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YEAST AS A CAUSE OF MASTITIS IN MILKING ANIMALS IN EL- BEHERA PROVINCE

HANAA F. FARAG and AMAL A. EL SAID
Department of Food Hygiene, Department of Bacteriology, Animal Health Research Institute, Damanhour Branche

ABSTRACT

Mastitis is one of the most expensive disease of milky cows, fungi, especially yeasts can be important etiological factor of mastitis. The aim of this work was to determine the frequency of yeasts isolation from milk of cows suffering from mastitis. A total of 80 milk samples from cows suffering from mastitis were examined. Yeasts were isolated from 50(62.5%) milk samples but 26(32.5%) samples were positive for other fungi (Aspragillus flavus, Aspragillus niger, Pencillium and Mucor) and only 4(5%) samples were negative for fungi isolation. About 20(40%) samples were pure yeast isolation, the other 30(60%) milk samples were mixed yeast and other fungi. Only 20(40%) strains of yeasts classified as Candida genus. Identification was adopted on the basis of morphological and biochemical characteristics including Germ tube test, urease production and carbohydrates assimilation. From the present study, prevention of mycotic mastitis requires management practices and hygienic management that prevent mycotic growth, advanced diagnosis for identifying yeasts for saving more antibiotics used in treatment. In which the use of antimicrobials for long period is pointed out the occurrance of mycotic mastitis.

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Key words: *Mastitis, Milk, Fungi, Yeast.*

INTRODUCTION

Mastitis in dairy cattle is defined as inflammatory reaction of the udder. This mammary gland infection is the most common disease in dairy cattle all over the world. It is also the most costly to the dairy industry. The symptoms of mastitis in milk may be abnormalities such as a watery appearance, flakes, clots or pus. Costa *et al.* (1998) and Krukowski *et al.* (2000), This disease can be identified by external symptoms of the udder such as swelling, heat, redness, hardness, or pain. Thereare many bacteria that are known to cause mastitis.

Likewise, yeast or yeast-like organisms have been reported to cause bovine mastitis. Sheena and Sigler (1995); Staroniewiez *et al.* (2007). Cryptococcus neoformans and Candida albicans are the most common cause, but other Candida species have also been associated with bovine mastitis. Mastitis is usually transmitted by the contact with the contaminted milking machine, and through contaminated hands or materials. Treatment is possible with long-acting antibiotics, but milk from such cows is not marketable until drug residues have left the cow's system. Antibiotics may be systemic, or they may be forced upwards into the teat through the teat canal. Antibiotic therapy, without identifying the mastitis causing organisms is frequently the veterinarian and dairy farmer's first choice of treatment for infected cows. As a result of this, cases of mastitis that are refractory to any type of treatment occur frequently. The incidence of mastitis due to yeast is usually rather low in dairy herds, but during last decade it increased significantly. It has usually been described as related to treatment directed toward another pathogens using contaminated syringes and canulas or contaminated antibiotic preparations.

Teat injuries may predispose to the establishment of a yeast infection. Yeast intramammary infections were reported to be responsible for not more than 10% of all clinical cases seen in a veterinary practice, Costa *et al.* (1998); Krukowski *et al.* (2000) and the majority of the cases are mild. Although antimycotic drugs have been used for treatment of yeast mastitis, there is no clear evidence of the effectiveness of this therapy. The aim of the present study was to isolate, identify and determine the prevelence of yeasts in milk samples from cows suffering from mastitis in El-behera Province.

MATERIALS and METHODS

Collection of samples

80 milk samples were taken from dairy farms in El-behera Province. The milk samples collected from separate quarters under aseptic conditions from dairy animals clinically suffering from mastitis and

examined for isolation and identification of microorganisms.

Isolation and identification of microorganisms:
The methods followed according to Cruickshank *et al.* (1975).

Direct smear:
From milk samples were taken and stained by gram stain after heat fixation smear. The samples were plated onto sabaurd's dextrose agar (Oxoid) with antibiotic according to Cruickshank *et al.* (1975). The plates were incubated at 37˚C. The incubation temperature of 37˚C was used because it has been observed that strains of yeast isolated from mastitis cases grow more abundantly at this temperature than lower temperature. While non pathogenic yeasts did not grow at 37˚C (Morse, 1961).

The plates were examined for growth at 24, 48 &72 hr and biweekly intervals and for 4 weeks after which the plates showing no growth were considered negative when yeast growth was noticed it was investigated by gram stain,Lactophenol cotton blue according to Raper and Fennel (1965). The yeast and other fungi were identified on the basis colony morphological charater (pseudohyphae, true hyphae, blastocondia and chlamydospores according to Raper and Fennel (1965); Frey *et al.* (1979).

