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Meta-analysis of the incidence of foodborne pathogens in vegetables and fruits from retail establishments in Europe

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In this study, a systematic review and meta-analysis were conducted to summarise available information on the occurrence of *Salmonella* spp. *Listeria monocytogenes* and shigatoxin-producing *Escherichia coli* (STEC) in fruits and vegetables sold at retail establishments in several European countries. Overall, *L. monocytogenes* was the main pathogen detected in all kinds of vegetables, packaged or not (3.4%; 95% CI: 2.1–5.4%) with *Salmonella* spp. being the pathogen of lowest incidence (0.9%; 95% CI: 0.5–1.2%). The pooled occurrence rate of pathogens in either packed or unpacked vegetables was estimated at 1.9% (95% CI: 1.2–3.1%), with 2.1% of prevalence (95% CI: 1.3–3.4%) for unpacked vegetables and 1.7% (95% CI: 0.9–2.9%) for packed ones. For the three pathogens, the category of spices and herbs was the most frequently contaminated with pathogens, whereas salads presented the lowest occurrence. The vegetable category with highest incidence of *Salmonella* spp. (1.7%; 95% CI: 0.7–4.1%) and *L. monocytogenes* (2.2%; 95% CI: 1.0–4.7%) is leafy greens whilst STEC is more frequently recovered from sprouts (1.9%; 95% CI: 0.5–5.9%). In the case of fruits, the pooled prevalence estimates for *Salmonella* spp., *L. monocytogenes* and STEC were 1.60% (0.54%; 95% CI: 0.55–4.60%), 1.91% (0.50%; 95% CI: 0.93–3.88%) and 4.71% (1.52%; 95% CI: 1.73–12.2%), correspondingly.

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Introduction

Compared to previous decades, today's society is increasingly more aware and concerned about their health and foods' impact on it, which has had two main consequences: the increase of vegetable and fruit consumption and the amplified offer of these products in retail establishments in varied forms (fresh, pre-washed, frozen, dried, etc.). However, these products can be contaminated by pathogens such as *Salmonella* spp., *Listeria monocytogenes* or shigatoxin-producing *Escherichia coli* (STEC), therefore, promoting the occurrence of foodborne diseases, a significant and widespread global public health threat. According to the European Food Safety Authority, in the European Union (EU) alone, over 320 000 human cases are reported each year, although the real figure is estimated to be much higher [1••]. In 2013, *Salmonella* was the most frequently detected causative agent of foodborne outbreaks (representing 22.5% of total outbreaks). In the same year, 73 outbreaks of VTEC and 13 of *Listeria* were also reported, thus demonstrating the impact these pathogens on public health [1••].

Fresh fruits and vegetables carry natural non-pathogenic microorganisms, yet, during growth, harvest, transportation and further handling, these products can become contaminated with pathogens [2]. During growth, contamination can arise from the use of organic fertilisers and poor quality of the irrigation water; during harvest and transportation, the use of contaminated equipment and containers, as well as poor storage conditions, with excessive humidity and temperatures, increase bacteria's opportunities of growth; during post-harvest, poor handling procedures may cause damage to the produce, thus opening a window for contamination if hygienic conditions from operators and equipment are not satisfactory.

The objective of this study was to summarise the incidences of *Salmonella*, *L. monocytogenes* and STEC in fruits and vegetables sold at European retail establishments and to present an overview of such contamination, broken down by type of produce and European region. In order to do so, separate multilevel meta-analysis model were adjusted.

Methodology

Meta-analysis is a statistical analysis of a vast collection of results from published primary studies, whose main purpose is to integrate and interpret the findings to achieve conclusions that the individual studies alone would not show clearly [3••]. In this study, the *population* is defined as fruits and vegetables surveyed at retail establishments in Europe while the *measured outcome* is the detection of pathogens. Literature search was conducted using Scopus and ISI Web of Knowledge and Web of Science databases for English written papers indexed since 2000 in Europe. For the searches, a formula that combined terms regarding the existence (prevalence, incidence, occurrence, concentration, count, microbiological quality) of pathogens (*Salmonella*, *L. monocytogenes* and *E. coli*) in the target products (fruit, produce, vegetable, leafy, seed, legume, spice, oilseed, nut, sprout, ready-to-eat, RTE) was applied, making proper use of the AND and OR logical connectors.

