# LISTERIA MONOCYTOGENES IN EGYPTIAN MILK AND DAIRY PRODUCTS

**A Thesis**

**Presented to the Graduate School**

**Faculty of Veterinary Medicine, Alexandria University**

**In Partial fulfillment of the**

**Requirement for the Degree**

**Of**

**Master of Veterinary Medical Sciences**

In

**Milk Hygiene**

By

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**January - 2011**

**بسم الله الرحمن الرحيم**

**{يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ}**

**صدق الله العظيم**

**سورة المجادلة ( أية : 11)**

# Listeria Monocytogenes in Egyptian Milk and Dairy Products

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For the Degree of

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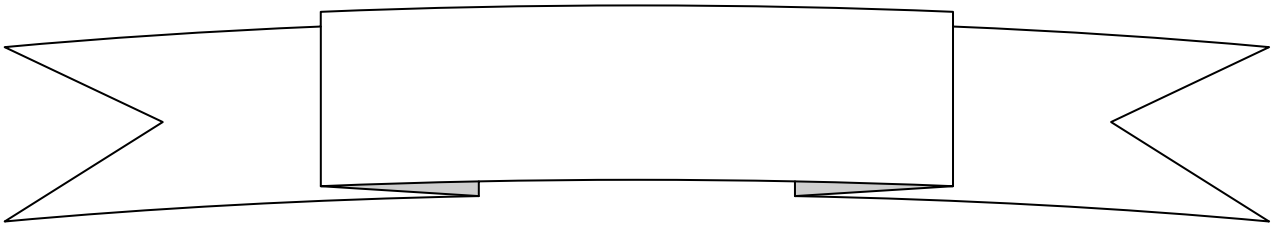
Dedicated to

*My Mother, My Father,*

*My Brother & My Sisters*

# ACKNOWLEDGEMENT



*First of all I am greatly indebted in my work and success to our merciful "**Allah**", who gave me the ability to finish this work.*

*Great appreciation, profound gratitude and deepest thanks are offered to **Prof. Dr. Ahlam Amin El-Leboudy,** Professor of Milk Hygiene, Head of food Department Faculty of Veterinary Medicine, Alexandria University for her kind supervision, valuable advice and continuous guidance during the course of the study.*

*Grateful thanks and deep sincere appreciation are also extended to **Dr. Amr Abdel-Moemen Amer**, Assistant professor of milk Hygiene, Faculty of Veterinary Medicine, Alexandria University for his supervision, cooperation and encouragement during the course of the study.*

*My appreciation goes to members of Food Hygiene Department, Faculty of Veterinary Medicine, Alexandria University for their help and the facilities they provided during the study.*

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# INTRODUCTION

Food-borne outbreaks due to consumption of dairy products constitute a chronic problem facing food hygienists. Milk and dairy products are subjected to different sources of contamination by the food poisoning pathogens either from endogenous origin or directly and indirectly from exogenous origin. The origin of contamination by food poisoning organisms varies with the type of product and the mode of production and processing. Treatment and processing of milk inhibits or encourages the multiplication of such organisms. All the nutritional components that make milk and milk products as an important part of the human diet also support the growth of these pathogenic organisms.

Although Listeria is widespread in the environment, it is only in recent years the publics have become aware of this organism. It has been involved in cases of food poisoning from the ingestion of contaminated milk, vegetables, meats, soft cheeses, ice cream and seafood, **(Adrian, 1992)**, Incidance of *Listeria monocytogenes* in milk was reported by **(El-Leboudy and Fayed, 1992).** *Listeria monocytogenes* is the most significant member that incriminated in many cases of food poisoning as the organism can grow at refrigerator temperatures, **(Forsythe and Hayes, 1998)**.

Prior to the 1980s, listeriosis, the disease caused by *Listeria monocytogenes,* was primarily of veterinary concern, where it was associated with abortion and encephalitis in sheep and cattle (Circling disease), **(Saudi, 2002).**

*Listeria monocytogenes* is considered emerging because the role of food in its transmission has only recently been recognized. In pregnant women, infections with *L. monocytogenes* can cause abortion and stillbirth, and in infants and persons with a weakened immune system it may lead to septicemia (blood poisoning) and meningitis. The disease is most often associated with consumption of foods such as milk and dairy products that are kept refrigerated for a long time because it can grow at low temperatures**. (Bell and Kyriakides, 1998)** Outbreaks of listeria have been reported from many countries, including Australia, Switzerland and the United States. and reported a twenty year overview of acute bacterial meningitis among adults in Iceland **(Siguardattir et al, 1997).**

This new food borne disease threats emerging for a number of reasons include international travel and trade, microbial adaptation and changes in the food production system, as well as human demographics and behavior.

