

Two hundred raw milk and dairy product samples (50 cow's milk and 25 each of sheep's milk, goat's milk, pasteurized milk, white soft cheese, plain yoghurt and fruit yoghurt) were collected from dairy farms and different localities, markets and shops in El Dakahlia Province, Egypt and examined for the presence of *Listeria* and *Yersinia* species as food poisoning and mastitis causing organisms. The incidence of *Listeria* spp. in raw milk and dairy product samples was 3.0% and their distributions were 6.0% in cow's milk, 8.0% in goat's milk, 4.0% in white soft cheese samples and can't be detected in sheep's milk, pasteurized milk and both fruit and plain yoghurt samples. The incidence of *Listeria monocytogenes* in raw milk and dairy product samples was 3 (1.5%), the other *Listeria* spp. were *L. innocua* 5 (2.5 %) and *L. welshimeri* 2 (1.0%). *Yersinia* spp. could be isolated from 41 (20.5 %) of examined raw milk and dairy product samples. The incidence percentages were 40.0% in cow's milk, 20.0% in sheep's milk, 32.0% in goat's milk, 4.0% in pasteurized milk, 8.0% in white soft cheese, 12.0% in fruit yoghurt and 8.0% in plain yoghurt. *Yersinia enterocolitica*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia frederiksenii* and *Yersinia pseudotuberculosis* could be detected in (5, 1, 2, 0, 1, 1 and 1); (10, 2, 0, 1, 2, 0 and 0); (3, 0, 0, 0, 0, 1 and 1); (0, 3, 4, 0, 0, 0 and 1) and (4, 0, 4, 0, 0, 2 and 0) of examined samples, respectively. The sanitary and public health importance of these organisms as well as control measures to improve the quality of dairy products and to safeguard the consumers from infection were discussed.

Key words: *Listeria*, *yersinia* milk, dairy products.

INTRODUCTION

Listeria is a ubiquitous gram-positive microaerophilic bacterium comprises seven species: *Listeria monocytogenes*, *Listeria innocua*, *Listeria ivanovii*, *Listeria welshimeri*, *Listeria seeligeri*, *Listeria grayi* and *Listeria murrayi*. Among them, *L. monocytogenes* which is a major pathogenic microorganism capable of causing severe Listeriosis infections in humans (encephalitis, meningitis and septicaemia especially in immunocompromised individuals) and in animals (mastitis, diarrhea and gastroenteritis) (Herman *et al.*, 1995; Vela *et al.*, 2001; Siegman-Igra *et al.*, 2002; McLauchlin *et al.*, 2004; Aygun and Pehlivanlar, 2006). and *L. ivanovii* which is rarely pathogenic for humans (McLauchlin, 1997 b; Swaminathan, 2001).

Listeriosis was discovered more than 70 years ago at the end of world war (McLauchlin, 1997 a). Since then, Listeriosis has emerged as an atypical foodborne illness of major public health concern because the severity of the disease, the high rate (20-30 % in some epidemic cases), the long incubation period and the predilection for individuals who have an underlying condition which leads to impairment of T-cell-mediated immunity (Franz, 2003). Listeriosis characterized by systemic illness such as premature birth, miscarriage, infection of newborn, stillbirth, septicaemia, meningitis, central nervous system infection, endocarditis, encephalitis and death. A person with listeriosis has fever, muscle aches and sometimes gastrointestinal symptoms such as nausea or diarrhoea. If the infection spreads to nervous system, symptoms such as headache, stiff neck, confusion, loss of balance or convulsions can occur. Risk groups are pregnant women, newborns, the elderly, persons with weak immune systems as AIDS, cancer, diabetes are also risk groups and sometimes healthy people.

Listeria monocytogenes has been reported to cause mastitis and can be shed in milk and feces. Shedding in milk can occur from both clinically affected and a symptomatic animals, thus constituting a potential public health hazard. Antimicrobials used as growth promoters in animal feed have reduced the impact of infectious diseases (diarrhea, skin and organ abscesses and mastitis) but led to the dissemination of antimicrobial-resistant *L. monocytogenes* into the environment (Teuber, 1999; Jansen *et al.*, 2003).

