Assiut University web-site: www.aun.edu.eg

INCIDENCE OF LISTERIA SPECIES IN SOME DAIRY PRODUCTS IN BENI-SUEF GOVERNORATE

SAADIA H.H. ELSHINAWAY[1]; ARAFA M.S. MESHREF[1]; MOHAMED M.A. ZEINHOM[1] and DALIA A.A. HAFEZ[2]

[1] Department of Food Hygiene, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.
[2] Animal Health Research Institute.

Received: 19 October 2016; **Accepted:** 21 November 2016

ABSTRACT

The present study was conducted to investigate the prevalence of *Listeria spp*. in some dairy products. A total of 240 samples of fresh cream, ice cream, butter milk cheese, Kareish cheese, Talaga cheese and yoghurt (40 of each) were collected randomly from different supermarkets, retail outlets and other markets outlets in Beni-Suef Governorate, Egypt. Out of 240 samples, 24(10%) were positive for *Listeria species*. The occurrence of *Listeria spp*. in fresh cream, ice cream, butter milk cheese and Kareish cheese was 6(15%), 8 (20%), 5 (12.5%) and 5(12.5%) respectively, while *Listeria species* couldn′t be detected in any of Talaga cheese and yoghurt samples. The most prevalent species was *L.grayii* (45.83%), followed by *L.monocytogenes* (33.33%), and *L.welshimeri* (12.51%) and *L. innocua* 2(8.33%). All 8 *L.monocytogenes* isolates showed the presence of the hly A gene suggesting the possibility of danger of food borne listeriosis among dairy products consumers. In conclusion, the results of this study emphasize the need forapplying more strict hygienic control measures especially during processing, storage and marketing of dairy products.

***Key words**: Listeria spp., Listeria monocytogenes, Dairy products, PCR assay.*

INTRODUCTION

Milk and dairy products, because of their nutritional value, are suitable for development of microorganisms including pathogenic bacteria as *Listeria species* (Kasalica *et al.*, 2011; El Marnissi *et al.*, 2013 and Abd El Tawab *et al.*, 2015).

Listeria species are ubiquitous bacteria, well adapted in the environment and can be isolated from soil, vegetables and natural waters as well as from healthy animal and man (Roberts and Weidmann, 2003; Cocolin *et al.*, 2005 and Liu 2008). In this manner contaminate milk and production plants (Leite *et al.*, 2006). Among the species of the genus *Listeria*, only *L. monocytogenes* is considered as one of the most significant food borne pathogen that induceserious and potentially life threatening illness known as listeriosis in humans and animals (Ryser and Marth, 2007 and Rahimi *et al.*, 2012). However occasional human infection due to *L.ivanovii* and *L.seeligeri* has also been reported (Gilot and Content 2002).

Corresponding author: Dr. DALIA A.A. HAFEZ
E-mail address: dalia_hafez@yahoo.com
Present address: Department of Food Hygiene, Faculty of Veterinary Medicine, Beni-Suef University, Egypt

Listeriosis has been recognized as one of the serious emerging bacterial zoonitic diseases. Several outbreaks and sporadic cases of listeriosis primarily associated with consumption of contaminated milk, soft or semi soft cheeses, uncooked and ready to eat foods, unwashed raw vegetables and fruits (Oliver *et al.*, 2005; Swaminathan and Gerner-Smidt, 2007; Rahimi *et al.*, 2010; Kevenk and Gulel, 2016).

Listeriosis can be life-threatening for newborns, elderly and immuno – compromised individuals (McLauchlin *et al.*, 2004 and Rahimi *et al.*, 2012). It may be ranged from non invasive febrile gastroenteritis or influenza like symptoms to persons with no predisposing conditions (Aureli *et al.*, 2000) to serious invasive sever symptoms which may lead to septicemia, meningitis and abortion with high mortality rate of 20-30% (Gandhi and Chikindas, 2007; Swaminthan *et al.*, 2007; Shamloo *et al.*, 2015; Kevenk and Gulel, 2016).

In dairy industry, many problems associated with *L. monocytogenes* contamination are related to minimally processed or post pasteurization contamination from plant environments (Gougouli *et al.*, 2008; Rosshaug *et al.*, 2012 and Olszewska *et al.*, 2015).

L.monocytogenes has the ability to survive or even grows in a wide range of adverse environmental conditions (Begley *et al.,* 2010 and Cheng *et al.,* 2015) such as refrigeration temperatures, high acidity and salinity and reduced water activity (Gandhi and Chikindas, 2007; Ahmed *et al.,* 2014) makes it a potential hazard in milk and other dairy products.

Therefore, the present study was carried out to satisfy an urgent need to provide data about the presence of *Listeria species* in some dairy products in Beni-Suef governorate and to clarify the virulence of *L.monocytogenes* isolated from dairy products.

MATERIALS AND METHODS

Collection of samples:

- A total of two hundred and forty samples of fresh cream, ice cream, butter milk cheese, kareish cheese, yoghurt and talaga cheese (40 of each) were collected from different supermarkets, retail outlet and other markets outlets in Beni-Suef governorate, Egypt.
- All samples were aseptically collected and transferred into individual sterile bags or flasks then transported to the laboratory in insulated coolers containing cold packs and were analyzed immediately.

