

## Prevalence and Identification of Yeasts Responsible for Mastitis in Dairy Cattle Farms in the Sidi Lahcene Region in the Wilaya of Sidi Bel abbes-Algeria

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### Abstract

Mastitis represents one of the main diseases in dairy cows. In Algeria, very few studies have been conducted on the prevalence of fungal mastitis in dairy cattle farms as well as various factors favouring their appearance and development. In most cases, the triggers this infection is bacteria. A growing number of fungi are currently associated with this pathology.

This is related to antibiotics too widely used in the treatment of these bacterial agents. So we set as objectives, determining the prevalence of mastitis caused by yeasts and the study of a number of risk factors in some dairy farms in the region of Sidi Lahcène, wilaya of Sidi Bel Abbes. The samples of this study were carried on farms of cows (mastitic cow and clinically healthy cow) belonging to two types of farms (farms with manual milking and mechanical milking farms).

The risk factors included, animal secretions, the teat cups, the hands of the milkers, the skin of the mammary gland, the drinker, the manger, the milk storage tank, the milk collection seal. Mycological analysis was conducted at the Laboratory of Parasitology-Mycolology from the Higher National Veterinary School-Algiers.

The isolated yeasts were identified using microscopic characterization, and auxanogramme realized in biochemical galleries (gallery Pasteur Institut Pasteur Algiers and testing API<sup>®</sup> AUX BioMerieux, France). Our results showed a prevalence of infection with yeast, a high frequency of isolation was attributed for the genus *Candida* sp. followed by the genus *Rhodotorula* sp. followed by the two genera *Cryptococcus* sp. and *Trichosporon* sp.

**Keywords:** Cattle; Mastitis; Yeasts; Milking machine; Trayeur; Antibiotics

### Introduction

Inflammation of the udder is one of the first three pathologies of dairy cows. This pathology can be in apparent (latent infection, subclinical mastitis), it is the most frequent (95 to 98% of the cases) and can be apparent (clinical mastitis), the latter only represents 2 to 5% of the cases [1].

Bovine mastitis is a multifactorial disease involving interrelationship between host, environment and infectious agents. It is considered the most widespread and infectious disease of dairy cows economically important on all continents, with annual losses estimated at \$35 trillion in the world dairy industry [2].

Several microorganisms are involved in the etiology of intramammary infections in cattle, represented mainly by bacteria, viruses, mycoplasmas, algae and yeasts [3]. Yeasts are found in moist places that are rich in organic matter, and are easily isolated from teats and milking equipment [4]

Mastitis foci caused by yeasts have been reported in intensively managed herds in which there were deficiencies in environmental hygiene or in combination with repeated intramammary treatment

[5,6]. Several species of yeasts of the genus *Candida*, *Cryptococcus*, *Rhodotorula* and *Trichosporon* have been associated with mastitis in dairy cows. *Candida* is usually the most frequently isolated genus, with large variations in prevalence and species identified [7,8].

Studies on fungal infections of the mammary glands in cows are becoming more frequent due to their increasing incidence. According to the literature, these infections account for 2%-13% of all cases of mastitis in cows [9-11]. For this reason we were interested in this pathology by conducting a study in a few dairy cattle farms in the region of Sidi Lahcène, wilaya of Sidi BelAbbes, by distributing a questionnaire to the veterinary practitioners of the region in order to identify this pathology by realizing milk simples and swabs in targeted dairy cattle for mycological analysis to identify responsible yeasts and determine the prevalence of this disease in the intended dairy herds.

### Materials and Methods

#### Description of the study region

Sidi Lahcene formerly Dètrie during the French colonization is one of the 52 communes of the wilaya of Sidi Bel Abbes. Located in the west of Algeria, 150 km from the Moroccan border.

### Distribution of a questionnaire

A total of 50 questionnaires were distributed to veterinary practitioners in the region, including the farms covered by our study. The questionnaire was distributed by the veterinary inspector of the wilaya of Sidi Bel Abbas to the veterinarians of the region of Sidi Lahcène which in turn are moved to the farms of the region as well as the farms included in the study and they filled the questionnaire on site, which allowed us to recover all distributed questionnaires (50 questionnaires).

The questionnaire focused on the characteristics of the different livestock farms, the practices of the milkers with regard to the cleaning of the milking equipment, the cleaning of their hands, the preparation of the udders to milking, the environment of dairy cows, litter, the type of housing, their pathological history (mastitis).

