



A systematic review and meta-analysis of the prevalence of *Listeria monocytogenes* in South-East Asia; a one-health approach of human-animal-food-environment

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ABSTRACT

Listeria monocytogenes is an important foodborne intracellular pathogen. The pathogen is the primary cause of human Listeriosis. The main source of human Listeriosis is through consumption of contaminated food products. Other modes of transmission include zoonotic and vertical transmission. The disease often presents in a mild form, but severe and fatal cases, as well as outbreaks, may occur. Despite these challenges, there has been little attempt at enumerating the burden of the disease in countries of Southeast Asia (SEA) and some developing countries. Thus, this study investigated the prevalence of *L. monocytogenes* in SEA using one health approach through a systematic review and meta-analysis (SR&MA) of the existing literature. In accordance with the PRISMA guidelines, an a priori protocol for the SR&MA was developed and registered in PROSPERO (ID=CRD42021288903). A systematic search of four electronic databases was performed for relevant citations. The identified publications were screened, and 17 studies were included in the review from where data was extracted. The pooling of the prevalence estimate (with the 95% confidence interval [CI]) was done using the random effect model by employing the double transformed arcsine method using MetaXL software. The overall determined prevalence for *L. monocytogenes* in SEA (in food, animal, and environmental sources) was 16% (95% confidence interval [CI]: 10–23). Further subgroup analysis revealed ready-to-eat food of vegetable origin with the highest prevalence of 21% (CI: 6–41). Also, seven virulence genes were identified to be prevalent in the subregion. The commonest identification method was found to be the polymerase chain reaction (PCR). The knowledge of the high prevalence of *L. monocytogenes* in SEA is relevant for informed decision making by clinicians, public health practitioners, and policymakers. To achieve the goal of the effective control and prevention of the disease in the subregion.

1. Introduction

Listeria monocytogenes is an invasive foodborne pathogen of humans and animals that causes the disease known as Listeriosis [1]. The organism is a ubiquitous Gram-positive, non-spore-forming, non-capsulated (unencapsulated), facultative anaerobic, rod-shaped bacterium [2]. There are 13 identified serotypes of *L. monocytogenes*, but only

1/2a, 1/2b, 1/2c, and 4b are of human importance [3]. In contrast to most other foodborne pathogens, *Listeria monocytogenes* can grow in food with reasonably low moisture content and high salt concentration [3]. Most importantly, *L. monocytogenes* thrives in refrigeration temperature in contrast to many other foodborne pathogens [3]. This capacity to persist and multiply in the food environment makes it especially difficult to control.

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Listeriosis often presents as a mild disease but can cause severe infection in ‘at-risk groups’ such as young and old individuals, pregnant women, and the immunocompromised [3]. The fatality rate of Listeriosis, particularly in pregnant women, can be as high as 30% [4], exceeding that of *Salmonella* and *Clostridium* species. The most common transmission mode is through the consumption of food and/or feed contaminated by the organism [4]. Also, vertical transmission from mother to fetus is another possibility [4]. Other likely but less frequent modes of transmission include from animal-to-human (zoonotic), by contact or nosocomial transmission as seen in cutaneous lesions among veterinarians [5].

Listeriosis is termed as either silage disease or circling disease manifesting commonly as meningoencephalitis in animals. Infections in animals are usually sub-clinical, but severe forms can also occur, leading to abortion in pregnant animals, food poisoning, and death [6]. Nevertheless, it can cause fatal disease in some animals, birds, fishes, and crustaceans, causing septicaemia and encephalitis predominantly [6].

Throughout the world, Listeriosis occurs in sporadic or epidemic forms [6,7]. Many studies have been reported on the prevalence of *L. monocytogenes* and Listeriosis in different parts of the world [8] and in humans and from food products of animal origin [9–11]. However, the true incidence of foodborne infection is unknown, and there has been little attempt in ascertaining the magnitude of the problem. This is due to the paucity of studies on the epidemiology and surveillance of the disease in some developing countries, including Southeast Asia (SEA).

Additionally, the complex nature of the relationship between animals and man is on the rise and factors responsible include; chemical, physical, biological and social. Furthermore, the newly emerging zoonotic infections have made bold headlines and heightened awareness of the role of wild and domestic animals in spreading diseases to man. Globally, the rapid explosion in the human population and unprecedented increase in numbers and density of animals raised for food production play an important role in spreading zoonotic diseases. Also, the transportation, rearing, marketing, and processing of these animals significantly affect the occupational health of the human beings working and rearing the animals. Thus, raising the risk of zoonotic transmission to the workers due to increased contact with the animals and their products (e.g., dead carcasses, blood, and other discharges). The implication is also applicable to the environment where the animals are kept and the entire ecosystem. Thus, emphasising the pivotal role of the ‘one health’ concept in combating the threat of emerging zoonotic infections. Therefore, the importance of *Listeria monocytogenes* cannot be under-emphasised as this may lead to substantial public health consequences in addition to economic losses in the livestock industry. Hence, this SR&MA was conducted to comprehensively investigate the prevalence of *L. monocytogenes* in SEA.

2. Methods

2.1. Study design

This systematic review and meta-analysis (SR&MA) study was designed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (S1 File). In addition, the study was preceded by a priori protocol (S2 File) following the PRISMA protocol (PRISMA-P) guidelines (S3 File). The protocol was then registered on the National Institute for Health Research International Prospective Register of Systematic Reviews (PROSPERO) (ID=CRD42021288903) available at: <https://www.crd.york.ac.uk/prospero/displayrecord.php?ID=CRD42021288903>.

