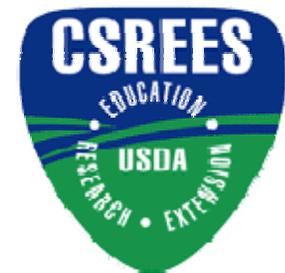
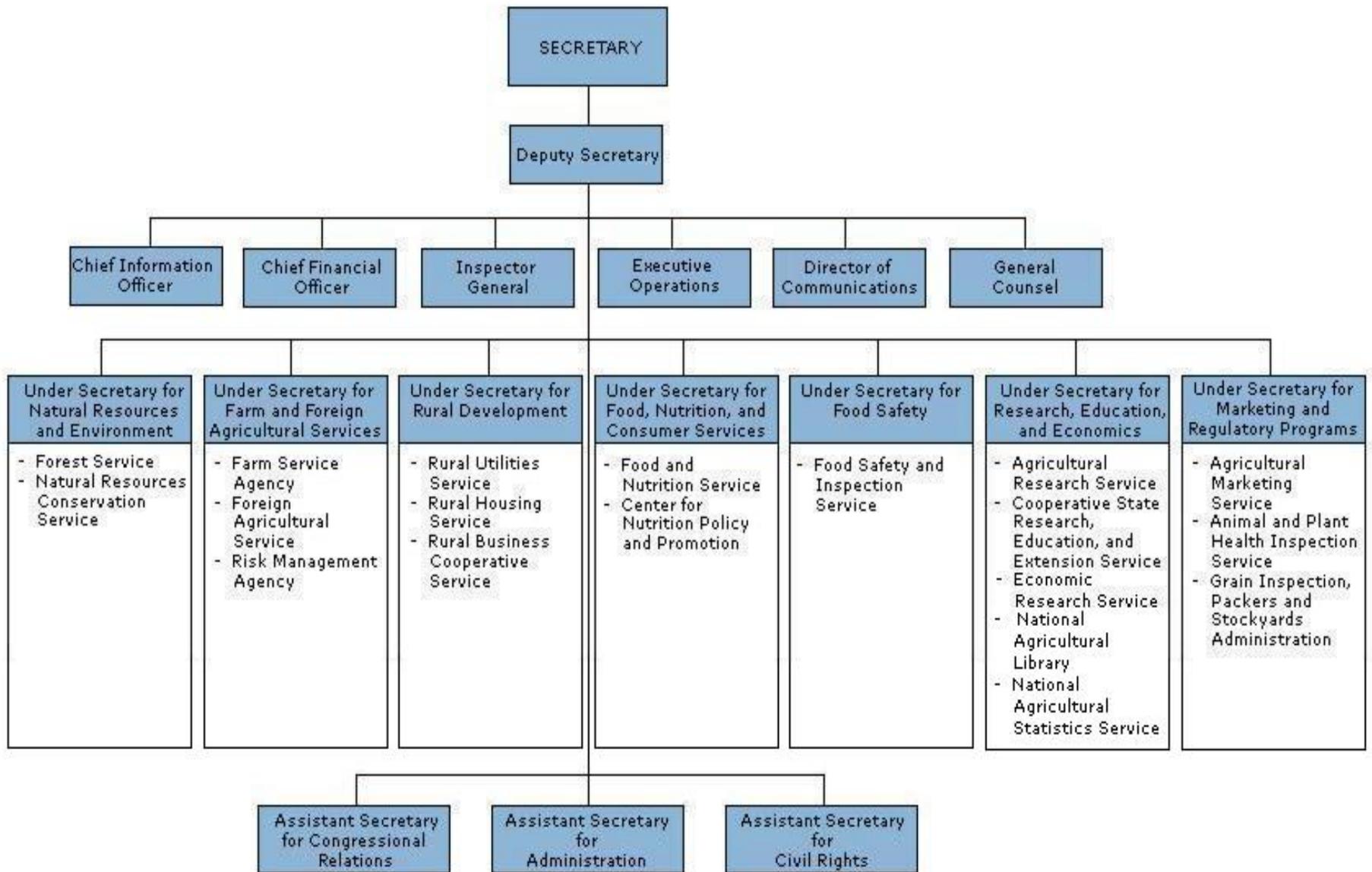


