (years)	≤ 3	18	5 (29)	
	4-6	79	11(14)	2.15*
	$\geq 7$	7	2 (33)	
Lactation stage (month)	1-2	61	9 (15)	3.54*
	3-4	26	3 (11.5)	
	>4	17	6 (35)	
Teat side	Right	208	23 (11)	3.25*
	Left	208	13 (6)	
Teat position	Anterior	208	13 (6)	3.25*
	Posterior	208	23 (11)	
Udder conformation	High	89	14 (16)	0.83*
	Low	15	4 (27)	
Quantity of milk litres per day	≤ 7	14	2 [14]	
	8-11	27	4 (16)	0.35*
	≥12	63	12 (19)	

\*. \*\*. \*\*\*/ Difference between rates statistical significantly at (\*: P < 5%, \*\*: P < 1%, \*\*\*: P < 0.1%)

kg/cow/day respectively reported by Belhadia et al. (2009), Boukhechem et al. (2019) and Kebbal et al. (2020).

#### Age

The frequency of mastitis varied significantly with age. There was a significant difference between age groups (P<0.05). Cows over 7 years had a higher risk of subclinical mastitis (33% compared to 14% in cows aged between 4-6 years and 3% in cows less than 3 years). Subclinical mastitis was found to increase with age. The effect of age has been reported by various authors (Bouchoucha, 2007; Busato et al., 2000; Saidi, 2014; Belmamoun 2017, Etifu and Tilahun, 2019), though the prevalence of subclinical mastitis detected by CMT test does not vary according to lactation order Bouaziz, (2005); Rakotozandrindrainy et al., (2007); Kalandi et al., (2017). Other authors have reported that the incidence of subclinical mastitis increases progressively with lactation rank (Benic et al., 2018; Cvetnić et al., 2021). Indeed, multiparas in the fourth lactation and above have been reported to be three times more affected than first-parouses (Sargeant et al., 2001; Mungube et al., 2005; Saidi et al., 2010; M'sadak et al., 2013) The higher predisposition to mammary infections in older cows could be the consequence of variables characterising the aging of animals: elongation of teats and, more specifically, reduced distance from the ground, lesions on the teat, loss of elasticity of the sphincter and increase in its permeability, which promote contamination (Hajmbarek et al., 2013).

#### Lactation stage

The distribution of subclinical mastitis according to lactation stage shows a frequency of 15% at the start of lactation followed by a decrease in the middle (11.5%) and a peak at the end of lactation (35%). A significant difference was noted between the cows at the beginning and in the middle of lactation and those at the end of lactation (P<0.05). These results are comparable to those reported by other studies in Algeria (Niar et al., 2000; Benmounah, 2002; Heleïli 2002; Hamlaoui et al., 2019). On the other hand, Saidi et al. (2010) report that the prevalence of subclinical mastitis was higher at the start (37%) than at the end of lactation (11%).

#### Quarter position

The distribution of quarters according to their position on the udder shows that the hind quarters were more susceptible to contamination, with 11% of cases of subclinical mastitis affecting the hind quarters, compared to 6% in the fore quarters % (P<0.05). M'sadak et al. (2014b) also found that the hindquarters had a higher infection rate (60%) than the forequarters. The hindquarters are a risk factor for subclinical mastitis with reports of a significant increase in the frequency of infection in the right and left hind quarters compared to the front quarters in lactating cows (Mekonnen et al., 2010). An explanation could be given by the fact that the hindquarters produce more milk and the teats tend to be closer to the ground, which exposes them to an increased risk of injury, but also to more contact with dirt. On the contrary, Kemp et al. (2008) do not report any significant differences in the risk of infection between the hindquarters and forequarters.

#### Quarter side

Right-side teats with a rate of 11% were more sensitive compared to the left side teats with a rate of 6% (P<0.05). Mekonnen et al. (2010) reported that the frequency of right-sided quarters testing positive was higher than of left-sided quarters (26.3% *vs.* 21.6%). On the other hand, M'sadak and Mighri (2015) did not

find a significant difference between the infections of the right quarters (C and D) and those of the left quarters (A and B)

#### **Udder conformation**

The distribution of infected quarters according to the conformation of the udder shows that the udders whose end of the quarter is below the hock are the most infected (27%) compared to udders whose end is above the hock (16%) (P<0.05). Any imbalance of the udder predisposes to mastitis. The main risk factor is the distance between the end of the teat and the ground (Mimoune et al., 2021). The shape of the teat orifice, the firmness of the sphincter, the length and diameter (and shape) of the teat (in relation to milking speed), and the anteroposterior balance of the quarters also play a role. In a study to investigate the impact of udder morphology, teat morphology and milking speed on udder health in first and second lactation cows, Haj Mbarek et al. (2013) reported that a decrease in the distance between the end of the teat and the ground was significantly associated both with an increase in somatic cell concentrations and with the occurrence of mastitis. The results obtained by Guarin et al. (2017) supported these findings. This is explained by the fact that a low udder is more exposed to dirt and injuries than a well attached udder.