Isolation and identification of the *Listeria species*:
The isolation of *Listeria species* is adopted according to (Roberts and Green wood, 2003). About 25 ml/g of each sample was aseptically homogenized in 225 ml of listeria selective enrichment broth (CM0862, Oxoid) supplemented with listeria selective enrichment agents (nalidixic acid, acriflavine and cyclohexamide) (SR0141, Oxoid) and incubated in 30°Cfor 24-48 h`. Aloopful of the incubated broth was streaked onto oxford listeria selective agar (CM0856, Oxoid) supplemented with listeria selective supplement (SR0140, Oxoid) which incubated for 48 h`at 30°C. The typical greyish green to brownish green colored colonies with black haloes of 1-3 mmin diameter of aesulin hydrolysis were presumed to be *Listeria*.

Five presumptive *Listeria* colonies were picked from each plates of selective agar and streaked ontotrypticasesoya agar (CM0131, Oxoid) containing (0.6% yeastextract) then incubated at 30°C for 24h for biochemical identification. Non spore forming, Gram positive coccobacilli isolates were tested for catalase test, umbrella growth in motility test medium at 25°C, nitrate reduction, MR/VP test, B.haemolysis activity, CAMP test (synergistic lyses of red blood cells) against *S. aureus* and acid production from fermentation of glucose, rhamnose, xylose and mannitol.

Detection of *Listeria monocytogenes* (hly A gene) by PCR technique:-
Listeria monocytogenes were screened for the presence of Listeriolysin O (hlyA) gene.

The primers used in the study (Aurora *et al.,* 2007)

Primers	Target gene	Length	Primer sequence	Amplification product (bp)
hlyA-F	hlyA	24	5’ GCAGTTGCAAGCGCTTGGAGTGAA 3’	456
hlyA-R	hlyA	24	5’ GCAACGTATCCTCCAGAGTGATCG 3’	456

Extraction Procedure: (QIA amp DNA Mini Kit):

- 1.5ml of enriched broths was taken into microcentrifuge tubes and bacteria were pelleted by centrifugation at 8000 rpm for 5 min. Re-suspend pellet in PBS to a final volume of 200µl. 20µl QIAGEN protease or proteinase K and 200µl buffer AL were added and mixed for 15S. followed by incubation at 56°C for 10min.200µl ethanol (96-100%) were added and mixed again for 15S.

- Carefully the mixture was applied and centrifuged at (8000 rpm) for 1 min. The QIA amp Mini spin column was placed in a clean 2ml collection tube (provided) and discarded the tube containing the filtrate. The QIA amp Mini spin column was opened and 500µl buffer AW1was added and centrifuged at (8000 rpm) for 1 min. The QIA amp Mini spin column was placed in a clean 2ml collection tube and discarded the tube containing the filtrate. 500µl buffer AW2was added and centrifuged at full speed (14.000rpm) for 3 min.

- The QIA amp column was placed in a new 2ml collection tube and discarded the old collection tube with the filtrate, then centrifuged at full speed for 10min. The QIA amp column was placed in a clean tube (not provided) and discarded the collection tube containing the filtrate. Carefully the QIA amp column was opened and added 100-200µl buffer AE or distilled water then incubated at (15-25°C) for 3 min and centrifuged at (8000 rpm) for 1 min.

Spectrophotometer:-
The concentration of DNA was measured by using spectrophotometer.

PCR Procedures:
There agents of PCR reaction were set up in a tube:

1 - 25 µlof 2X of PCR Master Mix containing: e**nzyme** (DNA polymerase, Taq); **dNTPs** (A, T, G, C) and b**uffer** (50 mMKCl; 10 mMTris-HCl; 1.5 mM MgCl2).

2 - 20 pmolprimer.

3 - 30-50 ngsample (DNA)

4 - If needed water DNA ase free water and 50 µltotalreaction volume.
Amplification was done in the following condition shown in the next table.

Cycling conditions used for the primers

Steps		Time and Temperature
• Initial denaturation		95°C for 3 min
• Denaturation	45 cycles	94°C for 40 sec
• Annealing		41°C for 40 sec
• Extension		72 °C for 1 min
• Final extension		72 °C for 10min
• Hold		4° C

The amplified DNA fragments were resolved by agarose gel electrophoresis, stained with ethidium bromide (e (0.5µg/ml) and the gels can be screened and pictured under UV light.

RESULTS

The genus *Listeria* is ubiquitous in the surrounding environment and therefore, control of this organism during food processing is difficult (Kells and Gilmour, 2004). Many outbreaks of *L. monocytogenes* infection associated with consumption of milk and dairy products have been reported by CDC (Centers for Disease Control and prevention) (2011) and Gaulin *et al.* (2012).

