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APPLICATION OF MICROBIAL RISK ASSESSMENT OF *ESCHERICHIA COLI* IN IRRIGATION WATER ON LETTUCE CROP

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Outline

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Introduction

Foodborne diseases

Food safety has been considered as an important global health issue for a long time now (Harris et al., 2003).

The Centers for Disease Control and Prevention (CDC) defines a “foodborne disease outbreak” as when 2 or more people get the same illness from the same contaminated food or drink (CDC, 2011).

In the recent years in the USA, produce has been a commonly reported source of foodborne outbreaks and surveillance reports suggest that produce may account for a higher proportion of multistate foodborne outbreaks compared with other food categories (Scallan E et al., 2011).

Fresh produce: food safety risk

Consumption of fresh fruits and vegetables is important for a balanced diet and healthy life-style.

- Fresh produce potentially poses an increased food safety risk because they are consumed raw or minimally processed.

Produce and leafy greens contamination routes

Leafy greens can become contaminated with:

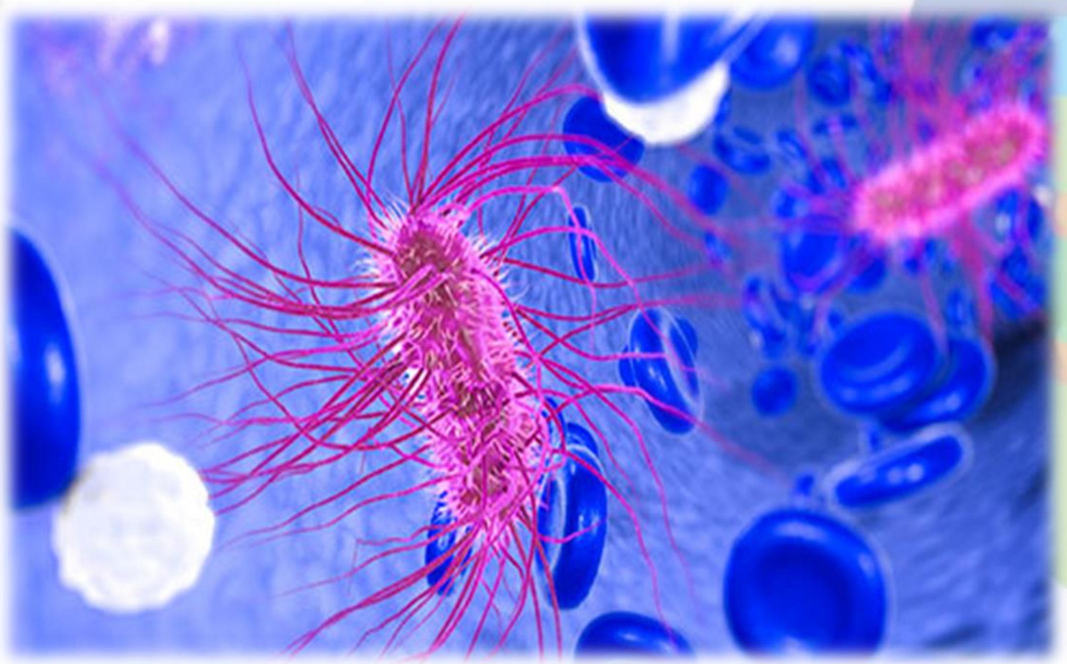
- Viral or bacterial pathogens in the field through:
    - soil,
    - feces,
    - irrigation water,
    - animals, insects
    - manure,
    - biosolids,
    - pesticides, and fertilizers,

- Harvest & postharvest operations (harvesting equipment, transport containers, and dust) food handlers in food service establishments, etc.

Irrigation with contaminated water

Irrigation with wastewater increases the health risk due to the presence of high concentrations of pathogens such as bacteria, viruses, protozoa and helminths (Toze, 2006).

Escherichia coli O157:H7 have been found to be one of the leading causes of the produce-related foodborne outbreaks (Olsen et al., 2000).



E. coli O157:H7 linked to leafy greens

- The 2006 outbreak linked to *E. coli*–contaminated spinach that resulted in 205 confirmed cases and three deaths served as a promoter for research efforts to ensure the safety of leafy greens (CDC, 2006).

