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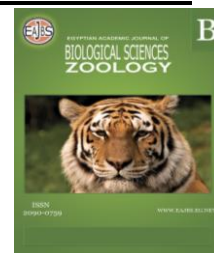


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## Study of some Factors Influencing the Occurrence of Subclinical Mastitis in Dairy Cattle in Western Algeria

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### ABSTRACT

Subclinical mastitis is an insidious disease of the mammary gland that negatively affects the production of the dairy cow. It is necessary to make a diagnosis in order to control them. The main objective is to determine the prevalence of subclinical mastitis and to identify the main risk factors related to the establishment of mammary infections, particularly subclinical mastitis with multi-resistant Staphylococcus in cattle farms in the region of Sidi-Bel-Abbès. The secondary objectives are to describe the characteristics related to the conduct and hygiene of milking that may increase the risk of intramammary infections. A total of 134 cows aged between 1 and 9 years, of Local Breed (n=3), Crossbreed (n=84) and *Holstein* Breed (n=47) in different stages of lactation. Milk samples collected from study cows were subjected to California Mastitis Test (CMT) for each udder quarter milk sample. All animals were clinically examined using a standardized questionnaire in an exhaustive manner and completed in real-time, interviewing the staff in charge of the farms and attending all stages of milking. All dairy cows were apparently healthy with clinically sound udder secreting apparently normal milk. The California Mastitis Test (CMT) was used to analyze the raw milk samples collected. The risk factors studied were either animal-related such as age, breed, lactation rank, stage of lactation (Early, Middle, Late) and parity, or environmental related such as season and collection area. Our results indicate that the prevalence of mastitis in crossbred cows (62.68%) and *Holstein* cows (35.07%) was significantly higher than that of homebred cows (2.23%) with a clear predominance at the age of 1 to 4 years. The result of CMT performed in the present study showed that the prevalence of subclinical mastitis in udder quarters was significantly higher in crossbred cows 204 (30.72%) than in *Holstein* cows 124 (36.9%) and local cows 8 (2.38%) respectively. The frequency of udder infections by *Staphylococcus* increases for both primiparous and multiparous. The analysis of variance shows that the occurrence of subclinical mastitis is influenced by the stage of lactation especially in early lactation compared to other stages ( $p<0.001$ ) and parity ( $p=0.041$ ) in crossbred cows. The Bacteriology was significantly influenced by stage of lactation ( $p=0.031$ ), breed ( $p=0.017$ ) and season ( $p=0.005$ ), respectively. The frequency of mammary infections by

Staphylococcus is increasing in both primiparous and multiparous cows. By comparing the sampling areas we can deduce that the western area of the region can be considered as a risk indicator compared to the northern area while the other two areas are not. On the other hand, the association of the two factors, i.e. stage of lactation and sampling zone, the risk of the western zone becomes insignificant ( $p=0.07$ ) while the middle of the stage of lactation persists as a non-risk phase. The present study allowed us to evaluate the frequency of subclinical mastitis and to identify the different bacterial strains of staphylococci responsible. In Conclusion, determining the risk factors for the development of Subclinical Mastitis allows us to establish a control program to improve the health of the udder of dairy cows and thus increase milk production.

## INTRODUCTION

Although several problems have hindered the livestock development in Algeria, diseases constitute a severe threat to the successful production of livestock and its industry. Therefore, any factor that adversely affects the quantity and quality of cattle milk is of great financial interest. Milk quality is mainly affected by bacterial contamination of the mammary gland, which causes mastitis (Boscós *et al.* 1996).

Cow milk production in Algeria is concentrated mainly in three zones ; Zone I (60% of dairy cows) is a coastal and a sublittoral with a humid and subhumid climate, Zone II (26% of dairy cows) is agropastoral and pastoral regions with a semi-arid and arid climate, Zone III (14%) is located in the Saharan region with a desert climate (Kali *et al.*, 2011). The Sidi-Bel-Abbès district in which this study is done belongs to Zone II.

Mastitis is one of the major problems of the dairy industry worldwide including in Algeria. It is to note that mastitis is the most common health problem with the highest economic impact in dairy farming (Seegers *et al.* 2003). Mastitis is a mammary gland infection, usually caused by bacteria that induce the inflammation of one or more quarters of the udder. However, so-called “aseptic” mastitis exists and it may be due to physiological disorders or local trauma, but it is much rare. Mammary gland infection may or may not be associated with clinical signs and are named as clinical mastitis and subclinical mastitis (SCM), respectively (Seegers *et al.* 2003).

The economic losses caused by mastitis are through disease control actions which include treatments and prevention and through other diverse losses, such as reduction in production, non-marketed milk, sales price penalties, mortality and anticipated reforms, bad milk quality. However, subclinical mastitis causes losses more than three times than clinical mastitis (Kayesh *et al.* 2014) because it is silent, more serious and causes much greater loss to the dairy industry (Abrahmsen *et al.* 2014).