2.2. Eligibility criteria

The following were defined as the eligibility criteria for the studies to be included in this SR&MA:

Inclusion criteria: Studies that meet the requirements outlined below

were included in this SR&MA:

- Study type: All observational studies (cross-sectional, cohort, case-control, prevalence surveys) conducted on human, animal, food, and environmental samples were included.
- Study location: All accessible published full articles from studies conducted in South-Eastern Asia countries were included.
- Time period: There was no time limitation on the period/year of publication.
- Age and sex: no restriction
- Language of publication: Only studies published in the English language were included.
- Publication type: Published peer-reviewed articles were included,

Exclusion criteria: The ensuing criteria were used to exclude studies from this SR&MA:

- Studies with incomplete data, no clear study design were excluded.
- Studies outside SEA countries were not considered.
- In silico studies, In vitro studies, Letters, books, dissertations, review articles, opinion papers, reports, and conference papers were excluded.
- Articles published in languages other than the English language were excluded.

2.2.1. Outcomes

Primary outcome: is to determine the overall prevalence of *Listeria monocytogenes* in humans, animals, food, and the environment in SEA countries.

Secondary outcomes:

- To identify the virulent genes in isolated *L. monocytogenes* in SEA.
- To determine the prevalent sample types/origin for the isolation of *L. monocytogenes* in SEA.
- To assess the frequent types of detection methods/techniques used to identify *L. monocytogenes* in SEA.

2.3. Search and selection strategy

A search strategy with specific search – terms was designed and applied on four selected electronic databases. Also, hand searching of references of selected (review) articles and conference proceedings was done after the electronic search. Additionally, a search was equally conducted on some non-specific internet search engines (Google Scholar and Google search) using specific terms for more literature.

2.4. Databases

The following databases were selected and searched: ProQuest, MEDLINE, PubMed, and Academic Search Complete. The specific search – terms used on each database are outlined in the study protocol (S2 file). First, however, the search algorithm used in the ProQuest database is given as follows; (Prevalence OR Occurrence OR Incidence) AND (Virulent factors OR Virulent genes OR Virulence Agents OR virulent strains) AND (*Listeria monocytogenes* OR Listeria OR Listeriosis OR Listeria species OR Listeria spp. OR *L. monocytogenes*) AND (human* OR animal* OR meat OR Vegetable* OR fruit* OR environment) AND (Malaysia OR Indonesia OR Singapore OR Thailand OR Cambodia OR Philippines OR Laos PDR OR Brunei OR Myanmar OR Vietnam OR East Timor).

2.5. Data management and selection process

The citations identified following the search were exported to the Mendeley reference manager for de-duplication. The de-duplicated

sources were then transferred to the Rayyan Intelligent Systematic Review software [12] for the title/abstract and full-text screening based on the study inclusion and exclusion criteria. Three independent reviewers undertook the screening process, with a fourth reviewer deciding on areas of dispute between the three reviewers.

2.6. Data collection process

The data extraction process began with creating an a priori data extraction form in a Microsoft Excel (MS) spreadsheet. Afterwards, the characteristics of the studies included, and other relevant data were retrieved and inputted in the data extraction form. Three independent reviewers carried out the complete data extraction procedure, and a fourth reviewer crosschecked to ascertain the accuracy of the extracted data.

2.7. Study quality assessment

Each article that meets the study inclusion and exclusion criteria was subjected to a quality assessment using the appraisal instrument developed for use in systematic reviews addressing questions of prevalence [13]. The appraisal tool has ten questions that were answered as either yes (Y), no (N), unclear (UN), or not applicable (NA). A score of 1 was given to questions with 'Y' answers, 0 was awarded to questions with 'N', and 'UC' answers and no score was awarded to 'NA'. The total scores were then calculated as percentages. Thus, studies with <50% score were termed low quality, those with >50%-to- < 70% were labelled medium quality, and those with $\geq 70\%$ score were high-quality studies. Two independent reviewers carried out the critical appraisal and were cross-checked by a third reviewer.

2.8. Meta-analysis

2.8.1. Statistical assessment

MetaXL software (add-in for Microsoft Excel) was used to analyse the extracted data quantitatively. The meta-analysis and pooling of the prevalence estimate (with the 95% confidence interval) were done using the random effect (RE) model to account for heterogeneity. This meta-analysis was done by employing (the transformed) double arcsine method.

2.8.2. Assessment of heterogeneity

Estimation of statistical heterogeneity among the included studies was done using the X^2 test, Cochran Q test, tau and I^2 statistics. An I^2 value of 0 to $\leq 40\%$ was considered low heterogeneity, $>40\%$ to 60% was considered moderate heterogeneity, $>60\%$ to 75% was deemed substantial heterogeneity, and $> 75\%$ to 100% was judged as high heterogeneity. Significant heterogeneity was considered for a $p < 0.10$.

2.8.3. Sensitivity analysis

Sensitivity analysis was done based on the leave-one-out model to identify the study that significantly influenced the meta-analysis result.

2.8.4. Subgroup analysis and meta-regression

Subgroup analysis and meta-regression were conducted to identify the factors that may contribute to heterogeneity among the studies. The factors considered for the analyses include population type studied (food or environment or animals or humans), year of publication, sample size, country of study and study quality. For meta-regression, univariate analysis was done for each of the covariates. Due to the low power of the test (meta-regression) and the fact that this study is considered as the first, 0.25 was considered significant.

2.8.5. Publication bias

A funnel plot of the double arcsine prevalence against standard error was constructed to examine publication bias. The observed asymmetry

on the funnel plot, indicating potential publication bias, was further assessed using the Doi plot to evaluate the degree of the asymmetry observed in the funnel plot. This was followed by Egger's regression test to test for the significance of the confirmed asymmetry.