The importance of dairy-based food as a vehicle for the transmission of various diseases has been documented; especially in countries where hygienic standards are not strictly enforced (Meyer-Broseta *et al.*, 2003). *L. monocytogenes* has been involved in many outbreaks and sporadic cases of disease primarily associated with the consumption of pasteurized milk, cheeses made from unpasteurized milk and other dairy based products that serve as good medium for the growth and survival of many pathogenic organisms in both industrialized and developing countries (Makino *et al.*, 2005; Manfreda *et al.*, 2005). The organism has been consistently can multiply in raw milk at a wide range of temperatures, including refrigeration. Also, it is more heat tolerant than many other pathogens, although current pasteurisation methods are considered to be effective. Post pasteurisation contamination with *Listeria monocytogenes* can be occurred. The outbreaks most often occurred from consumption of raw milk and dairy products because the *Listeria* organism capable of slow multiplication in refrigerated foods

(Fleming *et al.*, 1985). Important characteristics of *L. monocytogenes* are its ability to grow at temperatures of 1–44°C, at pH values of 5.0 and above, in high salt concentrations, and are relative resistance to freezing and drying (Lovett, 1989). Attention has therefore been directed to listeriosis, both as a clinical entity of increasing importance and as a substantial problem for the food industry.

Yersinia enterocolitica is an ubiquitous facultative anaerobic, Gram-negative, non spore forming, short rod shaped bacterium potentially pathogenic for man and has been recognized as a cause of acute gastroenteritis and mesenteric adenitis, and a variety of extraintestinal disorders like erythema nodosum or polyarthritits in humans (Robins-Browne, 1997). Human yersiniosis in Europe involves only few isolates of bio/serogroup 1B/O: 8, 2/O: 5, 27, 2/O: 9 and 4/O: 3 (Bottone, 1997). Although pigs are regarded as major reservoirs of pathogenic *Yersinia enterocolitica*, this microorganism has been isolated in different countries particularly from raw milk and pasteurised milk which are being implicated in several outbreaks of yersiniosis but the occurrence in derived dairy products was rarely reported (Schiemann, 1978; Larkin *et al.*, 1991). Food has been proposed to be the main source of intestinal yersiniosis, although pathogenic isolates have seldom been recovered from food samples. The psychrotrophic nature of this organism is a particular significance in milk and milk products that are normally stored at low temperatures. In raw milk *Yersinia enterocolitica* strains were able to survive in the presence of high numbers of competing microorganisms and were able to maintain the virulence plasmid during extended storage at refrigeration temperature (Larkin *et al.*, 1991). This study was performed to reveal out the prevalence of *Listeria* and *Yersinia* spp. in different types of milk and some dairy products purchased at the retail level in El-Dakahlia Province, Egypt, and to shed the light on the prevention of human Listeriosis and Yersiniosis.

MATERIALS and METHODS

1- Collection of samples (A.P.H.A., 1992):

a- Raw milk: One hundred raw milk samples (50 cow's milk, 25 each of sheep's and goat's milk) were collected from the dairy farms in El Dakahlia Province, Egypt. Each sample (about 50 mL) was collected in a clean, dry and sterile sampling bottle then labeled to identify the source, site and date of sampling.

b- Dairy products: One hundred random samples of dairy products (25 each of pasteurized milk, white soft cheese, plain yoghurt and fruit yoghurt) were collected in their retail packs from different localities, markets and shops in El Dakahlia Province, Egypt. All collected samples were placed in an insulated sampling case containing ice to ensure a storage temperature $<5^{\circ}\text{C}$ and transported to the laboratory without delay for microbiological examination.

2- Isolation and identification of *Listeria* species (Yoshida *et al.*, 1998):

a- Enrichment: 3 mL. or gm. of milk and dairy products were enriched by adding 30 mL. of *Listeria* Enrichment Broth Modified (**Difco**) and incubating at 30°C for 48 hrs.

b- Plating: 0.1 mL. of each enriched culture was streaked on PALCAM agar plate (Merck) supplemented with *Listeria* selective supplement (Merck) according to Van-Netten *et al.* (1989) which was incubated at 37°C for 24 to 48 hr. Typical *Listeria*-like colonies having developed on PALCAM agar plates (grey-green, 2 mm. in diameter and have black, sunken centers were selected. Suspicious colonies were subcultured on Tryptone-Soya Agar (Nissui) supplemented with 0.5 % yeast extract (Oxoid) for characterization and purification. The plates were incubated at 37°C for 24 hr.

c- Identification: Colonies are bluish grey by normal illumination. *Listerial* isolates were biochemically identified by using API-*Listeria* test (Bio Merieux, La Balme-Les-Grottes, France) which was specifically designed for the genus *Listeria* and include 10 biochemical differentiation tests in a microtube format (Bille *et al.*, 1992).