A parameterisation of the *effect size* was then determined to allow direct comparison and summation of primary studies. Because the occurrence of microbial hazards in fruits/vegetables is a binary trait (a sample tests either positive or negative for the pathogen), the parameter to measure the effect size θ was the raw proportion p (number of successes or positive samples, s, divided by the total sample size, n). In order to restrict the range of the effect size or pathogen's incidence from 0 to 1, and to stabilise the variance, the logit transformation of p was chosen as the effect size measure θ [4••].

After assessing all the information from the recovered publications, fifty-three primary studies [1••,5–33,34•,35–42,43•,44•,45,46–55,56•] published from 2001 until May 2017 were considered appropriate for inclusion for having used approved microbiological methods and presenting sufficient and extractable data. From each study, the total number n and number of positive samples s were extracted, as well as the country, year of the survey, packed/unpacked condition, food class, sample weight (g) for microbiological analysis and microbiological method. The fruit classes defined were: berries, drupes, nuts, pepo, pome, tropical and non-classified (assorted fruits or non-specified), while vegetables were classified as: leafy greens, lettuce only, spices and herbs, salads, sprouts and non-classified vegetables (assorted vegetables or non-specified).

Several multilevel random-effect meta-analysis models were fitted to appropriate data subsets in order to estimate overall or pooled incidences for: first, pathogens in packed and unpacked vegetables as a whole; second, pathogens in packed and unpacked disaggregated vegetable classes; third, pathogens by vegetable class; fourth, pathogens by country; fifth, pathogens by European region and sixth, pathogens in fruits. For a detailed explanation on multilevel meta-analysis modelling for prevalence data, refer to Xavier *et al.* [3••] and Viechtbauer *et al.* [4••]. Meta-analysis models and graphs were built in R Studio version 1.0.136 using the 'metafor' package.

Results and discussion

Following study quality checking, a total of 384 observations of positive and negative results of incidence of foodborne pathogens in vegetables and 69 observations in fruits were excerpted. Information on the distribution of observations by fruit and vegetable class can be found in Table 1. A breakdown of number of primary studies by ranges of publication year is shown in Table 2. From 2013 onwards, more surveys on the incidence of pathogens in fruits and vegetables have been published in comparison to five-year spans between 2001 and 2012.

Incidence of pathogens in packed and unpacked vegetables at retail level

For this meta-analysis, only the categories Salads, Spices and Herbs, Sprouts and Non-classified Vegetables were

Table 1

Number of observations (*n*) of foodborne pathogens in fruits and vegetables by class and by packaging type (*n*; packed/unpacked) extracted from published survey studies

Type of product		*Salmonella* spp.	*L. monocytogenes*	STEC
Fruits	Berries	3; 0/3	3; 0/3	4; 0/4
	Drupes	0	1; 0/1	0
	Nuts	5; 0/5	0	5; 0/5
	Pepo	4; 0/4	5; 0/5	0
	Pome	2; 0/2	1; 0/1	1; 0/1
	Tropical	3; 0/3	3; 0/3	0
	Non-classified	6; 1/5	12; 3/9	11; 2/9
	Total	23; 1/22	25; 3/22	21; 2/19
Vegetables	Leafy Greens	15; 0/15	21; 5/16	17; 0/17
	Lettuce	14; 1/13	16; 0/16	16; 0/16
	Salads	21; 13/8	33; 24/9	12; 7/5
	Spices & herbs	64; 5/59	13; 6/7	11; 2/9
	Sprouts	13; 2/11	10; 3/7	22; 3/19
	Non-classified	10; 3/7	21; 9/12	55; 8/47
	Total	137; 24/113	114; 47/67	133; 20/113

Table 2

Number of primary studies retrieved sorted by publication year range

Publication year	No. primary studies
[2001–2004]	9
[2005–2008]	12
[2009–2012]	12
[2013–2017]	20

used, since the classes Leafy Greens and Lettuce did not present sufficient data for the two packaging conditions to allow comparisons. For that reason, only 40 primary studies were used, which furnished a total of 284 observations. In most of the data (78%), there was no indication as to the type of packaging, while in the other 22%, authors stated that modified atmosphere packaged produce was sampled.