3. Results

3.1. Characteristics of included studies and study selection process

The literature search from the selected electronic databases returned 1867 citations. Additionally, six studies were identified from other searches conducted (manual search of references and other internet search engines). After de-duplication of the total citations, 1217 publications were screened on title/abstract and 48 were then selected for full-article review (Table S1). Finally, 17 publications were included in this SR&MA (Fig. 1 and S4 File) with a total sample size of 7160 (food, animal, and environmental samples). Of the included studies, 53% (9) were conducted in Malaysia. Other countries include Thailand, Indonesia, and Singapore, with no studies identified from the remaining seven countries of SEA. No study sampled humans in all the included publications. One study sampled food, animals, and environment [14], one sampled food and environment [15] and another [16] worked on animal and environmental samples. All other remaining studies sampled only food. Other characteristics of the included studies are outlined in Table 1.

3.2. Risk of bias (quality) assessment

The result of the study quality assessment is provided in Table S2. Of the included studies, 88% (15) were high quality, and the remaining two studies were medium quality. There was no study with low quality.

3.3. Outcomes

Primary outcome: the primary outcome was to pool the overall prevalence of *L. monocytogenes* in humans, animals, food, and the environment (one health concept). In this review, prevalence (proportion) is defined as the number of cases (positive *L. monocytogenes*) in the (sampling) population, divided by the population number (sample size) [17]. So, all the included studies (17) assessed the primary outcome. However, *L. monocytogenes* prevalence was not determined for humans (samples) in all the included studies. Prevalence results for food (only) samples were determined in 15 [14,18–31] of the included studies (Table 1). One study [15] had a result for the environmental (only) sample and one [16] for environmental and animal samples. Thus, all 17 studies were included in the meta-analysis of the primary outcome. The overall (RE pooled) prevalence estimate for *L. monocytogenes* in SEA was determined to be 16% (95% confidence interval [CI]: 10–23). To test for heterogeneity, the following statistics were computed: Cochran Q value ($Q = 1403.354$), $X^2 p < 0.0001$, and $I^2 = 98.9\%$. The forest plot (Fig. 2) gives the graphical presentation of the result, and Table S3 summarises the meta-analysis result.

3.4. Sensitivity analysis

A sensitivity analysis was done by removing the study with the largest weight [14] to determine its influence on the overall pooled prevalence. The removed study had no much impact on the pooled result (Fig. 3), indicating the stability of the meta-analysis.

3.5. Subgroup analysis and meta-regression

Subgroup analysis was used to explore the observed high heterogeneity in order to determine the predictors (source(s) of high heterogeneity). The factors examined in the subgroup analysis were sampled population (food, environment, and animals), country of study, year of

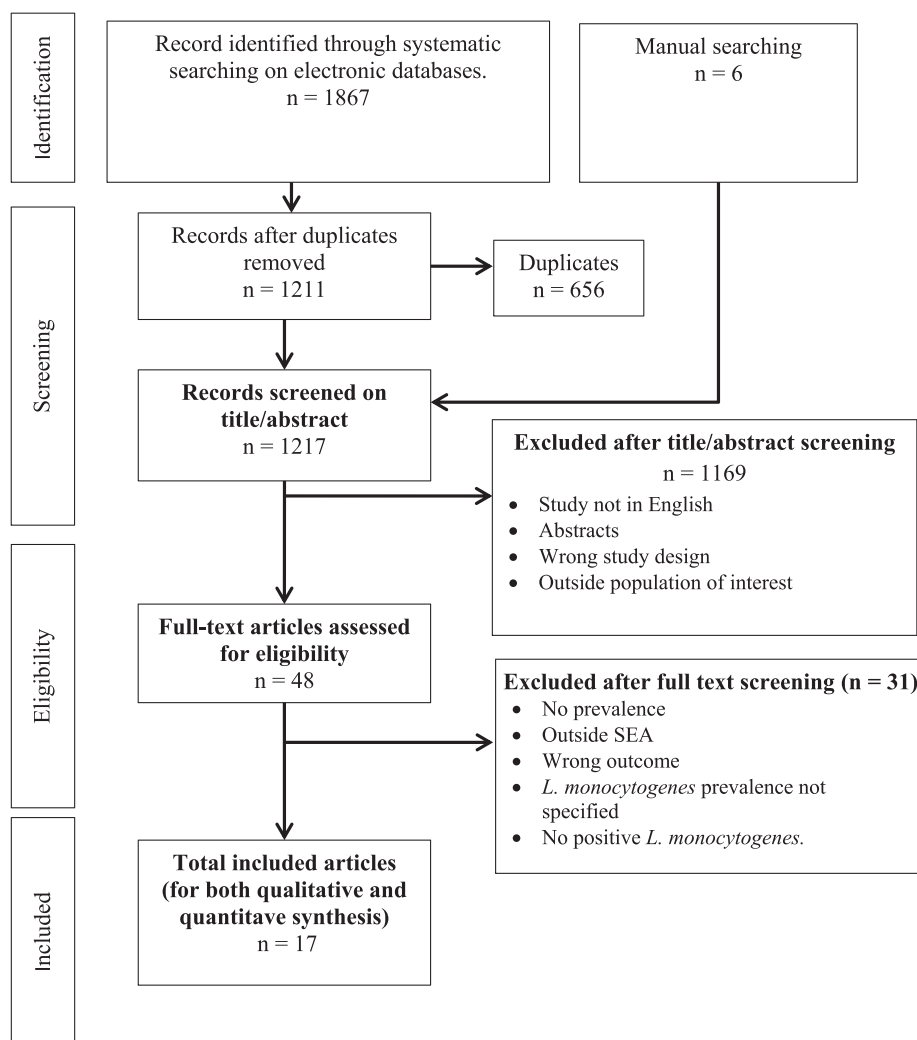


Fig. 1. PRISMA flow diagram.

publication, detection methods, and sample size. Table 2 gives the summary result of the subgroup analysis for each of the factors while the forest plots are presented in the S5 File. In order to determine the effect of the covariates as moderators of the cumulative prevalence, a univariable meta-regression was conducted. The covariates used in the subgroup analysis were used as the moderators in the meta-regression. Table 3 shows the proportion (R^2) of each of the moderators' effect on heterogeneity with their corresponding p values. Country of study (study location) is the only predictor with significant value, and it accounted for 64% of the detected heterogeneity. A multivariable meta-regression was not done because only one variable was significant.

