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Prevalence and Concentration of Bacterial Pathogens in Raw Produce and Minimally Processed Packaged Salads Produced in and for The Netherlands

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ABSTRACT

Recent outbreaks with vegetable or fruits as vehicles have raised interest in the characterization of the public health risk due to microbial contamination of these commodities. Because qualitative and quantitative data regarding prevalence and concentration of various microbes are lacking, we conducted a survey to estimate the prevalence and contamination level of raw produce and the resulting minimally processed packaged salads as sold in The Netherlands. A dedicated sampling plan accounted for the amount of processed produce in relation to the amount of products, laboratory capacity, and seasonal influences. Over 1,800 samples of produce and over 1,900 samples of ready-to-eat mixed salads were investigated for *Salmonella enterica* serovars, *Campylobacter* spp., *Escherichia coli* O157, and *Listeria monocytogenes*. The overall prevalence in raw produce varied between 0.11% for *E. coli* O157 and *L. monocytogenes* and 0.38% for *Salmonella*. Prevalence point estimates for specific produce/pathogen combinations ranged for *Salmonella* from 0.53% in iceberg lettuce to 5.1% in cucumber. For *Campylobacter,* this ranged from 0.83% in endive to 2.7% in oak tree lettuce. These data will be used to determine the public health risk posed by the consumption of ready-to-eat mixed salads in The Netherlands.

In recent decades, outbreaks with pathogenic microorganisms on fruit and raw produce (e.g., *Escherichia coli* O157:H7 in spinach in the United States [2006] and *Salmonella* in tomatoes in the United States [2008]) have increased interest to characterize the microbiological hazards associated with fresh fruits and vegetables *(2, 4, 5, 9, 10, 12–14, 17–19, 28, 29, 31, 37–39, 40)*. Literature review in the European Union provides, however, no clear data regarding foodborne infections related to fresh produce. Moreover, reports are not standardized between countries for commodity-specific outbreak investigations. Both the Centers for Disease Control in the United States and the Rapid Alert System for Food and Feed of the European Union report approximately 4% of reported food infections to be attributable to vegetables *(6, 9)*. Recently, 46% of the foodborne illnesses in the United States was attributed to produce *(33)*. General figures show that the consumption of raw vegetables does not pose an increased microbiological health risk based on epidemiological data from the European Union and the United States. Furthermore, the probability of an outbreak due to the consumption of raw vegetables is comparable with other product groups, such as eggs, milk products, and shell and shellfish. However, due to the high number of patients per outbreak, vegetables become the second most important commodity, after meat and meat products, for severity of microbiological infections. The rise in the production and consumption of ready-to-eat foods can increase the potential risk even though reported prevalences are low *(1, 6, 16, 36)*. Surveys related with these food commodities revealed the prevalence of pathogens in the range of 0 to 4.5% *(15, 20, 21, 34)*.

Recently reported outbreaks, together with increasing production figures, resulted in a framework for the survey presented in this article. The primary goal was to trace bacterial contamination sources through the investigation of raw produce (mainly leafy greens) used for the production of minimally processed packaged salads. In addition, this survey was intended to provide insight into the dynamics of selected pathogenic bacteria throughout the production chain by investigating the contamination from raw produce up to minimally processed mixed salads at retail level.

The pathogens of concern were *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella enterica* serovars, and *E. coli* O157. This selection was based on literature review on the occurrence of pathogenic microorganisms on fresh produce and their biological characteristics (e.g., contamination via soil, survival, and growth at low temperatures) *(27)*. The survey was carried out in close collaboration with the five laboratories of the Food and Consumer Product Safety Authority.

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TABLE 1. *Numbers and variety of raw produce selected for sampling at the entrance hall at two Dutch processing companies*

Raw produce (n = 13)	No. of samples investigated per wk (n = 50/wk)
Iceberg lettuce	15
Endive	12
Lollo rosso (*Lactuca sativa* var. Luberon)	5
Curly endive	4
Lollo bionda (*Lactuca sativa* var. Casabella)	3
Red pepper	2
Green oak leaf lettuce	2
Red oak leaf lettuce	2
Baby leaf	1
Cucumber	1
Red lettuce	1
Radicchio rosso	1
Rucola (arugula)	1

MATERIALS AND METHODS

Sampling plan. To meet the specific project goals, the study required a sampling approach specific for the ready-to-eat vegetable production chain. Thus, a thorough sampling plan was developed considering the relevant steps in the production chain of ready-to-eat salads, space and time effects, specific produce and product selection, laboratory capacity, and number of samples to be analyzed.