Meta-analysis summarised the global mean incidence of pathogens in either packed or unpacked vegetables to be 1.93% (95% CI: 1.19–3.11%), with unpacked vegetables having higher prevalence of pathogens (2.07%; 95% CI: 1.26–3.37%) than packed ones (1.68%; 95% CI: 0.97–2.89%). Pooled prevalence estimates for this meta-analysis are compiled in Table 3. *L. monocytogenes* is the pathogen of greatest concern as it bears the highest pooled incidences in packed vegetables (2.49%; 95% CI: 1.50–4.12%) and unpacked vegetables (4.42%; 95% CI: 1.79–10.53%). *Salmonella* spp. has the lowest prevalence in either packed (0.55%; 95% CI: 0.31–0.97%) or unpacked vegetables (0.98%; 95% CI: 0.37–2.61%). For each of the three pathogens under study, the unpacked/bulk vegetables presented consistently higher pooled prevalences than the packed vegetables (Table 3).

Among the four vegetable categories, unpacked or packed, Spices and Herbs (2.08%; 95% CI: 1.15–3.75%) were the most frequently contaminated by either STEC, *L. monocytogenes* or *Salmonella* spp. disregarding the Non-classified category (for being unknown and assorted) while Salads were the least contaminated (1.49%; 95% CI: 0.85–2.58%). A breakdown of these results by packaging condition is shown in Table 4. When all incidence measures across pathogens were brought together, *L. monocytogenes* was found to be the main one in all four vegetable categories (3.40%; 95% CI: 2.11–5.44%) while *Salmonella* spp. presented the lowest frequency of detection (0.86%; 95% CI: 0.52–1.21%) (Table 5).

Table 3

Meta-analysis of the incidence of pathogens in packed and unpacked vegetables surveyed at retail in Europe

	Microorganism	Pooled prevalence (%)	95% CI pooled prevalence (%)
Packed	*Salmonella* spp.	0.545	[0.305–0.971]
	L. monocytogenes	2.491	[1.496–4.122]
	STEC	1.235	[0.682–2.226]
Unpacked	*Salmonella* spp.	0.983	[0.366–2.614]
	L. monocytogenes	4.424	[1.789–10.53]
	STEC	2.215	[0.817–5.864]

Table 4

Meta-analysis of the incidence of pathogens in different vegetable categories, either packed or unpacked, surveyed at retail in Europe

	Pooled prevalence (%)	95% CI pooled prevalence (%)
Salads	1.488	[0.853–2.583]
Packed	1.464	[0.815–2.615]
Unpacked	1.526	[0.827–2.800]
Spices & Herbs	2.084	[1.148–3.754]
Packed	2.001	[0.624–6.225]
Unpacked	2.087	[0.633–6.650]
Sprouts	1.643	[0.849–3.154]
Packed	1.597	[0.494–5.035]
Unpacked	1.665	[0.501–5.383]
Non-classified veg.	2.916	[1.645–5.100]
Packed	2.817	[0.931–8.204]
Unpacked	2.936	[0.945–8.751]

Table 5

Meta-analysis of the incidence of pathogens in salads, spices and herbs, sprouts and undefined vegetables surveyed at retail in Europe

Microorganism	Pooled prevalence (%)	95% CI pooled prevalence (%)
STEC	1.905	[1.129–3.197]
L. monocytogenes	3.399	[2.108–5.435]
Salmonella spp.	0.860	[0.520–1.419]

Incidence of *Salmonella* spp., *L. monocytogenes* and STEC in vegetables at retail level

A meta-analysis on *Salmonella* spp. in packed and unpacked vegetables using vegetable class as moderator revealed high prevalence of this pathogen in Leafy Greens (1.74%; 95% CI: 0.74–4.07%) compared to the other categories under study (pooled prevalences from 0.47 to 1.24%; Table 6). The food class with the lowest incidence of this pathogen was Salads (0.47%; 95% CI: 0.24–0.934%; Table 6). In relation to *L. monocytogenes*, disregarding the Non-classified vegetables, Leafy Greens presented the highest incidence (2.25%; 95% CI: 1.05–4.74%). On the opposite side, the category with lowest incidence of *L. monocytogenes* turned out to be Spices and Herbs (1.06%; 95% CI: 0.43–2.61%; Table 6). Excluding the Non-classified category, Sprouts presented the highest pooled prevalence of STEC (1.86%; 95% CI: 0.56–5.96%; Table 6), while Lettuce presented the lowest frequency of detection (0.67%; 95% CI: 0.19–2.32%; Table 6).

