Full Length Research Paper

Yeasts in the mammary environment of the cattle in the region of Sidi M'hammed Ben Ali, Wilaya of Relizane, Algeria

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Mastitis is one of the principal pathologies in the dairy bovine exploitation. Most of the cases are caused by bacteria, but some are caused by fungi. The objective of our study was to evaluate the occurrence of these fungi in mammary glands of 39 cows (mastitic and clinically healthy cows) belonging to two farms (four exploitations using manual milking and three exploitations using milking machine) in the area of Sidi M'hammed Ben Ali, Wilaya of Relizane and to assess some risk factors (the tubes of drug, animal excretion, goblets-milkers, the milkers' hands and the litter). For this purpose, 150 samples of milk and 94 swabs were used. Our results reveal the presence of a heavy load of fungi cells in healthy and mastitic milks, with a strong frequency of *Trichosporon* sp. (43, 58%) followed by *Candida* sp. (30.76%). The same yeasts were isolated from swabs.

Key words: Mastitis, fungi, antibiotics, milking machine, the milker, Algeria.

INTRODUCTION

Mycotic mastitis was described from the beginning of the last century (Klein, 1901). This mastitis aroused some skepticism and numerous debates because the incriminated agents are often contaminants of the outside or the common saprophytes. Although still inadequately known, they seem to draw the attention of pathologists, especially since the acceptance of everyday treatment (intra-mammary antibiotic). The rates of the observed mycotic mastitis vary from 0.34 (Loftsgard et al., 1960) to 3.9%. Swinne-Desgain (1971) and Fortier (1990) said yeasts are responsible for 1.76 % cases of mastitis (clinic and sub-clinic). Milk from a healthy udder does not

contain either mushrooms or bacteria. It is better to speak about a fungal basic flora, resulting from the environment (dust resulting from feeds, equipment of collection as well as those of animals and even man). It is very common to find in the unpasteurized milk yeasts of the genre, *Candida* and mold, *Penicillium*, which can alter some dairy products.

Mycotic mastitis is split into two big groups according to the moment of appearance: primary mycotic Mastitis (bacterial preliminary mastitis) and secondary mycotic mastitis. The latter appears often straightaway, without antibiotic treatment or generally follows a bacterial

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Table 1. Sampling plan.

Nature of the sampling	Number of samples		
	Manual milking	Milking machine	Total
Number of cows	17	22	39
Numbers of milk samples	65	85	150
Numbers of anal swabs	17	22	39
Numbers of vaginal swabs	13	22	35
Number of swabs	0	2	2
Number of swabs on the hands of the milker	3	0	3
Number of swabs on creams Antibiotics	12	0	12
Numbers of litter samples	0	3	3

mastitis or an intramammary administration of antibiotics by diathétique way. According to some authors (Bertslinger et al., 1964), the first ones would represent 30% cases and the second 70% cases.

In Algeria, very few studies have been done on the prevalence of the fungal mastitis in the dairy bovine farms as well as on various factors favoring their appearance and development (Mebarki, 2007; Ksouri, 2008). So, the objective of the study is to determine the prevalence of mastitis caused by yeasts and to know the number of risk factors in some dairy bovine farms in Relizane.

MATERIALS AND METHODS

Distribution of a questionnaire

Pre-investigation was done in the last quarter of the year 2007 and the first half of 2008 to estimate the epidemiological situation of this pathological entity within the dairy beef herd in the region of Relizane. For that purpose, a questionnaire was distributed to the veterinarian practitioners. This investigation is on the breeding technique, frequency of the clinical mastitis in this breeding and the percentage use of antibiotics in the treatment of clinical mastitis.

Choice of farms

Four dairy farms with manual milking and tree with machine milking were used in this study. This selection was based on the comparison of both types of milking. All the farms exist in the same region- Sidi M'hammed Ben Ali, Wilaya de Relizène.

Nature and number of samples

A total of 244 samples were collected by the veterinarian practitioners of Sidi M'hammed Ben Ali's region. That is 150 samples of milk taken from 39 existing cows in 7 farms. The samples were obtained with different mammary glands health status: 19 cows with healthy mammary glands, 15 cows with subclinical mastitis as determined by the California Mastitis Test (CMT) and 05 cows with clinical mastitis, defined as follows: swelling, reduced milk flow and abnormal milk appearance, 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 1. This corresponds with an

appearance of viscous milk that does not adhere to the bottom of CMT plate, and correlates with 400,000-1,500,000 somatic cells/ml (Scott et al., 1986) (6 milk sampling emptied of their tube because they were badly kept), 91 swabs [39 anal swabs and 35 vaginal swabs of which four were badly kept), two swabs on milking machine, 3 on the hands of the milkers and 12 swabs of antibiotic creams. And at the end, three samples of litter were got back.

