FEED THE FUTURE
The U.S. Government's Global Hunger & Food Security Initiative

EatSafe: Evidence and Action Towards Safe, Nutritious Food

Food Safety Hazards and Risk Associated with Fresh Vegetables:

Assessment from a Traditional Market in Southern Ethiopia

October 2023

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This EatSafe report presents evidence that will help engage and empower consumers and market actors to better obtain safe nutritious food. It will be used to design and test consumer-centered food safety interventions in informal markets through the EatSafe program.

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ACRONYMS AND ABBREVIATIONS

Below is a list of all acronyms and abbreviations used in the report.

Avg	Average
CFU	Colony-forming units
DALYs	Disability-Adjusted Life Years
EatSafe	Evidence and Action Towards Safe, Nutritious Food
FBD	Foodborne disease
FERG	WHO Foodborne Disease Burden Epidemiology Reference Group
ILRI	International Livestock Research Institute
Max	Maximum
Min	Minimum
MPN	Most Probable Number
RA	Risk assessment
SDV	Standard Deviation
WHO	World Health Organization
#	Number of

EXECUTIVE SUMMARY

Feed the Future's EatSafe (Evidence and Action Towards Safe, Nutritious Food) aims to improve the safety of nutritious foods sold in traditional markets. The majority of communities in low- and middle-income countries use traditional markets to access fresh food. For traditional markets, poor infrastructure and limited regulations heighten the risk of spreading foodborne disease (FBD) and thus represent an important point for intervention. In Ethiopia, EatSafe operates in Hawassa, a city in the southwestern region of Sidama, and focuses on three nutritious and highly consumed fresh vegetable commodities: tomatoes, kale, and lettuce.

This report includes results from several EatSafe activities conducted in a large market in Hawassa, Ethiopia from April to December 2022, including:

- A market reconnaissance study to document market dynamics;
- A survey of 385 consumers and focus group discussions with over 20 consumers to understand food handling and preparation practices;
- Microbiological analysis of food samples to estimate frequency of occurrence and levels of generic *E. coli, Salmonella,* and Total Coliform bacteria; and
- A risk assessment (RA) for *Salmonella* to quantitatively assess the relative exposure and risk of illness from consuming tomatoes, kale, and lettuce.

Results indicate the key commodities were frequently purchased from the market for home consumption. Lettuce is almost exclusively consumed raw (95% of consumers) while kale is consumed cooked (99%). By contrast, tomatoes are eaten both cooked (97%) and raw (77%).

Of 328 kale, tomato, and lettuce samples collected from 178 vendors, 22 (7%) and 117 (35%) were found positive for *Salmonella* spp. and generic *E. coli,* respectively. Contamination prevalence for both bacteria was highest for kale, followed by lettuce, and lowest for tomatoes. Total Coliforms, an indicator often associated with fecal contamination, were detected in 89% of the samples at high levels. *Salmonella* concentrations were low to medium. These findings suggest widespread and ongoing contamination rather than isolated high-risk incidents.

Based on the scenarios considered in the risk assessment, the daily risk of falling ill with *Salmonella* after consuming raw lettuce, raw tomatoes, or under-cooked kale was estimated to be 4.5 cases, 0.8 cases, and 1.0 case in 10,000 individuals that consume each commodity, respectively. The corresponding average annual risk, expressed as individual probability of becoming ill, was 11.7%, 1.3%, and 2% respectively. Risk could be higher for individuals and households that consume multiple commodities in a day. For comparison, this level of risk is approximately 40 to 220 times the risk of salmonellosis illness from consuming vegetables in the U.S.

Risk estimates are consistent with the prevalence and concentrations observed for the three commodities. Risk was found to be potentially impacted by practices including washing, cooking, and storage.

In summary, all key commodities were found to harbor *Salmonella*, a key foodborne pathogen, at frequency and levels that warrant attention. Salmonellosis risk estimates, while not extreme, are a cause for concern and highlight the risk of becoming ill from consumption of these commodities, in particular if eaten raw. In addition, the relatively high prevalence of *E.coli* and the high prevalence and levels of fecal coliforms suggest hygiene issues and cross-contamination from animal manure or sewage. While source tracking was outside the scope of the study, the microbial patterns observed are compatible with contamination events at both the market and along the supply chain. Overall, findings indicate the need for corrective actions as well as the potential for improvements in the market to support contamination prevention and reduction.

1. INTRODUCTION

Feed the Future's Evidence and Action Towards Safe, Nutritious Food (EatSafe) aims to improve the safety of nutritious foods sold in traditional markets by increasing consumer demand for food safety. While the markets exist in almost all countries, they are particularly important in low- and middle-income countries, where traditional markets provide vendors with sustainable livelihoods and consumers with affordable and nutritious foods. However, the markets often have inadequate infrastructure and limited regulations, increasing the risk of foodborne disease (FBD) (1). In Ethiopia, EatSafe operates in Hawassa, a city in the southwestern region of Sidama, and focuses on kale, tomatoes, and lettuce – all of which are sold in traditional markets, commonly eaten in the community, and highly nutritious (2).

EatSafe's formative research included qualitative and quantitative studies that identified consumers' and vendors' knowledge, attitudes, and practices around food safety, as well as the larger enabling policy, physical, and social environment for food safety (3). Under this formative research, EatSafe conducted a systematic literature review that examined FBD hazard occurrence in foods and beverages consumed in Ethiopia (4). The review confirmed the presence of *Salmonella* spp. in multiple foods sold in traditional food markets; however, only six studies focused specifically on the contamination of fresh vegetables, and only one focused on bacterial contamination. Furthermore, no study examined viral occurrence. Together, these findings highlight the evidence gap of the presence and risks of bacterial hazards in vegetables sold in traditional markets in Ethiopia, critical to public health (4).

Seeking to address this gap, EatSafe conducted a rapid risk assessment (RA) to estimate the risk of contracting salmonellosis from consuming kale, lettuce, or tomatoes purchased from EatSafe's target market in Hawassa. In this report, EatSafe first describes its methodological approach, including three field data collection activities that provided context-focused inputs needed for the RA. EatSafe then describes the results of the RA, including the relative risk ranking among the three commodities. This report concludes by discussing the implications of the results for the EatSafe program as well as other programs seeking to select interventions to improve food safety in traditional markets.

1.1. HAZARD SELECTIONS AND RESEARCH QUESTION

Estimates from the Foodborne Disease Burden Epidemiology Reference Group (FERG), a global group of experts convened by the World Health Organization (WHO), indicate that *Salmonella* and *E. coli* represent the highest FBD burden among bacterial hazards in the African sub-region, which contains Ethiopia (5). These pathogens, including the pathogenic types of *E. coli*, are estimated to result in

9,103,518 Disability-Adjusted Life Years (DALYs), equivalent to 62% of the burden due to bacterial causes of diarrhea (5). One DALY is equivalent to one year of a "healthy" life that is lost (4). Furthermore, both *Salmonella* and *E. coli* serve as proxies for other pathogens transferred via fecal contamination that cause serious illnesses, particularly among children, the elderly, and other vulnerable groups (the acronym YOPI - young, old, pregnant, immunocompromised - is often used to summarize these categories) (5). EatSafe chose to focus on *Salmonella* for the RA detailed in this report because it is a highly virulent foodborne pathogen, and as little as 1 to 10 cells can cause salmonellosis (6). It was also a pathogen of interest in EatSafe work in Nigeria.

While bacterial pathogens such as *Salmonella* appear to be prevalent in foods consumed in Ethiopia, the risk of illness due to ingesting these foods is not well understood. To fill this gap, EatSafe's research question addressed by the *Salmonella* RA was: What is the relative risk of becoming ill with salmonellosis from eating food made from raw fresh vegetables purchased in a traditional market in Hawassa, Ethiopia?

2. RISK ASSESSMENT METHODOLOGY

Considered the gold standard for evaluating the impact of FBD on public health, RAs provide estimates on the probability, extent, and uncertainty of harm given exposure to a certain hazard (7,8). In the sections that follow, EatSafe describes its RA approach. First, a transmission model was developed that visualizes the potential contamination pathways of *Salmonella* on the focus foods from purchase to consumption and the associated health outcomes. EatSafe then developed a mathematical model, customized to each of the three key commodities (kale, lettuce, and tomato), to represent how handling and preparation practices result in risk to the consumer. This model "follows" portions of the food and the associated pathogen loads through the relevant stages of the food chain, from sale at the market to exposure upon consumption. Using inputs from the field data collection at the study site, EatSafe developed and applied a Monte Carlo software simulation model to estimate the risk of illness associated with the consumption of vegetables. EatSafe ran separate models for each commodity, developing a risk ranking to determine the relative risk of each of EatSafe's key commodities.

