Public health significance of \textit{Listeria} as a foodborne pathogen

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2013
بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلَّاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

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Dedication

To

My father
( Asking Allah accept him in his paradise )

My mother
( May ALLAH accept this work as a knowledge that benefits the humanity and reward her for it ).

My sisters and brothers

My friends ( Zeinab and Mervat )

Everyone encouraged me
Acknowledgement

I am extremely grateful to **ALLAH** for helping, prosperity and kindness and under whose willing this work was carried out.

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I would like to dedicate this modest effort to the memory of **Prof. Dr. Wafaa W. M. Reda**, Professor of zoonoses Fac. Vet. Med., Cairo University. Asking **ALLAH** grant her mercy and forgiveness her.

I extend sincere thanks to **Dr. Mudar Abd elazim**, I hope to **ALLAH** makes what he do in the balance of his good deeds.

It is a great pleasure to record my kind gratitude to all members of zoonoses Dept., Fac. Vet. Med., Cairo University.

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Abstract

This work was carried out to study the occurrence of Listeria species in different food, animal and human samples. A total of 1386 samples were collected from Cairo and Giza governorates (553 different food samples, 504 fecal samples and 257 vaginal swabs from different animals and 72 human samples). The occurrence of Listeria spp. in the examined food samples was 12.10%, 0.62%, 2.94%, zero and 1.89%, and the occurrence of Listeria monocytogenes in the examined food samples was 2.55%, 0.62% and 0.94% in the examined milk samples from dairy shops, milk samples from farms and eggs, respectively. On the other hand failed to isolate L. monocytogenes from cheese and meat products. The occurrence of Listeria spp. in the examined fecal samples of sheep and goat in this study was 2.33% and 1.45%, respectively and failed to isolate Listeria from vaginal swabs of different animals. The occurrence of Listeria spp. in stool samples of neonates and children, immunosuppressed persons and farm workers was 4.35%, 5.56% and zero, respectively. The isolated Listeria spp. was L.monocytogenes (4.35%) from neonates and children and L.livanovii (5.56%) from immunosuppressed persons. By PCR only six samples were positive for L. monocytogenes. Four milk samples (2.55%) from dairy shops were positive, one milk sample (0.62%) sample from farms and one stool sample (4.35%) of neonates and children, but by CHROMagar seven samples were positive for L. monocytogenes those detected by PCR in addition to another one milk sample from dairy shops and by conventional method eight samples were positive for L. monocytogenes those detected by PCR beside one (2.50%) from hen’s egg shell and one (0.58%)from fecal sample of sheep. PCR primer targeting the hemolysin (hly A) gene was used for confirmation the isolation of L. monocytogenes. The sensitivity and reliability of PCR was comparable with CHROMagar and conventional methods. As knowledge of molecular and applied biology of L. monocytogenes increases, progress can be made in the prevention and control of human infection.

(Keywords: Listeria species-Food-Animal -Human- CHROMagar-PCR)
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Introduction

Among the genus *Listeria*, which causes the infection of listeriosis in both animal and man, *Listeria monocytogenes* is a major pathogenic microorganism (Aygun and Pehlivanlar, 2006). *Listeria monocytogenes*, a human foodborne pathogen responsible for listeriosis, is widely distributed in the environment and therefore can be found in unprocessed foods of animal origin such as raw milk, meat, poultry, fish and other seafoods, and in some processed and ready-to-eat foods such as cheese, ice-cream and meat products due to post processing contamination (Berrada et al., 2006).

*Listeria monocytogenes* was found in at least 37 species of mammals, both domestic and wild, as well as up to 10% of humans may be intestinal carriers. It had been detected in 17 species of birds, some species of fish and shellfish, and is especially pathogenic to high risk populations, such as newborn, pregnant women, elderly, and immunocompromised individuals (Mugampoza et al., 2011).

Although *Listeria monocytogenes* is the primary human pathogen, there have been several reports of illnesses caused by *L. seeligeri*, *L. ivanovii*, *L.innocua*, *L. welshimeri* and *L. gyayi* in human (Guillet et al., 2010).

In the last years listeriosis has become one of the most dangerous foodborne diseases with a high mortality rate: 20-30% (Dmowska and Osek, 2010) representing the third leading cause of death among major pathogens transmitted commonly by food. The United States has a zero tolerance policy regarding the presence of *L.monocytogenes* in food, while Canada allows only 100 cfu/g of food. (Churchill et al., 2006).

The ability of *L. monocytogenes* to persist in food-processing environments and multiply under refrigeration temperatures makes it a significant threat to public health (Jemmi and Stephan, 2006).
Listeria monocytogenes infection (listeriosis), recognized as a foodborne illness in 1980 (Schlech et al., 1993), leads to invasive disease during vulnerable stages of life (Lund and O'Brien, 2011). Elderly and persons with immunocompromising conditions are at higher risk for Listeria bacteremia and meningitis which can be fatal. Listeriosis usually is a mild illness in pregnant women, but it can cause severe outcomes for the fetus or newborn infant, including fetal loss, preterm labor, and neonatal sepsis, meningitis, and death. In people with no predisposing factors, invasive listeriosis is rare, and the most typical symptom is mild gastroenteritis with fever, headache, nausea, diarrhoea, and abdominal pain (Patricia et al., 2012). Cutaneous and eye infections have rarely been reported, mainly in farmers and veterinarians in direct contact with after-births and infected fetuses (Tay et al., 2008).

ISO 11290 describe methods for the isolation of L. monocytogenes from food samples. After a two stage enrichment procedure, using half Fraser and Fraser broth, isolation takes place on two media: PALCAM and Oxford agar. These media have the disadvantage that L. monocytogenes cannot be differentiated from non-pathogenic Listerias. Recently, media have been described which distinguish L. monocytogenes from other Listeria spp., based on haemolysis or on a chromogenic substrate (ISO, 1996). Using chromogenic agars, the presumptive identification of L. monocytogenes is possible after 24 h, compared with 3-4 days using Oxford and other conventional agars (Greenwood et al., 2005).

PCR primers targeting the hemolysin (hly) gene for confirming the isolation of L. monocytogenes (Lampel et al., 2000). DNA-based methods such as conventional PCR have been developed as safe, useful, sensitive, and accurate methods for the detection of L. monocytogenes in clinical specimens (O' Grady et al., 2010).
So, the present work was carried out in different localities of Cairo and Giza governorates to:

1. Determine the occurrence of *Listeria species* in different human groups under risk and animals.
2. Determine the occurrence of *Listeria species* in different food samples (milk, cheese, meat products and commercial eggs).
3. Throw light on the public health hazard of *Listeria monocytogenes* as a foodborne pathogen.
4. Find out the actual association between human listeriosis and milk contaminated with *Listeria monocytogenes*.
5. Compare between the conventional method and the molecular method based on PCR for the identification of *Listeria monocytogenes*.
6. Elucidate the ecology of *L.monocytogenes* serotypes and their relationship to humans.
1. History and taxonomy of *Listeria* species:

**Hülphers (1911)** isolated a gram-positive bacterium from the necrotic liver of a rabbit in Sweden. He named the bacterium *bacillus hepatis* because of its affinity to the liver, from where he had isolated it.

**Dumont and Cotoni (1921)** reported that in 1918 the bacterium, albeit wrongly identified, was isolated from a human patient suffering from meningitis.

**Murray et al. (1926)** isolated the same organism from rabbits and guinea pigs in Cambridge (England) and gave it the name *Bacterium monocytogenes* because the disease was characterized by an increase in the number of circulating monocytes.

**Pirie (1927)** stated that during investigations of unusual deaths observed in gerbils near Johannesburg, South Africa, he discovered a new microorganism, agent of what he called “The Tiger River disease” as it was discovered near the Tiger River in South Africa. He named this new agent *Listerella hepatolytica* because of the liver lesions it produced.

**Nyfeldt (1929)** first recognized *Listeria monocytogenes* as a human pathogen in Denmark, but the route of transmission was unclear until the 1980 when a series of outbreaks indicated that *L. monocytogenes* was transmitted by food.

**Gill (1931)** in New Zealand credited the first isolation of *L.monocytogenes* from an infected domestic sheep and described a type of ovine encephalitis 'circling disease'.
Becker (1939) pointed out that the generic name *Listerella* had already been used for a mycetozoon. Pirie (1940) suggested that new name *Listeria*, be used. This name, however, was not adapted at the time because it had already been given to a certain plant group. He changed the name to *Listeria monocytogenes* (*L.monocytogenes*).


2. Epidemiology of listeriosis:

2.1. General characters of *Listeria* species:

Seeliger and Jones (1986) mentioned that there are 15 *Listeria* somatic (O) antigen subtypes (I–XV) whereas flagellar (H) antigens comprise four subtypes (A–D).

Cole et al. (1990) found that optimal growth of *L. monocytogenes* occurs between 30° C and 37° C.

Galsworthy et al. (1990) recorded that at 20-25°C peritrichous flagella are formed and cause the *Listeria* to be motile, whereas at 37°C the organism is weakly or non- motile.

Schuchat et al. (1991) described an unusual example of *Listeria* transmission in a nosocomial outbreak involving neonates, whom became infected through contact with contaminated mineral oil that was being used to bathe the infants within a specific neonatal unit.
Hudson et al. (1994) reported that the growth of *L. monocytogenes* has been to occur in temperatures from -1.5°C to approximately 45°C, at pH of 4.3–9.6, and in aw down to 0.90.

Wiedmann et al. (1997) classified *L. monocytogenes* into three broad evolutionary groups, termed phylogenetic lineages I, II and III.

Bunning et al. (1998) stated that *L. monocytogenes* can survive under adverse conditions although the organism is not heat-resistant and can be inactivated by pasteurization at 72°C for 15 seconds.

Kathariou (2002) found that the serotypes of individual *Listeria monocytogenes* were determined by their unique combinations of O and H antigens. At least 13 serotypes (i.e. 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7) have been recognized in *L. monocytogenes*.

Lukinmaa et al. (2003) reported that all serovars have the propensity to cause disease; however a small number (serotypes 4b, 1/2a and 1/2b) have been implicated in the majority of human *L. monocytogenes* infections.

Doumith et al. (2004) published that serotype 1/2a (lineage II) is also implicated in a large number of human listeriosis cases, second only to serotype 4b, however these are generally sporadic rather than epidemic cases.

Gray et al. (2004) defined a correlation between lineage and isolate source; with lineage I strains significantly more common from human clinical sources (largely due to the presence of the epidemic serotype 4b in the lineage), lineage II strains significantly more common from food sources, and lineage III isolates rarely recovered from either food or humans.
Nightingale et al. (2005) reported that the lineage I cluster includes *L. monocytogenes* serotypes 1/2b, 3b, 4d, 4e and most strains of serotype 4b. Serotypes 1/2a, 1/2c, 3a and 3C cluster within lineage II, while the lineage III cluster includes serotypes 4a, 4c and some serotype 4b strains.

Dieterich et al. (2006) mentioned that most bacteria grow poorly when temperature below 4°C. while *Listeria* survives in temperature from below freezing (-7 °C) to body temperature, a temperature range including that used for refrigeration. As a result, *Listeria* may be transmitted in ready- to- eat foods that have been kept properly refrigerated. It’s ability to grow in such diverse environments is just one of the many challenges presented by this dangerous bacterium.

Jemmi and Stephan (2006) mentioned that the ability to persist in food-processing environments and multiply under refrigeration temperatures makes *L. monocytogenes* a significant threat to public health.

Liu (2008) classified *L. monocytogenes* into different serovars based on the listerial somatic (O-factor) and flagellar (H-factor) antigen serological reactions. *L. monocytogenes* serovars most commonly associated with clinical cases are 4b, 1/2a, 1/2b and 1/2c.

Pal et al. (2008) exhibited that a considerable significance for food safety, as chilling can not be relied upon to prevent the growth of *L.monocytogenes* to dangerous levels.

Chan and Wiedmann (2009) declared that *L.monocytogenes* can survive temperatures well below freezing and can proliferate at temperatures from -0.4°C to 45°C, making it a psychrotroph and a mesophile.
Freitag et al. (2009) stated that although *L. monocytogenes* is non-spore forming, it is extremely hardy, surviving adverse conditions and even growing in environments specifically designed to inhibit bacterial growth, for example, in the presence of heavy metal ions, high salt concentrations, and in extremes of pH.

Lungu et al. (2009) mentioned that *L. monocytogenes* is a facultative anaerobe capable of growth in anaerobic, microaerobic and aerobic environments.

McLaughlin and Rees (2009) mentioned that *L. monocytogenes* belongs to the family Listeriaceae in the class Bacilli of the phylum Firmicutes, together with *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welchimeri*, *L. grayi*.

Sukhadeo and Trinad (2009) mentioned that *L. monocytogenes* is catalase and L-Rhamnose positive, oxidase negative and motile at 10 to 25°C.

Al-Zeyara et al. (2011) reported that despite the tolerance of *Listeria* to wide ranged temperature, pH, and aw, it is relatively fastidious in nutrient requirements and competing microbes that inhibit its growth.

Abram et al. (2012) mentioned that *L. monocytogenes* can multiply at refrigeration temperatures, thereby challenging an important defense against food-borne pathogens, refrigeration.

Pontello (2012) published that in Europe, as elsewhere in the world, about 95% of *L. monocytogenes* strains isolated from clinical and food samples belongs to serovars 1/2a, 1/2b, 1/2c and 4b.

Lang Halter et al. (2013) stated that all *Listeria* species are small, regular rods, 0.5 μm in diameter and 1–5 μm in length that do not form spores or capsula. They produce catalase but not oxidase. Except for *L. fleischmannii*, *Listeria* spp. are motile with peritrichous flagella under 30°C.
2.2. Nature of listeriosis:

Charpentier et al. (1995) found that *L. monocytogenes* is problematic due to its resistance to multiple antibiotics, which makes it difficult to treat.

Arimi et al. (1997) emphasized the relation between ingestion of *Listeriae*-contaminated silage, mastitis in dairy cattle and subsequent asymptomatic shedding of *Listeriae* in milk destined for human consumption.

Dalton et al. (1997) reported that as *L.monocytogenes* is killed by heat treating, the main sources of *L.monocytogenes* transmission include unpasteurised dairy products, smoked fish and post processing contamination of food stocks.

Fenlon (1999) evidenced that in all farm systems, the prevalence of *L. monocytogenes* in soil was higher than that seen in feed, indicating that soil may serve as a source of animal feed contamination by *L. monocytogenes*.

Pritchard and Donnelly (1999) reported that cattle farms play an important role in the spread of *Listeria* between animals or people rather than small ruminant farms.

Cossart and Bierne (2001) published that about one-third of *Listeria* cases are pregnancy-associated.

Vazquez-Boland et al. (2001) found that ruminant farm animals play a key role in the persistence of *Listeria* spp. in the rural environment via a continuous fecal-oral cycle.

Rodriguez – Lazaro et al. (2004) found that individuals who are particularly susceptible to listeriosis are immune-compromised individuals as in HIV/AIDS infection, pregnant women, persons with low stomach acidity,
newborn babies, cancer patients, alcoholics, drug abusers, patients with corticosteroid therapy and the elderly.

MacDonald et al. (2005) found that the most common route of infection in human listeriosis is ingestion of contaminated food, although vertical and nosocomial transmission have also been described.

Murphy et al. (2005) assumed that L. monocytogenes may cross-contaminate ready-to-eat (RTE) meat and poultry products during post-processing steps such as slicing, peeling and packaging.

Jemmi and Stephan (2006) mentioned that although L. monocytogenes infection is usually limited to immunocompromised individuals, the high mortality rate associated with human listeriosis makes L. monocytogenes the leading cause of death among bacterial pathogens acquired primarily through the consumption of contaminated foods.

Leite et al. (2006) mentioned that Listeria spp. are widely distributed in the rural environment and may in this manner contaminate milk and production plants.

Lyautey et al. (2007) reported that L.monocytogenes is ubiquitous in the environment and has been isolated from varied sources such as soil, silage, sewage, water and faeces of healthy animals.

Sauders and Wiedmann (2007) mentioned that L.monocytogenes is widely spread in the environment, being found in plants, soil, wastewater, stagnant water supplies, grazing areas, animal feed, and the intestines of healthy animals and humans. Although L. monocytogenes can be found anywhere, contaminated untreated silage/feed is the most probable cause of Listeria infections in farm animals. They mentioned that contaminated, untreated manure can be a source of
human listeriosis. For example, the coleslaw outbreak in Canada in 1981 was traced back to the sheep manure used for cabbages in the field.

**Posfay-Barbe and Wald (2009)** found that vertical transmission from mother to neonate can occur transplacentally or the infant can become infected during delivery through contact with organisms in the birth canal.

**Sukhadeo and Trinad (2009)** reported that the natural habitat of *L. monocytogenes* is thought to be decomposing plant matter, in which they live as saprophytes.

**Berzins et al. (2010)** mentioned that *L. monocytogenes* had been found in decaying vegetation, cultivated and non-cultivated fields, forests, aquatic environments, food, feed, animal and human faeces, and food-processing environments.

**Dmowska and Osek (2010)** mentioned that *L. monocytogenes* was widely distributed in food, especially in meat, smoked fish, raw milk, cheese, eggs and raw vegetables.

**Oevermann et al. (2010)** mentioned that ruminants were reservoirs of *L. monocytogenes* strains pathogenic for humans.

**Glinski and Kostro (2012)** stated that many domestic and wild mammals, birds, fish and crustaceans can be carriers of the bacteria. They found that not only contaminated food products of animal origin but also vegetables and fruits can be the source of *Listeria* organisms.

**Silk et al. (2012)** assumed that the main predisposing factor in listeriosis is a decrease in cell-mediated immunity because of an underlying disease or pregnancy, and the risk of listeriosis is increased also in neonates and the elderly.
In susceptible people and animals *L. monocytogenes* can cause a life threatening, invasive disease.

### 2.3. Incidence of listeriosis:

**Gellin et al.** (1991) described that listeriosis is more common in children at a rate of 10 cases per 100,000 person, and the elderly with 1.4 cases per 100,000 persons.

**Mead et al.** (1999) mentioned that although the actual number of *L. monocytogenes* infections is low, a mortality rate, which can be as high as 20-30% regardless of antimicrobial treatment, shows the danger of the presence of *L. monocytogenes* in foods. The mortality rate is considerably higher than the more common infections from other food-borne pathogens such as *Escherichia coli O157:H7* (*E. coli*), *Campylobacter* species and *Salmonella* species.

**Centers for Disease Control and Prevention (CDC)** (2002) recorded a multi-state outbreak of *L. monocytogenes* infections linked to eating turkey deli meat in the US in 2002 caused 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages. A total of 12.4 million kg of fresh and frozen ready-to-eat turkey and chicken products were recalled as a result of this outbreak.

**Ireton** (2006) mentioned that the incidence of listeriosis is relatively low and represents less than 0.1% of all food-borne illnesses but causes infections with very high mortalities (20 to 30% deaths).

**Kaur et al.** (2007) showed that prevalence of listeriosis in general population is 0.7 in 100000 but this prevalence is 12 in 100000 in pregnant women (that is a 17-fold increase).
Sauders and Wiedmann (2007) concluded that sporadic cases of listeriosis appeared to be more common than outbreak cases.