Fresh and Fresh-Cut Produce Food Safety Research

USDA-Cooperative State Education and Extension Service

**Dr. Mary E. Torrence
National Program Leader
Food Safety and Epidemiology**

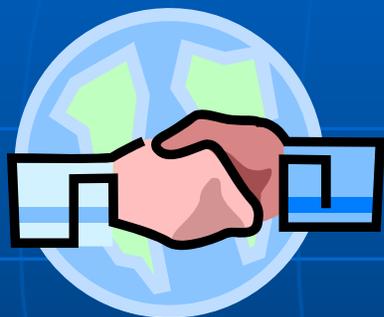




REE Mission

- USDA's leader for the "discovery of knowledge spanning the biological, physical, and social sciences, and involving agricultural research, economic analysis, statistics, outreach, and higher education". REE's scientific research, economic and statistical analysis, and education programs result in sound information and data used by USDA in making policy decisions to benefit all citizens of the Nation.
- **Four REE agencies:**
 - *Agricultural Research Service (including the National Agriculture Library),*
 - *Economic Research Service*
 - *National Agricultural Statistics Service*
 - *Cooperative State Research and Extension Service*

CSREES



- External research arm of USDA
- Provides a partnership of research, education, and extension with land-grant university system to fulfill USDA missions
- National Research Initiative (NRI) is the major competitive grants program (\$180 million)
- Other grants include special research grants and integrated programs (section 406)

Other CSREES Governmental Partners

In various ways food safety team cooperates with:

- Other USDA agencies, e.g. ARS, FSIS, ERS
- Other federal entities, e.g. CDC, NIH, FDA, DHS
- States and Counties, e.g. agriculture, public health, extension



CSREES Non-government Partners

- Industry- e.g. commodity groups, drug companies
- Professional organizations, e.g. AVMA, IFT, NASULGC, ASM, IAFP, IFT, IFIC
- Consumers
- Academia- e.g. multi-state regional committees



Major FS Granting Programs

- ***32.0 Ensuring Food Safety (NRI)***
 - 2007, \$1.8 million for produce (35%)
 - Total budget is \$4.7 million
- ***32.1 Epidemiologic Approaches for Food Safety (NRI)***
 - 2007, \$470,000 (13%)
 - 2006, \$1.2 million (35%)
 - Total budget is \$3.9 million

32. 0 Ensuring FS areas

- Enteric viruses related to vegetables
- Interaction of microbes with protozoa in fresh produce
- Detection and validation methods
- Impact of irrigation water quality
- Potential role of composted manure

Epidemiologic Approaches for FS

- Epidemiology and ecology of *E. coli* and produce in Salinas Valley (other areas)
- Earlier studies on produce, packing sheds, and processing practices in Texas and also in Mexico

Major FS Granting Programs

- **Integrated Food Safety Program (NIFSI) (Section 406 program)**
 - 2007- 2 major grants awarded for special emphasis areas, \$2.5 million each \$5 million (35%)
 - Total budget is \$14 million

NIFSI Areas

- **Multi-disciplinary approach to enhance adoption of vegetable safety behavior from farm to table**
- **A systems approach related to *E. coli* and leafy greens**

Food Safety Cap

- Consortium of over 17 universities, 50 researchers (funded in 2004, \$5 million over 4 years)
- To provide a multi-disciplinary approach for food safety issues



FSRRN

Food Safety Research & Response Network

17 Universities
50+ Food Safety Researchers
and Support Specialists

Cornell Univ.

Iowa State Univ.

McMasters Univ.

Mississippi State Univ.

N. Dakota State Univ.

The Ohio State Univ.

Tuskegee Univ.

