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PREVALENCE OF SOME FOOD POISONING ORGANISMS IN RAW MILK AND ICE CREAM WITH SPECIAL REFERENCE TO ENTEROPATHOGENIC *ESCHERICHIA COLI.*

(With 3 Tables)

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مدى تواجد بعض ميكروبات التسمم الغذائي باللبن الخام و الايس كريم مع الاشارة الخاصة للميكروب القولونى العصوى الممرض

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اجرى البحث على خمسة وسبعون عينة من كل من اللبن الخام والأيس كريم بنوعيه صغار المنتجين وهم الباعة الجائلين وانتاج المصانع الكبرى بواقع ٢٥ عينة لكل منهم وقد جمعت العينات عشوائيا من أماكن متفرقة بمدينة الأسكندرية وذلك لفحصها معمليا ومدى تلوثها بميكروبات التسمم الغذائي. وقد أسفرت النتائج عن تواجد الميكروبات المحبة للبرودة ومجموعة القولونية المعوية , ميكروب المكور السبحى و ميكروب المكورالعنقودى بمتوسط عد كلى كالتالى (٤و٩×١٠^٨±١و١ × ١٠^٨) و (٧,٥×١٠^٥±٢,٤×١٠^٥) و (٣×١٠^٢±٠,١×١٠^٢) و (١,٠×١٠^٤±٠,٥×١٠^٤) و (٥,٦× ١٠^٢±٢,٢×١٠^٢) و (٠و٠) و (١٦,٣٨ × ١٠^٣ ± ١,٥٣ × ١٠,^٣) و (٨,٩٧ × ١٠^٣ ± ١,٣١ × ١٠^٣) و(٠و٠) (٦,٩١×١٠^٢±١,٦٧×١٠^٢) و (١,٦٤×١٠^٢±٠,٣٨×١٠^٢) و(٧,٣٠×١٠±١,٦٧×١٠) من عينات اللبن الخام والأيس كريم بنوعيها على التوالى كما تم عزل وتصنيف كلا من الميكروبات القولونية العصوية الممرضة , والميكروبات القولونية المعوية و ميكروبات السيدوموناس , و ميكروبات اليرسينيا والايروموناس هيدروفيلا بنسب مختلفة كما أمكن عزل ميكروبات المكور العنقودى والمكور السبحى بنسب مختلفة ايضا. ثم تم عمل تصنيف سيرولوجى لميكروبات القولونية العصوية الممرضة وعزل ٨ عترات منها هذا وقد تم مناقشة النتائج من الناحية الصحية والاقتصادية وتوضيح الاجراءات الصحية الواجبة لضمان جودة المنتج وحماية المستهلك.

SUMMARY

75 samples of raw milk, small scale ice cream and large scale ice cream (25 each) were collected from different supermarkets and street vendors in Alexandria city, Egypt and examined bacteriologically for the presence of some food poisoning organisms. Psychrotrophic, Coliform, Staphylococcus and Enterococci were

present with a mean value of ($9.4x10^8$, $7.5x10^5$and $3x10^2$), ($1.0x10^4$, $5.6x10^2$ and 0.0), ($16.38x10^3$, 8.9 x 10^3 and 0.0) and ($6.9x10^2$, $1.6x10^2$ and 7.3x10) in the examined raw milk, small scale and large scale ice cream samples, respectively. *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Yersinia* spp., *Aeromonas hydrophila* improving the quality of the product were discussed. *Staphylococcus aureus* and Enterococci could be isolated at varying percentages from the examined samples. The Enteropathogenic *E. coli* could be isolated from 96% and 20% from raw milk and small scale ice cream samples and identified serologically as O_{119} : K_{66}, O_{112}: K_{66}, O_{111}: K_{58}, O_{114} : K_{90}, O_{78} : K_{80}, O_{124} : K_{72}, O_{128} : K_{67} and O_{26} : K_{60}. The public health significance and economic importance of existing food poisoning organisms as well as suggestive measure for improving the quality of the product were discussed.

Key words: *Milk, ice cream, E. coli, Aeromonas, Yersinia*

INTRODUCTION

Milk is a complex biological fluid, containing a wide variety of constituents and possessing unique physical characteristics. It consists largely of lipids, proteins, carbohydrates, minerals and vitamins, so it is a well-known vehicle of a number of human pathogens whatever, it is raw or processed (Aisha *et al.,* 2002).

Ice cream is considered as one of the most favourite frozen dairy products all-over the world. It is a delicious, highly nutritious food and has a therapeutic value for persons suffering from irritation and infection of mouth and throat due to its coldness (Robinson, 1994).

Although, ice cream is a wide spread dairy food consumed by people of all ages, yet it may be subjected to contamination by various microorganisms at different stages of manufacturing, handling and packaging. Furthermore, the processing of ice cream requires a variety of heat treatment, handling and frequently adding substances as sugar, fruits, raw eggs or other products of animal origin. These additives may contribute to food poisoning outbreaks gastroenteritis in human beings or other bacterial problems either by adding microflora or by creating conditions favourable for the growth and survival of bacteria (Kumari *et al.,* 1996; El-Prince and Hussein, 2000).