#### **Milk production**

The prevalence of mastitis increased with daily milk production. A significant difference was observed in cows producing more than 11 litres of milk (19%) per day compared to cows producing less than 7 litres (14%) (*P*<0.05). The same results were reported by Rakotozandrindrainy et al. (2007) and Saidi al. (2010). Mekonnen et al. (2010) report that a high milk production of primiparous cows (> 8000 kg / lactation) is strongly associated

with mammary infections by major pathogens.

#### Impact of the state of cleanliness of farms and cows on the occurrence of subclinical mastitis

The results of the study of the impact of the state of cleanliness of farms and cows on the occurrence of subclinical mastitis are recorded in Table 2. The study of the effect of cleanliness of cows showed a significant difference between the frequency of mastitis in cows with poor cleanliness (37%) and rates recorded in cows with good (7%) to average cleanliness (12%) (*P*<0.05).

Concerning the cleanliness of the udder, the results show a very significant difference between the frequency of mastitis in cows with poor cleanliness (55.5%) and rates in cows with good (5.5%) to average cleanliness (13 %) (P<0.001).

These results are in line with those reported by M'sadak et al. (2014c) showing that cows with poor cleanliness recorded a higher average individual cell concentrations (ICC) than cows with good to average cleanliness (P<0.05).

The cleanliness of the cow is an element of appreciation of general hygiene and constitutes a concrete synthesis of the dirt brought by the environment and the pathogenic factors linked to them. The cleanliness of the cow has a significant impact on its mammary health. Indeed, any decrease in cleanliness increases the cell number of the milk, thereby increasing the risk of mastitis (Lévesque, 2006).

Hygiene conditions in the barn also have an effect on the occurrence of subclinical mastitis. Statistical analysis showed a significant difference in mastitis rates between farms with poor hygiene (31%) and farms with good (8%) and medium hygiene conditions (20%) (P<0.01). The cleanliness of the general condition is an index of the hygiene of the litter (Benic et al., 2018; Tomanić et al., 2023).

# Impact of housing conditions on the occurrence of subclinical mastitis

The results of the effect of housing conditions on the occurrence of subclinical mastitis are presented in Table 2. The results obtained show a significant difference in the rates of subclinical mastitis between semi-intensive farming and extensive farming (18% *vs.* 17%) (*P*<0.001). Statistical analysis shows that for housing conditions, litter and scraping both have a significant effect on udder health status.

For the type of stall, subclinical mastitis was more common in tie stalls (22%) than in free stalls (14%) (*P*<0.05). These results are in line with those found by Pluvinage et al. (1991) who showed that subclinical mastitis was most frequent in tie-stalls. Bouraoui et al. (2014) found that the highest cell count is associated with tie-stalls: 691 x 10<sup>3</sup> ± 305 x 10<sup>3</sup> cells/mL for TCT (Tank Cell Rate) and  $5.29 \pm 0.49$  cells/ mL for SCS (Cell Score Somatic), compared to a TST of  $550 \times 10^3 \pm 258 \times 10^3$  cells/ mL and SCS of  $4.78 \pm 0.97$  cells/mL for the free stall. (There the high level of somatic cells means more rate of infection)

On the other hand, M'taallah et al. (2002) found that herds housed in tiestalls had lower TCTs than herds housed in free-stalls ( $521 \times 10^3 \pm 298 \times 10^3$  cells/mL  $vs 643 \times 10^3 \pm 448 \times 10^3$  cells/mL). However, the difference between the two means was statistically insignificant. According to Haj M'barek et al. (2013), the results of the analysis of variance of the ICC (Individual Cell Count) showed that rearing in free stall did not differ much from that in tie stall.

#### **Construction materials**

The characteristics of construction materials had a significant influence on the occurrence of subclinical mastitis (*P*<0.001). In fact, mastitis rates of 47% and 33% were noted respectively in cows in buildings built of wood and reeds, and in greenhouses compared to those in barns built of concrete blocks (9%) or stone and wood (14%). Saidi et al. (2010) reported mastitis prevalence detected by CMT of 67%, 50% and 40%, respectively in buildings constructed of concrete block, wood and brick.