Univ. of Arizona

Univ. of Calif. Davis

Univ. of Calif. Berkley

Univ. of Florida

Univ. of Illinois

Univ. of Kentucky

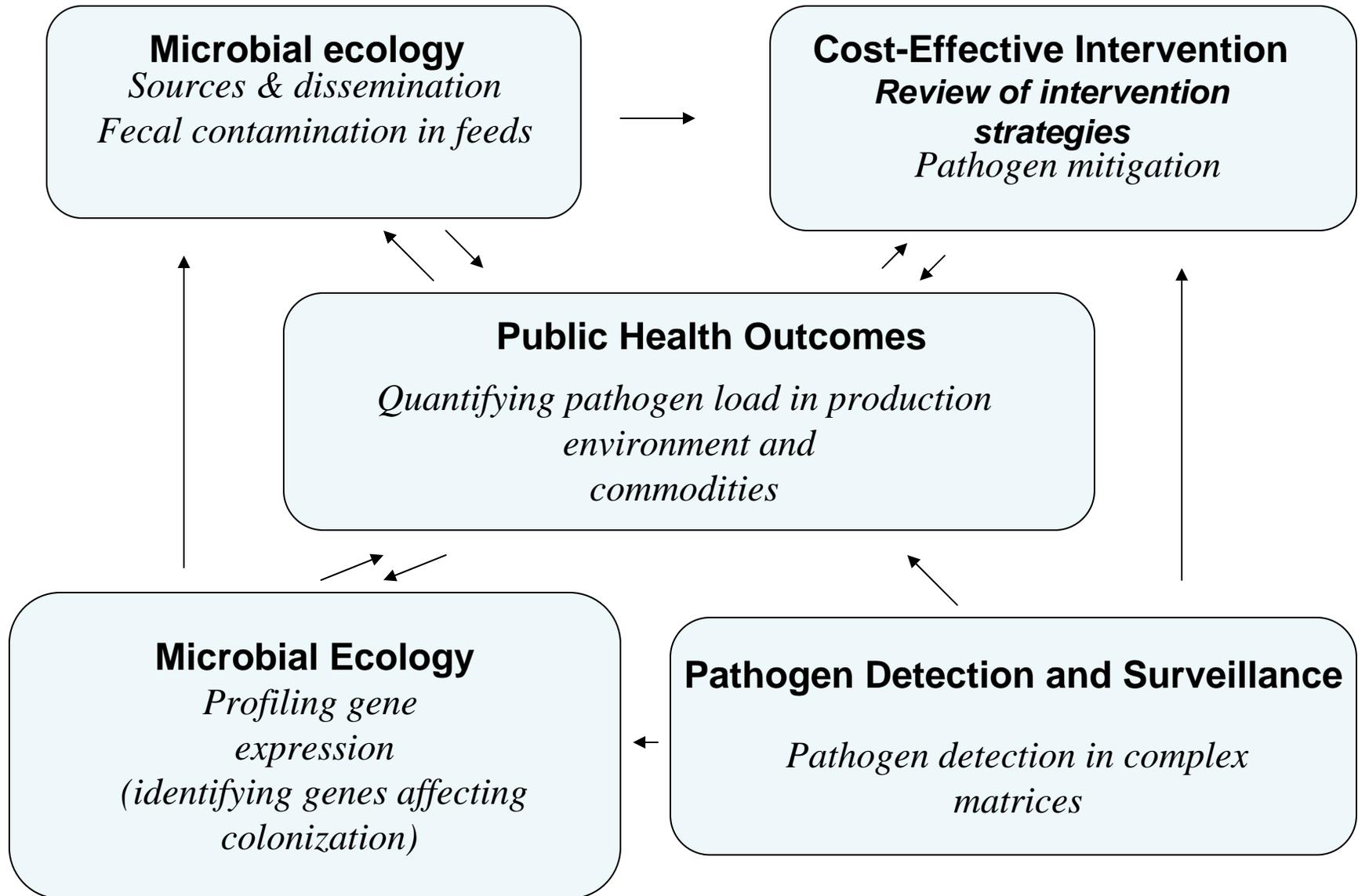
Univ. of Minnesota

Univ. of Montreal

Washington State Univ.

