



Salmonella spp. and *Escherichia coli* O157:H7 prevalence and levels on lettuce: A systematic review and meta-analysis

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ABSTRACT

Lettuce (*Lactuca sativa*), one of the most consumed leafy vegetables in the world, is frequently implicated with foodborne disease (FBD) outbreaks, with *Salmonella* spp. and *Escherichia coli* O157:H7 being the most common bacteria to cause this illness. Estimates of prevalence and levels of these pathogens on lettuce are scarce in developed or in developing countries, which hinders risk assessment attempts. In here, we present a systematic review and meta-analysis of reported prevalence and levels of *Salmonella* spp. and *E. coli* O157:H7 on lettuce using the worldwide available data. Literature was reviewed and examined the results for inclusion of articles in the meta-analysis. Data (prevalence and/or concentration of *Salmonella* spp. and *E. coli* O157:H7 on lettuce, sample characteristic, country of origin, and *Salmonella* identified serovars) were extracted, and meta-analysis was performed using Open Meta-Analyst, Task Order # 2 software. Although only one work reported the presence of *E. coli* O157:H7 on lettuce, several reports indicated the presence of other, distinct enterohemorrhagic *E. coli* (EHEC) strains, with a mean prevalence of 0.041 (95% CI: 0.005–0.078) and concentration varying from < 3.0 MPN/g to > 1100 MPN/g. Furthermore, the mean prevalence of *Salmonella* spp. on lettuce was 0.041 (95% CI: 0.030–0.052), with reported concentrations varying between 0.054 ± 0.058 CFU/g to 218.78 MPN/g. In addition, subgroup analysis of the presence of *Salmonella* spp. in lettuce revealed a mean prevalence of the bacteria of 0.028 (95% CI: 0.014–0.042) in developed nations and 0.064 (0.041–0.087) in developing nations, with reports varying from 0.001 in Japan to 0.5 in Burkina Faso. Despite a relatively low prevalence, consumption of lettuce is inherently risky because it usually is eaten raw, without thermal treatment to inactivate pathogens. This potential risk further supports performance of quantitative risk assessments to quantify the probability of FBD caused by *Salmonella* spp. and *E. coli* O157:H7 transmitted to lettuce.

1. Introduction

Leafy green vegetables are an important component of a healthy diet, providing essential vitamins, minerals, and fibers. As such, there has been an international increase in their production and consumption, aiming at promoting better human nutrition (Taban and Halkman, 2011). Along with the increased consumption, the number of foodborne outbreaks caused by pathogens associated with leafy green vegetables has increased worldwide in recent decades. The proportion of these outbreaks, however, has augmented beyond that which could be explained by an increased consumption, or even a better reporting system (Callejon et al., 2015).

Despite the development of strategies to prevent the contamination

of fresh produce, produce-related disease outbreaks still occur in many countries, and leafy green vegetables are thought to pose the greatest risk (WHO/FAO, 2008). Lettuce, which is the most consumed leafy vegetable in the world, and usually eaten raw, is frequently implicated with such outbreaks. Moreover, *Salmonella* spp. and *Escherichia coli* O157:H7 are the most common bacteria identified in such cases (Ilic et al., 2012; Wadamori et al., 2017).

Humans and animals are the primary reservoirs of *Salmonella* species, but these microorganisms are abundant in nature as well. *Salmonella* can contaminate fresh produce both during the production through water, soil, insects or other animals contaminated with fecal matter, and during the preparation, through cross contamination (equipment, surfaces, food handlers). Moreover, depending on the

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Table 1
Electronic search strategies for research databases and search results.

| Database | <i>Salmonella</i> | <i>E. coli</i> O157:H7 | Search strategy |
|---|-------------------|------------------------|-------------------------|
| PubMed MEDLINE | 334 | 298 | Abstract |
| Web of Science (Science Citation Index) | 996 | 933 | title/keywords/abstract |

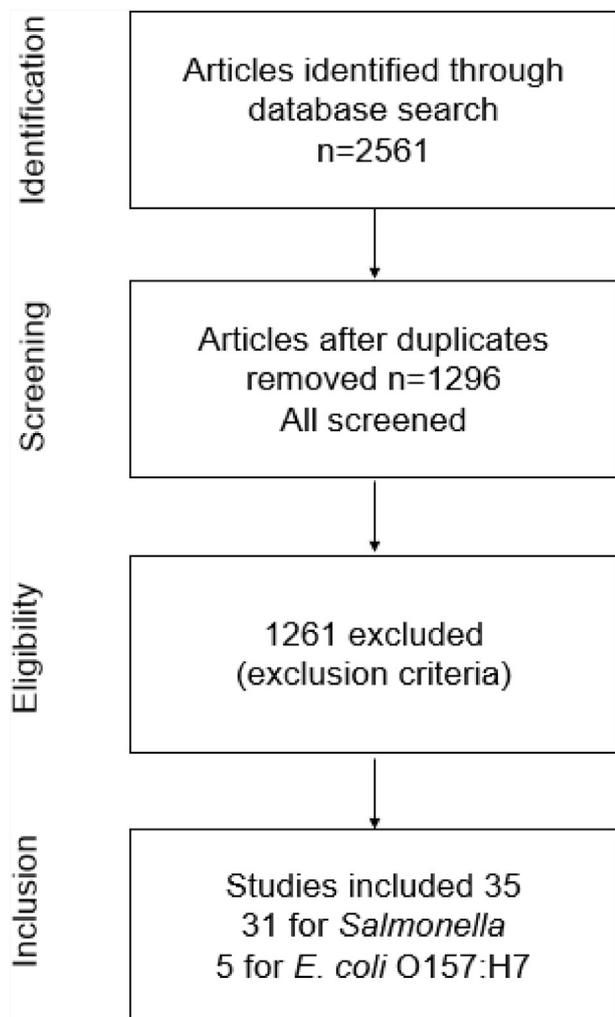


Fig. 1. Flow diagram of the literature search and selection of eligible studies.

serotype, *Salmonella* can be tolerant to low or high temperatures and acidic environments, making disinfection difficult (Yeni et al., 2016).

Currently, there are six pathogenic groups of *E. coli* - Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC),

Enterotoxigenic *E. coli* (ETEC) and Diffusely Adherent *E. coli* (DAEC) -, that may be tolerant to low pH in a similar way to *Salmonella*. The most severe diseases reported during produce-related outbreaks, including bloody diarrhea, thrombotic thrombocytopenic purpura, hemorrhagic colitis, and hemolytic-uremic syndrome are caused by enterohemorrhagic *E. coli* (EHEC) group, which also includes the Shiga-toxin-producing *E. coli* (STEC) serotypes or verocytotoxin-producing *E. coli* (VTEC). As primary reservoirs are ruminants, especially cattle, these pathogens can contaminate fresh produce during both the production phase through contaminated water with infected animal feces, or during the preparation phase through cross contamination (equipment, surfaces, handlers) (Yeni et al., 2016). Furthermore, *E. coli* serovar O157:H7 is a prevalent foodborne pathogen that has caused numerous outbreaks of human gastroenteritis linked to the consumption of lettuce in the United States, Europe, and other industrialized countries (Erickson et al., 2017).

Although studies have identified *Salmonella* spp. and *E. coli* O157:H7 on lettuce, data of prevalence and concentration of contamination are scarce in developed and developing countries. These data are indispensable to carry out quantitative microbial risk assessments, which are necessary to quantify the potential risk involving lettuce consumption and improve the evidence base for food safety regulations and public health policies. Therefore, the objective of this systematic review and meta-analysis is to estimate the prevalence and concentration of *Salmonella* spp. and *E. coli* O157:H7 on lettuce using available data worldwide.

2. Materials and methods

Systematic review and meta-analysis were used to estimate *Salmonella* spp. and *Escherichia coli* O157:H7 prevalence and concentration on lettuce, identifying data gaps.