3.5.1. Secondary outcomes

Virulence gene profile: one of the secondary outcomes was to assess the different virulent genes identified from *L. monocytogenes* isolates across SEA. A total of seven (*hly*, [*hly A-F*, and *hly A-R*, *LLO*] *prs*, *prfA*, *actA*, *flaA*, *iap*, *inlA*, *B*,) different virulence genes (Table 4) were identified from 14 (out of the 17) included studies in this review. In addition, virulence genes were reported from Malaysia (in seven studies), Thailand (in four studies), Indonesia (in two studies), and in one study from Singapore. The *hly* gene is the most frequently reported virulence gene, reported in 86% of the 14 studies.

Prevalent sample types: Food being the predominant sampled source of *L. monocytogenes* in all the included studies, a further meta-analysis was done by classifying the food sources into different classes. Details

of classification are outlined in the Table S4. The obtained pooled prevalence estimates for the various food classifications are ready-to-eat (RTE) foods of aquatic origin (16% CI: 6–29), RTE-food products of animal origin (18% CI: 2–43), RTE-foods of vegetable origin (21% CI: 6–41), and other products (25% CI: 16–35). Also, meta-analysis was done for raw foods of aquatic origin (13% CI: 1–30), raw food products of animal origin (19% CI: 9–31). All results are graphically presented in S6 File.

Detection techniques: this outcome was set to outline the various methods used to isolate and identify *L. monocytogenes*. Different detection techniques were used; culture, biochemical, serology, and molecular (Table 4). The polymerase chain reaction (PCR) is the most common method used to identify *L. monocytogenes*. The PCR was used in 82% of the included studies. However, most of the included studies used a combination of two or more methods for identifying *L. monocytogenes*. Combination of culture and biochemical methods was used in 18% of the studies. Three (18%) studies combined culture, biochemical, and PCR methods. An additional 12% combined four methods (culture, biochemical, serology, and PCR), and PCR alone was used in two studies. While 41% of the included studies used culture and PCR to isolate and identify *L. monocytogenes*.

3.6. Publication bias

A lack of symmetry was observed from the constructed funnel plot,

Table 1
Characteristics of included studies.

Author	Year of sampling	Country	Study design	Sample size	Studied population				Overall reported prevalence %			
					Human	Animal	Environment	Food	Human	Animal	Environment	Food
Minami et al., 2010	2006–2007	Thailand	Prevalence survey	388	N	N	N	Y	–	–	–	6
Arumugaswamy et al., 1994	1993	Malaysia	Prevalence	234	N	N	N	Y	–	–	–	43.2
Wong et al., 2012	2009	Malaysia	Prevalence	112	N	N	N	Y	–	–	–	22.3
Vongkamjan et al., 2016	2013	Thailand	Prevalence	200	N	N	N	Y	–	–	–	7.5
Kuan et al., 2013	2010–2011	Malaysia	Prevalence	216	N	N	N	Y	–	–	–	26.39
Sugiri et al., 2014	2012–2013	Indonesia	Prevalence	184	N	N	N	Y	–	–	–	15.8
Marian et al., 2012	2008	Malaysia	Prevalence	140	N	N	N	Y	–	–	–	16.4
Vongkamjan et al., 2017	2013–2014	Thailand	Prevalence	595	N	N	Y	Y	–	–	3.7	–
Goh et al., 2012	2011–2012	Malaysia	Prevalence	210	N	N	N	Y	–	–	–	20
Jamali et al., 2013	2006–2012	Malaysia	Prevalence	396	N	N	N	Y	–	–	–	11.4
Chau et al., 2017	2011–2015	Singapore	Prevalence survey	527	N	N	N	Y	–	–	–	12.7
Aksono et al., 2020		Indonesia	Cross sectional	60	N	N	N	Y	–	–	–	5
Indrawattana et al., 2011	2007	Thailand	Prevalence	104	N	N	N	Y	–	–	–	15.4
Hassan et al., 2001		Malaysia	Prevalence	101	N	N	N	Y	–	–	–	23.8
Kanarat et al., 2011	2004–2009	Thailand	Prevalence	3600	N	Y	Y	Y	–	–	–	1.7
Lesley et al., 2016	2012	Malaysia	Prevalence	30	N	Y	Y	N	–	33.3	–	–
Kuan et al., 2013b	2010–2011	Malaysia	PS	63	N	N	N	Y	–	–	–	33.3

CS: cross sectional, N: No, not included, PS: Prevalence survey, Y: Yes, include.