From a tracing and tracking perspective, the supply chain of fresh produce in The Netherlands is very complex. Therefore, both the raw produce (separate raw vegetables) and products (minimally processed packaged salads) in two vegetable processing companies in The Netherlands were sampled. For the incoming produce of these companies, raw produce was sampled at the storage hall; products were sampled at two sites further down the production chain for tracking purposes (i.e., following the dynamics of microbes throughout the chain). These two sites were at the end of the packing stage in the processing companies where samples were collected on the same day as the produce samples (to assess the effect of food handling) and the retail samples (to assess further distribution effects, such as transport and storage). Retail samples were obtained from supermarkets directly related to the processing companies. These supermarkets were situated in the areas surrounding the five departments of the Food and Consumer Product Safety Authority.

Because of the substantial variety of minimally processed packaged salads, the selection of the produce and products to be investigated was based on the sales volumes of the two processing companies of minimally processed packaged salads containing at least two types of leafy produce.

This approach resulted in a sampling strategy consisting of 13 types of vegetables and 12 types of minimally processed packaged salads produced by the two companies. Both raw produce and products were sampled proportionally to production numbers, which resulted in 13 vegetable types being sampled at the entrance hall of the companies (Table 1). Actual sample sizes will be further specified in the next section.

The presumed prevalence range, the capacities for sample analysis at the participating laboratories, and the accuracy of the resulting prevalence estimates formed the basis to decide the amount of samples to be taken during this 1-year trial. A preliminary, nonexhaustive, literature search revealed a prevalence range from 0 to 4.5% *(15, 20, 21, 34)*. The accuracy of prevalence estimates could be quantified using a previously described methodology *(42)*. The basic principle here is to consider a binomial process describing the number of positive samples (''successes''), using a known number of samples (n) and prevalence (d). The properties of this process can be used to calculate the number of samples that need to test negative to assess an upper prevalence level with some confidence (P). Assuming a worst-case scenario in which all samples tested negative, the question to be answered is how many samples should be taken (and subsequently tested negative) to make sure (with a reasonable confidence level) that the upper level of the prevalence estimate will be below the 4.5% already known from literature. The equation for this is

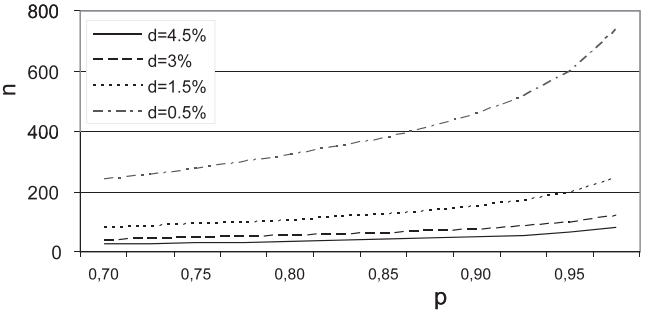

FIGURE 1. *Number of consecutive samples to be tested negative,* n*, to assess the upper prevalence level,* d*, with confidence* P.

$$n = \frac{^{10}\log(1-P)}{^{10}\log(1-d)} \quad (1)$$

where P is the level of confidence (value between 0 and 1), d is the prevalence (value between 0 and 1), and n is the number of samples.

Figure 1 illustrates the number of samples that need to test negative for a pathogen according to equation 1 to assess an upper level for the prevalence with some confidence. This figure illustrates, for example, that 600 samples need to be tested negative to assess a prevalence level between 0 and 0.5%, with 95% confidence.

A point estimate for the prevalence can be assessed if a positive sample is found during the predefined sampling period. Again, the binomial distribution forms the basis for this estimate, whereas the uncertainty about d, due to sampling variability, can be assessed with a beta distribution *(42)*. Both the prevalence (d) and the number of positive samples (k) affect the choice for a sample size (n). The total number of samples is determined by the presumed prevalence. That is, to be able to estimate a prevalence of 0.5%, one should collect at least 200 samples, because one positive sample would then result in this 0.5% point estimate for the prevalence. However, the accuracy of this estimate is determined by the number of positive samples from the total number collected. The relative confidence interval (representing the accuracy) decreases from 265 to 117% as the number of positive samples increases from 1 to 10 for a constant d. All these considerations resulted in the following initial sampling plan: approximately 1,900 raw produce samples at the entrance hall of two processing companies, approximately 780 product samples at the end of the processing chain in the companies, and approximately 1,500 product samples at retail. This would, at least, result in the prevalence estimates, as shown in Table 2. This survey will result in useful new insights in the microbial contamination of fresh produce, in addition to current knowledge.

