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SOME STUDIES ON THE MICROBIOLOGICAL CAUSES AND BIOCHEMICAL CHANGES IN MASTITIC MILK WITH EMPHASIS ON FUNGI AND MYCOPLASMA

(With 7 Tables)

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بعض الدراسات عن الأسباب الميكروبيولوجية والتغيرات البيوكيميائية فى اللبن

الناتج من التهاب الضرع وبصفة خاصة الأصابة بالفطريات والميكوبلازما

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شمل البحث جمع عدد ٩٠ عينة لبن من الأبقار المصابة بالتهاب الضرع والذى لم يستجب للعلاج بالمضادات الحيوية من مناطق محافظتى الجيزة وكفر الشيخ . هذا وقد تم تجميع عدد ٣٠ فقط ثلاثون عينة لبن من أبقار سليمة صحيا من الناحية الظاهرية . وقد اثبت الفحص المعملى الميكروبيولوجى وجود عدد ٢٣ عترة من فطر الخميرة (٢٥,٥ من أجمالى العينات) ، عدد ٨ عترات (٨,٨ من أجمالى العينات) من فطر العفن. هذا وبالتحليل لهذة الفطريات اتضح أنها كانديدا البيكانز ، كانديدا بارابسيللوزيز ، كانديدا تروبيكاليز ، كانديدا كروزاى ، تريكوسبورون ، تورولويسزجلابراتا ، رودوتوريلاروبرا ، اسبيرجيللاس نيجر، اسبيرجيللاس فيوميجاتس ، بنسلين ، ميوكر ، أنواع الابسيديا . بالأضافة الى كل هذا تم عزل أيضا ميكروب الميكوبلازما البقرى من عدد ٤٢ عينة (٤٦,٧ من أجمالى العينات) ، بينما ميكوبلازما بوفيجينيتاليم تم عزلها من عدد ١٥ حالة (١٦,٧ من أجمالى العينات) . نفس النتائج تم الحصول عليها بأختبار التلزن الدموى الغيرمباشر . وقد تم أيضا الحصول على خليط من الفطريات المسببة لألتهاب الضرع مع الميكوبلازما فى عدد ٤ عينات من محافظة كفر الشيخ . وتم فحص عينات اللبن المأخوذة لمعرفة النسبة المئوية للكلوريدات ، سكر اللاكتوز ، الكالسيوم ، الفسفور ، بالأضافة الى الماغنسيوم. علاوة على ذلك تم تعيين الأس الأيدروجينى والعد الكلى لخلايا الدم فى المليمتر المكعب من الدم.

SUMMARY

A total of 90 milk samples were collected from non-responding cow mastitic cases for antibiotics treatment from Giza and Kafr El-Sheikh Governorates. In addition, thirty samples were collected from apparently healthy cows.

The mycological examination of all samples revealed isolation of 23 (25.5 %) strains of pathogenic yeasts and 8 (8.8 %) strains of moulds from mastitic samples. These strains were identified as *Candida albicans*, *C. parapsillosis*, *C.tropicalis*, *C. krusei*, *Trichosporon* species, *Torulopsis glabrata*, *Rhodotorula rubra*, *Aspergillus niger*, *A. fumigatus*, *Penicillium* species, *Mucor* spp. and *Absidia* spp . *Mycoplasma bovis* was isolated from 42 (48.8%) while *Mycoplasma bovigentalium* was isolated from 15 (16.7 %) of mastitic milk. The same results were detected by the indirect haemagglutination test. Mixed infection of fungi and mycoplasma were observed in 4 samples from Kafr El-Sheikh area. Chlorides, lactose, calcium, phosphorous, magnesium percentages, pH value and total cell count were examined.

***Key words:** Mastitic milk - Microbiology - Biochemistry.*

INTRODUCTION

Mastitis is a disease complex having different causes, different degrees of intensity and variations in duration and residual effects.

The economic aspect of this problem is highly important as it causes great losses in the dairy products due to losses in milk and fat production.