-Germ tube test
Germ tube test was used for differentiation between Candida spp. and other yeast spp. In which a very light suspension of yeast like organisms in 0.5-1.0 ml of sterile rabbit serum can be used. Incubation was occurred at 37˚C for no longer than 3 hrs, then one drop of yeast-serum mixture was placed on a slide slip and was examined microscopically for germ tube production.

-Urease production test
Urease production test also was used for differentiation of yeast like fungi in which urease positive organism produce an alkaline reaction indicated by a pink-red color within 72 hrs.

-Assimilation test
Assimilation sugars were used in which carbon and nitrate were used. These biochemical tests were made according to Koneman *et al.* (1997).

RESULTS

From 80 milk samples, 76 samples were positive and only 4 samples were negative. 50 samples were positive for yeast isolation and 26 samples were positive for other fungi (Aspergillus flavus, Aspergillus niger, Mucor and Pencillium). From 50 positive yeast samples, only 20 samples were pure for yeasts and other 30 samples were mixed yeasts and other fungi. From 50 positive yeast Samples only 28 strains were classified as Candida genus among them 16 strains of Candida albicans (+ve germ tube test), 10 strains were Candida krusi (+ve urease) and 2 strains Candida pseudotropicals (+ ve lactose).

Table1: Percentage of yeasts and other fungi isolated from milk samples.

Number of examined milk samples	+ve		-ve		Yeasts only		Yeasts +other fungi		Other fungi only	
	No.	%	No.	%	No.	%	No.	%	No.	%
80	76	95%	4	5%	20	25%	30	37.5%	26	32.5%

-ve: milk sample.
+ve: milk sample
No.:number of milk

Table 2: Frequenceyof yeast isolated from milk samples.

Total number of yeasts	No. of *G.Candida	%
50	28	56%

* G.: Genus

Table 3: Results of Germ tube test.

Total number of yeasts (G.Candida)	+ve	-ve
28	16	12

Table 4: Results of urease production test.

Total number of yeast (G.Candida)	+ve	-ve
28	10	18

Table 5: Results of sugar assimilation.

Total number of yeasts (G.Candida)	Dextrose +ve		Lactose +ve	
	No.	%	No.	%
28	28	100%	2	7.1%

Table 6: Frequencey of Candida strains isolated from milk samples.

Total no. of Candida strains	C. albicans		C. krusi		C. pseudotropicalis	
	No.	%	No.	%	No.	%
28	16	57.1	10	35.7	2	7.1

*C.:Candida.

Table 7: Percentage of other fungi spp isolated from milk samples.

Total number of examined milk samples	Asp. flavus		Asp. niger		Pencillium		Mucor	
	No.	%	No.	%	No.	%	No.	%
80	32	40	36	45	20	25	20	25

DISSCUSION

The colonies of Candida spp. Generally grow well on sabauroud dextrose agar at 37˚C, usually forming colonies within 24-48 hours, colonies are opaque, often white or yellowish and at first usually smooth, their texture is creamy or pasty, and on microscopic smear appear to consists of oval to round budding blastospore. In our examination, we have observed that the yeast from mastitic milk need more time to form the colony. Most colonies appear at 48-72 hours but some strains need near week to grow on the media. It may be due to the abnormal condition which found in milk samples and fungi need more time for adaptation in new environment.

In this examination we have obtained a high ratio of isolation of fungi from milk samples about 95%. Yeasts isolated from about 62% of examined samples, we have obtained mixed yeasts &other fungi. Molds infection were 60%, of positive samples. Sole yeasts isolation were found in 40% of positive samples, and also we have obtained sole mold isolation (Aspergillus flavus, Aspergillus nigar, Mucor, Pencillium) of about 32.5% from all examined samples.

Among positive results of cultivation, the yeasts from Candida genus were isolated in higher frequency percentage 56% (table 2) Simillar results were described by Krukowski *et al.* (2000) and Casia dos Santos and Moacir (2005).

By Germ tube test and Assimillation test. We have found that C. albicans was isolated in about 32% of positive yeast samples and 57% of positive Candida samples, Candida krusi was 20% of positive yeast samples and 35% of positive Candida samples and Candida pseudotropicals 4% of positive yeast samples and 7% of positive Candida samples. These results agree with Sheena and Sigeer. (1995)

The percentage of fungal isolation in surveys carried out in many countries varies considerably with 6.1% rates described by Awad *et al*. (1980). 1.3% in Denmark reported by Aalbaek *et al*. (1994) and 12.07% in Brazil showed by Costa *et al.* (1998) and Casia dos Santos and Moacir Marin (2005) were isolated fungi in (32%) cases, with 17.3% of the fungi being Candida spp.