2.1. RISK ASSESSMENT MODEL STRUCTURE

As shown in **Figure 1**, the concentration of *Salmonella* in a simulated food portion was estimated sequentially from purchase at the market until consumption. The model included the following main steps that could affect *Salmonella* concentrations, as applicable to individual commodities: i) *Salmonella* growth, based on storage and preparation practices post-purchase (described in terms of temperature and time); ii) *Salmonella* reduction by cooking, and iii) consumption amounts (i.e., portion size).[1] Exposure was expressed in terms of the number of cells ingested with each portion of food (dose), calculated by multiplying the concentration by the portion size.

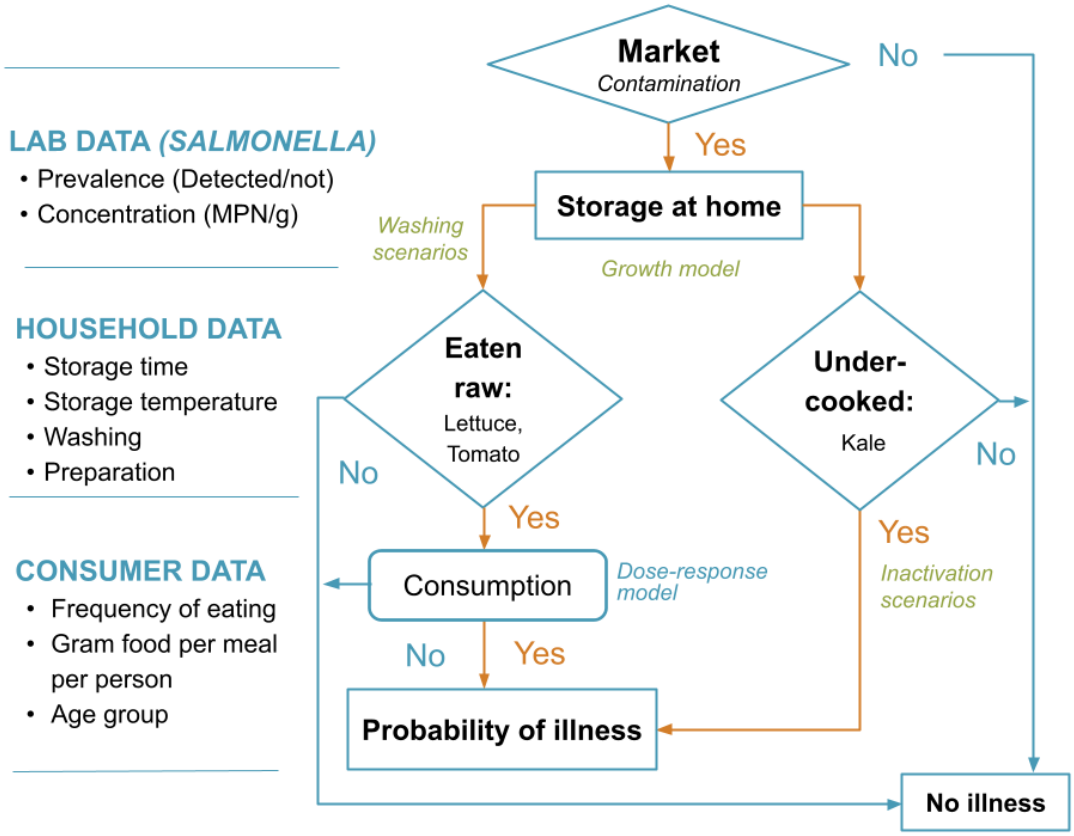

Figure 1. *Salmonella transmission model from retail to consumption*

Following the development of the transmission model, EatSafe conducted an exposure assessment, or an estimation of consumers' likely intake of *Salmonella* – a key step in the risk assessment process. The probability of illness following consumption was determined using an established dose-response equation (9). Risk was expressed as both daily and annual (based on consumption patterns reported by consumers) probability of illness. For all variables, variability was included in the model by randomly resampling either data or variable distributions using a Monte Carlo algorithm. The model was developed and run in @Risk, a simulation add-on to Microsoft Excel™, set to run a maximum of 10,000 for all input parameters until all input parameters converged (10).[2] EatSafe also conducted a sensitivity analysis to

[1] Because EatSafe is focused on markets, cross-contamination at the household level was not explored. However, results will inform which products need risk mitigation at the market, the risk of bringing contaminated products or introducing cross-contamination into the home may be reduced.

[2] For the convergence test, a 3% tolerance on the 95% percentiles was used as cutoff.

determine how input parameters influence the main output, i.e., the probability of illness from consumption of each product.

2.2. MODEL INPUT PARAMETERS

Exposure assessments require input variables and parameters, including data on consumer food handling and storage practices, as well as the frequency and intake of potentially contaminated products (11). EatSafe used a mixed-methods approach to gather these inputs, including field data collection (Section 3) and consolidated evidence from the literature as input parameters into the model. The Methods are more fully described in Appendix 3.

Results provide estimates for the mean dose ingested (cells/per person/per day) with accompanying ranges based on probability intervals that reflect the variability and uncertainty of the underlying data. These estimates allow ranking of transmission routes, either by commodity or from highest to lowest risk. In each step of the RA model, parameters were calculated to answer the following questions:

- Given the overall proportion of portions found contaminated, what is the probability that one specific portion is contaminated?
- How is the product stored (time and temperature) from purchase at the market to consumption? (How much bacterial growth, if any, can occur during storage?)
- Is the product cooked before consumption? (How much bacterial reduction can occur, due to cooking?)
- What is the bacterial dose ingested from eating one portion of the commodity?
- What is the probability of illness from eating one portion of each commodity, per day and over the course of one year?

3. MARKET DATA

EatSafe developed a mixed-method approach to collect necessary data for inputs into the RA model, described in the sections below: a reconnaissance study; a consumer survey and focus group discussions; microbiological food sampling, and subsequent laboratory analyses. EatSafe received IRB approval via ILRI's ethics review board (IREC), as amendments to previously approved activities.[3]

3.1. FINDINGS FROM RECONNAISSANCE STUDY

In April 2022, EatSafe conducted a reconnaissance visit to the market to understand its operations, estimate the vendor population, meet with market stakeholders, and pilot data collection tools. Key findings are highlighted here, while additional details of market operations and management are in Appendix 1. The reconnaissance

[3] ILRI-IREC 2020 51/4 for reconnaissance study and 2020 51/5 for the main data collection activity.

findings formed the basis for the formative phase of the program, including activities reported here and elsewhere (2,3,12,13).

Market operations. The market operates every day, with peaks on Mondays and Thursdays (i.e., "market days," perceived as offering the greatest variety of commodities at the lowest prices).

Vendor counting and stall structure. During a visit on a market day, EatSafe identified 245 vendors who sold the program's key commodities (kale, tomatoes, and lettuce; see **Figure 1**). The vast majority of vendors are women (87%). In particular, both men and women were seen selling tomatoes and kale, but all lettuce vendors were women. Most vendors (89%) selling the key commodities had stalls that were not raised off the ground (89%) and the structures were not fixed (69%).

Key commodity availability. While tomato sales do not vary significantly over time, kale and lettuce sales are higher during the fasting periods of the orthodox calendar, when vegetables are in high demand. Kale is mostly available during and after the rainy season (June-July). Vendors indicated that most lettuce and kale sales occur in the morning, and both quality and price tend to decrease in the late afternoon.

Consumer counting. EatSafe observed that the number of consumers varied significantly, with highs on Mondays and Thursdays, the main market days, and lows on Sundays. Local informers reported that several thousand consumers visit the market on the main market days. Daily, up to 30% of the total consumers who visit the market in a day shop during peak times (i.e., 9 - 11 AM and 4 - 6 PM). Notably, the majority of consumers who shop at the market are women.

Market facilities. The study market infrastructure has significant gaps that can impact food safety risks and potential interventions:

- ***Toilets.*** Though the market has four public toilets, only one has tap water and it requires a relatively high price to access (5 Birr); the remaining three facilities are not separated by gender and are not utilized frequently.
- ***Water.*** Water is not readily available in the market. Besides limited paid access to a tap-in-one toilet facility, there is no water source at the market. Some vendors purchase limited quantities of water from homes neighboring the market and transport them to the market in jerry cans.
- ***Waste management.*** There is no system for waste management or collection; individual vendors dispose of their unsold items or spoiled commodities haphazardly, which results in deterioration of hygiene in the market, bad odor, and increased risks of food contamination.
- ***Drainage***. While some walkways are paved with cobblestones, a significant amount of mud covers the walkways and accumulates during rain events.