From the microbiological aspect of view, the mammary gland can be expected to provide a favorable environment for a great variety of microbes. The continued extensive use of intramammary infusion, often adminstered carelessly, can be expected to lead to an over increasing list of infective agents involved in the mastitis complex. Although bacteria are the major microorganisms involved in this complex, fungi mainly yeasts and mycoplasma have been described as present problems, Abou-Zaid and Bahout (1993) isolated *Candida tropicalis* and *Penicillium* spp. from mastitic milk while El-Ebeedy et al. (1986) isolated *Mycoplasma bovivenitalium* from a dairy bovine herd suffering from mastitis.

The aim of this work was directed to evaluate the incidence of mastitis due to the mycological and mycoplasmal agents and to record the biochemical changes in such cases .

MATERIALS and METHODS

Milk samples were collected aseptically in the early morning from 90 clinically mastitic and 30 apparently normal Friesian cows in Giza and Kafr

El-Sheikh Governorates. The mastitic animals were previously treated with antibiotics (terramycin (Pfizer) and gentamycin) without response.

Each milk sample was centrifuged at 2000 r.p.m for 15 minutes and the sediment was cultured on Sabouraud's dextrose agar and incubated at 25°C for 2-5 days. Yeast colonies were examined macroscopically and microscopically. Identification was done by rice agar technique, fermentation and assimilation of glucose, galactose, sucrose, inositol and nitrate production according to Barnett et al. (1983) . Identification of mould was carried out according to Al-Doory (1980) .

Standard culture methods adopted by Sabry (1968) for isolation of Mycoplasma were employed by making indirect culture; 0.1 ml milk was inoculated into broth and broth to broth passages and subcultures to agar plates were made twice at 48 hours intervals with incubation at 37°C . Daily examination of agar plates was carried out and the final reading was made on the 7 th day ; samples were accepted as negative after five transfers that did not show growth .

Mycoplasma cultures were maintained in the form of agar blocks in broth (Sabry, 1971) after 48 hours incubation at 37°C ; then purification of the isolates was applied according to Sabry (1968) which needs at least four days . Digitonin sensitivity test was also applied as described by Erno and Stipkovits (1973) which needed 48 hours to differentiate between mycoplasma and acholeplasma .

Biochemical characterization was screened and it took a duration of 48 hours and for sero-identification growth inhibition test was applied (Clyde, 1964) .

Chloride content of milk samples was estimated according to Coles (1974) technique. The pH values were performed using portable Beckman Chemi-Mate pH meter. Estimation of lactose percent was adopted as El-Naggar (1983) method. Calcium and magnesium contents of milk were estimated according to Joshi et al. (1976).

RESULTS and DISCUSSION

The obtained results in Table (1) showed that 28 out of 90 mastitic milk samples collected from Giza and Kafr El-Sheikh harbourd both yeasts and moulds or one of them with a percentage of 31.1 %. Yeasts were isolated from 6 and 14 samples while moulds were isolated from 2 and 3 samples out of 40 and 50 samples from Giza and Kafr El-Sheikh, respectively. Three samples from Kafr El-Sheikh contained both yeasts and moulds. Similar results were perviously reported by Sharama et al. (1977), Amemiya and Tashiro (1978) and Costa et al. (1993).

From Table (2), it is clear that the percentages of different yeast isolates were *Trichosporon* species (8.9 %) , *Torulopsis glabrata* (5.6%) , *Candida parasillosis* (3.3%), *C. tropicalis* (3.3%), *Rhodotorula rubra* (2.2%), *C. albicans* (1.1 %) and *C. krusei* (1.1%). These isolates were previously recovered from bovine mycotic mastitis by Farid et al. (1975) , Abdel-Halim (1979) , Yeo and Choi (1982), Abou-Zaid and Bahout (1993) and Elad et al. (1995) .