The importance of microbiology to the dairy industry has been demonstrated by recent outbreaks of food-borne illness associated with consumption of milk and dairy products that had been contaminated with pathogenic organisms or toxins. Undesirable microorganisms constitute the primary hazard to safety, quality, and wholesomeness of milk and dairy foods. Consequently, increased emphasis has been placed on the microbiological analysis of milk and dairy products designed to evaluate quality and to ensure safety and regulatory compliance **(Vasavada, 1993).**

Owing to the continuous demand for the milk and dairy products, it is extremely necessary not only to increase the production of milk and its products but also to safeguard consumers against health hazard.

As the contamination of raw milk, kariesh cheese and ice cream with Listeria species constitutes a great problem for food producers, consumers and concerned authorities. Therefore, the present study was planned out to cover the following topics:

**1-Compositional quality tests:**

**1.1 Quality test, (Sanitary tests):**

1.1.1 Acidity precent of raw milk and kareish cheese.

1.1.2 pH value of raw milk and kareish cheese.

1.1.3 Methylene Blue Reduction Test (MBRT) for milk and ice cream.

**1.2 Chemical examination:**

Sodium chloride percent for kareish cheese.

**2- Isolation of Listeria species.**

**3- Isolation and Identification of *Listeria monocytogenes*.**

**4- Serotyping of isolated *Listeria monocytogenes.***

**5- Polymerase chain reaction for detection (Listeriolysin O gene) of *L.monocytogenes.(PCR).***

# 2 .0 REVIEW OF LITERATURE

## 2.1. Compositional quality tests

### 2.1.1. Raw milk:

**Moustafa (1978)** examined chemically 115 milk samples collected from Assiut province and reported that the titratable acidity of dairy shops samples was 0.17 ±0.047 .

**EL-Kholy (1981)** found that the titratable acidity of raw milk samples ranged from 0.11–0.17 with a mean value of 0.14. The highest frequency distribution was 52.2% lied between 0.14 - 0.16.

**Nelson and Trout (1981)** stipulated a standard for the quality of fluid milk used for manufacturing of various dairy products as follows:

| Test | Grade I | Grade II | Grade III | No grade |
|---|---|---|---|---|
| MBRT | > 5.5 hr | 2.5–5 h | 20 min–2.5 h | < 20 min. |

**Mansour (1982)** examined 100 samples of raw milk and found that the mean titratable acidity was 0.17%.

**El–Sagheer (1983)** recorded that the titratable acidity of examined 100 milk samples ranged from 0.17 to 0.19% with a mean value of 0.18%.

**Salam et al. (1983)** reported that the average acidity percent of the examined bulk milk samples was 0.17.

**Moustafa (1988)** examined forty raw milk samples collected from different localities in Mansoura, Egypt and found that the mean titratable acidity was 0.18%.

**El–Leboudy et al. (1992)** examined one hundred samples of raw milk and found that the pH value ranged from 6.3–6.8 with a mean value of 6.54 ± 0.018 for the cow's milk samples and from 6.3–6.9 with a mean value of 6.57 ± 0.28 for buffalo's milk samples.

**Morhan and Fahmy (1992)** examined 356 milk samples from individual cows and buffaloes in Assiut Province (Egypt) and reported that the mean value of titratable acidity was 0.174%.

**Connolly and Brieu (1994)** reported that Methylene blue reduction test is a rapid and simple method for quality grading of milk. The test is based on reduction of a dye to a colorless compound by reducing system setup in the milk. The reduction is due largely to bacterial activity caused by faulty method of production and handling of the milk or to mastitic milk and the greater the number of living organisms in milk, the rapid reduction occurs.

**El–Sayed (1997)** examined 60 raw milk samples collected from Sharkia Governorate and found that the mean value of acidity percent was 0.18 ± 0.05 .

**Grage and Mandokhot (1997)** tested 86 raw milk samples (67 from local vendors, 6 from vendors at organized dairy units and 13 from local milk plant), all had titratable acidity within the legal limit of 0.17%.

**Gosavi et al. (1998)** examined 50 samples of raw milk collected from vendors, bottling plants, organized and unorganized dairies and found that the highest methylene blue reduction time was determined in organized dairy milk while the lowest one was in bottling plant samples.

**Mosleh (2004)** examined 100 random samples of raw milk collected from dairy farms in Alexandria Governorate and found that the mean value of acidity percent was 0.13 ± 0.004 and 76.7% of examined samples had Grade II by using MBRT.

**Khair–Allah (2006)** examined 50 random samples of raw milk collected from street–vendors at El–Berhera Governorate and found that the mean acidity percent and pH values were 0.17 ± 0.004 and 6.33 ± 0.04, respectively. The author reported that 36, 44 and 20% of examined samples had Grade I, II and III by using MBRT, respectively.