West Texas A&M Univ.

Interactions between project themes





The FSRRN tackles pre-harvest food safety issues that are too broad to be tackled by a single investigator.

The Network's Research Response Team can be mobilized to address specific issues identified by agricultural commodities.

Network investigators are working with the tomato industry in three states to improve production practices



Potential Impacts

- Specific response to emerging issues and to other agency needs:
 - CDC, FDA- Tomato and *Salmonella* contamination
 - Field surveys in Florida and California

Related Programs

- **Water and Watershed Program (NRI)**
 - One focus is the source, fate, and transport of pathogens in soil and water

Examples of Current Research

- **“Clean Greens Study”** funded in early 2000 to look at epidemiology and production practices of produce in Texas, and Mexico
- **Detection methods in fresh and fresh cut produce**

Table 2. Comparison of the levels of various microorganisms of domestic and imported herbs within packing sheds

Range (Mean) Log CFU/g

Produce	Bin	Wash	Rinse	Box
Imported n = 165				
APC	5.95-6.09 (6.03) ^{a*A**}	NA	NA	5.68-7.50 (6.64) ^{bH}
Coliforms	1.60-2.29 (1.97) ^{cC}	NA	NA	0.70-4.32 (1.75) ^{cI}
<i>E. coli</i>	0.70-0.70 (0.70) ^{dD}	NA	NA	0.70-1.93 (0.84) ^{eK}
Enterococci	2.13-2.40 (2.25) ^{fF}	NA	NA	0.70-4.04 (2.26) ^{fM}
Domestic n = 57				
APC	4.61-7.48 (6.44) ^{gB}	6.12-7.43 (6.87) ^g	4.19-7.85 (6.59) ^g	4.42-7.71 (6.50) ^{gH}
Coliforms	0.70-4.48 (2.56) ^{hC}	0.70-4.11 (2.22) ^h	0.70-4.22 (2.51) ^h	0.70-4.37 (2.54) ^{hJ}
<i>E. coli</i>	0.70-3.79 (1.26) ^{iE}	0.70-4.01 (1.31) ⁱ	0.70-3.85 (1.20) ⁱ	0.70-3.19 (1.27) ^{iL}
Enterococci	0.70-5.29 (3.05) ^{jG}	2.30-4.74 (3.71) ^j	0.70-5.37 (3.13) ^j	0.70-5.42 (3.09) ^{jN}

* Different lower case superscripts denote statistically significant differences ($p < 0.05$) between sample locations e.g. “Wash” vs. “Rinse”

** Different upper case superscripts denote statistically significant differences ($p < 0.05$) between imported vs. domestic produce at each sample location

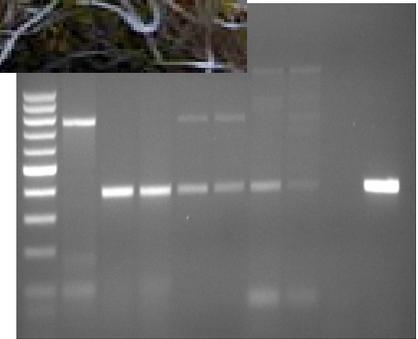
Table 3. Comparison of the levels of various microorganisms of domestic and imported cabbage within packing sheds

Produce	Range (Mean) Log CFU/g		
	Bin	Conveyor Belt	Box
Imported n = 43			
APC	NA	6.42-6.81 (6.63) ^{a* A**}	5.30-7.46 (6.33) ^{aF}
Coliforms	NA	1.95-3.20 (2.42) ^{bB}	0.70-3.48 (1.82) ^{bH}
<i>E. coli</i>	NA	0.70-0.70 (0.70) ^{cC}	0.70-3.23 (0.86) ^{dI}
Enterococci	NA	3.71-4.23 (4.00) ^{eE}	0.70-4.43 (3.06) ^{eJ}
Domestic n = 66			
APC	6.08 (5.38-6.46) ^f	3.95-6.36 (5.61) ^{fA}	4.33-6.40 (5.80) ^{fG}
Coliforms	1.70-3.24 (2.53) ^g	0.70-2.28 (1.92) ^{ghB}	0.70-3.42 (1.43) ^{hH}
<i>E. coli</i>	0.70-2.81 (2.10) ^j	0.70-2.48 (1.31) ^{jkD}	0.70-3.53 (0.96) ^{klI}
Enterococci	2.48-4.58 (4.10) ^m	2.57-4.58 (4.02) ^{mE}	1.00-4.45 (3.07) ^{nJ}