3- Isolation and identification of *Yersinia* species (Landgraf, 1993):

a- Enrichment: 25 mL. or gm. of milk and dairy products were homogenized with 225 mL. of SB broth (phosphate-buffered saline, sorbitol 1 % and bile salts 0.15 %) and incubated at 4°C for up to 21 days (Aulisio and Stanfield, 1984).

b- Plating: The selective enrichment medium (0.1 mL. of each culture) was plated on Cefsulodin-Irgasan-Novobiocin (CIN) agar plate (Merck) supplemented with *Yersinia* selective supplement (Merck). The plates were incubated at 30°C for 24 to 48 hrs. After 24 hr. of incubation the typical colonies of *Yersinia* appear as bulls eye colonies (1.5 mm. in diameter, deep red or purple center with sharp edge and surrounded by translucent border (Hamama *et al.*, 1992). The plates were read again after 48 hr. and presumptive colonies were removed for further testing.

c- Identification: Colonies were inoculated onto Nutrient Agar for purification then all isolates were identified by using the API-20E (Bio Merieux, Nürtingen, Germany) system which still accepted as the gold standard for the rapid identification of *Yersinia enterocolitica* (Neubauer *et al.*, 1998). The API-20E strips were inoculated following the manufacturer's instructions. The API-20E system was incubated for 18-20 hr. at 25°C (Bercovier *et al.*, 1980).

RESULTS

Table 1: Incidence of *Listeria* spp. in raw milk and dairy products.

Examined samples		No. of examined samples	No. of positive samples	%
Raw milk	Cow's milk	50	3	6.0
	Sheep's milk	25	0	0.0
	Goat's milk	25	2	8.0
Dairy products	Pasteurized milk	25	0	0.0
	White soft cheese	25	1	4.0
	Plain yoghurt	25	0	0.0
	Fruit yoghurt	25	0	0.0
Total		200	6	3.0

Table 2: Incidence of different types of *Listeria* spp. microorganisms in raw milk and dairy products.

Examined samples	Products	No. of samples	No. of samples positive for		
			<i>L. monocytogens</i>	<i>L. innocua</i>	<i>L. welshimeri</i>
Raw milk	Cow's milk	50	1	3	1
	Sheep's milk	25	0	0	0
	Goat's milk	25	1	2	0
Dairy products	Pasteurized milk	25	0	0	0
	White soft cheese	25	1	0	1
	Fruit yoghurt	25	0	0	0
	Plain yoghurt	25	0	0	0
Total		200	3 (1.5%)	5 (2.5%)	2 (1.0%)

Table 3: Incidence of *Yersinia* spp. in raw milk and dairy products.

Examined samples	Products	No. of examined samples	No. of positive samples	%
Raw milk	Cow's milk	50	20	40.0
	Sheep's milk	25	5	20.0
	Goat's milk	25	8	32.0
Dairy products	Pasteurized milk	25	1	4.0
	White soft cheese	25	2	8.0
	Plain yoghurt	25	3	12.0
	Fruit yoghurt	25	2	8.0
Total		200	41	20.5

Table 4: Incidence of different types of *Yersinia* spp. microorganisms in raw milk and dairy products

Examined samples	Products	No. of samples	No. Of samples positive for				
			<i>Y. enterocolitica</i>	<i>Y. intermedia</i>	<i>Y. kristensenii</i>	<i>Y. fredriksonii</i>	<i>Y. pseudotuberculosis</i>
Raw milk	Cow's milk	50	5	10	3	0	4
	Sheep's milk	25	1	2	0	3	0
	Goat's milk	25	2	0	0	4	4
Dairy products	Pasteurized milk	25	0	1	0	0	0
	White soft cheese	25	1	2	0	0	0
	Fruit yoghurt	25	1	0	1	0	2
	Plain yoghurt	25	1	0	1	1	0
Total		200	11 (5.5%)	15 (7.5%)	5 (2.5%)	8 (4.0%)	10 (5.0%)

