



***Listeria monocytogenes* in Dairy Products of the Middle East Region: A Systematic Review, Meta-Analysis, and Meta-Regression Study**

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Abstract

Background: The contamination of food products by *Listeria monocytogenes* as a pathogen bacterium, threatening public health and raised a global concern for a long time. Dairy and meat products and ready-to-eat foods are recognized as the most common carriers for *L. monocytogenes*.

Methods: The related reports of the prevalence of *L. monocytogenes* in dairy products in Middle East countries from 2009 to 2020 were screened through some of the international databases such as Science Direct, Web of Science, Scopus, PubMed, and Google Scholar. While a random effect model was applied to estimate pooled or overall prevalence, 95% confidence intervals (95%CI) were used.

Results: Results showed severe heterogeneity (84.2%) in studies and estimated the overall prevalence of *L. monocytogenes* dairy food products from the Middle East region of 3.5% (CI: 2.2-5). The highest and lowest prevalence was associated with Jordan (17.6% CI: 9.8-26.9) and Iraq (1.6% CI: 0.3-3.7), respectively. Based on the type of product, the highest and lowest prevalence of *L. monocytogenes* was recognized for raw cow milk (5.8% CI: 2.7-9.7) and pasteurized cow milk (1.1% CI: 0-8), respectively.

Conclusion: There is no justification for severe heterogeneity (I²) of subgroups as prevalence is heterogenic innately, but Jordan and row cow milk subgroups were found to have a considerable effect on overall pooled prevalence. Thus, they were the reason for prevalence changes.

Keywords: *Listeria monocytogenes*; Dairy products; Middle East; Systematic review; Meta-analysis



Introduction

Listeria is a genus from the *Listeriaceae* family that includes more than 20 species categorized into three main clades (1). It is a facultatively anaerobic, gram-positive, non-sporulating, and psychrotrophic bacterium (2). Some *Listeria* species are recognized as pathogenic such as *L. monocytogenes*, known as pathogens for both humans and animals, and *L. ivanovii* is considered pathogenic for animals (3). *L. monocytogenes* are highly important in terms of public health and grow easily at temperatures ranging from -0.4 °C to 45°C and tolerate osmotic stress up to 14% salt concentration. It also can undergo a broad zone of pH 4.0 to 9.6 (optimum pH 6–8). The water activity of 0.90 is distinguished as the lowest level of water activity that *L. monocytogenes* can survive (4, 5).

Listeriosis resulted from the consumption of contaminated food products by *L. monocytogenes* such as cheese, non-pasteurized milk, unwashed raw vegetables, and undercooked meat, and it is identified as one of the primary foodborne diseases for humans (6-10). The contamination of milk by *L. monocytogenes* is attributed to both infected and healthy animals besides poor hygiene conditions or practices (11, 12). Therefore, among different foods, milk, mainly raw milk, and other kinds of dairy products are particular sources for *L. monocytogenes*.

L. monocytogenes threaten human health by causing listeriosis. At the same time, diarrhea, vomiting, fever, headache, gastroenteritis, and myalgia are mild symptoms. Moreover, septicemia and meningitis that commonly resulted in stillbirth and abortion in pregnant women are the common invasive symptoms (8, 10, 13). According to the Center for Diseases Control (CDC), 467 listeriosis cases and 83 deaths were reported between 2011 and 2017 in the different states of the USA (14), while the number of confirmed cases of listeriosis reported by EU/EEA countries in 2016 was 2555 (15). Available data about the listeriosis epidemiology in the Middle East, especially Iran, are rare. Because in the Iranian health system, listeriosis cases are not reported precisely (16).

Studies for investigating the prevalence of *Listeria* spp. have been conducted based on different groups of foods, including meat, seafood, milk, dairy products, ready-to-eat foods, and eggs (4, 17). Contamination is different in various regions due to differences in livestock procedures conditions, storage and hygiene conditions, and technological practices (17). Several studies were carried out on the prevalence of *L. monocytogenes*. These studies do not have a specific pattern, and researchers have been reported inconsistencies in different regions like Iran, Egypt, and Italy. For instance, an Iranian research group represented 37% of raw milk samples contaminated with *L. monocytogenes* (18). However, some other researchers (19-21) described no contamination for *L. monocytogenes* in raw milk. Moreover, *L. monocytogenes* was detected in fifty percent of cheese samples (22), while other authors (20, 23) could not find *L. monocytogenes* in Iranian cheese samples. Besides, Kabuki et al isolated *L. monocytogenes* in 6.3% of Iranian cheese samples (24). Inconsistent statistics in *L. monocytogenes* prevalence in dairy products are also reported in other countries. For instance, in Egypt, *L. monocytogenes* range from 3.3 to 6.6% in milk products (25), and researchers in other countries like Syria reported that *Listeria* spp. exists in 10.96% of raw milk samples (26), or *L. monocytogenes* was positive in 4.8% of cheese samples in Turkey (27). Dairy product contamination with *L. monocytogenes* has been reported for European countries as well. For example, contamination of 2.2% was reported for Italian raw milk with *L. monocytogenes* (28). Furthermore, meta-analysis research conducted in Europe using EFSA reports and prior studies about the prevalence, contamination of *L. monocytogenes* in different cheese types showed the prevalence from 0.8 to 11.8% in European countries (29).

