Iranian Journal of Clinical Infectious Diseases
2009;4(4):214-218

ORIGINAL ARTICLE

Role of *Listeria monocytogenes hlyA* gene isolated from fresh cheese in human habitual abortion in Marvdasht

Mohammad Kargar*, Abdolazim Ghasemi
Department of Microbiology, Islamic Azad University, Jahrom Branch, Jahrom ,Iran

ABSTRACT

Background: *Listeria monocytogenes* is the causative agent of listeriosis, a highly fatal opportunistic food borne infection. Listeriolysin O is a major virulence factor in this bacterium, which is encoded by *hlyA*. The aim of the present study was to determine the relation of *Listeria monocytogenes hlyA* gene isolated from fresh cheese with habitual abortion in Marvdasht.

Materials and methods: In this cross-sectional study, 428 fresh cheese samples from four geographical area of Marvdasht were collected, then cold enriched and cultured in Hicrome *Listeria* Agar and Palcam Agar. Specific biochemical and sugar fermentation tests were used for identification of probability bacteria. Finally, *hlyA* gene was determined by PCR method.

Results: Of 428 samples, 56 (13.1%) *L. monocytogenes* were isolated, among which 91.7% were revealed to encode *hlyA*. Data analysis revealed significant association between months of sampling and isolated bacteria (p<0.004). Also, there was a significant association between bacteria and *hly A* gene with human abortion.

Conclusion: Fresh cheese and unpasteurized milk contaminated with *L.monocytogenesis* can be one of the reasons of habitual abortion in Marvdasht. Therefore pregnant women and immunocompromised people are strongly recommended not to consume fresh cheese and unpasteurized milk.

Keywords: *Listeria monocytogenes, hlyA gene, Fresh cheese, Habitual abortion.*
(Iranian Journal of Clinical Infectious Diseases 2009;4(4):214-218).

INTRODUCTION

The gram-positive bacterium *Listeria monocytogenes* is the causative agent of listeriosis, a highly fatal opportunistic food borne infection. Pregnant women, neonates, the elderly, and debilitated or immunocompromised patients in general are predominantly affected, although the disease can also develop in normal individuals. Clinical manifestations of invasive listeriosis are usually severe and include abortion, sepsis, and meningoencephalitis (1,2). Infection in utero may induce labor, resulting premature bright of an infected or stillborn fetus. Symptoms in mother usually subside following the delivery of infected infant and the placenta. Fetal survival is partly determined by the length of gestation, with spontaneous abortion occurring when the infection is acquired early in pregnancy, and neonatal infection resulting when the infection is required later (3). In 1941, Harvey and Faber demonstrated for the first time the production of a soluble hemolysin by *L. monocytogenes*.

Received: 21 September 2008 *Accepted*: 5 September 2009
Reprint or Correspondence: Mohammad Kargar, PhD. Department of Microbiology, Islamic Azad University, Jahrom Branch, Jahrom ,Iran..
E-mail: mkargar@jia.ac.ir

In 1985, Vicente et al. reported the molecular cloning of a chromosomal fragment from *L. monocytogenes* that conferred hemolytic activity on *E. coli*. These authors were the first to use molecular genetics in the study of listerial virulence factors. Finally, in 1987, Geoffroy et al. provided the first unambiguous evidence that the hemolysin of *L. monocytogenes* is an SLO-related cytolysin belonging to the family of cholesterol-dependent, pore-forming toxins. This toxin was given the name listeriolysin O (LLO). In addition to the emergence of *L. monocytogenes* as a major food-borne pathogen, the 1980s also marked the start of investigations into the molecular mechanisms underlying *Listeria* virulence. The attention of researchers was first drawn to hemolytic activity, these studies led between 1986 and 1989 to the discovery of the hemolysin gene, *hly*, and to elucidation of the key role that hemolysin plays in escape from destruction inside phagosomes, a prerequisite for intracellular bacterial proliferation (1). In 1981, a listeriosis outbreak involving 41 people occurred in Canada. Totally, 34 of the cases involved prenatal infections. There were 9 stillbirths. Of 23 infected infants that were born alive nearly one-third died. Of 7 non-pregnant adults who developed full symptomatic disease nearly 30% died. The source of the outbreak was coleslaw produced by a local manufacturer. In 1985 in California 142 people who consumed a certain braned of soft cheese developed symptomatic listeriosis. Of these 93 were prenatal cases and 49 were adult cases. Thirty fetuses or newborn infants died, and 18 adults died. Of 49 adult cases, 48 occurred in people who were immunocompromised or elderly. The source of the outbreak was cheese (2). Because *L. monocytogenes* is effectively inactivated by commercial heat treatments (pasteurization) used in the dairy industry, the food processing environment seems to represent a major source of finished product contamination. Whereas *L. monocytogenes* has been reported to be regularly isolated from food and dairy processing environments, a better knowledge of the spread and survival of *L. monocytogenes* in food processing environments and the contributions of different environmental sources to finished product contamination are necessary to understand the transmission of *L. monocytogenes*. In the processing environment, detection and isolation of *L. monocytogenes* followed by molecular subtype characterization provides an opportunity to study the molecular ecology of *L. monocytogenes* in food processing plants. A thorough understanding of the molecular ecology of food borne pathogens is necessary for the design of rational and science-based methods and approaches for the control of *L. monocytogenes* and other food borne pathogens (4).