Several risk factors such as host-related factors (age, parity stage of lactation, body condition), management practices (udder hygiene, husbandry system, floor disinfection and handwashing) and environmental factors (season and housing) have been reported to be important in the prevalence and epidemiology of subclinical mastitis (Bartlett *et al.* 1992, Almaw *et al.* 2008). To date, however, information is not available on the occurrence of subclinical mastitis nor is there information on the prevalent risk factors for mastitis on dairy farms in the country. The present study was therefore conducted to determine the prevalence of subclinical mastitis and its association of risk factors on farms and to assess their relationship to the occurrence of subclinical mastitis in lactating dairy cows.

## MATERIALS AND METHODS

The study was conducted from May 2019 to July 2021. A field survey for the prevalence of subclinical mastitis was carried out at all the districts of Sidi-Bel-Abbès Region (Western Algeria). The entire laboratory investigations were performed at the Microbiology Laboratory at the Departement of Agronomic Sciences, University of Sidi-Bel-Abbès, Algeria. The study was carried on dairy lactating cows belonging to the government and private dairy farms in all districts of Sidi-Bel-Abbès Region of Algeria. The 25 dairy farms are located in the perimeters of the region of Sidi-Bel-Abbès situated in the west of Algeria, 433 km from Algiers the capital. The dairy cows have differed from the breed point of view as 3 Local breeds, 84 cow Cross Breed and 47 *Holstein* breed. All dairy cows were apparently healthy with clinically sound udder secreting apparently. All animals were subjected to clinical and physical examinations with special interest towards the udder and teats. A simple random sampling method was considered to select the individual dairy cow. A questionnaire with the primary objective of identifying risk factors for subclinical mastitis was administered at every farm visited. The questionnaire was pre-tested prior to their final use. The questionnaire standardized has already been used in the study by Elmoslemany *et al.* (2010). It was completed in real-time, by interviewing staff in charge of the farms and assisting at all stages of milking. Some of the factors investigated included farm management and practices including host and environmental factors influencing the incidence of mastitis.

The udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed with cotton moistened with 70% ethyl alcohol. A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in CMT paddle. Then an equal amount of the CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. A cow or a quarter was considered to have subclinical mastitis if CMT score is 1, 2, or 3 and the California Mastitis Test (CMT) was carried out according to the method described by Quinn *et al.* (1999). All milk samples from cows with  $CMT \geq 2$  were subjected to further bacterial examination. The Techniques on culture methods, colonies morphology, differentiation of bacteria between Gram + and Gram – to identify isolated bacteria according to the International dairy Federation, 1981 (Waage *et al.* 1994).

The data was compiled and analysed with Statistical Package for Social Sciences (SPSS statistical package version 17). Prevalence estimation of positive cows was determined using standard formulae (that is, the number of positive animals/samples divided by the total number of animals/samples examined). Descriptive statistics such as percentages and frequency distributions were used to describe/present the nature and the characteristics of the data.

## RESULTS AND DISCUSSION

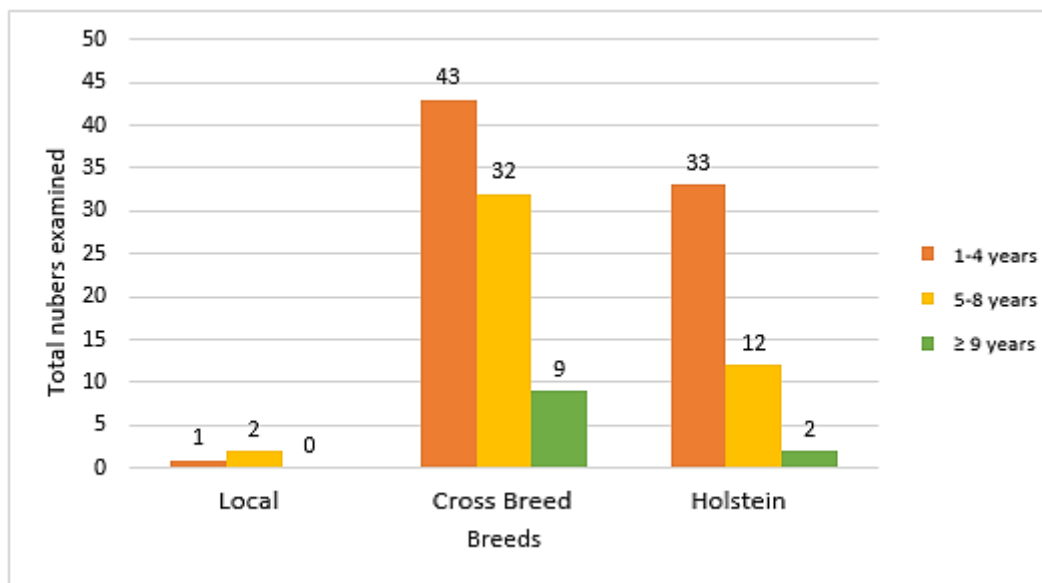
The present study was conducted to investigate the prevalence of subclinical staphylococcal mastitis isolated from raw milk of lactating cows in western Algeria, using the CMT test and to identify associated risk factors.

In our study, farmers are not at all familiar with the concept of mastitis because of the lack of visible signs. Therefore, cows were never screened by a screening test or sampled for bacteriological culture to detect subclinical mastitis. Veterinary assistance was requested only in the case of severe clinical mastitis. Therefore, informing farmers and raising awareness about subclinical mastitis in our region remains a major goal.