Although many studies have estimated the prevalence value of *Listeria* spp. in different food samples, no systematic review or meta-analysis regarding *L. monocytogenes* contamination of milk products in the Asian or the Middle East coun-

tries was reported cohesively. Hence, summarizing research from different databases that studied *L. monocytogenes* contamination in the Middle East countries in dairy products and considering the risk of bias assessment was the objective of this work.

Methods

Search scheme

According to the Cochrane protocol (PRISMA guideline), this systematic review and meta-analysis study was carried out (30). In the first step, Science Direct, Web of Science, Scopus, PubMed, and Google Scholar databases were used to retrieve published original studies in the Middle East countries between 2009 and 2020. The following search keywords and MeSH terms were used: (Cheese OR Cream OR Kashk OR Milk OR "Dairy Products" OR "Ice cream" OR Butter OR Yoghurt OR Ghee OR Buttermilk OR Kefir OR Koumiss OR Whey) AND ("*Listeria monocytogenes*" OR "*L.monocytogenes*") AND ("The Middle East" OR Afghanistan OR Bahrain OR Iran OR Iraq OR Israel OR Jordan OR Kuwait OR Lebanon OR Oman OR Qatar OR Saudi Arabia OR Syria OR Turkey OR United Arab Emirates OR Yemen) AND (Prevalence OR Outbreak OR Incident OR Contamination). Searching in the mentioned databases and screening was conducted. The title, abstract, and full text of retrieved articles were screened for eligibility. Moreover, to cover almost all papers, the references list of reviewed articles was considered.

Inclusion/exclusion criteria

The articles' selection was based on our research criteria by two reviewers separately after screening the databases with the same keywords and procedures. In this step, each author was responsible for reviewing the papers in all parts (titles, abstracts, and full texts) to select the articles having the inclusion criteria; any differences were supposed to be resolved by consensus. Articles included in this study based on inclusion criteria

(Eligibility criteria) including only dairy product samples, researches were done and reported in the middle east countries, available full-text in the English language; cross-sectional descriptive studies; *L. monocytogene* detection done with high accuracy technique (culture and PCR techniques); and prevalence data was presented clearly in the results of papers. Moreover, any other food samples like meat products or ready-to-eat-foods, reported prevalence of *L. monocytogene* in other countries, genetic, ecological, animal studies, case reports, thesis, books, non-English articles, and review articles were excluded (Fig. 1).

Data extraction and risk of bias assessment

Required data from selected articles including first author, publication year, method of *L. monocytogenes* detecting, type of dairy products, country, study area (province), sample size, and positive samples were extracted by two authors separately. Additionally, assessing the risk of bias of included studies was judged independently by the first author based on the JBI checklist with a minor modification (31).

Meta-analysis of data

In this meta-analysis, we applied random effect models for estimating pooled or overall prevalence and 95% confidence intervals (95%CI). The positive samples (p_i) divided into the total sample (n_i) indicates the prevalence of *L. monocytogene* in the dairy products ($I = p_i / n_i$). Overall prevalence was estimated using a Freeman-Tukey double arcsine transformation. Estimation of prevalence *L. monocytogene* was performed using the Meta-prop command (32). I^2 statistics and Cochran's Q Heterogeneity were used for exploring heterogeneity among studies. I^2 is a variance variable (prevalence) divided by Q value. I^2 ranges between 0-100 percent, and $I^2 > 70\%$ were considered heterogeneous (33, 34). A Forest plot was used to show *L. monocytogene* prevalence. In this study, subgroup analysis and meta-regression were performed to explore heterogeneity sources (35). Publication bias was not tested in this study because its aim is not to estimate the association between exposures and outcomes (35). Meta-

analysis was done by STATA 14.0 (2015; STATA 14.0 Statistical Software, College Station, TX,

USA). Statistical significance was selected less than 0.05 ($P < 0.05$).