Table 6

Meta-analysis of the incidence of *Salmonella* spp., *L. monocytogenes* and STEC in different vegetable categories surveyed at retail in Europe

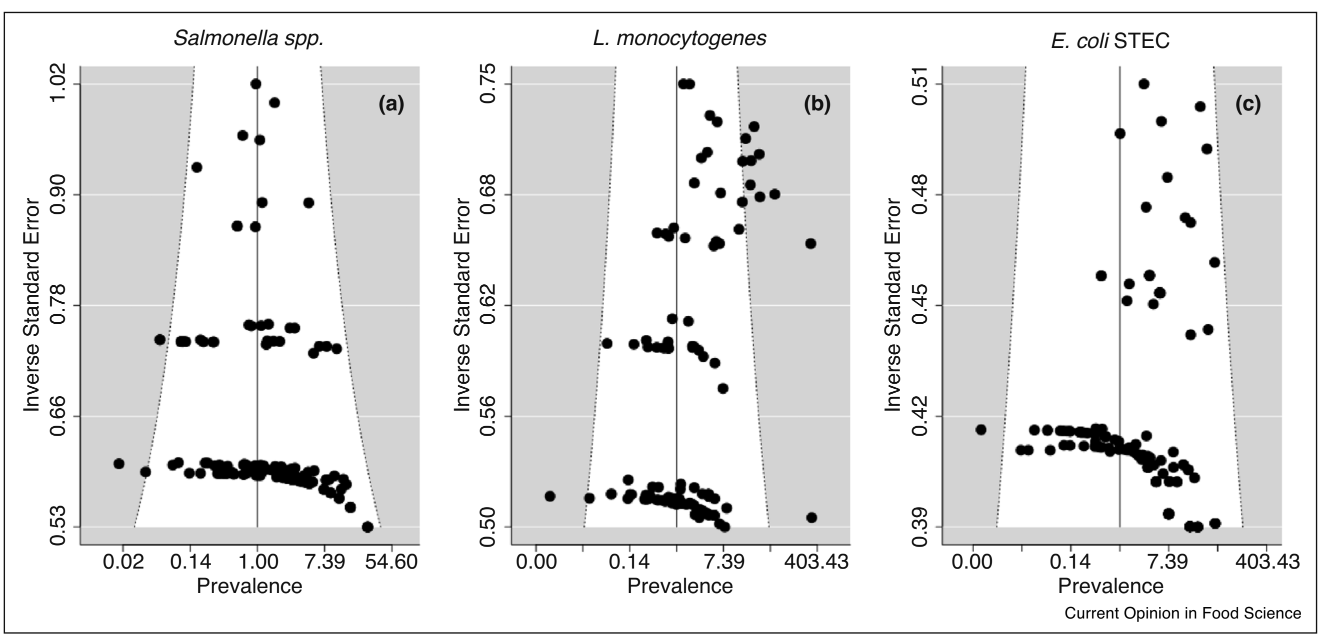
Microorganism	Product	Pooled prevalence (%)	95% CI pooled prevalence (%)
Salmonella spp.	Leafy Greens	1.742	[0.737–4.066]
	Lettuce	1.168	[0.469–2.877]
	Salads	0.471	[0.237–0.933]
	Spices & herbs	1.241	[0.644–2.380]
	Sprouts	0.593	[0.248–1.413]
	Non-classified veg.	0.955	[0.393–2.300]
L. monocytogenes	Leafy greens	2.245	[1.048–4.744]
	Lettuce	1.796	[0.842–3.791]
	Salads	1.752	[0.898–3.388]
	Spices & herbs	1.063	[0.429–2.608]
	Sprouts	1.495	[0.547–4.019]
	Non-classified veg.	3.340	[1.659–6.609]
STEC	Leafy greens	1.360	[0.412–4.396]
	Lettuce	0.672	[0.192–2.317]
	Salads	0.739	[0.237–2.280]
	Spices & herbs	1.012	[0.252–3.967]
	Sprouts	1.858	[0.562–5.963]
	Non-classified veg.	4.335	[1.132–15.21]