Subclinical mastitis has a multifactorial nature that involves a clear interaction between the host, the causative agent and the environment (Thrusfield, 2005). For this reason, the factors studied here were identified as hypothetical risk factors affecting mastitides such as breed (Bendixen *et al.* 1988), season, age (Hultgren, 2002), management, environment (McDougall, 2003) and hygiene (Ward *et al.* 2002).

#### The Relationship Between Subclinical Mastitis and Age:

The highest prevalence of SubClinical Mastitis was recorded in the age group of 1–4 years which 43 (55.84%) of crossbred cows, 33 (42.86%) of Holstein cows and 1 (1.29%) Local breed followed by the group of cows with the age group of 5–8 years which 32 (69.57%) crossbreed and 12 (26.09%) *Holstein* cows, 2 (2.17%) Local breed and the least was recorded in the group of cows with age greater than 09 years when tested with CMT test which 9 (81.82) % and 2 (18.18%) in crossbreed and Holstein cows, respectively (Fig. 1).



**Fig.1:** Distribution of dairy cows with Subclinical Mastitis by Breed and Age (Years)

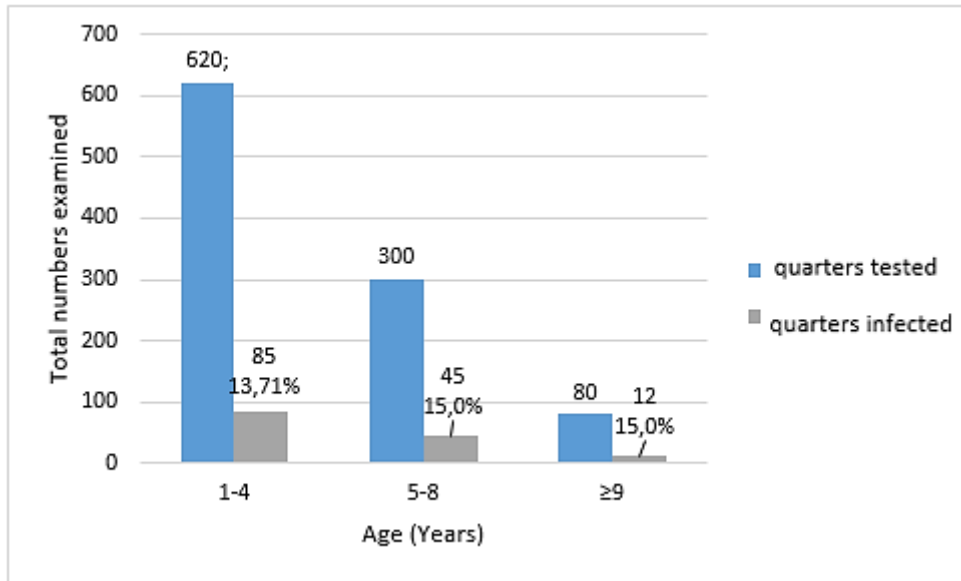
The Cows can get udder infections at different ages. Cows also vary in their ability to overcome an infection, once established. The occurrence of mastitis may be influenced by certain hereditary characteristics such as milk-producing capacity, teat structures and conformation (Schutz *et al.* 1993, Radostits *et al.* 1994).

Our results are in disagreement with those reported by these authors since the 1-4 year age group was more susceptible to subclinical mastitis than those of 5-8 years and older and in agreement with that of (Elbably *et al.* 2013) who reported that the 3-5 year age group is the most affected by subclinical mastitis. However, studies in other regions have reported a higher prevalence of heifers compared to older cows; this may be due to differences in management practices and poor hygiene during calving (McDougall *et al.* 2009).

The prevalence of staphylococcal infected quarters in the present study is higher in the 1-4 year age group with 85 (13.71%) quarters because staphylococci are adapted to survive in the udder and establish subclinical and chronic infections (Abera *et al.* 2010).

The prevalence of SCM of cows detected by CMT test indicated that the highest incidence of bovine mastitis was in a single quarter followed by two and four and the least was recorded in the three quarters. The prevalence of udder quarters infected with staphylococci in the present study was higher in the group of 1- 4 years with 85 (13.71%)

infected udder quarters because *S. aureus* is adapted to survive in the udder and establish subclinical and chronic infections (Abera *et al.* 2010) (Fig. 2).



**Fig 2.:** The relationship between infected udder quarters and Age (Years)

Haftu *et al.* (2012) showed that the prevalence of udder quarters infected increases with age. This can be attributed to the moment and prolonged duration of infection, especially in a herd without a mastitis control program. The possible difference in mammary pathogenicity of organisms involved in intramammary infection among genotypes may also exist (Gonzalez-Rodriguez *et al.* 1995). The high prevalence of clinical mastitis in older cows may be due to decreased immunity and bacterial resistance to antibiotics that have been used indiscriminately for mastitis treatment during previous infections (Kurjogi *et al.* 2014).

