Background

- Multi-state outbreaks of salmonellosis associated with the consumption of raw tomatoes and other produce have increased in recent years
  - Estimated number of human illnesses: 3,000-80,000
  - 2008: *Salmonella* Saintpaul outbreak alone caused ~1,500 human illnesses

- There is a critical public health need to:
  - Understand how and where tomatoes are contaminated in the farm-to-fork continuum
  - Develop and implement innovative, cost-effective solutions that can prevent/reduce contamination events and minimize the burden of disease
GAPs: A Possible Solution?

- GAPs assessment survey was developed by CFSAN and administered at tomato farms in 2007, but no environmental samples were analyzed simultaneously.
  - Does the implementation of GAPs prevent or reduce bacterial contamination on tomato farms?
  - Are GAPs cost-effective to growers?

1998: CFSAN Guide To Minimize Microbial Food Safety Hazards For Fresh Fruits And Vegetables

2004: CFSAN and North American Tomato Trade Work Group action plan to prevent bacterial contamination of fresh produce

Good Agricultural Practices (GAPs)
Univ. of MD/CFSAN Study

**Purpose:**
- To conduct a 2-year on-farm study on Mid-Atlantic tomato farms

**Goals:**
- To understand the impacts of GAPs implementation on levels of on-farm bacterial contamination
- To determine costs to tomato growers of implementing GAPs and to evaluate whether costs present a barrier to implementation
Specific Aims:

1. To modify the CFSAN GAPs assessment survey to include questions about the costs of GAPs implementation, and to administer the new survey at tomato farms in the Mid-Atlantic region

2. To determine the prevalence of *Salmonella* and *Enterococcus* spp. in environmental samples collected from the same tomato farms

3. To evaluate associations between specific GAPs, the costs of the GAPs, and the prevalence of *Salmonella* and *Enterococcus* spp. in environmental samples
Overall Approach:

9 Tomato Farms

- Winter Sampling Trip (Completed)
  - Collect and Analyze Environmental Samples
  - Administer GAPs Survey
  - Statistical and economic analysis

- Spring Sampling Trip (Completed)
  - Collect and Analyze Environmental Samples
  - Administer GAPs Survey
  - Statistical and economic analysis

- Summer Sampling Trip (Completed)
  - Collect and Analyze Environmental Samples
  - Administer GAPs Survey
  - Statistical and economic analysis

- Fall Sampling Trip (Completed)
  - Collect and Analyze Environmental Samples
  - Administer GAPs Survey
  - Statistical and economic analysis

Feedback to growers
Sampling Trips
Collection of GAPs/Cost Data

“Tomato Farm Sampling Questionnaire”:
- General farm information
- Meteorological conditions
- Tomato field characteristics
- Water sources
- Worker hygiene
- GAPs implementation
- GAPs costs

Administered during each sampling trip

Tomato Farm Sampling Questionnaire
JIPSAN - UMD/UFFM Tomato Farm Study 2019

1. General Information
1.1 Sample collection date (mm/dd/yyyy) ____________________________
1.2 What is the name of the packing company? (please specify) ________________
1.3 What is the name of the specific tomato farm? (please specify) ________________
1.4 What is the field name or number? ____ (On each field, we will assign a unique field code. The same field will be sampled on all subsequent sampling trips)
1.5 Are there any animals or birds in or around the tomato field that is sampled at the time of sampling?
   a) Yes
   b) No
   If YES describe __________________________________________
1.6 Is there any evidence of animals or birds in the tomato field that is sampled (fence, tractor, etc)?
   a) Yes
   b) No
   If YES describe __________________________________________
1.7 Are there fences around the field that is sampled?
   a) Yes - completely fenced
   b) No
   c) Partially fenced
   d) Other describe __________________________
1.8 Are there ditches, ponds, streams or other water where birds, reptiles, amphibians, etc can inhabit directly adjacent to the tomato field that is sampled?
   a) Yes
   b) No
   If YES describe __________________________________________
1.9 Are there forested, wooded or marsh areas adjacent to the field that is sampled?
   a) Yes
   b) No
   If YES describe __________________________________________
1.10 Are there any chicken, barn or other large scale animal production facilities in close proximity to the farm?
   a) Yes
   b) No
   If YES describe __________________________________________
Environmental Sample Analysis

- **Head-to-head comparison with Pathatrix (Ben Tall, CFSAN)**
- **Water**
  - Standard Membrane Filtration
- **Soil**
  - Enrichment in Lactose broth and Hajna TT, or Enterococcosel broth, followed by plating
  - ID of *Salmonella* and *Enterococcus* with the Vitek 2.0 Compact System
  - Antimicrobial Susceptibility Testing of *Salmonella* and *Enterococcus* with the Vitek 2.0 Compact System
- **Sediment**
- **Tomatoes**
- **Phyllosphere**
- **Surface Swabs**
  - Serotyping of *Salmonella* using serological methods, Premi® test and multiplex PCR
GAPs Analysis

- A scoring system based on the GAPs questionnaire was developed
  - A point is given for each practice that is NOT consistent with GAP's
  - A score of 0 implies a high level of consistency with GAP's and a score of 24 implies a low level of consistency with GAP's

- These scores will be used in a logistic regression analysis that analyzes the association between GAPs implementation and on-farm bacterial contamination
## Results: *Salmonella* Contamination on Participating Tomato Farms