#### Litter and scraping of the concrete area

Littering and scraping have a significant impact on udder health. Indeed, the rate of mastitis is higher on straw bedding (19%) than on the Gerflex mat (17%) or concrete floor (16%) (*P*<0.05). In farms where litter is not sufficient, the rate of subclinical mastitis was 20% compared to farms where litter was sufficient (18%) (P < 0.05). This is justified by the fact that the quality (humidity) and maintenance of litter are major risk factors for mastitis. Indeed, if the litter is defective, it leads to the multiplication of environmental bacteria responsible for mastitis. Insufficiently maintained litter increases the risk of subclinical mastitis infection (Benic et al., 2018). Mulching of litter is considered satisfactory when it is carried out every day at the rate of 4 to 5 kg per cow in free stalls; 3 kg per cow in tie-stalls. In fact, mulch must be daily and in sufficient quantity for better cow hygiene (Bareille et al., 2003). Furthermore, the difference between the rate of mastitis on farms that comply with a sufficient scraping frequency (i.e., twice a day or more) and those that do not comply is significant. Statistical analysis showed that the frequency of scraping causes a significant reduction in the rate of mastitis in farms that practice three or more scrapings per day (11%, 14%) compared to farms that practice two scrapings (18%) or only one scraping (36%) (P<0.05). Other authors

Factor	Characteristics	Cows <i>N</i> (%)	Cows with mastitis <i>N</i> (%)	Ρ
Type of farming	Semi-intensive	57	10 (18)	8.84**
	Extensive	47	8 (17)	
Animal hygiene	Good	28	2 (7)	3.51*
	Middle	49	6 (12)	
	Bad	27	10 (37)	
	Good	54	3 (5.5)	13.39***
Udder hygiene	Middle	30	4 (13)	
	Bad	20	11 (55,5)	
	Good	40	4 (10)	9.29***
Thig and flank	Middle	35	6 (18)	
nygiene	Bad	29	8 (28)	
	Concrete floor	32	5 (16)	0.24 *
Type of litter	Straw	37	7 (19)	
	Gerflex carpet	35	6 (17)	
Season	Summer	20	4 (20)	
	Winter	25	5 (20)	0.86*
	Autumn	35	6 (17)	
	Spring	24	3 (12.5)	
Stable hygiene	Good	50	4 (8)	8.54**
	Middle	25	5 (20)	
	Bad	29	9 (31)	
Housing Type	Tie-stall	45	10 (22)	1.32*
	Free stall	49	8 (14)	
Construction materials	Concrete block	57	5 (9)	
	Green house	10	3 (33)	11.35***
	Stone + wood	22	3 (14)	
	Wood + reeds	15	7 (47)	
Scrapping of stables times/day	1	14	5 (36)	
	2	33	6 (18)	3.5*
	3	22	3 (14)	
	>3	35	4 (11)	

#### Table 2. Effect of different building and environmental factors on subclinical mastitis

\*. \*\*. \*\*\*/ Difference between rates statistical significantly at (\*: P < 5%, \*\*: P < 1%, \*\*\*: P < 0.1%)

Factor	Characteristics	Cows <i>N</i> (%)	Cows with mastitis <i>N</i> (%)	Р
Milking method	Manual	13	3 (23)	2.64*
	Mechanical	91	15 (16)	
Milking machine hygiene	Good	31	2 (6)	
	Average	41	3 (7)	15.02***
	Poor	19	10 (53)	
Hygiene	Good	46	5 (11)	9.4***
	Average	31	3 (10)	
	Poor	14	7 (50)	
	<42	20	4 (20)	0.86*
Vacuum level	42-45	11	1 (8)	
(ry pascal	>45	60	10 (17)	
	< 55	6	1 (17)	1.24*
Pulse frequency (Beats/minute)	55 – 60	28	3 (12)	
	> 66	57	11 (19)	
Draining	Yes	80	9 (11)	11.31***
	No	9	5 (56)	
	Occasionally	15	4 (27)	
Elimination of the first jets	Yes	84	12 (14)	2.13*
	No	20	6 (30)	
On	Under the animal	49	8 (16)	0.42*
	In the recipient	35	4 (11)	
Washing udder prior to milking	Disinfection with collective wiping	78	14 (18)	
	Water without wiping	7	3 (43)	8.19**
	Disinfection with individual wiping	19	1 (5)	
Dipping	Yes	28	2 (7)	4.96**
	No	76	16 (21)	

#### Table 3. Effect of milking practices and hygiene on the prevalence of subclinical mastitis

\*. \*\*. \*\*\*/ Difference between rates statistical significantly at (\*: P < 5%, \*\*: P < 1%, \*\*\*: P < 0.1%)

have reported high cell counts in farms that do not respect the scraping frequency compared to those that respect the standards (M'taallah et al., 2002; Bouraoui et al., 2014; Knežević et al., 2021). According to Lévesque (2004b), the high cellular rate of farms that do not respect the standards for the frequency of scraping is explained by the accumulation of manure which causes the production of heat and humidity favouring the multiplication of bacteria.

#### Season

For the season, the percentages of subclinical mastitis observed were 20% for winter and summer, 17% for autumn and 12.5% for spring. The differences between these seasonal prevalences were statistically significant (P < 0.05). The high frequency of mastitis in summer and winter could be explained by the high humidity and temperature levels during the summer, and the high rainfall rate in winter, in addition to air currents that stress the animals while reducing their immunity (Bareille et al., 2000). M'sadak et al. (2014a) found a significant percentage of subclinical and clinical mastitis in the spring season.