DISCUSSION

Data presented in Table 1 indicate an overall *Listeria* spp. incidence of 3.0% in raw milk and dairy product samples. Proportions of 6.0% in cow's milk, 8.0% in goat's milk and 4.0% in white soft cheese samples were positive for presence of *Listeria* spp. This corresponding to an incidence level that previously reported by Breer and Schopfer (1989) and Yoshida et al. (1998). While higher levels of contamination were detected by Farber *et al.* (1988); Hassan *et al.* (2000) and Aysel *et al.* (2006). It is interesting to note that *Listeria* spp. can't be detected in sheep's milk, pasteurized milk and both fruit and plain yoghurt samples

indicating that the hygienic standards were extremely applied during the production stages.

Table 2 showed that among cow's milk samples positive for *Listeria monocytogens* one of three contained, in addition to this organism another species of *Listeria*: Three samples had *L. innocua* and one has *L. welshimeri*. Nearly similar finding of *L. monocytogens* in milk was reported by Farber *et al.* (1988). Higher prevalence of *L. monocytogens* was reported by El-Sherbini (1990) and Jayarao and Henning (2001). Terplan *et al.* (1986) and Stone (1987) failed to detect *L. monocytogens* in cow's milk. Two goat's milk samples contained *L. innocua* and *L. monocytogens*. Løken *et al.* (1982) recovered *L. monocytogens* from goat's milk (11.4%). Only one sample of white soft cheese has *L. monocytogens* and *L. welshimeri*. Higher percentages of *L. monocytogens* in white soft cheese were reported by Pini and Gilbert (1988) 10.0%; Breer and Schopfer (1989) 3.0%; El-Sherbini (1990) 16.0% and Aysel *et al.* (2006) 4.0%.

Previously mentioned findings revealed that among the examined dairy products, only white soft cheese was contaminated by *Listeria monocytogens* and this result is in agreement with that reported by Pini and Gilbert (1988) and Breer and Schopfer (1989). As an explanation to that, contamination may occurred during the ripening process due to the higher pH of cheese at the later stages of ripening which should be considered a hygienic problem.

The results recorded in Table 3 declared that *Yersinia* spp. could be isolated from 41 (20.5 %) of the examined raw milk and dairy product samples. The incidence percentages were variable as follow: 40.0% in cow's milk, 20.0% in sheep's milk, 32.0% in goat's milk, 4.0% in pasteurized milk, 8.0% in white soft cheese, 12.0% in fruit yoghurt and 8.0% in plain yoghurt samples. These findings of raw milk are in agreement with that reported by Franzin *et al.* (1984); Umoh *et al.* (1984); Ibrahim and MacRae (1991) and Awad (2002). While higher results obtained by Vidon and Delmas (1981); Walker and Gilmour (1986); MacManus and Lanier (1987); Adriana *et al.* (1994) and Ozbaz (2000). Lower results were recorded by Fukushima *et al.* (1986); Syed *et al.* (1989); Hamama *et al.* (1992); Mohamed *et al.* (2004) and Jayarao *et al.* (2006). While Quaglio *et al.* (1988), Desmasures *et al.* (1997) and Ramesh *et al.* (2002) could not isolate *Yersinia* from raw milk sample. Results of pasteurized milk are lower than that mentioned by Hamama *et al.* (1992) and Adriana *et al.* (1994). While Syed *et al.* (1989) and Mohamed *et al.* (2004) failed to detect *Yersinia* in pasteurized milk.

Hamama *et al.* (1992) reported nearly similar findings of white soft cheese and higher values with yoghurt samples.

The presence of *Yersinia* spp. in pasteurized samples can be explained either by post-pasteurization contamination or by the presence of a heat resistant strain. Insufficiently cleaned milk equipment was the most frequently incriminated source of pasteurized milk contamination with *Yersinia* spp. While, in fermented milk its presence can be explained by the use of initially contaminated milk for their preparation or due to fermented milk are consumed within 36 hrs. period following their manufacture, this period of time might not be sufficient to destroy all *Yersinia* present in these acidic products as *Yersinia* was found to survive for one week in yoghurt pH (4.5). *Yersinia* spp. in fresh cheese could be attributed to different factors such as the use of raw milk and the eventual contamination from human handlers, environment and water Hamama *et al.* (1992).

