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**SOME STUDIES ON THE OCCURRENCE
OF YERSINIA MICROORGANISMS IN RAW MILK
AND SOME SOFT CHEESES SOLD
IN ALEXANDRIA GOVERNORATE**

(With 5 Tables)

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بعض الدراسات عن تواجد ميكروب اليرسينيا فى اللبن
وبعض أنواع الجبن الطرى فى أسواق مدينة الإسكندرية

علا عبد العزيز باشا ، فتح الله على الشابورى ، أحمد حسن فايد

يعتبر اللبن الخام ومنتجاته من أكثر الأغذية استهلاكاً نظراً لقيمته الغذائية العالية إلا أنه قد يكون مسبباً لكثير من الأمراض عن طريق التلوث بالميكروبات أثناء الانتاج والنقل والتداول والاستهلاك. ومن هذه الميكروبات ميكروب اليرسينيا الذى يلعب دوراً خطيراً فى التأثير على الصحة العامة للإنسان لما قد يسببه من حالات تسمم غذائى مصحوبة بإضطرابات معدية ومعوية حادة ومزمنة. ولقد إشتملت الدراسة على فحص عدد ١٥٠ عينة من الألبان ومنتجاته عبارة عن ٥٠ عينة لبن خام ، ٥٠ عينة جبن قريش ، و ٥٠ عينة جبن دمياطى وتم تجميع هذه العينات خلال ربيع عام ٢٠٠٦ من محلات وأسواق محافظة الإسكندرية وقد أسفر العزل البكتريولوجى عن تواجد ميكروب اليرسينيا بنسبة ٢٠% فى عينات اللبن وبنسبة ١٤% فى عينات الجبن القريش وبنسبة ٦% فى عينات الجبن الدمياطى. وفى لبن المزارع تم عزل ميكروب اليرسينيا إنتيروكوليتيكا بنسبة ١٢% بينما تم عزل نفس الميكروب من الجبن القريش بنسبة ٦% ولم يتم عزل هذا الميكروب من الجبن الدمياطى وبدراسة اختبار الحساسية لميكروب اليرسينيا إنتيروكوليتيكا وجد أن أكثر العقاقير تأثيراً عليه هى الكلورامفينيكول والسلفاميثوكسازول والإستربتومايسين بينما يقاوم المضاد الحيوى النيومايسين والنورفلوكساسين. هذا وقد تمت دراسة الأهمية الصحية والإقتصادية والمقترحات الواجب اتباعها للمحافظة على اللبن ومنتجاته من التلوث بهذه الميكروبات.

SUMMARY

A total of 150 samples including 50 samples of raw milk, 50 samples of Kareish cheese and 50 samples of Damietta cheese, all were collected randomly from Alexandria markets and street vendors and examined

bacteriologically for the detection and identification of existing *Yersinias*. The result recorded in this investigation declared that *Yersinia* species could be isolated from raw milk, Kareish cheese and Damietta cheese in percentages of 20, 14, and 6% respectively. *Yersinia enterocolitica* which is pathogenic for human as it is responsible for food poisoning and other gastrointestinal symptoms was isolated from raw milk in 6 cases (12%), from Kareish cheese in 3 isolates (6%) and could not be isolated from Damietta cheese. The presence of *Yersinias* in this study reflected the lack of hygienic supervision and poorly cleaned and sanitized dairy farm equipments and processing plant equipments. Moreover, *Yersinia enterocolitica* isolated in this study was tested for susceptibility to 10 antimicrobial drugs. All isolates were highly susceptible to Chloramphenicol, Sulphamethoxazole, Tetracyclin, and Streptomycin but resistant to Ampicillin, Neomycin, and Norfloxacin. The public health hazard of *Yersinia enterocolitica* with other *Yersinia* species and the sanitary measures for improving milk quality beside proper handling and distribution of milk and milk products must be done under the most possible hygienic conditions.