3.2. FINDINGS ON CONSUMER PRACTICES

To support this risk assessment, EatSafe conducted two data collection activities involving consumers: a questionnaire adapted from a prior EatSafe RA in northwest Nigeria (14), and a focus group discussion. Both activities occurred during August 2022. Results from these two activities are synthesized in the section below, with methods and additional details in Appendix 1.

Food acquisition practices. While purchasing practices were not directly relevant to the risk assessment, and hence not included as a survey question, what consumers were purchasing on the day of the survey provides qualitative insights. Forty-four percent (170 of 385) of the surveyed consumers had bought one of the key commodities, 39% had bought two, while 17% had all three. Among those who bought only one, 52% had bought kale, 41% tomato, and 7% lettuce. Of the respondents who bought two vegetables, 70% had bought kale and tomato, 23% tomato and lettuce, and 7% kale and lettuce. Nearly all consumers bought the commodities for personal consumption, and most (94% lettuce; 88% kale; 77% tomatoes) ate it the same day.

Food preparation practices. Tomato was the only commodity that consumers reported eating in both raw and cooked forms; lettuce was consumed raw while kale was cooked (**Table 1**). All consumers reported washing tomatoes and lettuce before consuming them. Storage methods included room temperature (57% kale; 44% lettuce; 33% tomato) and refrigeration (66% tomato; 48% lettuce; 38% kale).

Table 1. *Preparation methods and amount consumed of the three key commodities*

	METHODS		AMOUNT CONSUMED [1]	
	Preparation Methods		As bought (g)	After Cooking (g)
	Raw	Cooked	Avg (min - max)	
LETTUCE	Yes	No	263	141 (83 - 192) [1]
TOMATO	Yes	Yes	503	503 (317 - 817)
KALE	No	Yes	534	313 (128 - 517) [2]

[1] The focus group discussions provided this data.

[2] Unlike the other two commodities, lettuce is only consumed raw. The decrease in weight from buying to consumption may reflect consumers' choices to discard leaves that appear unclean (e.g., not fresh, have spots, look wilted).

[3] Kale tends to shrink considerably after cooking.

Consumption frequency and amounts. The frequency of consumption of each commodity by age group varied (**Table 2**). On average, lettuce, tomato, and kale were prepared three, five, and four times a month respectively. In focus groups (not shown in tables), consumers reported consuming the three commodities, either prepared raw or cooked, on average, between 11 and 18 times out of 100 meals. In terms of the amount consumed in a meal (**Table 3**), kale was consumed in the largest amount, followed by lettuce and tomato. Across commodities, people aged 15-60 years had the highest intake, followed by youth (5-15 years), while children less than 5 years old had the least. Less data were available for older adults.

Table 2. *Consumption frequency of raw key commodities, by age group*

AGE GROUP [1]	FREQUENCY (NO. OF DAYS PER 30 DAYS) [2]		
	MEAN	SDV	MIN - MAX
LETTUCE (n=117)			
Children	0.8	3.0	0 - 20
Youth	4.8	5.5	0 - 21
Adult	7.9	5.2	0 - 30
Older Adult	0.1	0.8	0 - 7
All ages	**3.4**	**5.2**	**0 - 30**
TOMATOES (n=210)			
Children	1.2	4.3	0 - 30
Youth	5.5	7.2	0 - 30
Adult	9.3	6.3	0 - 30
Older Adult	0.7	3.2	0 - 30
All ages	**4.2**	**6.5**	**0 - 30**
KALE (n=12) [3]			
Children	0.3	0.9	0 - 3
Youth	4.9	8.0	0 - 28
Adult	6.7	7.6	0 - 28
Older Adult *	NA	NA	NA
All ages	**3.9**	**6.8**	**0 - 28**

[1] Age groups are as follows: children (≤5 years), youth (>5-15 years), adults (>15-60 years, and older adults (≥60 years). Note that these age categories are different than those used by Feed the Future (15).

[2] Data comes from the consumer survey, with sample sizes noted per commodity.

[3] Only 12 respondents reported consuming raw kale. While data for raw kale is shown here, it was not an input in the RA model, which considered undercooked kale. Consumption frequency data for cooked kale was derived from focus group discussions (see Table A3).
* The value for all kale among elders was not available.

Table 3. *Consumption amounts of key commodities per meal, by age group*

AGE GROUP [1]	CONSUMPTION PER MEAL (GRAMS) [2]
LETTUCE (RAW)	
Children	18
Youth	39
Adult	47
Older Adult [3]	47
TOMATO (RAW)	
Children	11
Youth	24
Adult	27
Older Adult [3]	27
KALE (COOKED)	
Children	33
Youth	47
Adult	69
Older Adult [3]	69

[1] Age groups are the same as in Table 2.

[2] Most likely amount consumed per meal. These data were derived from three consumer focus groups (sample size: 18 to 24 consumers).

[3] Due to scarcity of data specific to this age group, it was assumed that amounts consumed per meal are the same as for adults.

3.3. MICROBIOLOGICAL FOOD SAMPLING AND ANALYSIS

EatSafe collected 328 food samples from October 5 – December 30, 2022 from 178 vegetable vendors. All samples were collected in sterile polythene bags and transported on ice to laboratory facilities at the Hawassa University College of Medicine and Health Sciences (Microbiology Laboratory) (n=150), School of Medical Laboratory Microbiology (n=163), and Southern Public Health Laboratory (n=15). Approximately one-third of samples were collected in the morning, while the remainder were collected in the afternoon (n=126; n=202).

Laboratory analysis included identification and enumeration of *Salmonella* spp. and generic *E. coli*, as well as Total Coliforms. Findings, shown in **Table 4**, indicate 35% (n=117) of samples tested positive for *E. coli*, while 7% (n=22) tested positive for *Salmonella* spp. Further quantitative analysis determined *Salmonella* concentrations in four kale and three lettuce samples, ranging from 0.3 to 29 MPN/g (Appendix 2). *E. coli* levels in positive samples were similar across commodities, ranging from a mean of 5.1 LogCFU/g for lettuce (SD: 0.4), 5.1 LogCFU/g for tomato (SD: 0.2), to 5.3 LogCFU/g for kale (SD: 0.3). While only a subset of *E. coli* strains is pathogenic, these are high levels that suggest possible fecal contamination and non-ideal hygienic conditions in at least some samples. Across all three commodities, 89% of samples were positive for Total Coliforms (96% for lettuce, 76% for tomatoes, and 99% for kale), with concentrations ranging from 3.7 to 6 Log CFU/g. This suggests widespread exposure of the commodities to environmental routes of microbial contamination.

Table 4. *Salmonella and E. coli prevalence in fresh vegetable samples*

	# VENDORS	# SAMPLES	POSITIVE SAMPLES			
			***Salmonella* spp.**		***E. coli***	
			n	%	n	%
LETTUCE	20	80	6	8%	41	51%
TOMATO	128	128	1	1%	2	2%
KALE	30	120	15	13%	74	61%
TOTAL	178	328	22	7%	117	35%

4. RISK ASSESSMENT RESULTS

Estimates of salmonellosis incidence from different vegetables show a higher probability of illness due to consuming raw lettuce (approximately 4.5 cases per 10,000 person-days), followed by under-cooked kale (1 case per 10,000 person-days) and raw tomato (0.8 cases per 10,000 person-days). Annual incidence of salmonellosis associated with eating raw lettuce, under-cooked kale, and raw tomato were 11.7%, 2.0%, and 1.3% respectively, i.e., 1,170, 200, and 130 cases per 10,000 individuals (**Table 5**). Incidence of salmonellosis was highest in the adult group. Although the risk of severe illness is higher for older adults, very limited consumption data was available, thus adding uncertainty to risk estimates for this group. See Appendix 4 for details of risk results.