In addition, ice cream has been incriminated as a transmitter of other pathogenic microorganisms. *Klebsiella, E. coli, Serratia, Proteus, Pseudomonas, Yersinia, Citrobacter, Enterobacter, Aeromonas species* were the most predominant organisms previously isolated by Abdel-Haleem (1995); El-Bagoury (1996); Kumari *et al.,* (1996) and Mansour *et al.,* (2000) from ice cream samples in varying percentages. The presence of these microorganisms in higher percentages have been implicated in many cases of food poisoning and other foodborne diseases (Varnam and Evans, 1991).

The most psychrotrophic bacteria in milk were readily killed by heat treatment but many species produced heat resistant extra-celluler proteinases and/or lipases which remained active in milk products (Cogan, 1980).

Proteinase enzymes produced by psychrotrophic bacteria cause un-desirable proteolytic changes, objectionable flavour and quality defects in dairy products after storage (Bigalke, 1985; Mansour *et al.,* 2000).

The growth of *Staphylococcus aureus* in milk constitutes a potential public health hazard since many strains of *Staphylococcus aureus* produce enterotoxins that cause food poisoning if ingested. Neither the absence of *Staphylococcus aureus* nor the presence of small numbers is complete assurance that a food is safe. Conditions inimical to the survival of *Staphylococcus aureus* may result in diminishing operation or death of viable microbial cells, while sufficient toxins remain to elicit symptoms of staphylococcal food poisoning (AOAC, 1984; Lancette and Tatini, 1992).

Aeromonas spp. consists of three species, *A.hydrophila, A.caviae* and *A.sorbia,* these species collectively referred to as motile or mesophilic aeromonads. Milk and milk products as ice cream have been surveyed by several investigators for the occurrence of *A. hydrophila* (Khalil, 1997; El-Prince, 1998; Ahmed *et al.,* 2001). Two types of diarrhea have been attributed to these organisms, a "cholera like" illness characterized by watery stools and a less common "dysentery like" illness, characterized by blood and mucus in the stool. Both types of diarrhea are usually mild and self-limiting, but either may be chronic or sever (Palumbo *et al.,* 1992; Abd El-Hady and Halawa, 1999). These organisms cause gastroenteritis and several diseases such osteomylitis; Septicemia; meningitis; endocarditis; skin infection; cellulitis, wound infections, pneumonia, urinary tract infections, endocarditis and ear infections (Koneman *et al.,* 1994). *Aeromonas hydrophila* produces a number of potential virulence factors including enterotoxins, cytotoxins, haemolysins, lipases and proteases. Therefore it posses a highly significant public health problem as well as economic importance (Trust and Chipman, 1979 and Abd El- Hady and Halawa, 1999).

Yersinia organisms are Gram-negative, psychrotrophic milk-borne enteric pathogens. These organisms are widespread in the environment and are indigenous to the gastrointestinal tracts of warm-blooded animals including dairy cattle (Marshall, 1992; Hussein and Ahmed, 2002; Bahout and Moustafa, 2004).

Y. enterocolitica causes enterocolitis in young children and adults while older children and young adult may be accompaind present with symptoms of appendicitis including fever, abdominal pain, tenderness in the right lower quadrant and leucocytosis. However, serious cases may occur with rectal bleeding and perforation of the ileum (Rabinovitz, 1987) Moreover, there may be secondary immunologically mediated complication such as arthritis, erythema nodosum and to a lesser extent Reiter´s syndrome, glomerulonephritis, myocarditis, exudative pharyngitis and septicemia (Butler, 1998; Amal and Sayed, 2003).

Hazardous bacteria that grow at refrigeration temperature include *Y. enterocolitica* and *Aeromonas hydrophila* are newly recognized as food borne disease organisms. These bacteria are a potential threat for food held at refrigeration temperatures, and they grow much faster at higher abuse temperatures (Corlett, 1989). Although, psychrotrophic bacteria could be destroyed by freezing, bacterial spores and many vegetative cells can survive freezing. Thus some psychrotrophic bacteria will grow in frozen foods as ice cream, if the food is subjected to temperature abuse, (Kornacki and Gabis, 1990; Mira *et al.,* 2001).

Pseudomonas species are widely distributed in nature, they have been found in external environmental conditions surrounding dairy animals such as water, soil, sewage, air, grass, hay, feces and bedding materials. These organisms represent the most common psychrotrophs that contaminate milk and

cause variety of defects including fruity, rancid, bitter and putrid flavor as well as color defects (Kraft, 1995; Al-Ashmawy *et al.,* 1997; Pearson *et al.,* 2000)

P. aeruginosa is considered all over the world as one of the dangerous organisms causing different diseases and capable of secreting many extracellular products such as lipopolysaccharide (LPS), fibrinolysin, haemolysins, exotoxins and enterotoxins. These products have a major role in the virulence of pathogenic strains of *P. aeruginosa* (Khalil, 1992; Champagne *et al.,* 1994; Beuchat, 2000; Aly and Zaki, 2001).

Escherichia coli (E. coli), is incriminated as an etiologic agent of foodborne illness involving a variety of foods. The organism contaminates food through both direct and indirect sources, as it is commonly found in the gastrointestinal tract of man and animals (Gad El-Said *et al.,* 2005). *E. coli* strains involved in foodborne illness could be placed into five groups which are referred to as diarrhoegenic groups: enteropathogenic *E. coli,* enterotoxigenic *E. coli*, enteroinvasive *E. coli,* enterohaemorragic *E. coli* and enteroadherent-aggrigative *E. coli* (El-Bagoury and Hammad, 2004; Hassan and Abosrea, 2005). They are frequently associated with outbreaks of diarrhea affecting persons at different ages specially infants and elderly (Todd, 1990).