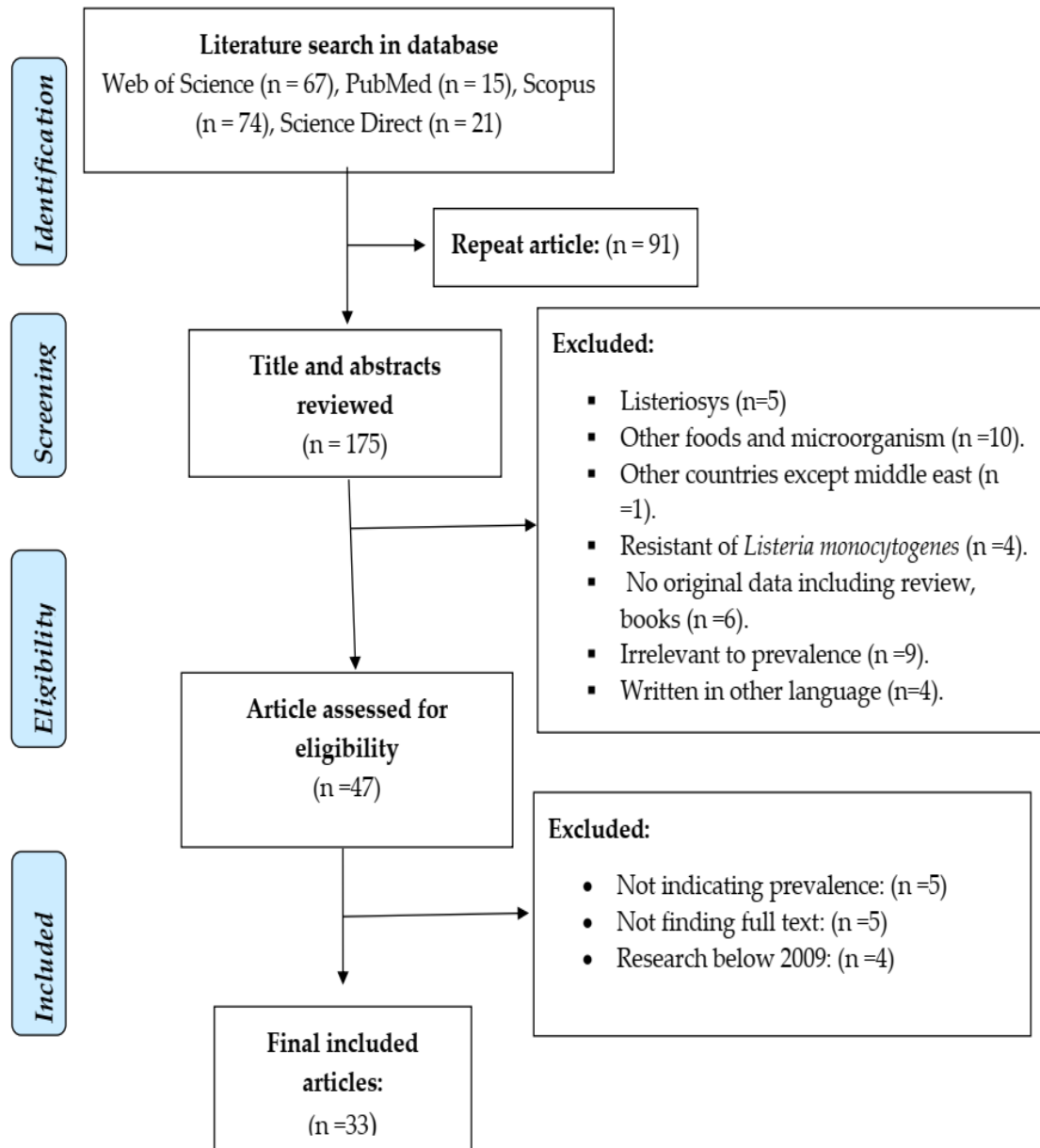


Fig. 1: The PRISMA flowchart diagram of the study.

Results and discussion

This systematic review and meta-analysis study was carried out on 76 key measures calculated from 32 papers related to dairy products. Articles included in this systematic review and meta-analysis from each country (the Middle East)

were summarized in Table 1. Iran (n=13) and Turkey (n=10) had the most considerable number of articles on the prevalence of *L. monocytogenes*. However, some other countries like Kuwait, Bahrain, and Yemen found no related publication in this field. In this research, dairy products were

divided into eight main sub-groups, in which most studies included cheese and raw cow milk, as well as the overall prevalence for the presence

of *L. monocytogenes* in dairy products, is illustrated in Fig. 2, which was 3.5 % (CI: 2.2%-5.0%).