Table 5. *Daily and annual incidence of salmonellosis of the key commodities, by age group*

AGE GROUP	LETTUCE		TOMATO		KALE	
	MEAN	5%-95% RANGE	MEAN	5%-95% RANGE	MEAN	5%-95% RANGE
Daily incidence of *salmonellosis* (illness cases per 10,000)						
Children (< 5 years old)	0.4	0 - 2.3	0.1	0 - 0	0.86	0 - 0.5
Youth (6-15 years old)	3.3	0 - 15.3	0.6	0 - 0.6	0.95	0 - 1.0
Adult (16-60 years old)	5.9	0 - 24.6	0.9	0 - 1.4	0.96	0 - 1.1
Older adult (>61 years old)	0.1	0 - 0	0.1	0 - 0	0.93	0 - 1.1
Overall daily incidence	**4.54**	**0 - 18.1**	**0.8**	**0 - 1.2**	**0.94**	**0 - 2.3**
Annual incidence of *salmonellosis* (%)						
Children (< 5 years old)	1.2	0 - 8.5	0.2	0 - 0	1.7	0 - 1.8

Youth (6-15 years old)	9.2	0 - 42.8	1.0	0 - 2.2	1.9	0 - 3.4
Adult (16-60 years old)	15.0	0 - 59.3	1.6	0 - 4.9	2.0	0 - 3.9
Older adult (> 61 years old)	0.2	0 - 0	0.2	0 - 0	2.0	0 - 3.9
Overall annual incidence	**11.7**	**0 - 44.4**	**1.3**	**0 - 4.1**	**2.0**	**0 - 7.7**

Lettuce. Risk estimates for lettuce eaten raw, point to this commodity posing the highest risk of *Salmonella* illness to consumers, among the foods considered. This is due to the fact that lettuce is always eaten raw, and was found to be contaminated at a high frequency (i.e. prevalence), even if concentrations were not extremely high in absolute terms. Risk is highest for adults, followed by youth; differences in risk between age groups are due to different consumption frequencies and amounts.

Tomato. The model considers tomatoes eaten raw, assuming that tomatoes eaten cooked (e.g., as part of soups or stews) pose a negligible direct risk. For the scenario considered, consumption of raw tomatoes poses a relatively lower risk compared to lettuce, and a risk comparable to undercooked kale. This is primarily due to the low observed prevalence and relatively lower concentrations of *Salmonella* in this product, as purchased at the market. Home washing scenarios were assumed to have the same effectiveness for lettuce and tomatoes; hence differences in risk estimates between these two commodities cannot be ascribed to differences in home washing. Similarly, the potential growth of bacteria during storage only accounts for a very small change in concentrations for all commodities.

Kale. This scenario includes the risk of consuming undercooked kale. Kale is never or only rarely consumed raw, hence this route of exposure was not included. Further, it is assumed that fully cooking kale, e.g., cooking for long times in soups or stews, can completely eliminate *Salmonella*. Variables related to undercooking and the associated partial reduction in *Salmonella* contamination contain a large uncertainty, due to a dearth of data; here a simplified assumption was used to illustrate a scenario including undercooking, in the hypothetical case where everyone that consumes kale consumes it undercooked.

Based on the results of the sensitivity analysis, which aims to assess how the variability in model inputs impacts the magnitude of the risk output, *Salmonella* prevalence in fresh vegetables as sold at the market (i.e. if a batch of product harbored *Salmonella* or not) had the highest correlation with magnitude of risk. Conversely, concentration did not impact risk as much, possibly because concentrations were clustered within a narrow range. The temperature at which vegetables were stored before being prepared for consumption (refrigeration vs. ambient temperature) was mildly associated with risk. This is also visible when comparing risk results including and excluding growth during storage, which differed by 20% on average for lettuce (0.8 cases per 10,000 for daily incidence), for all age groups combined. Hence, results including growth (shown in **Table 5**) should be considered worst-case scenarios in terms of bacterial growth that could occur between purchase and consumption.

Washing could have a substantial impact on risk. However, a range of washing practices may be followed by consumers (e.g. with water of variable quality, for different duration or intensity, one vs. multiple washing steps, with running water vs. in a container), whose characterization was out of the scope of this activity. As a result, risk estimates contain a large uncertainty in washing effectiveness. When simulating different degrees of washing effectiveness separately (**Figure A7** in Appendix 4), going from “not washing” to a “medium” degree of washing resulted in an average 44% decrease in risk. Implementing the “best” degree of washing resulted in a 72% risk reduction.

5. DISCUSSION AND CONCLUSIONS

The objective of this activity was to assess contamination levels from the pathogenic bacterium *Salmonella* and indicator bacteria (generic *E. coli* and Total Coliforms) in EatSafe priority commodities in one traditional market in Ethiopia and to estimate the risk of *Salmonella* infections among consumers of these products.

From the findings presented here, it can be concluded that foodborne hazards are present in foods sold in traditional markets in Hawassa, Ethiopia. Most importantly, the observed levels of microbial hazards (namely *Salmonella*) can pose a considerable risk, given prevailing handling, storage, and preparation practices both in the market and in households where the foods are consumed. While data and assumptions included in this rapid RA would need further refinement before these estimates can be validated, this effort provides a synthesis of context-specific data on key risk-relevant parameters, and a snapshot of absolute and relative risk among commodities commonly sold in traditional markets in Ethiopia. For an approximate comparison, salmonellosis risk from consuming each of the three focus vegetables estimated in this study (4.5, 0.8, and 1.0 cases per day per 10,000 individuals, for lettuce, tomato, and kale respectively) is by far higher than risk in the U.S. market, namely 40 (for tomato) to 220 (for lettuce) times higher than an approximate estimate of salmonellosis risk from consuming all vegetables in the U.S. (16,17).

The relatively high prevalence of both *Salmonella* and *E. coli* in lettuce and kale, combined with high levels of Total Coliforms, point to ongoing widespread contamination of these commodities as sold at the market, rather than occasional high-level incidents. Total Coliforms, while not indicators of pathogenicity, are associated with contamination from human or animal feces, directly or via soil, water, or contact surfaces. Leafy greens are particularly vulnerable to such contamination as they are in contact with the soil and often with water when grown in the field. In addition, all vegetables might be stored on the ground, and in markets are often displayed on or close to the ground.

The hazard occurrence findings presented here are generally consistent with evidence available in the published literature, which points to a medium-high frequency in contamination of a broad range of hazards in a variety of foods and beverages across supply chains in Ethiopia, from production to retail (4). However, in the evidence review on foodborne hazards in Ethiopia conducted by EatSafe (4) only a handful of studies were identified that assessed microbial contamination in fresh fruits and vegetables: four studies on parasites (18–20) and two on bacteria (21,22). In one of the studies investigating bacteria in lettuce and green peppers (each n=40), *Salmonella* was detected in 10% of samples and *Shigella* in 30%, while coliform counts were above 4 Log CFU/g in 48% and 35% of lettuce and green pepper samples (21). The second study detected *E. coli* O157:H7 (a pathogenic species of *E. coli*) in lettuce sold at Addis Ababa markets at a relatively low (0.5%) prevalence (22), lower than the prevalence observed in the EatSafe study market. No study on pesticides or other chemical hazards in fresh vegetables was identified.

The detection of *E. coli* and coliforms in Ethiopian drinking water sources (23) reported in the literature suggests many water sources may have been contaminated with feces, pointing to the importance of the fecal-oral route of transmission. These water sources could have been used to irrigate or even wash fresh vegetables. In particular, the detection of *Salmonella* and parasites with both human and animal hosts such as *Giardia* spp. and *Cryptosporidium* spp. points to the potential role of animals as contributors to water contamination, and hence vegetable contamination. The novel EatSafe data presented in this report is consistent with this point.

Handling and preparation practices before and after purchase make a difference in the risk a food may pose to consumers. The most effective practices vary by commodity. For instance, washing with potable-quality water can reduce bacterial contamination, although it will not completely eliminate it. At the same time, information is lacking on washing practices and their effectiveness in real-world settings. In the estimates presented here, a range of washing scenarios was included to account for this uncertainty. The degree of washing effectiveness had a noticeable impact on risk, although modest compared to the impact of initial prevalence. Storage conditions, in particular temperature, can also have an impact on risk as they may allow for bacterial growth (24–26). Growth is more likely or more rapid if the product is wet, cut, or damaged, pointing to the importance of separating damaged from intact product, and to not pre-cut vegetables at the market (27,28).

Washing, storage, and other practices at the market can also affect bacterial levels, positively or negatively. The RA results highlight the potential for risk mitigation actions, both at the market and at home.

Findings also highlight the importance of proper cooking. While cooking at sufficiently high temperatures and/or for sufficiently long times can inactivate *Salmonella* and other bacteria, partial cooking ("undercooking") can result in a non-negligible risk, as illustrated here for kale. While not included in this RA, it is also important to highlight the importance of minimizing cross-contamination, even for products that will be fully cooked. For example, if food to be cooked (e.g. raw meat or kale) is handled on the same surfaces or with the same utensils (including unwashed hands) as food to be eaten raw such as lettuce or tomatoes, cross-contamination can increase the risk of pathogen spread via the surfaces. Hence, selecting safer foods at the market and not "bringing home the germs" is a valid message even for commodities that will be fully cooked, in addition to messages on avoiding cross-contamination.