Drevets and Bronze (2008) recorded that worldwide incidence of listeriosis is being low, with 0.1–1.1 cases per 105 individuals.

Goulet et al. (2008) recorded that from 1999 through 2005, the incidence of listeriosis in France declined from 4.5 to 3.5 cases/million persons but in 2006, it increased to 4.7 cases/million persons.

Jalali and Abedi (2008) stated that the approximate mortality rate of listeriosis is 30% that may increase up to 75% in high risk groups, such as pregnant women, neonates and immune-compromised adults.

Chen et al. (2009) published that in China, the prevalence of *L. monocytogenes* in retail food was raised to 25.3% during 2000-2007 and *L. monocytogenes* was recognized as a serious risk to food safety.

Schuppler and Loessner (2010) demonstrated that although listeriosis is relatively rare and annual incidences are decreasing, it is still one of the most deadly foodborne pathogens, with around one third of all clinical manifestations resulting in mortality.

Centers for Disease Control and Prevention (2011) recorded that 1600 cases of listeriosis occur annually, of them 260 cases are fatal.

Centers for Disease Control and Prevention (2013a) reported that 1,651 invasive listeriosis cases to the *Listeria* Initiative from 2009 through 2011; 292 deaths or fetal losses were reported (CFR: 21%). Most cases (58%) were in adults aged ≥65 years, and 14% were pregnancy-associated and the average annual incidence was estimated to be 0.29 cases per 100,000 populations in FoodNet. In
adults aged ≥65 years, the incidence was 1.3 cases per 100,000 populations. The highest rates were among pregnant women (3.0 per 100,000), especially Hispanics (7.0 per 100,000). Compared with the population as a whole, rates were four times higher for adults aged ≥65 years, 10 times higher for pregnant women, and 24 times higher for pregnant Hispanic women.

2.4. Pathogenesis of Listeria monocytogenes:

Chakraborty et al. (1992) mentioned that L. monocytogenes contains a virulence gene cluster which is regulated by the main positive regulatory factor A regulon (PrfA). The PrfA gene is essential for the virulence of L. monocytogenes. It acts as the master regulator of virulence and virulence-like genes to varying degrees.

Coffey et al. (1996) mentioned that in L. monocytogenes, lecithinase is necessary for the breakdown of the two plasma membranes that surround the bacterium after cell-to-cell spread in the human host during infection.

Kuhn and Goebel (1999) demonstrated that virulence gene cluster contains the majority of the known virulence genes which are involved in the invasion and intracellular cycle of the pathogen. The cluster encodes six genes, prfA, plcA, hly, mpl, actA, plcB, and three additional small open reading frames (orfs) X, Y, and Z.

Chakraborty et al. (2000) mentioned that the virulence genes on the virulence gene cluster result in the protein products of listeriolysin O (LLO) by hly, a phosphatidylinositol-specific phospholipase C (PIPLC) by plcA, a phosphatidylcholine-specific phospholipase C (PC-PLC) by plcB, a metalloprotease (mpl), an actin polymerization protein (ActA) by actA, and three genes, X, Y, and Z which functions are currently unknown.
Gregory and Liu (2000) found that disturbed cell-mediated immunity may enable the passage of the bacterium from the liver to the central nervous system and placenta, leading to the appearance of symptoms of invasive listeriosis.

Vazquez-Boland et al. (2001) mentioned that in the stomach, the *Listeriae* are exposed to low gastric pH, which reduces the number of viable cells. They found that the surviving cells pass into the intestine, passively cross the intestinal wall, proliferate mainly in Payer’s patches, and spread to neighbouring enterocytes basolaterally. They thought that the massive invasion of *L. monocytogenes* to epithelial cells causes the symptoms of gastroenteritis. They found that following passage through the intestinal barrier, the bacterium enters the liver and, less extensively, the mesenteric lymph nodes and spleen through the lymph and blood.

Allerberger (2003) emphasized that *L. monocytogenes* is the only species that is pathogenic for humans, causing listeriosis, while *L. ivanovii* constitutes an animal pathogen.

Robbins and Theriot (2003) showed that *L. monocytogenes* moves rapidly through the cytoplasm with the help of the formed actin tails with a speed of up to 1.5 μm/sec. Quite surprisingly, *L. monocytogenes* rotates around its long axis as it is propelled by actin polymerization.

Lecuit et al. (2004) showed that *L. monocytogenes* pathophysiology in humans is linked to their ability to cross three important barriers in their host. These are the intestinal barrier, blood brain barrier and foetal-placental barrier.

Pamer (2004) assumed that the ability of *L. monocytogenes* to replicate in the cytosol of infected host cells and to spread from cell to cell enables it to avoid humoral immune responses.
Hamon et al. (2006) demonstrated that *L. monocytogenes* infection and pathogenesis include the following stages: internalization, escape from vacuoles, intracellular growth, and cell-to-cell spread.

Liu (2006) explained that variations in virulence and pathogenicity of *L. monocytogenes* strains occur and can be evaluated by investigating the presence of virulence-associated genes or by serological typing.

Pizarro-Cerda and Cossart (2006) explained that InlA binds E-cadherin, a host cell adhesion molecule, whereas InlB binds to the hepatocyte growth factor receptor, Met; binding to these receptors enables *L. monocytogenes* to gain entry into host cells through the use of the host endocytic machinery. Within the host cell cytosol, the bacteria replicate using nutrients that are acquired from the host. *L. monocytogenes* then moves through the cell and into adjacent cells using actin polymerization as a motility force, which it directs through it’s surface protein actin assembly-inducing protein (ActA).

Seveau et al. (2007) showed that a number of bacterial surface proteins, including the internalins InlA and InlB contribute to bacterial invasion of host cells. Once internalized, *L. monocytogenes* mediates its escape from the membrane-bound vacuole by secreting a pore-forming cytolysin, known as listeriolysin O (LLO), and two phospholipases, which work together to break down the phagosome in which it resides. The bacteria enter adjacent cells and secrete LLO and the broad-specificity phosphatidylcholine phospholipase C (PC-PLC) to escape from the double-membraned secondary vacuoles that are formed as a result of cell-to-cell spread.

Bonazzi et al. (2009) found that adhesion of internalin InlA to E-cadherin has recently been recognized as the first step that enables *L. monocytogenes* to cross the intestinal barrier.
Toledo-Arana et al. (2009) stated that the bacteria reach the brain through haematogenous dissemination and cause the meningo-encephalo pathology associated with acute listeriosis.

Bahey-El-Din et al. (2010) mentioned that the pore-forming haemolysin listeriolysin O (LLO), the main virulence factor of *L. monocytogenes*, allows bacteria to escape from the harsh environment of the phagosome to the cytoplasm of the infected cell.

Melton-Witt et al. (2012) showed that *L. monocytogenes* colonized the liver in all asymptomatic animals. Spread to the liver occurred as early as 4 h after ingestion via a direct pathway from the intestine to the liver. This direct pathway contributed significantly to the bacterial load in the liver and was followed by a second wave of dissemination via the mesenteric lymph nodes (indirect pathway). They pointed out that the mesenteric lymph node represents a considerable barrier which *L. monocytogenes* have to reach.

2.5. Listeriosis outbreaks:

Riedo et al. (1994) in 1989, isolated identical strains of *L. monocytogenes* from blood samples from two febrile pregnant women and from the stool samples of a person with diarrhea; they proposed the possibility that *L. monocytogenes* might cause gastroenteritis. The 3 people had attended the same party. A total of 47% of attendees of that party (17 of 36) reported at least 1 gastrointestinal symptom. With the exception of the stool sample from the 1 person who reported diarrhea and had a positive result of culture 42 days after the party, all stool samples (which were collected 6 weeks after the party) had negative results of culture for *L. monocytogenes*, as did food samples and environmental specimens.

Dalton et al. (1997) reported an outbreak of febrile gastroenteritis that was associated with the consumption of contaminated chocolate milk. Symptoms developed in 75% of persons (45 of 60) who drank chocolate milk that had been
served at a picnic. Indistinguishable strains of *L. monocytogenes* were isolated from unopened cartons of chocolate milk, from environmental specimens from the dairy that supplied the milk, and from the stool samples of 14 symptomatic persons.

**Miettinen et al. (1999)** mentioned that febrile gastroenteritis in five healthy persons was associated with the consumption of vacuum-packed cold-smoked rainbow trout containing *Listeria monocytogenes*. *L. monocytogenes* isolates from the incriminated fish product lot and the stool samples were all of serotype 1/2a.

**Aureli et al. (2000)** mentioned that the largest documented listeriosis outbreak occurred in 1997, when 1566 students and staff members from 2 primary schools in northern Italy developed febrile gastrointestinal illness after eating cafeteria food that had been prepared by the same caterer. A total of 292 persons were hospitalized. Cultures of one blood sample and 123 stool samples from hospitalized patients yielded *L. monocytogenes* strains that were identical to strains isolated from food and environmental specimens at the catering plant.

**Centers for Disease Control and Prevention (2000)** reported that since May 2000, 29 illnesses caused by a strain of *Listeria monocytogenes* have been identified in 10 states: New York (15 cases); Georgia (three); Connecticut, Ohio, and Michigan (two each); and California, Pennsylvania, Tennessee, Utah, and Wisconsin (one each). Dates of *Listeria monocytogenes* isolation ranged from May 17 through November 26 with 26 (90%) infections occurring since July 15. This report summarized the investigation, which linked these cases of listeriosis to eating deli turkey meat. Eight perinatal and 21 non-perinatal cases were reported. Among the 21 non-perinatal case-patients, the median age was 65 years (range: 29-92 years); 13 (62%) were female. The 29 cases had been associated with four deaths and three miscarriages/stillbirths.
Centers for Disease Control and Prevention (2003) reported that *L. monocytogenes* has been implicated in multiple large outbreaks worldwide. Each year in the United States, the incidence of listeriosis is estimated to range from 3.4 per million to 4.4 cases per million.

MacDonald et al. (2005) mentioned that in 2000, an outbreak of listeriosis among Hispanic persons was identified in Winston-Salem, North Carolina. They identified 13 patients, all of whom were Hispanic, including 12 females who were 18-38 years of age. Eleven case patients were pregnant; infection with *L. monocytogenes* resulted in 5 stillbirths, 3 premature deliveries, and 3 infected newborns. Case patients were more likely than control subjects to have eaten fresh, unlabeled, Mexican-style cheese sold by door-to-door vendors.


Swaminathan and Gerner-Smidt (2007) suggested that the most commonly incriminated vehicles for listeriosis have been ready-to-eat (RTE) meat and dairy products.

Centers for Disease Control and Prevention (2011) declared that *Listeria* contaminated cantaloupe caused the deadliest U.S. foodborne disease outbreak in nearly 90 years.

Centers for Disease Control and Prevention (2012a) mentioned that a total of 22 persons infected with the outbreak-associated strain of *L. monocytogenes* were reported from 13 states and the District of Columbia. Twenty ill persons were hospitalized, four deaths were reported. Public health officials determined that two of these deaths were related to listeriosis. One fetal loss was reported. Collaborative investigation efforts of local, state, and federal
public health and regulatory agencies indicated that Frescolina Marte brand ricotta salata cheese imported from Italy and distributed by Forever Cheese, Inc. was the likely source of this outbreak.

**Centers for Disease Control and Prevention (2012b)** reported that one of the largest listeriosis outbreaks, affecting 147 persons in 2011, was caused by contaminated cantaloupe from a single farm in USA. In total, 33 deaths from outbreak-associated cases of listeriosis have been reported to CDC. In addition, one woman pregnant at the time of illness had a miscarriage.

**Food and Drug Administration (FDA) (2012)** stated that in Los Angeles, 1985, a large-scale listeriosis outbreak occurred due to the consumption of contaminated Mexican-style soft cheese. Human listeriosis cases were 142, among them, 93 cases occurred in pregnant women or their offspring, and the remaining cases occurred in non-pregnant adults. The outbreak led to 48 deaths, including 20 fetuses, 10 neonates, and 18 non-pregnant adults. An investigation of the cheese plant suggested that the cheese was commonly contaminated by unpasteurized milk. The outbreak strain was serotype 4b. In United States, 1989, 2000: A serotype 1/2a strain was isolated from a single case of human listeriosis in 1989, which was caused by the consumption of processed meat. Eleven years later, the same strain isolated from sliced turkey produced by the same processing plant which was implicated in a listeriosis outbreak. This provided a powerful illustration of *L. monocytogenes*’s tenacity and prolonged survival in food-processing environments. In United States from 1998 to 1999, a large scale multistate outbreak of listeriosis caused at least 50 cases in 11 states. Six adults died, and two pregnant women had spontaneous abortions. Contaminated hot dogs were linked to this outbreak. All *L. monocytogenes* isolates from these cases were serotype 4b. In United States 2002, a multistate outbreak of listeriosis in the Northeastern U.S. resulted in 46 cases, including 7 deaths and 3 stillbirths or miscarriages in eight states. The outbreak was linked to eating sliceable turkey deli meat. *L. monocytogenes* was isolated from one food product and 25
environmental samples from a poultry-processing plant. The isolate from the food product had a PFGE pattern different from the outbreak strain; however, two environmental isolates from floor drains had an identical PFGE pattern to that of outbreak patient isolates, suggesting that the plant might have been the source of the outbreak. The outbreak strain was serotype 4b. In Canada, 2008, a widespread outbreak of listeriosis occurred in Canada and was linked to deli meat produced by a Maple Leaf Foods plant in Toronto, Ontario. The outbreak caused 57 confirmed cases in seven provinces, and 22 people died. The outbreak strain was serotype 1/2a *L. monocytogenes* has caused significant outbreaks worldwide over the past decades.

Silk *et al.* (2012) reported that in Europe, Australia, and the USA, approximately 0.3 listeriosis cases per 100,000 population are reported annually and the worldwide incidence seems to be increasing.

CDC (2013a) reported that nationwide, 1,651 cases of listeriosis occurring during 2009–2011, the case-fatality rate was 21%. Most cases occurred among adults aged ≥65 years (950 [58%]), and 14% (227) were pregnancy-associated. At least 74% of non-pregnant patients aged <65 years had an immune-compromising condition, most commonly immunosuppressive therapy or malignancy. The average annual incidence was 0.29 cases per 100,000 populations. Compared with the overall population, incidence was markedly higher among adults aged ≥65 years and pregnant women. Twelve reported outbreaks affected 224 patients in 38 states. Five outbreaks investigations implicated soft cheeses made from pasteurized milk that were likely contaminated during cheese-making (four implicated Mexican-style cheese, and one implicated two other types of cheese). Two outbreaks were linked to raw products.

CDC (2013b) mentioned that a total of six persons infected with the outbreak strain of *L monocytogenes* were reported from five states linked to Crave Brothers Farmstead Cheeses. The number of ill persons identified in each state
included: Illinois (1), Indiana (1), Minnesota (2), Ohio (1), and Texas (1). All six ill persons were hospitalized, one death was reported in Minnesota. In addition, one illness in a pregnant woman resulted in a miscarriage. No new ill persons were reported since the last update on August 22, 2013.

3. Listeriosis:

3.1. Listeriosis in animals:

Killinger (1970) mentioned that infected and asymptomatic carrier sheep excrete the organism in feces and manure; these materials along with spoiled silage are used as fertilizer, which constitutes the most significant source of transmission of the organism to man and animals as well as contamination of food such as raw milk.

Gitter et al. (1980) established that cows suffering from listeric mastitis or those clinically normal may produce milk containing large numbers of such organisms.

Maaten (1989) mentioned that ruminant animals are particularly susceptible to Listeria infection and that sheep are most susceptible, followed by goats and cattle.

Kirkbride (1993) showed that the L. monocytogenes was a causative agent for 3.58% of the cases of bovine abortion.

Wesley (1999) published that while it has been hypothesized that, as ecological systems, livestock farms may function as a natural reservoir for L. monocytogenes and ultimately, as a primary source of food processing plant is of environment contamination.

Wesley et al. (2002) indicated that most listeriosis cases have been reported in sheep, among which L. ivanovii is also a significant cause of listeric infections, and also cows and goats, causing encephalitis, abortion, or septicemia.
Wiedmann and Evans (2002) stated that \textit{L. monocytogenes} can cause eye infections and keratitis in ruminants; these symptoms have been linked to direct inoculation of the eye with \textit{L. monocytogenes} present in feeds, especially silage.

Roberts and Wiedmann (2003) stated that latent carrier animals on a farm may shed \textit{Listeria} in their feces, thereby continually contaminating the farm environment and serve as a reservoir for \textit{Listeria}.

McLauchlin \textit{et al.} (2004) mentioned that \textit{L. ivanovii} and \textit{L. innocua} are known to cause listeriosis in domestic animals such as sheep, cattle, and goats.

Nightingale \textit{et al.} (2004) suggested that the epidemiology and transmission characteristics of \textit{L. monocytogenes} differ between small-ruminant and cattle farms. Cattle contribute to amplification and dispersal of \textit{L. monocytogenes} into the farm environment. The ruminant, and particularly the bovine, farm ecosystem maintains a high prevalence of \textit{L. monocytogenes}, including subtypes linked to human listeriosis cases and outbreaks, and may thus constitute a significant natural reservoir for \textit{L. monocytogenes}. \textit{L. monocytogenes} subtypes may differ in their abilities to infect animals and to survive in farm environments.

Center for Food Security and Public Health (CFSPH) (2005) explained that the central nervous system (CNS) involvement of listeriosis includes facial paralysis with profuse salivation, circling, in-coordination, and head pressing or turning of the head to one side.

Shakuntala \textit{et al.} (2006) reported that \textit{L. monocytogenes} is a well-recognized cause of mastitis, abortion, repeat breeding, infertility, encephalitis, and septicaemia in cattle.
Wesley (2007) showed that in livestock, listeriosis is associated with indoor housing and consumption of bad quality feed, especially silage.

Esteban et al. (2009) reported that the highest prevalence of listeriosis, up to 30%, has been reported in cattle followed by other ruminants, whereas companion animals seldom carry this disease.

Hellström et al. (2010) mentioned that symptomless faecal carriage of *L. monocytogenes* has been reported in primates, other mammals, and birds.

Glinski and Kostro (2012) explained that listeriosis affects the nervous system and is characterized by either meningo-encephalitis and a syndrome of circling, facial paralysis, somnolence, endophthalmitis, head pressing, and abortion or by septicemia in the newborn animals.

### 3.2 Listeriosis in human:

Gellin and Broome (1989) mentioned that early-onset listeriosis in a newborn often presents with sepsis and may progress to a syndrome known as granulomatosis infantisepticum. This syndrome is often characterized by widely disseminated granulomas, premature birth, respiratory distress, and circulatory failure.

Farber and Peterkin (1991) defined late-onset listeriosis in a newborn between 8 to 30 days of life. Usually late-onset neonates are born apparently healthy and at full-term. Meningitis rather than sepsis is more common in late-onset neonates.

Hof and Rocourt (1992) reported a case of human listeriosis caused by *Listeria seeligeri*. 
Silver (1998) declared that physiological reduction in cell-mediated immunity in pregnant women may result in listeriosis with influenza-like symptoms and miscarriages.

Mead et al. (1999) reported that, 99% of human listeriosis cases are foodborne. Although listeriosis is a very small fraction of all illness due to known foodborne pathogens, it is an important cause of severe illness, accounting for 3.8% of foodborne disease hospitalizations and 27.6% of foodborne disease deaths.