* Different lower case superscripts denote statistically significant differences ($p < 0.05$) between sample locations e.g. “Wash” vs. “Rinse”

** Different upper case superscripts denote statistically significant differences ($p < 0.05$) between imported vs. domestic produce at each sample location

The Direct Detection of *Salmonella*
and *Escherichia coli* O157:H7 from
Raw Alfalfa Sprouts and Spent
Irrigation Water by Use of Polymerase
Chain Reaction

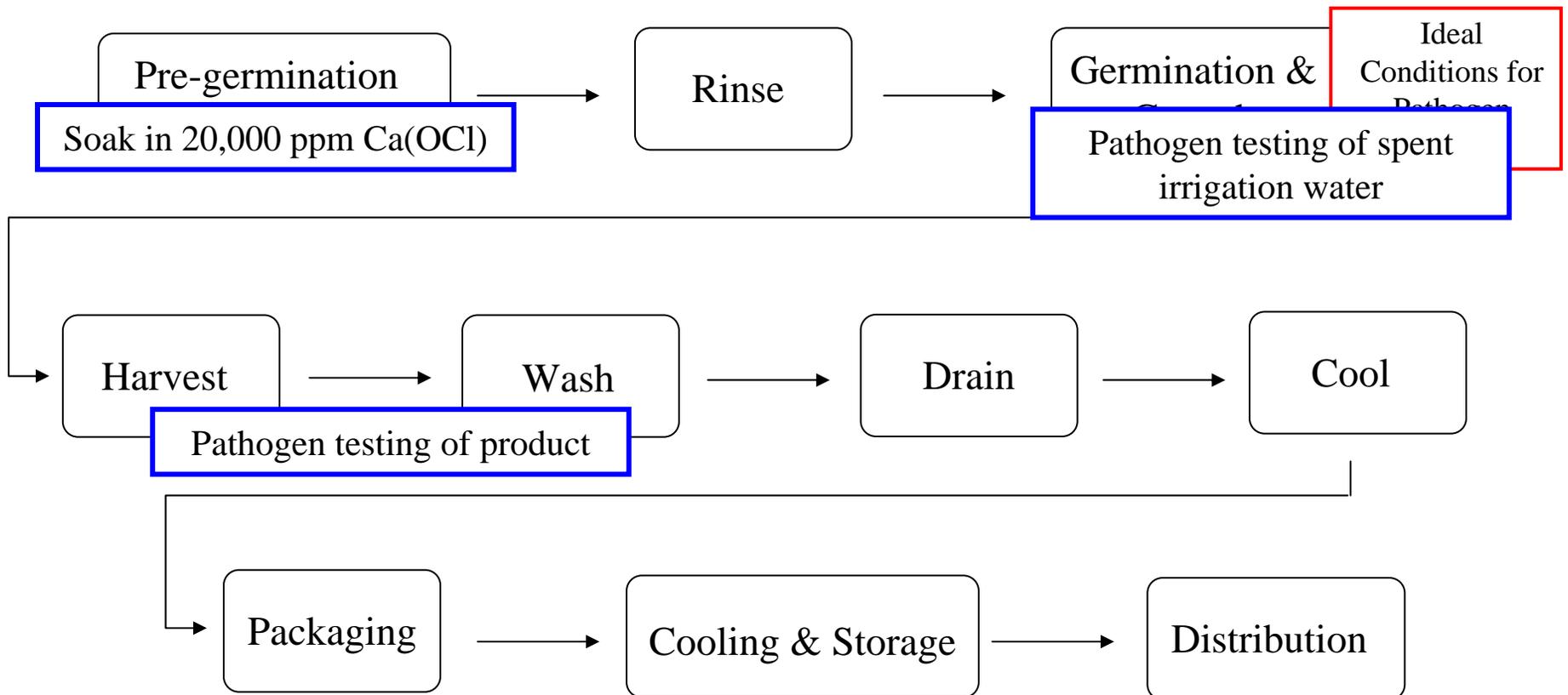


Lynette Johnston, Driss Elhanafi, MaryAnne Drake, and Lee-Ann Jaykus

North Carolina State University, Raleigh, NC



Sprouting Process





Microbiological Testing

- Each Production Lot at or After 48 Hours from Start of Process
 - *Salmonella* and *E. coli* O157:H7
 - Spent irrigation water
 - 100 ml
 - Sprout product
 - 25 g
 - Screening and Confirmation by Cultural, Immunological, or Molecular Methods
 - Requires overnight enrichment
- Screening: 3 days into process
Confirmation: 7 to 10 days into process



Pathogen Detection Methods

- Cultural Methods
 - Lengthy cultural enrichment steps required
 - May fail to detect low levels of pathogens and/or sporadic contamination
 - Pathogenic organisms must be cultured on site
 - ELISA
 - DNA Hybridization
 - PCR
- Detection limits have remained high (10^3 - 10^5 CFU/ml)
 - Continue to require enrichment steps
 - Food components may inhibit enzymatic reactions
 - Small amplification volumes (representation)



