Veterinary
Science & Technology

Akdouche et al., J Veterinar Sci Technolo 2014, 5:2
http://dx.doi.org/10.4172/2157-7579.1000172

Research Article Open Access

Importance of Yeasts in the Mammary Infection of the Cattle in the Region of Sidi M'Hamed Ben Ali, Wilaya of Relizane, Algeria

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Abstract

The mastitis is one of the principal pathologies in the dairy bovine exploitation. The majority of the cases are caused by bacteria, but there are also cases caused by fungi. The objective of our study was to evaluate the occurrence of these fungi in mammary glands of 39 cows (mastitic cows and clinically healthy cows) belonging to two types of farms (4 exploitations using manual milking and 3 exploitations with 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 excretions, goblets - milkers, the milker hands and the litter). For this purpose, 150 sample of milk and 94 swabs were carried out. Our results revealed the presence of a heavy load of fungi cells in healthy and in the mastistic milks; with a strong frequency of the *Trichosporon* sp. (43, 58%) followed by the *Candida* sp. (30.76%). The same yeasts were isolated from swabs.

Keywords: Mastitis; Fungi; Antibiotics; Milking machine; The milker; Algeria

Introduction

Mycotic mastitis was described from the beginning of the last century [1].This mastitis aroused some scepticism and numerous debates because the incriminated agents are often contaminants of the outside or the common saprophytes. Although still badly known, they seem to draw the attention of the pathologists, especially since the everyday acceptance of treatments (intra-mammary antibiotic). The rates of the observed mycotic mastitis vary of 0.34% [2] in 3.9% [3,4] accused yeasts of being responsible for 1,76% of the cases of mastitis (clinic and sub-clinic). Milk resulting from a healthy udder, does not contain either mushrooms or bacteria. It's better to speak about a fungal basic flora, resulting from the environment (dusts resulting from feeds, equipments of collection as well as by those of the animals and even the man) because a healthy milk does not contain, in the physiological state, germs of fungal origin [4]. It is very frequent to find in the unpasteurized milk, the yeasts belonging to the genre *Candida* and the molds *Penicillium* which can alter some dairy products.

Mycotic mastitis are split into two big groups according to the moment of appearance: primary mycotic Mastitis [5-7] bacterial preliminary mastitis and secondary mycotic mastitis [8,9] appear often straightaway, without antibiotic treatment or: generally follow upon a bacterial mastitis or upon an intramammary administration of antibiotics by diathélique way. According to some authors [10], the first ones would represent 30% of the cases and second 70% of the cases.

In Algeria, very few studies were led on prevalence of the fungal mastitis in the dairy bovine farms as well as various favoring factors their appearance and their development [11,12]. So, we settled as objectives, the determination of prevalence of mastitis caused by yeasts and the study of a number of risk factors in some dairy bovine farms of the region of Relizane.

Material and Methods

Distribution of a questionnaire

Pre-investigation was realized during the last quarter of year 2007 and the first half of year 2008 to estimate the epidemiological situation of bovine mycotic mastitis in the region of Relizane. For that purpose, a questionnaire was distributed to the veterinarians' practitioners of said region. This investigation concerns the breeding technique, the frequency of the clinical mastitis in these breedings and the percentage of use of antibiotics in the treatment of the clinical mastitis.

Choice of the followed farms

Four dairy farms with manual milking and tree with machine milking were retained for our study; this selection was based on the comparison of two types of milking. These farms are situated in Sidi M' hammed Ben Ali's region, Daira de Oued Rhiou, wilaya of Relizane, west of Algeria.

Nature and number of samples

A total of 244 samples were collected by the veterinarians practitioners of Sidi M' hamed Ben Ali's region. That is 150 samples of milk taken from 39 existing cows in 7 farms, all the farms exist in the same region which is the region of Sidi M'hammed Ben Ali ,wilaya de Relizène.