Table 1: Summary of the studies reporting the Prevalence of *Listeria monocytogenes* in dairy products in the Middle East countries

<i>Id</i>	<i>Assessment technique</i>	<i>Food groups</i>	<i>Country</i>	<i>Region</i>	<i>Year</i>	<i>Sample size</i>	<i>Positive samples</i>	<i>Quality assessment</i>	<i>Ref.</i>
1	PCR	Cheese Cream Kashk	Iran	Tehran	2012-2015	70 20 17	5 2 2	7 7 6	(46)
2	PCR	Raw cow milk Cheese Ice cream Cream	Turkey	Samsun	2011-2012	100 70 20 20	5 9 0 0	7 7 7 7	(47)
3	PCR	Raw cow milk Cheese	Iran	-	2013-2015	37 130	1 4	7 7	(48)
4	PCR	Raw cow milk Cheese Cream Yogurt	Turkey	Southern Marmara	2009-2010	42 140 12 2	0 0 0 0	7 7 6 6	(49)
5	PCR	Raw cow milk Raw sheep Milk	Iran	Tehran	2008-2010	240 206	13 5	7 7	(41)
6	Culture	Cheese	Turkey	Van otlu	2011	120	34	7	(50)
7	Culture	Raw cow milk Cheese	Turkey	Sakarya	2008-2010	8 8	0 0	8 8	(51)
8	Culture	Raw cow milk Pasteurized cow milk Yogurt Cheese Cream	Turkey	Aydin	2013	20 20 40 50 10	5 1 2 5 1	7 7 7 7 7	(52)
9	Culture	Raw cow milk	Iran	Tabriz	2014	18	9	7	(22)
10	Culture	Raw cow milk Cheese Yogurt	Jordan	Karak	2011	20 20 20	9 7 8	7 7 7	(36)
11	PCR and Culture	Raw cow milk Raw sheep Milk	Iran	Fars and Khuzestan	2010-2011	156 104	3 2	8 8	(10)
12	PCR and Culture	Raw cow milk	Egypt	Sharkia Governorate	2015-2016	100	25	8	(22)
13	PCR	Kashk Cheese	Lebanon	Bekaa Valley	2009	83 81	6 17	8 8	(43)
14	Culture	Raw cow milk Pasteurized cow milk	Iran	Sari	2013	100 100	0 0	8 8	(19)
15	PCR	Pasteurized cow milk	Iran	-	2011	596	61	8	(54)

		milk							
16	PCR	Raw cow milk	Egypt	-	2013	100	1	5	(55)
17	PCR	Cheese	Turke	Erzurum	2015	104	17	6	(56)
	Culture	Pasteurized cow milk	Turke	-	2012	10	0	7	(57)
18		Cheese		-		60	1	7	
		Ice cream		-		20	0	7	
		Cream		-		10	0	7	
19	PCR	Raw cow milk	Iran	-	2015	60	0	7	(58)
20	Culture	Cheese	Jordan	Amman	2017	150	8	7	(59)
21	PCR and Culture	Raw cow milk	Iran	Yazd	2016	140	11	8	(8)
		Cheese				220	10	8	
		Cream				100	1	8	
		Ice cream				85	0	8	
22	Culture	Ice cream	Iran	Kermans hah	2013	67	0	8	(21)
		Pasteurized cow milk				59	0	8	
		Cheese				59	0	8	
23	PCR and Culture	Raw cow milk	Iran	Kerman	2011	100	5	6	(60)
24	PCR and Culture	Raw cow milk	Iran	Isfahan	2007-2009	138	1	8	(10)
		Raw sheep milk				122	6	8	
		Cheese				90	9	8	
		Ice cream				136	2	8	
		Yogurt				130	0	8	
		Cream				40	1	8	
		Kashk				71	0	8	
25	Culture	Yogurt	Turke	Balikesir	2010-2011	100	3	7	(61)
		Cheese	y			100	5	7	
26	PCR	Cheese	Egypt	El Giza	2013-2014	50	0	8	(62)
		Raw cow milk		Governorate		50	4	8	
27	Culture	Raw cow milk	Iran	Noorabad	2010	120	3	7	(63)
		Cheese				60	3	7	
		Yogurt				180	0	7	
28	PCR	Cheese	Jordan		2011	350	39	7	(64)
29	Culture	Pasteurized cow milk	Turke	Bursa	2012	10	0	6	(38)
		Cheese				60	1	7	
		Ice cream				20	0	7	
		Cream				10	0	6	
30	Culture	Row cow milk	Jordan	-	2016	305	32	8	(65)
31	PCR	Cheese	Turke	Bolu	2016	40	0	8	(66)
		Ice cream	y			27	0	8	
32	Culture	Cheese	Turke	Hatay	2019	20	0	8	(67)
			y						
33	PCR	Cheese	Iraq	Dehok	2016-2017	121	1	8	(68)
		Raw sheep milk				118	3	8	