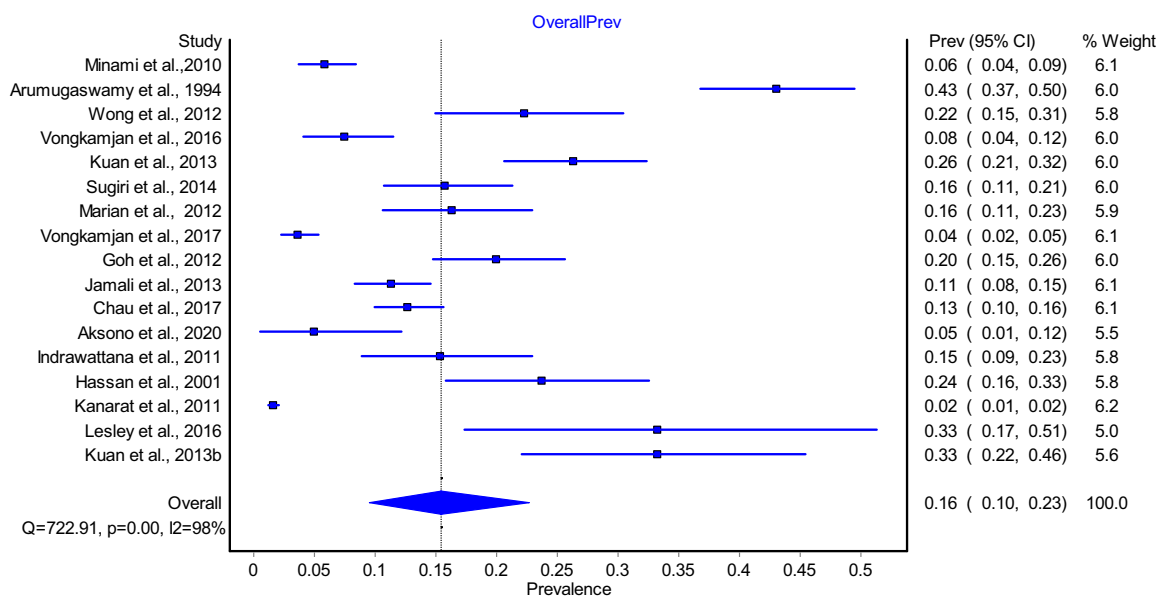


Fig. 2. Forest plot of overall meta-analysis of *L. monocytogenes* (for the animal, environment, and food) in SEA.

which illustrates potential publication bias (Fig. 4). To this effect, a Doi plot was constructed to determine the level of asymmetry. The LFK index of 4.9 (Fig. 5) observed from the Doi plot indicates the presence of major asymmetry. To quantify the level of asymmetry Egger's regression test was conducted which was not significant ($p = 0.052$).

4. Discussion

One Health is defined as an integrated, unifying strategy with the goal of enhancing the health of people, animals, and ecosystems in a sustainable manner [32]. This concept of One Health is increasingly gaining recognition as a viable tool in strengthening and supporting global health security. The acceptance of the One Health concept further buttresses the interconnectivity of human health to animal health and

the common environment. [33]. In recent years the world has seen disease outbreaks of emerging and resurging pathogens due to increased human-animal-environment interaction [34]. This close interaction has provided more opportunities for the emergence of zoonotic diseases. One such pathogen is the *L. monocytogenes* transmitted directly from infected animals and contaminated food products to humans [35]. This organism has a unique ability to withstand extreme food preservation conditions, thus making it a serious food safety threat [36]. Thus, addressing the threat of this pathogen will require robust data (from human, animal, food, and environment sources) on the prevalence of this pathogen at a regional level.

Therefore, this review included studies of food, environment, and animal samples (as there was no data on human samples) with relatively large sample size. The findings of this SR&MA revealed a high (16%)

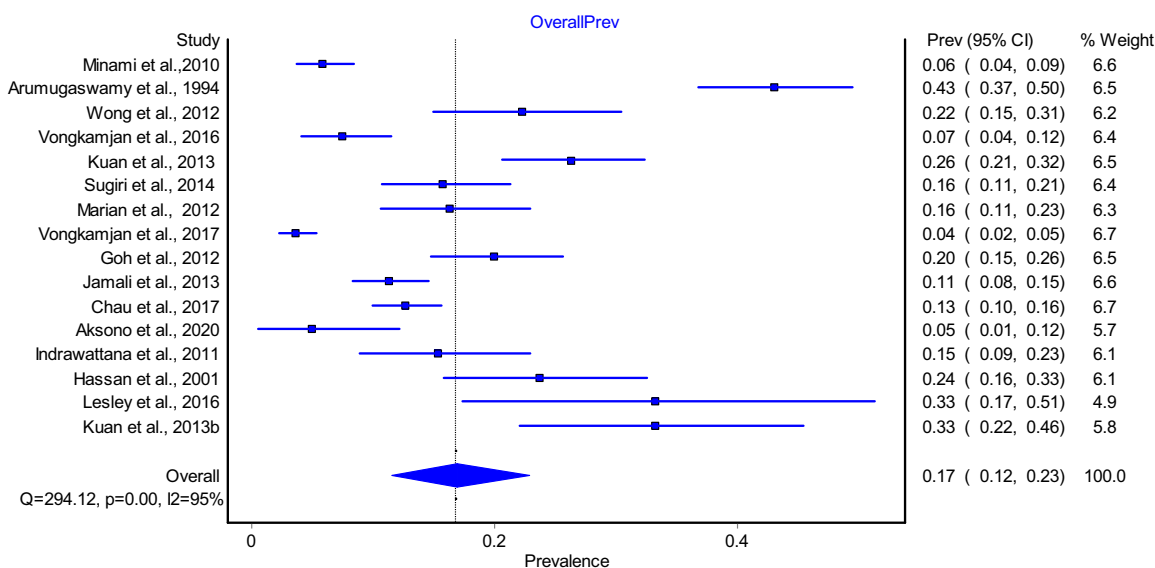


Fig. 3. Forest plot of sensitivity analysis of *L. monocytogenes* in SEA.

Table 2
Summary of subgroup analysis result.