# Impact of milking conditions on the occurrence of subclinical mastitis

The results of the impact of milking conditions on the occurrence of subclinical mastitis are presented in Table 3. The study of the effect of milking mode revealed that the rate of subclinical mastitis in cows was higher (23%) during manual milking than during mechanical milking (16%) (P<0.05). These results are contradictory with those reported by Saidi et al. (2010) who found that subclinical mastitis was more common in cows milked mechanically than in those milked manually.

# Preparation of the udder before milking

#### Cleaning the udder

Cleaning the udder with water alone without wiping was associated with an increase in the incidence of subclinical mastitis by around 43%. In farms using an individual cloth, the mastitis rate was 5%, while on farms using a collective cloth, the mastitis rate was around 18%.

The differences between the three rates are statistically significant (*P*<0.01).

These results are in line with those of M'taallah et al (2002), who found that farms using an individual cloth had an average TCT of  $272 \times 10^3 \pm 104 \times 10^3$  cells/mL. On the other hand, those not using a cloth have an average TCT of 788  $10^3 \pm 539$  10<sup>3</sup> cells/ml. The use of a collective wipe occupies an intermediate position with an average of  $555 \times 10^3 \pm 197 \times 10^3$  cells/mL. The differences between the three means are statistically significant.

According to Roussel and Ribaud (2000), in their study on mastitis, the absence of cleaning and disinfection after milking a cow is associated with an increase in the prevalence of subclinical mastitis. Thorough teat wiping removes bacteria-contaminated water. The absence of wiping of the teats after cleaning implies the runoff of dirt towards the teat liners which will be sucked up during milking, thus constituting a factor favouring the contamination of the udder through the teats (Lévesque, 2003). According to Bouraoui et al. (2014), the practice of wiping is linked to the lowest cell counts, namely a TST of the order of 335 x 10<sup>3</sup>± 128.3 x 10<sup>3</sup> cells/mL. Whereas for farms where wiping is not practiced, their TCT is around 704 x  $10^3 \pm 354$  x  $10^3$ cells/mL. The use of a collective dishcloth for all cows promotes the risk of milk contamination and the transmission of pathogenic germs from potentially infected quarters to healthy quarters (Noireterre, 2006), and consequently, the spread of mastitis from an animal sick to a healthy animal. In Tunisia, M'taallah et al. (2002) showed the influence of teat cleaning and wiping on TST.

#### **Observing the first jets**

The observation of the first milk jets has several advantages, in particular the

detection of clinical mastitis and the improvement of the cell count, by reducing the cells of the herd's milk, since the first jets are the most loaded with bacteria (Lévesque, 2003).

The elimination of the first jets of milk before milking is a practice linked to a low frequency of mastitis (14%). On farms that neglect this practice, the prevalence of subclinical mastitis is double (30%) (P<0.05). These results are in agreement with those found by M'taallah et al. (2002) and Bouraoui et al. (2014), who reported a significant difference between the mean of herd cell counts and those of individual cell counts on farms where the control of the first milking is practiced than in those which neglect this practice. This difference is statistically significant, and is explained by the fact that the first jets contain high concentrations of bacteria and their elimination prevents them from passing into the milking machine and therefore reduces subsequent contamination of the udders by the machine. The nonelimination of the first jets showed a significant influence (P < 0.05) on the average ICC (M'sadak et al., 2013).

The rate of subclinical mastitis was higher on farms where the elimination of the first drafts was performed on the ground than in farms where this practice is done in a container (16%) vs 11%) (P<0.05). This practice is commonly done on the ground under the cow (89%), which leads to contamination of the environment, and thus presents a risk factor for contamination of the lying surface of the cow. The first squirts of milk carry a significant microbial load which can constitute one of the main pollutants of the mixed milk, it is therefore essential to eliminate them (Boudry, 2005). Disposal should be done in a bowl with a black bottom. In addition, the farms that practice the elimination of the first jets of milk before the cups are placed have an average TCT of 298 x  $10^3 \pm 117 \times 10^3$ cells/mL. The average of those who do not use it is 703 x  $10^3 \pm 442 \times 10^3$  cells/mL. The difference between the two means is statistically significant.

### Teat disinfection after milking

Statistical analysis showed that the absence of teat dipping after milking is associated with a high prevalence of subclinical mastitis (21% vs 7%) (P<0.01). This result is in line with that reported by Saidi (2014) who found that the frequency of subclinical mastitis detected by the CMT test is higher in farms practicing dipping than in farms where this practice is neglected (46% vs 100%). M'taallah et al. (2002) reported that farms that practice teat dipping have an average TCT (Tank Cell Rate) of 387 x 10<sup>3</sup>± 185 x 10<sup>3</sup> cells/mL as opposed to an average of 722 x  $10^3 \pm$  $464 \times 10^3$  cell/mL on those farms that do not apply this practice. The difference between the two means is statistically significant. According to Noireterre (2006), this is due to the important role of soaking in the disinfection of teats after milking. Disinfection of teats after milking has an antiseptic action against existing germs on the skin of the teat, and a dermatological action to limit physical attacks and a physical barrier effect against the penetration of bacteria (Capon, 2010).

