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OCCURRENCE OF LISTERIA AND YERSINIA SPECIES IN MILK AND SOME MILK PRODUCTS

(With 4 Tables)

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مدي تواجد ميكروبات الليستيريا واليرسينيا في اللبن وبعض منتجاته

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أجريت هذه الدراسة علي ٢٠٠ عينة من اللبن الخام وبعض منتجاته وتشتمل علي ١٠٠عينة من اللبن الخام (٥٠ من لبن الأبقار و٢٥ من كل من لبن النعاج والماعز) و١٠٠ عينة من منتجات الالبان (٢٥ من كل من اللبن المبستر والجبن الأبيض الطري والزبادي العادي والزبادي بالفاكهة) والتي تم تجميعها من مزارع الألبان والمحلات المختلفة بمحافظة الدقهلية واشتملت الدراسة علي فحص هذه العينات لتواجد ميكروب الليستيريا واليرسينيا كمسبب للتسمم الغذائي والتهاب الضرع. وأسفرت النتائج عن تواجد ميكروب الليستيريا في العينات المفحوصة بنسبة ٣،٠ % و كان توزيعها كالتالي: ٦،٠ % في لبن الأبقار و ٨ % في لبن الماعز و ٤،٠ % في الجبن الأبيض الطري .كما اوضحت انه لم يتم عزل هذا الميكروب من لبن النعاج واللبن المبستر والزبادي العادي والزبادي بالفاكهة. وبتصنيف هذه المعزولات بيوكيميائيا تم عزل ميكروب الليستيريا مونوسيتوجينز بنسبة ٣ (١،٥ %) وميكروب الليستيريا اونوكوا بنسبة ٥ (٢،٥ %) وميكروب الليستيريا ولشيميري بنسبة ٢ (١،٠ %). كما تم عزل ميكروب اليرسينيا من عينات اللبن ومنتجاته التي تم فحصها بنسبة ٤١ (٢٠،٥ %). و كانت نسب التواجد كالتالي: ٤٠،٠ % في لبن الأبقار و٢٠،٠ % في لبن النعاج و٣٢،٠ % في لبن الماعز و٤،٠ % في اللبن المبستر و٨،٠ % في الجبن الأبيض الطري و١٢،٠ % في الزبادي العادي و٨،٠ % في الزبادي بالفاكهة. وبتصنيف هذه المعزولات بيوكيميائيا تم عزل ميكروب اليرسينيا انتيروكوليتيكا وميكروب اليرسينيا انترميديا وميكروب اليرسينيا كريستن سيناي وميكروب اليرسينيا فريدريك سيناي وميكروب اليرسينيا سيدوتوبركلوسس من العينات المفحوصة علي التوالي كالأتي (٥ و ١ و ٢ و ٠ و ١ و١و١) و (١٠ و ٢ و ٠ و ١ و ٢و ٠ و ٠) و (٣ و ٠ و ٠ و ٠ و ٠ و ١ و ١) و (٠ و ٣ و ٤ و ٠ و ٠ و ١) و (٤ و ٠ و ٤ و ٠ و ٠و ٢ و ٠). وقد تمت دراسة أهمية هذه الميكروبات الصحية وإمكانية التحكم في نقلها من الحيوان للإنسان والتسبب في الإصابة بالأمراض بالإضافة إلي الشروط الصحية الواجب توافرها لإنتاج منتج صحي عالي الجودة خالي من الأمراض.

SUMMARY

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 monocytogens* 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 monocytogens 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 monocytogens* 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 adentitis, and a variety of extraintestinal disorders like erythema nodosum or polyarthritis 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°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- Platting: 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- Platting: 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		
			L. monocytogens	*L. innocua*	*L. welshimeri*
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				
			Y. entero-colitica	*Y. inter-media*	*Y. kristen-senii*	*Y. fredrik-senii*	*Y. psudo-tuberculosis*
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 decleared 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 *Yesinia 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 erythma 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.

REFERENCES

A.P.H.A. (1992): Standard methods for the examination of dairy products. 16th Ed., Amer. Pub. Health Assoc., New York.

Adriana, D.R.T.; Bernadette, D.G.F. and Mariza, L. (1994): Incidence of Yersinia spp. in food in Sao Paulo, Brazil. Int. J. of Food Microbiol., 21: 263-270.

Aulisio, C.C.G. and Stanfield, J.T. (1984): Yersinia enterocolitica and Yersinia pseudotuberculosis. In: US Food and Drug Administration. Division of Microbiology. Bacteriological Analytical Manual. 6th Ed., Washington, DC, AOAC.