Subgroups	Number of studies	Pooled prevalence		Heterogeneity	
		%	95% CI	I ²	p
Food-environment-animal	18.0				
➤ Food	15.0	16.0	9.0–24.0	98.0%	<0.00001
➤ Environment	2.0	15.0	0.0–60.0	87.0%	<0.00001
➤ Animal	1.0	32.0	14.0–53.0	–	–
Country of study	17.0				
➤ Indonesia	2.0	10.0	2.0–22.0	81.0%	<0.00001
➤ Malaysia	9.0	25.0	17.0–33.0	91.0%	<0.00001
➤ Thailand	5.0	5.0	2.0–10.0	94.0%	<0.00001
➤ Singapore	1.0	13.0	10.0–16.0	–	–
Sample size	17.0				
➤ < 100	3.0	22.0	2.0–46.0	90.0%	<0.00001
➤ 100–500	11.0	18.0	12.0–25.0	94.0%	<0.00001
➤ >500	3.0	5.0%	0.0–12.0	98.0%	<0.00001
Year of publication	17.0				
➤ 1994–2001	2.0	33.0	15.0–53.0	92%	<0.00001
➤ 2002–2015	10.0	15.0	7.0–25.0	98.0%	<0.00001
➤ 2016–2020	5.0	10.0	4.0–17.0	91.0%	<0.00001
Detection methods	17.0				
➤ Culture/Biochemical/Serology/PCR	2.0	10.0	2.0–20.0	88.0%	<0.00001
➤ Culture/Biochemical/PCR	3.0	11.0	7.0–16.0	64.0%	<0.00001
➤ Culture/Biochemical	3.0	19.0	0.0–61.0	99.0%	<0.00001
➤ Culture/PCR	7.0	16.0	9.0–24	94.0%	<0.00001
➤ PCR	2.0	23.0	17.0–30.0	59.0%	0.12

overall prevalence estimate (for food, environment, and animal samples) of *L. monocytogenes* in SEA. The pooled prevalence estimate in this study is similar to but slightly lower than the calculated average prevalence of 22.2% recorded from a review study in Africa [37]. The obtained result suggests that *L. monocytogenes* is highly prevalent in food, environment, and animals in this subregion. Thus, implying that the human population might be at an increased risk of infection by this

Table 3
Univariate meta-regression.

Covariates	R ² (%)	p value
Country of study	64.00	0.011
Detection methods	18.30	0.775
Population type	18.80	0.423
Sample size	28.00	0.029
Study quality	0.633	0.762
Year of publication	67.90	0.401

R²: explains the proportion of between study variance (the effect of covariates on heterogeneity).

pathogen. In the presence of a clear indication of increased risk, there is the need for robust prevention and control strategies. Also, improved surveillance and focused research is required to address the threat of this pathogen in the subregion.

Further subgroup analysis equally showed high pooled prevalence for each of the sampled sources (food: 16%, environmental: 15% and animal:32%). There was, however, only one study that reported prevalence from animal origin [16] and only from one country. Hence likely the reason for the high prevalence observed from animal sources in this review. The observed prevalence (32%) from this study [16] is higher than the observed pooled prevalence of 7% from a meta-analysis conducted in Iran [38]. In the cases of environmental and food sources, it might be out of place to compare the observed prevalence in this study to what was obtained in the Iran study. Due to the fact that the Iran study [38] is a country level pooled prevalence. Comparing the result from this meta-analysis with the results of other meta-analyses is challenging because most of the available meta-analyses were conducted at the country level. We are yet to come across any regional level SR&MA that comprehensively pooled *L. monocytogenes* prevalence from different sampled sources (the One Health approach) as done in this current study.

At country level, this study revealed that the highest prevalence (25%) was observed in Malaysia. The prevalence observed in Malaysia is higher than what was obtained from Indonesia, Singapore, and Thailand. However, the observed prevalence from the other three countries was also relatively high, further confirming that *L. monocytogenes* is highly prevalent in the subregion. The observed variation in the prevalence between countries could be because Malaysia has the highest number of studies included in this SR&MA. Other possible reasons may include sample size variation, differences in identification

Table 4
Methods for the detection of *L. monocytogenes* and virulent genes targeted.

Study	Sample type	Mode of sample collection	Detection methods				Virulence gene (target gene)
			Culture (type of media)	Biochemical	Serology	Molecular	
Minami et al., 2010	Meat and seafood	Vendors' bare hand	PALCAM, CHROMagar, BHI, TSA	Rhamnose, Mannitol, Dextrose	Seroagglutination	PCR	<i>hly</i>
Arumugaswamy et al., 1994	Raw foods, RTE foods	Purchased	PALCAM	MR, VP, Rhamnose, Xylose, Mannitol	–	–	–
Wong et al., 2012	Raw burger patties	Purchase	PALCAM, TSA	–	–	PCR	<i>hlyA</i> , 16S <i>rRNA</i>
Vongkamjan et al., 2016	RTE foods	Purchase	BHI, CHROMagar	–	–	PCR, <i>sigB</i> sequencing	<i>Prs</i> , <i>hly</i>
Kuan et al., 2013	Raw chicken offal	Purchase	–	–	–	PCR,	<i>hlyA</i> , 16S <i>rRNA</i>
Sugiri et al., 2014	Raw chicken carcass	N/S	PALCAM, CHROMagar, BHI, TSA	CAMP-test	–	PCR	<i>Prs</i> , <i>prfA</i> , ORF2819
Marian et al., 2012	Raw foods, RTE foods	Purchase	PALCAM, TSA	–	–	PCR	<i>hlyA</i> , 16S <i>rRNA</i>
Vongkamjan et al., 2017	Food, Environmental	Sponge-stick swab	CHROMagar, BHI	–	–	PCR	<i>hly</i>
Goh et al., 2012	Raw chicken meat	N/S	N/S	–	–	PCR	<i>hlyA</i> , 16S <i>rRNA</i>
Jamali et al., 2013	RTE foods	N/S	PALCAM, TSA, BHI, CHROMagar	MR-VP, catalase, oxidase. Urea, SIM, and TSI	–	PCR	16S <i>rRNA</i> , <i>LLO</i>
Chau et al., 2017	RTE foods	Purchase	PALCAM, OAA	–	–	PCR, MLST	<i>Prs</i> , <i>inlA</i>
Aksono et al., 2020	Raw chicken meat	N/S	PALCAM,	MR-VP, SIM, and TSIA, CAMP	–	PCR, PGA	<i>hlyA</i>
Indrawattana et al., 2011	Raw meat	N/S	Chrom agar, PALCAM, TSA, TSYEA	CAMP,	Listeria antisera, for O and H antigen	PCR,	<i>hlyA</i> , <i>actA</i> , <i>flaA</i> , <i>iap</i> , <i>inl A</i> , <i>B</i> , <i>prf A</i>
Hassan et al., 2001	Frozen beef or meat	Purchase	TSYEA,	TSI, MR-VP, catalase, oxidase, CAMP	–	–	–
Kanarat et al., 2011	Soil litter, chicken feed, water, meat, RTE	Swab with sterile, gauze	ALOA, PALCAM, TSA	Catalase, Oxidase, CAMP	–	–	–
Lesley et al., 2016	Bats, birds, rodents, shrew, feces, water & sediment	Mist nets, cage traps, by scooping & dipping, anal & coecal by cotton bud swab	PALCAM	–	–	PCR	<i>hly A-F</i> , <i>hly A-R</i>
Kuan et al., 2013b	Raw beef offal	Purchased	TSA	–	–	PCR	16S <i>Rna</i> , <i>LLO</i>