Enterotoxigenic *E. coli* (ETEC) is considered among the most prevalent organisms associated with infantile diarrhea in developing countries and a leading cause of mortality among children less than five years of age (Ryan, 1991). ETEC produces diarrhea by the elaborated in a heat-labile enterotoxin (LT), a heat-stable enterotoxin (ST) or both toxins, that act on the intestinal mucosa resulting in fluid outpouring into the, intestinal lumen (El-Shishnagui and Nazem, 1999; Al- Hawary, 2005).

The present study was planned to reveal the incidence of some food poisoning organisms in raw milk and ice cream and high light the public health importance and suggested measure for their control in these products.

MATERIALS and METHODS

Collection of Samples:

Seventy five random samples of raw milk and ice cream (25 raw milk samples, 250 ml of each milk sample were collected in sterile screw capped glass jars, 25 each of small scale and large scale producers) were collected from different localities in Alexandria city, Egypt. The collected samples were transferred to the laboratory with a minimum of delay, in a thermos containing ice to be immediately examined..Each sample of ice cream was left to stand in a water bath at 40ºC for not more than 15 minutes to be melted.

Bacteriological examination:

1-Coliform count (MPN) and isolation:

The count was determined by using 3-tube MPN technique as described by Harrigan (1998). Streaks from positive tubes (gas after 48h.) were evenly spread onto violet red bile lactose agar plates. The plates were incubated at 30ºC for 48 h. and five colonies were selected and picked on nutrient agar slant for biochemical and serological tests.

Isolation and identification of Enteropathogenic *E. coli* (EPEC):

Twenty five ml of milk or ice cream were added to 225 ml trypton phosphate broth as pre-enrichment fluid then incubated for 4-6 h at 37ºC. Mossel´s enteric enrichment broth tubes (10 ml) were inoculated by 1ml from the pre-enrichment tryptone phosphate broth medium (Mehlman and Romero, 1982). Tubes were incubated at 44ºC for 24h to permit the growth of pathogenic *E. coli* .One loopful from the inoculated tube was inoculated onto MacConkey-Sorbitol agar and Eosin methylene blue agar, and then incubated at 37ºC for 24h. The suspected colonies were picked and streaked onto soft and slope nutrient agar for detection of motility and fully identification of *E. coli* according to Quinn *et al.,* (2002).

Serotyping of *E. coli* isolates:

E. coli isolates were subjected to serological identification according to Edwards and Ewing (1972) by using polyvalent. Antisera used for typing of *E. coli* were Coli test sera poly I, Coli test sera poly II and Bacto *E. coli* antisera.

2-Psychrotrophic counts: The technique was recommended by APHA (1992).

Isolation and identification of:

***a) Aeromonas* species:** Isolation was done according to the technique recommended by Palumbo *et al.* (1985) and identified according to Popoff (1984).

***b) Yersinia enterocolitica*:**

The technique was recommended by APHA (1992). *Yersinia enterocolitica* isolation was done as described by Donald and George (1992). The suspected colonies were identified according to the technique recommended by APHA (1992).

c) *Pseudomonads species:*

Loopfuls from the prepared samples were streaked onto Pseudomonas CNC agar medium plates for isolation of *P. aeruginosa* and Pseudomonas CFC agar medium plates for isolation of *P. fluorescens* (Uraz and Citak, 1998). Inoculated plates were incubated at 37ºC for 24 hrs and 25ºC for 48 hrs for CNC and CFC agar media, respectively. Suspected colonies were picked up and streaked onto nutrient agar slopes, then incubated at 37ºC and 25ºC, respectively for 24 hrs to obtain pure culture for further identification according to Howard *et al.* (1993) and Elmer *et al.* (1994).

3- *Staphylococcus aureus* presumptive count/ml according to APHA (1992):

0.1 ml from each of the previously prepared decimal dilutions was transferred onto duplicate plates of Baird-Parker medium (Baird-Parker, 1982) and incubated at 37ºC for 24 hrs. Suspected colonies on countable plates (black and shiny colonies greater than 1mm in diameter with narrow white margin surrounded by clear zone extending into opaque medium) were counted.

Isolation and identification of *Staphylococcus aureus*:

Suspected colonies were picked up onto slants of nutrient agar and incubated at 37ºC for 24hrs. Isolated strains were purified before being subjected for further identification according to Kowalski (1977) and Sonnewirth and Jarett (1980).

4-Enterococci count (MPN): The sample was done according to **ICMSF (1982)**

Isolation of Enterococci: according to Efthymiou *et al.* (1974) and Quinn *et al.* (1994).

RESULTS

Table 1: Incidence of organisms isolated from the examined samples.

Isolated Organisms	Raw milk		Ice Cream			
			Small scale		Large scale	
	No.of+ve samples	%	No.of+ve samples	%	No.of+ve samples	%
Coliforms						
E .coli	24	96	5	20	٠	٠
Klebsiella spp.						
Kleb.oxytoca	5	20	2	8	٠	٠
Kleb.pneumoniae	2	8	1	4	٠	٠
Enterobacter spp.						
Enter.gergoviae	2	8	1	4	٠	٠
Enter. agglomerans	1	4	-	-	٠	٠
Enter. aerogenes	1	4	1	4	٠	٠
Pseudomonas spp.						
P. aeruginosa	11	44	5	20	3	12
Yersinia spp.						
Yersinia enterocolitica	4	16	1	4	٠	٠
Aeromonas spp.						
A. hydrophila	15	60	4	16	٠	٠
A . caviae	10	40	5	20	٠	٠

A. sobria	7	28	2	8	٠	٠
Staph .aureus	3	12	1	4	٠	٠
Enterococci	2	8	1	4	1	4

Table 3: Frequceny distribution of Serotyping of Enteropathogenic *E. coli* isolates recovered from raw milk and ice cream (small scale).