**The Relationship Between Subclinical Mastitis and Breed:**

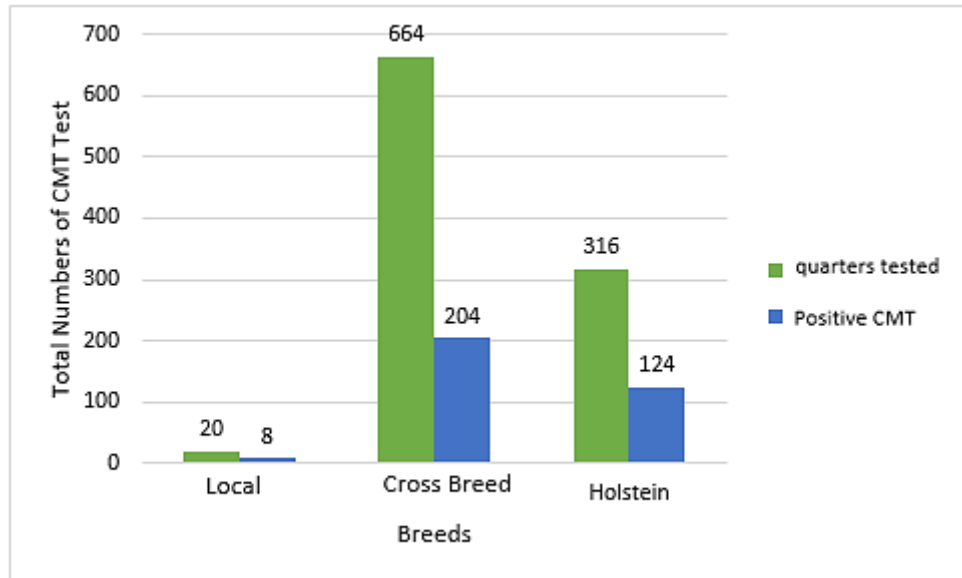
In the present study, it was found that subclinical mastitis was significantly influenced by the breed of cows ( $P = 0.026$ ) (Table 1).

Mastitis has a multifactorial nature that predominates with a clear interaction between host, agent and environment (Thrusfield, 2005). For this reason, the studied factors here were determined as hypothesized risk factors affecting mastitis. After studying the breed factor, it is found that the prevalence of subclinical mastitis is lower in the local breed of cows (2.38 %) than the crossbred cows (60.71%) and the *Holstein* (36.90%) ( $P = 0.026$ ).

**Table 1:** The relationship between Subclinical Mastitis and Breed.

Breed	Quarters tested	Positive CMT (%)	$\chi^2$ (p)
Local	20	8 (2, 38%)	7,337 (0,026)
Cross breed	664	204 (60, 71%)	
<i>Holstein</i>	316	124 (36, 90%)	
Total	1000	336 (100%)	





**Fig 3:** Distribution of positive CMTs by Breed

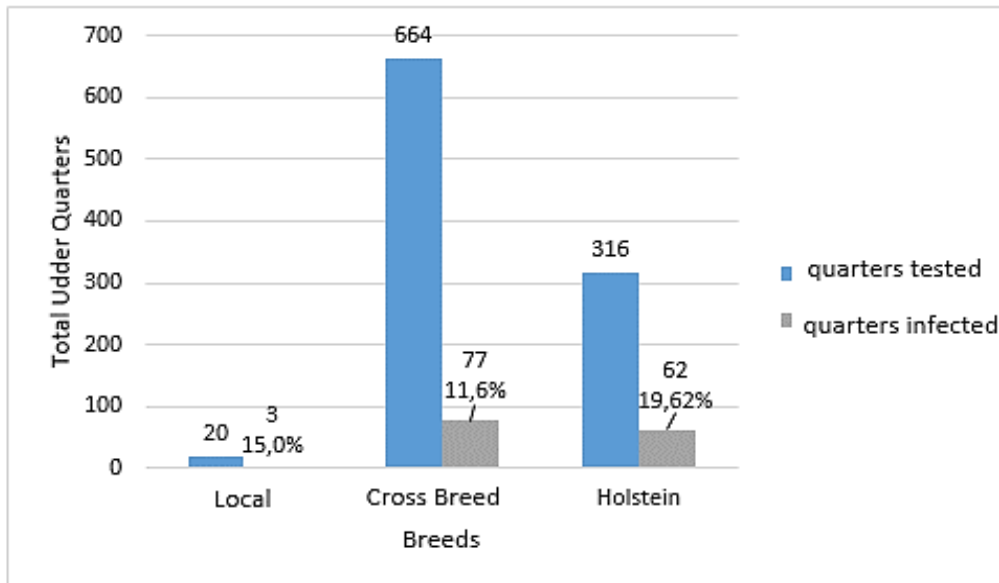
The results of the CMT performed in the present study showed that the prevalence of subclinical mastitis at the level of quarters was significantly higher in crossbred cows 204 (30.72%) quarters than in *Holstein* cows 124 (36.9%) and local cows 8 (2.38%) quarters respectively (Table 1 and Fig. 3). This high prevalence may be attributed to the type of breed of the animals since most of the cattle involved in this study were crossbred. On the other hand, this difference between breeds could be due to other uncontrolled factors, such as farm management, rather than a true breed difference, since not all cows in this study are subjected to similar rearing conditions ; this is consistent with the study by (Lakew *et al.* 2009) who found this same difference.

