Using of PCR assay for identification of Listeria monocytogenes recovered from table eggs

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Abstract

The purpose of this study was to determine the incidence of *Listeria monocytogenes* contaminating egg shells and contents of table eggs sold in Assiut city, Egypt. A total of 300 fresh table eggs were collected randomly from different markets in which every 3 eggs from each market were represented as one egg pooled sample. Each of egg shell and content was subjected to procedures of isolation of *L. monocytogenes* followed by PCR assay for the prfA gene for identification. It was found that egg shells were contaminated by 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. It can be concluded that it was uncomfortable result to find *L. monocytogenes* by this degree of contamination in table eggs and how extent the zoonotic view is meaningful. Future control strategies need to consider variations in the epidemiologies of food-borne zoonotic infections, and apply a quantitative risk analysis approach to ensure that the most cost-effective programs are developed.

Key words: Table egg, *Listeria monocytogenes,* Polymerase Chain Reaction, Zoonosis.

Introduction

Table eggs are one of the few foods that are used among the popular dishes consumed by people at home, restaurants and convenience stores in their natural states with no artificial additives. Most freshly laid eggs are sterile, at least from inside in case of good flock management and absence of vertical transmission also by the presence of cuticle, shell membranes and the antimicrobial properties of eggs (Yadava and Vadehra, 1977). But eggs may constitute, if contaminated with pathogens, a public health hazard.

The genus Listeria is composed of a number of gram positive bacterial species (*L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, L. grayi*) (Vazquez-Boland et al., 2001). *L. monocytogenes* is the most pathogenic species to humans and animals causing severe food-borne infections that leading to meningitis, encephalitis and gastroenteritis (Doganay, 2003). Despite its low incidence in western countries, *L. monocytogenes* is ranked first among the food-borne pathogenic bacteria in terms of death rate (40%), far ahead of *Campylobacter, Salmonella* and *E. coli O157:H7*. This high mortality rate has made L. monocytogenes and its spread the subject of intensive research.

Identification of Listeria is laborious and time consuming, requiring a minimum of 5 days to recognize Listeria species and about 10 days to identify *L. monocytogenes* by confirmatory tests (Amagliani et al., 2007), while immediate action should be taken in case of contamination since it is of fundamental importance to ensure the safety of food. In the last few years, molecular techniques have appeared as promising alternatives in food microbiology. Nucleic acid based amplification techniques, of which the polymerase chain reaction (PCR) has been so far the most extensively employed, offer several advantages over the classical microbiological methods such as shorter time of analysis, low detection limits, specificity and potential for automation (Germini et al., 2009).

Due to the growing interest in healthy foods and the lacks in information about contamination rates in listeriosis associated with table eggs in Assiut city, there was a need to run this investigation to put our hands on the incidence of *L. monocytogenes* in table eggs and to how extent listeriosis can occur.

Materials and Methods

Samples: Three hundreds fresh table eggs were collected randomly from different markets in Assiut city, Egypt. Every 3 eggs from each market were represented as one egg pooled sample. All 100 egg pooled samples were examined for incidence of *L. monocytogenes* in their shells and contents. Egg shells were tested by surface rinse method as described by Moats (1980) then egg contents were prepared and evacuated according to Speck (1976).

Microbiological analysis for Listeria isolation: Enrichment procedure was done using Listeria enrichment broth followed by isolation on Oxford agar plates (Curtis et al., 1989) and identification was performed according to FDA bacteriological analytical manual (Hitchins, 1995).

Genomic DNA Extraction: For each Listeria strain, a 10-ml culture was grown to mid-log phase in Tryptose Soya (TSY) broth, and 1 ml of cells was pelleted by centrifugation (13,000 xg for 5 min). The cell pellets were resuspended in 1 ml of sterile phosphate buffer saline. The resuspended cells were re-centrifuged at 12,500 xg for 15 min. The pelleted cells were then used for DNA extraction. Genomic DNA from suspected Listeria strains was extracted using the Wizard genomic DNA purification kit (Promega, USA) as recommended by the manufactures. DNA samples were stored at -20 °C until use.