Sampling process. Raw produce samples were randomly collected from incoming trays at the processing companies and

TABLE 2. *Estimated prevalence based on the investigation of raw produce samples, product samples collected at the end of the processing chain in the companies, and product samples collected at retail*

Sample type	No. of samples	Prevalence (%) estimate (95% confidence interval)	
		$k = 0$[a]	$k = 1$
Raw produce	1,900	≤0.16	0.10 (0.013, 0.29)
Product at company	780	≤0.38	0.26 (0.031, 0.71)
Product at retail	1,500	≤0.20	0.13 (0.016, 0.37)

[a] k, number of positive samples.

packed in separate bags by the responsible quality manager. Samples were collected evenly over 1 year from October 2006 through October 2007, to reveal possible seasonality influences on contamination levels. The sampling consisted of a cycle of 3 weeks, with sampling on Mondays and Wednesdays and the analysis starting on Tuesdays and Thursdays; this was followed by 1 week without sampling. Between sampling and the start of the analyses, the samples were kept refrigerated. In total, 50 raw produce samples were collected at the two processing companies in each sampling week (see Table 1 for numbers per produce).

The same procedure was followed for the product sampling at the end of the processing line (22 samples over 12 different products per week) and investigated at one of the five Food and Consumer Product Safety Authority departments.

Product sampling in the supermarkets was done on a monthly basis because this fitted the regular sampling protocols of the Food and Consumer Product Safety Authority. Each of the five departments was to collect 25 samples (over 13 different products) at supermarkets in their region with a direct link to the processing companies.

Following this scheme would result in 1,950 samples of raw produce, 858 product samples collected at the processing companies, and 1,500 product samples collected in retail.

Sample analysis. As low numbers of microorganisms were expected, detection and quantification of contamination levels was carried out with the most-probable-number (MPN) approach for *Campylobacter* spp., *Salmonella,* and *E. coli* O157. To keep the number of samples to be analyzed manageable, a modified MPN method was used: two portions of 25 g and two portions of 2.5 g were investigated. This setup was related to portion size at consumption, storage space and labor time in the participating laboratories, and the ability to come to an improved risk estimate over current knowledge from earlier studies. For detection and quantification of *L. monocytogenes* contamination, an enumeration method was used. In the following, detailed descriptions of the analytical methods are presented.

Preprocessing of the vegetables was carried out in accordance with the methods used by the production companies. In brief, the following actions were taken with the various produces before mincing and homogenization of the samples in a K-650 Combimax food processor (Braun, Kronberg/Taunus, Germany). For lettuce and endive heads, the stems were cut off and discarded, and the outer leaves were removed and discarded. Red peppers were sliced in half, and the seeds and membranes were removed. For cucumbers, the ends were cut; iceberg lettuce heads and radicchio rosso heads were cut in half, with the stalk and outer leaves removed and discarded. Rucola and baby greens were minced and homogenized without preprocessing. All materials for preprocessing the samples, in accordance with the methods used by the production companies (mixing bowls of the food processor, knives, and chopping boards), were decontaminated by submersion into boiling water for 1 min before each use to prevent cross-contamination. After mincing and homogenization, samples were examined using methods (described below in detail) based on International Organization for Standardization (ISO) 6579 for *Salmonella,* ISO 10272 for *Campylobacter* spp., ISO 16654 for *E. coli* O157, and ISO 11290-2 for *L. monocytogenes*, respectively. Minimally processed packaged salads, collected at the end of the lines at the production companies and at the retail level, were minced and homogenized without preprocessing and investigated as described below.

Unless stated otherwise, all media/materials were from Biotrading, Mijdrecht, The Netherlands. For *Salmonella*, 225 and 22.5 ml of buffered peptone water were inoculated in duplicate with 25 and 2.5 g of the homogenized sample, respectively, and incubated at 37°C for 18 to 20 h. Subsequently, modified semisolid Rappaport-Vassiliadis plates were inoculated with 100 μl of buffered peptone water culture divided over three drops and incubated at 41.5°C. Plates were evaluated after 24 and 48 h, and if suspected for *Salmonella,* brilliant green agar was inoculated and incubated at 37°C for 24 h. Modified semisolid Rappaport-Vassiliadis was regarded negative if, after 48 h of incubation, no suspected colonies had developed. Biochemical confirmation was carried out with triple sugar iron agar, urea agar, and L-lysine decarboxylase medium. Confirmed isolates were serotyped by the Laboratory for Infectious Diseases and Perinatal Screening of the National Institute for Public Health and the Environment.

For *Campylobacter,* 225 and 22.5 ml of Bolton broth with laked horse blood were inoculated in duplicate with 25 and 2.5 g of the homogenized samples, respectively, and incubated at 41.5°C for 48 h in a microaerophilic atmosphere (10% O_2). Subsequently, a sample from the Bolton broth culture was plated with charcoal cefoperazone deoxycholate agar and incubated for another 48 h in a microaerophilic atmosphere. Suspected colonies were tested for their microscopic appearance (motile corkscrew-like microorganisms) and oxidase reaction. Further determination was done with a *Campylobacter* test kit (Oxoid, Basingstoke, UK), according to the manufacturer's instructions.