2.1. Systematic review search strategy and selection criteria

A search was carried out using the terms “lettuce” OR “*Lactuca sativa*” AND “*Salmonella*” OR “*Escherichia coli* O157:H7” (“STEC” and “VTEC” were also considered) in the PubMed and Web of Science platforms in June 2017. No date restrictions were applied. Endnote version X6 (Clarivate Analytics) was used to collect publications (Table 1). All articles found were checked for duplicates using Endnote and Mendeley (<https://www.mendeley.com/>). The search focused on the prevalence and concentration of *Salmonella* spp. and *E. coli* O157:H7 on lettuce. Articles were collected and included when they were

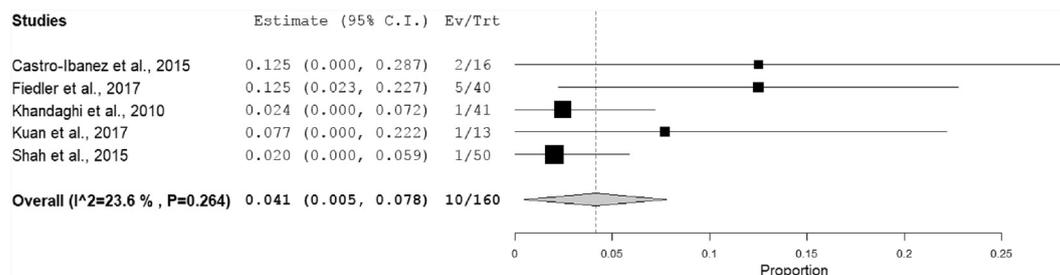


Fig. 2. Prevalence of *E. coli* (including O157:H7, VTEC, STEC) on lettuce (Random Effects Model, $I^2 = 23.6\%$, $p = 0.264$). X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number (Castro-Ibanez et al., 2015; Fiedler et al., 2017; Shah et al., 2015).

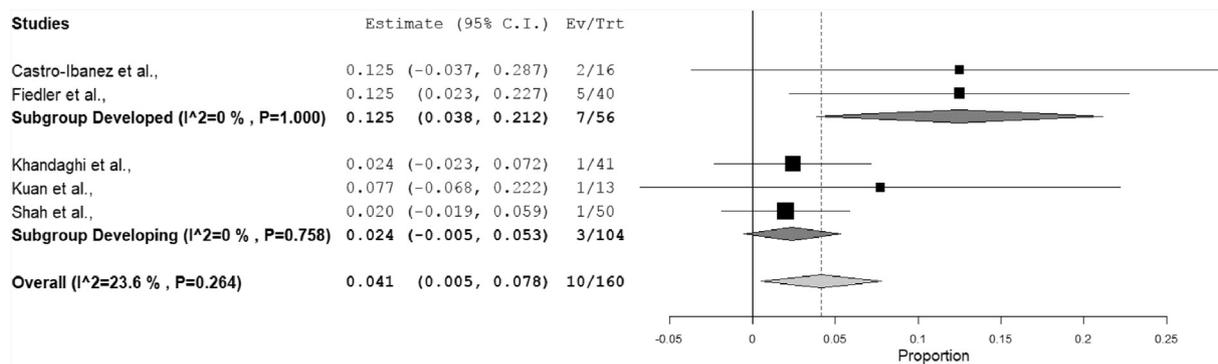


Fig. 3. Prevalence of *E. coli* (including O157:H7, VTEC, STEC) on lettuce in relation with country economic status. X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number (Castro-Ibanez et al., 2015; Fiedler et al., 2017; Shah et al., 2015).

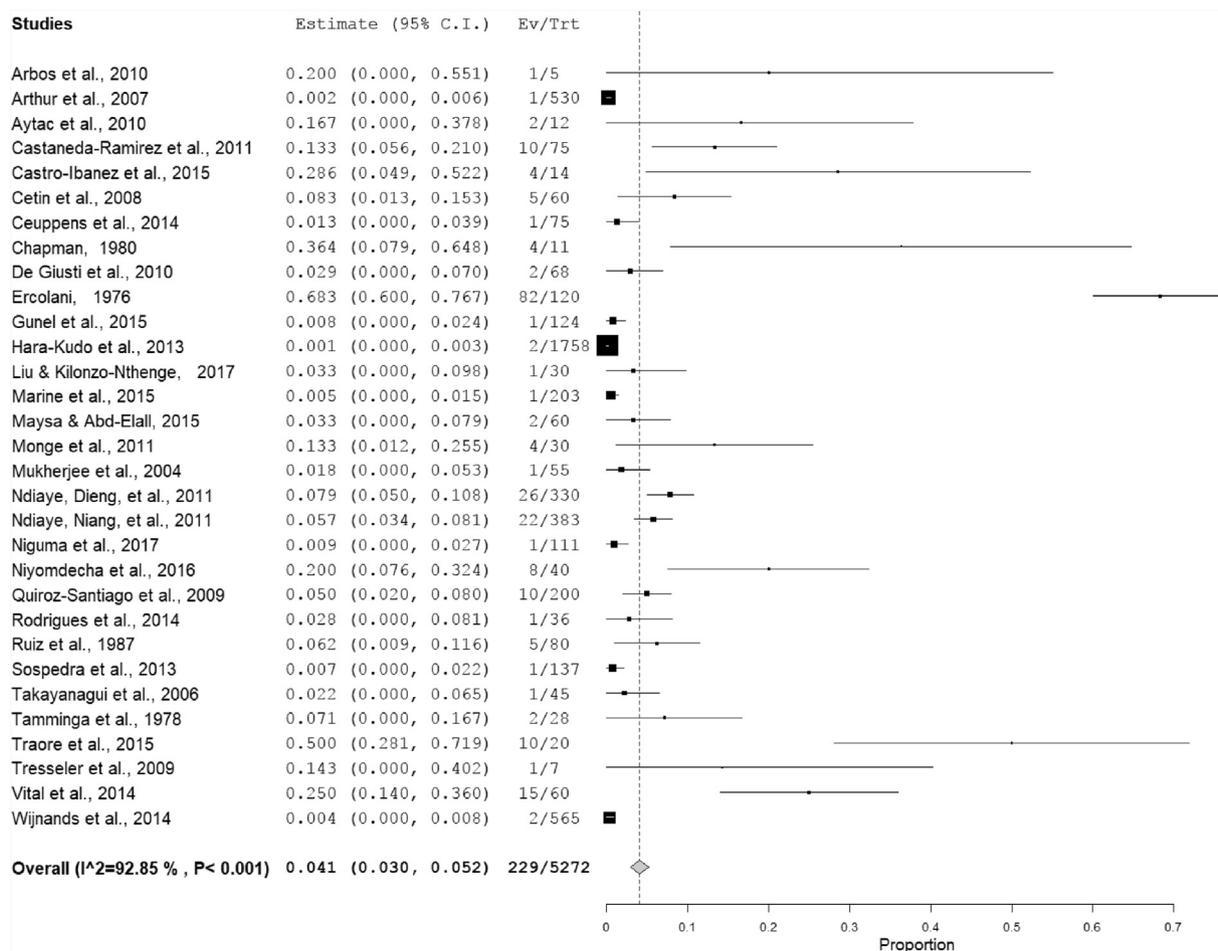


Fig. 4. Prevalence of *Salmonella* on lettuce (Random Effects Model, I² = 92.85%, p < 0.001). X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number (Arbos et al., 2010; Arthur et al., 2007; Aytac et al., 2010; Castaneda-Ramirez et al., 2011; Castro-Ibanez et al., 2015; Cetin et al., 2008; Ceuppens et al., 2014; Chapman, 1980; De Giusti et al., 2010; Fiedler et al., 2017; Gunel et al., 2015; Hara-Kudo et al., 2013; Liu and Kilonzo-Nthenge, 2017; Marine et al., 2015; Maysa and Abd-Elall, 2015; Monge et al., 2011; Mukherjee et al., 2004; Ndiaye et al., 2011; Niguma et al., 2017; Niyomdecha et al., 2016; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Rodrigues et al., 2014; Shah et al., 2015; Sospedra et al., 2013; Takayanagui et al., 2006; Tamminga et al., 1978; Traoré et al., 2015; Tresseler et al., 2009).

published in English, Spanish or Portuguese, and relevant search terms appeared in the title, abstract, or key words. Publications were excluded if they were review articles and book chapters; had incomplete information on the prevalence and concentration of pathogens on lettuce; or used sanitized lettuce or artificial contamination (Fig. 1).