Key findings and conclusions from this activity include:

- Contamination with *Salmonella* was observed in all three commodities sampled, providing evidence that raw vegetables sold in traditional markets can be significantly contaminated with pathogenic bacteria. Contamination was most frequent in lettuce (8%) and kale (13%) compared to tomatoes (1%).
- *E. coli* bacteria, which may indicate fecal contamination, were frequently observed, in particular on lettuce (51%) and kale (61%). Other coliforms were also reported, which suggests that fresh vegetables were not protected from fecal contamination at or before reaching the market.
- Samples positive for *Salmonella* were most often contaminated at concentrations that may cause illness if no reduction measures take place before consumption.
- The risk of salmonellosis was highest for consumers of raw lettuce, with a lower but still concerning risk for tomatoes. Kale, while primarily consumed cooked, can pose a significant risk if undercooked and may cross-contaminate other foods.
- Estimated risk was highest for adults, but it also warrants attention in children and youth; consumption data is lacking for older adults.
- Results justify the selection of fresh vegetables as focus commodities for EatSafe in Ethiopia. The frequent presence of *Salmonella* on foods not of animal origin indicates that cross-contamination may be occurring at one or multiple points in the supply chain, including possibly at the market.

- The relatively lower microbial levels in tomatoes, compared to the other commodities examined, suggests that actors along the tomato supply chain - including vendors - may already be implementing some food safety practices that could be built upon.
- Findings substantiate the need for interventions to reduce microbial risk, in the market and other supply chain stages, as well as the potential for available intervention to reduce risk (e.g. washing).
- Produce washing can have a significant role in reducing bacterial contamination, and hence should be considered as a potential practice to leverage as part of interventions; however, it is essential to use water of potable quality and avoid cross-contamination.
- Current infrastructure at the study market, in particular, the absence of a source of water and lack of sanitary waste management, can significantly hinder risk reduction efforts.

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7. APPENDICES

7.1. APPENDIX 1: ADDITIONAL DETAILS ON FIELD DATA ACTIVITIES

RECONNAISSANCE STUDY

During the reconnaissance study, EatSafe was accompanied by a representative from the Trade office who provided local support.

Vendors and commodities sold. EatSafe estimates that approximately 2,200 vendors are registered in the focus market, though approximately an additional 800 vendors are unregistered who operate inside and outside the boundaries of the market under temporary shades. Additionally, children under the age of 18 also sell commodities, generally in walkways of the market using movable shades or carts. As noted in Section 3, EatSafe identified approximately 245 vendors of EatSafe's key commodities, most of whom are women.

Market structure and management. While the market has no clear boundaries, there are four main gates. Each commodity has its own selling location, with stalls that vary in cleanliness and hygiene, as well as permanent and moveable structures. In the temporary stalls and movable carts, food items are exposed to sun and dust. The entrances of the market are the busiest areas. About 30 different food products are sold at the market, but EatSafe's key commodities, vegetables, are sold in four areas: one at gate 1, one at gate 4, and two in the center of the market. During the rainy season, the walkways in the center of the market often become muddy; these pathways are repaired by market management by spreading red ash that absorbs water. Beyond this specific service, market management does not regulate or supervise vendors' activities in the marketplace. Additionally, recent challenges faced by the target market include:

- Fire accidents, leading to destruction of market facilities and vendors' supplies
- Fluctuation of commodity supply to the market
- Inflation in the cost of commodities compared to the amount approved by trade office (6%) which was reported to impact market operations;
- Inadequate access to water for both washing products and personal use;
- Poor roads and walkways within the market, which hinder foot traffic, particularly during the rainy season;
- Inadequate drainage system, which is a particular concern for commodities sold on the ground in non-raised structures;
- Lack of proper toilet and waste disposal facilities.

Commodity transportation, storage, and display in the market. Delivered to the market in sacks, vendors wash lettuce, and then put it in a raised bucket containing water so as to avoid wilting. Lettuce requires careful handling, given that quality can deteriorate quickly with poor handling. Similarly, kale is transported to the market by porters who carry product in sacks on their back, covered with a flat sheet. Vendors then remove kale from the sacks and place product on raised surfaces, usually in movable carts. For registered vendors, tomatoes are delivered in boxes or crates in vehicles, which are offloaded and placed on the ground near vendors' stalls; for those who are unregistered, vendors generally bring in smaller quantities of tomatoes in sacks. Both types of vendors then spread the tomatoes onto a flat sheet which may or may not be raised off the ground. A few vendors were observed to use a cloth to wipe off dust and to keep tomatoes shiny.

Time mapping activity. Using a calendar matrix to understand supply and demand dynamics throughout the year, EatSafe identified the levels of supply per month for each commodity (**Table A1**). During January to April, the low supply of lettuce and kale leads to the highest prices.

Table A1. *Time mapping activity for EatSafe in Ethiopia's key commodities*

	SUPPLY BY MONTH (H = HIGH, M = MEDIUM, L = LOW)												# OF MONTHS		
	Ja	Fe	Ma	Ap	May	Ju	Jul	Au	Se	Oct	Nov	Dec	Low	Med	High
Lettuce	L	L	L	L	M	M	M	M	H	H	M	M	4	6	2
Tomato	H	M	M	H	H	M	M	M	M	M	M	M	0	9	3
Kale	L	L	L	H	H	H	H	H	H	H	M	M	3	2	7

Notable public holidays impacting supply and demand dynamics include:

- January: Epiphany
- April: Lent, Easter, Ramadan
- May: Current Ethiopian Government came to power
- Between May-June: Sidama New Year
- September: Ethiopian New Year and Finding of True Cross
- December: Christmas

Agricultural practices (i.e., plowing, sowing, weeding, and harvesting) occur from April to November and reduces farmers' presence in the market.

CONSUMER SURVEY AND FOCUS GROUP DISCUSSIONS

Enumerators were locally recruited and trained prior to data collection. Tools were developed in English and translated to Amharic at the time of the interview. EatSafe obtained informed consent from participants prior to data collection. EatSafe used Open Data Kit on tablets to administer the questionnaires. Inclusion criteria for both studies required participants to be above 18 years of age and to have previously purchased any commodity in the market during the prior month. Participants were compensated for their time at the end of the session.

Focus group discussion. EatSafe conducted three focus group discussions, with 6-8 consumers per group, to understand food handling and preparation practices in consumer's homes. All participants were women, with ages ranging from 20 to 58 years old. Their households had an average of four members, ranging from two to six. Their occupation varied from government workers, housekeepers, NGO/private employees, students, housewives, cleaners and retirees.

Consumer survey. A total of 385 consumers were interviewed using a structured questionnaire, over a six-day visit. Nearly all (96%) were women aged 31 ± 12 years (range 18-75 years). Approximately 30% of respondents were not working outside the home, 25% were self-employed, 19% were employed by the government, and 16% were students. About one-third of the participants had attained primary school education. Over two-thirds (68%) of participants lived in households with 3-6 members (range 1-12 people), and half of the households had children (<5 years, and 6-15 years); by contrast, few households had members over the age of 60 years.

7.2. APPENDIX 2: QUANTITATIVE ANALYSIS OF SALMONELLA

Among samples tested for *Salmonella*, the following were positive at screening:

- Lettuce: 6 out of 80 samples (8%)
- Tomato: 1 out of 128 samples (0.8%)
- Kale: 15 out of 120 samples (13%)

All screen-positive samples were further analyzed using an MPN (Most Probable Number of cells) approach based on serial dilutions to estimate concentrations. In this approach a series of dilutions are prepared from the original sample (e.g. 1:100, 1:1000, 1:10,000) and each dilution is inoculated into three replicate vials containing liquid growth medium specific to the target organism. If a vial contains cells from the target organism, the cells will grow and change the color or turbidity of the liquid in the vial. After incubation, each vial is scored as "positive" or "non-detected" across the three dilutions. An algorithm is then used to estimate the concentration in the original sample, based on the pattern of positive and non-detected vials.

Table A2 presents the enumeration results for the subset of samples that yielded a concentration estimate via the MPN assay. The remaining screen-positive samples analyzed by MPN resulted in non-detects (i.e. a MPN pattern of 0-0-0), i.e. concentration estimates below 0.3 MPN/g. These include the tomato sample positive at screening. **Appendix 3** shows how prevalence and concentration data were included in the risk model.