Table 1: Incidence of *Listeria spp*. in some dairy products

Products	No of samples	Positive samples (%)	
		No of positive samples	%
Fresh cream	40	6	15%
Ice cream	40	8	20%
Butter milk cheese	40	5	12.5%
Kareish cheese	40	5	12.5%
Talaga cheese	40	0	0
Yoghurt	40	0	0
Total	240	24	10%

Table 2: Distribution of *Listeria* species isolated from various dairy products samples

Type of product	*L.monocytogenes*	*L.welshimeri*	*L. grayii*	*L. innocua*	*L. ivanovii*	*L.seeligeri*	Total
Fresh cream	2(8.33%)	0	4(16.66%)	0	0	0	6 (25%)
Ice cream	3(12.5%)	1(4.16%)	3(12.5%)	1(4.16%)	0	0	8(33.34%)
Butter milk cheese	2(8.33%)	1(4.16%)	1(4.16%)	1(4.16%)	0	0	5(20.83%)
Kareish cheese	1(4.16%)	1(4.16%)	3(12.5%)	0	0	0	5(20.83%)
Talaga cheese	0	0	0	0	0	0	0
Yoghurt	0	0	0	0	0	0	0
Total	8(33.33%)	3(12.5%)	11(45.83%)	2(8.33%)	0	0	24(100%)

In the present study, of the 240 samples of some dairy products, 24(10%) were positive for the presence of *Listeria species* (Table 1). The prevalence of *Listeria spp*. in fresh cream, ice cream, butter milk cheese and Kareish cheese was 6 (15%), 8 (20%), 5(12.5%) and 5(12.5%) respectively, whereas ice cream was the most contaminated product tested. *Listeria spp.* couldn't be isolated from any of Talaga cheese and yoghurt samples.

From the inspection of Table 2, *L. grayii* with (45.83%) was the most prevalent species isolated followed by *L. monocytogenes* with (33.33%), *L.welshimeri* with (12.51%) and *L. innocua* (8.33%), while other species of *Listeria* pathogen couldn't be isolated from any of the examined samples. All the eight isolates of *listeria monocytogenes* which identified biochemically were genetically confirmed by PCR technique represented in 2(5%), 3(7.5%), 2(5%) and 1(2.5%) in fresh cream, ice cream, butter milk cheese and Kareish cheese respectively (Table 3 and Fig1).

Table 3: Results of PCR method

Type of samples	No. of samples	Positive results for L. monocytogenes	
		Positive PCR samples	%
Fresh cream	40	2	5%
Ice cream	40	3	7.5%
Butter milk cheese	40	2	5%
Kareish cheese	40	1	2.5%

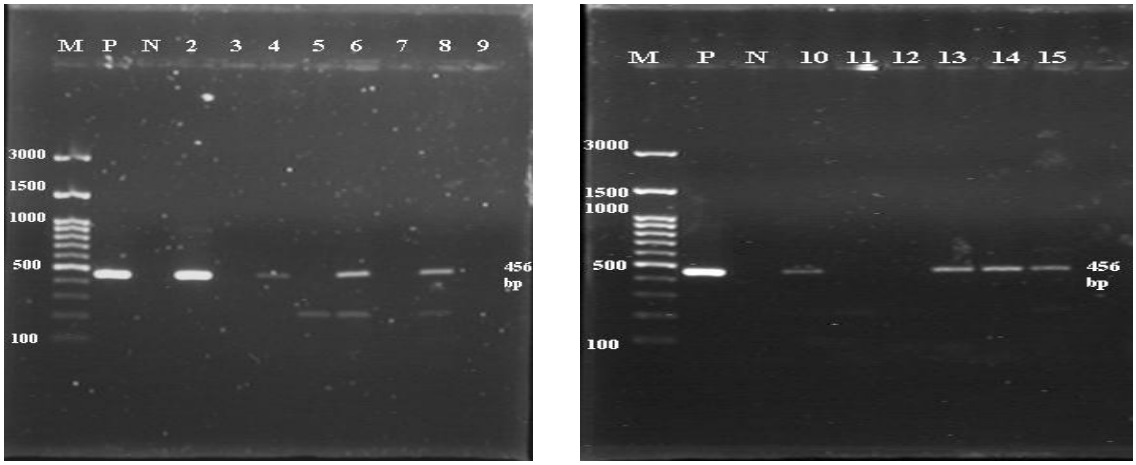

Fig. 1: Agarose gel electrophoresis of hylA gene specific for *L.monocytogenes* by enrichment PCR method
Lane M: DNA marker Ladder (100-3000 bp)
Lane P: Control positive for (hlyA) at 456 bp
Lane N: Control negative
Lane 2-15: Isolates of *Listeria monocytogenes* which biochemically identified

DISCUSSION

In the current study, 6(15%) of fresh cream samples were contaminated with *Listeria spp.*, whereas *L. grayii* was the most frequent 4(16.66%) species identified in fresh cream samples followed by *L.monocytogenes* which detected in only 2(8.33%) of the examined samples. Higher rate of contamination with *Listeria spp*. 10(20%) was obtained by Metwally and Ali, (2014), while Shamloo *et al.* (2015) reported low rate of contamination 3(11.11%) with *Listeria spp*.

Unlike to our results, Gunasena *et al.* (1995); El Habib *et al.* (2014) and Shamloo *et al.* (2015) couldn't detect *L.monocytogenes* in all examined fresh cream samples. The occurrence of *Listeria spp.* in fresh cream could be due to the use of raw milk, environmental contamination and insufficient hygiene during production.

Ice cream is one of the widely accepted dairy products that dominate interest of large segments of population (Anonymous 2012). Frequent occurrence of *L. monocytogenes* in ready to eat foods as ice cream as a result of its psychrotrophic genetic ability, implies increased public health risk hazard. The present study revealed that out of 40 examined ice

cream samples, 8(20%) samples yielded growth of *Listeria species*. The prevalence of *L. monocytogenes, L. welshimeri, L. grayii and L.* innocua detected in ice cream samples in this study were 3(12.5%), 1(4.16%), 3(12.5%) and 1(4.16%), respectively. Ice cream samples showed the highest incidence of *Listeria species* among all examined samples.