Full-text articles were accessed whenever possible, and when these

were not available, abstracts and article titles were evaluated for relevance. When abstracts were the only text available but deemed relevant, additional effort was done in order to access full articles; otherwise articles were excluded.

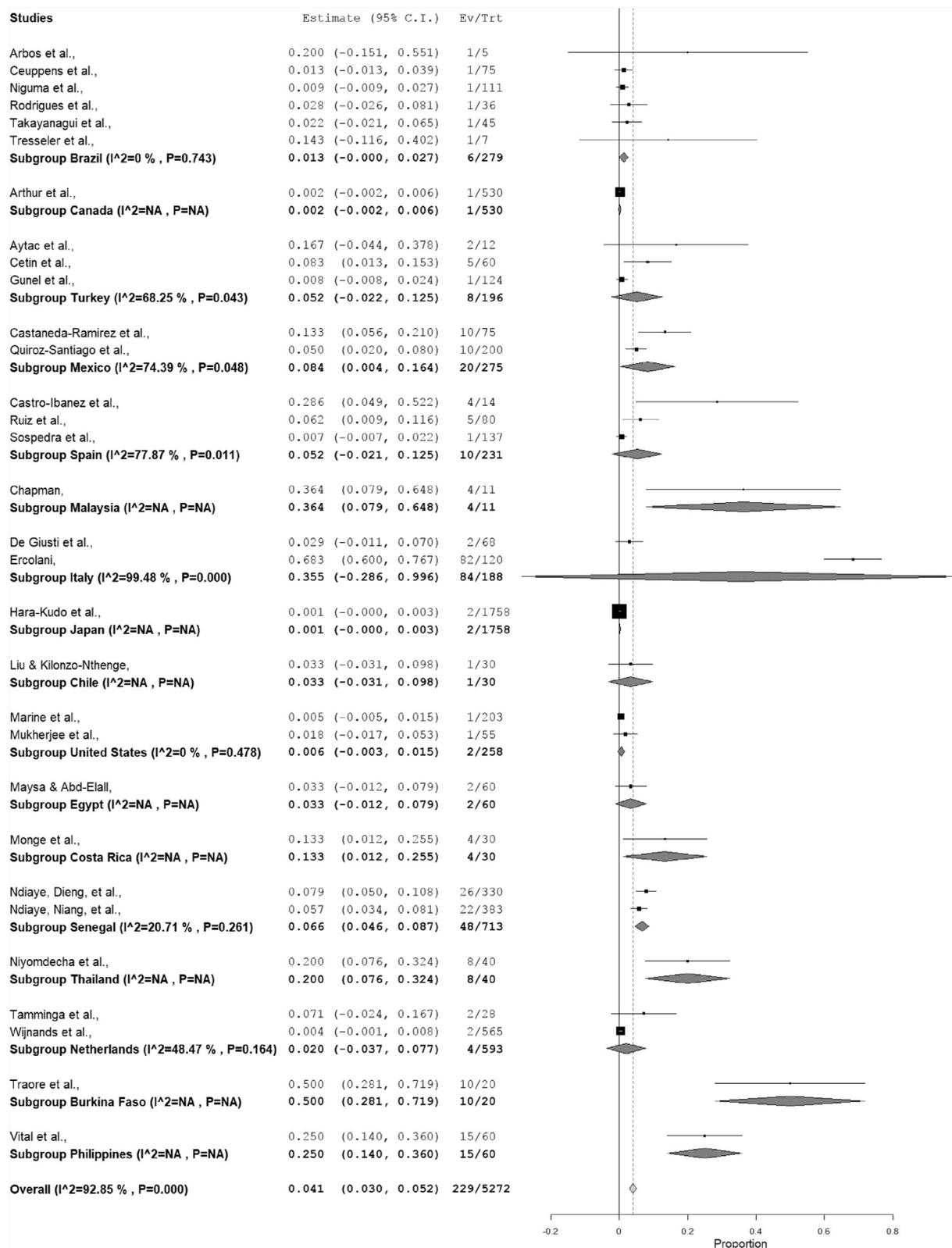


Fig. 5. Prevalence of *Salmonella* on lettuce in relation with country of origin. X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number (Arbos et al., 2010; Arthur et al., 2007; Castaneda-Ramirez et al., 2011; Castro-Ibanez et al., 2015; Cetin et al., 2008; Ceuppens et al., 2014; Chapman, 1980; De Giusti et al., 2010; Fiedler et al., 2017; Gunel et al., 2015; Hara-Kudo et al., 2013; Liu and Kilonzo-Nthenge, 2017; Marine et al., 2015; Maysa and Abd-Elall, 2015; Monge et al., 2011; Mukherjee et al., 2004; Ndiaye et al., 2011; Niguma et al., 2017; Niyomdecha et al., 2016; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Rodrigues et al., 2014; Shah et al., 2015; Sospedra et al., 2013; Takayanagui et al., 2006; Tammaing et al., 1978; Traoré et al., 2015; Tresseler et al., 2009).