According to (Moges *et al.* 2012), the prevalence of subclinical mastitis was high in all breeds. In addition, Kurjogi *et al.* (2014) stated that there was no significant relationship between cow breed and subclinical mastitis. However (Radostits *et al.* 2007) stated that high-yielding cows are more susceptible to mastitis than low-yielding ones. This may be due to the ease with which injuries are sustained in large udders, so foci for pathogen entry are created and the stress associated with high milk yield can disrupt the animal's defense system.

The influence of breeds on the prevalence of mastitis can be attributed to the difference in certain physiological and anatomical characteristics of the mammary glands (Biffa *et al.* 2005), than to hereditary characteristics and immunity (Kurjogi *et al.* 2014).

In Algeria, Ghazi and Niar (2006) reported that local breeds are more resistant compared to the imported ones. This is primarily due to the genetic resistance, the inherent low milk productivity and also the bad adaptation of these cows to the local environment and climate (Payne and Wilson, 1999).

In our region, the imported *Holstein* breed has a low milk yield compared to their country of origin, and it encounters many more health problems. This is mainly due to genetic resistance and also to the poor adaptation of these cows to the local environment and climate (Payne and Wilson 1999 ; De Vlieghe *et al.* 2012).



**Fig 4:** Frequency and Number of infected udder quarters (%) by Breed

Our results indicate that there were no significant differences between breeds and the frequency of infected udder quarters which 77 (11.60%) of the quarters were infected with staphylococci in the crossbred breed, 62 (19.62%) in the *Holstein* imported breed and 3 (15%) in the Local breed (Fig. 4). Our findings were similar to the report of Harouna *et al.* (2009) who indicated that probably related to the similarity in environment and livestock practices.

Further studies are needed to assess the breed difference and to shed light on the infection rates between local and imported breeds.

#### **The Relationship Between Staphylococci Isolated from Subclinical Mastitis and Lactation Rank:**

In the present study, the frequency of mammary infections in dairy cows by *Staphylococcus* increases for both primiparous and multiparous (Table 2). This could be explained by the neglect of cases of subclinical mastitis while allowing them to develop further. However, Rakotozandrindrainy *et al.* (2007) and Saidi *et al.* (2010) found that multiparous cows are more affected by mastitis than primiparous ones.

The age-multiparous-high prevalence is explored to be due to all increases in teat patency and degree and frequency of previous exposure in multiparous old cows (Harmon, 1994 ; Radostits *et al.*, 1994). According to Erskine (2001), primiparous cows have a more effective defense mechanism than multiparous cows.

According to (Waage *et al.*, 1999) the proportion of *S. aureus* did not differ between cows during prepartum and postpartum. *S. aureus* is a contagious organism, and transfer during milking is considered an important mechanism for the spread of this organism from one cow to another (Bramley *et al.* 1984). Roberson *et al.* (1998) suggested that milk from infected cows in herds was the most likely source for *S. aureus* IMI in heifers. *S. aureus* can be encountered at any time during lactation, suggesting that infection spreads from infected to healthy quarters during milking (Guerin, 1998). It has been reported that *S. aureus* has adaptive mechanisms that allow it to shed on the udder and cause intra-mammary infections during the milking process (Radostits *et al.*, 1994).

Coagulase-negative staphylococci have been isolated from the udder of empty, pregnant, or freshly calved cows (Trinidad *et al.*, 1990 ; Aarestrup *et al.*, 1999 ; De Vliegher *et al.*, 2003). This means that coagulase-negative staphylococci are important in cows of all ages (Taponen *et al.*, 2006).



**Table 2:** The relationship between a mastitis-causing pathogen (Staphylococci) isolated from subclinical mastitis in dairy cows and lactation rank.

Bacterial strain	Rank								Total
	1	2	3	4	5	6	7	8	
<i>Xylopus</i>	2	1	2	3	1	1	0	1	11
<i>Hominis</i>	0	1	4	0	0	0	1	0	6
<i>Caprae</i>	2	2	0	0	1	0	0	1	6
<i>Intermedius</i>	2	1	2	0	0	1	0	0	6
<i>Auricularis</i>	0	0	1	0	0	1	0	0	2
<i>Aureus</i>	2	2	3	3	1	2	1	7	21
<i>Chromogenes</i>	1	1	1	1	0	1	0	0	5
<i>Simulans</i>	0	1	1	1	0	0	0	0	3
<i>Scuiri</i>	0	0	0	1	0	0	0	0	1
<i>Epidermis</i>	1	1	1	2	0	1	0	0	6
<i>Warneri</i>	0	2	0	0	0	0	0	0	2
<i>Capitis</i>	1	1	0	0	0	0	0	0	2
<i>Hemolyticus</i>	3	0	0	1	0	0	1	0	5
<i>Hyicus</i>	0	2	0	0	0	0	0	0	2
<i>Lentus</i>	2	0	0	0	0	0	0	0	2
Total	16	15	15	12	3	7	3	9	80

Chronic Staphylococcal infections are common and likely to recur in later lactations (Almaw *et al.* 2008, Aphis 2008 and Haftu *et al.* 2012). This is due to the continuous exposure to infectious pressure during different lactations and the decline in natural defenses in the mammary gland of older cows (Badinad, 2003).