The aim of this study was to investigate fresh cheese contamination to *L. monocytogenes* and the relation of *hly* gene with habitual abortion in Marvdasht.

PATIENTS and METHODS

In this cross sectional study, 428 fresh cheese samples were collected from four geographical area of Marvdasht (Markazi, Seidan, Dorodzan, and Komfiroz). Briefly, 25-g samples of cheese were aseptically added to 225ml of Triptic Soy Broth with Yeast Extract (TSBYE) and homogenized in a Stomacher. The homogenized samples were incubated at 4°C. After one week incubation, aliquots from TSBYE were streaked onto PALCAM Agar and Hicrome Listeria Agar. Typical Listeria-like colonies from PALCAM and Hicrome Listeria Agar were purified on Neutriant Agar and identified using morphological, cultural, and biochemical criteria. In particular, Gram stain, catalase test, motility, ß-hemolysis, and production of acids from rhamnose and xylose were used, as described in Bergey's manual, to identify the species belonging to the genus (3). Finally, with PCR method present of virulence gene *hlyA* has been tested. *L. monocytogenes* was cultured in

laboratory and DNA was extracted by boiling method. Briefly, bacterial suspension was transferred to micro tube and lysed by lyses buffer (330mM Glucose, 10mM Tris , 5mM $MgCl_2$ %2 Triton X-100 , %2 SDS) for one hour at 37°C and boiled for 10 minutes. Bacterial lysate was centrifuged at 5000g for 5 minutes and supernatant transferred into a new micro tube and subjected for PCR amplification. PCR was performed in a final volume of 25µl containing 2.5µl PCR Buffer, 1µl $MgCl_2$, 0.25µl (each) deoxynucleoside triphosphates, 0.1µl of *Taq* polymerase, and 0.5µl (each) primers targeting the *hlyA* gene encoding the invasion-associated hemolysin in *L. monocytogenes*. Primers were named forward (5'-TGTTAATGAACCTACAAGACCTTC-3') and reverse (5'-TAGTTCTACATCACCTGAGAC AGA-3'). The samples were subjected to amplification in a MiniCycler (Genenco, Florence, Italy) using the following program: 95°C for 5 min; 30 cycles of 95°C for 1 min, 62°C for 45 sec, and 72°C for 1.5 min; and, at the end , 72°C for 5 min. Finally, 5µl of PCR product was transferred to the %2 gel containing ethidium bromide which of the electrophoresis, was investigated by UV transilluminator (5).

The data were then entered and analyzed using SPSS soft ware (version 14. SPSS Inc., USA) and p value was calculated using chi-square and Fisher's exact tests to find the significant relationship. P value less than 0.05 was considered statistical significant.

RESULTS

Of 428 samples, 56 (13.1%) were revealed to be infected with *L. monocytogenes*. Among 56 isolated bacteria, 51 (%91.1) entailed *hlyA* gene, according to PCR results (figure 1).

Table 1 represents the frequency of *L. monocytogenes*, and human and animal habitual abortion in Marvdasht. Among the aforementioned regions, contamination was more commonly observed in Kamfiroz (%4.7) and less commonly in Seidan (%1.9). On the other hand, of 22 reported human abortions, 8 (36.4%) occurred in Markazi area. Meanwhile, 10 cases of simultaneous human and animal abortion was also detected (table 1). Data analysis revealed significant association between months of sampling and isolated bacteria (p<0.004). Also, there was a significant association between bacteria and *hly A* gene with human abortion.

Table 1. Frequency of *Listeria monocytogenes*, human and animal habitual abortion in Marvdasht

Sampling Site	*Listeria monocytogenesis*	Animal & human simultaneous abortion	Human habitual abortion
Markazi	11 (2.57)	2 (0.46)	8 (1.87)
Seidan	8 (1.87)	2 (0.46)	5 (1.17)
Dorodzan	17 (3.97)	3 (0.7)	4 (0.93)
Kamfiroz	20 (4.67)	3 (0.7)	5 (1.17)
Total	56 (13.08)	10 (2.33)	22 (5.14)

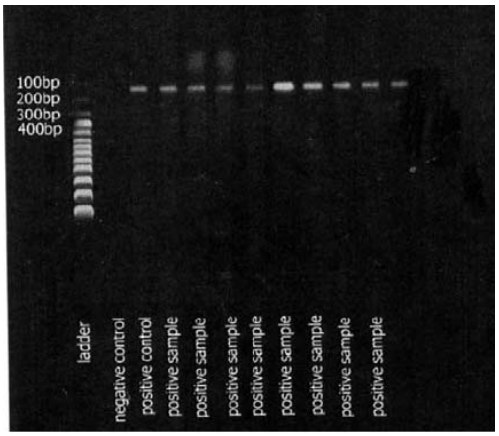

Figure 1. PCR products of hlyA gene (224 bp) on %1.5 agaros gel stained with Ethidium bromide (Ladder 100bp).

