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META-ANALYSIS

Extraintestinal infection of *Listeria monocytogenes* and susceptibility to spontaneous abortion during pregnancy: A systematic review and meta-analysis

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Abstract

BACKGROUND

Listeria monocytogenes (*L. monocytogenes*) is one of the most important zoonotic bacteria that is transmitted to humans through infected animal products and is the cause of human listeriosis. Pregnant women and immunocompromised patients are more susceptible to the bacterium than healthy people. Recent studies have reported extensive evidence on the role of *L. monocytogenes* infection and the risk of spontaneous abortion.

AIM

To evaluate the possible connection with *L. monocytogenes* in the risk of spontaneous abortion in pregnancy.

METHODS

We conducted a systematic literature review using several databases to search the relevant case-control studies on the association between *L. monocytogenes* infection and spontaneous abortion. Finally, the impact of infection with *L. monocytogenes* and risk of spontaneous abortion was assessed *via* odds ratio at corresponding 95% confidence intervals.

RESULTS

In the present study, we evaluated the data of 4059 pregnant women who had a spontaneous abortion, and interestingly their colonization rate of *L. monocytogenes* was about 20.5%.

CONCLUSION

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Therefore, based on statistical analysis, we found that there is a significant relationship between the infection with *L. monocytogenes* and spontaneous abortion.

Key Words: *Listeria monocytogenes*; Meta-analysis; Pregnancy; Spontaneous abortion; Women

Core Tip: *Listeria monocytogenes* is one of the most common bacterial infections among developing countries. Acquisition of this bacterium during pregnancy is dangerous for the health of neonates. Our results suggested that *Listeria monocytogenes* can significantly increase the risk of spontaneous abortion during pregnancy.

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INTRODUCTION

Spontaneous abortion is one of the biggest health challenges that has increased in recent decades, especially due to *in vitro* fertilization methods, such as *in vitro* fertilization, gamete intrafallopian transfer, and intracytoplasmic sperm injection[1]. About 1% of women suffer from recurrent miscarriage, so this phenomenon can be considered a serious threat to population decline[1,2]. The prevalence of spontaneous abortion in the world is reported to be 10%-20%, and many factors such as blood type, ectopic pregnancy, smoking, obesity, history of cesarean section, fertility treatment, and infectious pathogens are involved in its occurrence[3,4]. According to the literature, several infectious microorganisms such as *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Listeria monocytogenes* (*L. monocytogenes*), herpes simplex viruses, adenoviruses, human polyomaviruses, and cytomegalovirus play an important role in individual susceptibility to spontaneous abortion[5,6].

L. monocytogenes is a gram-positive, non-spore forming, facultative anaerobe, and motile bacterium that naturally resides in sources such as water, soil, vegetables, dairy products, and processed food[7]. These bacteria enter the human body through the fecal-oral route and can withstand harsh environmental conditions such as acidic pH, high salt concentration, and low temperature[8]. This bacterium can cross the intestinal barrier and enter various tissues through hematogenous dissemination, particularly the placenta-fetal unit and the cerebrospinal fluid[9,10]. Studies show that pregnant women are about 17 times more likely to be infected with *L. monocytogenes* than the general population[11]. Numerous studies have been performed on the effects of infection of *L. monocytogenes* and abortion in humans and animals[12]. The aim of this study was to investigate the effects of extraintestinal infection with this pathogen and susceptibility to spontaneous abortion. We estimated the frequency of *L. monocytogenes* colonization in the women who had a spontaneous miscarriage.

MATERIALS AND METHODS

Search strategy and study selection

Initially, a systematic search of global databases such as PubMed, Scopus, Google Scholar, and SID was conducted to collect all articles on the effects of *L. monocytogenes* infection on spontaneous abortion, regardless of publication date or language restrictions. Search terms were selected based on Medical Subject Headings browser including "*Listeria monocytogenes*," "Spontaneous abortion," "Pregnant women," and "Pregnancy." Articles published in Persian, Arabic, and English were the only articles

evaluated. After reviewing the titles and abstracts of eligible original articles (case-control studies, cross-sectional, and longitudinal studies), we selected those studies that were related to the effect of *L. monocytogenes* infection on spontaneous abortion. The process of searching and evaluating articles was done by two authors independently, and the discrepancies were examined by the third author. Duplicate articles, insufficient and vague information, case reports, and review articles were excluded from the study (Figure 1).

