International Journal of Medical Laboratory 2018;5(4):294-302.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

Distribution and Characterization of Dominant Serovars of *Listeria monocytogenes* Strains Isolated from Spontaneous Human Abortion in Tehran

Maryam Rezaei[1]M.Sc., Nadia Kazemi Pour[1*]Ph.D., Jalil Vandyousefi[2]Ph.D. Farokh Rokhbakhsh Zamin[1]Ph.D., Gholamreza Irajian[3]Ph.D.

[1]Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran.
[2]Shila Medical Diagnostic Laboratory, Tehran, Iran.
[3]Department of Microbiology, Iran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Article history
Received 23 Jan 2018
Accepted 15 Jul 2018
Available online 29 Nov 2018

Key words
L. monocytogenes
Serovars
Spontaneous abortion

Background and Aims: The aim was to determine the role of dominant serovars of *Listeria monocytogenes* (*L. monocytogenes*) in spontaneous abortions, using isolation methods and polymerase change reaction (PCR).

Materials and Methods: A total of 258 samples comprising of placental tissue, vaginal swabs and blood were collected from 123 patients with spontaneous abortion. *L. monocytogenes* was identified and confirmed by culture, biochemical reactions, serological tests, API system, CAMP (Christie, Atkins, Munch and Petersen) test, and hemolysis on sheep blood agar. Phosphatidyl inositol specific phospholipase C (PI-PLC) assay, followed by multiplex PCR was applied for detection of serotypes 1/2a and 4b.

Result: Out of 258 samples, 28 isolates of *L. monocytogenes* were identified by different methods. All of the isolates were confirmed by PCR. Of 28 isolated strains, 14(50%) belonged to serovar 1/2a, 10(35.7%) to serovar 4b and 4(14.3%) to other serovars.

Conclusions: Based on our study, serovars 1/2a and 4b are dominant serovars as causative agents of human spontaneous abortion due to *L. monocytogenes* in pregnant women.

[*]**Corresponding Author:** Department of Microbiology, Kerman branch Islamic Azad University Kerman, Iran. **Tel:**+989021202236, **Email:** nadia_kazemi@yahoo.com

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]

Introduction

Listeria monocytogene (L. monocytogenes) is a gram positive, non-sporulating, facultative, intracellular, pathogenic bacterium that causes morbidity and mortality in human and livestock. It is a significant food-borne pathogen due to its widespread distribution in nature, ability to survive in a wide range of environmental condition, and ability to grow at refrigerator temperature. *L. monocytogenes* has been found in 10% of normal healthy people usually in the gut. All the 13 serovars of *L. monocytogenes* are reported to cause human listeriosis, but serovars 1/2a, 4b and 2c are implicated in most cases. Pregnant women are particularly prone to infection, and placenta provides protective niche for its growth, thereby resulting in spontaneous abortion, stillbirth neonatal infection, severe necrotizing hepatitis, placental necrosis and increased risk of post implantation loss. Latent listeriosis in pregnant women leads to habitual abortion [1, 2]. *L. monocytogenes* causes both invasive and noninvasive infections. Invasive listeriosis is a severe disease mainly associated with groups of people specifically at risk, including fetus, neonates, immunocompromised individuals and persons in contact with animals [3, 4]. Mild non-invasive infection can also occur in about 10% or more healthy persons usually in the gut. Non-invasive infection of *L. monocytogenes* in pregnant women causes abortions, stillbirth and fetal death [5]. The incidence of listeriosis in general population is 0.7 in 100000 but its prevalence in pregnant women is 12 in 100000 [6, 7].

L. monocytogenes, a high-risk emerging food pathogen, has recently assumed great interest as a result of its association with several outbreaks of listeriosis across the world including a wide variety of foods, both raw and processed [3, 4, 7]. Its ability in survival and growth in many foods during processing and storage, has been attributed to its ubiquitous nature, resistance to diverse environmental conditions such as, low and high salt concentration and its microaerobic and psychrophilic nature [6, 8-10]. The Food and Drug Administration (FDA) definition of zero tolerance for the organisms in processed ready-to-eat foods has emphasized the need for development of molecular-rapid methods for detection of *L. monocytogenes* and its different serotypes and genes [11-13]. Serotyping is an ordinary accepted subtyping method for *L. monocytogenes*. Identification of the strain serotype permits differentiation between important food-borne strains and provides "gold standard" for comparing isolates analyzed in different labs with different techniques. According to some reports in Iran, serovars 1/2a, 4b, 2b, 4a and 2c have been isolated from animal products (raw-processes) [14-16]. The occurrence of *L. monocytogenes* in Tehran, Iran has been underreported in many cases because of the inefficient surveillance and monitoring systems. Nevertheless, different serovars of

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]

L. monocytogenes have been isolated from food and humans [8 ,17].

The present study tried to detect dominant serovars (1/2a, 4b) in pathogenic *L. monocytogenes* isolated from women with spontaneous abortion in Tehran.