The simples were obtained with different mammary glands health status:19 cows with healthy mammary glands, 15 cows with subclinical mastitis as determinated by the California Mastitis Test(CMT) and 05 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 stady ,milk simples from gland affected with subclinical mastitiswere included when the reaction to CMT was at least grade 1,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, (6 milk sampling emptied of their tube because badly kept), 91 swabbings [39 anal swabs and 35 vaginal

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Received March 03, 2014; **Accepted** May 07, 2014; **Published** May 09, 2014

Citation: Akdouche L, Aissi M, Zenia S, Saadi A (2014) Importance of Yeasts in the Mammary Infection of the Cattle in the Region of Sidi M'Hamed Ben Ali, Wilaya of Relizane, Algeria. J Veterinar Sci Technol 5: 172. doi:10.4172/2157-7579.1000172

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swabs (4 vaginal swabs badly kept), 2 swabbings on milking machines, 3 on the hands of the milkers and 12 swabbings of creams antibiotics] and at the end 3 samples of litter were got back.

On every cow in lactation: 04 takings of milk (a taking of milk of every trayon), an anal swab, a vaginal swab, 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), recovery of the tubes of creams antibiotics (factor of release) used for the treatment of cows with clinical (clinical mastitis: redness, heat, pain) and collect a sample of litter just before its renewal (factor of enrichment). Same taking were made in the breedings with machine milking except swabbing of tumblers milkers of the milking machine (factor of contamination) (Table 1).

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 Guerin and Guerin-faublee [13], 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; some milliliters of milk were taken and record the flask; every udder were disinfected before taking the milk of the corresponding district; finally, the takings of milk are kept in - 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 Veterinary graduate school - Algiers. It consists of a direct examination of the samples of milk after coloring with the blue of the lactophenol. Milk samples were centrifuged and the sediment was inoculated on the surface of Sabouraud Dextrose Agar (SDA) (Pasteur institute of Algiers) added of chloramphénicol and incubated for 3 days at 25 °C. Finally, isolated yeasts and filamentous fungi were identified using microscopic characterization and an auxanogramme performed in a miniature biochemical gallery (Pasteur gallery) (Pasteur institute of Algiers).

Nature of the taking	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

Table 1: Sampling plan.

Genre	%	Species	number
Breeding with manual milking			
Candida sp	25,7	*Candida zeylanoides*	1
		Candida pseudotropicalis	5
		Candida guilliermondii	1
		Candida tropicalis	1
		Candida parapsilosis	1
Trichosporon sp	48,6	*Trichosporon cutanium*	10
		Trichosporon capitatum	7
Rhodotorula sp	8,6	*Rhodotorula rubra*	3
Cryptococcus sp	2,9	*Cryptococcus terreus*	1
Torulopsis sp	2,9	*Torulopsis pulcherrima*	1
Penicillium sp	8,6	*Penicillium sp*	3
Aspergillus sp	2,9	*Aspergillus sp*	1
Total	100		35
Breeding with machine milking			
Candida sp	34,9	*Candida zeylanoides*	1
		Candida pseudotropicalis	2
		Candida guilliermondii	7
		Candida tropicalis	4
		Candida lusitaniae	1
Trichosporon sp	39,5	*Trichosporon cutanium*	8
		Trichosporon capitatum	6
		Trichosporon fermentens	3
Rhodotorula sp	9,3	*Rhodotorula glutinis*	4
Cryptococcus sp	4,6	*Cryptococcus terreus*	2
Torulopsis sp	2,3	*Torulopsis glabrata*	1
Penicillium sp	7	*Penicillium sp*	3
Aspergillus sp	2,3	*Aspergillus sp*	1
Total	100		43
Total			78

Table 2: Various species of yeasts and molds isolated.

Yeast identification was performed taking into consideration morphological characteristics, like formation of chlamydoconidium, pseudohyphae and germinal tube development.

The identification of the fungi colonies were realized as previously. For the genre and the species identification of yeasts, the key of identification of yeasts proposed by Drouhet and Dupont [14].

The anal, vaginal swabs and the material of milking

The vaginal and the anal excretions were collected by swabbing in the region of the perineum and in the vaginal region. Sowings were carried out by direct scattering of the swab on the surface of the SDA plates added of chloramphenicol. After incubation for 3 days at 25°C, the colonies of yeasts are 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 left resting for 30 mn, some gouts of the sediment are then inoculated on SDA added of chloramphénicol. Cultures were incubated for 3 days at 25 °C.

Results and Discussion

Fungal cultures were observed in 68 samples of milk (Table 2).

During period spreading out from December, 2007 till May, 2008, a study was realized on the mammary infection to dairy cows in exploitations of Sidi M' Hamed Be Ali's region (wilaya of Relizane).