Aspergillus fumigatus was isolated from one mastitic milk sample (Table 3). Fenzia et al. (1975) isolated *A. fumigatus* pathogenic for laboratory animals from milk of five apparently healthy cows with a relatively low milk yield. They suggested that *A. fumigatus* acts as a primary invador of bovine udder while Thompson et al. (1978) isolated *Aspergillus fumigatus* from one cow with mycotic mastitis . *Aspergillus niger* and *Penicillium* species were also isolated from 3 (3.3%) and 1 (1.1 %) mastitic milk samples, respectively. Similar findings were previously reported by Abou-Zaid and Bahout (1993), Costa et al. (1993) and Kuo and Chang (1993).

Table 1: Prevalence of fungi in mastitic milk samples .

Governorate	No. of samples	+ve Yeast		+ve mould		Mixed		Total	
		No.	%*	No.	%*	No.	%*	No.	%*
Giza	40	6	6.7	2	2.2	-	0	8	8.8
Kafr El-Sheikh	50	14	15.5	3	2.3	3	3.3	20	22.2
Total	90	20	22.2	5	5.5	3	3.3	28	31.0

* Percentages were calculated in relation to total samples (90 samples) .

Table 2: Yeasts isolated from mastitic milk samples .

Isolates	No.	% *
C.albicans	1	1.1
C. parapsillosis	3	3.3
C. tropicalis	3	3.3
C. krusei	1	1.1
Trichosporon	8	8.9
Torulopsis glabrata	5	5.6
Rhodotorula rubra	2	2.2
Total	23	25.5

* Percentages were calculated in relation to total samples (90 samples) .

Table 3: Moulds isolated from mastitic milk samples .

Isolates	No.	% *
A. niger	3	3.3
A. fumigatus	1	1.1
Penicilium	1	1.1
Mucor sp.	2	2.2
Absidia sp.	1	1.1
Total	8	8.8

* Percentages were calculated in relation to the total number of samples (90 samples).

The results in Table (4) showed that , out of 40 and 50 examined milk samples from Giza and Kafer El-Sheikh , respectively 16 (40 %) and 26 (52 %) were positive for Mycoplasma and identified as *Mycoplasma bovis* while *Mycoplasma bovigenitalium* were isolated from 3 (7.5%) and 12 (24 %) samples, respectively . These results of isolation were supported by the serological test of IHA as shown in Table (5). Similar results were previously reported by El-Ebeedy et al., (1985 and 1986), Gad et al., (1987) and Laila El-Shabiny et al. (1989) .

Mixed infections of *Mycoplasma bovis* and *C. parapsilosis*, *C. tropicalis* and *Trichosporon* species were shown in Table (6) .

Table 4: Isolation of Mycoplasma from mastitic milk samples .

Governorates	No. of samples	*M. bovis*		*M. bovigenitalium*	
		No.	%	No.	%
Giza	40	16	40	3	7.5
Kafr El-Sheikh	50	26	52	12	24
Total	90	42	48.8	15	16.7

5: Indirect Haemagglutination test for identification of Mycoplasma from mastitic milk samples .

Governorate	*M. bovis* (No. +ve)	*M. bovigentalium* (No. +ve)
Giza	16	3
Kafr El-Shikh	26	12

N.B. +ve $\frac{1}{32}$, suspect $\frac{1}{16}$, negative > $\frac{1}{8}$.

Table 6: Mixed Mycoplasma and yeast from Kafr El-Sheikh samples .