Key words: *Milk, milk products, cheese, Yersinia.*

INTRODUCTION

Yersiniosis is considered one of the most important bacterial foodborne infections as their incidence among humans had increased dramatically. *Yersinia* species are common environmental Gram negative, rod shaped bacteria that include several species from which *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are pathogenic to humans and animals and are recognized as foodborne pathogens. (Tassinari *et al.*, 1994). It can be isolated from environment, faeces of cattle and goats, flies and insects (Shayengani *et al.*, 1983). *Yersinia* can contaminate milk and milk products during handling, transportation, production, preparation and processing as it can adhere and colonize the dairy processing surface that, come in direct contact with dairy products. Milk and milk products have been responsible for numerous outbreaks of Yersiniosis, in Canada (Kasatiya, 1976), in America (Anon, 1976 and Black *et al.*, 1978), in Ireland (Walker and Gilmour, 1986), in England (Barret, 1986) and in Morocco (Hamama *et al.*, 1992). In Egypt several microbiological surveys of milk and milk products such as El-Kholy (1990), El-Sherbini *et al.* (1993), El-Gamal (1993), El-Prince and Sabreen (1998), Hussein and Ahmed (2002), El- Prince and Sayed (2002) and Bahout and Mustafa (2006) showed

that *Yersinia* species were present with variable percentages. *Yersinia* can produce a variety of clinical symptoms in human such as food poisoning associated with acute and chronic gastroenteritis, pseudo-appendicitis, arthritis, erythema and septicemia which lead to death (International Dairy Federation, 1994). *Yersinia* can survive and grow at refrigeration temperature (Kirov *et al.*, 1993) as well as can withstand freezing and surviving for extended periods in frozen food, even after repeated freezing and thawing (Toora *et al.*, 1992). Therefore, the aim of the present study was conducted to determine the incidence of *Yersinia* species in milk and some milk products like Damietta and Kareish cheeses and the relation of these microorganisms to public health importance in Alexandria Governorate.

MATERIALS and METHODS

A total of 150 samples including raw milk (50), Kareish cheese (50), and Damietta cheese (50) were collected randomly from Alexandria markets and street vendors. All samples were transferred to the laboratory in an ice box without delay for bacteriological examination in relation to *Yersinia* microorganisms.

Preparation of samples:

All milk samples were tested by peroxidase test to exclude heat-treated milk according to Lampert (1975). Kareish and Damietta cheeses samples were thoroughly mashed and mixed in sterile mortar. The methods recommended by APHA (1985) was followed.

Pre- Enrichment:

Twenty five ml. or gm. of each prepared sample were added to 225 ml. of sterile Trypticase-soya-broth at 22 °C for 24 hours (Schiemann, 1983), followed by selective enrichment in bile sorbitol broth by adding one ml. of pre-enrichment broth to 9 ml. of sterile peptone – sorbitol bile broth (International Standardization Organization (ISO/DIS1073) 1993) supplemented with Novobiocin (10 µg/ml) and Polymixin (5 I.u/ml). All inoculated broth tubes were incubated at 22 °C for 2-3 days (Landgraf *et al.*, 1993).

Isolation of Yersinias:

Streak from each peptone-sorbitol-bile broth tube was done on duplicate plates of Cefsulodin- Irgasan- Novobiocin agar. The plates were incubated at 30°C for 18-20 hours. Characteristic colonies (dark red center "Bulls eye like" with transparent border) were selected and picked into nutrient agar slants. All nutrient slants were incubated at

25°C for 24 hours and kept in refrigerator for complete conformation (Walker and Gilmour, 1986).

Identification of isolated cultures:

The presumptive *Yersinia* isolates were confirmed according to the scheme adopted by (Seeliger and Jones, 1986) and the differentiation between *Yersinia* strains were done by using sugar fermentation scheme adopted by (Simmonds *et al.*, 1987).