Materials and Methods

Study site and sample collection

During May 2016 to November 2017, a total of 258 human clinical samples including placental tissues (n=118) , vaginal swabs (n=87), and 5 milliliters of blood (n=53) were collected from 123 hospitalized women with spontaneous abortion in 4 private and 4 government-sponsored hospitals in Tehran, Iran. The abortions had occurred during the second and third trimesters of pregnancy. All samples were collected aseptically at the day of abortion and were quickly transported on an ice pack to the microbiology department and processed within 24 hours of collection [7, 9]. The necessary ethical clearance was obtained from University Ethics Committee. The ethical permossions was taken for collection and processing of human clinical samples.

Isolation of *Listeria*

All samples were homogenized in trypticase soy broth (TSB) with 0.6% yeast extract and placed at a 4˚C cold enrichment for a period of 4 or 6 weeks.

After 4 days the green shiny colonies surrounded by diffuse dark shadow around them on PALCAM agar, and grey shiny colonies surrounded by alpha hemolytic colonies appeared on blood agar.

Approval of isolates were performed by standard microbiological and biochemical tests such as gram staining, catalase reaction, oxidase test, tumbling motility at 20-25˚C, Methyl red-Voges-Proskauer (MR-VP) reaction, nitrate reduction, Chrisite-Atkins- Munch and Petersen (CAMP) test, phosphatidyl inositol specific phospholipase C (PI-PLC) assay, Application Programming Interface (API) and congored adsorption (Table 1) [3, 18-19]. The confirmed *L. monocytogenes* were stored in TSB including TSB 10% and glycerol 5%.

Mice inoculation test

The pathogenicity testing of *Listeria* was performed by mice inoculation as described by Menudier et al. Briefly, all isolates of *Listeria* were grown on Tripticase Soy Agar (TSA) slants at 37˚C for 24 hrs. The bacteria were harvested with a sterile normal saline solution, and the capacity of inoculum was adjusted to Macfarland nephelometric tube number one. The mice weighting 18-20 gram were inoculated interperitoneally with 0.4 mL of inoculum having approx 10^7 colony forming units (CFU) [20, 21]. The inoculated mice were observed for mortality over a period of 5 days, any *L. monocytogenes* isolates causing death after 5 days of inoculation were assumed as pathogenic [20, 22].

DNA Extraction

One milliliter of an overnight culture was incubated with penicillin G (500 $U.mL^{-1}$) for one hour at 37˚C and then transferred to 1.5 mL microfuge tubes and centrifuged at 800 rpm for 5 minutes. The supernatant was then discarded and 500 µL of cetyltrimethyl ammonium bromide buffer at 60˚C was added

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]

to the microfuge tube containing bacterial pellet. Afterwards the mixture was held in a water bath at 64˚C for 20 min., and briefly mixed several times during incubation. After incubation 500 µL of chloroform/octanol (24:1) was added and mixed vigorously followed by centrifugation at 3000 rpm for 15 min. [22]. The supernatant was transferred to clean microfuge tube and an equal volume of ice-cold isopropanol was added and kept on ice bath for 2 hrs. Precipitate of the solution was centrifuged at 8000 rpm for 8 min, the aqueous phase was discarded, and the DNA pellet was rinsed with 80% ethanol air-dried and resuspended in 50-100 mL distilled water and used for PCR. The primers for the detection of *L. monocytogenes* and dominant serovars 1/2a and 4b were used in this study synthesized by Cinagen Iran. The primer sequence are shown in Table 2 [23-24]. DNA amplification was performed in a DNA thermal cycler (Eppendorf-Nathel-Germany). The amplification conditions for identification of *L. monocytogenes* in PCR assays were those described by other researchers [24-25].

The Multiplex PCR assay was standardized for the detection of two major dominant serovars of *L. monocytogenes* namely 1/2a and 4b following the methodology described by Doumith et al [26] (Fig. 1).

PCR products were analyzed by 1.5% agarose gel electrophoresis and specific DNA bands were visualized using ethidium bromide staining under UV illumination.