From every cow during lactation, four takings of milk (a taking of milk of every trayon), an anal swab, and a vaginal swab were taken once during all the period.

In every breeding with manual milking, swabbing was done by the hands of the milker before the milking (factor of contamination), there was recovery of the tubes of antibiotic cream (factor of release) used for the treatment of cows clinically and a sample of the litter was collected just before its renewal (factor of enrichment). Some takings were made in the breeding with machine milking except swabbing of the milkers' tumblers of the milking machine (factor of contamination) (Table 1).

Milk sampling

The correct realization of the sampling procedure was a necessity, in terms of the ubiquity of fungi which can contaminate the milk. The characteristics of the surrounding atmosphere were noted. The cows' environment was not loaded with dust (hays moving nearby and agitated animals). If such was the case, the animals of the dusty premises must have gone out. The milk sampling was realized according to the protocol of Guerin and Guerrin-Fauble (2007), which consists of washing the milkers' hands with a disinfecting soap, identifying the flask (in wide opening) with 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 for rinsing the canal of the udder were eliminated (not more than 2 jets, otherwise there would be risk of the taking having germs). The teat canal of the udder was disinfected with a compress soaked with alcohol at 70°C; the sterile flask was opened to maintain the openings managed downward. The rubber was kept in the same hand without touching the inside; some milliliters of milk were taken and recorded in the flask. Every udder was disinfected before taking the milk of the corresponding district. Finally, the takings of milk were kept in - 20°C until the day of their analysis.

Mycological analyses of the milk

Mycological analysis was realized in the laboratory of Parasitology - Mycology of the Veterinary Graduate School- Algiers. It consists of a direct examination of the samples of milk, after vital staining. Milk

samples were centrifuged and the sediment was inoculated on the surface of Sabouraud Dextrose Agar (SDA) (QUELAB, Laboratories INC and code: QB-39-3806) added of chloramphénicol (QUELAB, Laboratories INC and code: QB-39-3806) and incubated for 3 days at 25 °C. Finally, isolated yeasts and filamentous fungi were identified using microscopic characterization. Yeasts isolated were identified by the gallery Pasteur (gallery Auxanogramme) (DIMED, code: 15300Algeria). This identification was performed taking into consideration morphological characteristics, like formation of chlamydoconidium, pseudohyphae and germinal tube development. This gallery is composite due to its various cultural middle and various tests for the precise identification of yeasts.

1. Middle Sabouraud/Chloramphénicol at 37°C: this test allows one to highlight the potential pathogenic character of the yeast when it develops in a temperature, bordering the corporal temperature.
2. Middle Sabouraud/Actidione; this test allows one to highlight colonies sensitive to Actidione (Cycloheximide). Colonies having grown on this middle are considered resistant to Actidione (R); colonies not having grown on this middle are considered sensitive to Actidione (S).
3. Middle with cream of rice (rice cream): this middle favors the production of chlamydoconidia characteristics of *Candida albicans* in anaerobic middle.
4. Middle with serum for blastèse: the serum of bovine is used as middle to favor the production of *Candida*'s typical germinal albicans tubes (test of germination).
5. Middle in the urea Indole: this test allows one to look for the hydrolysis of the urea. The change of the middle colour of yellow-orange to purple-red corresponds to the secretion of an uréase. The yeast which turns the middle to red in 4 h is *C. neoformans*.

A quantitative search for mushrooms (counting of colonies) and qualitative search (the various tests for identification) are made. For the identification of the genre and species of yeasts, the key of identification of yeasts proposed by Drouhet and Dupont (1985) was used.

The anal, vaginal swabs and the material of milking

The vaginal and the anal excretions were collected by swabbing in the perineum and vaginal regions. Swabbing of goblets was done-milkers of milking machines only in two dairy cow farms and swabbing of the hands of the milking men before the milking.

Swabbings were made by direct scattering of the swab on the surface of the SDA plates added to chloramphenicol. After incubation for 3 days at 25°C, the colonies of yeasts were identified as previously.

The litter samples

The collected litter was deposited in one sterilized conical glass cup containing sterile physiological water, then the whole was homogenized and the rest was left for 30 min. Some gouts of the sediment are then inoculated on SDA added to chloramphénicol. Cultures were incubated for 3 days at 25°C.

RESULTS

Results of the questionnaire distributed to the veterinarian practitioners

The veterinarians in charge of the follow-up of the bovine breeding note that the measures of hygiene are absent.