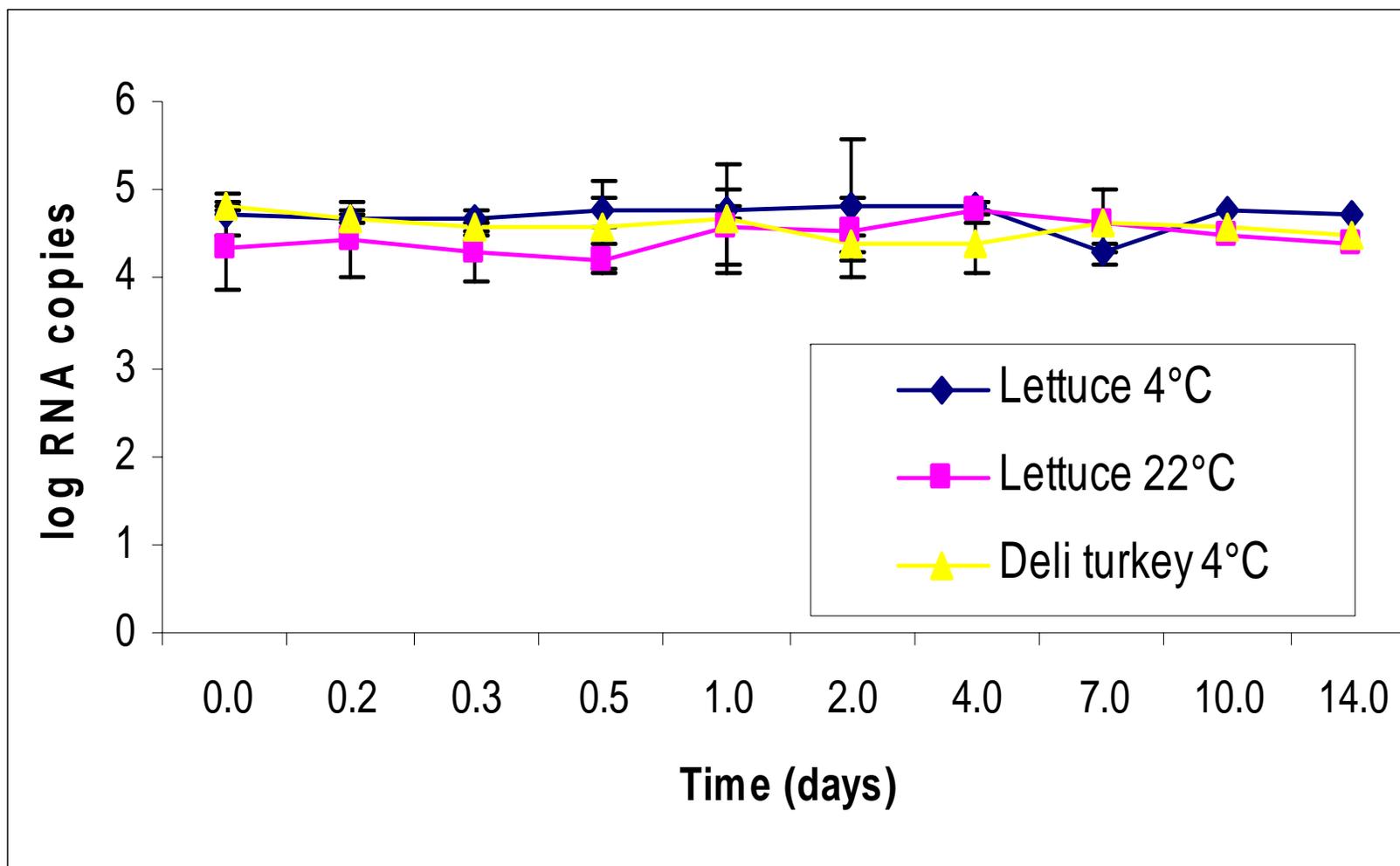
Improvement of Rapid Detection Methods

- Bacterial Separation and Concentration
 - Separation of bacteria from food particles
 - Reduced matrix-associated inhibition
 - Concentration of bacteria
 - Increased sample representation (reducing volume)
 - Increased target cell numbers
 - Increase sensitivity
 - Improved signal-to-noise ratio
- Non-specific and Inexpensive
- Reduced Detection Time



Conclusions

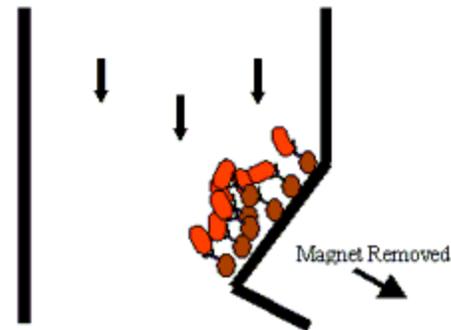
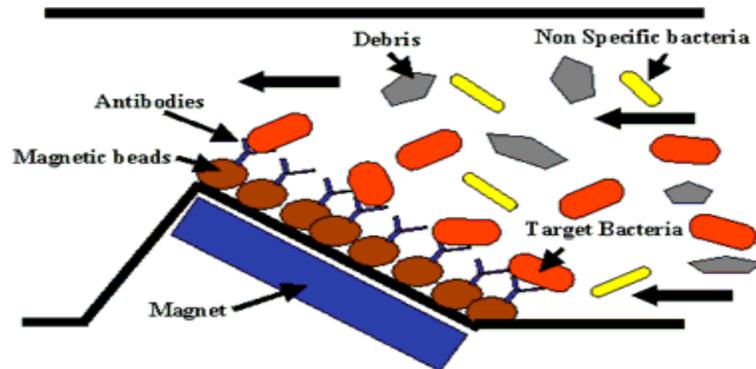