Results

The types and number of spontaneous abortion samples analyzed in this study are presented in Table 3. Among 258 samples from spontaneous abortion, 28 (18.8%) isolates were identified as *L. monocytogenes* infection by microbiological tests. Contamination rate was 21 (17.7%), 2 (3.7%) and 5 (5.7%) for placental tissue , blood and vaginal swabs, respectively. The standardized PCR allowed amplification of dominant serotypes of *L. monocytogenes* namely 1/2a and 4b. All of the 28 isolates of *L. monocytogenes* were found to be pathogenic by PI-PLC and pathogenicity test by mice inoculation. Serovars 1/2a (50%) and 4b (35.7) were dominant in samples.

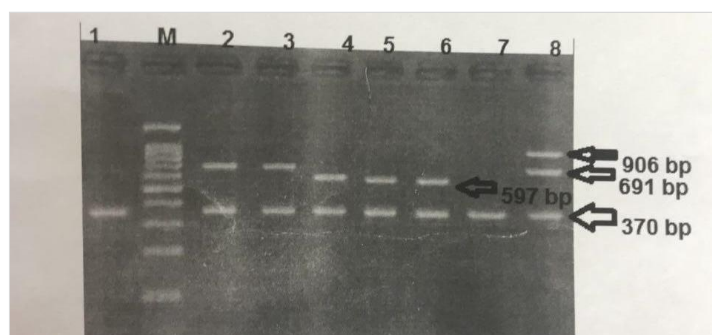

Fig.1. Multiplex PCR amplification of the dominant PCR: Lane M: DNA ladder 1 Kb, Lane 1, 7: Positive control *(L. monocytogenes* ATCC7644), Lane 2,3: 1/2a, Lane 4-6: 4b, lane 8: 2c.

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]

Table 1. Pathogenicity and biochemical reaction of *L. monocytogenes* isolates from spontaneous abortion

Isolates	Source	CAMP(S*)	API	PI-PLC	Mice lethality	C. red test	Tumbling test
1	Placental tissue	+	+	+	+	+	+
2		+	+	+	W4	+	+
3		+	+	-	+	+	+
4		+	+	+	+	+	W4
5		+	w	+	+	+	+
6		W4	+	+	W4		+
7		+	+	+	+	-	+
8		+	+	+	+	+	+
9		+	+	W4	+	+	+
10		+	+	+	W4	+	+
11		+	+	+	W4	+	+
12		+	+	+	+	+	+
13		+	W4	W4	+	+	+
14		+	-	+	+	+	+
15		+	+	+	+	-	+
16		+	+	+	+	+	+
17		+	+	W4	+	+	+
18		+	+	W4	+	+	+
19		+	+	+	+	+	-
20		+	+	+	+	+	+
21		+	+	+	W4	+	+
22	Blood	+	+	+	W4	+	+
23		+	+	+	+	+	+
24	Vaginal swab	+	+	+	+	+	+
25		+	+	+	+	+	+
26		+	+	+	+	+	-
27		+	+	+	W4	+	+
28		+	W4	+	+	+	+

*CAMP with staphylococcus aureus ATCC=25923; API= Application programming interface; PI-PLC= Phosphatidyl inositol specific phospholipase C; W4= Weakly positive after 4 days

Table 2. Identities and nucleotide sequences of serotypes (1/2a,4b*)* *L. monocytogenes* primes

Name	Sequences (5'-3')	Molecular weight	nmol	Temperature	GC%	Length
Prs	F: GCTGAAGATTGCGAAAGAAG	6881.5	19.18	58.39	45	22
	R: CAAAGAAACCTTGGATTGCGG	6783.4	17.03	58.39	45	22
Imo737	F: AGGGCTTCAAGGACTTACCC	6102	21.63	59.35	55	20
	R: ACGATTTCTGCTTGCCATTC	6033.9	21.88	55.25	45	20
ORF2110	F: AGTGGACAATTGATTGGGTGAA	6549.3	17.64	53.97	38	21
	R: CATCCATCCCTTTACTTTGGAC	6292.1	15.73	57.87	48	21

Table 3. The type and number of spontaneous abortion samples analyzed in this study

Source (various samples)	No. of samples (%)	No. of positive isolates	Serovar 2a	Serovar 4b	Other serovars
Placental bits	118 (45.8)	21	9	8	4
Blood	53 (20.5)	2	2	-	-
Vaginal swabs	87 (33.7)	5	3	2	-
Total	258 (100)	28	14	10	4

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]

Discussion

L. monocytogenes as one of the most virulent food-borne pathogens are transmitted to humans through consumption of food and induce septicemia, meningitis and abortion. They might be spread out in two forms: sporadic or epidemic. In pregnant women, self-limited flu-like symptoms are first presented, then spontaneous abortion, stillbirth, premature birth and other symptoms in infants will appear. In aged and immunity-challenged individuals it causes listeriosis [25, 27]. The incidence of listeriosis in pregnancy is 12 per 100000. With a regular rate of 0.7 per 100000 in the general population, one-third of listeriosis infections belong to spontaneous abortion [6, 7, 28].