Aureli et al. (2000) mentioned that non-invasive listeriosis demonstrates milder symptoms, often referred as febrile gastroenteritis, and is caused by the ingestion of a high-dose *L. monocytogenes* by immunocompetent people.

Donnelly (2001) estimated that 5% of healthy humans harbor *L. monocytogenes* in their gastrointestinal tract.

Franciosa et al. (2001) denoted that human infection by *L. monocytogenes* can result in invasive or non-invasive disease.

Maijala et al. (2001) reported that prolonged consumption of food contaminated with as few as 1.4─2.2 x 10^3 colony forming units (cfu) of *L. monocytogenes* per day may be sufficient to infect susceptible people.

Vazquez-Boland et al. (2001) considered that the clinical symptoms of invasive listeriosis typically begin 20─30 days after the ingestion, even though incubation period can be up to 72 days. They recorded that listeriosis affecting the central nervous system accounts for around 55-75% of cases whereas septicaemia accounts for 15-20% with non-typical infections making up the remainder and about 20% of invasive listeriosis cases are fatal.
Malik et al. (2002) pointed out that infection acquired in early pregnancy may lead to abortion, stillbirth or premature delivery, while listeriosis acquired late in pregnancy can be transmitted transplacentally leading to neonatal listeriosis.

Choi and Hong (2003) stated that listeriosis caused by L.monocytogenes has increased drastically in recent years.

Doganay (2003) detected that upon contracting listeriosis 20-30% of the cases result in death.

Grif et al. (2003) evidenced that about 1% of asymptomatic humans occasionally excrete L.monocytogenes in their faeces.

Food Agricultural Organisation / World Health Organisation of the United Nations (2004) stated that in pregnant women, the very old, the very young, immunocompromised individuals, listeriosis infection may be acquired more easily and can result in invasive disease.

McLauchlin et al. (2004) declared that the exact infective dose in human listeriosis is still unknown. Furthermore, the infective dose appears to vary depending on the strain and on the host susceptibility. They suggested that a non-invasive infection may occur with a higher dose (> 10^5 cells/g) and the invasive form of the disease may occur with fewer than 1,000 cells in susceptible populations. They reported that L. ivanovii and L. seeligeri cause rare illness in humans.

Santagada et al. (2004) confirmed that L.monocytogenes is a potentially fatal pathogen to humans.
Campos (2005) mentioned that the main clinical presentations of listeriosis suggest that the bacterium has a tropism to the placenta and central nervous system, causing the following diseases: infection during gestation, neonatal infection, bacteremia, meningitis, brain abscesses, endocarditis, localized infections, and gastroenteritis.

CDC (2005) reported that in USA, about one-third of reported human listeriosis cases happen during pregnancy, which may result in spontaneous abortion in second–third trimester.

Gasanov et al. (2005) mentioned that L. monocytogenes is the primary human pathogen although there have been rare reports of illnesses caused by L. seeligeri, L. ivanovii and L. innocua.

Ooi-Say and Lorber (2005) reported that the development of listeriosis seemed to be dose-dependent and that in non-susceptible people, food containing $1.9 \times 10^5$ cfu/g cause gastroenteritis, although clearly higher infectious doses have also been reported. They found that symptoms of gastroenteritis typically began 24 hours or less, but it ranged from 6 h to 10 days after ingestion of the bacterium and usually lasted 2 days to over 80 days in the case of Listerial bacteraemia. It was often asymptomatic or presented as a self-limiting influenza like infection characterized by fever, diarrhea, headache, muscle and joint pain and emesis.

Hamon et al. (2006) considered the resistance of L. monocytogenes to acidic conditions and to bile salts makes it particularly adept at infecting the gastrointestinal tract.

Ramaswamy et al. (2007) reported that adult infection is characterized by septicaemia and meningitis. Listerosis induced meningitis is often exacerbated by encephalitis which is very unusual for a bacterial infection.
Swaminathan and Gerner-Smidt (2007) stated that outbreaks causing gastroenteritis in healthy patients were associated with foods contaminated with high levels of the pathogen, $> 10^3 - 10^9$ cfu g$^{-1}$.

Drevets and Bronze (2008) demonstrated that the subsequent ingestion of contaminated food delivers the pathogen to the gastro-intestinal tract, its target site for starting invasive infection in the populations at risk, and gastroenteritis in healthy persons.

Tay et al. (2008) mentioned that cutaneous and eye infections have rarely been reported, mainly in farmers and veterinarians in direct contact with afterbirths and infected foetuses.

Chan and Wiedmann (2009) believed that a very high dose ($10^6$ cells / g) of *L. monocytogenes* is generally required for non-invasive infection; however doses as low as $10^2$ cells / g have been associated with invasive infection, and strain variation in infective potential is reported.

Sukhadeo and Trinad (2009) mentioned that clinical manifestations of invasive listeriosis are usually severe including abortion, sepsis, meningoencephalitis, neuro-encephalitis, chorioamnionitis, gastroenteritis and bacteraemia.

Allerberger and Wagner (2010) mentioned that *L. monocytogenes* is a saprophytic gram positive bacterium responsible for human listeriosis, a foodborne disease characterized in immune-compromised individuals by severe septicemia and meningoencephalitis; in pregnant women, placental infection can lead to meningoencephalitis of the newborn and abortion.
Guillet et al. (2010) mentioned that *L. monocytogenes* is the primary human pathogen although there have been several reports of illnesses caused by *L. seeligeri, L. ivanovii, L. innocua, L. welshimeri* and *L. gyayi* in human.

Melton-Witt et al. (2012) suggested that ingestion of *L. monocytogenes* by healthy adults may lead to intestinal villi and liver colonisation without causing any overt symptoms. However, conditions of immune compromise or pregnancy may promote further dissemination and severe infections such as meningitis and preterm labor.

Patricia et al. (2012) stated that in people with no predisposing factors, invasive listeriosis is rare, and the most typical symptom is mild gastroenteritis with fever, headache, nausea, diarrhoea, and abdominal pain.

Silk et al. (2012) found that most cases of invasive listeriosis are sporadic, leading to meningitis, encephalitis, sepsis, and abortion, and are reported in people with another severe underlying disease.

4. Occurrence of *Listeria* species in food:

Vasseur et al. (1999) mentioned that in industrial plants, the microorganisms are subjected to various stressing agents during food processing or cleaning and disinfection of surfaces. The effects of these treatments on *L. monocytogenes* are of great interest as they could influence its response and ability to survive.

Norton (2002) stated that the detection of *L. monocytogenes* in foods is hampered by the high population of competitive microflora, the low levels of the pathogen, and the interference of inhibitory food components.
Salyers and Whitt (2002) explained the trait that makes *L. monocytogenes* difficult to control during food processing is that it can multiply over a wide range of temperatures, especially at refrigerator temperatures.

Waak *et al.* (2002) found that the contamination of meat, poultry, fish, and dairy products by *L. monocytogenes* is common, with a reported prevalence of up to 69, 62, 50 and 20% respectively.

Roberts and Greenwood (2003) showed that foods containing 100 CFU/ g *Listeria* or greater should be considered adulterated.

Sakate *et al.* (2003) stated that different foods have been implicated in isolated cases and listeriosis outbreaks, but some types of food products are considered to be more important with respect to *L. monocytogenes* contamination. Products that are ready-to-eat and refrigerated and that boast a longer shelf-life are considered to pose a higher risk for foodborne disease. Foods with pathogen counts exceeding 100 CFU/g or CFU/mL are also considered to be at a high risk of causing disease.

Lappi *et al.* (2004) mentioned that contamination of food by *L. monocytogenes* can occur at any stage of the food production and processing cycle. *L. monocytogenes* may be introduced directly from the farm or other environment, or on the raw products used to prepare processed foods.

Churchill *et al.* (2006) mentioned that the United States has a zero tolerance policy regarding the presence of *L. monocytogenes* in food, while Canada allows only 100 cfu/g of food.

Rantsiou *et al.* (2008) published that the food safety regulations of most countries required zero tolerance of *L. monocytogenes* in RTE food, especially food produced for specific subgroups of the population that are at risk.
Watkins and Sleath (2008) reported that natural contamination of soil and plant matter forms a part of the organism’s ecological cycle.

Maklona et al. (2010) mentioned that foods commonly contaminated by *L. monocytogenes* include dairy products (particularly soft cheeses), meats (particularly ready-to-eat meat), fish (particularly gravlax and smoked fish), prepared salads, fresh fruit and vegetables.

### 4.1 In milk and cheese:

Breer and Schopfer (1989) mentioned that since some outbreaks of listeriosis have been traced to various foods, the contamination rate of the foodstuffs is of great interest. Out of 1708 samples of milk and milk products, 105 samples (6.1%) proved to contain *Listeria* spp. *L. monocytogenes* was isolated from 54 specimens (3.0%) in St. Gallen, Switzerland.

Destro et al. (1991) detected *L. monocytogenes*, *L. innocua*, *L. seeligeri* and *L. welshimeri* at percentages 5, 20, 2.5 and 2.5 respectively in raw milk and cheese samples (20 samples per product), in contrast to *Listeria*-negative results of pasteurized milk obtained from retail stores in Campinas, S. Paulo, Brazil.

Farber and Peterkin (1991) mentioned that milk and dairy products, because of their high nutritional value, are very suitable for development of microorganisms, including pathogenic bacteria.

El Shamy et al. (1993) found that *L. monocytogenes* was inactivated completely from milk at 60 °C for 15 minutes.

Jacqueta et al. (1993) in the period 1988-1990 in France, analyzed 340 samples collected in a dairy plant from equipments, dairy production facilities and different types of cheeses, for presence of *Listeria*. *L. monocytogenes* was isolated
in 44 tested samples of cheese, also from equipment and dairy production facilities. Cheese was contaminated during the ripening process.

Moura et al. (1993) evaluated the incidence of Listeria spp. in raw and pasteurized milk from a Brazilian dairy plant. Ten samples of each type of milk were collected monthly from October 1989 to September 1990 (except in December), comprising 440 samples (110 samples of each type of milk). Overall, 12.7% of raw milk samples, 0.9% of pasteurized milk samples and 6.8% of total of milk samples were positive for Listeria spp., while 9.5% of raw milk samples, none of the pasteurized milk samples and 4.8% of total milk samples, were positive for L. monocytogenes. Raw milk also contained L. innocua (9.5%), L. welshimeri (0.9%) and L. grayi (0.4%). Pasteurized milk contained only L. innocua (0.9%).

Sanaa et al. (1993) attributed the occurrence of Listeria spp. in the raw milk samples to contamination of milk from the producing animal, spoiled silage, feces, inadequate cleaning of cow’s exercise area, poor cow cleanliness, inadequate cleaning and disinfection of milking utensils and equipment, as well as due to neglected hygienic measures during milking, handling, transportation and distribution of milk.

Lončarević et al. (1995) found that in Sweden, in samples (333) of soft and semi-soft cheeses collected from retail stores, L.monocytogenes was isolated in 6% of samples, of which 42% were produced from raw milk, and 2% from heat treated milk.

Gaya et al. (1996) analyzed 1445 samples of caprine milk representing bulk tanks of 405 farms in Central Spain for Listeria spp. once per season over a one year period. L. monocytogenes and L. innocua were detected in 2.56 and 1.73% of samples, respectively. L. ivanovii (0.21%) and L. seeligeri (0.07%) were rarely isolated.
Nour (1996) tested four hundreds and fifty samples, (two hundreds raw milk and fifty each of Damietta cheese, kariesh cheese, hard cheese, processed cheese and ice cream) from different markets at Cairo, Giza and Kaliobia Governorates, Egypt. Listeria species proved to be present in 23.5, 14, 6, 10, 4 and 12% of raw milk, Damietta cheese, kariesh cheese, hard cheese, processed cheese and ice-cream samples, respectively. L. monocytogenes could be isolated in 4.5, 4, 2, zero, zero and 2 % from raw milk, Damietta cheese, kariesh cheese, hard cheese, processed cheese and ice-cream samples, respectively. L. welshimari, L. innocua, L. murrayi, L. monocytogenes, L. ivanovii, L. seeligeri and L. grayi could be isolated from examined samples at variable percentages.

El Sherbini et al. (1998) tested 138 raw milk samples from apparently normal cows from Sharkia government dairy farm, Egypt. Five samples (3.6%) were contaminated with Listeria. Both L. ivanovii and L. seeligeri were detected in two samples each (1.4%), while L. welshimeri was found in one sample (0.7%).

Vardar-Ünlü et al. (1998) tested 100 raw milk samples and the incidence of Listeria species in Sivas, Turkey was 6%. L. monocytogenes was found in 4% while L. innocua was found in 2% of the samples. One sample contained two serotypes of L. monocytogenes.

Bottarelli et al. (1999) discussed that the presence of L. monocytogenes in cheese can be associated with type of cheese, the manufacturing, inadequate pasteurization, post-pasteurization contamination, inadequate production, ability to multiply during storage at low temperatures and resistance to sanitation preparations.

El Kholy and Ahlam (1999) collected 100 cows’ and buffaloes’ milk samples (50 of each) from individual clinically normal cows and buffaloes to
detect the incidence of *L. monocytogenes* in such milk. The organism was detected in 2% of cows’ milk, while no buffaloes’ milk contained this pathogen.

**Abd EL-Shaheed (2000)** mentioned that 300 samples of which 200 milk samples were randomly collected from dairy shops and supermarkets in Alexandria governorate, Egypt. *Listeria* spp. were found in 6% of milk samples. *L. monocytogenes, L. innocua, L. murrayi, L. weishimeri* and *L. seeligeri* could be detected in 2.5, 4.5, 1.5, 1 and 0.5% of examined milk samples, respectively.

**Barbuddha et al. (2000)** In India tested 87 milk samples (64 from goats and 23 from ewes). They isolated *L. monocytogenes* from 1.56% of goat samples while ewes' samples were negative.

**Bassma (2000)** recovered 31 *Listeria* isolates from 500 raw milk and milk product samples in Egypt (100 raw milk, 100 Kareish cheese, 50 Talaga cheese, 50 Dommitty cheese, 50 hard cheese, 50 semi hard cheese and 100 ice-cream samples). The recovery rate of *Listeria* spp. from the examined samples was 6.2%. The highest incidence of *Listeria* spp. was recorded from low salt soft cheese (12%) followed by raw milk, ice-cream and kareish cheese samples with incidence of 11%, 7%, 7% respectively.

**Food Safety and Inspection Service (2000)** reported that the European community directive on milk and milk-based products specifies zero tolerance for *Listeria* spp. in soft cheese and the absence of the organism in 1 g of the product.

**Nahed (2002)** tested (100) raw milk, (40) soft cheese (Damietta and kareish cheese) samples in Assiut governorate, Egypt and detected *Listeria* spp. in 6 (6%) and 2 (3.3%) of the examined raw milk and kareish cheese, respectively.

**Abdel- Ghany (2004)** investigated the occurrence of *Listeria* spp. among 338 ruminant animals, 90 milk samples from dairy shops and 216 humans in
Beni-Suef governorate, Egypt for the occurrence of *Listeria* spp. He found that the occurrence of *Listeria* spp. in animals (1.48%) and humans (0.93%) was very low in comparison with that in dairy shop milk samples (5.56%). Moreover, *L. monocytogenes* was not isolated from any of the animal or human samples, whereas it was recovered from 3.33% of dairy shop milk samples.

**Amagliani et al. (2004)** revealed that several outbreaks of listeriosis were proven to be associated with the consumption of milk resulting in a great concern in the dairy industry due to the number of cases and the nearly 30% overall mortality rate of these outbreaks.

**Makino et al. (2005)** published that in February 2001 *L. monocytogenes* serotype 1/2b was isolated from a washed-type cheese during routine *Listeria* monitoring of 123 domestic cheeses. Further samples from products and the environments at the plant that produced the contaminated cheese were examined for *L. monocytogenes*. *L. monocytogenes* serotype 1/2b was detected in 15 cheese samples. Studies with people who had consumed cheese from the plant revealed 86 persons who had been infected with *L. monocytogenes*. Thirty-eight of those people had developed clinical symptoms of gastroenteritis or the common cold type after the consumption of cheese. From the epidemiological and genetic evidence, it appeared that the outbreak was caused by cheese. This was the first documented incidence of food-borne listeriosis in Japan.

**Aygun and Pehlivanlar (2006)** investigated the presence of *Listeria* spp. in a total of 157 raw milk and dairy product samples sold in Antakya, Turkey. The prevalence of *Listeria* spp. in raw milk and Turkish white cheese samples was found to be 2.12% and 8.23%, respectively. *L. monocytogenes* was not isolated from raw milk and was found in only two cheese samples (2.35%).

**Bassma (2006)** isolated 79 *Listeria* species from 650 samples (500 dairy products, 50 minced meat and beef burger, 50 chicken fillet, and 50 seafood) in
Egypt. She identified 25 isolates as *L. monocytogenes*. 14 selected *L. monocytogenes* isolates (5 from dairy products, 5 from sea food, 2 from meat and 2 from chicken fillet).

_Hamdi et al. (2007)_ estimated _Listeria monocytogenes_ in raw milk, whey and curdled milk produced and collected in the region of Algiers and Blida between September 2003 and July 2004. Four out of 153 (2.61%) farm milk samples and 6 out of 80 (7.50%) tankers' samples tested positive for *L. monocytogenes*. All samples of whey and curdled milk (12) tested negative for *L. monocytogenes*, but 2 of 22 (9%) samples of whey were contaminated by *L. innocua*. The results indicated that raw milk, and raw milk products are potential sources of the *L. monocytogenes* and represent a potential risk for consumers.

_Kaur et al. (2007)_ showed that, in most cases, animal products such as unpasteurized milk, soft cheeses, raw milk, butter, chocolate milk, and even poultry products are the main sources for human listeriosis.

_Swaminathan and Gerner-Smidt (2007)_ declared that cheeses have often been implicated in outbreaks of listeriosis and are considered risk products.

_Abd elhafiz (2010)_ tested 100 different food stuffs (milk, cheese and meat) in Zagazig governorate, Egypt. Fourteen milk and cheese specimens (17.5%) were presumptively diagnosed as _Listeria_ species on Oxford agar media and one (5.0%) from meat specimens. Six (7.5%) _L. monocytogenes_ out of 80 examined milk and cheese specimens but none from meat specimens were detected by PCR. The conventional methods detect 4.0% from 100 examined food samples versus 6.0% detected by PCR as _L. monocytogenes_. The author considered PCR to be more reliable than conventional identification since it is based on stable genotypic characteristics rather than relying on biochemical or physiological traits, which can be genetically unstable.
Frece et al. (2010) identified raw milk as a source of *L. monocytogenes*, but environmental and faecal contaminations during the transportation of milk and its storage had also been reported.

Jami et al. (2010) recorded that from 100 bulk tank milk samples, a low prevalence (4%) of *L. monocytogenes* was found in Mashhad, Iran.

The frequency findings of *L. monocytogenes* (0-5%) in bulk tank milk samples have been reported from different countries such as Austria 1.5% (Deutz et al., 1999), Spain 3.6% (Gaya et al., 1998), India 1.7% (Adesiyun et al., 1996), USA 4.1% (Rohrbach et al., 1992), Canada 1.9% (Fedio and Jackson, 1990) and Iran 1.6% (Moshtaghi and Mohammadpour, 2007).