## 2.1.2. Kareish cheese

**El–Leboudy (1985)** examined chemically 30 Kareish cheese samples and found that the average acidity percentage was 0.334 ± 0.037% while Sodium chloride content was 4.51 ± 0.26%.

**Abd El–Hakiem (1986)** examined 40 Kareish cheese samples in Assiut city and found that the titratable acidity was 0.948 ± 0.033%, while the mean Sodium chloride% was 5.17 ± 0.23.

**El–Kholy (1986)** examined 40 Kareish cheese samples collected from El–Behera Governorate and reported that the mean value of Sodium chloride in the examined samples was 3.95 % .

**Abd El-Tawab et al.(1988)** examined Kareish cheese for general composition and found that the acidity and salt % were 0.6 and 4.90, respectively .

**Moustafa (1988)** examined 40 Kareish cheese samples at El–Mansoura City and mentioned that the mean salt content was 9.08%, while the mean titratable acidity in the examined samples was 0.63%.

**El–Leboudy (1989)** examined 65 samples of Kareish cheese and found that the mean acidity% was 0.88 ± 0.007, while the mean Sodium chloride content was 4.79 ± 0.34%.

**Saleh (1989)** examined 35 samples of Kareish cheese and found that the mean titratable acidity value was 2.0 (0.45 to 4.05), while the mean Sodium chloride % was 8.00 (5.8 to 11.3 %).

**Zaki (1990)** reported that the mean Sodium chloride content of Kareish cheese was 2.85%, while the pH value was 4.40.

**Nazem (1991)** examined 25 Kareish cheese samples collected from Alexandria province. He found that the mean titratable acidity was 1.94± 0.069%, while the mean Sodium chloride content was 4.160 ± 0.266.

**Mahmoud (1993)** examined 40 Kareish cheese samples and reported that the mean value of titratable acidity was 1.79 ±0.11 % While that of sodium chloride was 8.72 ± 0.12 %.

**Abd El-Shaheed (1996)** reported that the mean acidity percent of farmer made Kareish cheese was 2.079, while the average salt percent was 3.873.

**Awad et al. (1998)** examined 50 samples of packed and unpacked kareish cheese and found that the mean values of pH, acidity, moisture content and NaCl were 4.4, 1.9, 69.2 and 1.6 for packed samples and 4.6, 1.6, 64.5, and 4.8 for unpacked samples, respectively.

**Egyptian Standards (2000)** stated that in Kareish cheese the Sodium chloride content should be not more than 7 %.

**Khair Allah (2000)** investigated 50 Kareish cheese samples collected from Alexandria Governorate for acidity and salt content. He found that the mean values of acidity percent and salt content were 1.85 ± 0.04 and 2.93± 0.08 , respectively.

**Nawar (2001)** examined 40 samples of Kareish cheese collected from street–vendors at Alexandria Governorate and found that the mean values of acidity percent, salt content and pH were 1.17 ± 0.062, 4.02 ± 0.22 and 4.07 ± 0.035, respectively.

**Aiad (2002)** examined 50 samples of Kareish cheese collected from Alexandria Governorate and found that the mean value was 2.05 ± 0.05% for acidity percent, 4.66 ± 0.094 for pH value, 2.56 ± 0.070% for salt content, 61.64 ± 0.77% for moisture percent and 8.99 ± 1.42 for Fat/DM.

**Amer (2002)** examined 30 random samples of kareish cheese collected from Alexandria and El–Behera Governorates and found that the mean values of acidity percent, salt content, pH value and moisture were 1.14 ± 0.081; 4.38 ± 0.028; 4.09 ± 0.042 and 68.4 ± 0.6, respectively.

**El–Agizy and Amer (2005)** examined 20 samples of Kareish cheese collected from street–vendors in Alexandria and El–Behera Governorates. They found that the mean values of salt content, moisture, acidity content and pH values were 3.65 ± 0.06; 60.46 ± 0.78; 1.14 ± 0.081 and 4.09 ± 0.042, respectively.

**Ibrahim and Sobeih (2006)** tested 60 random samples of kareish cheese manufactured by traditional method, direct acidification and lactic acid bacteria starter culture (Twenty of each) collected from Kalyobia Governorate. They found that the mean pH values were 4.47 ± 0.05; 5.04 ± 0.05 and 4.78 ± 0.04, respectively.

## 2.1.3. Ice cream

**Barton (1981)** reported that the Methylene blue reduction test (MBRT) was still considered to be a very useful laboratory test for monitoring the hygienic condition of ice cream. 21, 44, 59 and 76% of ice cream samples graded as 1, 2, 3 and 4, respectively.