Indeed, 60% of the milkers do not disinfect their hands before and after every milking. The udder is not disinfected before the milking in 45.71% cases. The majority of the breeding are done in hindered stall (54.85%); 57.14% of the breeders use the same rag for the disinfection of the udder and in 65.71% cases, this rag is not disinfected after each use; 25.71% of the farmers disinfect their milking material once a week; 17.14% of the breeders change the cow litters only once a week.

This report thus incited us to start a study on prevalence of the mastitis of fungal origin.

Results of the mycological analysis of the samples collected

Of the 244 samples realized, 91 are positive, that is, 37.3% and 78 fungal species were identified: 35 species in the breeding with manual milking namely *Candida* sp. (25.7%), *Trichosporon* sp. (48.6%), *Rhodotorula* sp. (8.6%), *Cryptococcus* sp. (2.9%), *Torulopsis* sp. (2.9%), *Penicillium* sp. (8.6%), *Aspergillus* sp. (2.9%) (Table 4); 43 species in the breeding with machine milking namely *Candida* sp. (34.9%), *Trichosporon* sp. (39.5%), *Rhodotorula* sp. (9.3%), *Cryptococcus* sp. (4.6%), *Torulopsis* sp. (2.3%), *Penicillium* sp. (7%) and *Aspergillus* sp. (2.3%) (Table 2).

DISCUSSION

Fungal cultures were observed in 68 samples of milk (Table 3). A study was done on the mammary infection of dairy cows in Sidi M' Hammed Be Ali's region, Wilaya of Relizane, from December 2007 to May 2008.

In our survey, the mycological examination of the milk samples and the realized swabs showed the presence of yeasts and filamentous fungi, with a higher frequency of yeasts (Table 3). This is in line with that of the literature. Indeed, the most frequent yeasts genus were *Candida* (30.76%) and *Trichosporon* (43.58%) (Table 4). Many authors noted that the fungal bovine mastitis is predominantly caused by yeasts (Swinne-Desgain, 1971; Kuo and Chang, 1993; Aalbaek et al., 1994; Watts, 1988; Lagneau et al., 1996; dos Santos and Marin, 2004).

The mycological analysis also revealed that, the same genre of yeasts was found in both types of exploitations namely *Candida*, *Trichosporon*, *Rhodotorula*, *Cryptococcus* and *Torulopsis* (Costa et al., 1993; Krukowski et al., 2001; Krukowski et al., 2006); with a higher frequency for *Candida* and *Trichosporon* genres (30.76; 43.58%) and then *Rhodotorula* (7.69%) and *Cryptococcus* (3.84%) (Table 4). All these fungal agents, with the exception of *Rhodotorula* have been detected before as pathogenic agents in numerous inquiries on fungal mastitis (Moulinier, 2003). Prevalence of the

Table 2. Various species of yeasts and molds isolated.

Genre	%	Species	Number
Breeding with manual milking			
	25.7	<i>Candida zeylanoides</i>	1
		<i>Candida pseudotropicalis</i>	5
<i>Candida</i> sp.		<i>Candida guilliermondii</i>	1
		<i>Candida tropicalis</i>	1
		<i>Candida parapsilosis</i>	1
<i>Trichosporon</i> sp.	48.6	<i>Trichosporon cutanium</i>	10
		<i>Trichosporon capitatum</i>	7
<i>Rhodotorula</i> sp.	8.6	<i>Rhodotorula rubra</i>	3
<i>Cryptococcus</i> sp.	2.9	<i>Cryptococcus terreus</i>	1
<i>Torulopsis</i> sp.	2.9	<i>Torulopsis pulcherrima</i>	1
<i>Penicillium</i> sp.	8.6	<i>Penicillium</i> sp	3
<i>Aspergillus</i> sp.	2.9	<i>Aspergillus</i> sp	1
Total	100		35
Breeding with machine milking			
	34.9	<i>Candida zeylanoides</i>	1
		<i>Candida pseudotropicalis</i>	2
<i>Candida</i> sp.		<i>Candida guilliermondii</i>	7
		<i>Candida tropicalis</i>	4
		<i>Candida lusitanae</i>	1
<i>Trichosporon</i> sp.	39.5	<i>Trichosporon cutanium</i>	8
		<i>Trichosporon capitatum</i>	6
		<i>Trichosporon fermentens</i>	3
<i>Rhodotorula</i> sp.	9.3	<i>Rhodotorula glutinis</i>	4
<i>Cryptococcus</i> sp.	4.6	<i>Cryptococcus terreus</i>	2
<i>Torulopsis</i> sp.	2.3	<i>Torulopsis glabrata</i>	1
<i>Penicillium</i> sp.	7	<i>Penicillium</i> sp	3
<i>Aspergillus</i> sp.	2.3	<i>Aspergillus</i> sp	1
Total	100		43
Total			78

Table 3. Frequency positive samples according to the milking procedure.