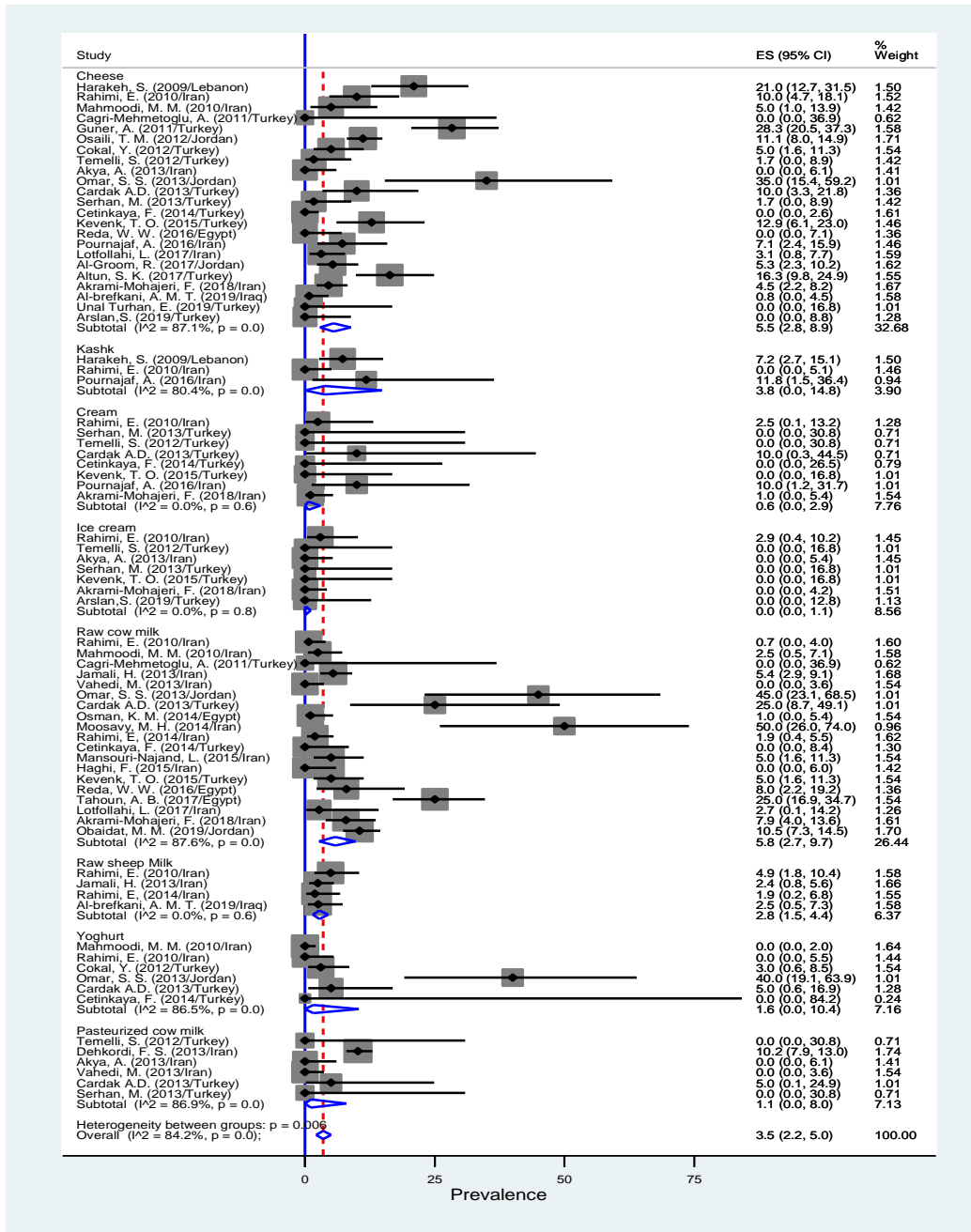


Fig. 2: The forest plot of prevalence of *Listeria monocytogenes* in dairy products based on food type/year of publication/country

Ice cream had the lowest mean prevalence of 0.0% (CI: 0.0-1.1%), followed by cream 0.6% (CI: 0.0-2.9%), pasteurized cow milk 1.1% (CI: 0.0-8.0%), yogurt 1.6% (CI: 0.0-10.4%), raw sheep milk 2.8% (CI: 1.5-4.4%), Kashk 3.8% (CI: 0.0-14.8%), cheese 5.5% (CI: 2.8-8.9%) and raw cow

milk had the highest *L. monocytogenes* prevalence of 5.8% (CI: 2.7-9.7%). On the other hand, the prevalence rank order in terms of country showed the following order: Iraq 1.6% (CI: 0.3-3.7%), Turkey 2.2% (CI: 0.3-5.1%), Iran 2.5% (CI: 1.2-4.0%), Egypt 5.8% (CI: 0-20.6%), Lebanon

13.3% (CI: 8.4-19%) and Jordan 17.6% (CI: 9.8-26.9%).

The highest level of *L. monocytogenes* contamination in cheese (Fig.3) was observed in Jordan with 35% contamination (CI: 15.4-59.2%) (36).