Awad, I.E. (2002): Studies on food poisoning bacteria with special reference to Campylobacter jejuni in milk. Ph.D Thesis, Fac. of Vet. Med., Zagazig Univ., Egypt.

Aygun, P. and Pehlivanlar, S. (2006): Listeria spp. in the raw milk and dairy products in Antakya, Turkey. Food Control; 17: 599–682.

Aysel, B.Ö.; Göktug, B.; Ahmet, E.C. and Gülderen, Y. (2006): Prevalence of L. monocytogens in some Turkish food stuffs. J. of Food Quality. 29: 76-86.

Bercovier, H.; Brenner, D.J.; Ursing, J.; Steigerwalt, A.G.; Fanning, G.R.; Alonso, J.M.; Carter, G.P. and Mollaret, H.H. (1980): Characterization of Yersinia enterocolitica sensu stricto. Curr. Microbiol., 4: 201-206.

Bercovier, H. and Mollaret, H.H. (1984): Yersinia In: Krieg. N. R. (ed), Bergey's Williams and Wilkins, Baltimore, pp. 489-506.

Bille, J.; Catimel, B.; Bannerman, E.; Jacquet, C.; Yersin, M. N.; Caniaux, I.; Monget, D. and Rocourt, J. (1992): API-Listeria, a new and promising one-day system to identify listeria isolates. Appl. and Environ. Microbiol., 58 (6): 1857-1860.

Black, R.E.; Jackson, R.J.; Tsai, T.; Medvesky, M.; Shayegani, M.; Feeley, J.C.; MacLeod, K.I.E. and Wakelee, A.M. (1978): Epidemic Yersinia enterocolitica infection due to contaminated chocolate milk. N. Engl. J. Med., 298: 76–79.

Bottone, E.J. (1997): Yersinia enterocolitica: the carisma continues. Clin. Microbiol. Reviews, 10: 257-276.

Breer, C. and Schopfer, K. (1989): Listeria in food. Schweiz Med. Wochenschr. 11; 119 (10): 306-11.

Cotton, L.N. and White, C.H. (1992): Listeria monocytogenes, Yersinia enterocolitica and Salmonella in dairy plant environments. J. Dairy Sci. 75: 51-57.

Desmasures, N.; Bazin, F. and Gueguen, M. (1997): Microbiological composition of raw milk from selected farms in the Camembert region of Normandy, J. Appl. Microbiol., 83 (6): 53-58.

El-Sherbini, M.A.A. (1990): Occurrence and behaviour of pathogenic microorganisms especially Listeria monocytogenes in milk and some dairy products. Ph.D Thesis, Fac. of Vet. Med., Zagazig Univ., Egypt.

Farber, J.M.; Sanders, G.W. and Malcolm, S.A. (1988): The presence of Listeria spp. in raw milk in Ontario. 34 (2): 95-100.

Fleming, D.W.; Cochi, S.L.; MacDonald, K.L.; Brondum, J.; Hayes, P.S.; Plikaytis, B.D.; Holmes, M.B.; Audurier, A.; Broome, C.V. and Reingold, A.L. (1985): Pasteurized milk as vehicle of infection in outbreak of Listeriosis. New Engl. J. Med., 312: 404-407.

Franz, A. (2003): Listeria: growth, phenotypic differentiation and molecular microbiology. FEMS Immunol. and Med. Microbiol., 35: 183-189.

Franzin, L.; Fantino, P. and Vidotto, V. (1984): Isolation of Yersinia enterocolitica and Yersinia enterocolitica-like organisms from raw milk in Italy. Current Microbiol., 10(1984): 357-360.

Fukushima, H.; Tsubokura, M.; Otsuki, K. and Kawaoka, Y. (1986): Suppression of Heat-stable Enterotoxin production by Y. spp. in milk. Veterinary Microbiol., 11: 163-172.

Hamama, A.; El Marrakchi, A. and El Othmani, F. (1992): Occurrence of Yersinia enterocolitica in milk and dairy products in Morocco. Int. J. of Food Microbiol., 16: 69-77.

Hanna, M.O.; Zink, Z.L. and Vanderzant, C. (1976): Yersinia enterocolitica-like organisms from vacuum-packaged beef and lamb. J. Food Sci., 41: 1254–1256.

Hassan, L.; Mohammed, H. O.; McDonough, P. L.; Gonzalez, R. A. (2000): A cross-sectional study on the prevalence of Listeria monocytogenes and Salmonella in New York dairy herds. J. Dairy Sci. 83 (11): 2441-7.