For *E. coli* O157, 225 and 22.5 ml of modified tryptone soya broth containing novobiocin was inoculated in duplicate with 25 and 2.5 g of the homogenized sample, respectively, and incubated at 41.5°C for 22 h. Subsequently, 1 ml of modified tryptone soya broth containing novobiocin culture was used for separation and concentration with Dynabeads anti–*E. coli* O157 test kit (Dynal Biotech ASA, Oslo, Norway), according to the manufacturer's instructions. Cefixime tellurite sorbitol MacConkey agar plates were used for detection. Presumptive colonies were confirmed with eosine methylene blue agar plates and Wellcolex *E.coli* O157 latex test (Remel Europe Ltd., Kent, UK).

For *L. monocytogenes,* 90 ml of buffered peptone water was inoculated with 10 g of the homogenized sample and left at room temperature for resuscitation for 1 h. Subsequently, 1.0 and 0.1 ml were plated in duplicate with agar *Listeria* (14 and 9 cm),

TABLE 3. *Results of the sample analysis from raw produce (A), products collected in the processing companies (B), and products collected at the retail level (C)*[a]

				95% confidence interval	
	k[b]	n[c]	d (%)[d]	Lower (%)	Upper (%)
A					
Salmonella	6	1,860	0.38	0.15	0.70
Campylobacter spp.	3	1,810	0.22	0.06	0.48
E. coli O157	1	1,833	0.11	0.01	0.30
L. monocytogenes	1	1,860	0.11	0.01	0.30
B					
Salmonella	0	751	<0.40	95% confidence level	
Campylobacter spp.	0	764	<0.39	95% confidence level	
E. coli O157	0	760	<0.39	95% confidence level	
L. monocytogenes	0	781	<0.38	95% confidence level	
C					
Salmonella	1	1,151	0.17	0.02	0.48
Campylobacter spp.	0	1,151	<0.26	95% confidence level	
E. coli O157	0	1,151	<0.26	95% confidence level	
L. monocytogenes	0	1,151	<0.26	95% confidence level	

[a] In those cases in which $k > 0$, the last two columns represent the lower (2.5%) and upper (97.5%) bound of the 95% confidence interval for d. In those cases in which $k = 0$, d gives the upper bound of the prevalence estimate for a 95% confidence level.
[b] k, the number of positive samples.
[c] n, the total number of samples analyzed per produce or product and pathogen combination.
[d] d, the prevalence point estimate.

according to Ottaviani and Agosti *(32),* respectively, and incubated at 37°C for 48 h. Confirmation of the suspected colonies was performed by means of a haemolysis test, a catalase reaction, motility test at 25°C, and the fermentation of L-rhamnose and D-xylose. From the number of counted and confirmed colonies, the number of CFU per milliliter was calculated.

RESULTS

The investigations of raw produce and minimally processed packaged salads collected in the processing company and at retail led to the finding of a number of pathogens. Based on the results of the modified MPN method (for *Salmonella, Campylobacter* spp., and *E. coli* O157) or the enumeration method (*L. monocytogenes*), the prevalence and the concentration of the various pathogens in raw produce and ready-to-eat products were calculated. Notably, a contaminated produce sample never contained more than one pathogenic species.

The actual number of investigated samples (n) of raw produce (A), products collected at the processing companies (B), and products collected at retail level (C), together with the number of samples positive for the respective microorganisms and the accompanying prevalence estimates (d) for the separate microbes, are shown in Table 3. The actual number of investigated samples differs from the desired number as shown in Table 2 due to circumstances, such as missing or insufficient produce/product samples or failed analysis. The overall prevalence point estimates for the microorganisms in raw produce varied from 0.11% for *L. monocytogenes* and *E. coli* O157 and 0.22% for *Campylobacter* spp. to 0.38% for *Salmonella*.

Because no microorganisms were detected in the products collected in the processing companies, only an upper prevalence level could be determined (in this case, the 95% confidence upper prevalence level was <0.40% for all pathogens).

Only one retail sample was found positive, which resulted in a *Salmonella* prevalence point estimate of 0.17% and an upper prevalence level estimate of <0.26% for the other pathogens (with 95% confidence). All *Salmonella* isolated from raw produce were typed as Typhimurium DT104, and the *Salmonella* isolated from the retail sample was typed as Montevideo.

In Table 4, the prevalence estimates of the separate microbes in the raw produce they were associated with are shown. Single pathogen/produce prevalence point estimates result in values up to 5.10% for *Salmonella* Typhimurium DT104 on cucumber (see Table 4). *Salmonella* was the most prevalent pathogen (responsible for six positive produce samples and one product sample), and endive appeared to be the most susceptible raw produce (six heads found positive with either *Salmonella* Typhimurium DT104, *Campylobacter* spp., or *E. coli* O157).