Table A2. *Quantitative analysis of Salmonella concentration in screen-positive samples*

	MPN pattern	Concentration (MPN/g)*
LETTUCE		
	2 : 1 : 2	27
	3 : 1 : 0	4.9
	0 : 3 : 0	0.94
KALE		
	3 : 0 : 0	2.3
	1 : 0 : 0	0.36
	0 : 0 : 1	0.3
	3 : 2 : 3	29

* The only tomato sample positive at screening was not detected in the MPN assay (<0.3 MPN/g).

7.3. APPENDIX 3: SUPPLEMENTAL INFORMATION ON RA METHODS

This section presents additional methods from the risk assessment on *Salmonella* in EatSafe's key commodities (Section 3). **Figure 1** visualizes the main risk-relevant steps that EatSafe in Ethiopia's key commodities go through from market to consumption. The variables relevant to these steps that were included in the risk assessment model are summarized in **Table A3.**

Table A3. *Parameters included in the risk assessment model*

VARIABLE	DISTRIBUTION	VALUE/PARAMETER	UNIT	SOURCE
Salmonella prevalence in vegetable (Pre)	Beta(s+1, n-s+1)	Lettuce, s=6, n=80 Tomato, s=1, n=128 Kale, s=15, n=120	NA	EatSafe data [1]
Number of Salmonella in one gram of vegetable (Npc) by commodities	Discrete Uniform (resampling from data); each sample: LogNormal(μ, sd)	μ: actual value of positive sample, sd=0.32 MPN/g Lettuce(0.94-4.7) Tomato (<0.3) Kale (0.3-29)	MPN/g	EatSafe data
Status of Salmonella contamination in cut vegetable (S) from market types	Binomial(1, Pre)	Pre for each vegetable	NA	EatSafe data
Temperature when storing raw vegetable in household refrigerator (Tre)	Number	4	°C	Based on common refrigerator settings
Temperature when storing raw vegetable at ambient temperature at household (Tro)	Normal(μ, sd)	Hawassa, Ethiopia: Mean (21.0), sd (6.0)	°C	Timeand date.com
Duration of storage for raw vegetable at household before cooking (Hst)	Discrete Uniform (resampling data)	If stored at room temperature - mean (min-max) Lettuce: 2.3(0-24) Tomato: 13.1(0.2-73) Kale: 3.2(0-24) If stored at refrigerated temperature: Lettuce:4.2(0-25) Tomato: 10.2(0.1-72) Kale: 6.7(0.1-48)	hours	EatSafe data
Salmonella grow rate in food (h0)	Normal(μ, sd)	μ =2.14, sd=0.71	LogCFU/g	(29)

VARIABLE	DISTRIBUTION	VALUE/PARAMETER	UNIT	SOURCE
Probability of Salmonella contamination after washing vegetable in scenario 1 (Pre_{sc1})	Beta(s+1, n-s+1)	s=80, n=100	[0,1]	(30,31)
Probability of Salmonella contamination after washing vegetable in scenario 2 (Pre_{sc2})	Beta(s+1, n-s+1)	s=70, n=100	[0,1]	(30,31)
Probability of Salmonella contamination after washing vegetable in scenario 3 (Pre_{sc3})	Beta(s+1, n-s+1)	s=60, n=50	[0,1]	(30,31)
Probability of Salmonella contamination after washing vegetable in scenario 4 (Pre_{sc4})	Beta(s+1, n-s+1)	s=50, n=100	[0,1]	(30,31)
Reduced Salmonella concentration (MPN/g) after washing vegetable in Scenario 1 (Worse case), 2, 3 and 4 (Best case)	PERT	Sc1=PERT(40, 50, 60) Sc2=PERT(65, 75, 85) Sc3=PERT(85, 90, 95) Sc4=PERT(95, 97.5,100)	% reduction	Assumption
Probability of Salmonella contamination after washing vegetable - All scenarios (Csc)	Binomial(1, Pre_{sc})	Pre_{sc1}, Pre_{sc2}, Pre_{sc3}, Pre_{sc4}	[0,1]	Calculation
Likelihood of each washing scenario	Scenario 1:2:3:4	14:36:36:14	%	Based on Eatsafe data
Probability of eating raw (tomato, lettuce) or cooked (kale) vegetable per meal (Frq) (0<Frq≤1)	Discrete Uniform (resampling data) or PERT	Non-parametric bootstrapping from household data - mean (min-max) Lettuce 0.11(0-1) Tomato 0.14(0-1) Kale: PERT (0, 0.18, 0,34)	times/ day	Eatsafe data
Status of eating raw/under-cooked vegetable in the meal (Seat) - Kale only	Binomial(1, Frq)	Frq: survey data from household (0,1)	[0,1]	EatSafe data

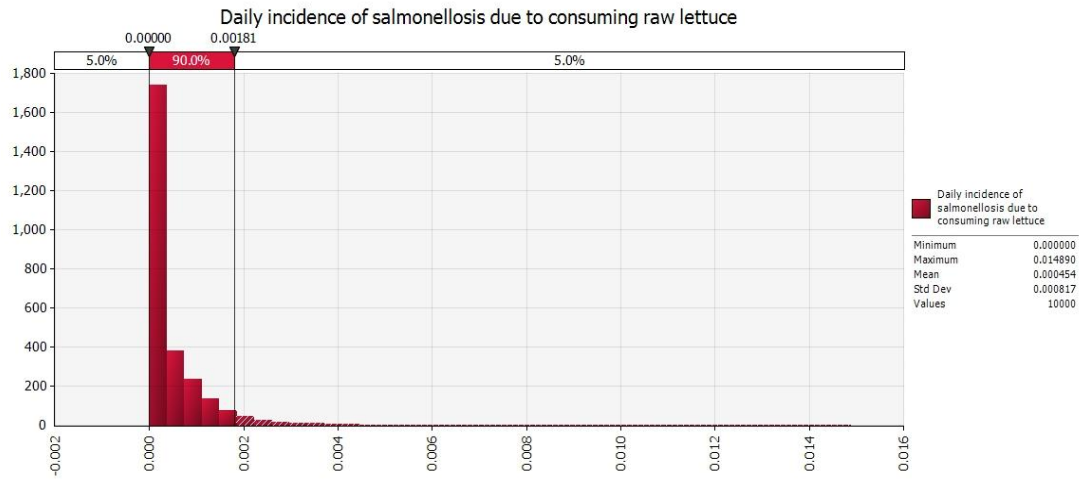
VARIABLE	DISTRIBUTION	VALUE/PARAMETER	UNIT	SOURCE
Quantity of raw/under-cooked vegetable consumed/meal (Qty)	Discrete Uniform (resampling data)	Non-parametric bootstrapping from household data, (min, most likely, max): Lettuce (0, 36, 96) Tomato (5, 22, 45) Kale (15, 55, 150)	g/meal	EatSafe data
Reduction during partial cooking - Kale only	PERT	PERT (0, 0.0025, 0.005)	Concen-tration after cooking	Assumption
Illness probability from dose response model (Ins)	Beta Poisson (α,β)	1-(1+dose/β)^(-α) α =0.00853 β= 3.14	Probability [0,1]	(9)
Probability of illness per day (Din)	Assumes 3 meals per day	1-(1-Ins)^3	Probability [0,1]	Calculation
Probability of illness per year (Ain)	Assumes 365 days per year	1-(1-Din)^365	Probability [0,1]	Calculation

[1] EatSafe data: data collected as part of EatSafe activities presented in this report.

7.4. APPENDIX 4: SUPPLEMENTAL INFORMATION ON RA RESULTS

This section presents additional results from the risk assessment on *Salmonella* in EatSafe's key commodities (Section 4). **Figures A1**, **A3**, and **A5** contains the summary outputs for the risk assessment for each key commodity. **Figures A2**, **A4**, and **A6** illustrate the results of the sensitivity analysis. **Figure A7** illustrates, for lettuce, how risk can change as a result of different degrees of washing, which can reduce prevalence and/or concentration before the produce is consumed raw. These scenarios are hypothetical, but compatible with washing effectiveness reported in the literature. The main estimates presented in **Table 5** include a combination of washing scenarios (not including "no washing") to represent the range of possible practices in the population.