Data extraction and quality assessment

The required information such as first author, publication year, country, number of cases with at least one spontaneous abortion, number of *L. monocytogenes* strains, frequency of *hlyA* gene, frequency of serotypes, diagnostic methods, and the reference number were extracted from the eligible studies and are presented in Table 1. An important evaluation checklist of the Joanna Briggs Institute was used to evaluate the quality of the studies in the present meta-analysis.

Statistical analysis

The comprehensive meta-analysis software ver 2.0 (Biostat, Englewood, NJ, United States) was used for data pooling as well as statistical analysis of the extracted data. The frequency of *L. monocytogenes* infection in women with spontaneous abortion was measured as the incidence rate (event rate) with 95% confidence intervals (CIs) using cross-sectional and case-control studies. In addition, the frequency of the *hlyA* gene as well as common serotypes isolated from women with spontaneous abortion were also examined as the incidence rate. The effect of infection with this bacterium on the susceptibility to spontaneous abortion was assessed according to odds ratio with the 95%CIs using eligible case-control studies. Finally, the frequency of resistance of *L. monocytogenes* strains to different antibiotics was calculated and reported as the incidence rate and heterogeneity between studies was determined using I^2 index and Cochran's *Q*-test. In cases with high heterogeneity (I^2 > 25% and Cochran's *Q*-test *P* > 0.05), the analysis was done using the random-effects model. The publication bias was also determined using Egger's *P* value, Begg's *P* value, and asymmetry of funnel plots.

RESULTS

Characteristics of eligible studies

Of the 732 initial studies collected, 24 studies were eligible and entered in our meta-analysis (Figure 1)[13-36]. Of these, 17 studies were conducted in Iran, 2 studies in India, 2 studies in Iraq, 1 study in Bosnia and Herzegovina, 1 study in Nigeria, 1 study in Nagpur, and 1 study in Senegal. Overall, in selected studies *L. monocytogenes* (strains were isolated from urine, blood, placenta, vaginal swabs, and cervix swabs). In general, microbiological methods such as cold-enrichment, culture on PALCAM agar, culture on blood agar, as well as Gram-staining, oxidase, catalase, carbohydrates fermentation, methyl red/Voges-Proskauer, and motility at 25 °C were used to isolate and identify *L. monocytogenes* strains. In addition, the authors had used polymerase chain reaction technique (for genes such as *hlyA*, *InlA*, *InlB*, *actA*, *iap*, *plcA*, and *PrfA*) and serological tests on blood samples in their studies (Table 1).

Statistical analysis of spontaneous abortion

The prevalence of *L. monocytogenes* infection in women undergoing spontaneous abortion was approximately 20.5% (18.9-22.3 with 95%CIs; *P* = 0.001; I^2: 93.85; *Q*-value: 374.16; *P* = 0.001; Egger's *P* = 0.05; Begg's *P* = 0.01) was estimated (Figure 2).

The frequency of the *hlyA* gene in *L. monocytogenes* strains isolated from patients was estimated to be about 28.0% (21.0%-36.3% with 95%CI; *P*= 0.001; I^2: 87.34; *Q*-value: 94.84; *P* = 0.001; Begg's *P* = 0.03; Egger's *P* = 0.001). Also, abundance of serotypes 1/2a, 12/b, and 4b were measured to be about 38.7% (26.8%-52.2% with 95%CIs), 26.5% (13.1%-46.3% with 95%CIs), and 49.5% (32.8%-59.5% with 95%CIs), respectively.

Furthermore, it was revealed that the infection with *L. monocytogenes* significantly increases the risk of spontaneous abortion in pregnant women (odds ratio: 2.778; 2.130-3.623 with 95%CIs; *P* = 0.001; I^2: 76.30; *Q*-Value: 21.01; *P* = 0.001; Egger's *P* = 0.38; Begg's *P* = 0.51) (Figure 3).