E.coli Serotype	Raw milk		Ice cream (small scale)		Strain characteristics
	NO	%	NO	%	
$O_{119}:k_{66}$	7	29.16	2	40	Untypable
$O_{112}:k_{66}$	5	20.83	1	20	Untypable
$O_{111}:k_{58}$	4	16.66	1	20	Enteropathogenic
$O_{114}:k_{90}$	2	8.33	-	-	Enterotoxigenic
$O_{78}:k_{80}$	2	8.33	-	-	Enterotoxigenic
$O_{124}:k_{72}$	2	8.33	1	20	Enteroinvasive
$O_{128}:k_{67}$	1	4.16	-	-	Enterotoxigenic
$O_{26}:k_{60}$	1	4.16	-	-	Enterohaemorrhagic
Total	24	100	5	100	

DISCUSSION

Ice cream is the most popular frozen dairy products widely used all over the world due to its high nutritive value and its palatability to consumers at all ages. However, it is worth to mention that ice cream may constitute a serious public health hazard due to its contamination with a variety of pathogenic microorganisms which may gain entrance to ice cream through using inferior quality raw material, insufficient heat treatment of milk or contaminated equipment used for its preparation and distribution.

Results reported in Table (1), reveal that the incidence of different Coliforms organisms could be isolated in variant percentages. These organisms were identified in raw milk, small scale ice cream and large scale ice cream as *E. coli* 96%, 20% and 0%, *Klebsiella oxytoca* 20%, 8% and 0%, *Klebsiella pneumoniae* 8%, 4% and 0%, *Enterobacter geroviae* 8%, 4% and 0%, *Enterobacter agglomerans* 4%, 0% and 0% and *Enterobacter aerogenes* 4%, 4% and 0%, respectively. Similar microorganisms could be isolated by El-Bassiony *et al.* (1985), El-prince and Hussein (2000) and Mansour *et al.* (2000).

Pseudomonas aeruginosa could be isolated from raw milk, small scale ice cream and large scale ice cream samples at varying percentages 44%, 20% and 12%, respectively (Table 1). Similar results of raw milk samples were recorded by Aly and Zaki (2001) with a rate of 48.57%. Comparatively, higher than those reported by Khalil (1992) and Madeha and Zaki (2003) with incidence of 17.03% and 16.67%, respectively. On the other hand, the obtained prevalence of small and large scale ice cream were lower than those

detected by Mansour *et al.* (2000) with a rate of 29.4% and 20%, respectively. The ability to produce extracellular hydrolytic enzymes (Proteases, Lecithinases, and Lipases) is common to *P. aeruginosa* (Madeha and Zaki, 2003). Those products play some role in its pathogenicity (Seddik *et al.,* 1987).

Yersinia enterocolitica isolated from the examined small scale ice cream with incidence (4%) Table (1)., similar finding have been reported by Mira *et al.,* (2001) was isolated *Yersinia enterocolitica* from small scale ice cream with incidence of 4%. Lower incidence was recorded by El-Prince and Hussein (2000) with a rate of 1%. Moreover, *Yersinia enterocolitica* could be isolated from 4 (16%) of the examined raw milk samples. Nearly similar results were obtained by Bahout and Moustafa (2004) with a rate of 14% and comparatively higher than those reported by Mercado and Ibanez (1986) and Mira *et al.* (2001) with incidence of 5.5% and 8% in finished product is most likely due to post pasteurization contamination. Also contamination of ice cream samples obtained from street vendors with species of Enterobacteriaceae could be taken as an index of fecal contamination and could be attributed to the unsanitary practices, poor hygienic quality of ingredients used and/or absence of pasteurization during manufacturing processes (Hussein and Ahmed, 2002).

Aeromonas hydrophila could be isolated from 60% of the examined raw milk samples. This result was higher than those reported by Abdel-Khalek (1997), Mira *et al.,* (2001) and Nashwa and Isis (2001) with a rate of 40%, 36% and 25%, respectively. On the other hand, *A.hydrophila, A. caviae* and *A. sobria* were recorded to be 60%, 40% and 28% of the examined milk samples which are lower than that reported by E-Shorbagy and Al- Ganzoury (2002) with a rate of 75%, 50% and 37.5%, respectively. The higher incidence of *Aeromonas* species in raw milk could be a result of bad sanitary measures. *A. sorbia* was more virulent in the cytotoxic activity than the *A. caviae* and *A. hydrophila* (Janda and Abott, 1998). Table 1 indicates that *A.hydrophila, A. caviae* and *A. sobria* were detected in 16%, 20% and 8% of the examined small scale ice cream, respectively. The obtained results are in a agreement with that reported by Ahmed *et al.* (2001) with incidence of 17.3%, 22.7% and 6.7%, respectively. Strains of *Aeromonas species* can cause gastroentrities where some individuals and consumers of ready to eat foods in Italy, are regularly exposed to many genetically distinct strains of *A. hydrophila, A. caviae* without evident signs of malaise. Therefore, few of these strains if any likely to be pathogenic (Villari *et al.,* 2000).