Figure A1. *Daily (top) and annual (bottom) of salmonellosis due to consuming raw lettuce*



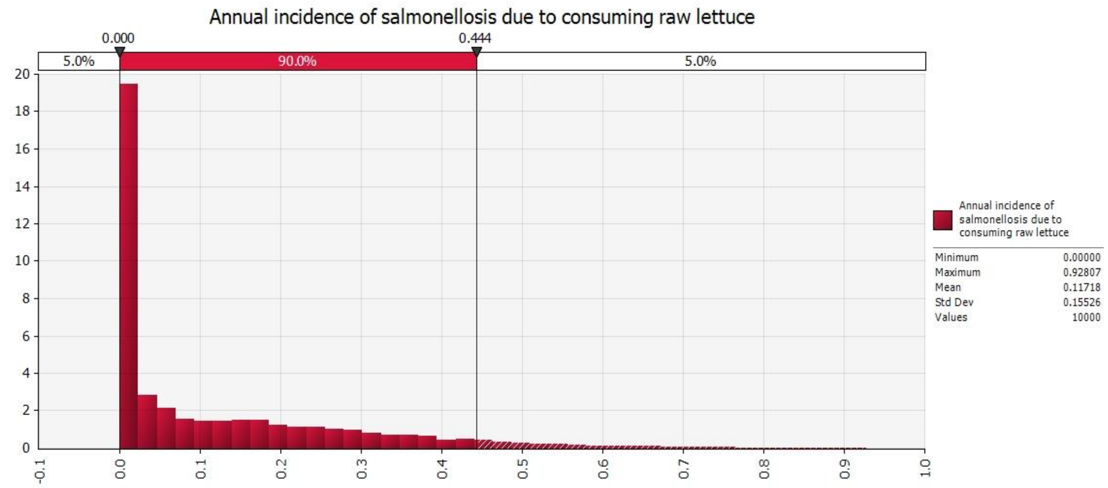


Figure A2. *Sensitivity analysis: factors affecting risk from consuming raw lettuce.*

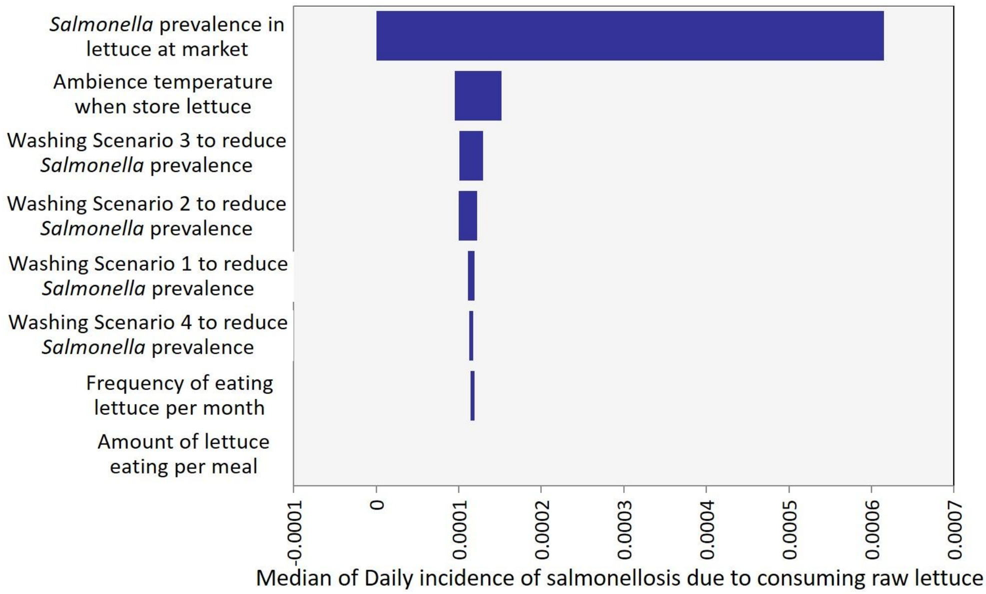
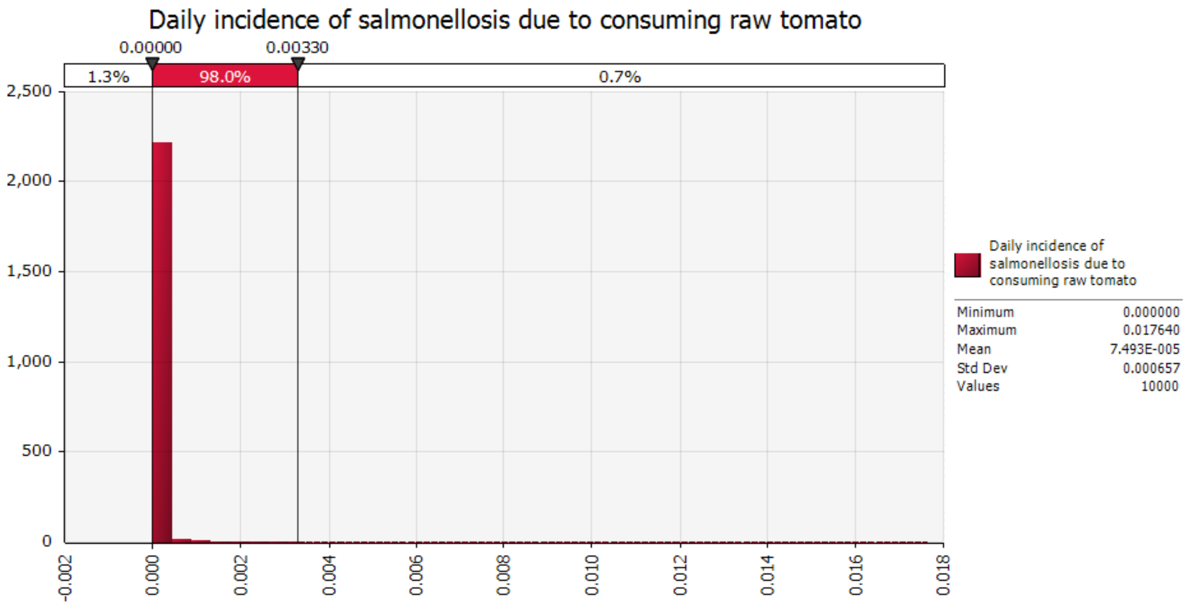


Figure A3. *Daily (top) and annual (bottom) incidence of salmonellosis due to consuming raw tomatoes*



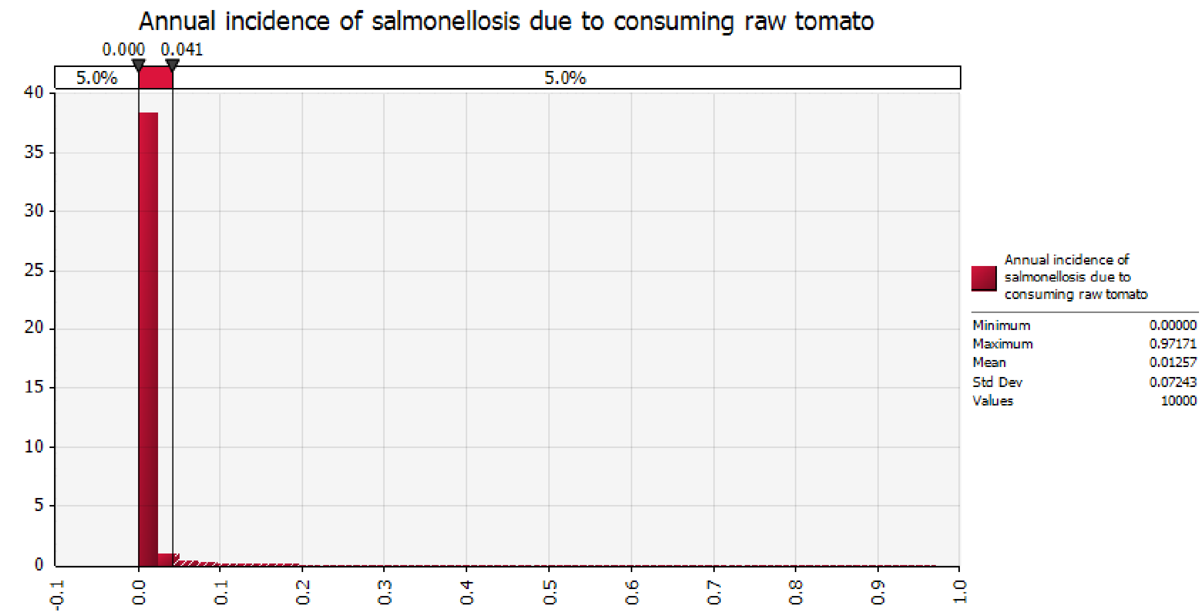


Figure A4. *Sensitivity analysis: factors affecting risk from consuming raw tomatoes*