#### Treatment at drying off

Statistical analysis showed that treatment in the dry period leads to a very significant reduction in the rate of mastitis in milked cows compared to untreated cows (7% vs 12%) (*P*<0.001). The absence of antibiotic treatment at drying off is strongly associated with high rates of subclinical mastitis (M'taallah et al., 2013; Maćešić et al., 2022).

# Assessment of cow milking characteristics

#### **Pulse frequency**

Pulse frequency revealed a significant influence on mastitis rates (P<0.05). In fact, the highest mastitis rates are those whose pulse frequency were the highest (>60 pulses/minute) or lowest (<55 pulses/minute) (19 and 17%). For a frequency of pulsations between 55 and 60 pulses/ minute, the rate of mastitis rises to 12%. Pulse frequency was high in 54% of the machines, as farmers sought to increase it to decrease milking time. A defective pulsation (>60 pulses/min) is related to the appearance of new infections and lesions of the teats (Mezine, 2006). Bouraoui et al. (2014) report that the highest cell counts are those with the lowest (<55/min) or highest (>60/min) pulse rate. Indeed, inadequate pulse frequencies are associated with mean TSTs of 986 x 10<sup>3</sup> cells/ mL for a pulse frequency <55/min and  $816 \times 10^3 \pm 264 \times 10^3$  cells/mL for a pulse frequency >60/min as compared to a TST of 433.5 x  $10^3 \pm 135$  x  $10^3$  cells/mL for a pulse frequency between 55 and 60 /min. According to M'sadak et al. (2010), the averages of the TSTs and ICCs associated with a pulse rate higher than 60 pulses/ min were higher than the other two averages (P<0.05).

# Conclusion

This study provided evidence of the prevalence of subclinical mastitis and information on risk factors of the disease in small Algerian dairies. There especially prevalence of contagious udders and cows (8.7% *vs* 17% respectively) associated with California mastitis test positive results (24% *vs* 10% for cows and udders respectively. Methods of prevention and therapy must be in according with con-

ventional procedures and results of risk factors impact study.

## Acknowledgement

Many thanks to the breeders who collaborated, to the staff of the Hiba analysis laboratory at El-Khroub in Constantina and the Staff of the microbiology laboratory of the ISVK institute at Khroub. Constantina. Algeria, also thanks to professor Lakhdara, N. for the translation of the text into English and corrections.

### References

- BAREILLE, N., M.B. KIEBERT-TOE, F. BEAUDEAU and H. SEEGERS (2000): Risk factors for elevated milk somatic cell count during early lactation in dairy heifers. Rens. Rech. Rumin. 7, 99-102.
- BELKHEIR, B., F. GHOZLANE, M. BENIDIR, N. BENHMED and A. BOUSBIA (2016): Screening of subclinical mastitis of dairy cattles in mounts area of Tizi Ouzou (Algéria). Renc. Rech. Rumin. 23, 314.
- BELMAMOUN, A. R. (2017): Microbiological, epidemiological and antibiotic resistance study of Staphylococcus aureus in the milk of cows suffering from mastitis. Doctoral theses in science. Biological sciences. University Djilali abes. Sidi Bel-Abbes.160 p.
- BENIC, M., N. MACESIC, L. CVETNIC, et al. (2018): Bovine mastitis: a persistent and evolving problem requiring novel approaches for its control - a review. Vet. arhiv 88, 535-557. 10.24099/vet. arhiv.0116
- BENMOUNAH, B. (2002): Etiological prevalence of subclinical mastitis in the wilaya of Constantine. Master's thesis. Mentouri University of Constantine, 94 p.
- BOUAZIZ, O. (2005): Contribution to the study of intramammary infections of dairy cows in eastern Algeria. Doctoral thesis, Mentouri University, Faculty of Sciences, Constantine, Algeria, 235 p.
- BOUCHOUCHA, B. (2007): Subclinical mastitis of dairy cattles in the region of Mila et Constantine. Thésis of Magister. University Center of El-Taref. 113 p.
- BOUDRY, B. (2005): Milking quality milk: daily attention, Milk quality and herd management, Study Day for the AREDBs of Aubel, Herve-Fléron-Visé and Montzen and the Walloon Region, Department of Development and popularization, 13.
- BOUFAIDA, A., Z. ASNOUN, M. J. BUTEL and R. OUZROUT (2012): Prevalence of germs responsible of subclinical mastitis in the region of north in Algeria [in French]. Rev. Elevage. Med. Vet. Des pays tropicaux 65, 5-9. 10.19182/remvt.10132