The results of the quantitative assessments are presented in Tables 3 and 4. The MPN estimates for *Salmonella, Campylobacter* spp., and *E. coli* O157 are shown in Table 5; the concentration of *L. monocytogenes* could be calculated based on the number of colonies on the 14- and 9-cm plates.

The MPN values were mainly in the range between 0.019 and 0.052 CFU/g; two extreme findings were a value of 0.281 CFU/g estimated for *Salmonella* Typhimurium DT104 on iceberg lettuce and a value of >0.281 CFU/g

TABLE 4. *Prevalence estimates of the separate microbes on produce level*

Microbe/produce	k[a]	n[b]	d (%)[c]	95% confidence interval[d]	
				Lower (%)	Upper (%)
Salmonella					
Endive	3	370	1.10	0.29	2.30
Cucumber	1	37	5.10	0.64	13.8
Iceberg lettuce	2	565	0.53	0.11	1.27
Campylobacter spp.					
Endive	2	360	0.83	0.17	2.00
Oak tree lettuce (green)	1	72	2.70	0.33	7.40
E. coli O157					
Endive	1	370	0.54	0.06	1.50
L. monocytogenes					
Curly endive	1	111	1.77	0.22	4.87

[a] k, the number of positive samples.
[b] n, total number of samples analyzed per pathogen/produce combination.
[c] d, the prevalence point estimate.
[d] The lower (2.5%) and upper (97.5%) bound of the 95% confidence interval for d.

estimated for *Salmonella* Typhimurium DT104 on endive. Assuming a lettuce head weighs approximately 500 g, point estimates in the range of 9 to 18 CFU on contaminated produce in general and estimates of ≥136 CFU for the *Salmonella* DT104 on iceberg lettuce and endive were calculated. *L. monocytogenes* was found on curly endive, which resulted in a point estimate of 250 CFU/g. Again, assuming a lettuce head weighs on average 500 g, this corresponds to approximately 10^5 *L. monocytogenes* cells on a head of curly endive.

The stratified sampling design in this survey revealed that source tracing for *Salmonella, Campylobacter* spp., *E. coli* O157, and *L. monocytogenes* in mixed salads should mainly focus on the primary production of the raw produce, i.e., at farm level. Produce sampling resulted in 11 positives of approximately 1,850 samples, whereas only 1 of 1,151 samples was found positive during the survey of mixed salads at retail. The consequences of these findings are discussed elsewhere *(35)*.

DISCUSSION

A complete comparison of the data presented here is hard because these investigations concern both the prevalence and concentration of four pathogens. Some comparisons, however, can be made. The U.S. Department of Agriculture investigated over a number of years the prevalence of pathogenic *E. coli* and *Salmonella* in ready-to-eat lettuce. The prevalence for the respective pathogens was 8 (0.52%) of 1,530 and 5 (0.33%) of 1,530 in 2006, 2 (0.19%) of 1,039 and 6 (0.58%) of 1,039 in 2007, 8

TABLE 5. *Concentration of pathogens per date and per product during the survey, number of positive (*k*) products/produce with a further serotype specification, and concentration estimates (MPN) together with lower (2.5%) and upper (97.5%) values of the 95% confidence intervals of the MPN for the positive samples throughout the sampling period*

	k	Product	Pathogen	MPN	Lower	Upper
2006						
10 Nov.	1	Oaktree lettuce	*Salmonella* Montevideo	0.02	0.001	0.11
2007						
2 May	2	Endive	*Campylobacter* spp.	0.024	0.0014	0.112
		Endive	*Campylobacter* spp.	0.024	0.0014	0.112
21 May	1	Oaktree lettuce	*Campylobacter* spp.	0.096	0.0133	0.518
20 June	1	Curly endive	*L. monocytogenes*[a]			
18 July	1	Endive	*E. coli* O157	0.052	0.0084	0.171
3 Sep.	2	Cucumber	*Salmonella* Typhimurium DT104	0.019	0.0011	0.082
		Endive	*Salmonella* Typhimurium DT104	0.019	0.0011	0.082
10 Sep.	1	Iceberg lettuce	*Salmonella* Typhimurium DT104	0.024	0.0014	0.112
17 Oct.	3	Endive	*Salmonella* Typhimurium DT104		0.281[b]	
		Endive	*Salmonella* Typhimurium DT104	0.024	0.0014	0.112
		Iceberg lettuce	*Salmonella* Typhimurium DT104	0.281	0.041	1.31

[a] Point estimate of 250 CFU/g, based on finding 26 and 28 colonies in the undiluted sample.
[b] In this case, only a lower limit could be estimated since all dilutions and replicates of the MPN were tested positive.