Nearly similar results (19.04%) was reported by Shamloo *et al.* (2015), while prevalence of *Listeria spp*. was much higher as compared to those observed by Wahba (2002); Rahimi *et al.* (2012); Akya *et al.* (2013); Muhammed *et al.* (2013) and Abd El Tawab *et al.* (2015). High incidence of *Listeria spp*. was recorded by Molla *et al.* (2004) and Mengesha *et al.* (2009).

Contrary to the postulated results, *Listeria spp*. couldn't be isolated from examined ice cream samples in studies performed by Dhanashree *et al.* (2003); Ambily and Beena (2012); El Habib *et al.* (2014); Kevenk and Gulel (2016). On the other hand, Muhammed *et al.* (2013); Akya *et al.* (2013); Metwally and Ali (2014) failed to isolate *L. monocytogenes* from the examined ice cream samples.

The higher incidence of *Listeria spp*. in ice cream samples could be attributed to the contamination of raw milk, low quality of ingredients used, use of polluted water supplies and lack of hygienic measures during processing and handling besides the absence of pasteurization, especially in case of small scale produced ice cream.

Egyptian cheese has a long history as an important part of the modern Egyptian diet with an extended shelf life and is more self stable over the raw material used in the production (Fox *et al.,* 2004). Soft cheeses have been involved in both outbreaks (Goulet *et al.,* 1995) and several sporadic cases (Farber *et al.,* 1990) of listeriosis and are thus considered as risk products.

The present study was conducted to detect the prevalence of *Listeria spp*. and *L.monocytogenes* in 3 types of soft cheeses, Kareish cheese, butter milk cheese and Talaga cheese, whereas the high moisture content of almost 50% of these cheeses make them more suitable for growth of microorganisms (Metwally and Ali, 2014).

Listeria spp. was identified in 5(12.5%) of each Kareish cheese and butter milk cheese, while none of the samples of Talaga cheese was found to contain *Listeria* microorganisms.

The results of Talaga cheese in the present study are in agreement with the results of Atil *et al.* (2011); Akya *et al.* (2013) and Ahmed (2013). In the studies conducted by Kevenk and Gulel (2016); Abd El Tawab *et al.* (2015); Wijendra *et al.* (2014), *L.monocytogenes* was isolated with very low incidence of 1(5%), 1(2%) and 4(5%) in the examined white cheese samples.

L. grayii was the most prevalent species isolated from Kareish cheese (12.5%), whereas *L.monocytogenes* and *L.welshimeri* were detected in (4.16%) for each. While in butter milk cheese *L. grayii, L. monocytogenes, L. welshimeri and L. innocua* were isolated from 4.16%, 8.33%, 4.16% and 4.16%, respectively.

In the study performed by Metwally and Ali (2014), *Listeria spp.* was detected in 11(22%) of the examined butter milk cheese from which 8% *L.grayii* followed by 2% *L.innocua* and 1% *L.ivanovii*, while *L.monocytogenes*, *L.welshimeri* and *L.seeligeri* couldn't be isolated from any of the examined samples.

On the other hand, high incidence of *Listeria spp*. in Kareish cheese was detected by Metwally and Ali (2014); Meshref *et al.* (2015), while lower incidence was reported by Derra *et al.* (2013); Muhammed *et al.* (2013); Ismail *et al.* (2014); Abd El Tawab *et al.* (2015).

In a study performed by Rahimi *et al.* (2010), *Listeria spp*. and *L.monocytogenes* were isolated in 18.9 and 10 of 90 cheese samples, while in another study conducted by Arslanand Ozdemir (2008) *Listeria spp*. was detected in 33.1% of homemade white cheese samples.

The type and composition of cheese, pH, % of moisture, % of salt, ripeness of cheese, storage conditions, starter cultures and virulence of pathogens influenced the reproduction of *L. monocytogenes* in cheese (Kovincic *et al.,* 1991). The high incidence of these organisms in different types of cheeses may be accounted for by insufficient hygiene during milking, manufacturing process, ineffective pasteurization and/or post pasteurization contamination.

The absence of *Listeria spp*. in Talaga cheese could be attributed to the presence of undesirable conditions during cheese manufacturing process such as high concentration of sodium chloride, low acidity, heat treatment and high moisture content and /or preservation in salted whey (Zamani-Zadeh *et al.,* 2011).

High incidence of *Listeria spp*. In Kareish cheese and butter milk cheese may be revealed to traditional homemade manufacturing technique utilized, whereas separating milk for Kareish cheese and the collection of butter milk cheese from butter churn are steps difficult to be controlled besides these cheeses don't contain salt content nor preserved in brine solution as to Talaga cheese.

Yoghurt is one of the most popular fermented dairy products which has a wide acceptance worldwide. In the present study, none of the yoghurt samples was found to contain *Listeria spp*. which may be attributed to antagonistic activity of starter and effective heat treatment Arquez *et al.* (2005) and Vermeulen *et al.* (2007).

The results obtained in the current study are almost similar to those of Arslan and Ozdemir (2008); AbdElAal and Atta (2009); Rahimi *et al.* (2012); Ismail *et al.* (2014) ; Metwally and Ali (2014); Shamloo *et al.* (2015).