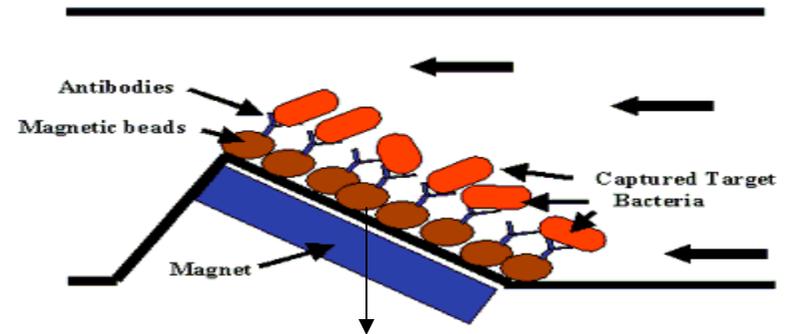
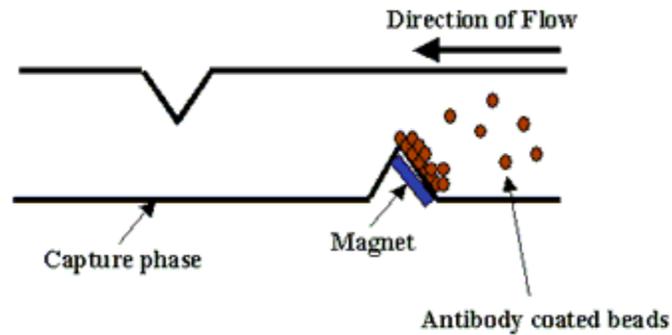
- Detection of *Salmonella* and *E. coli* O157:H7 at low levels
 - Alfalfa sprouts: 10^1 CFU/g (10^2 CFU/25 g)
 - Spent irrigation water: 10^{-1} CFU/ml (10^1 CFU/400 ml)
- Concentration of bacteria
 - Elimination of enrichment step
 - Confirmation of pathogens from sprouts/spent irrigation water within 48 hours
 - Increased sample size for testing (representation)
 - 400 mls versus 100 mls
 - Non-specific assay
 - Applicable to new detection technologies