(0.38%) of 2,078 and 25 (1.2%) of 2,078 in 2008, and 5 (0.21%) of 2,336 and 1 (0.04%) of 2,336 in 2009. In this study a prevalence of 0.17% was found in the ready-to-eat salads obtained at the retail level *(3, 4, 7, 8)*. Szabo et al. (2000) investigated the prevalence of *L. monocytogenes* at the retail level and found a prevalence of 2.5% *(41),* while here a prevalence of <0.26% was found. Whether the difference in isolation method is relevant for this discrepancy is not known, as no comparing experiments were carried out. Nguyen-the and Carlin (1994) reviewed studies undertaken in France, England, and Germany *(30)*. In Canada, 466 fresh produce samples, including 43 cabbage samples, were investigated for *L. monocytogenes, E. coli* O157:H7, *Salmonella,* and *Shigella* and an overall prevalence was found of 1% for *L. monocytogenes* and 7% for *L. monocytogenes* in cabbage only *(22)*. Earlier, the same group found a prevalence of 0, 0, and 0.7% for *L. monocytogenes, E. coli* O157:H7, and *Salmonella,* respectively, after investigating 398 samples of leafy greens, herbs, and cantaloupe *(23)*. In a study on the microbiological condition of various types of fresh produce sold in markets in Canada, none of the investigated samples contained *Salmonella, Campylobacter,* or *E. coli* O157 *(11)*. However, none of these publications contains data on concentrations of the various pathogens, which are needed for proper risk assessments. In this article, concentrations of pathogens have been calculated, and these data will be used to draw up a risk assessment for the consumption of ready-to-eat mixed salads *(35)*.

An important reason for sampling all year was to investigate possible seasonal influences. The incidence of foodborne infections caused by *Campylobacter* is highest during spring and early summer. In addition, the prevalence of *Campylobacter* in broiler flocks in various European countries is highest between June and November *(25, 26)*. Even though we found only three raw produce samples positive for *Campylobacter,* all three in spring (May), these findings confirm the seasonality of this pathogen. Six *Salmonella*-positive raw produce samples were demonstrated in September and October 2007 and one positive product in November 2006, but this finding cannot be linked with known data on seasonal incidence of *Salmonella* in general. In addition, for *L. monocytogenes,* it is impossible to say anything on seasonal influence because we only found one positive raw produce sample for each pathogen. For *E. coli* O157, nothing can be said about any seasonal influence because, first, only one positive sample was found, and second, no data are available on seasonal incidence in cattle compared with incidence in lettuce. The only seasonal connection for *E. coli* O157:H7 is described by Williams et al. (2010), who linked incidence in cattle with the incidence in ground beef *(43)*.

Recently, it was found that stratified sampling may give better insight in the distribution of contamination in case of point contamination *(24)*. This might be a good strategy for future sampling experiments involving raw produce for ready-to-eat salads.

This study is the first to determine both the prevalence and concentration of pathogens in raw produce and the related minimally processed packaged salads. The data from this study were used to assess the risk for the public health due to the consumption of such commodities, with a few exceptions *(35)*. The result for *L. monocytogenes* was not used for determination of the public health risk for two reasons. First, because *L. monocytogenes* is able to grow at storage temperatures for raw produce, leading to different dynamics compared with the other microorganisms. Second, no relevant dose-response data are available.

A single sample of minimally processed packaged salad appeared to be contaminated with *Salmonella* Montevideo. This single finding was not used to determine the public health risk as no dose-response data are available for *Salmonella* Montevideo.