Yeast especially Candida is commonly viewed as an opportunistic yeast pathogen and sources of infection may be the skin of the udder, milker´s hand, milking machines and other equipments, floors, straw, feed,drugs and sanitizing mixtures Costa *et al.* (2004) and Seker Esra (2010). Under the immunosuppersion condition, the yeast mainly together with the fungi are

able to break the udder defence, and there was an increase of the number of udder infections caused by Candida species was reported in recent years by Krukowski *et al.* (2004).

In our investigation the most frequent isolate species, Candida albicans (10 strains) confirmed by positive Germ tube test and the next Candida krusi (5 strains) and these results agree with Costa *et al.* (1993, 1998, 2004) and Vinitha and Ballal (2007), but disagree with Santos and Marin (2005) and Seker (2010) who reported that Candida krusi is the most predominant strian isolated.

CONCLUSION

From the present study, prevention of mycotic mastitis requires management practices and hygienic management that prevent mycotic growth, also more advanced diagnosis for identifying yeast by several kits must be available, for saving more antibiotics used in treatment. In which the use of antimicrobials for long period is pointed out the occurrance of mycotic mastitis. Candida albicans produces toxins and can colonize in mouth and intestine, infected animals can be danger for men who consume this mastitic milk. The presence of yeasts and yeast-like fungi affect the quality and organoleptic characteristics influencing the shelf-life of the product. The yeasts found in bovine milk may be part of the normal microbes or might cause damage to the mammary gland. Although it is not expected that these microorganisms can survive the thermal treatment, milk may be a carrier for a great diversity of agents that could be harmful to public health.

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الخمائر كمسبب من أسباب ألتهاب الضرع فى الحيوانات الحلابه فى محافظة البحيرة

هناء فتحى فرج ، أمل عبدالمنعم السيد

يعتبر التهاب الضرع من الامراض التى تصيب الحيوانات الحلابه فى موسم الحليب وتسبب خسارة أقتصاديه كبيرة نتيجة الأستخدام الزائد للمضادات الحيوية وبعض الأدوية الأخرى التى تضر بصحة الأنسان وأجريت هذه الدراسة لعزل الخمائر كنوع من أنواع الفطريات المسببه لألتهاب الضرع فى الحيوانات الحلابة وتصنيف هذه المسببات ومعرفة العلاقة بينه وبين المسببات الأخرى. لذا اجريت هذة الدراسة على ٨٠ عينة لبن من حيوانات مصابة بالتهاب الضرع من مزارع فى محافظة البحيرة من خلال الفحص المباشر للعينات تم تصنيف عترات الخمائر والعترات المصاحبة من خلال زراعتها على وسط السبرود ثم بعد ذلك عمل أختبار أنبوبة الأستنبات وذلك خصيصا لتصنيف الكانديدا وقد وجد الأتى: حوالى ٥٠ عينه لعزل الخمائر بنسبة ٦٢.٥% و٢٦ عينه ايجابية للفطريات الأخرى بنسبة ٣٢.٥% و٤ عينات كانت سلبية تماما بنسبة ٥% من العينات التى تم فحصها. من العينات الأيجابية لعزل الخمائر وجد ٣٠ عينة بنسبة ٦٠% أيجابية للخمائر مع بعض الفطريات الأخرى و٢٠ عينة بنسبة ٤٠% كانت أيجابية للخمائر دون غيرها ووجد أن ٢٨ عترة بنسبة ٥٦% من المعزولات صنفت من سلالة الكانديدا. ووجد أن١٦ عترة من هذة الكانديدا صنفت البيكانز بمعدل ٥٧% و ١٠ عترة صنفت كانديدا كيرشنى بمعدل ٣٥% و٢ عترة صنفت كانديدا سيدوتوبيكالز بمعدل ٧%. ومن هذه الدراسة يتلخص الآتى: يجب اتباع الأشتراطات الصحية السليمة فى انتاج الألبان للحصول على ألبان عالية الجودة. وكذلك التشخيص المبكر لحالات ألتهاب الضرع بأستخدام الأختبارات الحقلية للكشف عن حالات ألتهاب الضرع الغير مرئى ،البحث عن طرق جديدة مثل الأليزا للكشف المبكر. الأعتدال فى أستخدام المضادات الحيوية حتى لا تؤدى الى تقليل المناعة وبالتالى الى الأصابة بالخمائر، التى قد تؤدى الى تدمير الضرع بالكامل. التعامل مع اللبن حراريا لتجنب الأصابة بالخمائر لما لها من تأثير عاى صحة الأنسان.