- *E. coli* has been also linked to foodborne outbreaks associated with the consumption of lettuce (Hilborn et al., 1999).

- Last year the FDA investigated the multistate outbreak of *E. coli* infections linked to romaine lettuce from Yuma growing region..

  - CDC laboratory testing identified the outbreak of *E. coli* O157:H7 in water samples taken from a canal in the Yuma growing region.
  - Whole Genome Sequencing (WGS) showed that the *E. coli*O157:H7 found in the canal water is related genetically to the *E. coli* O157:H7 from ill people.
  - FDA is continuing to investigate how the *E. coli* bacteria could have entered the water and ways this water could have contaminated romaine lettuce in the region.

E. coli outbreaks investigations in leafy greens by Year

February 13, 2019	Outbreak Investigation of *E. coli* O157:H7 Linked to Romaine Lettuce Grown in CA	Total Illnesses: 62 Hospitalizations: 25 Deaths: 0
November 1, 2018	FDA Investigated Multistate Outbreak of *E. coli* O157:H7 Infections Linked to Romaine Lettuce from Yuma Growing Region	Total Illnesses: 210 Hospitalizations: 96 Deaths: 5
February 28, 2018	FDA Ends Investigation of *E. coli* O157:H7 Outbreak Likely Linked to Leafy Greens	Total Illnesses: 25 Hospitalizations: 0 Deaths: 0
December 11, 2013	Lettuce: FDA Investigation Summary - Multistate Outbreak of *E. coli* O157:H7 Illnesses Linked to Ready-to-Eat Salads	Total Illnesses: 33 Hospitalizations: 0 Deaths: 0
December 10, 2012	FDA Investigates *E. coli* O157:H7 Illnesses Linked to Organic Spinach and Spring Mix Blend	Total Illnesses: 33 Hospitalizations: 0 Deaths: 0

Quantitative microbial risk assessment (QMRA)

Water from surface sources should not pose a risk of infection from waterborne pathogens **greater than 1:10,000 per year** according to the U.S. Environmental Protection Agency (EPA, 1989).

This value has been used to evaluate risk associated with the quality of irrigation water (Petterson et al 2001).

Quantitative Microbial Risk Assessment (QMRA) is an approach that allows the quantitative expression of risk in terms of infection, illness, or mortality from microbial pathogens.

It is a key factor in all decision making for determining the urgency of problems and the allocation of resources to reduce risks. (Haas et al., 1999).

Materials and Methods

The present study focuses on the risk to consumers of lettuce irrigated with *E. coli*-contaminated water

Risks for farmers and workers are not in the scope of this work.

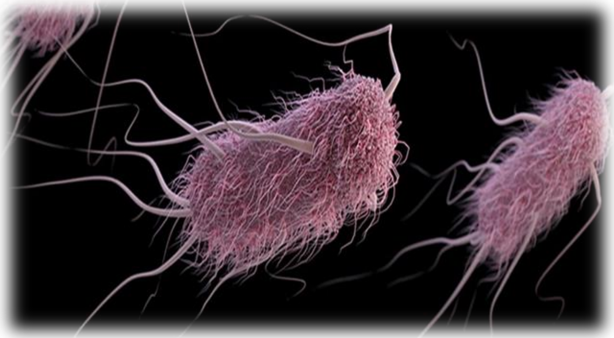
Decay of microorganisms during storage and processing were not considered either.

Standard QMRA techniques (Haas et al. 1999) were used to estimate risks of infection from model pathogen-ingestion scenarios.