While studies performed by Shahbazi *et al.* (2013); Muhammed *et al.* (2013); Seyoum *et al.* (2015) *L.monocytogenes* was detected in only one sample.

In study of Faleiro *et al.* (2003) revealed absence of *L.monocytogenes* in all examined samples of Iranian fermented milk drink to the combined inhibitory effect of low pH and the antimicrobial activity of some compounds that probably secreted by lactic acid bacteria present in these fermented products.

Detection of *Listeria monocytogens*hly A gene by using PCR technique:

Molecular biology method basedon Polymerase chain reaction (PCR) assay was characterized by high specificity, sensitivity ,rapidity and it may permit direct detection of pathogen without need for isolation of pure culture (Zhou and Jiao 2005).

Thus, this study was attempted for further confirmation of *L. monocytogenes* isolates through the detection of one of its virulence factors. PCR was carried out targeting the virulence factor Listeriolysin O (LLO) which encoded by hlyA gene and present only in virulent strains of the species and required for virulence Jay, (1996); Ueda *et al.* (2005); Aurora *et al.* (2007); Suriyapriya *et al.* (2016).

In this study, the result of PCR technique was represented in Table 3 and Figure 3 confirmed the presence of *L.monocytogenes* in 2(5%), 3(7.5%), 2(5%) and 1(2.5%) of fresh cream, ice cream, butter milk cheese and Kareish cheese samples, respectively.

Abd El Tawab *et al.* (2015) detected that the (hlyA) gene was amplified in 5(100%) *L.monocytogenes* strains. Also Nayak *et al.* (2015) detected virulence listeriolysin O(hlyA) gene in the all three *L.monocytogenes* which isolated from raw milk samples.

In a study conducted by Al Ashmawy *et al.* (2014) Enrichment PCR investigated hly A gene in 28 and 32 of the examined Damietta cheese and Kareish cheese, respectively. In another study performed by Wijendra *et al.* (2014) *L.monocytogenes* was positively detected by using PCR technique in 10(12%), 8 (10%), 4 (5%) and 3(4%) of ice cream, curd, cheese and yoghurt samples respectively, while Derra *et al.* (2013) reported that only one sample (1.7%) of cottage cheese was positive for *L.monocytogenes* by PCR.

PCR results showed that detection of virulent (hlyA) gene of *L. monocytogenes* which present only in the virulent strains in fresh cream, ice cream, Kareish cheese and butter milk cheese suggested the presence of a significant public health hazard linked to the consumption of these products.

CONCLUSION

This study indicates that some dairy products sold in Beni-suef markets may be considered as a threat to consumers. They are significant vehicles of *L. monocytogenes* which regularly causing listeriosis outbreaks. Therefore clear risk factors and people that are susceptible for acquiring listeriosis should not consume such products. This indicates importance and need for permanent control, and detection of potential sources of contamination. Introduction of HACCP (Hazard Analysis and Critical Control Points), as away of control in the process of production and processing the risk of contamination of products with these pathogen micro organisms is reduced.

REFERENCES

Abd El Aal, S.F.A. and Atta, M.A.H.B. (2009): Occurrence of *Listeria* and *Yersinia species* in milk and some milk products. Assuit Vet. Med. J., 55(123):45-60.

Abd El-Tawab, A.A.; Maarouf, A.A.A. and Mahy, Z.A.M. (2015): Bacteriological and Molecular studies of *Listeria species* in milk and milk products at El-Kaliobia Governorate, Benha. Veterinary Medical J., 29(2):170-180.

Ahmed, Hanaa, S. (2013): Public health significance of Listeria as a food borne pathogen. M.V. Sci. Thesis, Faculty. Vet. Med. Cairo Univ., Egypt.

Ahmed, L.I.; Morgan, S.D.; Hafez, R.S. and Abdel-All, A.A.A. (2014): Influence of yoghurt starter culture on viability of some pathogenic microorganisms in yoghurt. Int. J. Dairy Sci., 9(3): 82-88.

Akya, A.; Najafi, F.; Moradi, J.; Mohebi, Z. and Adabagher, S. (2013): Prevalence of food contamination with *Listeria spp*. in Kermanshah, Islamic Republic of Iran. Eastern Mediterranean Health J., 19(5): 474-477.

Al-Ashmawy, Maha, A.M.; Gwida, Mayada, M. and Abdelgalil, K.H. (2014): Prevalence, detection

methods and antimicrobial susceptibility of listeria monocytogenes isolated from milk and soft cheeses and its zoonitic importance. World Applied Sciences J., 29(7): 869-878.

Ambily, A. and Beena, A.K. (2012): Bacteriological quality of ice cream marketed in Thrissur town, Kerala, India. Vet. World J., 5(12): 738-741.

Anonymous, (2012): Over 50 children took ill; food poisoning suspected. The Hindu on the Web. 1 March 2012.

Arques, J.L.; Rodriguez, E.; Gaya, P.; Medina and Nunez, M. (2005): Effect combinations of high pressure treatment and bacteriocin producing lactic acid bacteria on the survival of *L.monocytogenes* in raw milk cheese. Int. Dairy J., 15:893-900.

Arslan, S. and Ozdemir, F. (2008): Prevalence and antimicrobial resistance of *Listeria spp*. in homemade white cheese. Food Control., 19(11): 360-363.