Jayamanne and Samarajeewa (2010) observed that the normal pasteurization treatments at 62.8°C for 30 min (LTLT; Low Temperature Long Time) and 71.7°C for 15sec (HTST; High Temperature Short Time) appear to be adequate to destroy a *Listeria* population of 10²CFU/ml in milk, but not a population of 10⁷cfu/ml.

Mahmoodi (2010) investigated 360 raw milk and dairy products samples including white cheese, yoghurt and Iranian yoghurt drink that were collected from two traditional dairy manufacturer in southern Iran. The prevalence of *L. monocytogenes* in raw milk and white cheese samples of manufacturer A was found to be 1.7% and 3.3%, respectively whereas it accounted for 3.3% and 6.7%, respectively in those of manufacturer B.

Tirziu et al. (2010) confirmed that milk and milk products have been classified as primarily responsible in cases of listeriosis due to consumption of foods.
Atil et al. (2011) indicated that a total of 46 Listeria spp. were isolated from 719 samples (milk, bulk tank swabs, cheese, feed, water, faeces and the environment) collected from 415 cattle and 304 sheep over 12 months (from February 2007 to January 2008) in Turkey. The prevalence of Listeria spp. was found to be 9.4% (39/415) in cattle farms and seven isolates were identified as L. monocytogenes (from faeces, feed, water and environment), seven were L. seeligeri (from milk, feed, water and environment), 17 were L. innocua, (from milk, faeces, feed, water and environment), five were L. grayi (from faeces, feed and environment) and three were L. welshimeri (from feed and water). The prevalence of Listeria spp. was found to be 2.3% (7/304) from feed, faeces and the environment of sheep farms and one isolate was identified as L. monocytogenes (from the environment), one was L. seeligeri (from feed), one was L. innocua (from the environment), two were L. grayi (from feed) and two were identified as L. welshimeri (from feed). Listeria ivanovii was not isolated from any of the samples. Listeria spp. were not isolated from cheese and bulk tank swabs. With regard to seasonal variations most Listeria spp. were isolated in the spring and winter seasons.

Kasalica et al. (2011) mentioned that in certain countries (USA and Switzerland) large outbreaks of listeriosis were associated with consumption of fresh cheeses and milk. Studies on presence of L. monocytogenes in raw milk, carried out in Europe, had shown that 2, 5-6% of samples can be contaminated with L. monocytogenes and according to research conducted by many authors, cheeses produced from raw milk are more often contaminated with L. monocytogenes, compared to cheeses obtained from pasteurized milk.

Kumar (2011) mentioned that healthy cows can serve as reservoirs for L. monocytogenes and secrete the organism in milk. Contamination of milk may also occur through accidental contact with faeces and silage.
Tantawy (2011) collected two hundreds and fifty samples of raw milk (100), kareish cheese (75) and ice-cream (75) from supermarkets and street vendors at Alexandria province, Egypt. The incidence of Listeria spp. in the examined raw milk, kareish cheese and ice cream samples was 3, 2.66 and 2.66%, respectively. The incidence of L. monocytogenes in the examined raw milk, kareish cheese and ice-cream samples accounted for 3, 2.66 and 2.66 %, respectively.

Abay et al. (2012) examined 350 samples for the presence of Listeria spp in Turkey. Listeria spp. were isolated from 22%, 10%, 48% and 16% of 50 cattle feces, 50 sheep feces, 25 beef minced meat and 25 sheep minced meat samples, respectively. No Listeria spp. were obtained from the sheep and cows’ milks, bulk tank milks and silage samples examined. In total, 32 Listeria spp. isolates were obtained. Of these isolates, 12 were identified as L. monocytogenes, 12 as L. innocua, 6 as L. grayi and 2 as L. welshimeri. Listeria carriers of animal feces should be considered as a potential risk for the epidemiology of listeriosis.

Scharff (2012) announced that the economic cost related with foodborne illnesses in the United States is higher than 50 billion of dollars per year involving more than 48 millions of persons.

Yakubu et al. (2012) collected one hundred and ninety two raw milk samples from lactating cows identified in Fulani herds and small scale dairy farms within Sokoto metropolis, Nigeria in order to investigate the presence of L. monocytogenes in the milk. Seventy six samples (39.58%) were positive for Listeria species, which upon biochemical characterization 39(51.3%) were Listeria innocua, 14(18.4%) Listeria ivanovii, 17(22.4%) Listeria monocytogenes, 4(5.3%) Listeria welshimeri and 2(2.6%) Listeria seeligeri.

Safarpour Dehkordi et al. (2013) published that in study a total of 596 milk, 619 feces, 443 vaginal swabs, and 522 urine samples from 101 bovine, 100
ovine, 102 caprine, 91 buffaloes, and 65 camel herds of Iran were tested for the presence of *L. monocytogenes*. The study showed that totally 186 of 2180 samples (8.53%) including 58 (2.66%) milk, 40 (1.83%) feces, 42 (1.92%) vaginal secretions, and 46 (2.11%) urine samples had positive results for *L. monocytogenes*.

4.2 In meat products:

Glass and Doyle (1989) mentioned that during further transformation processes of raw meat into meat products, *L. monocytogenes* can be introduced, where the amount depends on the extent of cross-contamination, personal and general hygienic measures and the process parameters. In addition, minced/chopped meat products as luncheon, by their nature, undergo extensive processing and handling during their production.

Destro et al. (1991) isolated *L. monocytogenes, L. innocua, L. welshimeri* and (*L. seeligeri* and *L. murrayi*) from meat and meat products (20 samples per product) at rates 71.7%, 80.0%, 3.3% and 1.7%, respectively.

Khattab (2002) recorded that out of 120 ready-to-eat meat samples, *L. monocytogenes* was detected in 4 out of 40 beef luncheon, and 3 out of 30 chicken luncheon samples.

Tompkin (2002) mentioned that *L. monocytogenes* is distributed in the environment, may be present in food processing and can contaminate sausages during peeling or packaging.

Borucki and Call (2003) found that cross-contamination, which can occur within environment of food-processing equipment, is considered to be a possible source of *Listeria* contamination in processed meat such as luncheon. *L. monocytogenes* is able to attach to and survive on various working contact surfaces.
Endang et al. (2003) found that *Listeria* spp. especially *L. monocytogenes* has been associated with a wide variety of food sources particularly meat and chicken.

Reij et al. (2004) discussed that RTE meats are especially vulnerable to recontamination from slicers, knives, peeling and other food contact surfaces after they have exited the thermal processing step.

Reham (2007) published that 160 random samples of beef burger, beef sausage, chicken burger and chicken nuggets (40 samples of each) were collected from Cairo, El-Kalyobia and El-Gharbia governorates, Egypt. The results were negative for *Listeria* spp. from any of the examined samples.

Jalali and Abedi (2008) collected that a total of 617 meat and meat products, diary, vegetable and ready-to-eat food samples in Isfahan, Iran. The incidence of *Listeria* spp. was 4.6% in all samples, of which *L. monocytogenes* was found in 1.2% of food samples. It was found that *Listeria* spp. was present in 6.7% of meat and meat product samples, 1.3% of diary samples, 1.2% of vegetable samples and 12% ready to eat samples. The results indicated the potential risk of eating ready-to-eat, raw and undercooked foods.

Usama (2009) tested two hundred samples of RTE meat products (40 of each) beef burger, kofta, Shawerma, Hawawshy and Chicken from fast food restaurants in Assiut governorate, Egypt. Two strains of *L. monocytogenes* (1%) were isolated from one Hawawshy sandwich and one chicken sample. *L. monocytogenes* was failed to isolate from all samples of beef burger, kofta and Shawerma.

Abd El-Malek et al. (2010) examined 25 luncheon meat samples in Assiut city, Egypt. *Listeria* spp. were detected in 8 samples (32%).
Mottin et al. (2011) suggested that luncheon meat may pose a potential hazard for consumers.

Hanaa et al. (2012) tested 105 random samples represented by 7 kinds of ready-to-eat meals (kofta, fried liver (kibda), luncheon, sausage, hotdog, cooked cheese with basturma and basturma) collected from different supermarkets and street vendors in Port-Said City, Egypt. L. monocytogenes was isolated from basturma only in 4 samples (26.6%). While it failed detection in all other samples.

Hendawy (2012) screened one hundred random samples of ready-to-eat meat products (frankfurter, salami, basturma, and luncheon) from different supermarkets and shops (25 collected of each type) in El–Behira and Alexandria governorates. L. monocytogenes could not be detected in all products of ready-to-eat meat samples.

4.3. In commercial eggs:

Yadava and Vadehra (1977) attributed the failure of detection of L. monocytogenes from egg contents to the unsuitability of pH of raw egg albumen for growth of L. monocytogenes. Furthermore, the antibacterial properties of eggs hydrolyze the polysaccharide bacterial cell wall causing cell lysis.

Adesiyun et al. (2005) conducted a survey of the microbial quality of table eggs sold in Trinidad. All 184 samples tested (Swabs of egg shells and egg content) were negative for Listeria spp.

Chemaly et al. (2008) referred the occurrence of L. monocytogenes in the egg shell to contamination with the bacteria present in the laying hen's environment. This is because L. monocytogenes is very frequently present in broiler poultry farms and flocks of laying hens.
Sayed et al. (2009) collected 300 fresh table eggs randomly from different markets in Assiut governorate, Egypt. They found that egg shells were contaminated by *Listeria* up to 7% while none of egg contents were contaminated, concluding that egg shell was more subjected to contamination with *L. monocytogenes* than egg content. The obtained results revealed the degree of contamination and public health hazard in the surroundings contacting eggs until reaching the markets and consequently the consumers.

Rivoal et al. (2010) mentioned that there have been no documented foodborne *L. monocytogenes* illnesses due to the consumption of eggs or egg products, even though the bacterium has been isolated from faeces, body fluid, and oviducts of asymptomatic laying hens. They detected *L. monocytogenes* in 25(17.3%) of the 144 raw egg samples collected in France.

Ateya (2011) collected 100 hens’ eggs from different shops and supermarkets in Assiut province, Egypt. Out of 50 egg shells examined, *Listeria* spp. were isolated from 20% including, 8%, 6%, 2% and 4% *L. monocytogenes, L. innocua, L. ivanovii and L. seeligeri*, respectively.

Mahdavi et al. (2012) tested a total of 525 egg samples randomly collected from various shops in Isfahan, Iran for the occurrence of *Listeria* in egg contents and on eggs shells. No *Listeria* isolates were found in the examined egg samples.

Jamali et al. (2013) published that out of 396 RTE food samples, *Listeria* spp. were detected in 71 (17.9%) samples in which 45 (11.4%) were positive for *L. monocytogenes*. Among the studied RTE foods, salads and vegetables had the highest prevalence (14.7%) of *L. monocytogenes*, followed by chicken and chicken products (13.2%), beverages (10%), eggs and egg products (9.5%), beef and beef products (6.7%), lunch boxes (6.7%) and seafood and seafood products (6.7%).
5. **Occurrence of *Listeria* in fecal samples and vaginal swabs of animals:**

*Ralovich et al. (1986)* recorded 90% of 50 healthy sheep investigated in Hungary during summer were excreting *Listeria* in their faeces, nasal mucus, vaginal mucus and milk.

High prevalence rates in cattle feces have been reported in Denmark 51 % *Skovgaard and Morgen (1988)*, Scandinavia 3.1 % (spring to autumn on pasture) to 9.2 % (winter indoors) *Husu (1990)*, Yugoslavia 19 % *Buncic (1991)* and *Vojinovic (1992)*, Canada 14.5 % *Fedio and Jackson (1992)*, Germany 33 % *Weber et al. (1995)*.

*Sanaa et al. (1993)* concluded that the presence of *Listeria* spp. in faeces was associated with the prevalence of these bacteria in feed.

*Hassanein (1994)* examined vaginal swabs taken from 33 animals including cows (11), ewes (8), does (7) and buffalo (7) in Assiut Governorate, Egypt, and failed to isolate any *Listeriae*.

*Mohamed (1997)* tested 110 animals of different species (30 cows, 60 sheep and 20 goats) for the presence of *Listeria* spp. in their faeces in Zagazig governorate, Egypt. Five animals of these (one cow and 4 sheep) were positive for *Listeria* spp. (4.54%), 3 of them (a cow and two sheep) yielded *L. seeligeri* (2.72%) and 2 (2 sheep) yielded *L. innocua* (1.81%). The examination of fecal samples of sheep and cattle revealed that the prevalence of *Listeria* spp. was 6.66% and 3.33% respectively.

*Bailey et al. (2003)* published that in a study of faeces from 475 slaughter-age cattle and sheep from 19 herds or flocks, *L. monocytogenes* was not isolated from any sample. *L. ivanovii* was cultured from one dairy cow but was not isolated from any sheep.
Chaudhari et al. (2003) published that out of the 125 samples each of fecal and vaginal swabs from buffaloes 8% and 4%, and 6.4% and 2.4% were positive for *Listeria* spp. and *L. monocytogenes*, respectively. *L. ivanovii* was confirmed from 0.8% vaginal sample.

Grif et al. (2003) explained that *L. monocytogenes* as a colonising organism in the vagina most likely originates from two sources: transperineal spread from gastrointestinal tract or contamination by an infected fetus/neonate, although the former is considered as the more probable source than the latter. It seems reasonable to assume that relatively low rate of faecal carriage as well as its short duration at least partly explains the low frequency of vaginal carriage of *L. monocytogenes*.

Kalender (2003) investigated faecal samples collected from 170 sheep and 130 cattle for the presence of *L. monocytogenes* in animals slaughtered at local abattoirs in Elazığ (Turkey). The prevalence of *L. monocytogenes* was 0.58% in sheep and 1.53% in cattle. Of the 12 *L. monocytogenes* isolates, seven (58.33%) and five (41.66%) belonged to serotypes 1 and 4, respectively. These results indicated that animal faeces can represent a source of *L. monocytogenes* contamination of carcasses at abattoirs. Thus, constituting a serious hazard to human health as it may lead to outbreaks of human listeriosis.

Roberts and Wiedmann (2003) stated that latent carrier animals on a farm may shed *Listeria* in their feces, thereby continually contaminating the farm environment. Thus, the farm may serve as a reservoir for *Listeria*. A case-control study involving 24 case farms with at least one recent case of listeriosis and 28 matched control farms with no listeriosis cases was conducted to probe the transmission and ecology of *L. monocytogenes* on farms. A total of 528 fecal, 516 feed, and 1,012 environmental soil and water samples were cultured for *L. monocytogenes*. The overall prevalence of *L. monocytogenes* in cattle case farms
24.4% was similar to that in control farms (20.2%), small-ruminant (goat and sheep) farms showed a significantly higher prevalence in case farms (32.9%) than in control farms 5.9%. *L. monocytogenes* isolated from clinical cases and fecal samples were more frequent in environmental than in feed samples, indicating that infected animals may contribute to *L. monocytogenes* dispersal into the farm environment.

Lyautey *et al.* (2007) investigated the prevalence and characteristics of *L. monocytogenes* isolated from livestock, wildlife, and human potential sources of contamination in 2 areas in Ontario, Canada. From February 2003 to November 2005, a total of 268 fecal samples were collected from different animals. *L. monocytogenes* was isolated using selective enrichment, isolation, and confirmation procedures, and 15 samples (6%) yielded to the isolation of 84 confirmed strains. *L. monocytogenes* was isolated from livestock (beef and dairy), wildlife (deer, moose, otter, and raccoon), and human (biosolids and septic) fecal sources. Thirty-two isolates were from serovar 1/2a, 34 from serovar 1/2b, 1 from serovar 3a, and 17 from serovar 4b.

Stepanovih *et al.* (2007) mentioned that the obviously rapid clearance of *L. monocytogenes* from the genital tract may also be considered as a factor contributing to a low vaginal carriage rate of *Listeria* species.

Akça and Şahin (2011) tested 500 samples of milk and vaginal swabs from 250 cows from herds with a recent history of abortion in the Province of Kars, Turkey. *L. monocytogenes* was present in only two of the milk samples (0.8%) while *Listeria* spp. were isolated from 14 (5.6%) of the vaginal swab samples. Among those 14 isolates, seven (2.8%), three (1.2%), two (0.8%) and two (0.8%) were identified as *L. monocytogenes, L. welshimeri, L. seeligeri* and *L. innocua*, respectively.
Dağ et al. (2013) cleared that by microbiological investigation of 96 vaginal swab samples, 5 (5.2%) were determined to be positive for Listeria spp.

6. Occurrence of Listeria in human stools:

Muller (1990) isolated 17 strains (1.7%) of L. innocua and 6 stains (0.6) of L. monocytogenes were isolated from 1000 samples of patients suffering from diarrhea. As a comparison 2000 fecal samples from healthy people contained 40 strains (2%) of L. innocua and 16 stains (0.8) of L. monocytogenes. He concluded that, were no differences of the frequencies of L. innocua and L. monocytogenes between patients and healthy persons.

Hof (2001) reported that the prevalence of asymptomatic stool carriage of L. monocytogenes in healthy adults is 1–5%.

Frye et al. (2002) mentioned that stool examination in an outbreak of acute febrile gastroenteritis among 16 of 44 healthy attendees of a catered party in Los Angeles County, California. Six stool specimens yielded L. monocytogenes, and all isolates were serotype 1/2a.

Sauders et al. (2005) mentioned that despite wide exposure to Listeria organism, little is known about the prevalence of L. monocytogenes in human stool, and it is not known whether human fecal dispersal contributes to human foodborne transmission. They cultured 827 stool specimens (well formed and loose-watery) from individuals from four large metropolitan areas of New York State for L. monocytogenes and found only 1 (0.12%) positive specimen.

Pichler et al. (2009) published that in September 2008, the Austrian Agency for Health and Food Safety learned of an outbreak of diarrheal illness that included a 71-year-old patient hospitalized for gastroenteritis with a blood culture positive for L. monocytogenes. Three stool specimens provided by seven of 19 persons attending a day trip to a foreign city, including a final break at an Austrian
tavern, yielded *L. monocytogenes*. All isolates were of serovar 4b and had fingerprints indistinguishable from each other. This study revealed that the outbreak of gastroenteritis occurred among 16 persons who had eaten dinner at the wine tavern on September 6. Of the 15 persons who ate from platters of mixed cold-cuts, 12 (80%) developed symptoms of febrile gastroenteritis within 24–48 h. The median age of those who became ill was 62 years. A 72-year-old patient recovered from gastroenteritis but was hospitalized with bacterial meningitis on day 19 after the dinner. The epidemiological investigation identified the consumption of mixed cold-cuts (including jellied pork) at the wine tavern as the most likely vehicle of the foodborne outbreak.

*Abd El-Malek et al. (2010)* recorded 7.14% as incidence of *Listeria* spp. among 28 stool cultures from hospitalized children with underlying disease in Assiut Univ. hospital, Egypt.

**7. Diagnosis of listeriosis:**

*Gray (1948)* introduced one of the earliest methods available, the cold enrichment technique, which remained the only available method for many years. In this method, samples are inoculated in a non-selective media and incubated at 4°C for 30 days to six months.