No. of samples	Mixed infection	%	No.	Isolates	
				Mycoplasma	yeast
50	4	8	1	*M. bovis*	*C. parasillosis*
			1	*M. bovis*	*C. tropicalis*
			2	*M. bovis*	*Trichosporon sp.*

Results recorded in Table (7) revealed that Mycoplasma infected samples showed that the mean chloride contents were 128.69 $\pm$ 1.31 and 132.61 $\pm$ 1.31 , lactose were 3.81 $\pm$ 0.02 and 3.8 $\pm$ 0.02 , total cell count 552938 $\pm$ 15550 and 787621 $\pm$ 12850 and pH values were 7.1 $\pm$ 0.03 and 6.9 $\pm$ 0.03 in Giza and Kafr El-Sheikh samples , respectively . While mycotic infected samples showed that the mean chloride contents were 145.74 $\pm$ 2.2 and 161.23 $\pm$ 2.32 , lactose 3.86 $\pm$ 0.03 and 3.77 $\pm$ 0.04 , total cell count 3547560 $\pm$ 23475 and 3762264 $\pm$ 21680 and pH values were 7.2 $\pm$ 0.05 and 7.4 $\pm$ 0.06 respectively . Milk from apparently healthy cows showed that the mean chloride content was 99.79 $\pm$ 0.44, lactose 4.56 $\pm$ 0.03, total cell count 235000 $\pm$ 5595 and pH value was 6.56 $\pm$ 0.02, respectively. It was noticed that the increase in chloride content , pH value and cell count and the decrease in lactose , calcium , phosphorous and magnasium contents were more in case of mycotic mastitis than mycoplasmal mastitis . These findings were in harmony with those reported by El Ebeedy (1985) , Larson (1985) and Abou El-Fotoh (1986) .

The increase of both chloride contents and pH values in mastitic cases may be due to transmission of sodium chloride and alkalinity to milk through blood as a result of alteration in permeability of the infected mammary gland (Peaker, 1977). The decrease in lactose content which was observed in mastitis is due to the effect in manufacture of lactose by the udder tissue as a result of inflammatory process (Larson, 1985).

From public health aspect, *Candida albicans* causes bronchopulmonary infection, secondary intestinal infections and less frequently septicemia, meningitis, endocarditis and pyelonephritis in human beings. *Candida tropicalis* and *C. parapsilosis* are responsible for pulmonary mycosis and deplitsation in man with septicemia (Jen et al., 1967 and Bruns et al., 1970) . *Candida parapsilosis* was isolated from normal milk samples . As this milk may be consumed unpasteurized or used for manufacuring of cheese or youghort, infection of consumers would be of high possibility especially in children .

Table 7: Chemical analysis of mastitic and non-mastitic milk samples .

	Giza		Kafr El-Sheikh		Normal
	Mycoplasma (19)	Fungi (8)	Mycoplasma (38)	Fungi (20)	Milk (30)
Chloride mg /100 ml	128.69 ± 1.3	145.74 ± 2.2	132.61 ± 1.31	161.23 ± 2.32	99.79 ± 0.44
Lactose g / 100 ml	3.81 ± 0.02	3.86 ± 0.03	3.8 ± 0.02	3.77 ± 0.04	4.56 ± 0.03
Calcium mg / 100 ml	0.21 ± 0.004	0.18 ± 0.003	0.19 ± 0.002	0.16 ± 0.002	0.298 ± 0.004
Phosphorous mg / 100 ml	0.092 ± 0.89	0.09 ± 0.49	0.089 ± 0.44	0.087 ± 0.29	0.116 ± 0.02
Magnesium mg / 100 ml	0.178 ± 0.001	0.175 ± 0.001	0.172 ± 0.003	0.170 ± 0.004	0.250 ± 0.004
Total cell count cell / ml	552938 ± 15550	3547560 ± 23475	787621 ± 12850	4 ± 21680	235000 ± 5595
pH value	7.1 ± 0.03	7.2 ± 0.05	6.9 ± 0.03	7.4 ± 0.06	6.56 ± 0.02

There is no effective therapy against mycoplasma mastitis and there is difficult effective therapy against mycotic mastitis (Ciba-Geigy report, 1983 and Laila El Shabiny et al., 1989) . Therefore, adequate and rapid diagnosis is considered to be one of the most important factors necessary for enhancing the control of the spread of the disease by quarantine and culling of infected animals and preventing the economic losses due to the uses of uneffected antibiotics and possibility of spreading the infection to others .

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