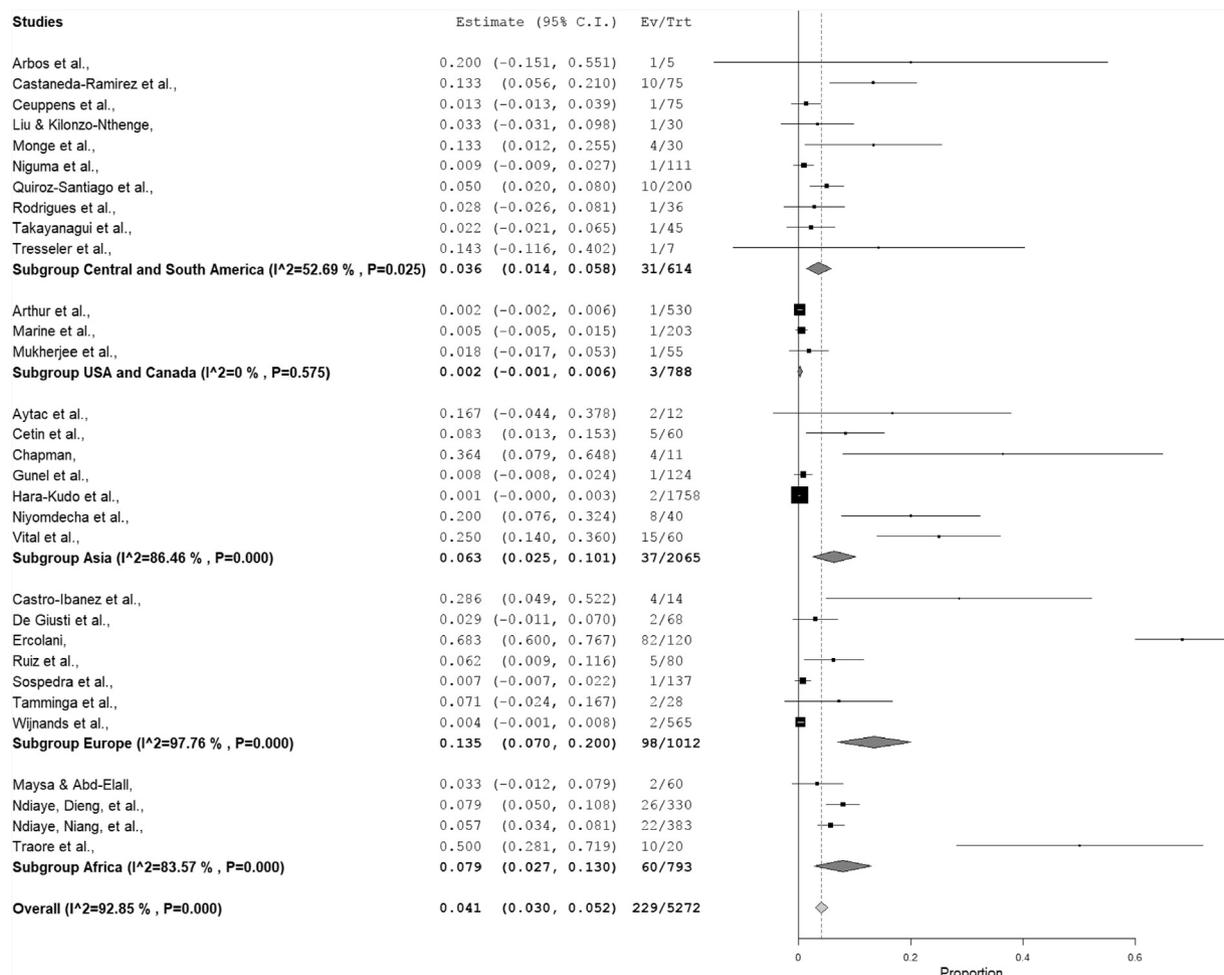


Fig. 6. Prevalence of *Salmonella* on lettuce in relation with region of origin. X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number (Arbos et al., 2010; Arthur et al., 2007; Aytac et al., 2010; Castaneda-Ramirez et al., 2011; Castro-Ibanez et al., 2015; Cetin et al., 2008; Ceuppens et al., 2014; Chapman, 1980; De Giusti et al., 2010; Gunel et al., 2015; Hara-Kudo et al., 2013; Liu and Kilonzo-Nthenge, 2017; Marine et al., 2015; Maysa and Abd-Elall, 2015; Monge et al., 2011; Mukherjee et al., 2004; Ndiaye et al., 2011; Niguma et al., 2017; Niyomdecha et al., 2016; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Rodrigues et al., 2014; Sospedra et al., 2013; Takayanagui et al., 2006; Tamminga et al., 1978; Traoré et al., 2015; Tresseler et al., 2009).

2.2. Data extraction

Data for *Salmonella* spp. and *E. coli* O157:H7 prevalence and concentration on lettuce were extracted from the studies identified through the systematic review of the literature and included in the study database independently by a single reviewer and validated by a second reviewer. Data extracted included type of lettuce, sample size, sample pooled, sample source/location of sampling, laboratory detection technique, country of origin, *Salmonella* spp. or *E. coli* O157:H7 prevalence on lettuce, level of contamination in CFU/g or MPN/g, and identified *Salmonella* serovars. Data on farms that were sampled repeatedly, and samples that were analyzed with different methods were included in the meta-analysis as unique values.

2.3. Data analysis

The meta-analysis and Forest plotting of pathogens prevalence as well as estimation of the subgroups effects were done using the Open Meta-Analyst, Task Order # 2 software (available at <https://www.brown.edu/academics/public-health/research/evidence-based-medicine/research-initiatives/software-0>). The data were analyzed in binary random model effects by the DerSimonian-Laird method at 95% confidence interval. Individual models were used for analysis of each

pathogen.

The heterogeneity across the studies estimated in the random-effects model was quantified using inverse variance index (I^2). The I^2 values at 25%, 50% and 75% were considered as low, moderate and high heterogeneity, respectively (Higgins et al., 2003). Subgroup analyses were performed, differentiating by the country of origin, region of origin, country economic status (it was considered per capita income, life expectancy, and literacy; dividing in developing countries with lower indexes and developed countries with higher ones), sample source, laboratory detection technique, sample size, and sample pooled.

Pathogen concentration values were also extracted when possible, however, limited availability prevented us from developing meta-analysis of such data.

3. Results and discussion

In this study, a rigorous and a transparent approach was used to identify publications reporting the prevalence and/or the concentration of *Salmonella* spp. and *E. coli* O157:H7 on lettuce. All 1296 articles were screened to search this information of pathogens on lettuce (Fig. 1). Most of these studies investigated but did not detect the pathogens on lettuce. This can be explained by the fact that the different works sampled lettuce at different stages of the food supply chain, and also