Listeriosis has been reported to occur either in sporadic or epidemic form; however, there are certain Asian countries where the disease has been underreported [28-31]. Research on *L. monocytogenes* as the main abortive agent is not yet fully explored in Iran. Scientific reports on this topic have not been covered by Iran's health and sanitation organizations as well. The main reason for this gap is the lack of access to the standard diagnostic methods in Iran. There have often been reports on *L. monocytogenes* isolated from raw milk, cheese, ready-to-eat meat and vegetables in which the serovars 1/2a and 4b are detected. However, the correlation between these serovars and spontaneous abortion was not identified. Among few reports on their genotypes which are conducted with PCR method, virulence genes are recorded [11, 16, 17, 30]. There have not yet been reliable statistics on spontaneous and habitual abortions in Iran. The reason for this weakness has mainly been the methods of isolation and detection. On the contrary, in this research the selected methodologies have been totally unique based on standard bacteriological, biochemical and molecular isolation techniques where by the resulted isolation percentage is distinct from the previous reports in Iran [5, 28, 29]. In this research 258 samples from 123 patients with spontaneous abortion were collected. Isolation resulted in 28 cases (18.8%) of *L. monocytogenes* from 118 (45.8%) placental bits, 53 (20.5%) blood samples and 87 (33.7%) vaginal secretions. *L. monocytogenes* isolation percentage is reported diversely in various countries. Kaur et al. (2007) isolated 4 *L. monocytogenes* samples from 305 specimens collecting from 67 patients [6]. In Belgrade, one *L. monocytogenes* isolate was found in 958 clinical samples [32]. Dhermendra et al. (2015) isolated 5 *L. monocytogenes* out of 300 clinical samples [33]. Lotfollahi et al. isolated 9 *L. monocytogenes* out of 100 clinical samples [15]. Eslami et al. (2014) reported 16 *L. monocytogenes* in 96 clinical samples of spontaneous abortion with the latest bacteriological and molecular techniques [34]. As *L. monocytogenes* is one of the virulent intracellular bacteria to causes spontaneous abortion, in this study for the first time, the pathogenic species were identified and

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]

evaluated with multiplex PCR. API assay was also followed to confirm the other experiments. In this study 28 (18.8%) *L. monocytogenes* with various serovars were isolated. The presence of *L. monocytogenes* in placental bits and blood, in this study, indicated the most and the least outbreaks in all clinical samples. Pournajaf et al. also confirmed *L. monocytogenes* isolated from vaginal secretion (14.5%) and in placental bits (7.5%) [35]. In that study on samples of placental bits, blood and vaginal secretions, the isolates belonged to two patients in their second trimester of the pregnancy with averaged age of 28.5 years. *L. monocytogenes* isolates in this study demonstrated virulence based on all confirmatory and pathogenicity tests in *in vivo* conditions. *L. monocytogenes* in one case was isolated from placental bits of a 37 years old woman with spontaneous abortion in the early trimester of pregnancy. *L. monocytogenes* was also obtained from vaginal secretions as well as placental bits in three patients with the average age of 30 years.

In 123 patients (17.7%) it was isolated only from placental bits. It is indicated that *L. monocytogenes* has a desire for placental tissue. A number of factors are implicated in the virulence of *Listeri*a. The first factor is PI-PLC which is expressed by pathogenic species. The goal in this was discrimination of virulent and l pathogenic *L. monocytogenes* from other non- pathogenic *L. monocytogenes* isolates which were confirmed by pathogenic tests on live mice so that 24 (85.7%) cases were positive for PI-PLC but 4 (14.2%) cases were getting positive after four days. Sattari and colleagues' study supported these results [36]. Another part of this study investigated dominant serotypes. Our results showed that serovars 1/2a and 4b were dominant. It is for the first time in Iran that serovars 1/2a and 4b are reported as dominant serovars isolated from spontaneous abortions. The correlation between the dominant serovars in spontaneous abortion in this research, indicated that these serovars play key roles in spontaneous abortion.