*Rocourt and Catimel (1985)* showed that *L. ivanovii* can be differentiated biochemically from *L. monocytogenes* and other *Listeria* species by its production of a wide, clear or double zone of haemolysis on sheep or horse blood agar, a positive Christie–Atkins–Munch–Petersen (CAMP) reaction with *Rhodococcus equi* but not with haemolytic *Staphylococcus aureus*, and fermentation of D-xylose but not L-rhamnose.

*Fraser and Sperber (1988)* mentioned that *Listeria* isolation media contain esculin as all *Listeria* spp. hydrolyze esculin and the inclusion of esculin
and ferric ion in enrichment or plating media results in the formation of an intense black colour. This is due to the formation of a complex between ferric iron with 6, 7-dihydroxycoumarin, the product of esculin cleavage by β-D-glucosidase, resulting in a black precipitate.

**Notermans et al. (1991)** stated that cleavage of L-α-phosphatidylinositol (PI) by PI-PLC resulted in the production of water insoluble fatty acids and the formation of an opaque white halo-like zone of precipitation around the colonies of the haemolytic species.

**Petran and Swanson (1993)** showed that the non-pathogenic species *L. innocua* outgrows the pathogenic species *L. monocytogenes* by a wide margin during enrichment in both UVM and Fraser broths. This makes selection of suspect colonies for confirmation from Oxford agar more difficult, since the incidence of non-pathogenic strains is higher than that of *L. monocytogenes* and all species demonstrates the same phenotype.

**Cooray et al. (1994)** noted that boiling *L. monocytogenes* cells for 5 min at 100°C was superior to heating at 95°C for releasing DNA from cells.

**Gouin et al. (1994)** mentioned that the virulence gene *plcA*, present on *L. monocytogenes, L. ivanovii* and *L. seeligeri*, encodes the synthesis of a phosphatidylinositol-phospholipase C (PIPL-C) which is generally employed for the differentiation of haemolytic and non-haemolytic *Listeria*

**Bansal (1996)** described the nucleic acid isolation by boiling method. Bacterial pellets were washed once with 1 ml phosphate buffered saline (PBS), pH 7.4, resuspended in a same volume of cold water and incubated in a boiling water bath for 10 min. The clear supernatants obtained after a 5 min centrifugation at 12000g were used for PCR reaction.
ISO (1996) mentioned that ISO 11290 describe methods for the isolation of *L. monocytogenes* from food samples. After a two stage enrichment procedure, using half Fraser and Fraser broth, isolation takes place on two media: PALCAM and Oxford agar. These media have the disadvantage that *L. monocytogenes* cannot be differentiated from non-pathogenic *Listeria*. Recently, media have been described which distinguish *L. monocytogenes* from other *Listeria* spp., based on haemolysis or on a chromogenic substrate.

Ryser et al. (1996) mentioned that some *Listeria*-positive samples go undetected due to overgrowth by other *Listeria* species and/or natural background flora during enrichment and differing abilities of *Listeria* strains to grow competitively. This may explain why negative results were obtained from PCR when using presumptive positive colonies from Oxford agar.

Hassouba (1997) found that PALCAM agar was a more efficient selective and differential medium than Oxford agar.

European Commission (EC) (1999) revealed that the most commonly used culture reference methods for the detection of *Listeria* in foods are the ISO 11290 standards.

Quinn et al., (1999) mentioned that in CAMP test *L. monocytogenes* is positive with the *S. aureus* streak and negative with *R. equi*, whereas the test with *L. ivanovii* gives the reverse reactions.

Wang et al. (1999) considered that food samples and enrichment media may be inhibitory to PCR and thereby can lower its detection capacity. Therefore subculturing on a non-selective medium often precedes the PCR.

Lampel et al., (2000) used PCR primers targeting the hemolysin (*hly*) gene for confirming the isolation of *L. monocytogenes*. 
Robinson et al. (2000) mentioned that *Listeria* species vary in their ability to haemolyse horse or sheep red blood cells, and produce acid from L-rhamnose, D-xylose and -methyl-D mannoside. They stated that *L. innocua* is distinguished from *L. monocytogenes* on the basis of its negative CAMP reaction and its failure to cause β-haemolysis or to show PI-PLC activity on chromogenic media. *L. welshimeri* is differentiated from other *Listeria* species by its negative β-haemolysis and CAMP reactions, and by its acid production from D-xylose and -methyl-D-mannoside.

Hitchins (2001) mentioned that CHROMagar™ *Listeria* is one of the medium recommended in the Bacteriological Analytical Manual for research of *L. monocytogenes* in food and was validated by AFNOR in 2001.

Jinneman and Hill (2001) described a mismatch amplification mutation assay (MAMA) PCR primers that allow *L. monocytogenes* strains to be easily classified into phylogenetic divisions.

Gilot and Content (2002) demonstrated that the coexistence of several *Listeria* species on the same food is usual, and often the incidence of *Listeria* species other than *L. monocytogenes* is higher than that of *L. monocytogenes* itself.

Norton (2002) showed that the detection of *Listeria* pathogen in food by the standard culture methods is made difficult by the sporadic or low levels of contamination (<100 cfu/g). This may attributed to the presence of a high level of background microflora and competitor organisms that could mask the presence of *L. monocytogenes*, and by interference due to food matrix components.

Borucki and Call (2003) mentioned that serotyping PCR primers were designed from variable regions of the *L. monocytogenes* genome. Three primer
sets were used in conjunction with Division III primer set in order to classify 122 *L. monocytogenes* strains into five serotype groups [1/2a(3a), 1/2b, 1/2c(3c), 4b(d,e), and 4a/c].

Ermolaeva et al. (2003) mentioned that *L. ivanovii* can be distinguished from *L. monocytogenes* by its strong lecithinase reaction with or without charcoal in the medium, in comparison to *L. monocytogenes*, which requires charcoal for its lecithinase reaction.

Hitchins (2003) reported that fermentation of different sugars producing acid without gas allows differentiation of the species of *Listeria*.

Akpolat et al. (2004) suggested that when several studies in various countries were compared, *L.monocytogenes* isolation rates seem to vary significantly. This wide variation may be explained in terms of geographic location, isolation methods and kinds of media employed.

ISO (2004) pointed that chromogenic media are now included in most protocols and standards for detection of *L.monocytogenes*.

Reissbrodt (2004) published that in recent years a number of selective chromogenic plating media for pathogenic *Listeria* spp. have been developed and marketed. Their advantages are direct detection and enumeration of pathogenic *Listeria* spp. utilizing cleavage of substrates by the virulence factor phosphatidylinositol-phospholipase C (PI-PLC) and, to a lesser extent, by phosphatidylcholin-phospholipase C (PC-PLC). Selective chromogenic *L. monocytogenes* plating media offer the attraction of rapid economic detection and enumeration of pathogenic *Listeria* spp. within 24 or 48 h of incubation at 36+/−1 °C.
El Marrakchi et al. (2005) mentioned that L. innocua can mask the presence of L. monocytogenes on both Palcam and Oxford agars because of the difference in growth rates of Listeria spp. in selective broths.

Gasanov et al. (2005) explained that Phosphatidylinositol phospholipase C is an enzyme produced only by L. monocytogenes and Listeria ivanovii which hydrolses a specific substrate added to the chromogenic medium, producing an opaque halo around the colonies.

Gouws and Liedemann (2005) referred the main limitation with PCR application to food contaminating microorganisms concerns to the presence of inhibitory substances that are coextracted with DNA and may be present in the sample, causing a failure in the amplification reaction which leads to false negative results. Therefore, quality and purity of extracted nucleic acids are the main requirements for a PCR-based detection assay at the meanwhile the selection of a proper extraction method is determinant for a successful and valid PCR analysis.

Greenwood et al. (2005) published that using chromogenic agars, the presumptive identification of L. monocytogenes is possible after 24 h, compared with 3-4 days using Oxford and other conventional agars.

Paoli et al. (2005) found that the presence of L. monocytogenes has been widely observed in foods, environmental and clinical samples. Its detection and identification in foods traditionally involve culture methods based on selective pre-enrichment, enrichment and plating. This is followed by the characterization of Listeria spp. using colony morphology, sugar fermentation and haemolytic properties.

Tavakoli (2006) announced that Listeria chromogenic media cultures (Chromoagar) with the help of advanced technology might be possibly significant
to direct isolation *Listeria* in one step. This test method sensitivity is 100% and in *L. monocytogenes* have special blue color and surrounded with white color halo due to the phospholipids activities, so easily identified. *L.innocua* is blue (without halo) and other *Listeria* species are colorless.

**Amagliani et al. (2007)** documented that the standard microbiological methods for identification of *Listeria* spp. are laborious and time consuming requiring a minimum of five days to recognize *Listeria* spp. and about 10 days to identify *L. monocytogenes* by confirmation tests. They proved that the PCR-based method be a reliable means of detecting the pathogen in food samples independently from the extraction procedure used, even for a contamination cell number of 1 cfu/g before culture enrichment.

**Hegde et al. (2007)** evaluated the performance of CHROMagar for detection of *L. monocytogenes* for its ability to isolate and identify *L. monocytogenes* from food and environmental samples in Canada. The medium was compared to non-chromogenic selective agars commonly used for *Listeria* isolation: Oxford, Modified Oxford, and PALCAM. From environmental samples, the *L. monocytogenes* confirmation rate was 100% for CHROMagar *Listeria* as compared to 50% for conventional agars tested. On CHROMagar *Listeria, L. monocytogenes* forms a translucent white precipitation zone (halo) surrounding blue-pigmented colonies of 2-3 mm in diameter, with an entire border. CHROMagar *Listeria* offers a high degree of specificity for the confirmation of suspect *L. monocytogenes* colonies, whereas non-chromogenic selective agars evaluated were not differential for *L. monocytogenes* from other *Listeria* species.

**Aragon-Alegro et al. (2008)** demonstrated that according to the producers, CHROMagar *Listeria* medium comes to facilitate the detection, differentiating *L. monocytogenes* from the other species, directly on the isolation process, and allowing final results in 72 h, while the traditional method takes up
to 10 days. A total of 120 food samples of sliced cooked ham, ground beef and frankfurter were analyzed. From 151 colonies presenting typical \textit{L. monocytogenes} characteristics on CHROMagar \textit{Listeria} (bluish, surrounded by a white halo), only 95 (62.9\%) were confirmed as \textit{L. monocytogenes}. The medium was highly sensitive to detect \textit{L. monocytogenes} in ground beef and frankfurter, but not in sliced ham.

\textbf{Aurora et al. (2009)} mentioned that for easy, rapid and efficient methods of isolating and identifying \textit{L. monocytogenes}, both standard culture and several PCR based techniques have been employed.

\textbf{Jami et al. (2010)} mentioned that the combined conventional culture and PCR method allows accurate detection of \textit{L. monocytogenes} in various food samples and could serve as a rapid screening method.

\textbf{Jeyaletchumi et al. (2010)} published that most regulatory agencies stipulate that isolation must be capable of detecting one \textit{Listeria} organism per 25 g of food. This can only be achieved through enrichment methods that employ antimicrobial agents to suppress competing microflora, prior to plating onto selective agars and confirmation of cultures. The selective agents that are normally used in enrichment are acriflavine which inhibits growth of other gram positive bacteria; nalixidic acid which inhibits gram negative bacteria and cycloheximide which inhibits fungi. They mentioned that different chromogenic media have been developed to enable identification of pathogenic \textit{Listeria} spp. and \textit{L. monocytogenes} based on enzymes produced by the pathogen and acids produced due to fermentation of sugars.

\textbf{O’ Grady et al. (2010)} reported that DNA-based methods such as conventional PCR and real-time PCR have been developed as safe, useful, sensitive, and accurate methods for the detection of \textit{L. monocytogenes} in clinical specimens.
Jamali et al. (2013) published that both *Listeria* selective agar and PALCAM agar displayed a low sensitivity and specificity in *L. monocytogenes* detection compared to CHROMagar *Listeria* in naturally-contaminated foods.
3. Material and Methods

I. Material

1. Samples:

A total of 1386 samples used in the present study were collected during the period from October 2012 to August 2013 from different localities in Cairo and Giza governorates.

Humans’ samples were obtained from Theodor Bilharz Research Institute in Giza, Abu Alreesh Children Hospital in Cairo and a medical examination laboratory in Giza. Data of names and ages were provided with collected human samples.

Fecal, vaginal swabs and some milk samples were collected from cattle, buffalo, sheep and goats from herds and farms in different locations in Cairo and Giza governorates, which included Saft Allaban, Kafr Tohormos, Nazlat El-Shawbak, Shinbab, Mit Rahina, El-Badrasheen clinic, faculty of agriculture farm, Cairo University and El Tunsiy farm.

Food samples were collected from different shops and street vendors in Cairo and Giza governorates.

1.1. Food samples:

- Milk samples:

A total of 319 raw milk samples, 157 raw milk samples (500ml) were randomly collected from dairy shops and 162 milk samples from farms were collected from different animals species (56 cows, 30 buffaloes, 36 sheep and 40 goats) either normal or abnormal (milk tinged with blood).

- Cheese samples:

Sixty eight samples of different cheese types were collected from dairy shops and street vendors {Kareish (17), Talaga (24), Feta (14) and processed cheese (13)}. 

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- **Meat products:**

  Sixty samples of different meat products were collected from supermarkets (20) samples for each of the following luncheon, beef burger and frankfurter.

- **Egg samples:**

  One hundred and six commercial egg samples were collected randomly (40 hens’ egg shell, 40 hens’ egg content, 13 ducks’ egg shell and 13 ducks’ egg content).

1.2. **Animal samples:**

  A total of 504 fecal samples, 257 vaginal swabs and 162 milk samples were collected from different animals.

Table (1): Data of the examined animals' samples:

<table>
<thead>
<tr>
<th>Items</th>
<th>Species</th>
<th>Cattle</th>
<th>Bufaloe</th>
<th>Sheep</th>
<th>Goat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces</td>
<td></td>
<td>142</td>
<td>121</td>
<td>172</td>
<td>69</td>
</tr>
<tr>
<td>Vaginal swabs</td>
<td></td>
<td>58</td>
<td>31</td>
<td>106</td>
<td>62</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td>56</td>
<td>30</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Health problem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td>3</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Mastitis</td>
<td></td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Nervous signs (circling)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vaginal infection (pus)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Still birth</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

1.3. **Human samples:**

  A total of 72 stool samples were collected from immunosuppressed persons (36), neonates and children (23) and farm workers (13).
Table (2): Sex, age groups and stool consistency of the examined humans.

<table>
<thead>
<tr>
<th>Items</th>
<th>Human group</th>
<th>Immunosuppressed Persons (36)</th>
<th>Neonates &amp; children (23)</th>
<th>Farm workers (13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>17</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>19</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Age group (Year)</td>
<td>&lt;1</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1-10</td>
<td>13</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>4</td>
<td>1 (12 years)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>21-30</td>
<td>6</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>31-40</td>
<td>5</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>41-50</td>
<td>4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>51-60</td>
<td>4</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Stool</td>
<td>Diarrheic</td>
<td>3</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Non diarrheic</td>
<td>33</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>

2. Media used:

2.1. Media used for isolation and cultivation:

2.1.1. Fraser broth base (Oxoid, CM0895)

It is an enrichment medium was prepared according to Fraser and Sperber (1988), for the isolation of Listeria spp. from food samples.

2.1.2. Half Fraser supplement (Oxoid, SR0166) and Fraesr supplement (Oxoid, SR0156):

It is a selective supplement according to Fraser and Sperber (1988) for the primary and secondary enrichment of Listeria spp. from food samples.

2.1.3. Listeria selective agar (Oxford Formulation) (Oxoid, CM0856):

According to Curtis et al., (1989). It was used as a selective medium for the detection of Listeria spp. from food and clinical samples.
2.1.4. *Listeria* selective supplement (Oxford Formulation) (Oxoid, SR0140):

According to Curtis *et al.*, (1989). The base media was sterilized by autoclaving at 121°C for 15 minutes, cooled to 50°C and aseptically the *Listeria* selective supplement was added.

2.1.5. PALCAM agar base (Oxoid, CM0877):

According to Van Netten *et al.*, (1988). It was used as a selective and differential medium for the detection of *L. monocytogenes* from food and clinical samples.

2.1.6. PALCAM selective supplement (oxoid, SR0150):

According to Van Netten *et al.*, (1988). The base media was sterilized by autoclaving at 121°C for 15 minutes, cooled to 50°C and aseptically the *Listeria* selective supplement was added.

2.1.7. *Listeria* enrichment broth base (oxoid, CM0862):

According to Lovett *et al.*, (1987). It was used as enrichment medium for the isolation of *Listeria* spp. from fecal samples and vaginal swabs.

2.1.8. *Listeria* selective enrichment supplement (oxoid, SR0141):

According to Lovett *et al.*, (1987). The base media was sterilized by autoclaving at 121°C for 15 minutes, cooled to 50°C and aseptically the *Listeria* selective supplement was added.

2.1.9. Motility test medium:

The base media consisted of tryptose 10g, NaCl 5g and agar 5g then dissolved in 1L of distilled water and then boiled to dissolve completely till obtaining a final clear and transparent medium. The medium distributed into test tubes. The tubes were autoclaved at 121°C for 15 minutes and, then, cooled in upright position.

2.1.10. Tryptic soy broth and agar (Difco):

Tryptic soy broth (Difco) with 0.6% yeast extract (TSB-YE) was used for cultivation and purification of *Listeria* spp.
2.1.11. Nutrient agar (Oxoid):

It was sterilized by autoclaving at 121°C for 15 minutes and tubes containing the media were allowed to cool in a slanted position and were used for preservation of the isolates.

2.1.12. Blood agar base (Oxoid):

The base media was sterilized by autoclaving at 121°C for 15 minutes and cooled to 50°C then 5% sheep blood or 7% horse blood was added. It was used for detection of hemolysis test as well as in CAMP test.

2.1.13. Brain heart infusion – egg yolk agar:

According to Coffey et al., (1996), the base medium sterilized by autoclaving at 121°C for 15 minutes and cooled to 50°C. Egg yolk 5% previously diluted in 0.15 M Nacl (1:2 vol/vol) was added. It was used for detection of lecithinase activity of the isolates.

2.1.14. CHROMagar™ Listeria (Chromagar):

According to Notermans et al., (1991) and used for detection of L.monocytogenes.

2.1.15. CHROMagar™ Listeria supplement (Chromagar):

According to Notermans et al., (1991), the base media was sterilized by autoclaving at 121°C for 15 minutes and cooled to 47°C ± 2°C and then CHROMagar™ Listeria supplement was added.

2.1.16. CHROMagar™ Identification Listeria (Chromagar):

According to Notermans et al., (1991), the base media was sterilized by autoclaving at 121°C for 15 minutes. It used for identification of L.monocytogenes.

2.1.17. CHROMagar™ Identification Listeria supplement (Chromagar):

According to Notermans et al., (1991), the base media was sterilized by autoclaving at 121°C for 15 minutes and cooled to 47°C ± 2°C, and then CHROMagar™ Listeria identification supplement was added.
2.2. Media used for biochemical identification of the isolate:

All media used were performed according to US-FDA bacteriological analytical manual Hitchins (2001):

2.2.1. Triple sugar iron (Oxoid)

It was sterilized by autoclaving at 121°C for 15 minutes and tubes containing the media were allowed to cool in a slanted position which were used for determination of sugar fermentation as well as detection of hydrogen sulphide.