Sensitivity test:

The antibiograms of the isolated *Yersinia enterocolitica* were done using disc diffusion method of Bauer *et al.* (1966). The interpretation of zones of inhibition were estimated according to the limits given by Finegold and Martin (1982). Different drugs discs were used such as Tetracyclin, Streptomycin Chloramphenicol, Sulphamethoxane, Ampicillin, Norfloxacin, Pencillin, Cefatoxin and Neomycin.

RESULTS

Bacteriological examination of 150 samples including 50 samples of raw milk 50 samples of Kareish cheese and 50 samples of white Damietta cheese revealed the isolation of *Yersinia* species in percentages of 20, 14, and 6% respectively Table (1). The organisms were Gram negative, motile, rod shaped that gave characteristic colonies dark red center "Bulls eye like" with transparent border on Cefsulodin-Irgasan-Novobiocin agar. The morphological and biochemical characteristics of *Yersinia* isolates are illustrated in Tables 2 and 3.

The results presented in Table (4) showed different *Yersinia* strains which were isolated from milk, Kareish and Damietta cheeses samples.

The results of drug sensitivity was studied and summarized in Table (5). From such Table we can notice that *Yersinia enterocolitica* was sensitive to Chloramphenicol and Sulphamethoxazole.

Table 1: Occurrence of *Yersinia* species in milk, Kareish and Damietta cheese samples.

Kind of samples	No. of samples examined	Positive samples	
		No.	%
Milk	50	10	20
Kareish cheese	50	7	14
Damietta cheese	50	3	6
Total	150	20	-

Table 2: Morphological and biochemical characteristics of *Yersinia* species.

Test	Reaction
Gram stain	-ve
Motility at 22°C	+ve
Indol 30 °C	+ve
Nitrate reduction	+ve
Vogus – proskauer 22°C	+ve
Urease	+ve
Lysine decarboxylase	-ve
Ornithine decarboxylase	+ve
Sucrose 22°C	+ve
Xylose 22°C	+ve
Salicin 22°C	+ve
Dextrose 22°C	+ve
Maltose 22°C	+ve
Malonate	-ve
Dulcitol	-ve

Table 3: Identification of *Yersinia* species by sugar reactions according to Simmond's *et al.* (1987).

<i>Yersinia</i> Species	Sucrose	Rhamnose	Rafinnose	Melibiose
<i>Yersinia enterocolitica</i>	+ve	-ve	-ve	-ve
<i>Yersinia frederiksenii</i>	+ve	+ve	-ve	-ve
<i>Yersinia kristensenii</i>	-ve	-ve	-ve	-ve
<i>Yersinia intermedia</i>	+ve	+ve	+ve	+ve

Table 4: Incidence of *Yersinia* species in milk, Kareish and Damietta cheese samples.

Identified <i>Yersinia</i> spp.	Milk samples		Kareish cheese samples		Damietta cheese samples	
	No.	%	No.	%	No.	%
<i>Yersinia enterocolitica</i>	6	12	3	6	0	0
<i>Yersinia intermedia</i>	1	2	2	4	1	2
<i>Yersinia frederiksenii</i>	1	2	1	2	1	2
<i>Yersinia kristensinii</i>	2	4	1	2	1	2
Total	10	20	7	14	3	6

Table 5: Drug sensitivity test for the isolated strains of *Yersinia enterocolitica*.

Drug Disc	Reaction
Tetracyclin (TE 30)	+++
Streptomycin (S10)	+++
Chloramphenicol (C30)	+++
Sulphamethoxazole (SXT 25)	+++
Ampicillin (AM10)	---
Pencillin (P10IU)	+
Norfloxacin (NOR10)	--
Cefotaxin(CTX30)	--
Neomycin (N30)	--
Gentamycin (GM10)	+++

DISCUSSION

Foodborne bacterial gastrointestinal infections are important causes of morbidity and mortality world-wide, and despite successful control programs in some developed countries, these infections continue to have a major impact on public health economy (Varnam and Evans, 1991). *Yersinia* species are one of the most reported pathogens incriminated in gastrointestinal illness (Kapperud, 1991) Recently *Yersinia enterocolitica* is considered one of intracellular bacteria, which localize and multiply in lymphatic system causing acute mesenteric lymphadenitis and terminal ileitis accompanied by fever, diarrhea and abdominal pain.