During our survey, the mycological examination of the samples of milk and the realized swabs, highlighted the presence of yeasts and filamentous fungi with a higher frequency of yeasts (Table 3), who concord with the literature [15-20]. Indeed, the most frequent yeasts genre were *Candida* (30, 76%) and *Trichosporon* (43, 58%) (Table 4).

Many authors note that the fungal bovine mastitis is predominantly caused by yeasts [15-20].

The mycological analysis also revealed that, the same genres of yeasts were found in both types of exploitations namely *Candida*, *Trichosporon*, *Rhodotorula*, Cryptocoques, and *Torolupsis* [21-23] with a hugher frequency for *Candida* and *Trichosporon* genres (30,76%; 43,58%), then *Rhodotorula* with 7,69%, Cryptocoques with 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 [24]. Prevalence of the fungal mastitis varies 1% to 44% according to authors' number [2,5,25-29].

Global frequency observed on the present study was considered at 45, 33% for the exploitations with clinical mastitis and subclinical mastitis (Table 5), similar results to those of Swinne-Desgain [15]. This frequency may be explained by the animal management put in place in the visited dairy farms in the region of Sidi M' hamed Ben Ali (results of the questionnaire): 60% of the milkers do not disinfect the hands before and after each milking; the udders were not disinfected before the milking in 45, 71% of the cases; the majority of the farms are hobbled Stalling (54,85%); 57,14% of the farmers use a collective rag for the disinfection of the udder and in 65,71% of the 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.

The genre *Trichosporon* was quoted by several authors as being a potential pathogenic fungi, in particular the species *Tr. capitatum*, and *Tr. cutaneum* [2,3]. The present study highlighted these species with a 43, 58% rate (23, 06% for *Tr. cutaneum*, 16, 66% for *Tr. capitatum*) widely upper to the rates found in a survey led by Mebarki [11] in the region of Algiers (19, 25%) in dairy exploitations presenting subclinical mastitis. Other authors pointed to lower rates, as Moretti et al. [30], that were able to isolated *Tr. capitatum* in 31, 2% of the cases and *Tr. cutaneum* in 18.72% in Italy. Aalbaek et al. [17] described 5 cases of mastitis caused by *Tr. capitatum* in Denmark, what is lower than the present results (13 cases). Costa et al. [21] have discribed 21 mastitis cases caused by Tr. *cutaneum,* in Brazil.

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

Table 3: Summary of the number of yeasts and filamentous fungi isolated from milk.

Genres	Percentage (%)
Candida spp.	30 ,76
Trichosporon spp.	43,58
Rhodotorula spp.	8 ,97
Cryptococcus spp.	3,84
Torulopsis spp.	2,56
Penicillium spp.	7,69
Aspergillus spp.	2 ,56

Table 4: Frequency of fungi isolations in the samples.

	Number of milk samples	Positive samples	Frequency
Manual milking	65	30	46,15%
Machine milking	85	38	44,70%
Total	150	68	45,3%

Table 5: Frequency positive samples according to the milking procedure.

Concerning the Candida genre, its strong predominance (30,76%) in the whole of the positive samples, confirm the importance of this yeast genre, often evoked as the main genre in the etiology of mycotic mastitis [3,16-18,20,26,31,32]. This frequency of *Candida* isolation was lower than that recorded in the region of Algiers by Mebarki [11] (52, 07%) and in the South of Brazil by Spanamberg et al. [33].

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%, what means that there is an independence between the positive milk samples and the milking procedure at the beginning of meaning (the difference is not significant, p>5) , It is the Chi-squared test of the independence, used for the comparison between both method (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 progress of the milking (the factors of enrichments, the factors of releases and the factors of contamination) (Table 5).

Conclusion

The frequency of fungal mastitis is underestimated in Algeria. The present study reports fungal mastitis found in two types of exploitation (using manual milking or machine milking). The Isolation of the same genre of fungi in an almost similar percentage in both milking systems, confirms the idea. This leads 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 arising 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 the clinical aspect of the mastitis.

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Citation: Akdouche L, Aissi M, Zenia S, Saadi A (2014) Importance of Yeasts in the Mammary Infection of the Cattle in the Region of Sidi M'Hamed Ben Ali, Wilaya of Relizane, Algeria. J Veterinar Sci Technol 5: 172. doi:10.4172/2157-7579.1000172

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