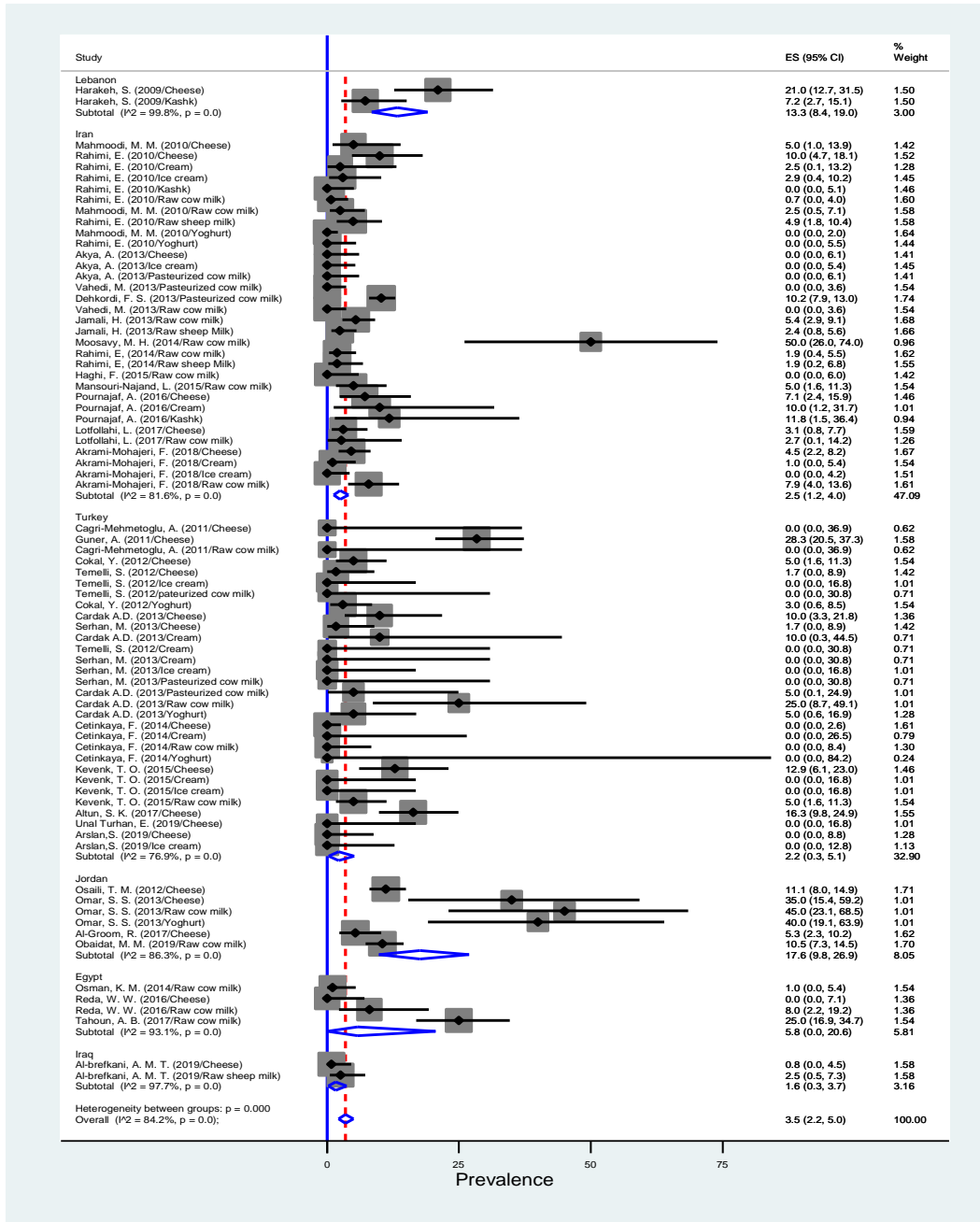


Fig. 3: The forest plot of prevalence of *Listeria monocytogenes* in dairy products based on country/year of publication/ food type

Moreover, the highest and lowest *L. monocytogenes* incidence in raw ship milk was observed in two

studies carried out (10, 37) in Iran, which found values of 4.9 and 1.9%. The main reason cheese