#### **Relationship Between the Frequency of Subclinical Mastitis, Stage of Lactation, Parity and Breed:**

In the present study, the prevalence of Subclinical Mastitis was significantly influenced by the stage of lactation ( $p < 0.001$ ) and parity ( $p = 0.041$ ) in crossbred cows and by the area of sampling ( $p = 0.043$ ) (Tables 3 and 4).

**Table 3:** Association between some of the factors (Stage of Lactation, Parity and Breed) with the occurrence of Subclinical Mastitis.

Breeds	Factors	Detected	Subclinical mastitis		X <sup>2</sup>	Ddl	P			
			N	%						
Local	Early	0	0	0,0	16,744	2	<0,001			
	Mid	1	1	33,3						
	Late	4	2	66,7						
Cross Breed	Early	12	9	10,7						
	Mid	87	31	36,9						
	Late	67	44	52,4						
<i>Holstein</i>	Early	9	7	14,9				3,427	2	0,180
	Mid	33	16	34,0						
	Late	37	24	51,1						
Local	Primiparous	0	0	0,0	4,172	1	0,041			
	Multiparous	5	3	100,0						
Cross Breed	Primiparous	21	15	17,9						
	Multiparous	145	69	82,1						
<i>Holstein</i>	Primiparous	18	8	17,0				2,191	1	0,139
	Multiparous	61	39	83,0						

According to Biffa *et al.* (2005), the stage of lactation affects the prevalence of mastitis significantly ( $P < 0.001$ ). An early-stage and period of involution of the mammary glands were the most sensitive phase and lowest for cows in mid-lactation.

A similar result was observed, by Bitew *et al.* (2010) who showed that a significantly higher infection was found in cows, In Ethiopia, with early and late lactation stages than the middle stage of lactation in crossbreeds ( $p < 0.05$ ). There was a significant difference in the prevalence of mastitis in cows with different parity numbers ( $p < 0.05$ ). Similar results were reported by the studies of Corbett (2009) and Lakshmi *et al.* (2009). Also, Elbably *et al.* (2013), showed that the stage of lactation had a highly significant effect on the prevalence of mastitis ( $p < 0.003$ ). This may be due to a lack of dry period therapy and calving influences (Abera *et al.*, 2010). The udder is more susceptible to subclinical mastitis during the period after parturition (Abrahmsen *et al.*, 2014).

The absence of dry cow treatment could be among the main factors contributing to the high prevalence of early lactation. During the dry period due to the low bactericidal and bacteriostatic quality of milk, pathogens can easily enter the teat canal and multiply; this can be replicated in the post-calving period (Biffa *et al.*, 2005).

In Ireland, Berry *et al.* (2005), explained that the increased prevalence of mastitis at an early stage of lactation can be attributed to changes in both the stage of lactation can be attributed to changes in both host defense mechanisms and host defense mechanisms and too non-specific host defense mechanisms in the peripartum period in the peripartum period in the dairy cow.

According to studies by (Mungube *et al.*, 2004; Getahun *et al.*, 2008; Almaw *et al.* 2008) in Ethiopia and in India (Sudhan *et al.*, 2005), cow quarters at the end of lactation were more susceptible to subclinical mastitis than cow quarters at the beginning of lactation.

Our results are in agreement with the research results of Busato *et al.* (2000) and Moges *et al.* (2012) who found that the risk of subclinical mastitis increases significantly in multiparous cows.

#### **Association Between the Prevalence of Positive Bacteriology and the Various Risk Factors:**

In the present study, the stage of lactation, the breed and the season have significantly influenced the results of bacteriology, respectively ( $p = 0.031$ ,  $p = 0.017$  and  $p = 0.005$ ) (Table 4). Guerin *et al.* (2007) showed that the new infections caused by *S. aureus* and coagulase-negative staphylococci, mainly occur during the first weeks of lactation and the onset of the dry period.

The dry period has been reported to inhibit the phagocytic action of neutrophils in the udder and, during the dry period, the ability of the udder quarters to provide phagocytic and bactericidal phagocytic and bactericidal activities is diminished, leading to a high infection rate (Sori *et al.*, 2005). Furthermore, Compton *et al.* (2007a) showed that the Intra-Mammary Infection (IMI) of pre-calving environmental pathogens affects host immunity and increases the risk of subclinical mastitis in the post-calving period. Although, Compton *et al.* (2007a) showed that the isolation of *S. aureus* in early lactation in lactating cows significantly increases the risk of subclinical mastitis during first lactation (Compton *et al.*, 2007b ; Paradis *et al.*, 2010 ; Piepers *et al.*, 2010).

The season also influences the frequency of udder infections, following the considerable increase in temperature (Rakotozandrindrainy *et al.*, 2007).