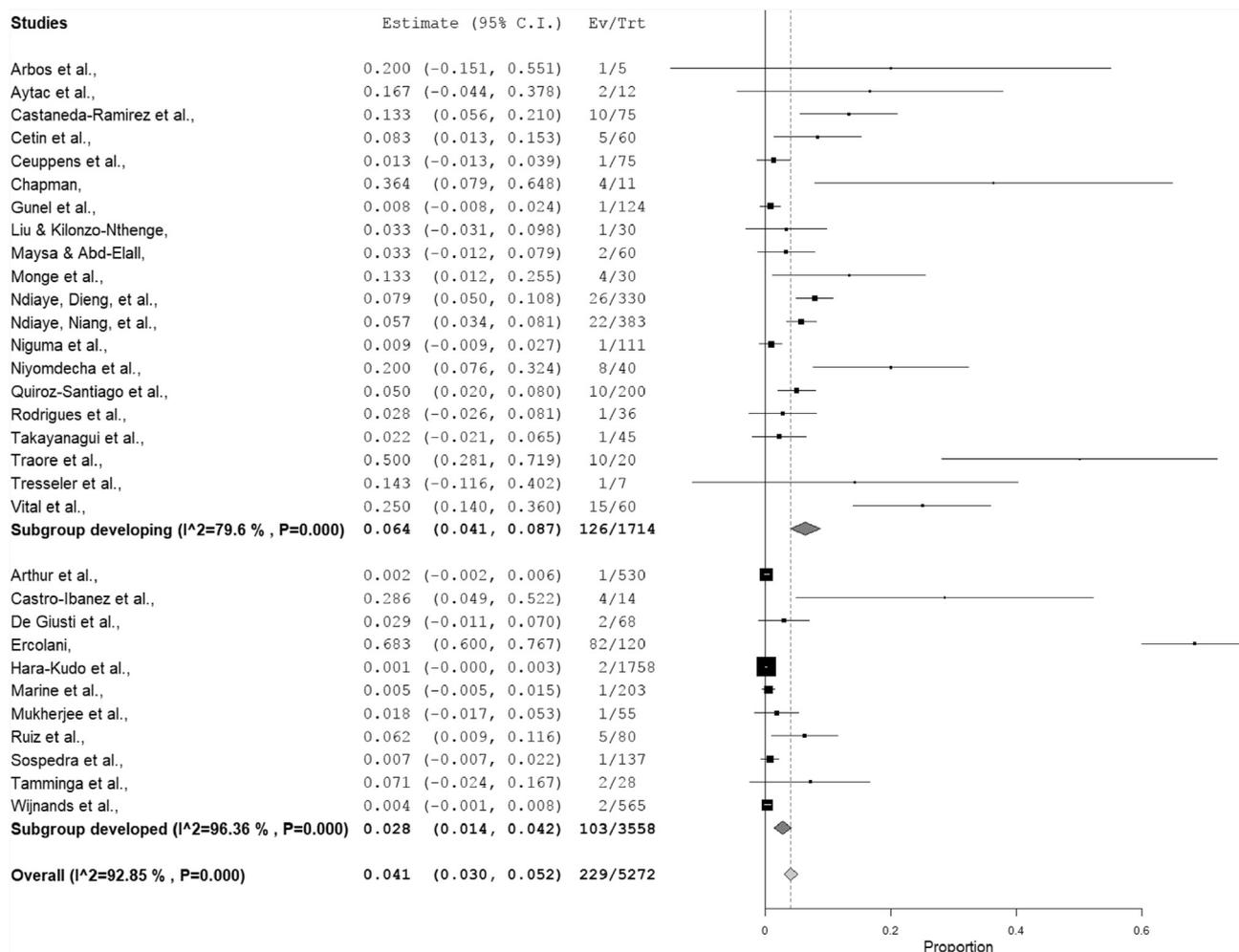


Fig. 7. Prevalence of *Salmonella* on lettuce in relation with country economic status. X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number (Arbos et al., 2010; Arthur et al., 2007; Aytac et al., 2010; Castaneda-Ramirez et al., 2011; Castro-Ibanez et al., 2015; Cetin et al., 2008; Ceuppens et al., 2014; Chapman, 1980; De Giusti et al., 2010; Gunel et al., 2015; Hara-Kudo et al., 2013; Liu and Kilonzo-Nthenge, 2017; Marine et al., 2015; Maysa and Abd-Elall, 2015; Monge et al., 2011; Mukherjee et al., 2004; Ndiaye et al., 2011; Niguma et al., 2017; Niyomdecha et al., 2016; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Rodrigues et al., 2014; Sospedra et al., 2013; Takayanagui et al., 2006; Tammaing et al., 1978; Traoré et al., 2015; Tresseler et al., 2009).

used diverse sampling approaches or detection methods. On the cases in which bacterial contamination was artificial, detection was reported on sanitized lettuce, or the type of vegetable in which the detection was reported was not clear, the articles were excluded from the analysis. After the screening, one study was also excluded due to be impossible to access the full article (Rude et al., 1984). We considered exclusively publications that detected *Salmonella* spp. and *E. coli* O157:H7 on lettuce *in natura*.

Only one article presented the detection of *E. coli* O157:H7 on lettuce (Khandaghi et al., 2010). Because of this, studies that found STEC or VTEC on lettuce were also considered. Thus, five articles published between 2010 and 2017, written in English, reporting studies carried out in five different countries (Spain, Germany, Iran, Malaysia, and Pakistan) were considered. The sample source were farms (2) and retail (3), and the analyses were done using traditional and molecular methods (Table S1 supplementary material). Only one of these five manuscripts showed results about concentration of STEC on lettuce, and the results ranged from less than 3.0 MPN/g to more than 1100 MPN/g (Kuan et al., 2017). These results reflect the scarcity of data on the detection and levels of *E. coli* O157:H7 on lettuce, indicating the importance of further studies focusing on these themes and making possible researches as microbiological risk assessments.

Fig. 2 shows that the average prevalence of *E. coli* (including

O157:H7, VTEC, STEC) on lettuce as calculated from the reviewed publications ($n = 5$) was 0.041 (95% CI: 0.005–0.078), which means approximately 4 positives samples in 100 tested (4.1%). The I^2 was 23.6% ($p = 0.264$), meaning low heterogeneity. In other words, all studies analyzed had similar prevalence of pathogen on lettuce. Moreover, although a small number of studies are currently available, they obtained similar results, which may suggest their application in future studies. The average prevalence in developed countries was 0.125 (95% CI: 0.038–0.212) ($n = 2$), while in developing countries it was 0.024 (95% CI: -0.005 –0.053) ($n = 3$, Fig. 3). In both cases the heterogeneity among the publications concerning prevalence of *E. coli* on lettuce depending on the economic country status was 0%. The developed countries showed a higher prevalence than those countries in development. This may be explained due to microbiological detection techniques used in developed countries being more sensitive than those applied in developing countries, providing higher positive results.

In relation to the prevalence of *Salmonella* on lettuce, 31 articles were found, published between 1976 and 2017. Out of these, 26 studies were written in English, four in Portuguese, and one in Spanish. The articles represent four different continents (Africa, America, Asia, and Europe), and 17 countries, and the sample sources were farms, retail, industry. The methods of analysis were based on microbial culture and molecular detection (Table S2 supplementary material).

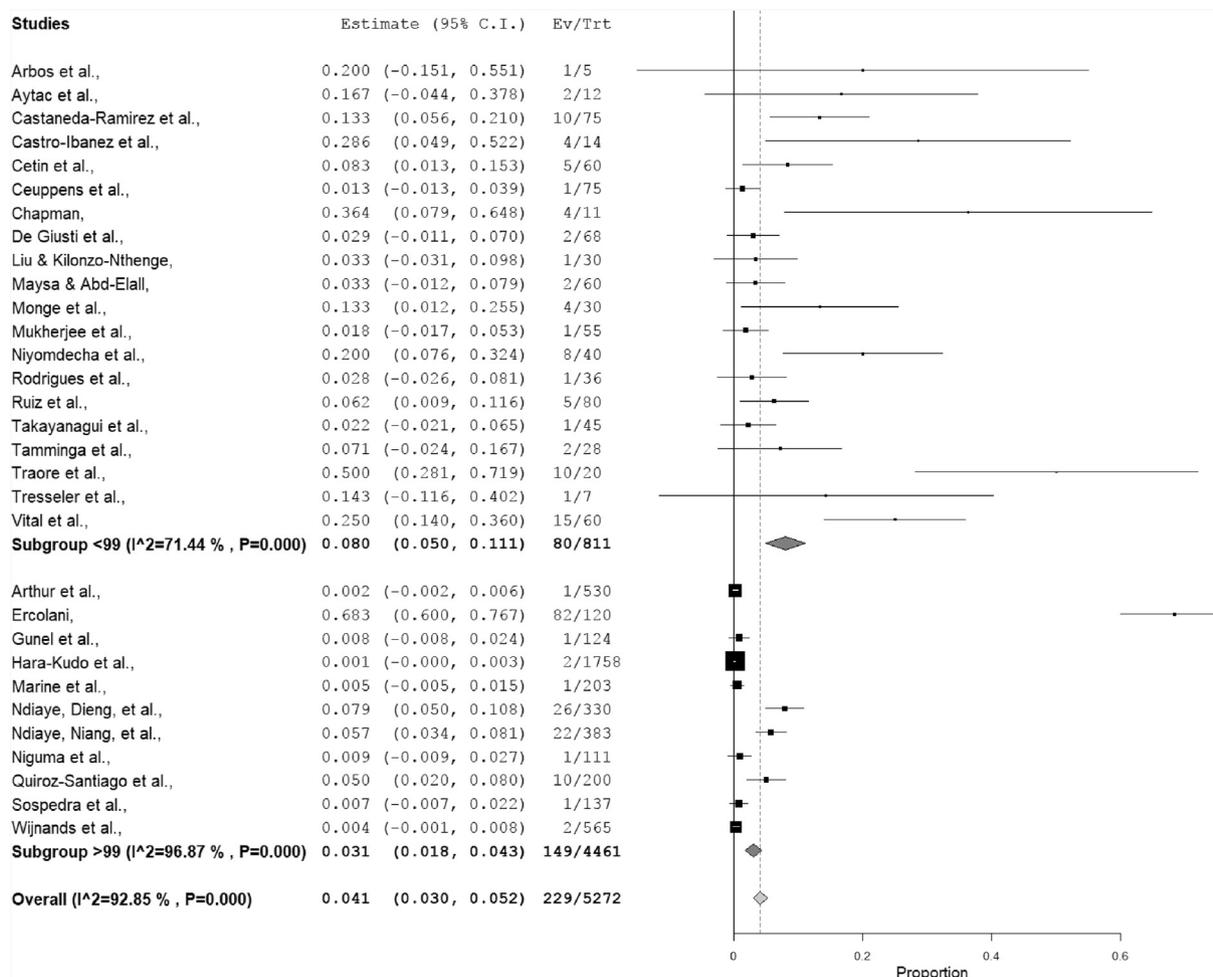


Fig. 8. Prevalence of *Salmonella* on lettuce in relation with sample size. X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number (Arbos et al., 2010; Arthur et al., 2007; Aytac et al., 2010; Castaneda-Ramirez et al., 2011; Castro-Ibanez et al., 2015; Cetin et al., 2008; Ceuppens et al., 2014; Chapman, 1980; De Giusti et al., 2010; Fiedler et al., 2017; Gunel et al., 2015; Hara-Kudo et al., 2013; Liu and Kilonzo-Nthenge, 2017; Marine et al., 2015; Maysa and Abd-Elall, 2015; Monge et al., 2011; Mukherjee et al., 2004; Ndiaye et al., 2011; Niguma et al., 2017; Niyomdechana et al., 2016; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Rodrigues et al., 2014; Shah et al., 2015; Sospedra et al., 2013; Takayanagui et al., 2006; Tamminga et al., 1978; Traoré et al., 2015; Tresseler et al., 2009).