Staph. aureus could be detected in raw milk with incidence of 12%. The obtained results are lower than that reported by Aisha *et al.,* (2002) with incidence of 64%. While, *Staph. aureus* could be isolated in small scale ice cream samples with incidence of 4%. This result was agree to Mansour *et al.* (2000) with a rate of 4%.

Enterococci species could be isolated from examined raw milk and (small and large scale) ice cream samples with incidence of 8%, 4% and 4%, respectively. The obtained results of small and large scale ice cream are nearly similar results recorded by Mansour *et al.* (2000) with incidence of 4% and 2%. Although microbial growth doesn't occur in frozen foods held below -10ºC, large numbers of proteolytic enzymes remain active at the usual storage temperatures for frozen products, consequently the deterioration in their quality during the frozen storage will be occurred. Therefore, freezing will be slow but not arrest the development of enzymatic spoilage after it has begun (ICMSF, 1980).

The data recorded in Table (2) recorded the Psychrotrophic, Coliform, Staphylococcus, and Enterococci counts. Nearly similar counts were reported by El-Shorbagy and Al-Ganzoury (2000); Mansour *et al.* (2000) and Aly and Zaki (2001).

The results recorded in Table (3) showed the most prevalent serogroups of entero-pathogenic *E. coli* revealed from the examined raw milk could be identified as O_{119} : K_{66} ; O_{112} : K_{66} ; O_{111}: K_{58} ; O_{114} : K_{90}; O_{78} : K_{80} ; O_{124} : K_{72} ; O_{128} : K_{67} and O_{26} : K_{60}, with the frequency percentages of 29.16, 20.83, 16.66, 8.33, 8.33, 8.33, 4.16 and 4.16, respectively. In ice cream (small scale) Table (3), 3 strains of enteropathogenic *E.coli* serotypes were belonged to : O_{112} : K_{66} ; O_{111}: K_{58} and O_{124} : K_{72}, with the same frequency percent (20%), but two strains were belonged to O_{119} : K_{66}, with percent of (40%). These isolated serovars were belonged to Enteropathogenic *E. coli* (EPEC) as O_{111}, O_{128}. Enterotoxigenic *E. coli* (ETEC) O_{114} and O_{128}. Enterohaemorrhagic *E. coli (*EHEC) as O_{26} and O_{111}. Enteroinvasive *E. coli* (EIEC) as O_{124} (Abram, 1995), who added that *E. coli* O_{112} and O_{119} serovars caused epidemic children enteritis. The same findings could be detected by (El-Bagoury and Hammad, 2004; Gad El-Said *et al.,* 2005; Hassan and Abosrea, 2005).

Pathogenic *E. coli* is one of the most significant groups of bacteria causing diarrhoea and extra-intestinal infections in humans and domestic animals. Five distinct groups of the organisms have been described associated with enteric disease of human. These include the toxin-producing strains, enterotoxigenic, enterohaemorrhagic and enteroinvasive and the non-toxin producing strains, enteropathogenic and enteroinvasive *E. coli.* These strains have been proposed as contributors to enteric and extra-intestinal disease in both human and animals (DeRycke *et al.,* 1990; Al-Hawary, 2005).

The public health importance of enteropathogenic *E.coli* in gastroenteritis was reported by Tulloch *et al.,* (1973). A milkborne outbreak of *E.coli* entritis among children has been reported in USSR (Matsievskii *et al.,* 1971). Enteropathogenic *E. coli* (EPEC) which were an important cause of infantile diarrhoea were originally incriminated epidemiologically as pathogens for infants and young children and Enterohaemorrhagic *E. coli* (EHEC) a cause of haemorrhagic colitis and haemolytic uremic syndrome (Levine, 1987).

Environmental contamination, bad hygienic measures during milking and transportation are the main sources of raw milk contamination which must be diminished as possible. Sanitary precautions in connection with handlers, utensils and surroundings during milking and processing must be adopted. Adequate cooling of raw milk, as well as efficient measures to preclude the multiplication of the organisms during preparation, service and storage of dairy products must be applied.

Although the results obtained show lower contamination rates of pathogenic microorganisms in large scale ice cream than small scale ice cream, both of them constitute a high-risk hazard to consumers. The finding of the study warrant the need to undertake safety measures to avoid potential threats and apply educational programs for both small and large scale ice cream producers about the risk of contamination, how to prevent it and how to apply strict hygienic measures during production, storage and distribution of ice cream. Moreover, regulation of small scale producing ice cream should be a part of a strategy to enhance producing of save and high quality ice cream in Alexandria city.

It is clearly evident from the previous results, that ice cream samples of street vendors were of inferior quality. Therefore, using of high quality raw ingredients, efficient heat treatment, proper cleaning and sanitation of equipment as well as good hygienic measures during manufacturing, handling, storage, transportation and distribution of ice cream are necessary to prevent its contamination and safeguard consumers against infections.

REFERENCES

Abd El-Hady, H.M. and Halawa, M.A. (1999): "Surveillance of *Aeromonas and Listeria species* in some Dairy products". J. Egypt. Med. Asso. No. (6): 1661-1670.

Abdel-Haleem, A.A. (1995): "Microbiological evaluation and sanitary improvement of ice cream". Ph. D. Thesis. Fac. Vet. Med., Assiut Univ.