PCR identification of *Listeria monocytogenes*: For L. monocytogenes PCR identification, 2 primers were selected based on the prfA (transcriptional activator of the virulence factor) gene for *L. monocytogenes*. Primer sequences used in PCR were listed in Table 1. All PCR reactions were performed in a final volume of 25 ul using 2 ul of extracted DNA as template. Each reaction mixture contained 12.5 µl GoTaq® Green Master Mix (Promega, M7122) 1 µl of 500 M forward primer (LIS-F); 1 ul of 500 M reverse primer (LIS-R) and 8 µl of Ultra-Pure DNase/RNase-Free distilled water (Gibco, Grand Island, NY, USA). The DNA amplification reactions were performed in thermal cycler (Techne Cyclgene, Germany). The cycling conditions for PCR were as follows: preincubation at 95°C for 5 min; 40 cycles consisting of dsDNA denaturation at 95°C for 30 s, primer annealing at 54°C for 30 s, primer extension at 72°C for 30 s; final elongation at 72°C for 10 min. All amplification products were resolved in 1% agarose gel, stained with ethidium bromide, detected under a short-wavelength UV light source, and photographed with EDVOTEK Gel documentation system. The 1-KB plus DNA Ladder (Invitrogen) was used as molecular size marker. In order to test the specificity of primers, PCR was carried out using purified bacterial DNA from E. coli.

Results

The microbiological analysis of 100 pooled egg samples revealed 7 isolates resembling Listeria species and no Listeria could be isolated from egg content. For species identification, the genomic DNA from the isolated strains was subjected to PCR. The prfA gene primers (Table 1) formed an expected band of 217 bp that revealed that all the isolated strains were *L. monocytogenes.* Negative control produced no detectable product.

Discussion

Why this study was run? The answer of this question represented in 3 views, firstly the public health hazard view as *L. monocytogenes* is one of the major pathogens (Doganay, 2003); secondly zoonotic view in which *L. monocytogenes* causing listeriosis through food borne transmission (Linnan et al., 1998); thirdly economic view which table eggs are highly consumed by nearly all peoples in the society especially the poor who substitute the expensive animal protein of meat with eggs.

The data reported in Table 2 revealed failure of detection of *L. monocytogenes* from all egg contents samples in the present study; Korashy et al. (2008); Saad and El-Prince (1995) and that may be attributed to the unsuitability of pH of raw egg albumen for growth of L. monocytogenes. Furthermore, presence of the antibacterial properties of eggs which hydrolyze the polysaccharide bacterial cell wall causing cell lysis (Yadava and Vadehra, 1977). While Table 2 showed that, the considerably high 7% incidence of *L. monocytogenes* on egg shells reflected the degree of environmental contamination and also revealed eggs as one of sources of listeriosis. The presence of *L. monocytogenes* in eggs most likely is due to contamination from the shells during the breaking process or from the processing environment (Foegeding and Leasor, 1989).

Hence, it is necessary to adopt strict hygienic measures for controlling *L. monocytogenes* in table eggs (Korashy et al., 2008) such as: 1) choosing of

Table-1. Oligonucleotide sequences used for identification of Listeria monocytogenes by PCR.

Target gene	Primer sequence (5'-3')	Amplified fragment length	Reference
prfA gene	LIS-F: TCA TCG ACG GCA ACC TCG G LIS-R: TGA GCA ACG TAT CCT CCA GAG T	217 bp	Germini et al. (2009)

Table-2. Incidence of Listeria monocytogenes in table eggs samples.

Egg samples	Positive samples recovered from shell		Positive samples recovered from content	
	No./total eggs	%	No./total eggs	%
The present study	7/100	7%	0/100	0%
Korashy et al. (2008)	0/90	0%	0/90	0%
Saad and El-Prince (1995)	8/45	17.78%	0/45	0%

healthy mother's hens (to obtain eggs-free pathogens), 2) hygienic measures in the farms during handling and storage, 3) using of hot soapy water with those come in contact with eggs and eggs containing foods in work areas, 4) eggs must be held at low temperature (5°C) to prevent proliferation of pathogens, 5) cleaning with sanitizer minimizes the contamination of shells, 6) educational programs for consumers informed the risks resulted from eating under cooked eggs particularly the elderly and immune-comprised persons who are more susceptible to infection, and 7) pasteurization of egg products as statutory requirements in many countries.

In conclusion, all controlling measures must be supervised by the authorities. Not only pasteurization of egg products must be supervised but also the rest of measures, why? Because the risk may be still persist and that what occurred when processing of egg white under the minimum conditions specified by the USDA was insufficient to eliminate *L. monocytogenes* which may be a potential problem in refrigerated pasteurized liquid egg white-based products (Muriana, 1997).

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