BHI; Brain Heart Infusion, CAMP; Christie Atkins-Munch-Peterson, CHROMagar™; Trade name, MLST; Multilocus Sequence Typing, MR-VP; Methyl Red and Vogues-Proskauer, NS; Not stated, PALCAM; Trade name for listeria culture agar. PCR; Polymerase chain reaction, PGA; Phylogenetic Analyses, RTE; Ready to Eat, SIM; Sulfide Indole Motility, TSA; Tryptic Soy Agar, TSATE; Tryptic Soy Agar Yeast Extract, TSI; Triple Sugar Iron. 16S – 16 sub-units, *actA*; – actin polymerization protein, *FlaA*; – Flagellin A, – *hlyA*; – Hemolysin O, *iap*; – associated protein, *Inl*; – internalin A, *LLO*; – Listeriolysin O, *ORF*; – Open Reading Frame, *plcA*; Phosphotidylinositol A. *PrfA*; – perforin A, *prs*; – pyrophosphokinase, *rRNA*; – ribosomal, Ribonucleic Acid.

methods or seasonal variability. It is also discovered from the meta-analysis that prevalence rises with decreasing sample size. However, the group with the smaller sample sizes (<100) expectedly has less precision with a higher margin of error. Additional prevalence estimation based on the year of publication showed a decreasing prevalence pattern from 1994 to 2020. The prevalence was highest between 1994-to-2001, and the lowest prevalence was recorded between 2016-to-2020. The observed reduction in the prevalence of *L. monocytogenes* across the years might not be unconnected with improved sanitary practices and food processing best practices. It was equally observed in this review that studies that used PCR alone had the highest prevalence of 23% (CI: 17–30). Although, this group had the least number of studies.

However, it is important to state that heterogeneity persisted within all the subgroups. Which indicates that the factors evaluated in the subgroup analysis did not completely explain the observed heterogeneity. Nevertheless, the univariate analysis shows that study location (country) can explain 64% of the observed variation in the meta-analysis. Other possible reasons for the observed heterogeneity might be due to some covariates that are not within the scope of this analysis. Such factors may include but not limited to temperature variation

during sample processing, sample contamination, sample transportation mode, and sample storage methods and conditions [39].

In terms of the virulence genes, many virulence genes were identified in this study, and the most frequent is the *hly* gene. The high-level presence of the *hly* gene in the isolates in the region may have an implication on potential outbreak occurrence and disease severity [40,41]. Also, our findings showed the prevalent sample types from food sources. The highest prevalence was observed in RTE-food of vegetable products followed swiftly by raw-food of animal origin. This information is of clinical and public health significance as it can be used to enumerate high-risk foods for *L. monocytogenes* infection [42].

In addition, our study provided information on various methods used to identify *L. monocytogenes*. The results showed that PCR is gaining more prominence as most recent studies used the technique. While culture and biochemical tests that are previously considered as the gold standard, are seldom used in most recent studies. This is probably due to the several limitations associated with these methods [43]. This information is relevant in clinical settings and the food industry. The demand for more sensitive, highly specific, and rapid techniques for identifying the pathogen is on the rise in clinical settings. Whereas in the food