Abdel- Khalek, A. (1997): "Enumeration and characterization of *Aeromonas species* isolated from farm bulk milk in Mansoura, Egypt". Alex. J. Vet. Sci., 13: 35-40

Abram, S.B. (1995): "Control of Communicable Diseases Manual". 16th Ed. An official Report of American Public Health Association (APHA).

Ahmed, A.A.H.; Abd El-Haleem, A.A. and Hussein, M.F. (2001): "Prevalence of *Aeromonas hydrophila* in some dairy products available in Assiut City". Assuit Vet. Med. J. 44, 88: 198-206.

Aisha, A. Mohamed; Nour, M.K. Hassan and Eman, M.S. Zaki (2002): "Astudy On some Enterotoxigenic Microorganisms in Raw and Boiled milk in Cairo and Giza markets". J. Egypt, Vet. Med. Ass. 62, 60: 71- 81.

Al- Ashmawy, A.M.; El- Gamal, A.M. and Abd El- Kalek, A. (1997): "Prevalence of Pseudomonas in farm Bulk-milk". Assiut, Vet. Med. J. 36, 72.

Al-Hawary, I.I. (2005): "Microbiological quality of Ewe's milk with special references to *Escherichia coli* O_{157}*:* H_7". J. Egypt Vet. Med. Assoc.65, No.2: 269- 278.

Aly, S.A. and Zaki, H.M. (2001): "Studies on *Pseudomonas species* in milk and feces of Dairy Cattle". Assiut. Vet. Med. J. 46, 91.

Amal, S.M. Sayed and Sayed, M. (2003): Prevalence of some zoonotic Enteropathogenic Bacteria in ice cream. 2nd ICOFHHH; 21-23 October 2003, Assiut- Egypt.

American Public Healh Association (APHA), (1992): "Compendium of methods for the microbiological examination of Foods 2nd Ed". Washington, DC, USA.

Association of Official Analytical Chemists (AOAC), (1984): "Bacteriological analytical manual 6th Ed. Edited by division of Microbiology Center For Food Safety and Applied Nutrition, Us Food and Drug Administration. AOAC, Arlington, Virginia, 22201.

Bahout, A.A. and Moustafa, A.H. (2004): "Occurrence of *Yersinia spesies* in Raw and Pasteurized milk in Dakahlia Governorate". Assiut, Vet. Med. J. 50, 101: 57- 63.

Baird-Porker, A.C. (1962): An improved diagnostic and selective medium forisolation of coagulase positive staphylococci-J Appl. Bact., 25(1): 12-19.

Beuchat, L.R. (2000): "Control of Foodborne Pathogens and spoilage microorganisms by naturally occurring microbials". In: Microbial food contamination (Wilson, C. L. and Droby, S. Eds) 5th ed. CRC London Press. P. 149-170.

Bigalke, D. (1985): "Biochemical changes in dairy products caused by Psychrotrophic microorganisms". I- Microbial enzyme. Dairy and Food Sanitation 5(9): 345- 346. Dairy Sci. Abst., 48(1), (1986).

Butler, T. (1998): Yersiniosis and Plague. In Palmer, S. R., Soulsby, L. and Simpson, D.I.H.Zoonoses. Biology, clinical practice and Public Health Control. Oxford University Press. pp. 281-293.

Champagne, C.; Laing, R.; Roy, D.; Mafu, A. and Griffiths, M., (1994): "Psychrotrophs in dairy products: effects and control". Crit. Rev. Food Sci. Nutr., 34(1): 1-30.

Cogan, T.M. (1980): "Heat resistant lipases and proteases and the quality of dairy products. Annu. Bull. Int. Dairy Fd. Document. 118: 26-32

Corlett, Jr.D.A. (1989): "Refrigerated Foods and use of Hazard Analysis and Critical Control Point Principals". Food Technol. (2): 91-94.

Donald, A.S. and George, W. (1992): "Yersinia. In: Compendium of methods for the Microbiological Examination Food. 3rd Ed., chapter 27 "APHA".

Edwards, P.R. and Ewing, N.H. (1972): "Identification of Enterobacteriaceae". 3rd Ed. Burgeon publishing. Co.; Atlanta. U.S.U., 208-339.

Efthymiou, C.J.; Baccash, P.; Labombard, V.J. and Epistein, D.S. (1974): "Improved isolation and differentiation of Enterococcus in cheese". Appl. Microb. 28: 417-422.

El Bagoury, A.M. (1996): "Bacteriological quality of market ice cream". Vet. Med. J. Giza, 44 (2A): 203-207

El- Bagoury, A.M. and Hammad, A.M. (2004): Prevalence of *E. coli* $O_{157:}$ H_7 and other verotoxigenic *E. coli* in raw milk and some dairy products". Alex. Vet. 21, 1: 266-275.

El- Bassiony, T.A.; Aboul- Khier, F.A. and Saad, N.M. (1985): Psychrotrophic bacteria in dairy products". Assiut Vet. Med., J., 15(29): 101-105.

Elmer, W.K.; Stephan, D.A.; William, M.J.; Paul, C.S. and Washington, C.W. (1994): "Introduction to Diagnostic Microbiology". J. B.Lippincott Co., Philadelphia.

El- Prince, E. (1998): "Incidence and characterization of *Aeromonas species* in Domiati and Kariesh cheese sold in Assiut Province". Assuit Vet. Med. J.; 39: 184-193.