Herman, L.; De Block, J. and Moermans, R. (1995): Direct detection of Listeria monocytogenes in 25 milliliters of raw milk by a two-step PCR with nested primers. Appl. Environ. Microbiol., 61: 817–9.

Hughes, D. (1979): Isolation of Yersinia enterocolitica from milk and a dairy farm in Australia. J. Appl. Bacteriol., 46: 125–130.

Ibrahim, A. and Mac Rae, I.C. (1991): Isolation of Yersinia enterocolitica and related from red meat and milk., J. Food Sci., 56 (6): 1524-1526.

Jansen, B.; Kohnen, W.; Obst, U. and Schwartz, T. (2003): Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS. Microbiol. Ecol., 43: 325–35.

Jayarao, B.M. and Henning, D.R. (2001): Prevalence of foodborne pathogens in Bulk Tank Milk. J. Dairy Sci., 84 (10): 2157-62.

Jayarao, B.M. ; Donaldson, S. C.; Straley, B. A.; Sawant, A. A.; Hegde, N. V.; Kells, J. and Gilmour, A. (2004): Incidence of Listeria monocytogenes in two milk processing environments and assessment of Listeria monocytogenes blood agar for isolation. Int. J. Food Microbiol., 91: 167–74.

Landgraf, M. (1993): An improved enrichment procedure for isolation of Yersinia enterocolitica and related species from milk. J. of Food Prot., 56: 447-450.

Larkin, L.L.; Vasavada, P.C. and Marth, E.H. (1991): Incidence of Yersinia enterocolitica in raw milk as related to its quality. Milchwissenschaft, 46: 500-502.

LoØken, T.; AspØy, E. and GrØnstØl, H. (1982): Listeria monocytogenes execution and humoral immunity in goats in a herd without breaks of listeriosis and in a healthy herd. Acta Vet. Scand. 23: 392-399.

Lovett, J. (1989): Listeria monocytogenes. In M.P. Doyle (Ed.), Foodborne Bacterial Pathogens (pp. 283–310). New York: Marcel Dekker.

Makino, S.I.; Kawamoto, K.; Takeshi, K.; Okada, Y.; Yamasaki, M. and Yamamoto, S. (2005): An outbreak of food-borne Listeriosis due to cheese in Japan, during 2001. Int. J. Food Microbiol., 104: 189–96.

Manfreda, G.; De Cesare, A.; Stella, S.; Cozzi, M. and Cantoni, C. (2005): Occurrence and ribotypes of Listeria monocytogenes in Gorgonzolla cheese. Int. J. Food Microbiol., 102: 287–93.

McLauchlin, J. (1997 a): The discovery of Listeria. PHLS Microbiol. Digest., 14: 76-78.

McLauchlin, J. (1997 b): The pathogenicity of Listeria monocytogenes: A public health perspective. Reviews in Medical Microbiology, 8: 1–14.

McLauchlin, J.; Mitchell, R.; Smerdon, W. and Jewell, K. (2004): Listeria monocytogenes and Listeriosis: a review of hazard characterization for use in microbiological risk assessment of foods. Int. J. Food Microbiol., 92: 15–33.

McManus, C. and Lanier, J.M. (1987): Salmonella, Campylobacter jejuni and Yersinia enterocolitica in raw milk. J. Food Prot., 50(1): 51-55.

Meyer-Broseta, S.; Diot, A.; Bastian, S. and Riviere, Cerf. O. (2003): Estimation of low bacterial concentration: Listeria monocytogenes in raw milk. Int. J. Food Microbiol., 80: 1-15.

Mohamed, M.S.D.; Alijan, T. and Koorosh, M. (2004): Comparison of four methods for isolation of Yersinia enterocolitica from raw and pasteurized milk from Northen Iran. Int. J. of Food Microbiol., 94: 87-91.

Moustafa, M.K.; Ahmed, A.A-H. and Marth, E.H. (1983): Occurrence of Yersinia enterocolitica in raw and pasteurized milk. J. Food Prot., 46: 276–278.

Neubauer, H.; Sauer, T.; Becker, H.; Aleksic, S. and Meyer, H. (1998): Comparison of systems for identification and differentiation of species within genus Yersinia. J. Clin. Microbiol., 36: 3366-3368.

Osman, E. (1996): Survival of virulent Y. enterocolitica during the manufacture and storage of Turkish Feta cheese. Int. J. of Food Microbiol., 33: 285-292.