Statistical analysis of antibiotic resistance

The current analysis showed that *L. monocytogenes* strains isolated from spontaneous

Table 1 Characteristics of included studies

Ref.	City	Number of patients		Number of positive culture for *L. monocytogenes*		Number of *hylA* positive strains	Frequency of serotypes	Diagnostic method
		Case	Control	Case	Control			
Aljicevićet *al*[13], 2005	Bosnia and Herzegovina	30	30	18	8	ND	ND	Serology
Kaur *et al*[14], 2007	India	61	ND	4	ND	3	ND	Convectional
Saeedi *et al*[15], 2009	Iran	118	99	9	3	ND	ND	IgG
Jamshidi *et al*[16], 2009	Iran	250	200	89	35	ND	ND	IgG
Tahery *et al*[17], 2009	Iran	102	102	82	20	ND	ND	IFA
Nazeri[18], 2011	Iran	512	ND	6	ND	ND	ND	PCR
Lotfollahi *et al*[19], 2011	Iran	100	ND	9	ND	ND	ND	Convectional
Jahangirisiskht *et al* [20], 2012	Iran	190	120	9	2	11/107	ND	Convectional
Jahangirsisakht *et al* [21], 2013	Iran	64	ND	7	ND	10.28	ND	PCR
Shindang *et al*[22], 2013	Nigeria	200	ND	14	ND	ND	4b (71%); 1/2a (28)	Convectional
Eslami *et al*[23], 2014	Iran	96	ND	16	ND	16	ND	PCR
Shoukat *et al*[24], 2014	India	141	ND	4	ND	4	ND	Convectional
Haghroosta *et al* [25], 2015	Iran	120	60	25	4	ND	ND	IFA
Pourkaveh *et al*[26], 2016	Iran	317	ND	54	ND	11		PCR
Bobade *et al*[27], 2016	Nagpur	113	ND	11	ND	11	4b (81%); 1/2b (18%)	PCR
Tajedini *et al*[28], 2017	Iran	58	ND	21	ND	ND	ND	IFA
Pour *et al*[29], 2018	Iran	123	ND	28	ND	28	1/2a (50%); 4b (35%)	PCR
Heidari *et al*[30], 2018	Iran	100	ND	4	ND	4	ND	Convectional
Al-dorri[31], 2018	Iraq	94	ND	11	ND	11	ND	PCR
Ohadi *et al*[32], 2019	Iran	96	ND	16	ND	16	1/2b (31%); 1/2a (25%); 4b (12.5%)	PCR
Mozaffari *et al*[33], 2019	Iran	130	100	35	11	ND	ND	IFA
Zahirnia *et al*[34], 2019	Iran	124	76	31	28	3	ND	PCR
Fall *et al*[35], 2020	Senegal	43	ND	2	ND	ND	ND	PCR
Al-Mayahi and Jaber[36], 2020	Iraq	90	ND	15	ND	15	ND	PCR

L. monocytogenes: *Listeria monocytogenes*; PCR: Polymerase chain reaction; ND: Not detected; IFA: Increased femoral anteversion; IgG: Immunoglobulin G.

abortion cases were resistant to various antibiotics. The resistance to different antibiotics was as follows: ampicillin 31.8% (25.5-38.9 with 95%CIs; I^2: 92.96; *Q*-Value: 56.84; *P* = 0.001), penicillin G 56.8% (51.4-62.0 with 95%CIs; I^2: 94.64; Q-Value: 74.67; *P* = 0.001), cotrimoxazole 33.0% (24.5-42.8 with 95%CIs; I^2: 0.00; *Q*-Value: 0.00; *P* = 1.00), cephalothin 50.0% (40.3-59.7 with 95%CIs; I^2: 0.00; *Q*-Value: 0.00; *P* = 1.00), tetracycline 29.2% (23.2-36.1 with 95%CIs; I^2: 91.75; *Q*-Value: 36.37; *P* = 0.001), erythromycin 15.0%