El-Prince, E. and Hussein, A.A. (2000): "Occurrence of Enterobacteriaceae in Ice cream with special reference to Salmonella species". Assiut, Vet. Med. J. 45, 89: 104-116.

El- Shishnagui, S.M.L. and Nazem A.M. (1999): Prevalence of Enterotoxigenic *Escherichia coli* in soft cheese, As determined by the polymerase chain reaction (PCR) Assay". Alex. J. Vet. Science. 15, 2: 451-462.

El- Shorbagy, I.M. and Al- Ganzoury, H.H. (2002): "Incidence of Aeromonas species in raw milk and yogourt in Zagazig city. J. Egypt. Vet. Med. Ass. 62, 4: 229-233.

Gad El – Said, W.A.; El- Jakee, J.K.; Xandel, M.M. and El- shabrawy, M. A. (2005): "Presence of *E. coli* O_{157} *:* H_7 in raw milk and meat samples". J. Egypt. Vet. Med. Assoc. 65, 3: 341-350.

Harrigan, W.F. (1998): "Laboratory methods in food microbiology". 3rd Ed. Toronto, London, New York.

Hassan, N.M.K. and Abosrea, N.A. (2005): "Experimental studies on the effect of Bifidobacterium bifidium on *Listeria monocytogenes* and *Echerichia coli* in Ice cream". J. Egypt. Vet. Med. Asso. 65, 5: 179-192.

Hussein, S.Z. and Ahmed, E.K. (2002): "Occurrence of *Yersinia enterocolitica* and *Aeromonas hydrophila* in pasteurized milk in Sohage city".Assiut vet. Med. J. 46, 92: 116-124.

Howard, B.J.; Keiser, J.F.; Weissfeld, A.D.; Smith, T.F. and Tilton, R.C. (1993): "Clinical and Pathogenic Microbiology 2nd Edition, P. 900.

I.C.M.S.F. (1980): "Microbiology of Foods. Vol. I: Factors affecting life and death of microorganisms. Academic Press, New York, London, Toronto, Sydny, San Francisco.

I.C.M.S.F. (1982): "International Committee on Microbiological Specification for Food: Microorganisms in Foods, Their Significance and Methods of Enumeration". 2nd Ed., Univ. Toronto Press, Toronto Buffalo London.

Janda, J.M. and Abott, S.L. (1998): "Evolving concepts regarding the genus Aeromonas: an expanding panorama pf species, disease Presentations and unanswered questions". Clin. Infect. Dis., 27: 322-344.

Khalil, N.G. (1992): "Occurrence, Delection and Significance of *Pseudomonas aeruginosa* in raw milk". Assiut Vet. Med. J. Vol. 28 (55): 152-157.

Khalil, N.G. (1997): "Incidence of *Aeromonas hydrophila* group in raw milk and some dairy products in Assiut City". Assuit Vet. Med. J., 37: 100-108.

Koneman, E.W.; Allen, S.D.; Janda, W.M.; Schreckenberger, P.C. and Winn, W.C.Jr. (1994): "Introduction to diagnostic microbiology. J. B. Lippincot Company, PP. 117-123.

Kornacki, J.L. and Gabis, D.A. (1990): "Microorganisms and refrigeration Temperatures". Dairy Food Environ. Sanit. 10: 192.

Kowalski, J.J. (1977): "Microbial agents and bovine mastitis". J.A.V.M.A., 170(10): 1175-1177.

Kraft, A.A. (1995): Spoilage of dairy products, fruits, vegetables and other foods. In: Psychrotrophic bacteria in foods, disease and spoilage (Kraft, A. A. ed.) CRC Academic Press, P. 115-121.

Kumari, V.; Sherikar, A.A. and Majee, S. (1996): "Microbiological profile of various Ice-creams sold in Mumbai region". Ind. J. Dairy Sci., 9(9): 619-624.

Lancette, G.A. and Tatini, R. (1992): "*Staphylococcus aureus".* Chapter 33. In: Compendium of methods or the microbiological examination of Foods 2nd ed. Vanderzant, C. and Splittoessr, D. (eds.) 3rd ed. American Public Health Association, Washington, Dc, USA.

Levine, M.M. (1987): "*E. coli* that cause diarrhoea, enterotoxigenic, enteropath ogenic, enteroinvasive, enterohaemorrhagic and enteroadherent". J. Infect. Dis., 155: 377-389.

Madeha, A.A. and Zaki, M.S.A. (2003): "Pseudomonas as potential source of contamination in raw milk and dairy products". SCVM J., Vol. (2): 13-26.

Mansour, M.A.; Bahout, A.A.; Abd-Allah, W. and Gomaa, M.M.S. (2000): "Proteolytic psychrotrophic bacteria in Ice cream marketed in Sharkia Governorate". 9th Sci. Cong. Fac. Vet. Med. Assiut Univ.: 13-20.

Marshall, R.T. (1992): "Standard Methods for the Examination of Dairy products. 16th ed., American Public Health Association, Washington, DC.

Matsievskii, V.A.; Logachev, A.V.; Fedorina, A.P. and Pisklova, A.S. (1971): "Outbreak of Food Poisoning caused by *Escherichia coli* O_{124} : K_{72}: B_{17}". Zhuhrnal Mikrobiologii, Epidemiologii Immunologii, 48(3): 137-139. Dairy Sci. Abst., 35(10): 398.