faced a high prevalence of *L. monocytogenes* is probably related to raw milk origin and survival ability of *L. monocytogenes* in high salt concentration and resistance to different pH (38). *L. monocytogenes* can survive in some cheeses like white cheese after several weeks, dangerous for public health. The estimated mean prevalence of *L. monocytogenes* in cheese products in the Middle East (5.5%) was more than results from European reports (2.3%) (3). The only study on *L. monocytogenes* contamination in Iraq reported a mean prevalence of 1.6% (CI: 0.3%-3.7%) in cheese and raw ship milk (39). Milk contamination of *L. monocytogenes* is not specific to raw cow milk, but also pasteurized cow milk could carry *L. monocytogenes* in case of inadequate pasteurization or post-pasteurization contamination. Furthermore, the high prevalence of *L. monocytogenes* in raw milk could be associated with animal infection or contamination of livestock feed, and contamination of raw milk during breastfeeding and milk storage could be another reason (40, 41). Similarly, in a study conducted by the EU's Rapid Alert System for Food and Feedstuff between 2004 and 2009, 97 dairy products were positive for *Listeria* and 34 of them were pasteurized, and 22 samples were raw milk products (42). Moreover, the highest occurrence rate of *L. monocytogenes* in yogurt was found in Jordan (36), that 40% of samples were contaminated with *L. monocytogenes*. Moreover, in the only study on *L. monocytogenes* contamination in Lebanon, they reported a mean prevalence of 13.3% (CI: 8.4-19%) in cheese and Kashk (43). According to the results of this meta-analysis, contamination of cream and ice cream by *L. monocytogenes* was low.

Generally, occurrence evaluation of *L. monocytogenes* is necessary for dairy products as they are vehicles for this pathogen transmission that led to the highest death rate among infec-

tious diseases (44). Preventing foods, especially dairy products, from being contaminated by *L. monocytogenes* is highly recommended. Additionally, adequate pasteurizing, forage quality, housing facilities, and milking hygienic conditions can affect milk's microbial quality (45).

The meta-regression test investigates potential contributing parameters that affect study heterogeneity. This test was used to examine the year of publication and the quality score for each included study in our study. The year of publication's meta-regression plot versus *L. monocytogenes* prevalence in dairy products showed no significant relationship ($C=0.0006$; $P=0.88$). As shown in Fig. 4, regarding the effect sizes of the research studies; there is a tendency to decrease in *L. monocytogenes* prevalence over the years between 2010 and 2020, which seems that during these years, various factors, including hygienic-sanitary quality of milk, storage conditions, and pasteurization method has improved as well as post-pasteurization contamination reduced. Generally, hygienic procedures in the Middle East have been introduced to some extent for quality improvement over the last decade. In this study, the quality assessment of research studies was conducted. We evaluated the methodological quality of the 32 research reports, using nine quality criteria relating to the prevalence studies. The items were determined to consist of sampling method, sample size adequacy, subject description in detail, data coverage analysis, applying valid method, response rate adequacy, using the standard method, and using appropriate statistical analysis. A meta-regression analysis was used to find the relationship between *L. monocytogenes* occurrence rate and the quality score of the included study. Figure 5 indicated that included papers' quality scores have no considerable effects on the prevalence of *L. monocytogenes* in dairy products.

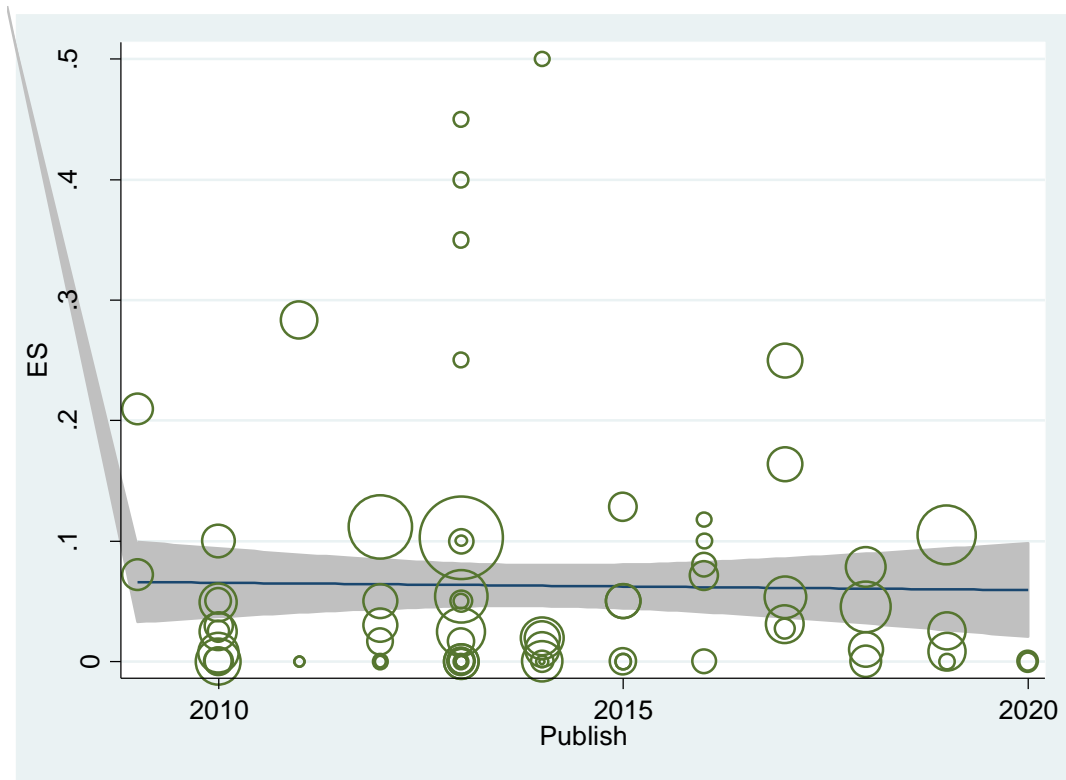


Fig. 4: The relationship between the prevalence *Listeria monocytogenes* with year of study