Fig. 4 shows that the calculated average prevalence of *Salmonella* on lettuce based on the results of 31 reviewed publications was 0.041 (95% CI: 0.030–0.052), which means approximately 4 positives samples in 100 tested, the same value found to *E. coli* (EHEC). The I^2 was 92.85%, ($p < 0.001$) meaning high heterogeneity among the publications was found concerning prevalence of *Salmonella* on lettuce.

Factors affecting variation (e.g., season, livestock presence, irrigation water type, flooding, fertilizer type, and hygiene practices) that were not identified by our data extraction may have caused this heterogeneity or there may have been few study inputs to reflect the true variation in prevalence. Besides, no studies were obtained from most populous nations such as China, India, Indonesia, Nigeria, Bangladesh, and Russia, which may be due to a large data gap or to the selection criterion of limiting the search to English, Portuguese and Spanish-language publications. Furthermore, the high heterogeneity is common for systematic reviews, because of the limited number of published reports (Christidis et al., 2016; Paudyal et al., 2017; Pintar et al., 2015).

Fig. 5 presents the average prevalence of *Salmonella* on lettuce in relation with country of origin. The prevalence range of 0.001 (95% CI: –0.000–0.003) ($n = 1$) in Japan to 0.5 (95% CI: 0.281–0.719) ($n = 1$) in Burkina Faso. The heterogeneity among the publications concerning prevalence of *Salmonella* on lettuce depending on the country of origin ranged from 0% (Brazil, United States) until 99,48% (Italy). In

countries where there was only 1 study, the heterogeneity could not be calculated. The country with higher number of studies was Brazil. This might have occurred, due to the language selection (English, Portuguese and Spanish).

Fig. 6 presents the average prevalence of *Salmonella* on lettuce in relation with region of origin. The higher prevalence was found in Europe 0.135 (95% CI: 0.070–0.200) (1012 samples tested), while the lower was in United States and Canada 0.002 (95% CI: –0.001–0.006) (788 samples tested), both regions are considered developed, which is related to higher per capita income, life expectancy, and literacy. Central and South America, Asia, and Africa - that are considered in developing regions - had similar prevalence 0.036, 0.063, and 0.079, respectively. The heterogeneity among the publications concerning prevalence of *Salmonella* on lettuce depending on the region of origin varied from 0% (United States and Canada) until 97,76% (Europe).

Fig. 7 demonstrated that the average prevalence of *Salmonella* in developing countries was 0.064 (95% CI: 0.041–0.087) ($n = 20$), while developed countries had average prevalence of 0.028 (95% CI: 0.014–0.042) ($n = 11$). In both cases, the heterogeneity was high (79,6% in developing and 96,36% in developed countries). The developing countries showed higher prevalence than those developed. This may have occurred due to good agricultural practices that are more frequently apply in developed countries.

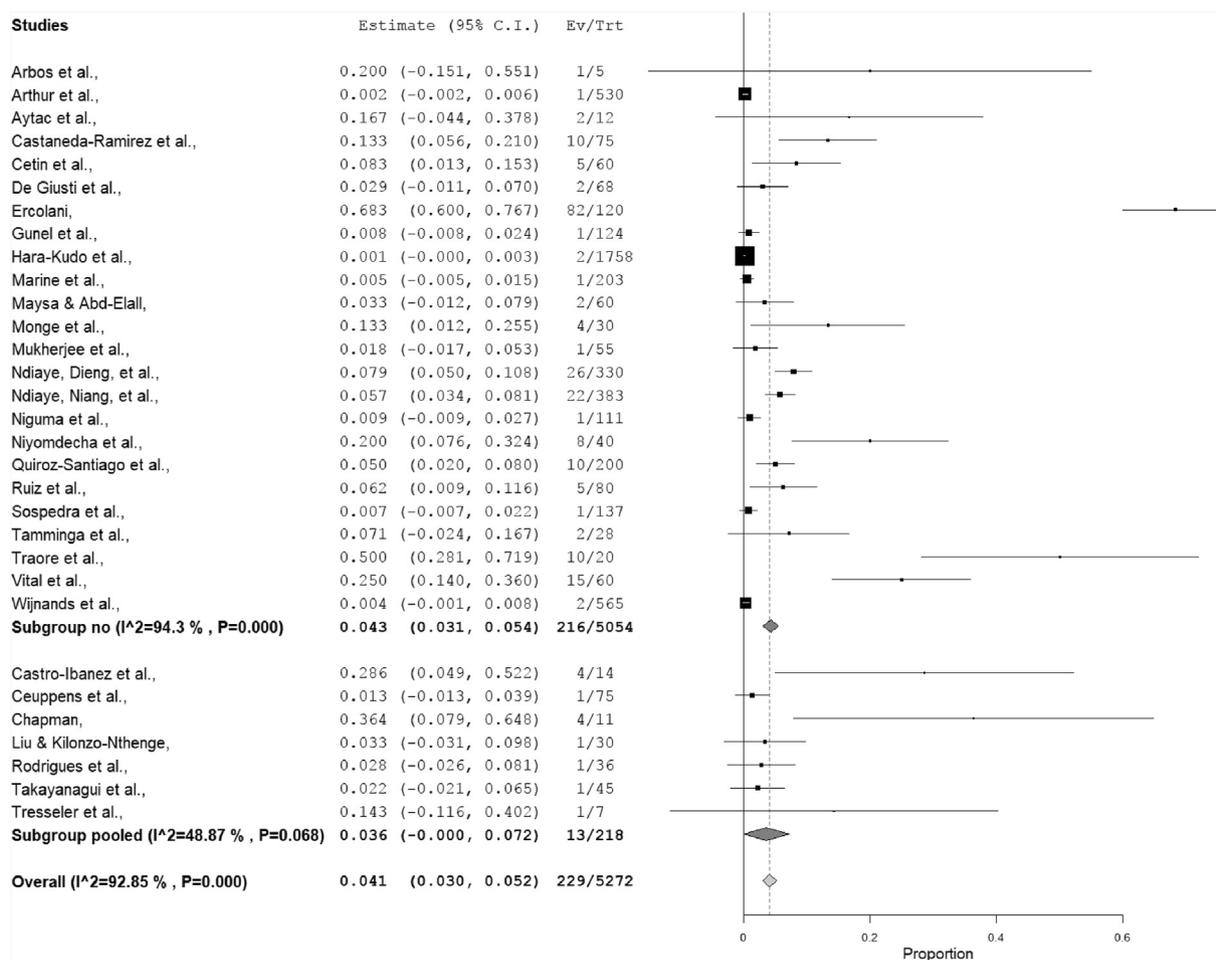


Fig. 9. Prevalence of *Salmonella* on lettuce in relation with sample pooled. X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number (Arbos et al., 2010; Arthur et al., 2007; Aytac et al., 2010; Castaneda-Ramirez et al., 2011; Castro-Ibanez et al., 2015; Cetin et al., 2008; Ceuppens et al., 2014; Chapman, 1980; De Giusti et al., 2010; Fiedler et al., 2017; Gunel et al., 2015; Hara-Kudo et al., 2013; Liu and Kilonzo-Nthenge, 2017; Marine et al., 2015; Maysa and Abd-Elall, 2015; Monge et al., 2011; Mukherjee et al., 2004; Ndiaye et al., 2011; Niguma et al., 2017; Niyomdecha et al., 2016; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Rodrigues et al., 2014; Shah et al., 2015; Sospedra et al., 2013; Takayanagui et al., 2006; Tamminga et al., 1978; Traoré et al., 2015; Tresseler et al., 2009).