Atil, E.; Ertas, H.B. and Ozbey, G. (2011): Isolation and molecular characterization of *Listeria spp.* from animals, food and environmental samples. Veterniary Medicine., 56(8): 386-394.

Aureli, P.; Fiorucci, G.C.; Caroli, D.; Marchiaro, G.; Novara, O.; Leone, L. and Salmaso, S. (2000): An outbreak of febrile gastroentroenteritis association with corn contamination by *L.monocytogenes*. New Engl. J. Med., 342: 1236-1241.

Aurora, Ritu; Prakash, A. and Prakash, S. (2007): A comparative study of conventional culture and PCR method for the detection of *L.monocytogenes* from artificially inoculated milk. Ind. J. of Dairy Sci., 60: 331-337.

Begley, M.; Cotter, P.D.; Hill, C. and Ross, R.P. (2010): Glutamatedecarboxylase-mediatednisinresistancein *L.monocytogenes*. Appl. Environ. Microbiol. 76: 6541–6546.

CDC (Centers for Disease Control and prevention) (2011): Multistate outbreak of listeriosis associated with Jensen farms cantaloupe-United States, Augest – September 2011. Morbidity and Mortality Weekly Report, 60: 1357-1358.

Cheng, C.; Yang, Y.; Dong, Z.; Wang, X.; Fang, C.; Yang, M.; Sun, J.; Xiao, L.; Fang, W. and Song, H. (2015): L. monocytogenes varies among strains to maintain intra cellular pH homeostasis under stresses by different acids as analyzed by a high throughput microplate based fluorometry. Frontiers in Microbiology. 6(15).

Cocolin, L.; Stellab, T.S.; Nappic, R.; Bozzettac, E.; Cantonib, C. and Comi, G. (2005): Analysis of PCR- based methods for characterization of *L.monocytogenes* strains isolated from different sources. Int. J. Food Microbiology, 103, 167-178.

Derra, F.A.; Karlsmose, S.; Monga, D.P.; Mache, A.; Svendsen, C.A.; Felix, B.; Granier, S.A.; Geyid, A.; Taye, G. and Hendriksen, R.S. (2013): Occurrence of *Listeria spp*. in retail meat and dairy products in the area of Addis Ababa, Ethiopia. Foodborne Pathogens and Disease, 10(6): 577-579.

Dhanashree, B.; Otta, S.K.; Karunasagar, I.; Goebel, W. and Karunasagar, I. (2003): Incidence of *Listeria spp*. in clinical and food samples in Mangalore, India. Food Microbiology., 20: 447-453.

El-Habib, F.; Mustapha, M.E.; El-Quardi, A.E. and Senouci, S. (2014): L. monocytogenes in food matrix: Frequency and effect of antagonist microbiology. Pakistan J. Nutrition., 13(3): 141-145.

El-Marnissi, B.; Bennani, L.; Cohen, N.; Lalami, A.E. and Belkhou, R. (2013): Presence of *L.monocytogenes* in raw milk and traditional dairy products marketed in the north –central region of Morocco. African J. Food Science. 7(5): 87-91.

Faleiro, M.L.; Andrew, P.W. and Power, D. (2003): Stress response of *L. monocytogenes* isolated from cheese and other foods. Int. J. Food Microbiol., 84: 207-216.

Farber, J.M.; Carter, A.O.; Varughese, P.V.; Ashton, F.E. and Ewan, E.P. (1990): Listeriosis traced to the consumption of alfalfa tablets and soft cheese. New England J. Medicine., 322, 338.

Fox, P.F.; McSweeney, P.L.H.; Cogan, T.M. and Guinee, T.P. (2004): Cheese: Chemistry, Physics and Microbiology, 3rd ed., Elsevier Academic Press, California.

Gandhi, M. and Chikindas, M.L. (2007): Listeria: A foodborne pathogen that knows how to survive. Int. J. Food Microbiol., 113, 1–15.

Gaulin, C.; Ramasay, D. and Bekal, S. (2012): Widespread listeriosis outbreak attributed to pasteurized cheese, which led to extensive cross-contamination affecting cheese retailers, Quebec, Canada, 2008. J. Food Protection. 75:71-78.

Gilot, P. and Content, J. (2002): Specific identification of *L.welshimeri* and *L.monocytogenes* by PCR assays targeting a gene encoding a fibronection – binding protein. J. Clin. Microbiol., 40 : 698-703.

Gougouli, M.; Angelidis, A.S. and Koutsoumanis, K. (2008): A study on the kinetic of *L.monocytogenes* in ice cream stored under static and dynamic chilling and freezing conditions. J. Dairy Science., 91: 523-530.

Goulet, V.; Jacquet, C.; Vaillant, V.; Rebiere, I.; Mouret, E.; Larente, C.; Maillot, E.; Stainer, F. and Rocourt, J. (1995): Listeriosis from

consumption of raw milk cheese. Lancet., 345: 1581-1582.

Gunasena, D.K.; Kodikara, C.P.; Genepola, K. and Widanapathirana, S. (1995): Occurrence of *L.monocytogenes* in food in Sri Lanka. J. Natn. Sci. Coun. Sri Lanka. 23(3): 107-114.