2.2.2. Nitrate broth:

It was used for nitrate reduction test.

2.2.3. Sugar fermentation medium:

The base medium was sterilized by autoclaving at 121°C for 15 minutes but the sugar (Xylose, Rhamnose and Mannitol) sterilized by filtration.

2.2.4. Glucose phosphate broth:

It was used for Voges Proskauer and methyl red tests.

2.2.5. BD BBL™ Taxo ™ N discs (231044, Becton, Dickinson and Company-Taxo):

Taxo N discs are 6mm discs made from high quality adsorbent paper impregnated with 6% ρ-aminodimethylaniline monohydrochloride. Used for oxidase test.

2.3. Chemicals and reagents:

2.3.1. Hydrogen peroxide 3%: for catalase test.

2.3.2. Reagents for nitrate reduction test: Reagent A of 8g of sulfanilic acid in one liter of 5N acetic acid and reagent B of α- Naphthylamine in one liter of 5N acetic acid.

2.3.3. Methyl red solution: as an indicator for methyl red test.
2.3.4. Voges Proskauer reagent: solution A of 5% α- Naphthol in absolute ethyl alcohol and solution B of 40% potassium hydroxide in distilled water.

3. Stain (Cruickshank et al. 1975):

   Gram's stain: was used for identification of the isolates.

4. Bacterial strains:

   4.1. *Listeria monocytogenes*:

       It was supplied from the Biotechnology Department, Animal Health Institute Dokki, Giza.

   4.2. *Staphylococcus aureus* for CAMP test:

       It was supplied from the Bacteriology Department, Faculty of Veterinary Medicine, Cairo University.

   4.3. *Rhodococcus equi* for CAMP test:

       It was supplied from Bacteriology Department, Faculty of Veterinary Medicine, Cairo University.
5. Materials used for DNA extraction, PCR and agarose gel electrophoresis:

1. Sterile PBS (pH 7.4).
2. Primers:
   Primers used for PCR amplification were synthesized in MetaBion (Germany).

Table (3): Primers sequences, their specific targets and amplicon size used in *Listeria monocytogenes* confirmation and serotyping:

<table>
<thead>
<tr>
<th>Primer set</th>
<th>Sequence 5′ → 3′</th>
<th>Product size</th>
<th>Anneal Temp (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>For confirmation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LM2   AAG-AGC-TTG-CAA-CTG-CTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse TTG-CTC-CAA-AGC-AGG-GCA-T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse AAT-TAG-AGG-AT C-GA C-CTT-CT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mama-C</td>
<td>Forward CAGTTGCAAGCGCTTGGAGT</td>
<td>268bp</td>
<td>55</td>
<td>Jinneman and Hill (2001)</td>
</tr>
<tr>
<td></td>
<td>Reverse GTAAGTCTCCGAGGGTTGCAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>Forward GCG-GAG-AAA-GCT-AT C-GCA</td>
<td>140bp</td>
<td>59</td>
<td>Borucki and Call (2003)</td>
</tr>
<tr>
<td></td>
<td>Reverse TTG-TTC-AAA-CAT-AGG-GCT-A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse AAG-AAA-AGC-CCC-TCG-TCC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. PCR master mix: Jena Bioscience Germany.

4. Nuclease free water.

5. Reagents used for agarose gel electrophoresis:

   - Agarose gel: Biotechnology Grade.
   - Electrophoresis buffer:
     
     |        | Stock solution |
     |--------|----------------|
     | 10X TBE|                |
     | 1 X TBE| Working solution|

   - Ethidium bromide (Bioshop Canada).

6. 6x DNA loading dye (Thermo scientific).

7. DNA marker:

   Gene ruler 100bp and 50bp plus DNA ladder ready to use, Jena Bioscience (Germany).

8. Apparatus and equipment used for PCR:

   1) Thermal cycler (BIO- RAD, MJ mini).
   2) Power supply.
   3) Ultra violet transillumination ( Vilber lourmat, 312nm).
   4) Safety hood (Spectroline).
   5) Microwave (Olympic electric).
   6) Digital camera (Sony, Japan).
   7) Water bath.
   8) Centrifuge (Beckman).
   9) Horizontal electrophoresis tank (GIBCO/ life technologies, INC).
II. Methods

1. Collection of samples:

The fecal, vaginal swabs and milk samples of animals were collected from different localities in clean sterilized equipment (sterile cups and sterile swabs) and taken under aseptic condition in an ice box. They were processed upon arrival.

2. Enrichment and isolation procedures:

2.1 Enrichment procedure:

2.1.1. For milk:

A total of 25ml of each sample was added to 225 ml of half Fraser broth and incubated at 30°C for 24 hrs, then 0.1 ml from the pre-enrichment culture (half Fraser broth) was transferred into 10 ml of Fraser broth and incubated at 37°C for 48 hrs. According to ISO 11290-1 (1996, 2004).

2.1.2. For cheese and meat products:

A total of 25g of each sample was added to a stomacher bag containing 225 ml of half Fraser broth. The mixture was homogenized using Stomacher for 2 minutes and incubated at 30°C for 24 hrs, then 0.1 ml from the pre-enrichment culture (half Fraser broth was transferred into 10 ml of Fraser broth and incubated at 37°C for 48 hrs. According to ISO 11290-1 (1996, 2004).

2.1.3. For raw egg:

The egg surface was rinsed with 100 ml tryptic soya broth in a sterile beaker then 1ml of that broth was transferred into 10ml of half Fraser broth and incubated at 30°C for 24hrs. Then the egg content was evacuated in a sterile petridish through making an opening in the shell by using sterile scissor and take 1ml of egg content into 10ml of half Fraser broth and incubated at 30°C for 24hrs. From the pre-enrichment culture (half Fraser broth) either from the
egg shell or egg content, 0.1 ml was transferred into 10 ml of Fraser broth and incubated at 37°C for 48 hrs. According to ISO 11290-1 (1996, 2004).

2.1.4. For feces:

One gram of each fecal sample was transferred into 10 ml Listeria enrichment broth containing selective supplement and incubated at 30 °C for 48 hrs. According to Kalender (2003)

2.1.5. For vaginal swabs:

Vaginal swab samples were transferred to Listeria enrichment broth containing selective supplement and incubated at 30°C for 48hrs. According to Lovett and Hitchins (1989)

2. 2. Isolation procedures:

It was performed according to the technique suggested by ISO 11290-1 (1996, 2004):

From each tube of Fraser broth or Listeria enrichment broth culture a loopful was streaked onto PALCAM and Oxford agar plates and incubated at 37°C for 24 to 48 hrs. Plates were then examined for typical Listeria colonies; Listeria spp. on PALCAM agar form colonies that are grey green in colour with sunken center and a black halo against a cherry red medium. Listeria spp. on Oxford plates are gray colored surrounded by black halo. Suspicious colonies were transferred onto tryptic soy agar with 0.6% yeast extract (TSA-YE) and incubated for 24 hrs at 37°C, then maintained at 4 °C for further investigation.
3. Genus identification:

3.1. Microscopic examination:

Films from the suspected colonies were stained by Gram's stain and examined microscopically. The regular Gram- positive short rods with rounded ends, non capsulated and non sporulated were further examined biochemically. According to Cruickshank et al., (1975)

The following tests were performed according to US-FDA bacteriological analytical manual (Hitchins, 2001):

3.2. Motility test in semisolid agar:

The top portion of a motility test tube was stabbed with bacteriological needle touched with the suspected pure culture and incubated at 30°C for 24-48 hrs. Listeria spp. show motility in semisolid agar in the form of an umbrella-like zone.

3.3. Catalase test:

A colony of suspected pure culture was emulsified in a drop of hydrogen peroxide (3.0%) on a glass slide. Immediate bubbling indicates a positive catalase test. Listeria spp. are catalase positive.

3.4. Oxidase test:

A number of Taxo N discs were placed in a petri dish and moisten with sterile distilled water using a platinum wire or a sterile wooden applicator stick; suspicious colonies were applied to the moistened discs. Change in colour within 5 min with the Taxo N discs turning dark purple to black indicate a positive result. Listeria spp. are Oxidase negative, giving no color change.
3.5. Triple sugar iron (TSI):

A TSI tube was inoculated by streaking the slant and stabbing the butt with a pure culture of the isolated organism, then incubated at 35°C for 24-48 hrs. The development of yellow color indicated acid in slant (lactose) and/or butt (glucose). A black color resulted from H$_2$S-positive reaction. *Listeria* spp. induce an acid slant/acid butt, with no H$_2$S.

3.6. Methyl red test:

Five ml of 48hrs glucose phosphate broth culture incubated at 35°C, 5 drops of Methyl red reagent were added. The development of red color indicated a positive reaction. *Listeria* spp. are methyl red positive.

3.7. Voges Proskauer test:

Five ml of 48hrs glucose phosphate broth culture incubated at 35°C, 5 drops of Voges Proskauer reagent were added. The development of red color indicated a positive reaction. *Listeria* spp. are Voges Proskauer positive.

4. Species identification:

4.1. Blood hemolysis:

The obtained isolates were streaked onto 5% sheep or 7% horse blood agar and incubated at 35°C for 24 hrs. Haemolytic and non-haemolytic isolates could be recognized by the detection of haemolytic zones around the colonies.

4.2. Sugar fermentation test (Xylose, Rhamnose and Mannitol):

Five ml sugar media tubes were inoculated with the tested organism and incubated at 35°C for 24-48hrs may reach to 6 days. The development of yellow color indicated acid production. Table (4)
4.3. Nitrate reduction test:

Nitrate broth tubes were inoculated with the tested organism and incubated at 35°C for 12-24 hr. Add 5 drops of each reagent A and reagent B. A positive reaction was indicated by the development of red color. Only *L.murrayi* is nitrate reduction positive.

4.4. CAMP test:

This test was done for detection of the synergistic reaction of hemolysin of *L. monocytogenes* with beta toxin of *Staphylococcal aures* which was streaked vertically on sheep blood agar plate and the isolate was streaked horizontally without quite touching, *Rhodococcus equi* was streaked vertically on sheep blood agar plate and *L. ivanovii* was streaked horizontally. The inoculated plates were incubated at 37°C for 24-48hrs. Positive reaction gave an arrow shape of hemolysis in the zone of influence of the vertical streaks. According to Federal Register (1988)

4.5. Detection of lecithinase activity:

Brain heart infusion agar containing 5% egg yolk inoculated with the isolate and incubated at 37 °C for up to 36hrs. The plates were examined for precipitation of degraded egg yolk surrounding the colonies. *L. ivanovii* gives a positive lecithinase reaction without charcoal in the medium. According to Coffey *et al.* (1996).
Table (4): Differential characteristics of *Listeria* species (Sneath *et al.* 1989)

<table>
<thead>
<tr>
<th>Test</th>
<th><em>L. monocytogenes</em></th>
<th><em>L. seeligeri</em></th>
<th><em>L. ivanovii</em></th>
<th><em>L. innocua</em></th>
<th><em>L. welshimeri</em></th>
<th><em>L. murrayi</em></th>
<th><em>L. grayi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>β</td>
<td>β</td>
<td>β</td>
<td>γ</td>
<td>γ</td>
<td>γ</td>
<td>γ</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CAMP with <em>S. aures</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CAMP with <em>R. equi</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urea hydrolysis test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H2s production</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Slant/butt</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Esculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) positive  
(-) negative  
(V) variable  
A/A acid slant/ acid butt  
B complete hemolysis  
γ non hemolytic
4.6. Identification through chromogenic media:

a. Chromogenic medium for detection of *L. monocytogenes*:

Suspected positive colonies by the conventional method confirmed by streaking onto CHROMagar™ *Listeria* and incubated for 24hrs at 37 °C. *L. monocytogenes* give blue colonies surrounded by white halo. According to Notermans *et al.*, (1991).

b. Chromogenic medium for identification of *L. monocytogenes*:

The positive colonies on CHROMagar™ *Listeria* (blue colony surround by white halo) were streaked onto CHROMagar™ identification *Listeria*. *L. monocytogenes* appear rose surrounded by a white halo. According to Notermans *et al.*, (1991).

3. Application of polymerase chain reaction (PCR):

3.1. Inoculum preparation:

For each isolate of *Listeria* spp. was streaked onto tryptic soy agar with 0.6% yeast extract (TSA-YE) and incubated for 24 hrs at 37°C, then colonies were collected into eppendorf contain 1 ml PBS.

3.2. DNA extraction (Bansal, 1996):

DNA extraction of *L. monocytogenes* was done by the boiling method:

Bacterial colonies were washed once with 1 ml phosphate buffered saline (PBS), pH 7.4, resuspended in a same volume of cold water and incubated in a boiling water bath for 10 min. The clear supernatants obtained after a 5 min centrifugation at 12000g were used for PCR reaction.
3.3. Gene amplification:

DNA samples were amplified in a total of 25µl of the following reaction mixture: 2 µl of DNA template, 1µl of 20 pmol of each primer and 5 µl of 5X of PCR master mix in separate reaction.

The reaction conditions for confirmation of *L. monocytogenes* were optimized to be 95°C for 5 min as initial denaturation followed by 30 cycles of 95 °C for 15 sec, 57 °C for 2 sec, 72 °C for 30 sec and final at 72 °C for 5 min. (Mengaud et al., 1988).

For serotyping:

The same thermal profile for confirmation of *L. monocytogenes* used, but the annealing temperature of each primer used as in table (3).

Only strains testing positive with D1 primers were tested with GLT primers and only strains testing positive with GLT primers tested using Mama-C primers. Also only strains testing positive with D2 primers were tested with FlaA primers Borucki and Call (2003), Diagram (1).

3.4. Detection of PCR products:

Five µl of PCR products were electrophoresed in 1.5% agarose gel as shown in the following steps:

A) Gel preparation:

1) The prepared 1.5 % agarose was melted using microwave, left to cool till 55°C then 1 µl ethidium bromide was added by and poured into the assembled horizontal gel tray after proper installation of the desired combs.
2) The gel was allowed to be solidified at room temperature, and then remove the comb.
3) TBE buffer used for preparation of the gel was added into the tank to a level 1-2 mm above the gel layer.

B) Sample loading:

Five µl of the PCR product added to 1µl of 6X DNA loading dye, mix well then loaded to specific lane. 5 µl of 100bp DNA ladder (marker) were loaded in the first well for *Listeria monocytogenes* confirmation. 5 µl of 50bp DNA ladder (marker) were loaded in the first well for *L. monocytogenes* serotyping.

C) Electrophoresis:

Assemble the horizontal gel tray, the tank was closed and the power supply was attached (connect the site of sample application to the cathode (black) & the other side to anode (red). Switch the power supply to 100V for 30 min.

D) Photograph:

Specific amplicons were observed under ultraviolet transillumination compared with the marker and photographed by a digital camera while the flash is off.
Table (5): Occurrence of *Listeria* species in the examined milk samples by conventional method:

<table>
<thead>
<tr>
<th>Listeria spp.</th>
<th>From dairy shops (157)</th>
<th>From farms (162)</th>
<th>Total (319)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>%</td>
<td>positive</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>4</td>
<td>2.55</td>
<td>1</td>
</tr>
<tr>
<td><em>L. ivanovii</em></td>
<td>1</td>
<td>0.64</td>
<td>0</td>
</tr>
<tr>
<td><em>L. seeligeri</em></td>
<td>1</td>
<td>0.64</td>
<td>0</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>2</td>
<td>1.27</td>
<td>0</td>
</tr>
<tr>
<td><em>L. welshimeri</em></td>
<td>4</td>
<td>2.55</td>
<td>0</td>
</tr>
<tr>
<td><em>L. grayi</em></td>
<td>2</td>
<td>1.27</td>
<td>0</td>
</tr>
<tr>
<td><em>L. murrayi</em></td>
<td>5</td>
<td>3.18</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19</td>
<td>12.10</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. (1): Occurrence of *Listeria* species in the examined milk samples by conventional method.
Table (6): Occurrence of *Listeria* species in the examined cheese samples by conventional method:

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>Kareish (17)</th>
<th>Talaga (24)</th>
<th>Feta (14)</th>
<th>Processed (13)</th>
<th>Total (68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*L.*momocytogenes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*L.*ivanovii</td>
<td>1</td>
<td>5.88</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*L.*seeligeri</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*L.*innocua</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*L.*welshimeri</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*L.*grayi</td>
<td>1</td>
<td>5.88</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*L.*murrayi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>11.76</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. (2): Occurrence of *Listeria* species in the examined cheese samples by conventional method.
Table (7): Occurrence of *Listeria* species in commercial eggs of hens and ducks by conventional method:

<table>
<thead>
<tr>
<th>Listeria spp.</th>
<th>Hens’ egg shell (40)</th>
<th>Hens’ egg contents (40)</th>
<th>Ducks’ egg shell (13)</th>
<th>Ducks’ egg contents (13)</th>
<th>Total (106)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
</tr>
<tr>
<td><em>L.momocytogenes</em></td>
<td>1</td>
<td>2.50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.ivanovii</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>L.seeligeri</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.innocua</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.welshimeri</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.grayi</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.murrayi</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1</td>
<td>2.50</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. (3): Occurrence of *Listeria* species in commercial eggs of hens and ducks.
Table (8): Occurrence of *Listeria* species and *L. monocytogenes* in the examined food samples by conventional method.

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>No. of samples</th>
<th><em>Listeria</em> spp.</th>
<th><em>L. monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ve</td>
<td>%</td>
</tr>
<tr>
<td>Milk from dairy shops</td>
<td>157</td>
<td>19</td>
<td>12.10</td>
</tr>
<tr>
<td>Milk from farm</td>
<td>162</td>
<td>1</td>
<td>0.62</td>
</tr>
<tr>
<td>Cheese</td>
<td>68</td>
<td>2</td>
<td>2.94</td>
</tr>
<tr>
<td>Meat products</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eggs</td>
<td>106</td>
<td>2</td>
<td>1.89</td>
</tr>
<tr>
<td>Total</td>
<td>553</td>
<td>24</td>
<td>4.34</td>
</tr>
</tbody>
</table>

Fig. (4): Occurrence of *Listeria* spp. and *L. monocytogenes* in the examined food samples by conventional method.
Table (9): Occurrence of *Listeria* species in the examined fecal samples of animals by conventional method.