Quantitative Microbial Risk Assessment (QMRA) paradigm

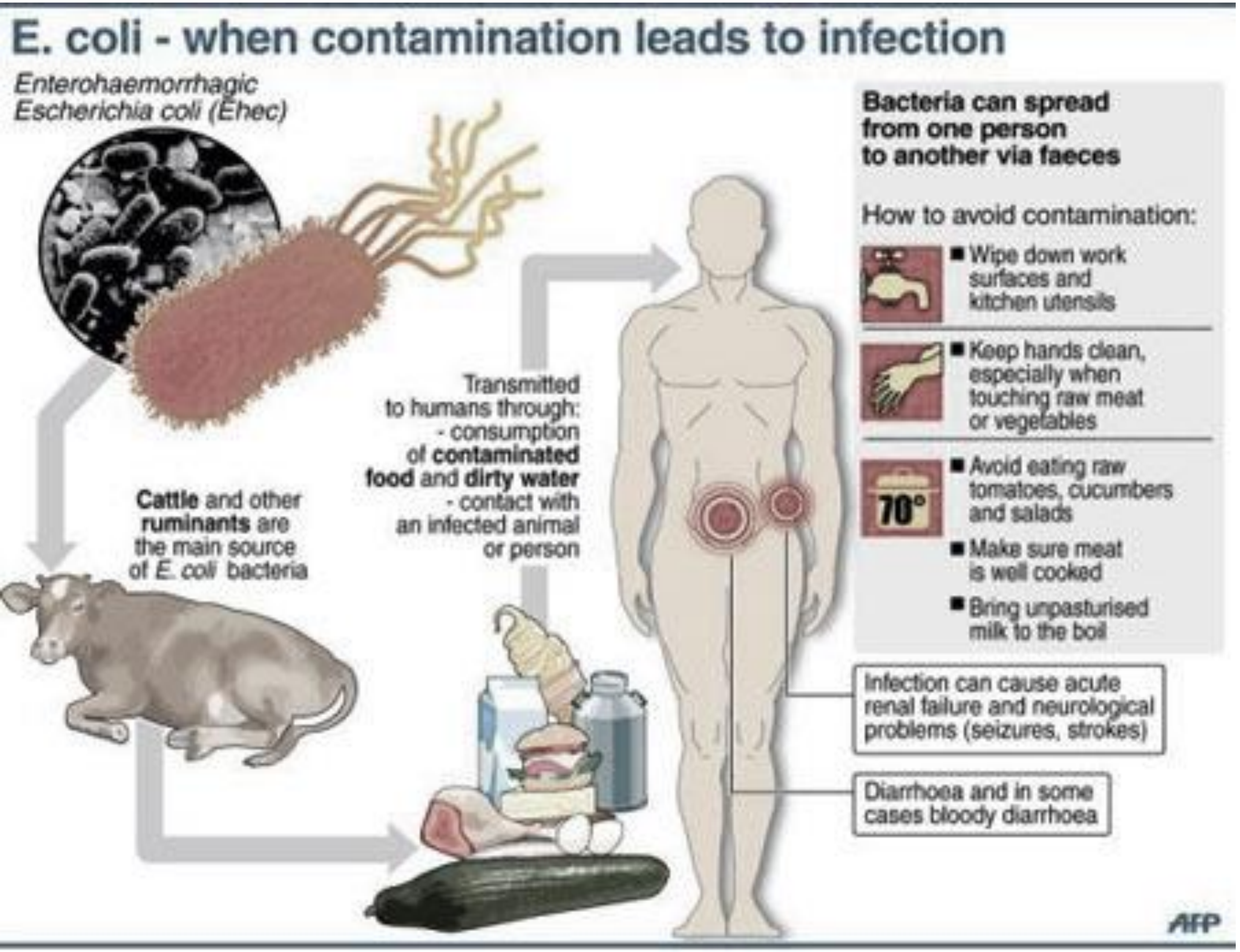
The risk assessment paradigm involves four steps:

1. Hazard identification
2. Exposure assessment
3. Dose response
4. Risk characterization



1. Hazard Identification: *E. coli*

- *Escherichia coli* (*E. coli*) is a Gram-negative, rod-shaped, facultative anaerobic bacterium.



https://healthlifemedia.com/healthy/food-poisoning-what-are-e-coli-breakouts/

Healthy cattle are a reservoir of *E. coli* O157:H7.

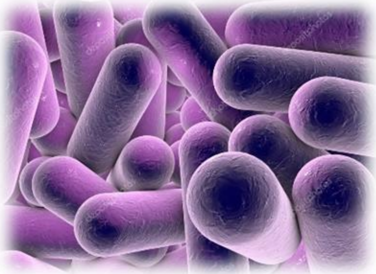
Common symptoms include:

- Severe bloody diarrhea
- Abdominal cramps
- Little or no fever

Complications

- Hemolytic Uremic Syndrome develops in about 5% of reported *E. coli* O157:H7 cases, most frequently in young children or the elderly.

2. Exposure assessment: Transfer of *E. coli* O157:H7 from water to Lettuce Plants



Transfer of low numbers of *E. coli* O157:H7 from water to growing lettuce plants was reviewed.