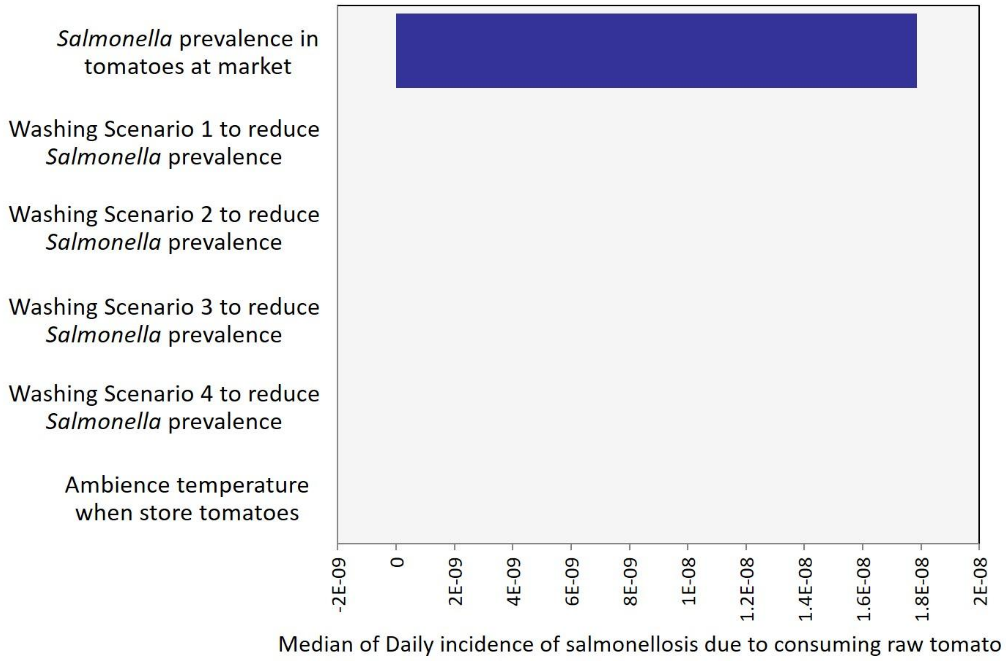
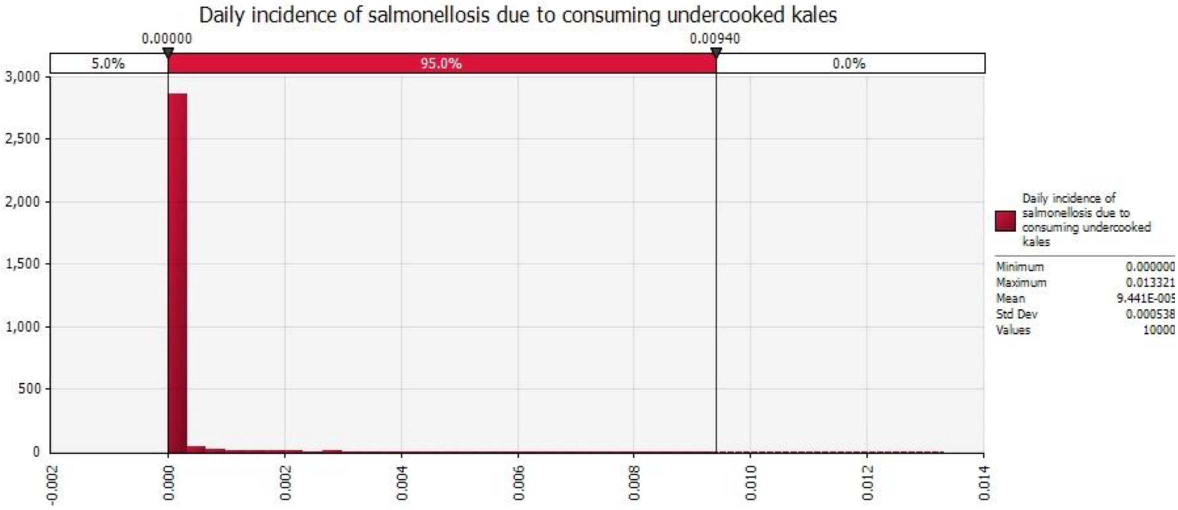


Figure A5. *Daily (top) and annual (bottom) incidence of salmonellosis due to consuming undercooked kale*



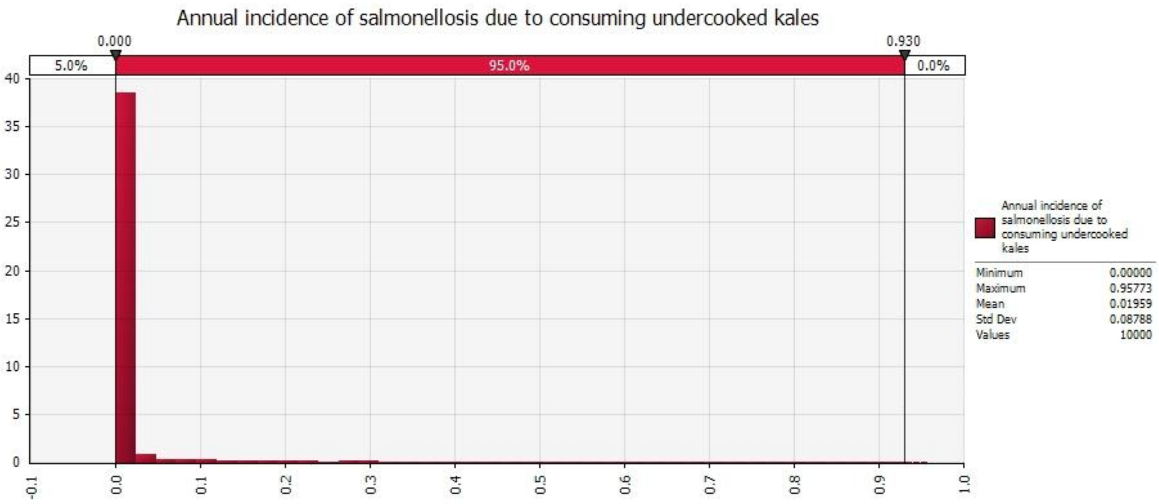


Figure A6. *Sensitivity analysis: factors affecting risk from consuming undercooked kale.*

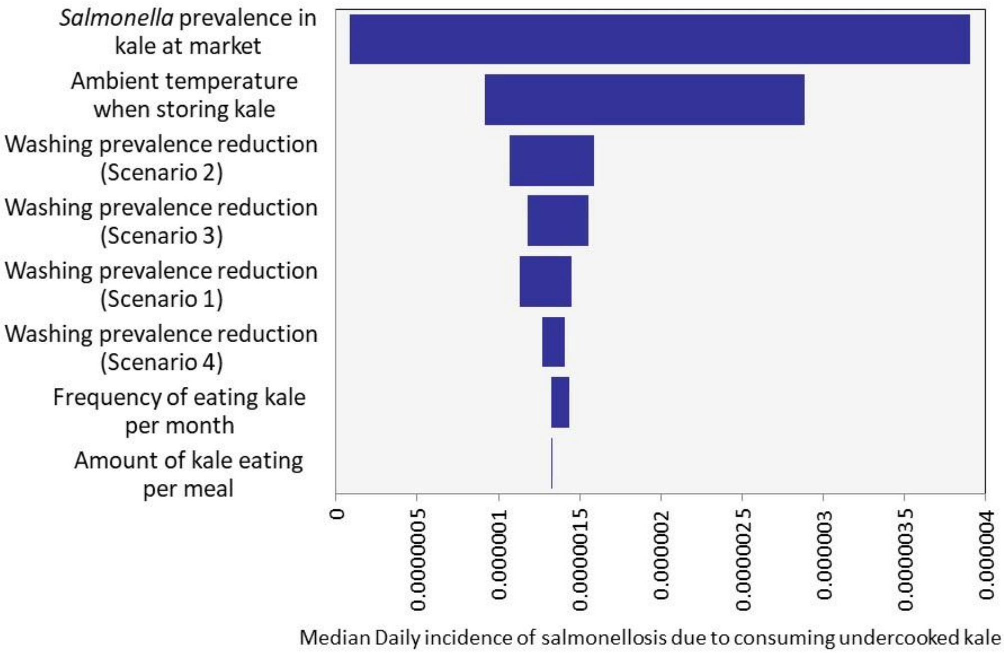


Figure A7. *Scenarios analysis showing the impact of washing effectiveness on risk.*