Ismaiel, A.A.; Ali, E.A. and Enan, G. (2014): Incidence of *Listeria* in Egyptian Meat and Dairy samples. Food Sci. Biotechnol., 23(1): 179-185

Jay, J. (1996): Modern food Microbiology. In: Heldman DR, Editor. Foodborne Listeriosis. New York: Chapman and Hall, pp: 478-506.

Kasalica, A.; Vukovie, V.; Vranjes, A. and Memisi, N. (2011): L.monocytogenes in milk and dairy products. Bacteriology in Animal Husbandry., 27(3): 1067-1082.

Kells, J. and Gilmour, A. (2004): Incidence of *L.monocytogenes* in two milk processing enviromentsand assessment of *L. monocytogenes* blood agar for isolation. Int. J. Food Microbiol., 91:167-174.

Kevenk, T.O. and Gulel, G.T. (2016): Prevalence, antimicrobial resistance and serotype distribution of *L.monocytogenes* isolated from raw milk and dairy products. J. Food Safety., 36:11-18.

Kovincic, I.; Vujicic, F.I.; Švabic-Vlahovic, M.; Vulic, M.; Gagic, M. and Wesley, V.I. (1991): Survival of *L.monocytoenes* during the manufactureand ripening of trappist cheese. J. Food Protect., 54, 6, 418-420.

Leite, P.; Rodriguez, R.; Ferreira, M.; Ribeiro, G.; Jacquet, C.; Martin, P. and Brito, L. (2006): Comparative characterization of *L. monocytogenes* isolated from Portuguese farm house ewe' cheese and from humans. Int. J. Food Microbiology., 106: 111-121.

Liu, D. (2008): Epidemiology. In D. Liu (Ed.) Handbook of *L.monocytogenes* (pp.27-30). New York: CRC Press.

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

Mengesha, D.; Zewde, B.M.; Toquin, M.T.; Kleer, J.; Hildebrandh, G. and Gebreyes, W.A. (2009): Occurrence and distribution of *L.monocytogenes* and other *Listeria species* in ready- to-eat and raw meat products. Berl. Munch. Tierarztl. Wochenschr., 122 (1-2_:20-24.

Meshref, A.M.S.; Zeinhom, M.M.A. and Abdel-Atty, N.S. (2015): Occurrence and distribution of *Listeria species* in some Egyptian foods. Alexandria. J. Vet. Sci., 46: 42-47.

Metwally, A.M.M. and Ali, Fatma, H.M. (2014): Listeria spp. in ready –to-eat dairy products from retailers and small shops. J. Food and Dairy Sci. Mansoura Univ., 5(10): 725-730.

Molla, B.; Yilma, R. and Alemayehu, D. (2004): L. monocytogenes and other *Listeria species* in retail meat and milk products in Addis Ababa, Ethiopia. Ethiop. J. Health Dev., 18(3): 208-212.

Muhammed, Wosila; Muleta, D.; Deneke, Y.; Gashaw, A. and Bitew, M. (2013): Studies on occurrence of *L. monocytogenes* and other species in milk and milk products in retail market of Jimma Town, Ethiopia. Asian J. Dairying and Food Research., 32(1): 35-39.

Nayak, D.N.; Savalia, C.V.; Kalyani, I.H.; Kumar, R. and Kshirsagar, D.P. (2015): Isolation, identification and characterization of *Listeria spp.* from various animal origin foods .Vet. World.,8(6): 695-701.

Oliver, S.P.; Jayarao, B.M. and Almeida, R.A. (2005): Food-borne pathogens in milk and the dairy farm environment: Food safety and public health implications. Foodborne Pathog. Dis., 2: 115–129.

Olszewska, M.A.; Panfil-Kuncewicz, H. and Łaniewska-Trokenheim, L. (2015): Detection of viable but nonculturable cells of *L. monocytogenes* with the use of directepifluorescent filter technique. J. Food Safety.,35: 86-90.

Rahimi, E.; Ameri, M. and Momtaz, H. (2010): Prevalence and antimicrobial resistance of *Listeria species* isolated from milk and dairy products in Iran. Food Control 21: 1448–1452.

Rahimi, E.; Momtaz, H.; Sharifzadeh, A.; Behzadnia, A.; Ashtari, M.S.; Zandi Esfahani, S.; Riahi, M. and Momeni, M. (2012): Prevalence and antimicrobial resistance of *Listeria species* isolated from traditional dairy products in Chahar Mahal and Bakhtiyari, Iran. Bulgarian J. Vet. Medicine., 15(2):115-122.

Roberts, A.J. and Weidmann, M. (2003): Pathogen, host and environment factors contributing to the pathogensis of listeriosis. Cell Mol. Life Sci., 60: 904-918.

Roberts, D. and Greenwood, M. (2003): Practical Food Microbiology., 3rd edition, by Black well Publishing Ltd, USA.

Rosshaug, P.S.; Detmer, A.; Ingmer, H. and Larsen, M.H. (2012): Modeling the growth of *L.monocytogenes* insoft blue-white cheese. Appl. Environ. Microbiol., 78:8508–8514.

Ryser, E.T. and Marth, E. (2007): Listeria, Listeriosis and Food Safety.3rd.Ed.Taylor and Francis. Boca Raton, Fl.

Seyoum, E.T.; Woldetsadik, D.A.; Mekonen, T.K.; Gezahegn, H.A. and Gebreyes, W.A. (2015): Prevalence of *Listeria* in raw bovine milk and milk products from central highlands of Ethiopia. J. Infect. Dev. Ctries., 5(11): 1204-1209.