- BOUKHECHEM, S., N. MIMOUNE, M. K. GHOZLANE, N. MOULA and R. KAIDI (2019): Status, characterization and typology of dairy cattle farms in northern Algeria. Bulletin UASVM Veterinary Medicine 76, 191-200. 10.15835/ buasvmcn-vm :2019.0022
- BOURAOUI, R., H. SELMI, A. MEKNI, I. CHEBBI and H. ROUISSI (2014): Impact of housing conditions and milking practices on mammary health and milk quality of dairy cows in Tunisia. Livest. Res. Rural. Dev. 26, 3.
- BUSATO, A., P. TRACHSEL, M. SCHALLIBAUM and J. W. BLUM (2000): Udder health and risk factors for Subclinical Mastitis in organic dairy farms in Switzerland. Prev. Vet. Med. 44, 205-220. 10.1016/S0167-5877(00)00104-5
- CAPON, S. (2010): Contribution to the study of teat lesions in dairy cows, Thesis Veterinary Lyon, France, 124 p.
- CVETNIĆ, L., M. SAMARDŽIJA, B. HABRUN, G. KOMPES and M. BENIĆ (2016): Microbiological monitoring of mastitis pathogens in the control of udder health in dairy cows. Slov. Vet. Res. 53, 131-140.
- CVETNIĆ, L. M. SAMARDŽIJA, S. DUVNJAK, B. HABRUN, M. CVETNIĆ, V. JAKI TKALEC, D. ĐURIČIĆ and M. BENIĆ (2021): Multi Locus Sequence Typing and spa Typing of Staphylococcus aureus Isolated from the Milk of Cows with Subclinical Mastitis in Croatia. Microorganisms 9, 725. 10.3390/microorganisms9040725
- CVETNIĆ, L., S. ŠPIČIĆ, G. KOMPES, B. HABRUN, V. KATALINIĆ-JANKOVIĆ, M. CVETNIĆ, M. ZDELAR-TUK, I. REIL, S. DUVNJAK, Ž. CVETNIĆ and M. BENIĆ (2022): Bovine mastitis caused by rapid-growth environmental mycobacteria. Vet. stn. 53, 493-501. 10.46419/vs.53.5.11
- ETIFU, M. and M. TILAHUN (2019): Prevalence of bovine mastitis, risk factors, isolation and antibio gram of major pathogens in Mid Rift valley, Ethiopia. Int. J. Livest. Prod. 10, 14-23. 10.5897/ IJLP2018.0517.
- FARTAS, H., Z. BOUZEBDA, F. AFRI and S. KHEMASSI (2017): Prevalence and impact of subclinical mastitis on the profitability of dairy cattle in the far east of Algeria. Livest. Res. Rural Dev. 29, 9.
- GUARIN, J. F., M. G. PAIXAO and P. L. RUEGG (2017): Association of anatomical characteristics of teats with quarter-level somatic cell count. J. Dairy Sci. 100, 1-10. 10.3168/jds.2016-11459
- HANZEN, C. H. (2016): Physio-anatomy and propaedeutic of the mammary gland Symptomatology, etiology and therapeutics. Individual and herd approaches to mastitis.Prof. Ch. Hanzen. Year 2015-2016. University of Liège. Faculty of Veterinary Medicine 170 p.
- HAJ-M'BAREK, R., L. MIGHRI and Y. M'SADAK (2013): Descriptive analysis of mastitis risk factors in soilless dairy herds in semi-arid environments (Tunisia). Rev. Mar. Sci. Agron. Vet. 3, 26-31.