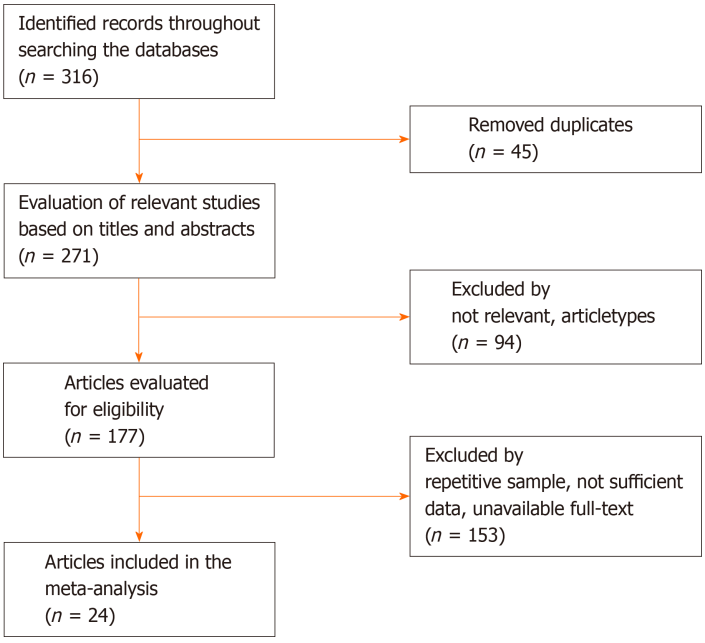

Figure 1 Flowchart of search strategy and study selection.

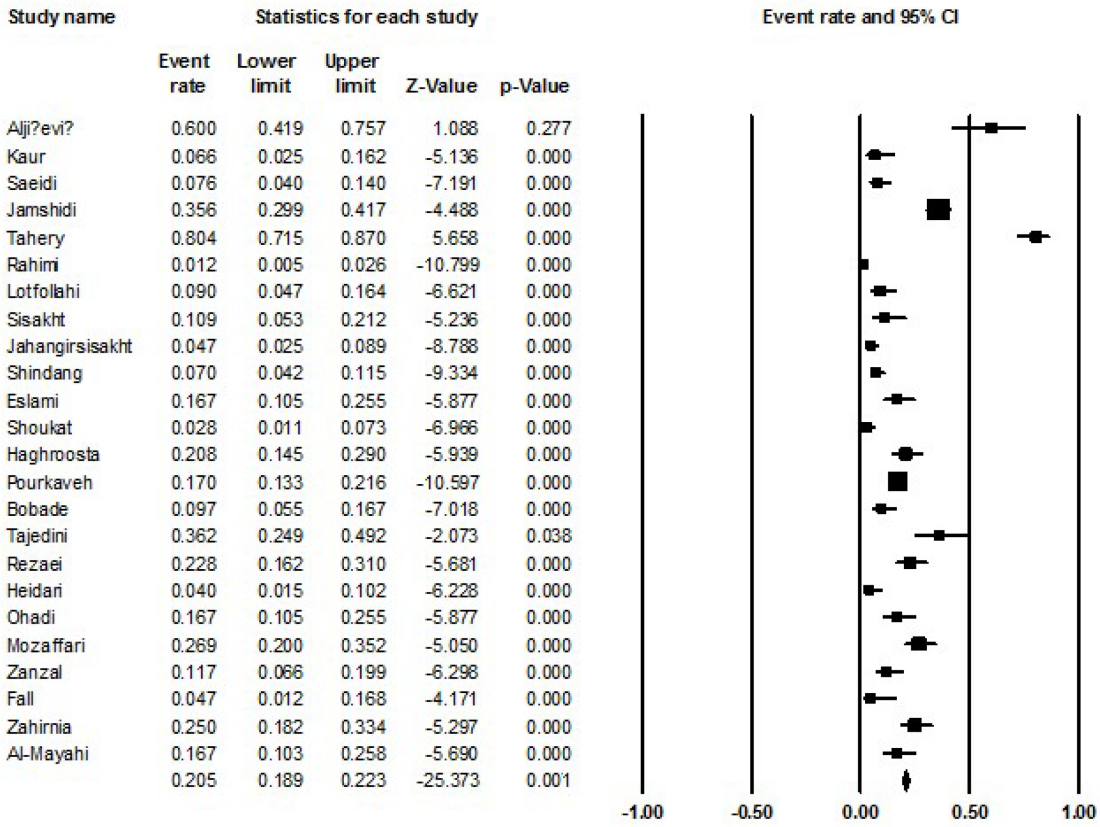

Figure 2 Forest plot for frequency of infection with *Listeria monocytogenes* among spontaneous abortion cases.