<table>
<thead>
<tr>
<th>Listeria spp.</th>
<th>sample</th>
<th>Cattle (142)</th>
<th>Buffalo (121)</th>
<th>Sheep (172)</th>
<th>Goat (69)</th>
<th>Total (504)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
</tr>
<tr>
<td><em>L.momocytogenes</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.58</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td><em>L.livanovii</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.58</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td><em>L.seeligeri</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.innocua</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.welshimeri</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.58</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td><em>L.grayi</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.58</td>
<td>1</td>
<td>0.40</td>
</tr>
<tr>
<td><em>L.murrayi</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>2.33</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Fig. (5): Occurrence of *Listeria* species in the examined fecal samples of animals by conventional method.
Table (10): Occurrence of *Listeria* species in different specimens of the examined animals by conventional method:

<table>
<thead>
<tr>
<th>Species</th>
<th>Fecal samples</th>
<th>Vaginal swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>+ve</td>
</tr>
<tr>
<td>Cattle</td>
<td>142</td>
<td>0</td>
</tr>
<tr>
<td>Buffaloe</td>
<td>121</td>
<td>0</td>
</tr>
<tr>
<td>Sheep</td>
<td>172</td>
<td>4</td>
</tr>
<tr>
<td>Goat</td>
<td>69</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>504</td>
<td>5</td>
</tr>
</tbody>
</table>

**Fig. (6):** Occurrence of *Listeria* species in examined animal samples by conventional method.
Table (11): Occurrence of *Listeria* species among the examined humans by conventional method:

<table>
<thead>
<tr>
<th>Human group</th>
<th><em>Listeria</em> spp.</th>
<th>Neonates and children (23)</th>
<th>Immunosuppressed persons (36)</th>
<th>Farm workers (13)</th>
<th>Total (72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
</tr>
<tr>
<td><em>L.momocytogenes</em></td>
<td>1</td>
<td>4.35</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Livanovii</em></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5.56</td>
<td>0</td>
</tr>
<tr>
<td><em>L.seeligeri</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.innocua</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.welshimeri</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.grayi</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L.murrayi</em></td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>4.35</td>
<td>2</td>
<td>5.56</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. (7): Occurrence of *Listeria* species among the examined humans by conventional method.
Table (12): Comparison of the methods used in identification *Listeria monocytogenes* from other *Listeria* species:

<table>
<thead>
<tr>
<th>Listeria spp.</th>
<th>No. of +ve <em>Listeria monocytogenes</em> via:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional method</td>
<td>CHROMagar</td>
</tr>
<tr>
<td>Source</td>
<td>No.</td>
<td>+ve</td>
</tr>
<tr>
<td>Food</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk from dairy shops</td>
<td>157</td>
<td>19</td>
</tr>
<tr>
<td>Milk from farms</td>
<td>162</td>
<td>1</td>
</tr>
<tr>
<td>Cheese</td>
<td>68</td>
<td>2</td>
</tr>
<tr>
<td>Eggs</td>
<td>106</td>
<td>2</td>
</tr>
<tr>
<td>Feces of animal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>172</td>
<td>4</td>
</tr>
<tr>
<td>Goat</td>
<td>69</td>
<td>1</td>
</tr>
<tr>
<td>Humans' stool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonates and children</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Immunosuppressed persons</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>8</td>
</tr>
</tbody>
</table>
Photo (1): *Listeria* species on Oxford agar showing gray colonies surrounded by black zone.
Photo (2): *Listeria* species on PALCAM agar forming grey green colonies with a black halo against a cherry red medium background.
Photo (3): Gram positive short rods *Listeria* (X100)
Photo (4): Motility test of *Listeria* species (umbrella shape).
Listeria monocytogenes show narrow zone of β-hemolysis on sheep blood agar.

Photo (5): β hemolytic Listeria on sheep blood agar.
Photo (6): Positive CAMP test *L. ivanovii* with *R. equi*

Photo (7): Positive CAMP test *L. monocytogenes* with *S. aures*
Photo (8): Lecithinase positive *L. ivanovii* on Brain heart infusion–egg yolk agar medium.
Photo (9): *Listeria monocytogenes* on Chromagar showing blue colony surrounded by white halo zone.
Photo (10): The results of PCR for *Listeria monocytogenes* confirmation.

*Listeria monocytogenes* specific band is 702bp.

Lane 1:6: positive *Listeria monocytogenes*.

Lane M: 100 bp DNA ladder.

Lane 7: positive control.
Photo (11): The results of PCR for *Listeria monocytogenes* serotyping (D2 primer).

Lane M: 50 bp DNA ladder.

D2 positive *Listeria monocytogenes* specific band is 140bp.

Lane 1, 2 and 5: positive *Listeria monocytogenes* for D2 primer.
Discussion

Listeriosis is one of the important emerging bacterial zoonotic diseases worldwide. *Listeria monocytogenes* is the causative organism of listeriosis, a debilitating and often fatal infection, which occurs mostly via food consumption. However, some foods such as dairy products, probably due to their nutritious nature and handling characteristics, are more prone to contamination and thus are relatively more culpable.

*L. monocytogenes* is the primary human pathogen although there have been several reports of illnesses caused by *L. seeligeri, L. ivanovii, L. innocua, L. welshimeri* and *L. gyayi* (Guillet et al., 2010).

Human infection primarily results from eating *L. monocytogenes* contaminated food and may lead to serious and potentially life-threatening listeriosis (Posfay-Barbe and Wald, 2009).

Raw milk was identified as a source of *L. monocytogenes*, but environmental and fecal contaminations during the transportation of milk and its storage have also been reported (Frece et al., 2010).

On the farm, the environmental conditions for the growth of *Listeria* are ideal. The organism can grow in soil, muddy and dusty conditions, in water and in dams. Cows can be carriers and mastitis can result from a *Listeria* infection. The feeding of poor quality silage is another problem area, as the organism can grow in poorly fermented silage.

The result in Table (5) and Figure (1) showed that 2.55%, 0.64%, 0.64%, 1.27%, 2.55%, 1.27% and 3.18% of the examined raw milk samples collected from dairy shops were positive for *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, L. grayi* and *L. murrayi*, respectively. Totally *Listeria* species were isolated from 12.10% of the examined raw milk samples collected from dairy shops. This result is near to that obtained by Bassma (2000) who isolated *Listeria* spp. (11%) from raw milk (100) in Cairo, Egypt.
This result is lower than that detected by Nour (1996) in Cairo, Giza and Kalbiobia governorates and Abd elhafiz (2010) in Zagazig governorate, Egypt at rates 23.5% and 17.5% respectively.

On the other hand our result is higher than that detected by Vardar-Ünlü et al. (1998) 6% in Sivas, Turkey, Abd EL-Shaheed (2000) 6% in Alexandria province, Egypt, Abdel-Ghany (2004) 5.56% in Beni-Suef governorate, Egypt, Aygun and Pehlivanlar (2006) 2.12% in Antakya, Turkey and Tantawy (2011) 3% Alexandria governorate, Egypt.

As in Table (5) and Figure (1) occurrence of L. monocytogenes at rate of (2.55%) in milk samples from dairy shops is similar to that detected by Abd EL-Shaheed (2000) 2.5% in Alexandria governorate, Egypt and nearly similar to that detected by Tantawy (2011) 3% in Alexandria governorate, Egypt.

This result is lower than that detected in Brazil (5%) by Destro et al. (1991), USA (4.1%) by Rohrbach et al. (1992), Egypt (4.5%) by Nour (1996), Spain (3.6%) by Gaya et al. (1998), Egypt (3.33%) by Abdel-Ghany (2004), Egypt (7.5%) by Abd elhafiz (2010) and Iran (4%) by Jami et al. (2010).

On contrast these results were higher than that reported from different countries such as Canada 1.9% by Fedio and Jackson (1990), India 1.7% by Adesiyun et al. (1996), Austria 1.5% by Deutz et al. (1999) and Iran 1.6% by Moshtaghi and Mohammadpour (2007).

The occurrence of Listeria spp. in the examined raw milk samples may be attributed to contamination of milk from the producing animal, spoiled silage, feces, inadequate cleaning of cow’s exercise area, poor cow cleanliness, inadequate cleaning and disinfection of milking utensils and equipment, as well as due to neglected hygienic measures during milking, handling, transportation and distribution (Sanaa et al., 1993).

The risk of Listeria spp. increases during the storage and transportation of milk or because of insufficient standards of hygiene which has been reported in previous studies (Atil et al. 2011).
The results indicate that raw milk is potential sources of the \textit{L. monocytogenes} and represent a potential risk for consumers. Raw milk is one of the most common paths for transmission of \textit{L. monocytogenes}.

In most cases, animal products such as unpasteurized milk, soft cheeses, raw milk, butter, chocolate milk, and even poultry products are the main sources for human listeriosis (Kaur \textit{et al.}, 2007).

When several studies in various countries including the current study are compared, \textit{L. monocytogenes} isolation rates seem to vary significantly. This wide variation may be explained in terms of geographic location, isolation methods and kinds of media employed (Akpolat \textit{et al.}, 2004). Moreover, it has been established that the cows suffering from listeric mastitis or those clinically normal may produce milk containing large numbers of such organisms (Gitter \textit{et al.}, 1980).

In the present study, Table (5) and Figure (1) showed the occurrence of 	extit{Listeria} species among milk samples from farms; only one milk sample was positive (0.62\%) for 	extit{Listeria} species. These results were similar to that reported by Akça and Şahin (2011) who isolated \textit{L. monocytogenes} from only two of the milk samples (0.8\%) in the Province of Kars, Turkey.

It should point out that healthy animals are often carriers of \textit{L. monocytogenes} and as such can be source of contamination of the environment or milk (Kasalica \textit{et al.}, 2011).

The isolated \textit{Listeria spp.} from the farm milk samples was \textit{L. monocytogenes} (1.79\%) which isolated from cattle’s milk (56), a result which is nearly similar to that reported in Egypt by El K holy and Ahlam (1999) who detected \textit{L. monocytogenes} in 2\% of cow’s milk samples, while no buffaloes’ milk samples contained this pathogen.

These results were lower than other findings in several previous studies as that obtained by El Sherbini \textit{et al.} (1998) who found that (3.6\%) of raw milk samples were contaminated with \textit{Listeria spp.} from Sharkia government
dairy farm, Egypt, and Hamdi et al. (2007) (2.61%) in the region of Algiers and Blida and much lower than that detected by Yakubu et al. (2012) (39.58%) in Sokoto metropolis, Nigeria.

Shifting to the occurrence of Listeria spp. in milk samples of sheep and goats was zero in this study, it is similar to that detected by Barbuddhe et al. (2000) who found that all the ewe's milk samples were negative for L. monocytogenes in India. On the other hand by Barbuddhe et al. (2000) in India recorded Listeria spp. in 1.56% of goats’ milk samples.

The occurrence of L. monocytogenes in the examined milk samples from dairy shops (2.55%) is higher than the examined milk samples from farms (0.62%) so the obtained results confirm that the major problems with the epidemiology of listeriosis are of environmental origin rather than being related to the animal itself as that mentioned by Abdel-Ghany (2004).

Cheeses have often been implicated in outbreaks of listeriosis and are considered risk products (Swaminathan and Gerner-Smidt, 2007).

Most dairy products are susceptible to Listeria spp. contamination if hygiene is not good during manufacture. In most cases, contamination has been found to result from contact with an environmental source within the factory premises after the milk has undergone pasteurization.

The findings in Table (6) and Figure (2) showed that the occurrence of Listeria spp. in different types of cheese purchased from dairy shops and street vendors was 11.76, zero, zero, zero of Kariesh, Talaga, Feta and processed cheese respectively. L. ivanovii and L. grayi were isolated from the examined Kariesh cheese in 5.88% of each. L. monocytogenes failed to be isolated from the examined cheeses.

With regard to kariesh cheese, the obtained results are higher than that detected by Nour (1996) who isolated Listeria spp. in 6% of Kariesh cheese and isolated L. monocytogenes in 2% in Egypt, Aygun and Pehlivanlar (2006) who isolated Listeria spp. in 8.23% of Turkish cheese and L. monocytogenes in
2.35% in Turkey, Tantawy (2011) who isolated Listeria spp. in 2.66% of Kariesh cheese in Alexandria governorate, Egypt, Bassma (2000) who isolated Listeria spp. from (7%) of kareish cheese samples and Nahed (2002) who isolated Listeria spp. in 3.3% of kareish cheese samples in Assiut governorate, Egypt.

The presence of L. monocytogenes in cheese can be associated with type of cheese, the manufacturing, inadequate pasteurization, post-pasteurization contamination, inadequate production, ability to multiply during storage at low temperatures and resistance to sanitation preparations (Bottarelli et al., 1999).

In the present study the occurrence of Listeria spp. in cheese produced from raw milk (11.76%) is higher than that obtained from pasteurized milk (zero) as that conducted by (Kasalica et al., 2011).

In industrial plants, the micro-organisms are subjected to various stressing agents during food processing or cleaning and disinfection of surfaces. The effects of these treatments on L. monocytogenes are of great interest as they could influence its response and ability to survive (Vasseur et al., 1999).

Although L. monocytogenes can survive under adverse conditions, the organism is not heat-resistant and can be inactivated by pasteurization at 72°C for 15 seconds (Bunning et al., 1998). Furthermore, El Shamy et al. (1993) found that L. monocytogenes was inactivated completely from milk at 60 °C for 15 minutes.

Occurrence of Listeria spp. in the examined meat products was zero as in Table (8) and Figure (4) which is similar to that detected by Hanaa et al. (2012) who could not isolate L.monocytogenes from any of the examined luncheon and sausage samples in Port-Said City, Egypt. Also Usama (2009) who failed to isolate L. monocytogenes from all samples of beef burger in Assiut government, Egypt, Hendawy (2012) who could not detect Listeria monocytogenes in all products of ready-to-eat meat samples including frankfurter and luncheon in El –Behira and Alexandria governorates and

The current results are lower than detected by Abd El-Malek *et al.* (2010) who tested 25 luncheon meat samples in Assiut city, Egypt and detected *Listeria* spp. in 8 samples (32%), Khattab (2002) who recorded that out of 120 ready-to-eat meat samples, *L. monocytogenes* was detected in 4 (40) of beef luncheon.

The detection of *L. monocytogenes* in foods is hampered by the high population of competitive microflora, the low levels of the pathogen, and the interference of inhibitory food components (Norton, 2002).

There have been no documented foodborne *L. monocytogenes* illnesses due to the consumption of eggs or egg products, even though the bacterium has been isolated from faeces, body fluid, and oviducts of asymptomatic laying hens (Rivoal *et al.*, 2010).

It should be mentioned that, eggs are being nearly sterile when laid. However, eggs have the potential to become contaminated with bacteria from the hen’s intestinal tract, feces, and from the surrounding environment.

The occurrence of *Listeria* spp. in commercial eggs (Table 7 and Figure 3) cleared that only one positive sample from egg shell of hen and another one of duck for *L.monocytogenes* (2.50%) and *L. ivanovii* (7.69%), respectively. On the other hand the egg contents of both hens and ducks were negative.

The egg shell contamination observed in this study might be due to contamination with the bacteria present in the laying hen's environment. This is because *L. monocytogenes* is very frequently present in broiler poultry farms and flocks of laying hens (Chemaly *et al.*, 2008).

The obtained results revealed the degree of contamination and public health hazard in the surroundings contacting eggs until reaching the markets and consequently the consumers. Sayed *et al.*, (2009) concluded that it was
uncomfortable result to find *L. monocytogenes* in table eggs and how extent the zoonotic view is meaningful.

The data reported in Table (7) revealed failure of detection of *L. monocytogenes* from all egg content samples, that may be attributed to the unsuitability of pH of raw egg albumen for growth of *L. monocytogenes*. Furthermore, the antibacterial properties of eggs which hydrolyze the polysaccharide bacterial cell wall causing cell lysis (*Yadava and Vadehra, 1977*).

However the current results are lower than that detected by *Ateya (2011)* who collected (100) hen’s eggs from different shops and supermarkets in Assiut governorate, Egypt. Out of 50 egg shell examined, *Listeria* spp. were isolated from 20% including, 8%, 6%, 2% and 4% *L. monocytogenes, L. innocua, L. ivanovii* and *L. seeligeri*, respectively and *Jamali et al. (2013)* who tested 396 RTE food samples, *Listeria* spp. were detected in 71 (17.9%) samples of which 45 (11.4%) were positive for *L. monocytogenes*. Nevertheless, the obtained data are much lower than that detected by *Rivoal et al. (2010)* who detected *L. monocytogenes* in 25 (17.3%) of the 144 raw egg samples in France.

The results in table (8) and fig. (4) showed the occurrence of *Listeria* spp. in the examined food samples as 12.10%, 0.62%, 2.94%, zero and 1.89%, also showed the occurrence of *L. monocytogenes* in the examined food samples as 2.55%, 0.62%, zero, zero and 0.94% in the examined milk samples from dairy shops, milk samples from farms, cheese, meat products and eggs, respectively.

It is important to take into account that the high percentage of *Listeria* spp. in this study occurred in milk samples from dairy shops represents a serious threat as emphasized by *Amaglani et al., (2004)* who showed that several outbreaks of listeriosis were proven to be associated with the consumption of milk and are causing great concern in the dairy industry due to the number of cases and the nearly 30% overall mortality rate of these
outbreaks. For this reason, the European community directive on milk and milk-based products specifies zero tolerance for soft cheese and the absence of the organism in 1 g of the product (Food Safety and Inspection Service, 2000) and the United States has a zero tolerance policy regarding the presence of *L. monocytogenes* in food, while Canada allows only 100 cfu/g of food Churchill *et al.* (2006).

Milk and milk products have been classified as primarily responsible in cases of listeriosis due to consumption of foods (Tirzii *et al.*, 2010).

The results illustrated in Table (9) and Fig. (5) indicated that zero, zero, 2.33% and 1.45% of fecal samples of cattle, buffaloe, sheep and goat, respectively were *Listeria*-positive. None of vaginal swabs examined from different animal species reacted positively for *Listeria* species.

These results support the concept that ruminant animals are particularly susceptible to *Listeria* infection and that sheep are most susceptible, followed by goats and cattle (Maaten, 1989). These results indicate that animal feces can represent a source of *Listeria*.

These results are lower than that obtained by Abay *et al.* (2012) who isolated *Listeria* spp. from 22% and 10% of 50 cattle feces and 50 sheep feces in Turkey and by Weber *et al.* (1995) who isolated *L. monocytogenes* from 33.3% fecal samples of 138 cattle and from 8% fecal samples of 100 sheep and much lower than that reported in Scandinavia 3.1 % (spring to autumn on pasture) to 9.2 % (winter indoors) by Husu (1990), and Canada 14.5 % by Fedio and Jackson (1992). In addition Ralovich *et al.* (1986) showed 90 % of 50 healthy sheep investigated during summer were excreting *Listeria* in their faeces, nasal mucus, vaginal mucus and milk.

However, these results are higher than that obtained by Kalender (2003) who investigated faecal samples collected from 170 sheep and found that the prevalence of *L. monocytogenes* was 0.58%.
Infected and asymptomatic carrier sheep excrete the organism in feces and manure, these materials along with spoiled silage are used as fertilizer, which constitute the most significant source of transmission of the organism to man and animals as well as contamination of food such as raw milk (Killinger, 1970).

Latent carrier animals on a farm may shed Listeria in their feces, thereby continually contaminating the farm environment. Thus, the farm may serve as a reservoir for Listeria (Roberts and Wiedmann, 2003).

The contaminated, untreated manure can be a source of human listeriosis. For example, the coleslaw outbreak in Canada in 1981 was traced back to the sheep manure used for cabbages in the field (Sauders and Wiedmann, 2007).

Ruminant farm animals play a key role in the persistence of Listeria spp. in the rural environment via a continuous faecal-oral cycle (Vazquez-Boland et al., 2001).

Table (10) and figure (6) showed that no Listeria spp. were isolated from vaginal swabs of all examined animals. This result is very similar to that obtained by Hassanein (1994) in Assiut Governorate, Egypt, but is lower than that obtained by Akça and Şahin (2011) who isolated Listeria spp. from 14 (5.6%) of the vaginal swab samples in the Province of Kars, Turkey and Dağ et al. (2013) who detected Listeria spp. in 5 (5.2%) of 96 vaginal swab samples.