- HAMLAOUI, M. W., F. Z. KAYOUECHE, A. BENMAKHLOUF, A. BADACHE and L. HAOUAR (2019): Influence of some intrinsic parameters related to the animal on the frequency of subclinical mastitis in dairy cows. Rev. Mar. Sci. Agron. Vet. 7, 433-436.
- HELEÏLI, N. (2002): Study of subclinical mastitis and the in vitro sensitivity of isolated germs to antibiotics. (Thesis of magister), Batna, Algeria, 202 p.
- KALANDI, M., A. SOW., V. MILLOGO, S. FAYE, A. G. OUEDRAOGO and G. J. SAWADOGO (2017): Prevalences & risk factors of subclinical mastitis in the traditional breeding of kaolack in Senegal. J. App. Biosci. 112, 10978-10984. 10.4314/jab. v112i1.2
- KARUMIRIBO, E. D., J. L FITZPATRIK, C. E. BELI., C. E. SWAI, D. M. KARUMBRAGE, M. J. OGDEN and N. P. FRENCH (2006): Clinical and subclinical mastitis in stallholder dairy farm in Tanzania: Risk, intervention and knowledge transfer. Prev. Vet. Med. 74, 84-98. 10.1016/j.prevetmed.2006.01.009
- KEMP, M. H., A. M. NOLAN, P. J. CRIPPS and J. L. FITZPATRICK (2008): Animal-based measurements of the severity of mastitis in dairy cows. Vet. Rec. 163, 175-179. 10.1136/vr.163.6.175.
- KENNEDY, B. W., M. S. SETHAR, A. K. W. TONG, J. E. MOXLEY, B. R. DOWNEY and P. LÉVESQUE (2003): The milking method reviewed: Cleaning the teats part 1, Le Producer du Lait Québécois, Canada, 28-29.
- KNEŽEVIĆ, K., V. DOBRANIĆ, D. ĐURIČIĆ, et al. (2021): Use of somatic cell count in the diagnosis of mastitis and its impacts on milk quality. Vet. stn. 52, 751-764. (In Croatian). 10.46419/vs.52.6.11
- LAMARI, I., N. MIMOUNE and D. KHELEF (2021): Effect of feed additive supplementation on bovine subclinical mastitis. Vet. stn. 52, 445-460. 10.46419/ vs.52.4.12
- 30. LEVESQUE, P. (2004a): The observation of the first jets. The Quebec milk producer. 43-44.
- LÉVESQUE, P. (2004b): Symposium on dairy bovine: how that logement and equipment influence the quality of milk? Conference Center of Reference on Agriculture & Agroalimentary of Québec (CRAAQ), Octobre 18 p.
- 32. LEVESQUE, P. (2006): Cows are They clean? The productor of milk Quebecois: 33-35.
- MAĆEŠIĆ, N., I. BAČIĆ, G. BAČIĆ, et al. (2022): Selective dry cow treatment. Vet. stn. 53, 735-743. (In Croatian). 10.46419/vs.53.6.8
- MEKONNEN, H. and A. TESFAYE (2010): Prevalence and etiology of mastitis and related management factors in market-oriented smallholder dairy farms in Adama. Ethiopia. Rev. Med. Vet. 161, 574-579.
- 35. MEZINE, D. M. C. S. (2006): Descriptive Analysis of risk factors related on mastitis in breeding of clientèle in Ardennes appliqueing la démarche GTV Parten aria. Thesis of Doctor Veterinary, veterinary high school of Alfort (France), 146 p.

- MIMOUNE, N., R. SAIDI, O. BENADJEL, D. KHELEF and R. KAIDI (2021): Alternative treatment of bovine mastitis. Vet. stn. 52, 639-649. 10.46419/vs.52.6.9
- M'SADAK, Y., L. MIGHRI and K. KRAIEM (2013): Study of factors of variation in level of cellular individual accompt of milk in small bovine herds above ground in Tunisia, Revue « Nature & Technology », B- Sciences Agronomies & Biology, N° 08, January 2013, 48-52.
- M'SADAK, Y., L. MIGHRI and K. KRAIEM (2014a): Study of cell counting of milk and descriptive analysis of risk factors of mastitis in small bovine herds above ground in Tunisia. Rev Nat tec. B. Scien. Agr. Biol. 10, 56-61.
- M'SADAK, Y., L. MIGHRI and K. KRAIEM (2014b): Health Situation of mammary and risk factors of mastitis in bovine dairy breeding in hors sol. Tunisia. Rev. Agri. 06, 47-53.
- M'TAALLAH, B., Z. OUBEY and H. HAMMAMI (2002): Estimation oof loss of milk and risk factors of subclinical mastitis with cell counting the tank milk of dairy bovine breeding. Revue Med. Vet. 153, 251-260.
- MUNGUBE, E. O., B. A. TENHAGEN, T. KASSA, F. REGASSA, M. N. KYUH, M. GREINER and M. P. BAUMANN (2004): Risk factors for dairy cow mastitis in the central highlands of Ethiopia, Trop. Anim. Heath Prod. 36, 462-472. 10.1023/b:trop.0000034999.08368.f3
- NIAR, A., K. GHAZI and S. Y. DAHACHE (2000): Incidence of mastitis on breeding of bovine in the wilaya of Tiaret, 4<sup>th</sup> Séminary International of Veterinary Medicine, Constantina, 21-22 November 2000.
- NMC (1987): Laboratory and field on handbook on mastitis. National Mastitis Council Inc., Arlington, VA22201, USA.