### The study period

The samples were taken during the second quarter of 2012 (March, April, and May 2012). The mycological analysis was carried out during the first half of 2013 (from January 2013 until July 2013).

### Nature and number of samples

A total of 562 samples were collected by the veterinarians' practitioners of Sidi Lahcene's region. That is 280 samples of milk taken

from 70 existing cows in 13 farms; all the farms exist in the same region, wilaya of Sidi Bel Abbas. And 275 swabs performed on 13 farms (65 swabs of the breeding equipment and 210 swabs on the cow).

The samples were obtained with different mammary glands health status: 10 cows with healthy mammary glands, 46 cows with subclinical mastitis as determined by the California Mastitis Test (CMT) (leucocytost, Symbiotic Europe, France) and 14 cows with clinical mastitis was defined by: swelling, reduced milk flow and abnormal milk appearance, additionally, other signs of infection as fever, inappetence, ataxia. CMT was used to identify subclinical mastitis on mammary gland of the cows. For this study, milk samples from gland affected with subclinical mastitis were included when the reaction to CMT was at least grade 2, corresponding with an appearance of viscous milk that does not adhere to the bottom of CMT plate and correlates to 400,000-1,500,000 somatic cells/ml.

On every cow in lactation: 04 takings of milk (a taking of milk of every trayon), an anal swab, a vaginal swab and swabs of the mammary gland, only once during all the period of the taking. In every breeding with manual milking, it made a swabbing of the hands of the milker before the milking (factor of contamination), a swabbing of the feeder, a swabbing of the milk bucket and it made water samples from the water trough. Same taking were made in the breedings with machine milking except swabbing of tumblers milkers of the milking machine (factor of contamination) (Table 1).

Nature of the taking	Number of samples		
	Manual milking	Milking machine	Total
Number of cows	18	52	70
Numbers of milk samples	72	208	280
Numbers of anal swabs	18	52	70
Numbers of vaginal swabs	18	52	70
Number of swabs of the mammary gland	18	52	70
Number of swabs of the feeder	06	07	13
Number of swabs of drinking trough	06	07	13
Number of water samples from the water trough	04	03	07
Number of milk bucket swab	06	07	13
Number of swab of the cistern	06	07	13
Number of swabs on the hands of the milker	06	00	06
Number of swabs of the milking machine	00	07	07
Total samples taken	160	402	562

**Table 1:** Sampling plan.

### Milk sampling

The correct realization of the sampling procedure was a necessity, regarding the ubiquity of fungi which can contaminate the milk. The

characteristics of the atmosphere surrounding were noted: The cow's environment was not loaded of dusts (hays moved nearby, agitated animals). If such was the case, the animals of the dusty premises were taken out. The milk sampling was realized according to the protocol of

[12]. which consists in washing itself hands with a disinfecting soap; identify the flask (in wide opening) with the indelible felt-tip: number of the cow, the mammary gland quarter (FR, FL, RR or RL), date and time; the udder was carefully washed and wiped; the rough drafts of milk to rinse the canal of the udder was eliminate (no more than 2 jets otherwise risk of very poor taking in germs); Disinfect the teat canal of the udder with a compress soaked with alcohol in 70°C; the sterile flask was opened maintaining the opening managed downward. The rubber were kept in the same hand without touching the inside; 08 milliliters of milk were taken and record the flask; every udder were disinfected before taking the milk of the corresponding district; finally. The milk samples were transported to the laboratory of Parasitology and Mycology of the National Veterinary graduate school of Algiers in a cooler (4°C). They were frozen at -20°C until the day of their analysis.

### Mycological analyses of the milk

The mycological analysis was realized in the laboratory of Parasitology-Mycology of the National Veterinary graduate school of Algiers. It consists of a direct examination of the samples of milk after colouring with the blue of the lactophenol. Yeasts are recognizable by their budding and their size by several microns, which avoids confusion with bacteria. A few drops of milk are seeded on Sabouraud Dextrose Agar (SDA) (Pasteur institute of Algiers) added of chloramphénicol (cast in petri dishes) and incubated in an oven at 27°C for 48 hours to 72 hours or more a week. Finally, isolated yeasts were identified using macroscopic characterization; this is a first step in the orientation of the mycological diagnosis. We used as criteria the shape, color, consistency and appearance of the colony. The yeast colonies of different appearance are sub cultured on Sabouraud/ chloramphenicol medium at 27°C for subsequent identification. In this phase, various culture media and tests are used for the precise identification of yeasts (Pasteur gallery) (Pasteur institute of Algiers) [13]. This identification was performed taking into consideration morphological characteristics, like formation of chlamydoconidium, pseudohyphae and germinal tube development.