Mootian et al. (2009) determined the transfer of *Escherichia coli* O157:H7 from water to growing green ice leaf lettuce (*Lactuca sativa* L.).

Lettuce plants, young (12 days of age at exposure to contaminated water) or mature (30 days of age at exposure to contaminated water), were irrigated with water containing 10^1, 10^2, 10^3, or 10^4 CFU *E. coli* O157:H7 per ml.

Harvested plants were processed to determine whether *E. coli* O157:H7 was associated with the leafy surfaces or within internal locations.

Young plants (12 days) were harvested at 1, 10, 20, and 30 days postexposure.

Mature plants (30 days) were harvested at 1, and 15 days postexposure.

The outer leaves were allowed to touch the soil, to closely model in-field conditions. (Tables 1 and 2).

2. Exposure assessment: Transfer of *E. coli* O157:H7 from water to Lettuce Plants

Table 1. Contamination of 12-day-old lettuce plants, after exposure to *E. coli*-contaminated irrigation water (Mootian et al., 2009).

Location	Day(s) after exposure	Concentration of *E. coli* (CFU/ml) in contaminated irrigation water			
		10^1	10^2	10^3	10^4
Leaf surface	1	0/6	0/6	1/6	3/6
	10	3/6	5/6	4/6	3/6
	20	0/6	1/6	0/6	0/6
	30	6/6	1/6	0/6	0/6
Internal	1	0	0/6	0/6	0/6
	10	1/6	1/6	0/6	0/6
	20	0/6	4/6	0/6	0/6
	30	0/6	0/6	0/6	0/6

*Samples tested positive. Values are number of positive plants/number of plants tested.

2. Exposure assessment: Transfer of E. coli O157:H7 from water to Lettuce Plants

Table 2. Contamination of 30-day-old lettuce plants, after exposure to *E. coli*-contaminated irrigation water (Mootian et al., 2009).

Location	Day(s) after exposure	Concentration of *E. coli* (CFU/ml) in contaminated irrigation water			
		10^1	10^2	10^3	10^4
Leaf surface	1	1/6	3/6	5/6	3/6
	15	5/6	6/6	3/6	1/6
Internal	1	0/6	2/6	0/6	0/6
	15	1/6	0/6	1/6	1/6

*Samples tested positive. Values are number of positive plants/number of plants tested.

3. Dose–response assessment

- Quantitating the adverse effects arising from exposure to *E. coli* based on the degree of exposure. This assessment is expressed mathematically as a plot showing the probability of infection to increasing doses (consumption *E. coli*).

- The steps taken to quantify the risk of microbial infection and the assumptions used are summarized in Table 3 with surface-irrigated 30-day-old lettuce contaminated by 10^4 CFU/ml of *E. coli* level as an example.

Table 3. Risk assessment steps and assumptions used to calculate the risk of infection of *E. coli* in lettuce.

Risk Assessment Steps	Assumptions
Calculation of the annual risk of infection	1:10,000
Amount of fresh produce consumed per capita/year	4416.5 g
Contamination rate of 30-day-old lettuce	3/6 samples tested positive
Concentration of E. coli in irrigation water to achieve 1:10,00 risk of infection	10^4 CFU/ml

4. Dose–response assessment

- The Beta-Poisson model can be used to quantify the risk of microbial ingestion. The model gives the following equation (Haas et al., 1999):

$$P_i = 1 - \left[1 + (d/N_{50})(2^{1/\alpha} - 1)\right]^{-\alpha} \qquad (1)$$

P_i is the risk of infection by ingesting pathogens in drinking water, d is the dose of microorganisms ingested, N_{50} is the microbial dose resulting in 50% infection, and α is a slope parameter.

- The best-fit dose-response parameters N_{50}, and α for ingestion of *E. coli* (DuPont et al.,1971) are reported in Table 4.

Table 4. Best fit dose-response parameters (DuPont et al.,1971).

Organism	Beta-Poisson model	
	N_{50}	α
Escherichia coli	2.11 x 10^6	0.155

4. Dose–response assessment

- The annual acceptable risk of infection (P_A) was determined with the following equation (Haas et al., 1999):

$$P_A = 1 - (1 - P_i)^{365} \qquad (2)$$

where P_A is the annual risk, which was assumed to be the U.S. Environmental Protection Agency (EPA) benchmark annual acceptable risk of infection of 1:10,000 for drinking water.