- 44. ROUSSEL, P. H. and D. RIBAUD (2000): Study of clinical and subclinical mastitis in primiparous calving patients. CR No. 2003112.
- RAKOTOZANDRINDRAINY, R., J. M RAZAFINDRAJAONA and G. FOUCRAS (2007): Rapid diagnostic in farm of subclinical mastitis in triangle of high lands of Madagascar. Rev. Vet. Med. 158, 105-108.
- SAIDI, R., D. KHELEF and R. KAIDI (2010): Evaluation of one rapid screening of subclinical mastitis test of dairy cows. Rev. Med. Vet. Trop. 63, 75-61. 10.19182/remvt.10098
- SAIDI, R. (2014): Quest on dairy mastitis in some bovine breeding in the center region (Algéria). Thesis of Doctorate. University of Blida. 230 p.
- SARGEANT, J. M., K. E. LESLIE, J. E. SHILEYI, B. J. PULKRABEK and J. H. LIM (2001): sensitivity and specificity of somatic cell count and CMT for identifying intramammary infections in early lactation. J. Dairy. Sci. 84, 2018-2024. 10.3168/jds. S0022-0302(01)74645-0
- 49. SIMON, D. and R. JEAN PHILIPPE (2005): Veterinary guide to investigation on the health of the mammary gland. Canadian Bovine Mastitis Research Network CBMRN, Faculty of Veterinary Medicine, University of Montreal, Canada, J2S 7C6, 26 p.
- TOMANIĆ, D. M. SAMARDŽIJA and Z. KOVAČEVIĆ (2023): Alternatives to Antimicrobial Treatment in Bovine Mastitis Therapy: A Review. Antibiotics 12, 683. 10.3390/antibiotics12040683
- TSURUTA, S., I. MISZATAL and T. J. LAWLOR (2004): Genetic correlations among production, body size, udder, and productive life traits over time in Holsteins. J. Dairy Sci. 69, 510-517. 10.3168/ jds. S0022-0302(04)73297-X

# Analiza utjecaja faktora rizika na pojavnost subkliničkog mastitisa na nekim farmama mliječnih krava na istoku Alžira

Brahim BOUCHOUCHA, Noureddine ZEGHILET, Rachida AIMEUR, Omar BOUAZIZ, GSPA Research Laboratory (Management of Animal Health and Productions), Institute of Veterinary Sciences of El-Khroub, Mentouri Brothers University of Constantine 1, Algeria; Nedjoua LAKHDARA, (Institute of Veterinary Sciences of El-Khroub, Mentouri Brothers University of Constantine 1, Algeria

Da bismo uočili učinak različitih faktora rizika na prevalenciju subkliničkog mastitisa, ispitali smo prevalenciju subkliničkog mastitisa u 104 (416 vimena) mliječnih krava iz 18 stada goveda koja se uzgajaju u tri provincije (wilaya) u istočnom Alžiru. Ispitivanje smo proveli pomoću kalifornijskog testa za mastitis (CMT) u kombinaciji s bakteriološkom analizom. Cilj je ovog istraživanja bio utvrditi učinak 27 faktora rizika povezanih sa životinjama, njihovim okolišem i praksom mužnje na prevalenciju, kao i učinak pasmine, dobi, faze laktacije, vrste staje i stelje, razine higijene, godišnjeg doba, proizvodnje mlijeka, razine vakuuma i eliminacije prvih mlazova na učestalost subkliničkog mastitisa. Provedena je anketa u svrhu prikupljanja podataka o broju krava, eksploatiranim pasminama, vrsti

uzgoja, staje, stelje i razini higijene na farmama, metodi mužnje i praksom mužnje. Proveden je CMT test u kombinaciji s bakteriološkim testiranjem za detekciju subkliničkog mastitisa. Chi 2 test je rabljen da bi se provjerila razlika između sredstava. Vrijednosti prevalencije bile su: 24 % prema CMT-u u usporedbi sa 17 % prema bakteriološkoj analizi. U svezi s vimenima, zabilježili smo stopu od 10 % u usporedbi s 8,7 %. Rezultati ankete pokazali su da je holstein pasmina najeksploatiranija i najviše zahvaćena (P<0,05); broj mliječnih krava varira od 3 do 20 krava. U ovoj studiji 56 % krava uzgajano je na farmama izgrađenima od opeke i one su imale najniže stope mastitisa u usporedbi s farmama izgrađenima od drva i trske (P<0,001). Staje za životinje imaju betonski pod za 33 % (životinja) u usporedbi s 36 % uzgajanih na podlozi od slame koje su bile više zahvaćene (P<0,001); stelja se podiže jednom na dan za 13 % krava, a to je uvjetovalo krave na ovu bolest. U usporedbi sa sredinom laktacije (P<0,05) učestalost je veća u ranoj i kasnoj laktaciji, niska morfologija vimena i desna strana vimena su utjecale su i na ovu bolest, uz higijenu životinja (P<0,05). Krave koje su proizvodile više od 12 L/dan bile su prijemčivije na bolest (P<0,05). Međutim, učestalost mastitisa malo je modificirana brojem laktacija krava. Visoki i niski vakuum strojeva za mužnju, učestalost impulsa više je uvjetovalo krave na ovu bolest (P<0,05). U ovoj studiji, krave koje su ručno mužene značajno su više bile pogođene ovom bolesti (P<0,05), posebice ljeti i zimi, pokazujući veće stope bolesti (P<0,05).

**Ključne riječi:** mliječna goveda, subklinički mastitis, istraživanje, CMT, bakteriološka analiza