Conclusion

We determined *L. monocytogenes* infection in women with history of spontaneous abortion. Serovars 1/2a and 4b are the most important in spontaneous abortion in Iranian women. Determination of serovars 1/2a and 4b in women with spontaneous abortion is recommended.

Conflict of Interests

There is no conflict to declare.

Acknowledgements

The authors are indebted to staff and member of the Department of Microbiology of Azad University Branch in Kerman and also Faculty of Medicine, Iran University of Medical sciences as well as members of the Shila Medical Laboratory.

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]

References

[1]. Ramaswamy V, Cresece VM, Rejita JS, Lekhshmi MU, Dharsana KS, Prasad SP, et al. Listeria review of epidemiology and pathogenesis. J Microbiol Immunol Infect. 2007;40(1): 4-13.

[2]. Salyers AA, Whitt DD. Bacterial pathogenesis. A molecular approach. 2nd ed. Washington DC: ASM press; 2002. pp. 398-406

[3]. Destro MT, Serrano AM, Kabuki DY. Isolation of Listeria species from some Brazilian meat and dairy product. Food Control. 1991; 2(2): 110-12.

[4]. Bakardjiey AI, Stacy BA. Growth of L. monocytogenes in guinea pig placenta and role of cell-to-cell spread in fetal infection. J Infect Dis. 2005; 191: 1889-897.

[5]. Aljicević M, Beslagić E, Zvizdić S, Hamzić S, Mahmutović S. L. monocytogenes as the possible cause of the spontaneous abortion in female of the fertile age. Bosn J Basic Med Sci. 2005; 5(4): 89-92

[6]. Kaur S, Malik SVS, Bhilegaonkar KN, Veidya VN. Barbuddhe SB. L. monocytogenes in spontaneous abortion in human and its detection by Multiplex PCR. J Appl Microbiol. 2007; 20: 145-52.

[7]. Bahador A, Sadeghi Kalani B, Valian F, Irajian GH, Lotfollahi L. Phenotypic and genotypic characteristics of L. monocytogenes isolated from dairy and meat products. J Accute Dis. 2015; 2(3): 321-26.

[8]. Gudarzi E, Yousefi J, Harzandi N. Survey of PCR efficiency in the detection of Listeria, Brucella and Mycoplasma in culture in negative samples obtained from women with abortion. J Mazandaran Univ Med Sci. 2013; 23(105): 61-9.

[9]. Hudson JA. Efficacy of high sodium chloride concentration for the destruction L. monocytogenes. Lett Appl Microbiol. 1992; 14(4): 178-80.

[10]. Low JC, Donachie WA. Review of *L. monocytogenes* and listeriosis. Vet J. 1997; 153(1): 9-29.

[11]. Mead PS, Slutsker L, Slutsker L, Dietz V, Mc Caig LF, Bresee JS, et al. Food-related illness and death in the United States.Emerg. Infect Dis. 1999; 5(5): 607-25.

[12]. Schmid M, Walcher M, Bubert A, Wagner M, Schleifer KH. Nucleic acid bases, cultivation in depended detection of *Listeria* spp. And genotypes of *L. monocytogenes*. Immun Medical Microbiol. 2003; 35(3): 215-25.

[13]. Ingianni A, Floris M, Palomba P. Madeddu M, Quartuccio M, Pompei R. Rapid detection of L. monocytogenes in foods , by a combination of PCR and DNA probe. Mol cell probes. 2001; 15(5): 275-80.

[14]. Seeliger H, Hohen K. Serotyping of L. monocytogenes and related species. Methods Microbiol. 1979; 13(11): 31-49.

[15]. Lotfollahi L, Nowroozi J, Irajian GH, Kazemi B. Prevalence and antimicrobial resistance profiles of L. monocytogenes in spontaneous abortion in humans. Afr. J Microbiol Res. 2011; 5(14): 1990-993.

[16]. Fagundes de Mello J, Einsfeldt K, Paula Guedes Frazzon A, da Costa M, Frazzon J .Molecular analysis of the iap gene of L. monocytogenes isolated from cheeses in rio grande do Sul, Brazil. Braz J Microbiol. 2008; 39(1): 169-72.

[17]. Vandyousefi J, Moradi S. Evaluation contamination of L. monocytogenes in row and pasteurized milk in Iran. J Sci Info Database 1992; 1(1): 57-65.