Mehlman, I.J. and Romero, A. (1982): "Escherichia *coli* recovery from foods". Foods Tech., 36(3): 73-79.

Mercado, E.C. and Ibanez, S.B. (1986): "Isolation of *Yersinia enterocolitica* from raw cow milk in Argentina. International Journal of Food Microbiology, 3237- 3242.

Mira, E.K.I.; Antown, I.G. and Abou- Zaid, S.M.A. (2001): "Psychrotrophic bacteria in milk and some milk products. J, Egypt. Med.Asso. 61, No. (6): 7-13.

Nashwa, M.H. and Isis, G.A. (2001): Prevalence of *Aeromonas species* in milk and some milk products". J. Egypt. Vet. Med. Ass., 61(4): 209-216.

Palumbo, S.A.; Maxino, F.; Williams, A.; Maxino, F.; Williams, A.C.; Buchnan, R.L. and Thayer, D.W. (1985): "Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. Appl. Environ. Microb. 50: 1027.

Palumbo, S.; Abeyto, C. and Stema, G. (1992): "Aeromonas *hydrophila* group". Chapter 30 in Compendium of Methods for the Microbiological Examination of Foods. 3rd Ed. Vanderzant, C. and Splittstoesser, D. F., APHA.Washington, D.C., 2005.

Pearson, J.P.; Feldman, M.; Iglewski, B.H., (2000): "Pseudomonas *aeruginosa* cell-to-cell signaling is required for virulence in a model of acute pulmonary infection". Infect Immun., 68(7): 4331-4433.

Popoff, M. (1984): Genus III. Aeromonas Kluver and river Niel PP. 545-548. In N.R. Krieg and J.G. Holt (eds) Bergey´s Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore.

Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter, G.R. (1994): "Clinical Veterinary Microbiology: The Streptococci and related cocci". Wolfe Publishing an important of Mosby. Yearbook, Europe Limited.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donelly, W. J. C. and Leonard, F.C. (2002): "Veterinary Microbiological and Microbial Diseases". Ist Iowastate University Press Blackwell Science.

Rabinovitz, M. (1987): Yersinia enterocolitica infection complicated by intestinal perforation. Arch. Internal Med. 147, 1662-1663.

Robinson, R.K. (1994): "Modern Dairy Technology". 2nd Ed. Chapman and Hall, London, New York.

Ryan, K.J. (1991): "Escherichia *coli* In Medical Microbiology". An introduction to infectious diseases 2nd Ed. Sherris, J. C. editor, PP. 360-369.

Seddik, I.; Ahmed, S.H. and Amani, G. Thabit, (1987): Preliminary studies on the exoproducts of *Ps. aeruginosa*. Assiut Vet. Med. J., 18, 35: 120-125.

Sonnerwirth, A.C. and Jarett, L. (1980): "Grad Wohl´s clinical laboratory methods and diagnosis. Vol. II, 8th Ed. The C.V. Mosby Co. St. Louis, Toronto, London, 2980.

Todd, E. (1990): "Food borne illness. Epidemiology of Foodborne illness: North. America. The Lancet. 336: 788-793.

Trust, T.T. and Chipman, D.C. (1979): Clinical involvement of *Aeromonas hydrophila*". Can. Med. Assoc. J., 120: 942-947.

Tulloch, E.F.; Ryan, L.J.; Formal, S.B. and Franklin, F.A. (1973): "Invasive enteropathogenic Escherichia *coli* dysentery, an outbreak in 28 adults". Annals of Internal Medicine, 79(1): 13- 17. Dairy Sci., Abst. 37: 253(1975).

Uraz, G. and Citak, S. (1996): The isolation of Pseudomonas and other gram-ve psychrotrophic bacteria in raw milk. J. Basic. Microbiol., 38(2): 124-134.

Varnam, A.H. and Evans, M.G. (1991): "Foodborne Pathogens". Wolfe Publishing LTD., England.

Villari, P.; Crispino, P. and Stanzione, S. (2000): "Prevalence and molecular charachterization of *Aeromonas species* in ready to eat foods in Italy." J. Food Prot., 63 (12): 175-1757.

Table 2: Statistical analytical results of different bacterial groups in examined

Bacterial Counts/ml	Raw milk			Ice cream Small scale			Ice cream Large scale		
	Min	Max	Mean±S.E	Min	Max	Mean±S.E	Min	Max	Mean±S.E ♀
Psychrotrophic	$9x10^3$	$6.8x10^{10}$	$9.4x10^8±1.1x10^8$	3x10	$1.4x10^7$	$7.5x10^5± 2.4x10^5$	10x4	$2x10^3$	$3x10^2± 0.1x10^2$
Coliform (MPN/ml)	$0.3x10^2$	$11x10^4$	$1x10^4±0.5x10^4$	$0.3x10^2$	$0.5x10^3$	$0.56x10^2±2.2x10^2$	-	-	-
Staphylococcus aureus	$8.95x10^3$	$26.5x10^3$	$16.38x10^3±1.53 x10^3$	$3.42x10^2$	$21.4x10^3$	$8.97x10^3±1.31x 10^3$	-	-	-
Enterococci (count/ml)	$3.7x10^2$	$1.14x10^3$	$6.91x10^2±1.67x 10^2$	8x10	$3.7x10^2$	$1.64x10^2±0.38x 10^2$	3x10	$2x10^2$	7.3x10 ±1.67x10