Identification of the species of yeast has been realized by assimilation of carbon sources (auxanogramme): We used the test API<sup>®</sup> 20 C AUX (*BioMerieux*, France) comprising 19 carbon sources [14]. API<sup>®</sup> 20 C AUX should not be used directly from clinical sample or other. The microorganisms to be identified must first be isolated on a culture medium adapted according to the usual techniques of bacteriology and mycology. After 48 hours of incubation or 72 hours, observe the growth of the yeast compared to the negative control well 0. A cupule more turbid than the control indicates a positive reaction to note on the results sheet.

### Mycological analysis of the water of the drinking trough

A few drops of the water are inoculated on Sabouraud medium with chloramphenicol added. The identification of the pushed colonies is carried out as before for the milk samples.

### Mycological analysis of swabs

We followed the same experimental steps as those used in the mycological analysis of milk except direct examination. A seeding on a petri dish by friction of the swab over the entire surface of the medium. The yeast colonies are sub cultured on Sabouraud medium for subsequent identification as mentioned earlier.

## Results

### Results of the questionnaire

The questionnaire sent to veterinary practitioners allowed us to carry out an initial analysis on the management of dairy cattle farms in the Sidi Lahcène region (Sidi Belabbes wilaya) before going on to laboratory analyses. The veterinarians in charge of follow-up of the bovine breeding note that the measures of hygiene are absent. The breeders change the litter every two days at a percentage of 25% and 20% change the litter once a week (7 days), 39% of the breeders disinfect their milking equipment once a week, but it is noted that there is a certain percentage of the breeders (22%) never disinfects their milking equipment, despite this a few percentage of breeders (11%) disinfecting this equipment twice a day. 78% of milkers do not disinfect their hands before and after each milking. Only 6% of the milkers use an individual cloth to disinfect the teats of the udder and 63% of the milkers use a single cloth for all cows.

### Results of the direct examination

Direct examination of the 280 milk samples and 07 water samples from the trough revealed under the optical microscope (Gr. X 100 and X 400), the presence in some samples of mycelial filaments, yeasts and inflammatory cells (Leukocytes) (Tables 2-5).

Number of samples examined directly (milk and water from the trough)	Number of samples Positive	Number of samples Negative
287	179	108

**Table 2:** Results of the direct examination of mycological analysis.

		Machine milking	Manual milking
Total number of samples taken	562	402	160
Number of positives samples	182	130	52
Number of positives samples for yeast	164	117	47
Number of positives samples for filamentous fungi	19	13	05

**Table 3:** Results of mycological analysis.

	Species	Number of isolation of the same species in our study	%
01	<i>Candida zeylanoides</i>	47	26.70
02	<i>Candida guilliermondii</i>	22	12.5
03	<i>Candida tropicalis</i>	20	11.36
04	<i>Candida glabrata</i>	01	0.57
05	<i>Candida albicans</i>	02	1.13
06	<i>Candida famata</i>	03	1.7
07	<i>Candida pseudotropicalis</i>	05	2.84

08	<i>Candida rugosa</i>	11	6.25	18	<i>Trichosporon capitatum</i>	03	1.70
09	<i>Candida boidinii</i>	16	9.09	19	<i>Trichosporon asahii</i>	02	1.13
10	<i>Candida lipolytica</i>	02	1.13	20	<i>Saccharomyces cerevicea</i>	02	1.13
11	<i>Candida parapsilosis</i>	01	0.57	21	<i>Kloeckera apis</i>	01	0.57
12	<i>Rhodotorula glutinis</i>	17	9.66	22	<i>Sporobolomyces salmonicolo</i>	01	0.57
13	<i>Rhodotorula rubra</i>	03	1.70	23	<i>Geotricum candidum</i>	01	0.57
14	<i>Cryptococcus humicola</i>	07	3.98		Total	176	100
15	<i>Cryptococcus terreus</i>	02	1.13				
16	<i>Cryptococcus uniguttulatus</i>	02	1.13				
17	<i>Trichosporon cutaneum</i>	05	2.84				