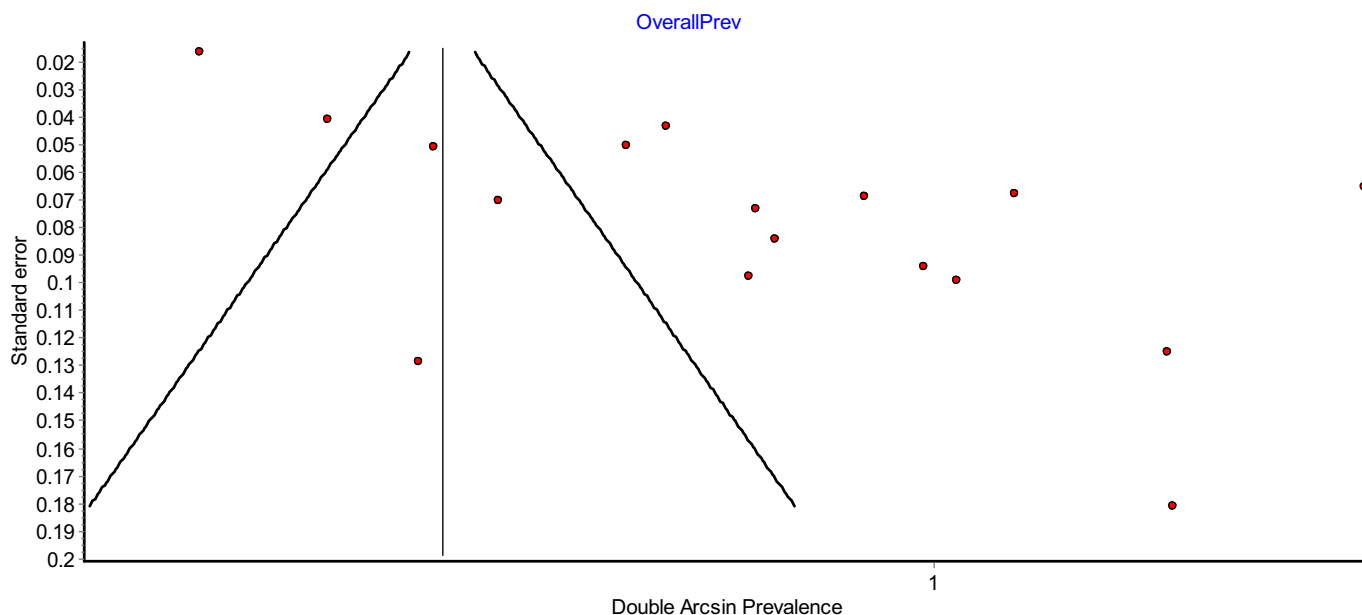


Fig. 4. Funnel plot of double arcsine prevalence against standard error showing observed asymmetry.

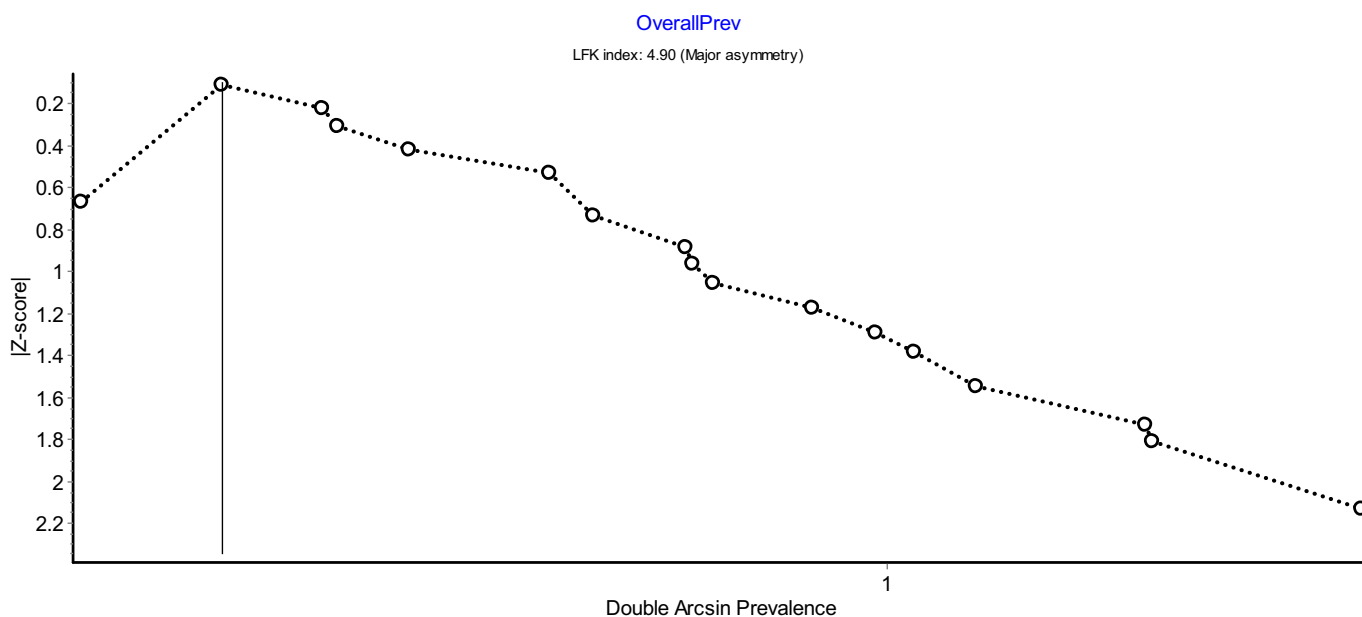


Fig. 5. Doi plot of double arcsine prevalence against Z-score showing evidence of major asymmetry.

industry, there is increased demand for contaminant-free foods [42]. Thus, the need for ultra-rapid, sensitive, and specific detection techniques cannot be overemphasized.

5. Strength and limitation of the study

It is indeed worthy of note that this is the first SR&MA to investigate the prevalence of *L. monocytogenes* in SEA. The study also comprehensively reviewed the prevalence (comprising food, environment, and animal sources) and related factors of *L. monocytogenes* in the subregion. This review also provided relevant information on virulence gene profile, prevalent sample types, and widely used identification methods for the pathogen in the subregion. The provided information will improve awareness to understand this essential foodborne pathogen better. Additionally, it will assist in informed – decision making in clinical

practice, public health intervention, and policy design for the prevention and control of the disease. However, the limitations of this study include the fact that only articles published in the English language are included in the review. Also, the meta-analysis result showed high heterogeneity between studies even in the subregion analysis.

6. Conclusion

Addressing the challenges of emerging and resurging pathogens requires intersectoral collaboration, coordination, and communication. The one health approach can help achieve this goal. Using this approach in this study has provided the desired information to address the challenges posed by *L. monocytogenes* in SEA. We now know that *L. monocytogenes* is highly prevalent in SEA with this approach. In addition, we are now aware of the high-risk foods, virulence gene profile, and

frequently used identification methods for *L. monocytogenes*. Similarly, there is a need for further research on the human origin of the pathogen in the subregion. Also, more studies are required from countries in the subregion with no reported studies.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Author contributions

Conception of research idea (ZJ), Literature review (GJ and YR), Research protocol design (YR and GJ), Study appraisal (ZJ, SN, and RM), Data extraction (GJ, YR, and SA), Data analysis and interpretation of results (ZJ, SN, RM, GJ, and YR), Manuscript drafting (GJ, SA and YR), and review of the initial and final draft of the manuscript (ZJ, SN, and RM).

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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