### Analysis of Univariate and Multivariate Logistic Regression of the Prevalence of Risk Factors for Subclinical Mastitis:

Our results show that the risk caused by the early stage of lactation cannot be excluded with a predominance of occurrence of subclinical mastitis during the early stage of lactation compared to the end. On the other hand, a value of 0.357 with a  $p < 0.001$  indicates that the cow is far from being affected by subclinical mastitis during the lactation stage.

**Table 4:** The association between the prevalence of subclinical mastitis, positive bacteriology and various risk factors.

		Bactériology		X <sup>2</sup>	ddl	Subclinical Mastitis		X <sup>2</sup>	Ddl	P	
		positive				N	%				
		N	%			N	%				
Age (Years)	1-4	55	59,78	0,474	2	0,789 <sup>NS</sup>	77	57,46	2,778	2	0,249 <sup>NS</sup>
	5-8	30	32,61				46	34,33			
	≥9	7	7,61				11	8,21			
Rank of Lactation	1	17	18,48	4,592	7	0,710 <sup>NS</sup>	23	17,16	6,529	7	0,480 <sup>NS</sup>
	2	23	25,00				34	25,37			
	3	17	18,48				22	16,42			
	4	13	14,13				19	14,18			
	5	6	6,52				14	10,45			
	6	7	7,61				9	6,72			
	7	3	3,26				4	2,99			
	8	6	6,52				9	6,72			
Stage of Lactation	Eraly	11	11,96	6,976	2	0,031*	16	11,94	19,212	2	<0,001***
	Mid	35	38,04				48	35,82			
	Late	46	50,00				70	52,24			
Breed	Local	2	2,17	8,035	2	0,017*	3	2,24	1,844	2	<sup>NS</sup> 0,432
	Cross breed	51	55,43				84	62,69			
	Holstein	39	42,39				47	35,07			
Season	Autumn	15	16,30	10,732	2	0,005**	21	15,67	3,568	2	0,168 <sup>NS</sup>
	Winter	0	,00				0	,00			
	Spring	28	30,43				51	38,06			
	Summer	49	53,26				62	46,27			
Parity	Primiparous	17	18,48	0,916	1	0,339 <sup>NS</sup>	23	17,16	0,537	1	0,464 <sup>NS</sup>
	Multiparous	75	81,52				111	82,84			
Area of sampling	North	15	16,30	6,978	3	0,073 <sup>NS</sup>	28	20,90	8,157	3	0,043*
	South	23	25,00				28	20,90			
	East	28	30,43				37	27,61			
	West	26	28,26				41	30,60			

<sup>NS</sup>: No significant; \*: Significant; \*\*: high significant; \*\*\*: highly significant; N: Number; %: percentage;  $\chi^2$ : value of chi deux; ddl: degree of freedom; p: signification.

By comparing the sampling areas, we can say that the Western area can be considered as a risk indicator compared to the Northern area while the other two areas do not. Finally, by combining the two factors, that is to say by taking into account both the stage of lactation and the sampling area, the risk of the western area is no longer significant ( $p = 0.07$ ) while the mid-stage of lactation persists as a risk-free phase (Table 5).

**Table 5:** Univariate and multivariate logistic regression analysis of the prevalence of subclinical mastitis risk factors.

Indicators of risk		Subclinical Mastitis		Analyse univariate				Analyse multivariate			
		N	%	p	OR	IC 95%		p	OR <sub>adj</sub>	IC 95%	
						BI	BS			BI	BS
Stage of Lactation	Early	16	11,94	0,316	1,737	0,590	5,110	0,329	1,725	0,578	5,151
	Mid	48	35,82	<0,001	0,357	0,209	0,611	<0,001	0,376	0,218	0,647
	Late <sup>a</sup>	70	52,24	<0,001				<0,001			
Constante				0,002	1,842						
Area of sampling	North <sup>a</sup>	28	20,90	0,045				0,123			
	South	28	20,90	0,936	1,029	0,509	2,084	0,888	0,949	0,456	1,974
	East	37	27,61	0,090	1,850	0,909	3,764	0,160	1,694	0,812	3,532
	West	41	30,60	0,021	2,330	1,136	4,775	0,070	1,990	0,945	4,193
Constante				0,379	0,800			0,330	1,350		

a : reference category ; OR : Odds Ratios ; IC 95% : intervalle de confiance à 95% ; BI: lower boundary ; BS: upper bound ; p :signification ; N : Number ; % : percentage.

## Conclusion

The present study allowed us to evaluate the frequency of subclinical mastitis and to determine the risk factors associated with the occurrence of subclinical mastitis in dairy cows. The relatively high prevalence reported in this study clearly indicated a lack of strategic control measures against the disease, as well as poor surveillance measures. Lack of maintenance of strict hygiene and a good sanitary environment may be contributory factors in the cause of subclinical mastitis. It is therefore important that farmers should ensure strict personal hygiene and that of animals, and general sanitary conditions of the farms should be improved and maintained. Furthermore, all dairy producers should know that early detection of intramammary infection is important for selecting and implementing proper therapy. Unfortunately, most infections are not detected until they become clinical, and by then, extensive and costly damages could result. Routine milk cultures should be an ongoing part of any mastitis control program. The sampling strategies for any ongoing program require the input of the herd veterinarian, as well as herd management. Finally, determining the risk factors for the development of Subclinical Mastitis allows us to establish a control program to improve the health of the udder of dairy cows and thus increase milk production.