Funnel plots of the incidence of the three pathogens in these six vegetable classes are displayed in Figure 1. None of the funnel plots showed strong evidence of publication bias, since there were no blank areas at the bottom of the funnels. However, the top of the funnel plots, in all cases, displays a large blank area, which hints the lack of published surveys with large sample size. That might influence the results, since it is likely that a small sample size will not have the statistical power to detect a positive food unit if the true prevalence is very low [3••]. Hence, in microbiological surveys of absence/presence of pathogens in foods, a large sample size should be used [3••]. In funnel plot C, most studies are concentrated to the right (indicating that most primary studies report positive results of STEC), which is consistent with the possibility that studies that failed to have positive results are missing.

Incidence of pathogens in vegetables at retail level by European country and region

From the initial subset of data (six vegetable categories), studies from countries with at least 8 observations were selected, thus creating a new subset of 340 studies from 12 countries. The number of observations and the estimated pooled incidences of all three pathogens in the six vegetables by country are presented in Table 7. This meta-analysis suggested that the highest overall frequencies of detection of pathogens in vegetables were reported in studies from Spain (8.94%; 95% CI: 6.66–11.90) and Czech Republic (6.89%; 95% CI: 4.29–10.89). On the other hand, the UK (0.27%; 95% CI: 0.16–0.43%) and Norway (0.87%; 95% CI: 0.45–1.69%) reported the lowest occurrence of pathogens.

Figure 1



Funnel plots of the meta-analyses of *Salmonella* spp. **(a)**, *L. monocytogenes* **(b)**; and STEC **(c)** in vegetables — encompassing leafy greens, lettuce, salads, spices & herbs, sprouts and non-classified vegetables–surveyed at retail in Europe.

Table 7

Meta-analysis of the incidence of pathogens in vegetables surveyed at retail in several European countries

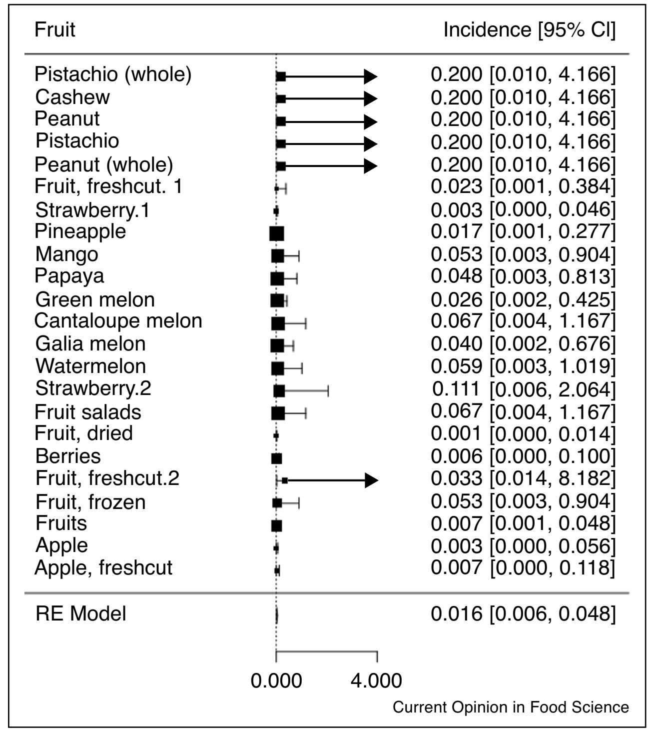
Country	Pooled prevalence (%)	*n*	95% CI of pooled prevalence (%)
Albania	2.600	8	[0.816–7.965]
Austria	4.180	18	[1.969–8.654]
Belgium	1.006	9	[0.458–2.193]
Czech Republic	6.891	41	[4.291–10.88]
Ireland	5.173	62	[3.509–7.565]
Italy	0.939	24	[0.485–1.810]
Norway	0.871	24	[0.448–1.687]
Portugal	2.384	14	[1.056–5.293]
Spain	8.938	77	[6.657–11.90]
Sweden	2.202	9	[0.848–5.597]
Turkey	3.641	24	[2.113–6.206]
United Kingdom	0.265	30	[0.163–0.430]