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REFERENCES

1. Anonymous. 2002. Risk profile on the microbiological contamination of fruits and vegetables eaten raw. Brussels (Belgium), Scientific Committee on Food. European Commission, Health and Consumer Protection Directorate-General. Available at: http://ec.europa.eu/food/fs/sc/scf/out125_en.pdf. Accessed October 2008.
2. Anonymous. 2005. Outbreaks of salmonella infections associated with eating roma tomatoes—United States and Canada, 2004. *Morb. Mortal. Wkly. Rep.* 54:325–328.
3. Anonymous 2007. Microbiological Data Program. Progress update and 2006 data summary. Washington, DC, United States Department of Agriculture. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5050633. Accessed October 2008.
4. Anonymous. 2008. Microbiological Data Program. Progress update and 2007 data summary. Washington, DC, United States Department of Agriculture. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5067866. Accessed October 2008.
5. Anonymous. 2008. Microbiological hazards in fresh fruits and vegetables. Meeting report. Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization. Available at: http://www.fao.org/fileadmin/templates/agns/pdf/jemra/FFV_2007_Final.pdf. Accessed October 2008.
6. Anonymous. 2008. The Rapid Alert System for Food and Feed (RASFF) Annual Report 2007. Available at: http://ec.europa.eu/food/food/rapidalert/report2007_en.pdf. Accessed October 2008.
7. Anonymous. 2009. Microbiological Data Program. Progress update and 2008 data summary. Washington, DC, United States Department of Agriculture. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5079908. Accessed October 2008.
8. Anonymous. 2011. Microbiological Data Program. Progress update and 2009 data summary. Washington, DC, United States Department of Agriculture. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5088761. Accessed October 2008.
9. Bean, N. H., J. S. Goulding, C. Lao, and F. J. Angulo. 1996. Surveillance for foodborne-disease outbreaks—United States, 1988–1992. *Morb. Mortal. Wkly. Rep. CDC Surveill. Summ.* 45:1–66.
10. Berger, C. N., S. V. Sodha, R. K. Shaw, P. M. Griffin, D. Pink, P. Hand, and G. Frankel. 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ. Microbiol* 12:2385–2397.
11. Bohaychuk, V. M., R. W. Bradbury, R. Dimock, M. Fehr, G. E. Gensler, R. K. King, R. Rieve, and P. R. Barrios. 2009. A microbiological survey of selected alberta-grown fresh produce from farmers' markets in Alberta, Canada. *J. Food Prot.* 72:415–420.