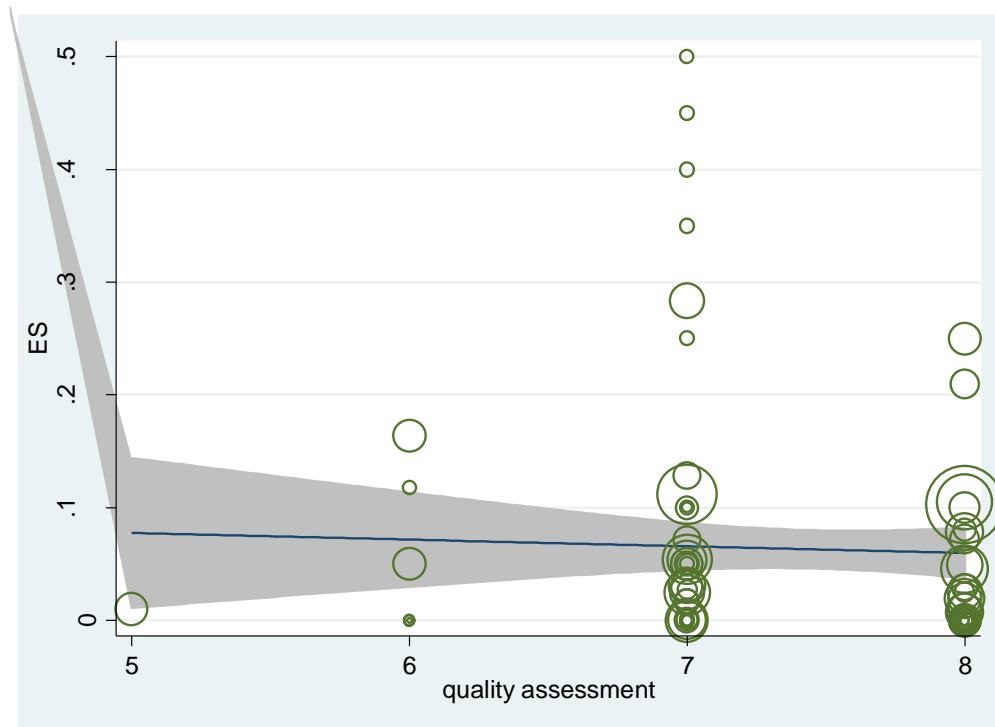


Fig. 5: The relationship between the prevalence of *Listeria monocytogenes* with quality score for included study

There are some limitations to this study. Although our search was comprehensive, there were limited studies in some countries or finding no studies on *L. monocytogenes* contamination. Moreover, no study was found about *Listeria* contamination in some samples, such as Kefir or Komis. Another limitation was that some of the available studies had limited sample sizes or a lack of data on *Listeria* contamination, or no full-text access. Despite these limitations, the authors tried to cover all the studies done in the Middle East region and showed a general view of the prevalence of *L. monocytogenes*. Moreover, researchers suggest that researchers aim to carry out secondary review articles (systematic review and meta-analysis study) to limit and make narrower the type of reviewed food samples and possibly widen reviewed regions. It helps to draw a much better image of the prevalence of *L. monocytogenes* in the world and finds critical and threatening points in *L. monocytogenes* contaminations affecting public health. Similarly, risk assessment studies for this pathogen in different dairy products in different regions and countries and even worldwide are suggested for future original research.

Conclusion

The prevalence of *L. monocytogenes* in dairy products in the Middle East based on defined subgroups was systematically reviewed and meta-analyzed. The order of contamination by *L. monocytogenes* among the investigated dairy products based on prevalence was raw cow milk > cheese > Kashk > raw sheep milk > yogurt > pasteurized cow milk > cream > ice cream, demonstrating the required further attention in the production process of some products such as cheese and Kashk. The high prevalence of *L. monocytogenes* in some dairy products is probably related to the unhealthy production method and lack of adequate pasteurization temperature in these products, as well as environmental contamination by animal wastes, which can be a reason for this contamination. Consumption of raw milk and its products, especially cheese produced with insuf-

ficient heat treatment and the lack of appropriate control measures, might cause serious health problems.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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