Fig. 8 presents the average prevalence of *Salmonella* in relation with sample size. Studies with less than 99 samples had prevalence of 0.080 (95% CI: 0.050–0.111) ($n = 20$). While articles with more than 99 had average prevalence of 0.031 (95% CI: 0.018–0.043) ($n = 11$). The heterogeneity among the publications concerning prevalence of *Salmonella* on lettuce depending on the sample size was high in large samples (96, 87%) and moderate in smaller samples (71.44%).

Fig. 9 presents the average prevalence of *Salmonella* on lettuce in relation with pooled samples. Articles with no pooled samples had prevalence of 0.043 (95% CI: 0.031–0.054) ($n = 24$), while studies with pooled samples had average prevalence of 0.036 (95% CI: –0.000–0.072) ($n = 7$). The heterogeneity among the publications concerning prevalence of *Salmonella* on lettuce depending on the pooled samples was high in no pooled sample studies (94, 3%) and moderate in pooled sample studies (48.87%).

Fig. 10 presents the average prevalence of *Salmonella* on lettuce in relation with sample source. When samples were collected from farms, the average prevalence of *Salmonella* was 0.021 (95% CI: 0.007–0.035) ($n = 11$). In studies in which the samples were collected from more than one spot, the prevalence was 0.019 (95% CI: 0.007–0.030) ($n = 5$). At retail it was 0.169 (95% CI: 0.103–0.236) ($n = 13$), while at the industry it was 0.010 (95% CI: –0.049–0.236) ($n = 2$). The heterogeneity among the publications concerning prevalence of *Salmonella*

on lettuce depending on the sample source varied from 9.84% (industry) until 96.17% (retail). Retail showed the higher prevalence, while other sources presented similar values.

Fig. 11 presents the average prevalence of *Salmonella* in relation with laboratory detection techniques. Studies that used only culture method had prevalence of 0.057 (95% CI: 0.040–0.074) ($n = 19$). While articles that used culture and molecular methods had average prevalence of 0.029 (95% CI: 0.013–0.044) ($n = 12$). In both cases the heterogeneity among the publications concerning prevalence of *Salmonella* on lettuce depending on the laboratory detection technique was high (94.91% in culture and 92.85% in culture and molecular methods). In general, molecular methods may overestimate the presence of live microorganisms by providing a false-positive result, whereas culture methods may underestimate prevalence due to the presence of viable but nonculturable cells. In this meta-analysis, more studies included the use of a culture detection method versus a molecular detection method, and the results showed the opposite of expected: the prevalence of culture methods was higher.

In general, the prevalence data had high and moderate heterogeneity among studies, and identifying the specific factors that may contribute to this is considerably challenging. The estimation of the effects of origin, economic status, size, pool, source and analyses methods on average prevalence also revealed that the findings from

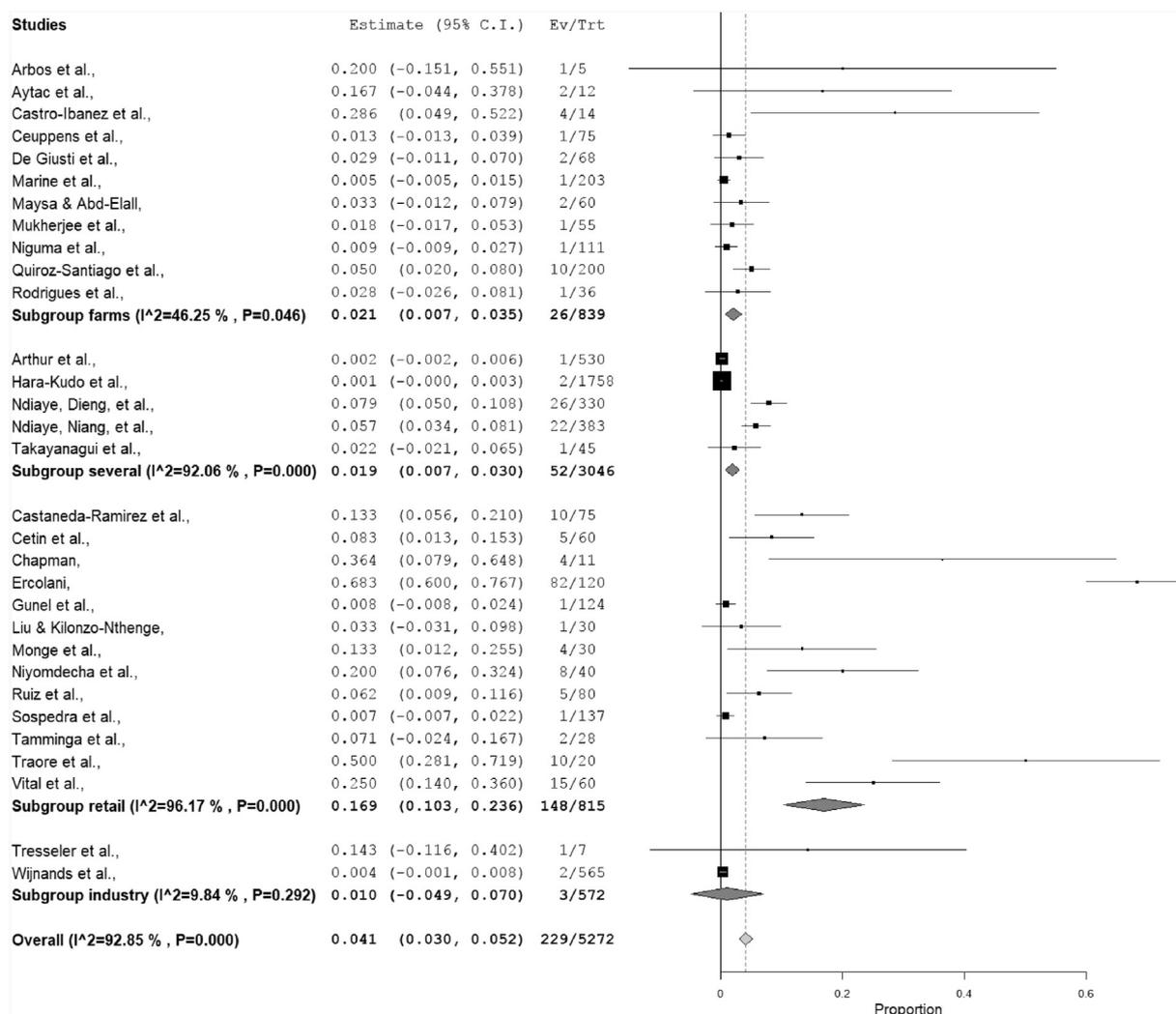


Fig. 10. Prevalence of *Salmonella* on lettuce in relation with sample source. X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number. Several = more than one source (Arbos et al., 2010; Arthur et al., 2007; Aytac et al., 2010; Castaneda-Ramirez et al., 2011; Castro-Ibanez et al., 2015; Cetin et al., 2008; Ceuppens et al., 2014; Chapman, 1980; De Giusti et al., 2010; Fiedler et al., 2017; Gunel et al., 2015; Hara-Kudo et al., 2013; Liu and Kilonzo-Nthenge, 2017; Marine et al., 2015; Maysa and Abd-Elall, 2015; Monge et al., 2011; Mukherjee et al., 2004; Ndiaye et al., 2011; Niguma et al., 2017; Niyomdecha et al., 2016; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Rodrigues et al., 2014; Shah et al., 2015; Sospedra et al., 2013; Takayanagui et al., 2006; Tamminga et al., 1978; Traoré et al., 2015; Tresseler et al., 2009).

most studies were highly heterogeneous. The prevalence data could be factual with extensive varieties depending on many issues of hygienic conditions. Besides this, high heterogeneity is common in meta-analysis studies (Higgins et al., 2003). Paudyal et al. (2017) suggests implementation of surveillance programs that use uniform laboratory protocols to attain homogeneous results.