**Table 4:** The different yeast species isolated in our study and their frequency.

Yeasts isolated in milk	Identification of the milk in which the yeast was isolated	Other samples analysed in which the same yeast was isolated
<i>Candida tropicalis</i>	Milk taken from 03 teats of cow No. 3 of breeding No. 8: right anterior, left anterior and left posterior	Water from the trough
<i>Candida rugosa</i>	Milk taken from the teats of the cows of the breeding No. 9: -left posterior teat of cow No. 3; -left posterior teat of cow No. 2; -right anterior teat of cow No. 1	
<i>Cryptococcus humicola</i>	Milk taken from the teat right anterior, of the cow No 1 in the breeding No. 4	
<i>Candida zeylanoides</i>	Milk taken from the teats of the cows of the breeding No. 6: -right anterior teat of cow No. 7 - right anterior teat of cow No. 6 - left posterior teat of cow No. 2 - left posterior teat of cow No. 2	Swabs from the hands of milkers
<i>Candida guilliermondii</i>	Milk taken from the teats of the cows of the breeding No. 12: - right anterior teat of cow No. 2 - left posterior teat of cow No. 1	Swabs of the bucket and the cistern

**Table 5:** The yeast species isolated in milk and in other samples analysed.

## Discussion

The survey conducted by questionnaire distributed to veterinarians in the region of Sidi Lahcène revealed the importance of respecting the hygiene of the animal's environment. Indeed, moisture from urine and milk as well as body heat, promote the development of fungi and more specifically the yeasts in the litter, hence the importance of regular renewal of the litter in a stable. Our survey showed that breeders change the litter every other day to 40% and 35% change the litter once a week (from 05 to 07 days).

According to the questionnaire responses, mastitis is common in high-producing cows at 72%, consistent with various studies that have shown positive correlations (0.30 to 0.44) between milk production and susceptibility to mastitis [15,16].

According to Slavolju et al. if no bacteriological research in the milk is carried out, fungal mastitis is suspected when antibiotic treatment fails.

Foci of mastitis caused by yeasts have been reported in herds managed with failures in environmental hygiene or in combination with antibiotic treatment in repetitive intra-mammary [5,6].

B/ Our study concerned the infection of the mammary gland in cattle by yeasts; it was carried out on 13 cattle farms comprising all 70 cows in the area of Sidi Lahcène, in the region of Sidi Belabbas. The samples were taken during the second quarter of 2012 and were preserved by freezing at -20°C until the day of analysis. The mycological analysis was carried out during the first half of 2013. The results of our study show that the prevalence of yeast infection is 29.54%.

This phenomenon is due to the frequent use of antibiotics, which was found by our survey, because in 66.67% of cases, mastitis persists after antibiotic treatment. They are used not only in treatment, but also in the dry period. Ant biotherapy leads to a disturbance in the homeostasis of the udder, inhibition of T lymphocyte and neutrophil activity and consequently to the stimulation of growth of yeast [17-20]. Half of the cows which the milk is contaminated by yeasts have been

treated with antimicrobial agents (penicillin, streptomycin, tetracyclin, lincomycin, and nystatin).

The etiological agents of mycotic mastitis are mainly yeasts, especially *Candida sp.* which is according to several authors found more frequently [21-30]. Our results are consistent with literature data; indeed, the genus *Candida sp.* was isolated in 73.86% of the isolated yeasts, which corresponds to about 130 isolated species.

Followed by the genus *Rhodotorula sp.* whose *Rhodotorula glutinis* (9.66%), *Rhodotorula rubra* (1-70%), in third place the genus *Cryptococcus sp.* whose *Cryptococcus humicola* (3.84%), *Cryptococcus terreus* and *Cryptococcus uniguttulatus* (1.13%). In fourth position the genus *Trichosporon sp.* with a frequency of 2.84% for the species *Trichosporon cutaneum*, 70% for *Trichosporon capitatum* et 1.13% for *Trichosporon asahii*. *Saccharomyces cervisea* is isolated in 1.13% and finally *Kloeckera apis* (0.57%), *Sporobolomyces salmonicolo* (0.57%), *Geotricum candidum* (0.57%). Most isolated yeast species in this study have also been reported by many authors as a cause of mastitis [31-35]. The four genus of yeast: *Candida*, *Rhodotorula*, *Cryptococcus*, *Trichosporon* were also isolated in the study conducted in Poland.