[18]. Notermans SHW, Dufrnne J, Leimeister-Wächter M, Domann E, Chakraborty T. Phosphatidylinositol- specific phospholipase C activity as a marker to distinguish between pathogenic and non-pathogenic species. Appl Envirin Microbiol. 1991; 57(9): 2666-670.

[19]. Setiani BE, Elegado FB, Perez MTM, Mabesa RC, Dizon EI, Sevilla CC. API Listeria rapid kit for confirmatory phenotypic conventional biochemical test of the prevalence L. monocytogenes in selected meat and meat products. Procedia Food Sci. 2015; 3(1): 445-52.

[20]. Monudier A, Bosiraud C, Nicolas JA. Virulence of L. monocytogenes serovars and Listeria spp. In experimental infection of mice.J Food Prot. 1991, 54,917-21.

[21]. Palerme JS, Pan PC, Parsons CT, Kathariou S, Ward T, Jacob ME. Isolation and characterization of atypical L. monocytogenes associated with a canine urinary tract infection. J Vet Diagn Invest. 2016; 28(5): 604-607.

[22]. Peik G, Suggs MT. Reagents, stains and miscellaneous test procedures. In manual of clinical Microbiology. 1974, P:933.

[23]. Ahmadzadeh Nia S , Hanifian S. Survival of L. monocytogenes strains in ultra-filtered white cheese: Effect of Lactobacillus plantarum and incubation period. J Food Process ad Preserv. 2017; 41(6): e13283.

[24]. Bubert A, Hein I, Rauch M, Lehmer A, Yoon BW. Detection and differentiation of Listeria spp. By a single reaction based on multiplex PCR. Appl Environ Microbiol. 1999; 65(10):4688-692.

[25]. Kargar M, Ghasemi A. Role of L. monocytogenes hlyA gene isolated from fresh cheese in human habitual abortion in Marvdasht. J Clinic Infect Dis. 2009; 4(4): 214-18.

[26]. Doumith M, Buchrieser C, Glaser P Jacquet C, Martin P. Differentiation of the Major L. monocytogenes Serovars by Multiplex PCR. J Clin Microbiol. 2004; 42(8): 3819-822.

[27]. Romana C, salleras L, Sage M. Latent Listeriosis may cause habitual abortion

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]

intrauterine deaths, fetal malformation. When diagnosed and treated adequately normal children will be born. ActA Microbiol Hung. 2002; 36(2): 171-72.

[28]. Mansouri MN, Kianpour M, Sami M, Jajarmi M. Prevalence of L. monocytogenes in row milk in Kerman, Iran. Vet Res. 2015; 6(3): 223-26.

[29]. Pourkaveh B, Ahmadi M, Eslami G, Gachkar L. Factors contribute to spontaneous abortion caused by L. monocytogenes in Tehran, Iran. Cell Mol Biol. 2016; 62(9): 3-10.

[30]. Low JC, Donachie W. A review of L. monocytogenes and listeriosis. Vet J. 1997; 153(1): 9-29.

[31]. Sedighimoghadam B. Assesment to indirect method of hemagglutinin diagnosis of L. monocytogenes and other comparison with indirect immunofluorescence method. J Semnan Med Sci. 2001; 12(1): 51-8.

[32]. Stepanovic S, Vukovic D, Djukic S, Cirkovic I, Svabic-M. Long- term analysis of L. monocytogenes carriage frequency in Belgrade, Serbia. Acta Microbiol Immunol Hungari. 2007; 54(2): 195-99.

[33]. Dhermandra KS, Durg VS, Suresh KD. Pregnancy- associated human listeriosis: Virulence and genotypic analysis of L. monocytogenes from clinical samples. J Microbiol. 2015; 53(9): 653-60.

[34]. Eslami G, Samadi R, Taherpanah R, Taherpor A, Baseri N. Detection of acta and InlB genes in L. monocytogenes isolated from women with spontaneous abortion. Novely in Biomedicine. 2014; 2(1): 18-21.

[35].Pournajaf A, Rajabnia R, Sedighi M, Kassani A, Moqarabzadeh V, Lotfollahi L, et al. Prevalence and virulence determination of L. monocytogenes strains isolated from clinical and non-clinical samples by multiplex polymerase chain reaction. Rev Soc Bras Med Trop. 2016; 49(5): 624-27.

[36].Sattari M, Forouzandeh M. Isolation and identification of L. monocytogenes in vaginal swabs by PCR. Pathobiol Res. 2009; 12(1): 51-8.

[Downloaded from ijml.ssu.ac.ir on 2025-03-11]

[DOI: 10.18502/ijml.v5i4.158]