Milking procedure	Number of milk samples	Positive samples	Frequency (%)
Manual milking	65	30	46.15
Machine milking	85	38	44.70
Total	150	68	45.3

fungal mastitis varies from 1 to 44% according to authors' number (Loftsgard et al., 1960; Monga et al., 1971; Swinne-Desgain, 1971; Farnsworth et al., 1972; Kumer et al., 1975; Fenizzia et al., 1976; Awad et al., 1980; Ramisse et al., 1982).

Global frequency observed on the present study was considered at 45.33% for the exploitations with clinical mastitis and subclinical mastitis (Table 3), which is similar to those of Swinne and Desgain (1971). This frequency may be explained by the animal management put in place in the visited dairy farms in the region of Sidi M'hammed Ben Ali (results of the questionnaire).

The genus *Trichosporon* was quoted by several authors as being a potential pathogenic fungus; in particular, *T. capitatum*, and *T. cutaneum* (Loftsgard et al., 1960; Fameree et al., 1970). The present study highlighted these species with a 43.58% rate (23.06% for *T. cutaneum*, 16.66% for *T. capitatum*), higher than the rates found in a survey done by Mebarki (2005) in Algiers (19.25 %) on dairy exploitations of subclinical mastitis. Other authors pointed to lower rates, such as Moretti et al. (1998), who isolated *T. capitatum* in 31.2% cases and *T. cutaneum* in 18.72% cases in Italy. Aalbaek et al. (1994) described five cases of mastitis caused by *Tr*

Table 4. Frequency of fungi isolations in the samples.

Genre	Percentage (%)
<i>Candida</i> spp.	30.76
<i>Trichosporon</i> spp.	43.58
<i>Rhodotorula</i> spp.	8.97
<i>Cryptococcus</i> spp.	3.84
<i>Torulopsis</i> spp.	2.56
<i>Penicillium</i> spp.	7.69
<i>Aspergillus</i> spp.	2.56

Table 5. Summary of the number of yeasts and filamentous fungi isolated from milk.

Fungi	Manual milking	Machine milking
Yeasts	40	46
Moulds	08	06
Total	48	52

capitatum in Denmark, which is lower than that of this present result (13 cases). Costa et al. (1993) have described 21 mastitis cases caused by *T. cutaneum* in Brazil.

Concerning the genus, *Candida*, its strong predominance (30.76 %) in the whole of the positive samples confirms the importance of this yeast, often evoked as the main genus in the etiology of mycotic mastitis (Fameree et al., 1970; Farnsworth et al., 1972; Richard et al., 1980; Yeh et al., 1988; Kuo and Chang, 1993; Aalbaek et al., 1994; Lagneau et al., 1996; dos Santos et al., 2004). This frequency of *Candida* isolation was lower than that recorded in the region of Algiers by Mebarki (2005) (52.07%) and in the South of Brazil by Spanamberg et al. (2008) (37.9%), but superior to that (17.3%) noted by Sailor et al. (2004) in Brazil.

Prevalence of the fungal mastitis according to the milking modality was almost the same: in the manual milking, it is 46.15% and in the machine milking, it is 44.70%. This means that there is independence between the positive milk samples and the milking procedure at the beginning. The difference is not significant ($p>5$). The Chi-square test of independence was used for the comparison of both methods (manual milking and machine milking). This indicates that the problem does not settle at the level of the method of milking but in the conditions of the milking progress (the factors of enrichments, factors of releases and factors of contamination) (Table 5).

Conclusion

The frequency of fungal mastitis is underestimated in Algeria. The present study shows cases of fungal mastitis found in two types of exploitations (manual milking or

machine milking). The isolation of the same genus of fungi in an almost similar percentage in both milking systems confirms the idea. This leads one to conclude that the problem of the fungal mastitis is not only connected to the milking modality but is connected to the conduct of farmers and the hygienic practices applied during the milking. The hygiene practices in the stables of the dairy farm do not have to be an additional act in the conduct of the farmers but a regular component of the farm management. With the aim of limiting the increase of the fungal mastitis, it is important to establish a specific diagnosis on healthy and pathological milk to modulate a treatment according to the etiology and clinical aspect of mastitis.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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