(9.5-22.8 with 95%CIs; I^2: 91.96; *Q*-Value: 37.31 ; *P* = 0.001), cefotaxime 77.0% (70.7-82.3 with 95%CIs; I^2: 0.00; *Q*-Value: 0.00; *P* = 1.00), chloramphenicol 11.0% (7.4-16.1 with 95 % CIs; I^2: 0.00; *Q*-Value: 0.00; *P* = 1.00), trimethoprim 38.3% (30.5-46.8 with 95%CIs; I^2: 95.47; *Q*-Value: 66.26; *P* = 0.01), ciprofloxacin 11.1% (6.8-17.7 with 95%CIs; I^2: 82.1; *Q*-Value: 11.18; *P* = 0.04), gentamycin 28.0% (20.0-37.6 with 95%CIs; I^2: 0.00; *Q*-Value: 0.00; *P* = 1.00), and streptomycin 11.0% (7.4-16.1 with 95%CIs; I^2: 0.00; *Q*-Value: 000; *P* = 1.00). No resistance has been reported to the three antibiotics amikacin, meropenem,

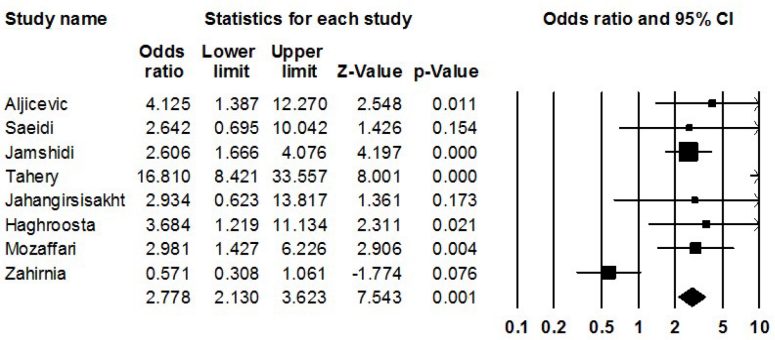

Figure 3 Forest plot for relationship between *Listeria monocytogenes* infection and susceptibility to spontaneous abortion.

and norfloxacin.

DISCUSSION

Listeriosis is one of the most well-known foodborne diseases that is transmitted to humans through the consumption of contaminated animal products. Several outbreaks of listeriosis have been reported worldwide, with children being the main victims[37]. According to studies, the prevalence of listeriosis *per* 100000 people in children, the elderly, and pregnant women is estimated at 3.4, 10.0, and 12.0, respectively. Pregnant women can pass listeriosis to their baby in the womb or at birth[38]. Latent listeriosis in pregnant women can have fatal effects on the fetus such as granulomatosis infantiseptica, spontaneous abortion, stillbirth, premature birth, and meningitis[39]. According to the Centers for Disease Control and Prevention, about 14% of human listeriosis occurs in pregnant women. Therefore, the diagnosis of listeriosis during pregnancy is essential, especially in developing areas (*Listeria* spp. are more likely to be contaminating food in these areas)[40].

Based on the current analysis, it was shown that the rate of colonization with *L. monocytogenes* in women who had spontaneous abortions was about 20.5%, although in some studies, *L. ivanovii* and *L. seeligeri* were the cause of infection and abortion. For the first time, in the current meta-analysis, we evaluated the association of colonization with *L. monocytogenes* in pregnant women with spontaneous abortion, and most of the studies considered in the current analysis were related to Iran. Iran as a developing country has a high rate of *L. monocytogenes* infection, which in turn is related to working conditions, lifestyle, and geographical location. In another meta-analysis study from Iran, the rate of colonization with *L. monocytogenes* in humans, animals, and food products was estimated at 10%, 7%, and 4%, respectively[41].

Cell-mediated immunity is somewhat suppressed during pregnancy, while humoral immunity is as active as ever. Therefore, pregnancy increases the risk of infection with facultative intracellular bacteria such as *L. monocytogenes*. Meanwhile, the human placenta also provides a protective niche for the growth and proliferation of this bacterium, which in turn increases the risk of spontaneous abortion[42]. We found that infection with *L. monocytogenes* can significantly increase the risk of miscarriage during pregnancy (odds ratio: 2.7; 2.1-3.6 with 95%CI; $P = 0.01$).