The results tabulated in Table 4 revealed that *Yersinia enterocolitica*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia frederiksenii* and *Yersinia pseudotuberculosis* could be detected in (5, 1, 2, 0, 1, 1 and 1%); (10, 2, 0, 1, 2, 0 and 0%); (3, 0, 0, 0, 0, 1 and 1%); (0, 3, 4, 0, 0, 0 and 1%) and (4, 0, 4, 0, 0, 2 and 0%) of the examined cow's milk, sheep's milk, goat's milk, pasteurized milk, white soft cheese, fruit yoghurt and plain yoghurt samples, respectively. Hamama *et al.* (1992) could identify the same isolates from cow's milk, white soft cheese and yoghurt. While, Syed *et al.* (1989); Fukushima *et al.* (1986); Catton and White (1992) and Jayarao and Henning (2001) could isolate only *Yersinia enterocolitica*.

High incidence of *Yersinia enterocolitica* in milk and its products was significantly related to high bacterial counts (Catton and White, 1992) and implies that these products are a likely sources of contamination with *Yersinia* (Adriana *et al.*, 1994). An increase in *Yersinia enterocolitica* number was observed during manufacture, although its number was decreased following salting and through out the storage period of cheese (Osman, 1996). Water is believed to be an important source of *Yersinia enterocolitica* as several studies indicate that *Yersinia enterocolitica* outbreaks in human are mainly caused by consumption of contaminated food and water. Raw milk and inadequately pasteurized milk and milk products have also been implicated in transmission of *Y. enterocolitica* infections to humans (Black *et al.*, 1978). Epidemiological studies in food microbiology revealed that refrigerated food stored over a long period pose an

additional risk, because *Yersinia*, as a psychrotrophic microbe, is able to grow at temperatures as low as 0 °C (Hanna *et al.*, 1976). These microorganisms have been isolated in different countries from raw milk: in Australia (Ibrahim and Mac Rae, 1991; Hughes, 1979), Canada (Schiemann, 1978), France (Vidon and Delmas, 1981), Ireland (Walker and Gilmour, 1986), Italy (Franzin *et al.*, 1984) and USA (Moustafa *et al.*, 1983). Although there are over 50 different serotypes of *Yersinia enterocolitica*, serogroups, 0:3, 0:5, 27, 0:8 and 0:9 are generally regarded as the most common human pathogens (Toora, 1995). *Yersinia enterocolitica* produce a heat-stable enterotoxin (ST), which shows serological cross-reactivity to *E.coli* ST. Usually it can not be produced above 30°C, can not be found in vivo and is common in non pathogenic environmental strains mainly O:3 can cause enteritis to children aged 1-4 years which is characterized by heavy watery and sometimes bloody diarrhea, abdominal cramps, fever and vomiting, symptoms which lasts normally only for 1-2 days while in people between 10-14 years old. It leads to pseudoappendicitis which seems to be typical for Yersiniosis. Arthritis of extremities is the most usual complication of Yersiniosis in young people while erythema nodosum as sequellae is known specially for women older than 40 years. Septicemia is rare due to invasion of *Yersinia enterocolitica* in the circulatory system, but result in a lethality rate of 30%.

Yersinia kristensenii are usually environmental strains and are not generally associated with human gastrointestinal infections but can act as opportunistic pathogens and cause extra intestinal infections (Bercovier and Mollaret, 1984).

From our conducted study we concluded that contamination of milk and dairy products by these pathogenic microorganisms can be of endogenous origin, following excretion from the udder of an infected animals or may be also of exogenous origin, through direct contact with infected herd or through environment (water and personnel). Attention toward the way by which the restriction of these microorganisms must be done because it is the primary concerns for safety assurance in the dairy industry. This can be achieved by hygiene in all aspects of milk handling (Farm hygienic measures), strict maintenance of refrigeration 4°C or lower, minimization of the storage time of raw milk, deficiencies in the hygienic measures of milk and dairy products storage particularly refrigeration that was not properly implemented should be corrected and a suitable method to kill or remove these microorganisms, where heat treatment and processing of milk can inhibit or encourage the

multiplication of these microorganisms followed up by an effective HACCP system.

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