DISCUSSION

L. monocytogenes is present as a contaminant in many kinds of food stuffs, including raw milk, raw vegetables, fish, poultry, and both fresh and processed meat. In contaminated food, colony counts of the organism may exceed 10^9 colony–forming unit (CFU)/g of food. Shedding of organism from the udder or contamination from the

environment can lead to the presence of *L.monocytogenes* in raw milk. Surveys have indicated that *L.monocytogenes* may be present in approximately 4% of raw milk samples examined in the U.S. The organism has also been recovered from unpasteurized milk and cheeses prepared from unpasteurized dairy products (3). In Silva et al. study, out of various types of cheese in Rio de Janeiro, 11 (%10.7) were contaminated by *L.monocytogenes* (6). In 2000, Waak et al. studied the incidence of Listeria species in raw whole milk from farm bulk tanks and from raw milk in storage at a Swedish dairy plant. *L.monocytogenes* was found in %1.0 and *L. innocua* was found in %2.3 of 294 farm bulk tank (farm tank) milk (7).

In 2006, Burton et al surveyed frequency of *hlyA* gene in isolated *L.monocytogenes* from foods, as well as environmental and clinical samples by PCR method (5). In Iran, Saadatzadeh et al (1965-1971) studied 208 cases of habitual abortion among which 26 Listeria spp. were isolated. Lashkari et al could isolate *L.monocytogenes* from blood culture of women with habitual abortion. Vand–Yosefi and Moradi studied a total 2216 serum samples of patients with habitual abortion, among which %42.5 were Listeria positive (8). Out of 720 dairy samples surveyed by Mojtahedi et al (2004) 70 were infected with *L.monocytogenes* (9).

Results revealed a notable contamination rate of fresh cheese with *L.monocytogenesis* (%13.1) and high rate of animal (%13.8) and human abortion (%5.1) in Marvdasht. Statistic analysis showed a significant association between human habitual abortion and these bacteria in studied areas. For this reason extended molecular epidemiology researches in other cities of Iran is strongly recommended.

Antibiotic treatment of pregnant women or immunocompromised people who have eaten food contaminated by *L.monocytogenesis* can prevent the most serious consequences of listeriosis, but only if the infection is diagnosed in time. Another complication is that *Listeria* is able to grow well at law temperatures. Thus, refrigeration is not as effective in preventing growth of *Listeria* in food as it is for most other bacteria that cause food-born disease (2).

In conclusion, fresh cheese and unpasteurized milk contaminated with *L.monocytogenesis* can be one of the reasons of habitual abortion in Marvdasht. Therefore pregnant women and immunocompromised people are necessary to avoid consuming fresh cheese and unpasteurized milk. Also, avoiding contamination of dairy products during processing would be an ideal solution.

ACKNOWLEDGEMENT

The authors wish to extend their thanks and appreciation to the Islamic Azad University, Jahrom Branch, for their financial and executive support of this project.

REFERENCES

1. Jose A, Vazquez B, Michael K, Patrick B, Trinad C, Gustavo B, et al. Listeria pathogenesis and molecular virulence determinants. J Clin Microbiol Rev. 2001;14: 584-640.

2. Salyers AA, Whitt DD, editors. Bacterial pathogenesis. A molecular approach. 2nd ed. Washington D.C: ASM press;2002:398-406.

3. Washington WJ, Stephen A, William J, Koneman E, Procop G, Scheckenberger P, editors. Color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams'& Wilkins;2006: 765-73.

4. Lecuit M, Cossart P. *Listeria monocytogenes*. In: Sussman M, editor. Molecular medical microbiology. 2nd edition. California: Academic Press;2002:1437-55.

5. Burtun W, Blais M. Identification of presumptive positive *Listeria monocytogenes* from foods and environmental samples by the polymerase chain reaction (PCR). Health Canada, Food & Nutrition 2006;3:22-7.

6. Silva D, Delgado MC, Anita T. Incidence of *Listeria monocytogenes* in cheese produced in Rio de Janeiro, Brazil. Journal of Food Protection 1998;61:354-6.

7. Waak E, Tham W, Danielsson TML. Prevalence and fingerprinting of *Listeria monocytogenes* strains isolated

fromk raw whole milk in farm bulk tanks and dairy plant receiving tanks. Appl Environ Microbiol 2002;68:3366-70 .

8. Vand–Yosefi J, Moradi S. Evaluation contamination of Listeria monocytogenes in raw and pasteurized milk in Iran. Journal of Pajouhesh and Sazandegi 1992;17:57-65. (In Persian)

9. Mojtahedi A, Tarahi MJ, Sepahvand A, Kakpour A, Radsari A, Tavasoli M, et al. The frequency of Listeria contamination in dairy products that referred to laboratory food and health administration in Lorestan province. Yafteh 2004;22:27-30. (In Persian)