In addition, it is interesting to note that in this study we found that:

- The same species was isolated from the four teats of the same animal (example: *Candida zeylanoides* and *Candida tropicalis*).
- Different species belonging to the same genus have been isolated from the teats of the same animal (example: *Candida zeylanoides*, *Candida tropicalis*, and *Candida parapsilosis*).
- Different genus of yeasts isolated from the teats of the same animal (example: *Candida zeylanoides* and *Trichosporon cutaneum*)
- Différents genus of yeasts isolated from the same teat (example: *Candida zeylanoides*, and *Cryptococcus humicola*)
- Yeasts isolated from two teats and the other two summers free of infection.

This indicates that several fungal agents can infect the same animal as in the bacterial infection and that each quarter of the udder is independent of the other.

The pathogenic yeasts as *Candida albicans*, *Candida guilliermondii*, *Geotricum candidum* and *Candida kefir* show a great biochemical activity. They change the pH in the udder by the fermentation of carbohydrates. Consequently, they acidify the environment and inhibit the development of bacterial flora [36]. In this study we isolated several species of *Candida*; to know; *Candida zeylanoides* (26.70%), *Candida guilliermondii* (12.5%), *Candida tropicalis* (11.36%), *Candida boidinii* (9.09%), *Candida rugosa* (6.25%), *Candida pseudotropicalis* (1.70%), *Candida famata* (1.70%), *Candida albicans* (1.13%), *Candida lipolytica* (1.13%), *Candida glabrata* (0.57%), *Candida parapsilosis* (0.57%). According to some authors, animal species and geographical variations may be the reason for this difference in the distribution of yeast species [37].

It is well established that yeasts of the genus *Candida*, for example, are able to use antibiotics such as penicillin and tetracycline as sources of nitrogen [38]. In addition, high doses of antibiotics can cause a reduction in vitamin A, causing lesions of the udder epithelium, facilitating the invasion of fungi [39]. It has been reported that *Candida* species can cause clinical mastitis characterized by pain, prolonged fever and inflammatory reaction in the mammary gland and lymph nodes associated with a reduction in milk production in animals [35]. They can also be isolated from animals suffering from

subclinical mastitis [40]. Besides the well-known virulence factors in *Candida* such as hydrolytic enzymes, toxins, dimorphism and hemolysin production.

## Conclusion

The literature review and the results we have obtained, allow us to complete the data collected on fungal mastitis in Algeria. Lack of demand for accurate laboratory tests during mastitis and non-specificity of symptoms in fungal mastitis and bacterial mastitis, results in poor management in this condition since in the field it is systematically treated with antibiotics and anti-inflammatories (diathelic pathway). Training thus, a possible rupture of the mycobacterial equilibrium, already disturbed and thus aggravate the inflammation of the udder of fungal origin. The particularity of fungal mastitis due to the fact that their epidemiological forms depend on the intervention of a number of factors and their combinations together, more than the causal agent. In addition, fungal mastitis most of the time has a tendency to self-limitation and spontaneous healing, these are other factors of intervention which generally ensure the persistence or the extension of the affection.

Analysis of the questionnaire distributed to veterinary practitioners in the region of Sidi Lahcène, wilaya of Sidi Bel Abbas, has highlighted numerous shortcomings in breeding management and in particular the respect of the general rules of the hygiene, the conditions of milking and the general atmosphere. The hygiene in the barns should not be an additional instrument in the conduct of breeding but be constitutive.

## Perspectives

Through our investigation, we found that the incidence of fungal mastitis varies widely across regions and investigations. In order to minimize these affections and the economic losses they cause, we propose the following recommendations:

A. The practitioner, faced with a problem of mastitis in a dairy livestock, must be alerted to face a next anamnesis: a recent intramammary antibiotic treatment followed by the appearance, persistence or even aggravation of clinical signs. Failure of such treatment should lead to suspicion of a fungal infection of the udder.

B. The environment is a crucial point in the genesis of mycotic mastitis, this makes it difficult to develop a control strategy to eradicate or reduce their incidence.

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