Oxoid (1990): The Oxoid Manual. 6th edition, Unipath. Ltd. U.K.

Ozbas, Z.Y.; Lehner, A. and Wagner, M. (2000): Development of multiplex and semi-nested PCR assay for detection of Yersinia enterocolitica and Aeromonas hydrophila in raw milk., Food Microbiol., 17: 197-203.

Pini, P.N. and Gilbert, R.J. (1988): The occurrence in the U. K. of Listeria species in raw chickens and soft cheeses. Int. J. of Food Microbiol., 6 (4): 317-26.

Quaglio, P.; Aggazzotti, G.; Montovani, G.; Messi, P. and Fabio, A. (1988): A study on the presence of Yersinia spp. in milk and bovine feaces., L'Igiene Moderna, 90: 585-596.

Ramesh, A.; Padmapriya, B.P.; Chrashekar, A. and Varadaraj, M.C. (2002): Application of convenient DNA extraction method and Multiplex PCR for the direct detection of Staphylococcus aureus and Yersinia enterocolitica in milk samples., Mol. Cell. Probes., 16 (4): 307-314.

Robins-Browne, R.M. (1997): Yersinia enterocolitica. In: Doyle, M. P.; Beuchat, L.R. and Montville, T.J. (eds), Food Microbiol.: Fundamentals and Frontiers, pp. 192-215. ASM press, Washington.

Schiemann, D. A. (1978): Association of Yersinia enterocolitica with the manufacture of cheese and occurrence in pasteurised milk. Appl. Environ. Microbiol., 36: 274-277.

Siegman-Igra, Y.; Levin, R.; Weinberger, M.; Golan, Y.; Schwartz, D. and Samra, Z. (2002): Listeria monocytogenes infection in Israel and review of cases worldwide. Emerg. Infect. Dis.; 8: 305–10.

Stone, D.L. (1987): A survey of raw whole milk for C. jejuni, L. monocytogens and Y. enterocolitica. N. Z. Dairy Sci. Technol., 22: 257-264.

Swaminathan, B. (2001): Listeria monocytogenes. In M. P. Doyle, L.R. Beuchat, & T.J. Montville (Eds.), Food Microbiology – Fundamentals and Frontiers (pp. 383–410). Washington: ASM Press.

Syed, T.; Gagandeep, S.; Ram, P.T. and Gurinder, S. (1989): Drug resistance and Lecithinase activity of Y. enterocolitica isolated from buffalo milk. Int. J. Food Microbiol., 9: 167-171.

Terplan, G.; Schoen, R.; Springmeyer, W.; Degle, I. and Becker, H. (1986): L. monocytogens in Milch und Milch produkten. Archiv fur Lebensmittel hygiene, 37, 129-156.

Teuber, M. (1999): Spread of antibiotic resistance with food-borne pathogens. CMSL Cell Mol. Life Sci., 56: 755–61.

Toora, S. (1995): Inactivation of pathogenic serotypes of Yersinia enterocolitica by chlorine., J. Food Prot., 58 (12): 1383-1385.

Umoh, V.J.; Dangana, A. and Umoh, J.U. (1984): Isolation of Yersinia enterocolitica from milk and milk products in Zaria, Nigeria. Int. J. Zoon., 11: 223-228.

Van-Netten, P.; Perales, I.; Van-De-Moosdijik, A.; Curtis, G.D.W. and Mossel, D.A.A. (1989): Liquid and solid selective differential media for the detection and enumeration of Listeria

monocytogenes and other Listeria spp. Int. J. of Food Microbiol., 8: 299-316.

Vela, A.; Fernandez-GarayzáBal, J.; Latre, M.; Rodriguez, A.; Dominguez, L. and Moreno, M. (2001): Antimicrobial susceptibility of Listeria monocytogenes isolated from meningoencephalitis in sheep. Int. J. Antimicrob Agents; 17: 215–20.

Vidon, J.M. and Delmas, C.L. (1981): Incidence of Yersinia enterocolitica in raw milk in eastern France., Appl. and Environ. Microbiol., 41 (2): 355-359.

Walker, S.J. and Gilmour, A. (1986): The incidence of Yersinia enterocolitica and Yersinia enterocolitica- like organisms in raw and pasteurized milk in Northern Ireland. J. Appl. Bacteriol., 61 (2): 133-138.

Yoshida, T.; Sato, M. and Hirai, K. (1998): Prevalence of Listeria species in raw milk from farm bulk tanks in Nagano prefecture. J. of Vet. Med. Sci., 60 (3): 311-314.