Incidence of *Yersinia* microorganisms in raw milk samples:

Results recorded in Table (1) showed that 10 isolates (20%) of the examined milk samples were positive for *Yersinia*. This obtained result was higher than those reported by Fukushima *et al.* (1984), El-Gamal, (1993) and Contantinue *et al.* (1998) who recorded an incidence of 4.3%, 6% and 1.5% respectively. Higher percentages of *Yersinia* species were reported by Franzin *et al.* (1984) 36.7%, Hamama *et al.* (1992) 36.6% and Tassinari *et al.* (1994) 45%.

Results summarized in Table (4) revealed that *Yersinia enterocolitica* could be isolated from 12% milk samples. The organism could be detected with lesser extent by El-Kholy (1990) 2%,

Abd-El-Hady (1993) 2%, Korashy (1997) 1%, and Hussein and Ahmed (2002) 4% but higher incidence rates (10-50%) were reported by Norberg (1981), Ray (1983), Hafez (1988) and Abd-El-Khalek (1998). *Yersinia intermedia*, *Yersinia frederiksenii* and *Yersinia kristensenii* were detected in 2%, 2%, and 4% of the examined samples respectively (Table 4). Contamination of milk with *Yersinia* species was explained by Meadows and Sudden (1982), they found that, *Yersinia* species might gain entry to milk during collection, contaminated water utensils, soil and milkers hands.

Incidence of *Yersinia* in Damietta cheese:

Yersinia enterocolitica failed to be detected in any of the examined Damietta cheese samples while 3 strains of *Yersinia* were identified as *Yersinia intermedia* (1), *Yersinia frederiksenii* (1) and *Yersinia kristensenii* (1) as shown in Table (4). Nearly similar findings were recorded by Korashy (1997), Abd-El-Khalek (1998) and Halawa and Abd-El-Hady (1998). Failure to detect *Yersinia* in Damietta cheese may be due to the process of aging which eliminate *Yersinia enterocolitica* (Shiemann, 1978). In addition El-Gamal (1993) failed to detect *Yersinia enterocolitica* in Damietta cheese where long period of ripening for several weeks before marketing lead to lowering in pH from 6.4 to 4.4.

Incidence of *Yersinia* in Kareish cheese:

In the present study *Yersinia* could be isolated from 7 out of 50 Kareish cheese samples out of 50 samples examined (14%) Table (1). The 7 strains of *Yersinia* isolates were identified as *Yersinia enterocolitica* (3) *Yersinia intermedia* (2) *Yersinia frideriksenii* (1) and *Yersinia kristensenii* (1) as presented in Table (4). Nearly similar findings were recorded by Ebrahim (1998), Halawa and Moawad (1999), and Bahout and Mustafa (2006). While, El-leboudy (1989) failed to detect the organism in Kareish cheese. The result of this investigation indicates that Kareish cheese collected from local markets and street vendor in Alexandria was of poor sanitary quality and could cause considerable risk to human health.

In-vetro drug sensitivity test was done against isolated strains of *Yersinia enterocolitica* using a panel of 10 drugs Table (5). The most effective antibiotics for all isolates of *Yersinia enterocolitica* were Chloramphenicol, Stryptomycin and Sulphamethoxazole, while it was resistant to Norfloxacin, Pencillin and Neomycin. This support the data obtained by Bottone (1977), Hussein and Ahmed (2002) and agree with the reports of International Dairy Federation (1994) which declared that

Yersinia enterocolitica is mainly sensitive to Gentamycin, Kanamycin, Streptomycin and Chloramphenicol and resistant to Penicillin. It is of value noting that, consumption of raw milk and milk products contaminated with *Yersinia enterocolitica* has a potential hazard.

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