Shahbazi, A.M.; Rashedi, M. and Sohrabi, R. (2013): Comparative contamination of *L.monocytogenes* in traditional dairy products in Esfahan province, Iran. African J. Microbiology Research.,7(16):1522-1526.

Shamloo, E.; Jalali, M.; Mirlohi, M.; Madani, G.; Metcalf, D. and Merasi, M.R. (2015): Prevalence of *Listeria spp*. In raw milk and traditional dairy products in Isfahan, Iran. Int. J. Environmental Health Engineering., 4:1.

Suriyapriya, S.; Selvan, P.; Porteen, K. and Suresh, S.K. (2016): Prevalence of *Listeria spp*. in traditional Indian dairy products from Chennai Metropolis, Tamil Nadu. International Conference of Sabaragamuwa University of Sri-lanka 2015. Procedia Food Science.,6: 230-234.

Swaminathan, B. and Gerner-Smidt, P. (2007): The epidemiology of human listeriosis "Microbes and Infection "., Vol. 9 (10): 1236-1243.

Swaminathan, B.; Cabanes, D.; Zhang, W. and Cossart, P. (2007): L.monocytogenes. In Food Microbiology Fundamentals and Frontiers, 3rd ed. (M.P. Doyle and L.R. Beuchat, eds.) pp: 457–491, American Society for Microbiology (ASM) Press., Washington DC.

Ueda, F.; Anahara, R.; Yamada, F.; Mochizuki, M.; Ochiai, Y. and Hondo, R. (2005): Discrimination of *L. monocytogenes* contaminated commercial Japanese meats. Int. J. Food Microbiology.,15: 455–462.

Vermeulen, A.; Gysemans, K.P.M.; Bernaerts, K.; Geeraerd, A.H.; Van Impe, J.F.; Debevere, J. and Devlieghere, F. (2007): Influence of pH, water activity and acetic acid concentration on *L.monocytogenes* at 7°C: Data collection for the development of a growth/no growth model. Int. J. Food Microbiol., 114: 332–341.

Wahba, Nahed, M. (2002): Studies on *Listeria species* in milk and some milk products in Assuit Governorate. Ph. D. Thesis. Faculty of Vet. Med., Assuit University. Egypt.

Wijendra, W.A.S.; Kulathunga, K.A.K.C. and Ramesh, R. (2014): First report of *L. monocytogenes* serotypes detected from milk and milk products in Sri Lanka. Adv.Anim.Vet.Sci.,2(55):11-16.

Zamani-Zadeh, M.; Sheikh-Zeinoddin, M. and Soleimanian-Zad, S. (2011): Prevalence and characterization of *Listeria species* in domestic and industrial cheeses of Isfahan Region. Iranian J. Publ. Health., 40(3): 98-104.

Zhou, X. and Jiao, X. (2005): Polymerase Chain Reaction detection of *L. monocytogenes* using oligonucleotide primers targeting actA gene. Food Control., 16(2): 125-130.

مدى تواجد انواع الليستيريا فى بعض منتجات الالبان فى محافظة بنى سويف

سعديه حلمى حسين الشناوى ، عرفه مشرف سليمان مشرف ، محمد معروف على زينهم ، داليا عبد الحافظ احمد حافظ

Email: dalia_hafez@yahoo.com Assiut University web-site: www.aun.edu.eg

اجريت هذه الدراسة لتحديد مدى انتشار ميكروب الليستيريا مونوسيتوجينس والانواع الاخرى لليستيريا فى بعض المنتجات اللبنية. تم تجميع ٢٤٠ عينة من القشدة الطازجة ، الآيس كريم، جبنة اللبن الخض، الجبنة القريش، والجبنة الثلاجة والزبادى (٤٠ عينة من كل منتج) عشوائيا من مختلف السوبرماركت ومنافذ البيع بمحافظة بنى سويف . وجد ان ٢٤(١٠%) من هذه العينات كانت تحتوى على ميكروب الليستيريا. وكانت نسبة تواجد ميكروب الليستيريا فى القشدة الطازجة ، الآيس كريم ، جبنة اللبن الخض والجبنة القريش هى ٦ (١٥%) ، ٨ (٢٠%) ، ٥ (١٢.٥%) و ٥ (١٢.٥%) بالترتيب ولم يتم عزل الميكروب من عينات الجبنة الثلاجة او الزبادى.وقد كان ميكروب الليستيريا جراى هو الاكثر تواجد بنسبة ٤٥.٨٣% يليه ميكروب الليستيريا مونوسيتوجينس بنسبة ٣٣.٣٣% ثم الليستريا ولشمرى بنسبة ١٢.٥% ثم ميكروب الليستيريا انكوا بنسبة ٨.٣٣% .وقد اظهر اختبار تفاعل البلمرة التسلسلى عن وجود احد الجينات المسئولة عن ضراوة ميكروب الليستيريا مونوسيتوجينس وهو الجين (hlyA) لجميع عترات الليستيريا مونوسيتوجينس وما ينتج عنه خطورة التعرض لداء الليستريات ولذلك فأن هناك ضرورة لتطبيق جميع الشروط الصحية اثناء تصنيع ، تخزين وتسويق هذه المنتجات.