So far, four lineages have been identified from *L. monocytogenes* strains, and it should be noted that lineages I (serotypes 1/2b and 4b) and II (1/2a) are responsible for most human infections. In this study, the frequencies of serotypes 1/2a, 1/2b, and 4b were reported as 38.7%, 26.5%, and 49.5%, respectively, which were similar to the distribution of serotypes in other human listeriosis infections[27,29]. In pathogenic strains, numerous virulence factors are encoded by pathogenicity island 1. The *hlyA* gene encodes hemolysin (listeriolysin-O), which is one of the most important virulence factors of *L. monocytogenes*[43]. According to our analysis, only 28% of strains isolated from women with spontaneous miscarriage had this gene, indicating the low diagnostic value of this gene for diagnosis of *L. monocytogenes* infection in pregnant women.

Based on the evidence, β-lactam antibiotics alone or in combination with aminoglycosides as well as cotrimoxazole are recommended treatment options for severe listeriosis infections. Although, nowadays, the resistance of *Listeria* spp. to β-lactams and aminoglycosides is increasing[44,45]. Some *L. monocytogenes* strains are also

resistant to other antibiotics such as fluoroquinolones, macrolides, and tetracyclines, and the emergence and spread of the drug-resistant strains in pregnant women can be life-threatening for both mother and child[44]. Our results showed that the antibiotic resistance of *L. monocytogenes* strains isolated from the women with spontaneous abortion was high. The highest resistance was related to cefotaxime (77%), and the lowest was related to chloramphenicol and streptomycin (both 11%); all strains were sensitive to norfloxacin, meropenem, and amikacin. In a recent meta-analysis study by Khademi and Sahebkar[44], resistance of *L. monocytogenes* strains isolated from Iranian patients to penicillin, ampicillin, and gentamicin was reported to be 56.8%, 29.5%, and 32.4%, respectively, which was close to the results of our study[44]. Quentin *et al*[46] also identified a multi-resistant strain of *L. monocytogenes* in a septic abortion[46]. It is important to note that increasing drug resistance of *L. monocytogenes* strains to β-lactams and aminoglycosides in the coming years will lead to the ineffectiveness of these antibiotics in the treatment of human listeriosis.

Our meta-analysis has several limitations as following: (1) The population sample size was low; (2) Heterogeneity was significant; (3) There is a slight publication bias; and (4) We included only available articles in Persian, Arabic, and English. Overall, our results showed that there is a significant relationship between *L. monocytogenes* infection and the increased risk of miscarriage during pregnancy. The results of the present analysis also showed that drug resistance is increasing among *L. monocytogenes* strains, which needs to be re-evaluate. If necessary, treatment guidelines should be updated to reduce the incidence of human listeriosis.

CONCLUSION

Overall, regarding the importance of microorganisms such as *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, L. monocytogenes*, herpes simplex viruses, adenoviruses, human polyomaviruses, and cytomegalovirus in the induction of spontaneous abortion, it is important that all aspects of these pathogens, such as diagnosis, treatment, control, and vaccination, be considered by all researchers around the world.

ARTICLE HIGHLIGHTS

Research background

Spontaneous abortion is one of the most important concerning issues in pregnant women, and it has been suggested that *Listeria monocytogenes* (*L. monocytogenes*) infection can play a key role in pathogenesis of this disease.

Research motivation

We conducted the present study to estimate the risk of infection with *L. monocytogenes* in the development of spontaneous abortion during pregnancy.

Research objectives

The aim of this study was evaluation of the probable connection between infection with *L. monocytogenes* and risk of spontaneous abortion in pregnancy.

Research methods

We conducted a systematic literature review using several databases to search the relevant case-control studies on the association between *L. monocytogenes* infection and spontaneous abortion. Finally, the impact of infection with *L. monocytogenes* and risk of spontaneous abortion was assessed *via* odds ratio at corresponding 95% confidence intervals.

Research results

The frequency of *L. monocytogenes* infection was significantly increased in pregnant women with spontaneous abortion in comparison with healthy subjects. There is significant association between infection with *L. monocytogenes* and development of spontaneous abortion in pregnant women (odds ratio: 2.778; 2.130-3.623 with 95% confidence interval).

Research conclusions

Our results suggested the infection with *L. monocytogenes* is a marker for prediction of the risk of development of spontaneous abortion during pregnancy.

Research perspectives

Regarding the importance of *L. monocytogenes* in the initiation and development of spontaneous abortion, it is important that all aspects of this pathogen, such as diagnosis, treatment, control, and vaccination, be considered by all researchers around the world.

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