12. Bowen, A., A. Fry, G. Richards, and L. Beauchat. 2006. Infections associated with cantaloupe consumption: a public health concern. *Epidemiol. Infect.* 134:675–685.
13. Burke, G. 2008. Mexican Peppers Posed Problem Long Before Outbreak. Associated Press Aug 18, 2008. Available at: http://www.marlerclark.com/case_news/detail/mexican-peppers-posed-problem-long-before-outbreak. Accessed October 2008.
14. Cooley, M., D. Carychao, L. Crawford-Miksza, M. T. Jay, C. Myers, C. Rose, C. Keys, J. Farrar, and R. E. Mandrell. 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. *PLoS One* 2:e1159. doi:10.1371/journal.pone.0001159.
15. Erickson, M. C., and M. P. Doyle 2007. Food as a vehicle for transmission of Shiga toxin-producing *Escherichia coli*. *J. Food Prot.* 70:2426–2449.
16. European Food Safety Authority. 2013. Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 1. Outbreak data analysis and risk ranking of food/pathogen combinations. *EFSA Journal* 11:3025.
17. Greene, S. K., E. R. Daly, E. A. Talbot, L. J. Demma, S. Holzbauer, N. J. Patel, T. A. Hill, M. O. Walderhaug, R. M. Hoekstra, M. F. Lynch, and J. A. Painter. 2008. Recurrent multistate outbreak of *Salmonella newport* associated with tomatoes from contaminated fields, 2005. *Epidemiol. Infect.* 136:157–165.
18. Ilic, S., J. Odomeru, and J. T. LeJeune. 2008. Coliforms and prevalence of *Escherichia coli* and foodborne pathogens on minimally processed spinach in two packing plants. *J. Food Prot.* 71:2398–2403.
19. Jackson, R. 2006. *Salmonella* outbreaks: CSPI reports produce to be a major cause. *Health Care Food Nutr. Focus* 23(3):12.
20. Jansen, H. A. P. personal communication.
21. Jansen, H. A. P. and P. H. In't Veld 2002. Pathogenic microorganisms in food, 2002 (in Dutch). Report SAZD/2002/50/23. Dutch Food and Consumer Product Safety Authority. Available at: http://www.vwa.nl/actueel/bestanden/bestand/10403. Accessed October 2008.
22. Johnston, L. M., L. A. Jaykus, D. Moll, J. Anciso, B. Mora, and C. L. Moe. 2006. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *Int. J. Food Microbiol.* 112:83–95.
23. Johnston, L. M., L. A. Jaykus, D. Moll, M. C. Martinez, J. Anciso, B. Mora, and C. L. Moe. 2005. A field study of the microbiological quality of fresh produce. *J. Food Prot.* 68:1840–1847.
24. Jongenburger, I., M. W. Reij, E. P. J. Boer, L. G. M. Gorris, and M. H. Zwietering. 2011. Random or systematic sampling to detect a localised microbial contamination within a batch of food. *Food Control* 22:1448–1455.
25. Jore, S., H. Viljugrein, E. Brun, B. T. Heier, B. Borck, S. Ethelberg, M. Hakkinen, M. Kuusi, J. Reiersen, I. Hansson, E. Olsson Engvall, M. Løfdahl, J. A. Wagenaar, W. van Pelt, and M. Hofshagen. 2010. Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997–2007. *Prev. Vet. Med.* 93:33–41.
26. Jorgensen, F., J. Ellis-Iversen, S. Rushton, S. A. Bull, S. A. Harris, S. J. Bryan, A. Gonzalez, and T. J. Humphrey. 2011. Influence of season and geography on *Campylobacter jejuni* and *C. coli* subtypes in housed broiler flocks reared in Great Britain. *Appl. Environ. Microbiol.* 77:3741–3748.
27. Long, S. M., G. K. Adak, S. J. O'Brien, and I. A. Gillespie. 2002. General outbreaks of infectious intestinal disease linked with salad vegetables and fruit, England and Wales, 1992–2000. *Commun. Dis. Public Health* 5:101–105.
28. Manuel, D. G., S. Neamatullah, R. Shahin, D. Reymond, J. Keystone, J. Carlson, C. Le Ber, B. L. Herwaldt, and D. H. Werker. 2000. An outbreak of cyclosporiasis in 1996 associated with consumption of fresh berries—Ontario. *Can. J. Infect. Dis.* 11:86–92.
29. Naimi, T. S., J. H. Wicklund, S. J. Olsen, G. Krause, J. G. Wells, J. M. Bartkus, D. J. Boxrud, M. Sullivan, H. Kassenborg, J. M. Besser, E. D. Mintz, M. T. Osterholm, and C. W. Hedberg. 2003. Concurrent outbreaks of *Shigella sonnei* and enterotoxigenic *Escherichia coli* infections associated with parsley: implications for surveillance and control of foodborne illness. *J. Food Prot.* 66:535–541.
30. Nguyen-the, C., and F. Carlin 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* 34:371–401.
31. Nuorti, J. P., T. Niskanen, S. Hallanvuo, J. Mikkola, E. Kela, M. Hatakka, M. Fredriksson-Ahomaa, O. Lyytikäinen, A. Siitonen, H. Korkeala, and P. Ruutu. 2004. A widespread outbreak of *Yersinia pseudotuberculosis* O:3 infection from iceberg lettuce. *J. Infect. Dis.* 189:766–774.
32. Ottaviani, F., M. Ottaviani, and M. Agosti. 1997. Differential agar medium for *Listeria monocytogenes*. Presented at the Quimper Froid Symposium, Quimper, France, 16 to 18 June 1997.
33. Painter, J. A., R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerg. Infect. Dis.* 19:407–415.
34. Park, C. E., and G. W. Sanders. 1992. Occurrence of thermotolerant campylobacters in fresh vegetables sold at farmers' outdoor markets and supermarkets. *Can. J. Microbiol.* 38:313–316.
35. Pielaat, A., F. M. van Leusden, and L. M. Wijnands. 2014. Microbiological risk from minimally processed packaged salads in the Dutch food chain. *J. Food Prot.* 77:395–403.
36. Pollack, S. L. 2001. Consumer demand for fruit and vegetables: the US example. *In* Changing structure of global food consumption and trade/WRS-01-1. Ed. A. Regmi. Washington, DC, Economic Research Service. US Department of Agriculture. Available at: http://www.ers.usda.gov/publications/wrs-international-agriculture-and-trade-outlook/wrs01-1.aspx. Accessed October 2008.
37. Quiroz-Santiago, C., O. R. Rodas-Suárez, C. R. Vázquez Q., F. J. Fernández, E. I. Quiñones-Ramírez, and C. Vázquez-Salinas. 2009. Prevalence of *Salmonella* in vegetables from Mexico. *J. Food Prot.* 72:1279–1282.
38. Scavia, G., M. Staffolani, S. Fisichella, G. Striano, S. Colletta, G. Ferri, M. Escher, F. Minelli, and A. Caprioli. 2008. Enteroaggregative *Escherichia coli* associated with a foodborne outbreak of gastroenteritis. *J. Med. Microbiol.* 57:1141–1146.
39. Sewell, A. M., and J. M. Farber 2001. Foodborne outbreaks in Canada linked to produce. *J. Food Prot.* 64:1863–1877.
40. Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot.* 67:2342–2353.
41. Szabo, E. A., K. J. Scurrah, J. M. Burrows, and L. Szabo. 2000. Survey of psychrotrophic bacterial pathogens in minimally processed lettuce. *Lett. Appl. Microbiol.* 30:456–460.
42. Vose, D. 2000. Risk analysis: a quantitative guide. John Wiley and Sons, Chichester, UK.
43. Williams, M. S., J. L. Withee, E. D. Ebel, N. E. Bauer, W. D. Schlosser, W. T. Disney, D. R. Smith, and R. A. Moxley. 2010. Determining relationships between the seasonal occurrence of *Escherichia coli* O157:H7 in live cattle, ground beef, and humans. *Foodborne Pathog. Dis.* 7:1247–1254.