Table 2 shows three articles reporting concentration of *Salmonella* on lettuce. The values varied from 0.054 ± 0.058 CFU/g until 218.78 MPN/g. In these studies, the levels were based on a total of 99 positive samples for *Salmonella*. Each study adopted different methods and units of measuring, making difficult to calculate mean values.

The risk of consuming *Salmonella* and *E. coli* O157:H7 through lettuce can be estimated with a quantitative microbial risk assessment by using the prevalence and levels determined in the present meta-analysis, adding information about lettuce consumption and washing. The worldwide prevalence found in this study was 0.041 for both pathogens on lettuce. When the countries were divided by social economic status, the prevalence of developed countries was 0.028 for *Salmonella* and 0.125 for *E. coli* (EHEC), while developing countries was 0.064 for *Salmonella* and 0.024 for *E. coli* (EHEC).

Although efforts have been made to detect pathogens in food, reliable data on food safety are still insufficient and fragmented (Paudyal et al., 2017; Ssemenda et al., 2018). Then, the results of this study can be used to identify and quantify risk associated with the consumption of lettuce when performing risk assessments, and to identify data gaps for future research. Efforts to systematically collect the available evidence are critical to inform public health risk management and decision-making, and prioritization of interventions.

Lastly, the present meta-analysis provides the estimates for the prevalence and levels of *Salmonella* and *E. coli* O157:H7 on lettuce. However, a significant gap exists in the enumeration of these pathogens on lettuce internationally, mainly on the *E. coli* O157:H7 data.

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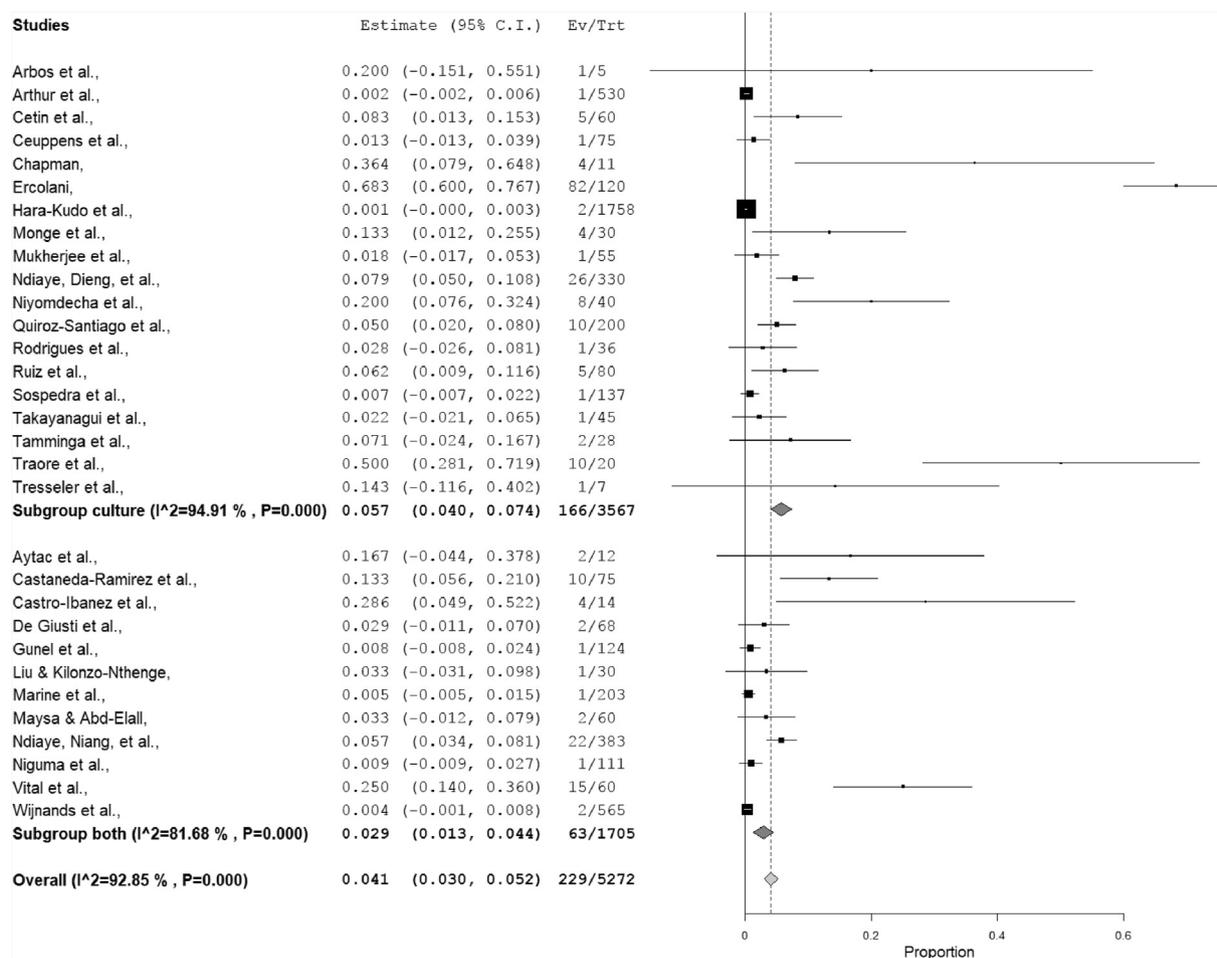


Fig. 11. Prevalence of *Salmonella* on lettuce in relation with laboratory detection technique. X-axis is the proportion of the organism reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval (CI). Studies given higher weights are indicated by larger marker. Ev = number of positive sample; Trt = total sample number. Both = culture and molecular (Arbos et al., 2010; Arthur et al., 2007; Aytac et al., 2010; Castaneda-Ramirez et al., 2011; Castro-Ibanez et al., 2015; Cetin et al., 2008; Ceuppens et al., 2014; Chapman, 1980; De Giusti et al., 2010; Fiedler et al., 2017; Gunel et al., 2015; Hara-Kudo et al., 2013; Liu and Kilonzo-Nthenge, 2017; Marine et al., 2015; Maysa and Abd-Elall, 2015; Monge et al., 2011; Mukherjee et al., 2004; Ndiaye et al., 2011; Niguma et al., 2017; Niyomdecha et al., 2016; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Rodrigues et al., 2014; Shah et al., 2015; Sospedra et al., 2013; Takayanagui et al., 2006; Tamminga et al., 1978; Traoré et al., 2015; Tresseler et al., 2009).

Table 2
Concentration of *Salmonella* on lettuce.

| Reference | Concentration of <i>Salmonella</i> on lettuce |
|------------------------|---|
| Ercolani (1976) | 0.054 ^a ± 0.058 CFU/g (fresh weight) |
| Vital et al. (2014) | 4.57–218.78 MPN/g |
| Wijnands et al. (2014) | 0.281 (0.041–1.31) CFU/g |

^a Mean.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fm.2019.05.001>.

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