- The dose (d) was determined with the following equation:

$$d = (L * P_p * N_{pc})/10{,}000 \qquad (3)$$

where L is the level of *E. coli* to achieve the levels 10^1, 10^2, 10^3, or 10^4 CFU/ml in irrigation water, P_p is the number of positive plants/number of plants tested, and N_{pc} the amount of produce items consumed which was determined by adjusted annual per capita consumption of lettuce of 4,416.5 g (Alum, A. 2001; Stine et al., 2005).

Results and Discussion

4. Risk characterization

- The worst-case scenario was found when produce is harvested the day after the last irrigation and maximum contamination level is used, for mature lettuce plants (Table 6).
- For young plants the worst-case scenario was found when produce is harvested one day and ten days after the last irrigation and maximum contamination level is used (Table 5).
- Both results indicate that the concentrations needed to achieve an annual 1:10,000 risk of infection were as low as 10^4 CFU/100 ml of *E. coli* in irrigation water.
- If the EPA guideline is applied to produce that is consumed raw, then the irrigation water should not contain any *E. coli* above concentrations of 10^3 CFU/ml at least.

4. Risk characterization

$$P_A = 1 - (1 - P_i)^{365}$$

Table 5. Calculated annual risk of infection from consumption of 12-day-old lettuce, with the four *E. coli* concentrations analyzed.

Location	Day(s) after exposure	Concentration of *E. coli* (CFU/ml) in contaminated irrigation water			
		10^1	10^2	10^3	10^4
Leaf surface	1	0	0	0.2	1
	10	0	0.1	0.5	1
	20	0	0	0	0
	30	0	0	0	0
Internal	1	0	0	0	0
	10	0	0	0	0
	20	0	0.1	0	0
	30	0	0	0	0

4. Risk characterization

$$P_A = 1 - (1 - P_i)^{365}$$

Table 6. Calculated annual risk of infection from consumption of 30-day-old lettuce, with four different E. coli concentrations.

Location	Day(s) after exposure	Concentration of E. coli (CFU/ml) in contaminated irrigation water			
		10^1	10^2	10^3	10^4
Leaf surface	1	0	0	0.6	1
	15	0	0.1	0.4	0.8
Internal	1	0	0	0	0.8
	15	0	0	0.2	0

4. Risk characterization

$$d = (L * P_p * N_{pc})/10{,}000$$

Table 7. Calculated *E. coli* dose (CFU/g) consumed from 12-day-old lettuces irrigated with different *E. coli* concentrations.

Location	Day(s) after exposure	Concentration of *E. coli* (CFU/ml) in contaminated irrigation water			
		10^1	10^2	10^3	10^4
Leaf surface	1	0	0	73.61	2208.25
	10	2.21	36.80	294.43	2208.25
	20	0	7.36	0	0
	30	4.42	7.36	0	0
Internal	1	0	0	0	0
	10	0.74	7.36	0	0
	20	0	29.44	0	0
	30	0	0	0	0