A separate meta-analysis by region revealed that the Northern European regions present the lowest pooled prevalence of pathogens in vegetables (1.10%; 95% CI: 0.46–2.6%). Since most cases of human salmonellosis, listeriosis and STEC infections are reported during the summer [57–59], this might indicate that lower temperatures during other seasons play some role in reducing the viability of pathogens. As a result, it is likely that in Northern European countries, whose average temperatures (per year) are lower, the growth of microorganisms on vegetables on farms is retarded. This, in turn, would lead to lower concentrations at the harvest and retail stages in comparison to the Southern European countries. In fact, according to a study appraising the impact of climatic determinants on foodborne diseases, elevated ambient temperatures were proven to augment the replication cycles of most foodborne pathogens [60], a conclusion that supports the hypothesis above.

Incidence of pathogens in fruits at retail level

For fruits, a meta-analysis was performed with no distinction of classes, since the data available within categories were very small (Table 1). On meta-analysis, the prevalence of *Salmonella* spp. in fruits is 1.60% (95% CI: 0.55–4.60%), whereas that of *L. monocytogenes* is slightly higher at 1.91% (95% CI: 0.93–3.88%). Interestingly, unlike the results for vegetables, STEC was the pathogen of greatest incidence in fruits, presenting a pooled incidence of 4.71% (95% CI: 1.73–12.2%). Forest plots were built to gather the incidence measures extracted from primary studies for *Salmonella* spp. (Figure 2), *L. monocytogenes* (Figure 3) and STEC (Figure 4) in fruits. None of the forest plots signalled strong heterogeneity in pathogens' prevalence among studies, although, due to the small sample size of some studies, wide confidence intervals were produced. The limited number of studies available for the fruits meta-analysis may have biased the results, so these pooled prevalences must be interpreted with caution.

Figure 2

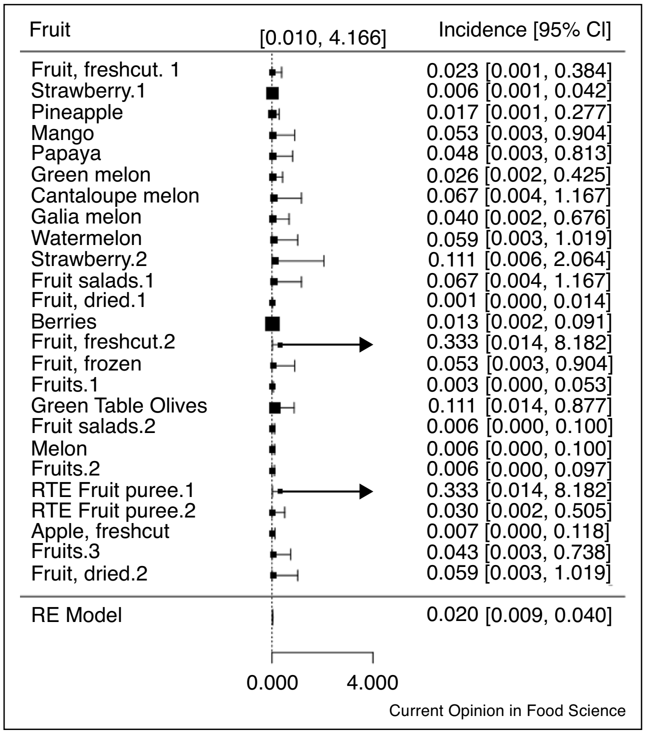


Forest plot of the incidence of *Salmonella* spp. in fruits surveyed at retail in European establishments.

Results from the EFSA report indicate that, amongst raw and minimally processed foods of non-animal origin, leafy greens, bulb and stem vegetables, tomatoes, melons, fresh pods, legumes, sprouted seeds and berries pose the highest risks in the EU [61]. According to this EU report, the top-ranking combinations of foods and pathogens are *Salmonella* and leafy greens eaten raw; *Salmonella* and bulb and stem vegetables; *Salmonella* and tomatoes; *Salmonella* and melons; and pathogenic *E. coli* and fresh pods, legumes or grains [61]. Although the food categories of highest prevalence in this study are in accordance with EFSA's results, it is interesting that, in our meta-analysis, *Salmonella* spp. emerged, in all cases, as the pathogen of lowest incidence. The reason for these findings could be that, in our meta-analysis, only the incidence in vegetables was used to draw conclusions, while the risk ranking combinations defined by EFSA were calculated by taking into account also the number of outbreaks in the EU population. With *Salmonella* stated as the most frequently detected causative agent in foodborne outbreaks occurred in 2013 [1••], it is expected that this pathogen turned out as the most important in the EFSA's top-ranking combinations.