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## ARABIC SUMMARY

دراسة بعض العوامل المؤثرة في حدوث التهابات الضرع تحت السريرية في أبقار الألبان في غرب الجزائر

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التهاب الضرع تحت السريري هو مرض خبيث يصيب الغدة الثديية ويؤثر سلبيًا على إنتاج بقرة الألبان. من الضروري إجراء التشخيص من أجل السيطرة عليها. الهدف الرئيسي هو تحديد مدى انتشار التهاب الضرع تحت الإكلينيكي وتحديد عوامل الخطر الرئيسية المتعلقة بتكوين عدوى الثدي، وخاصة التهاب الضرع تحت السريري مع المكورات العنقودية متعددة المقاومة في مزارع الأبقار في منطقة سيدي بلعباس. الأهداف الثانوية هي وصف الخصائص المتعلقة بسلوك ونظافة الحلب التي قد تزيد من خطر الإصابة بالعدوى داخل الثدي. ما مجموعه 134 بقرة تتراوح أعمارهم بين 1 و 9 سنوات، من السلالات المحلية (ن = 3) ، هجين (ن = 84) وهولشتاين سلالة (ن = 47) في مراحل مختلفة من الرضاعة. تم إخضاع عينات اللبن التي تم جمعها من أبقار الدراسة لاختبار كالفورنيا لالتهاب الضرع (CMT) لكل عينة من ربع لبن الضرع. تم فحص جميع الحيوانات سريريًا باستخدام استبيان موحد بطريقة شاملة وتم إكمالها في الوقت الفعلي، وإجراء مقابلات مع الموظفين المسؤولين عن المزارع وحضور جميع مراحل الحلب. يبدو أن جميع أبقار الألبان تتمتع بصحة جيدة مع وجود ضرع سليم سريريًا يفرز حليبًا طبيعيًا على ما يبدو. تم استخدام اختبار كالفورنيا لالتهاب الضرع (CMT) لتحليل عينات الحليب الخام التي تم جمعها. كانت عوامل الخطر المدروسة إما مرتبطة بالحيوان مثل العمر، والسلالة، ورتبة الإرضاع، ومرحلة الرضاعة (المبكرة، والمتوسطة، والمتأخرة) والتكاثر، أو مرتبطة بالبيئة مثل الموسم ومنطقة التجميع. تشير نتائجنا إلى أن انتشار التهاب الضرع في أبقار الهجين (62.68%) وأبقار هولشتاين (35.07%) كان أعلى بكثير من انتشار الأبقار المحلية (2.23%) مع انتشار واضح في سن 1 إلى 4 سنوات. أظهرت نتيجة CMT التي أجريت في هذه الدراسة أن انتشار التهاب الضرع تحت الإكلينيكي في الضرع كان أعلى بشكل ملحوظ في الأبقار الهجين (30.72%) منها في أبقار هولشتاين (36.9%) والأبقار المحلية (2.38%) على التوالي. يزداد تواتر عدوى الضرع بالمكورات العنقودية لكل من المولودات والولادة. يوضح تحليل التباين أن حدوث التهاب الضرع تحت الإكلينيكي يتأثر بمرحلة الرضاعة خاصة في فترة الرضاعة المبكرة مقارنة بالمرحلة الأخرى ( $P < 0.001$ ) والتكاثر ( $p = 0.041$ ) في الأبقار المهجنة. تأثرت البكتيريا معنويًا بمرحلة الإرضاع ( $E = 0.031$ ) ، السلالة ( $E = 0.017$ ) والموسم ( $E = 0.005$ ) على التوالي. يتزايد تواتر الإصابات الثديية بالمكورات العنقودية في كل من الأبقار البدائية والمتعددة. من خلال مقارنة مناطق أخذ العينات يمكننا أن نستنتج أن المنطقة الغربية من المنطقة يمكن اعتبارها مؤشرًا للمخاطر مقارنة بالمنطقة الشمالية بينما المنطقتين الأخرين ليست كذلك. من ناحية أخرى، فإن ارتباط العاملين، أي مرحلة الإرضاع ومنطقة أخذ العينات، يصبح خطر المنطقة الغربية ضئيلاً ( $P = 0.07$ ) بينما يستمر منتصف مرحلة الرضاعة كمرحلة غير خطيرة. سمحت لنا الدراسة الحالية بتقييم وتيرة التهاب الضرع تحت الإكلينيكي والتعرف على السلالات البكتيرية المختلفة للمكورات العنقودية المسؤولة. في الختام، فإن تحديد عوامل الخطر لتطور التهاب الضرع تحت الإكلينيكي يسمح لنا بوضع برنامج تحكم لتحسين صحة ضرع الأبقار الحلوب وبالتالي زيادة إنتاج الحليب.

**الكلمات المفتاحية:** التهاب الضرع تحت السريري، اختبار التهاب الضرع في كالفورنيا، علم البكتيريا، سيدي بلعباس، الجزائر.