Listeria monocytogenes as a colonising organism in the vagina most likely originates from two sources; transperineal spread from gastrointestinal tract or contamination by an infected fetus/neonate, although the former is considered as the more probable source than the latter (Grif et al., 2003).

The obviously rapid clearance of L. monocytogenes from the genital tract may also be considered as a factor contributing to a low vaginal carriage rate of this bacterium Stepanovih et al. (2007). Vaginal carriers of L.
monocytogenes are uncommon, likely due to the antibacterial action of vaginal mucus (Souza et al., 2008).

Table (11) and figure (7) showed the occurrence of Listeria spp. in stool samples of neonates and children, immunosuppressed persons and farm workers as 4.34%, 5.55% and zero respectively. The isolated Listeria spp. were L. monocytogenes (4.34%) from neonates and L. ivanovii (5.55%) from immunosuppressed persons. These results are lower than that obtained by Abd El-Malek et al. (2010) who examined 28 stool cultures from hospitalized children with underlying disease in Assiut University hospital, 2 (7.14%) were found positive for Listeria spp.

Our results are higher than that detected by Sauders et al. (2005) in USA, who found L. monocytogenes in one (0.12%) fecal specimen out of 857 and Muller (1990) who detected L. innocua and L. monocytogenes in fecal specimens. 17 strains (1.7%) of L. innocua and 6 strains (0.6%) of L. monocytogenes were isolated from 1000 patient samples.

These results comparable to Silk et al. (2012) who assumed that the main predisposing factor of listeriosis was a decrease in cell mediated immunity because of underlying disease or pregnancy, and the risk of listeriosis is increased also in neonates and the elderly and in susceptible people and animals.

In addition, periods in which L. monocytogenes is excreted in the faeces appear to be rather short. The bacterium is excreted in faeces for maximum four days (Grif et al., 2003).

CHROMagar Listeria offers a high degree of specificity for the confirmation of suspect L. monocytogenes colonies, whereas non-chromogenic selective agars are not differential for L. monocytogenes from other Listeria species. Aragon-Alegro et al. (2008) demonstrated that according to the producers, CHROMagar Listeria medium comes to facilitate the detection, and differentiating Listeria monocytogenes from the other species. L. monocytogenes characteristics on CHROMagar Listeria are bluish, surrounded by a white halo.
The pore-forming haemolysin listeriolysin O (LLO), the main virulence factor of *Listeria monocytogenes*, allows bacteria to escape from the harsh environment of the phagosome to the cytoplasm of the infected cell (Bahey-El-Din *et al.*, 2010).

In the present study, based on the primers targeted to the *L. monocytogenes* hlyA gene which encode listeriolysin O was highly species specific and provided a means for easily differentiating *L. monocytogenes* from other hemolytic species of *Listeria* (Deneer and Boychuk, 1991). As in photo (10) and table (12) by PCR only six samples were positive for *Listeria monocytogenes*. Four (2.55%) samples were positive from the examined milk samples from dairy shops (157), 1(0.62%) sample from the examined milk samples from farms (162) and 1(4.35%) sample from the examined stool samples of neonates and children (23), but by CHROMagar seven samples were positive for *Listeria monocytogenes* those detected by PCR in addition to another one milk sample from dairy shops, and by conventional method eight samples were positive for *Listeria monocytogenes* those detected by PCR beside one (0.94%) from (40) hen’s egg shell and one (0.58) from (172) fecal sample of sheep. So PCR proves to be sensitive technique to be included in the procedure of detection of *L. monocytogenes*.

These findings come in accordance with Amagliani *et al.* (2007) who documented that the standard microbiological methods for identification of *Listeria* spp. are laborious and time consuming requiring a minimum of five days to recognize *Listeria* spp. and about 10 days to identify *L. monocytogenes* by confirmation tests. They proved that the PCR-based method be a reliable means of detecting *L. monocytogenes* in food samples.

Some *Listeria*-positive samples go undetected due to overgrowth by other *Listeria* species and/or natural background flora during enrichment and differing abilities of *Listeria* strains to grow competitively. This may explain why negative results were obtained from PCR when using presumptive positive colonies from Oxford agar (Ryser *et al.*, 1996).
The main limitation with PCR application to food contaminating microorganisms concerns to the presence of inhibitory substances those are coextracted with DNA and may be present in the sample, causing a failure in the amplification reaction which leads to false negative results. Therefore, quality and purity of extracted nucleic acids are the main requirements for a PCR-based detection assay (Gouws and Liedemann, 2005).

Different chromogenic media have been developed to enable identification of pathogenic *Listeria* spp. and *L. monocytogenes* based on enzymes produced by the pathogen and acids produced due to fermentation of sugars. Different antimicrobials are added to the media to obtain sufficient selectivity. Chromogenic media represent the most popular culture confirmation method because of its easy preparation and interpretation. They enable presumptive identification of *L. monocytogenes* after 24 hours. Most of these media have been tested on a wide range of different foods (Reissbrodt, 2004) and are now included in most protocols and standards (Hitchins, 2003; ISO, 2004). The introduction of chromogenic media has efficiently improved the isolation of *L. monocytogenes* (Jeyaletchumi et al., 2010).

In this study when using multiplex PCR for serotyping *Listeria monocytogenes* there was a primer dimer so I used conventional PCR and did each primer set separately with the thermal profile used for *Listeria monocytogenes* confirmation but using the specific annealing temperature for each primer as in table(3). The results showed in photo (11) clarified three raw milk samples from dairy shops that were positive for D2 primer and when tested with FlaA primer were negative so these samples’s serotype group was (1/2c, 3c), but the other three positive samples were untypeable as these samples may be rare serotypes. The D1 and D2 primers designed for the most common serotypes (1/2a, 1/2b, 1/2c and 4b) as mentioned by Borucki and Call (2003). The isolated serotype is belonging to lineage II which is more common from food sources. This result improved our understanding on the
ecology and transmission dynamics of *Listeria* spp. in general and the human pathogen *L. monocytogenes* in particular, but further researches are needed on the development of PCR primers that distinguish the rare *Listeria monocytogenes* serotypes.
Conclusion

According to the results obtained, the following can be concluded:

- Our results showed that the highest occurrence of *Listeria* species specially *Listeria monocytogenes* was recorded in milk samples from dairy shops which indicates the insufficient standards of hygiene during transportation and storage of milk so raw milk sold in Cairo and Giza governorates may occasionally represent a serious threat to public health and it is considered as a risk factor in the manufacture of various dairy products.

- The detection of *L. monocytogenes* in foods is hampered by the high population of competitive microflora, the low levels of the pathogen, and the interference of inhibitory food components.

- Infected and asymptomatic carrier animal excrete the organism in feces and manure, these materials along with spoiled silage are used as fertilizer, which constitute the most significant source of transmission of the organism to man and animals as well as contamination of food.

- The introduction of chromogenic media has efficiently improved the isolation of *L.monocytogenes* in this study.

- *hlyA* gene was highly species specific and provides a means for easily differentiating *L. monocytogenes* from other hemolytic species of *Listeria*.

- PCR was more rapid and sensitive method, useful for the rapid detection of *L. monocytogenes* in a reasonable period of time which may avoid costly recalls and reducing outbreaks.

- PCR used for serotyping of the isolated *Listeria monocytogenes* and three of the isolated *L. monocytogenes* were serotype group 1/2c (3c).

Ongoing efforts are needed to further reduce the incidence of listeriosis, so we recommend that:

1. The manure must be properly removed, and it should be emphasized that fields should not to be fertilized with untreated manure.

2. Choosing of healthy mother’s hens (to obtain eggs-free pathogens), in addition to hygienic measures in the farms during handling and storage.
3. Periodical examination of refrigerators and tanks of milk during production and storage.

4. Control strategies are needed in the food chain from preharvest through consumption to minimize the contamination by *L. monocytogenes* and to prevent the growth of the organism to high numbers by:

- Good manufacturing practices, sanitation standard operating procedures, and hazard analysis critical control point (HACCP) programs to minimize environmental *L. monocytogenes* contamination and to prevent cross-contamination in processing plants and at retail.
- Using postpackaging treatments to destroy *L. monocytogenes* on products.
- Pasteurization of milk by HTST (high temperature short time) pasteurization of milk, a minimum of 72°C for 15 seconds is essential. The same method is strongly advised in milk for cheese manufacture.
- Cleaning with sanitizer minimizes the contamination of egg shells.

5. Education of consumers, especially those who fall into the high risk groups, about high-risk foods:

- Educational programs for consumers informed the risks resulted from eating under cooked foods and proper heating of ready-to-eat food particularly the elderly and immune-comprised persons who are more susceptible to infection.
- It is imperative that the population be enlightened on the need to handle eggs with good sanitary practices and to consume only properly cooked eggs or egg products.
- It should be mentioned that cheese made from raw milk (Kareish) is of great threat to public health and should be avoided.

6. All controlling measures must be supervised by veterinary and public health authorities and must include reports about the incidence of listeriosis in Egypt.

7. Further researches are needed on the development of PCR primers that distinguish the rare *Listeria monocytogenes* serotypes.
Summary

*Listeria monocytogenes* has become one of the most important food-borne pathogens in recent years and a major concern both for the food industry and public health. In fact, it causes severe illnesses associated with the contamination of various categories of foods, particularly milk, and dairy products with high mortality rate documented by several outbreaks.

So, this study was conducted to evaluate the occurrence of *Listeria* spp. in different food samples, animals, and humans and explain the role of animals in transmitting *Listeria* to humans.

The present study evidenced that the occurrence of *Listeria* species in the examined raw milk samples from dairy shops was 12.10%. Meanwhile the occurrence of *L. monocytogenes* was 2.55% in the same samples.

On the other hand the occurrence of *Listeria* species in farm milk samples from different animals spp. revealed only one cattle milk sample was positive (0.62%) for *Listeria* and failed to isolate any *Listeria* spp. from buffaloes, sheep and goats.

Concerning, *Listeria* spp. occurrence in different types of cheese collected from dairy shops and street vendors was, 11.76 in Kareish, *L. ivanovii* and *L. grayi* could be isolated from examined Kariesh cheese each in 5.88%. *L. monocytogenes* failed to be isolated from the examined cheese.

The attempt to isolate *Listeria* spp. from meat products evidenced no *Listeria* spp. could be isolated from the examined meat products.

The occurrence of *Listeria* spp. in commercial eggs cleared there are only two positive samples; one from egg shell of hen and another one from egg shell of duck for *L. monocytogenes* (2.50%) and *L. ivanovii* (7.69%), respectively. On the other hand the egg contents of both hen and duck were negative.
The examination of animal fecal samples for isolation *Listeria* spp. cleared that sheep and goats harboured *Listeria* spp. by incidence of 2.33% and 1.45% respectively. *L.monocytogenes* in fecal samples of sheep was 0.58%.

The occurrence of *Listeria* spp. among the examined vaginal swabs of cattle, buffalo, sheep and goat in this study was zero in all the examined samples.

The occurrence of *Listeria* spp. in the examined stool samples of neonates and children, immunosuppressed persons and farm workers was 4.35%, 5.56% and zero respectively. The isolated *Listeria* spp. was *L.monocytogenes* (4.34%) from neonates and children, and *L.ivanovii* (5.56%) from immunosuppressed persons.

By PCR only six samples were positive for *Listeria monocytogenes*, Four (2.5%) samples were positive from the examined milk samples from dairy shops (157), 1(0.61%) sample from the examined milk samples from farms (162) and 1(4.34%) sample from the examined stool samples of neonates and children (23). Meanwhile by CHROMagar seven samples were positive for *Listeria monocytogenes* those detected by PCR in addition to another one from milk sample from dairy shops while by conventional method evidenced eight samples positive for *Listeria monocytogenes* those detected by PCR beside one (2.5%) from hen’s egg shell(40) and one (1.72%)from fecal sample of sheep(172).

Three samples from the six samples positive for *Listeria monocytogenes* by PCR were positive for serotype group (1/2c, 3c). Meanwhile the other three positive samples were untypeable.
الملخص العربي

أصبحت الليستيريا مونوسيتوجينز في السنوات الأخيرة واحدة من أهم مسببات الأمراض التي تنتقل عن طريق الغذاء، ومصدر قلق كبير سواء بالنسبة للصناعة الغذائية أو الصحة العامة. حيث أنها تسبب أمراض خطيرة مرتبطة بتلوث أنواع مختلفة من الأطعمة، وخاصة اللبن، ومنتجاته مع ارتفاع معدل الوفيات التي وقعت في حالات تفشي المرض.

لذلك، تم إجراء هذه الدراسة وتقديم معدل وجود فصائل الليستيريا في عينات الغذاء المختلفة والحيوانات والإنسان وشرح دور الحيوانات في نقل الليستيريا إلى الإنسان.

وجد أن نسبة تواجد فصائل الليستيريا في عينات اللبن المجمعة من المحلات التجارية 12.01% و معدل وجود الليستيريا مونوسيتوجينز في هذه العينات 2.20%.

أما بالنسبة لمعدل وجود فصائل الليستيريا بين عينات اللبن من المزارع من الحيوانات المختلفة (الأبقار، الجاموس، الأغنام، والماعز) فقط أظهرت عينة لبن واحدة فقط من الأبقار إيجابية 0.25% لفصائل الليستيريا وصنفت فصيلة الليستيريا المعزولة من هذه العينات هي الليستيريا مونوسيتوجينز 17.95% من لبن الأبقار.

وبفحص عينات الجبن المختلفة لمعدل وجود فصائل الليستيريا وجد أن الجبن القريش يحتوي على نسبة 0.75% و تتمي فصائل الليستيريا المعزولة للليستيريا إيفانوفي في الليستيريا جراي بنسبة 0.62% لكل منهما.

وجدير بالذكر أنه لم يتم عزل أي من فصائل الليستيريا من منتجات اللحوم.

وسجلت نتائج فحص عينات البيض التجارية أن اثنين فقط من عينات سطح البيض إيجابية إحداهما من قشرة البيض من الدجاج و أخرى من البط، لكلا من لستيريا مونوسيتوجينز 2.50% وليستيريا إيفانوفي 7.69% على التوالي. من ناحية أخرى محتويات البيض من الدجاج و البط على حد سواء كانت سلبية.
وبفحص عينات براز الحيوانات المختلفة لتحديد معدل وجود فصائل الليستيريا وجد أن

2.32 ٪ و1.48 ٪ سجلت في الأغنام والمازع على التوالي بينما لم يمر عزل أي فصيلة من

فصائل الليستيريا في براز الأبقار والجاموس، كذلك لم يتم عزل أي من فصائل الليستيريا

في المساحات المهبلية لكل الحيوانات محل الدراسة.

وعند فحص براز الأطفال وحديثي الولادة والأشخاص ناقصى المناعة وعمال المزارع

وجد أن معدل وجود الليستيريا هو 4.23 ٪، 5.56 ٪ وصفر على التوالي. وفصائل الليستيريا

المعزولة هي الليستيريا مونوسيتوجينز 4.35 ٪ من الأطفال حديثي الولادة وليستريا

إيفانوفي. 5.94 ٪ من الأشخاص ناقصى المناعة.

باستخدام تفاعل البلمرة المتسلسل تم الحصول على 4 عينات ليستريا مونوسيتوجينز:

4 عينات منهم 2.22 ٪ من عينات اللبن من المحلات التجارية وعينة 2.22 ٪ من عينات

لبن المزارع وعينة 2.22 ٪ من عينات براز حديثي الولادة والأطفال، ولكن باستخدام

ekروم أجارت الحصول على 7 عينات موجبة لليستيريا مونوسيتوجينز وهم العينات الموجبة

باستخدام تفاعل البلمرة المتسلسل بالإضافة إلى عينة لبن أخرى من المحلات التجارية أما

بالطرق التقليدية تم الحصول على 8 عينات موجبة لليستيريا مونوسيتوجينز وهم العينات

الموجبة باستخدام تفاعل البلمرة المتسلسل بالإضافة إلى عينة 2.22 ٪ من عينات قشرة

بيض الدجاج وعينة 0.58 ٪ من عينات براز للأغنام.

وباستخدام تفاعل البلمرة المتسلسل لمعرفة أي مجموعة تنتمي إليها الليستريا

مونوسيتوجينز وجد أن ثلاث عينات لبن من العينات الموجبة لليستيريا مونوسيتوجينز التي تم

عزلها من محلات اللبن التجارية تنتمى إلى مجموعة (c/02,1/02,3/02) ولكن الثلاث عينات

الموجبة الأخرى غير معروفة المجموعة.
جامعة القاهرة
كلية الطب البيطرى
قسم الأمراض المشتركة

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تاريخ الميلاد: 28/4/82
الجنسية: مصرية
التخصص الدقيق: أمراض المشتركة

عنوان الرسالة: أهمية الصحية لميكروب الليستريا

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المستخلص العربي

لمعرفة مدى تواجد فصائل الليستريا في العينات المختلفة من الأغذية والعينات الآدمية والحيوانية تم تجميع 1386 عينة من محافظتي القاهرة والجيزة (355 عينة غذائية مختلفة، 505 عينة براز و255 مسحة مهبلية من الحيوانات المختلفة و273 عينة آدمية). فان معدل تواجد فصائل الليستريا المعزولة باستخدام الطرق التقليدية هو 16.10% و11.50% و10.50% من عينات الليستريا مونوسيتوجينز هو 5.55، 6.22 و 5.94%، وتواجد الفصائل الأحادية كانت acknowledgment هو 2.4% و 1.89% و 1.47% في عينات البن والليمون وعينات الأغذية المختلفة. بينما لم يتم عزل هذا الفصيل من عينات البن والليمون. كما تم عزل فصائل الليستريا من برز كلا من الأشخاص الأول والثاني بنسبة 2.3% و 1.45% على التوالي، ولكن لم يتم عزل أي من فصائل الليستريا من المسحات المهبلية من الأشخاص الأول والثاني. وجد أن معدل تواجد فصائل الليستريا في عينات البراز الآدمية من حديثي الولادة والأطفال والأشخاص ذوي المناعة المنخفضة ومن عمال المزارع هو 4.35% و5.06% و6.5% و1.50%، وتتنوع الليستريا مونوسيتوجينز المعزولة بنسب مختلفة من العينات (2.75% من عينات البراز، 10.30% من عينات البن، 3.60% من عينات البن والليمون و 2.20% من عينات البن). ولذا موضحه الدراسة أن استخدام كروم أجار بارد يتطلب تفاعل البلمرة المتسلسل في عينة من عينات البن والليمون وعينات البن والليمون وعينات البن والليمون. بالإضافة إلى ذلك، يمكن استخدام تقنيات مبكرة لمعرفة الفصائل نوعية موجبة للليستريا مونوسيتوجينز. وهم الفصائل الموجبة باستخدام تقنيات مبكرة للتعرف على العينات. وهم العينات التي يتم إنتاجها من عينات البراز بطرق مختلفة. وهم العينات التي يتم إنتاجها من عينات البن والليمون وعينات البن والليمون وعينات البن، بالإضافة إلى عينات البن، وعينات البن والليمون، وعينات البن والليمون.

الكلمات الدالة (فصائل الليستريا - الأغذية - الحيوان - الآدمي - الكروم أجار - تفاعل البلمرة المتسلسل).
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