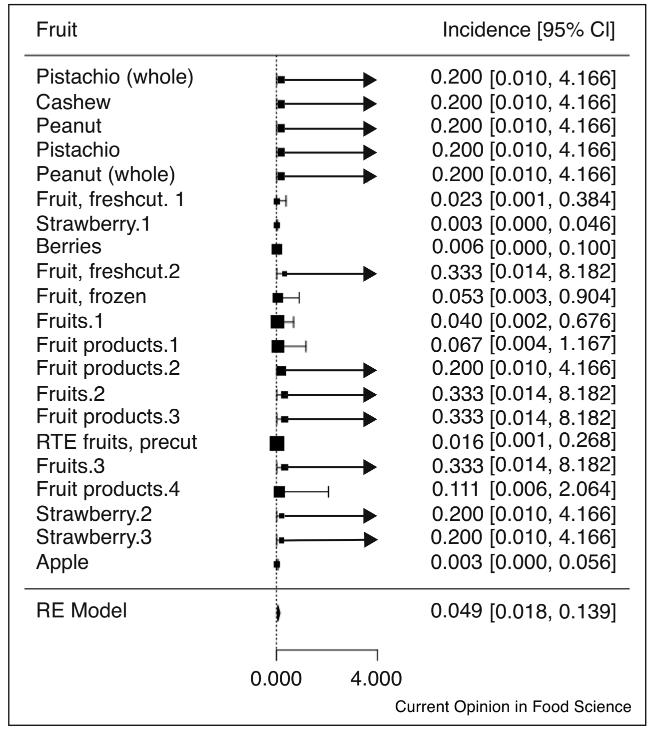
As most fruits and some vegetables can be eaten raw by the consumers after washing, a step sometimes disregarded or not properly performed, it is important for

Figure 3



Forest plot of the incidence of *L. monocytogenes* in fruits surveyed at retail in European establishments.

Figure 4



Forest plot of the incidence of shigatoxin-producing *E. coli* in fruits surveyed at retail in European establishments.

the food industry to assess the microbial content of its products in order to protect consumers' health and reduce the risk of food poisoning. Being responsible for providing safe food, processing plants should reinforce the implementation of good hygiene practice sand monitoring plans, proper maintenance of the cold chain (from factory to retail) and the use of visible labelling for use-by date. Simultaneously, the food industry should be encouraged to be compliant with guidelines and rules created by countries' governments and food safety organisations.

Despite industries' responsibility to provide safe food, the consumer can also take preventive measures to avoid pathogens in fruits and vegetables, such as: scrubbing the skin of pepos (i.e. cantaloupe and other melons) with water and a brush before cutting it; washing the fruit or vegetable even if it will be peeled; washing fruits and vegetables with running water while using some friction (instead of soaking); using clean utensils and work surfaces; washing hands after handling raw products and avoiding cross-contamination. Consumers also need to be aware of the proper storage of foods: the refrigerator, for instance, must be kept below 5 °C. Cooking is another way to attain safer foods, but a certain temperature must be achieved to eliminate pathogenic bacteria. For *Salmonella* spp. and *L. monocytogenes*, 66 °C and 77 °C, respectively, must be reached while for STEC, temperatures around 69 °C are expected to provide at least a 5-log reduction [62].

Concluding remarks

Meta-analyses on prevalence data from surveys in Europe indicated that *L. monocytogenes* is currently the main pathogen contaminating vegetables, while STEC is the one most frequently detected in fruits. Further research focused on reducing the levels of pathogens in fruits and vegetables by minimal processing technologies should be undertaken. In addition, challenge tests and predictive microbiology are scientific resources that researchers and food companies can take up in order to guarantee safe products and prevent outbreaks. As control of pathogens in fruits and vegetables sold at retail may not be easy, the food industry and food safety agencies must continue taking surveillance and training actions to guarantee products' quality and the well-being of consumers. Finally, the consumers themselves must be educated on how to properly handle, wash and store vegetables and fruits prior to consumption.

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- • of special interest
- •• of outstanding interest

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