Persistence of HAV (in days) on foods (lettuce at 4°C, lettuce at 22°C, and deli-turkey at 4°C) as evaluated by quantitative real-time RT-PCR.

Pathatrix System--Matrix BioSciences

Device intended to concentrate pathogens from large sample sizes using combined magnetic capture and slow but continuous sample pumping



How we identify funding priorities

- Emerging issues, current literature
- Stakeholder input (includes government, industry, academia, professional groups, public meetings)
- National priorities

Impacts

- Research on-going when produce outbreak occurred (CA)
- Ability to do field work on farm to identify risk factors and to advise on interventions (tomatoes and *Salmonella* sp)
- Only program in US that funds epidemiologic research – needed for data gaps, and to understand problems in field
- Can provide needed education and extension programs

Epidemiology

- Provides the expertise for GIS
- Sampling methodologies, mathematical modeling and risk
- Population based, longitudinal
- Risk factors
- Outcome measurement/measurement of interventions/preventions
- Understanding environmental influence

CSREES and ARS Collaborations

- Ecology and epidemiology of *E. coli* 0157:H7 in fresh produce production regions of Salinas, CA (in Central Valley)
- White paper for emerging issues and needs for future budgets
- Briefings for Congress, other stakeholder meetings
- Coordination of research plans, expertise

Specific Examples of Coordination around Produce

- Numerous inter-agency meetings, telephone conferences (included updates on current activities and short and long-term plans)
- 3 National Meetings in the last 8 months (research on *E. coli* in lettuce, 2 on tomatoes)
- Project with FDA and DHS on detection and validation of methods in complex matrices.

Limitations

- **Funding**
- **Competitive grants**
- **Scientific issues-**
 - **detection and validation of methods in complex matrices, sampling whole fields, complexity of multiple risk factors**

Next steps

- **Microbial fate and transport as a function of contaminated water and/or soil**
- **GIS and microbial source tracking**
- **Need to know which agricultural, processing, and handling practices contribute most to contamination**
- **In order for GAPS to work, need most critical, specific points of contamination**

Next Steps

- Tomato exposed at various phases of growth through various means (soil, etc)
- Development of mitigation strategies, both pre-and post- to reduce microbial load
- Measurement of impact of mitigations, interventions, management practices
- Enteric viruses
- Training (education and extension) of GAPs and Guidelines