4. Risk characterization

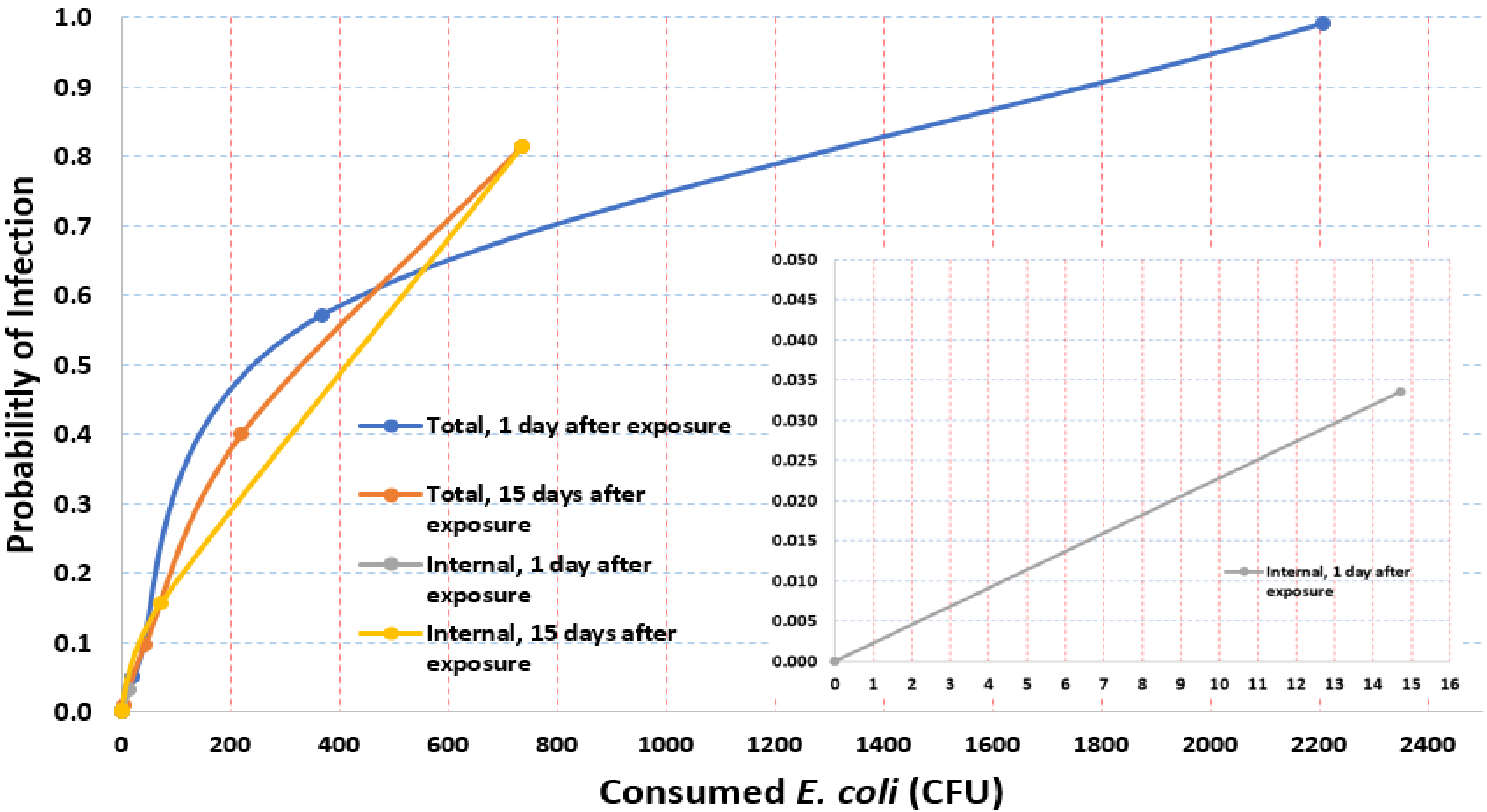
$$d = (L * P_p * N_{pc})/10{,}000$$

Table 8. Calculated *E. coli* dose (CFU/g) consumed from 30-day-old lettuces irrigated with different *E. coli* concentrations.

Location	Day(s) after exposure	Concentration of E. coli (CFU/ml) in contaminated irrigation water			
		10^1	10^2	10^3	10^4
Leaf surface	1	0.74	22.08	368.04	2208.25
	15	3.68	44.17	220.83	736.08
Internal	1	0	14.72	0	0
	15	0.74	0	73.61	0

4. Risk characterization

Figure 1. Dose-Response Model for consumption of *E. coli* (CFU).



Conclusions

QMRA is an important tool for assessing the risk involved in irrigation water, both in terms of the formulation of the risk analysis problem and in predicting the probability of infection in different scenarios.

Results suggest that lettuce exposed to and grown in the presence of low numbers of *E. coli* O157:H7 may become contaminated and thus present a human health risk.

Concentrations needed to achieve an annual 1:10,000 risk of infection were as low as 10^4 CFU/100 ml of *E. coli* in irrigation water.

Contamination of lettuce close to harvest may increase the risk of the pathogen being present on the crop.

If the EPA guideline is applied to produce that is consumed raw, then the irrigation water should not contain any *E. coli* above concentrations of 10^3 CFU/ml at least.

Future efforts must center on avoiding human pathogen contamination of produce.

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