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Feb</th>
<th>May</th>
<th>July</th>
<th>Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground water</td>
<td>0/3</td>
<td>0/3</td>
<td>0/5</td>
<td>0/4</td>
</tr>
<tr>
<td>Pond water</td>
<td>0/7</td>
<td>1/7</td>
<td>1/7</td>
<td>1/7</td>
</tr>
<tr>
<td>Filter back wash</td>
<td>-</td>
<td>-</td>
<td>1/1</td>
<td>-</td>
</tr>
<tr>
<td>Pond sediment</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
<td>1/7</td>
</tr>
<tr>
<td>Soil</td>
<td>2/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
</tr>
<tr>
<td>Control soil</td>
<td>1/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
</tr>
<tr>
<td>Irrigation ditch soil</td>
<td>1/5</td>
<td>0/5</td>
<td>0/6</td>
<td>0/4</td>
</tr>
<tr>
<td>Irrigation ditch water</td>
<td>-</td>
<td>-</td>
<td>1/4</td>
<td>0/2</td>
</tr>
<tr>
<td>Phyllosphere</td>
<td>-</td>
<td>0/2</td>
<td>0/6</td>
<td>0/2</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>-</td>
<td>-</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Harvest trays</td>
<td>-</td>
<td>-</td>
<td>0/1</td>
<td>0/3</td>
</tr>
<tr>
<td>Port-a-potty swabs</td>
<td>-</td>
<td>0/2</td>
<td>0/1</td>
<td>0/1</td>
</tr>
</tbody>
</table>

*Blue – BAM method  
Green – Pathatrix*
# Results: Persistence of *Salmonella* on Participating Tomato Farms?

<table>
<thead>
<tr>
<th>Farm Code</th>
<th>February</th>
<th>May</th>
<th>July</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF 9</td>
<td>Soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF 25</td>
<td>Soil Control Soil</td>
<td>Pond Water Filter Back Wash</td>
<td>Pond Sediment</td>
<td></td>
</tr>
<tr>
<td>TF 32</td>
<td></td>
<td>Ditch Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF 37</td>
<td>Pond Water</td>
<td></td>
<td></td>
<td>Pond Water</td>
</tr>
</tbody>
</table>
## Results: *Enterococcus* Contamination on Participating Tomato Farms

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>February</th>
<th>May</th>
<th>July</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground water</td>
<td>0/3</td>
<td>0/3</td>
<td>0/5</td>
<td>0/4</td>
</tr>
<tr>
<td>Pond water</td>
<td>1/7</td>
<td>5/7</td>
<td>3/7</td>
<td>6/7</td>
</tr>
<tr>
<td>Filter back wash</td>
<td>-</td>
<td>-</td>
<td>1/1</td>
<td>-</td>
</tr>
<tr>
<td>Pond sediment</td>
<td>5/7</td>
<td>5/7</td>
<td>5/7</td>
<td>5/7</td>
</tr>
<tr>
<td>Soil</td>
<td>9/9</td>
<td>7/9</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>Control soil</td>
<td>7/9</td>
<td>5/9</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>Irrigation ditch soil</td>
<td>5/5</td>
<td>3/5</td>
<td>6/6</td>
<td>4/4</td>
</tr>
<tr>
<td>Irrigation ditch water</td>
<td>-</td>
<td>-</td>
<td>2/4</td>
<td>2/2</td>
</tr>
<tr>
<td>Phyllosphere</td>
<td>-</td>
<td>0/2</td>
<td>5/6</td>
<td>2/2</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>-</td>
<td>-</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Harvest trays</td>
<td>-</td>
<td>-</td>
<td>1/1</td>
<td>3/3</td>
</tr>
<tr>
<td>Porta-potty swabs</td>
<td>-</td>
<td>2/2</td>
<td>1/1</td>
<td>1/1</td>
</tr>
</tbody>
</table>
Results: *Enterococcus* spp. Distribution on Tomato Plants

**FIGURE 1.** *Enterococcus* spp. distribution on the bottom, middle and top portions of tomato plants on (A) large- and (B) small-scale farms.

Micallef SA, et al. 2010 [Submitted]
Results: Antimicrobial Susceptibility of *Enterococcus* spp. Recovered from Tomatoes

**FIGURE 3.** Resistance to individual antibiotics for all strains isolated is shown by species. The number of isolates per species is in parentheses.

Micallef SA, et al. 2010 [Submitted]
Preliminary Results: GAPs and Cost Data

- GAPs and cost data are being cleaned and analyzed
- Some variability in GAPs implemented on participating farms
- To be completed:
  - Logistic regression on GAPs, cost and microbiological data
  - Geographical analysis of participating farms
**Additional Work to be Done**

- Confirm serotyping on *Salmonella* isolates
- Completion of manuscripts
- Additional feedback to (and from) growers
- Development of additional hypotheses to be tested
Acknowledgements

- JIFSAN
- Steve Rideout (Virginia Tech)
- Tomato packers and growers
- The Virginia Institute of Marine Science (VIMS)
- Ben Tall, OARSA, CFSAN
- UMD Students
  - Ashish George
  - Zenas Chang
  - Norman Wang
- CFSAN
  - Samir Assar
  - Jack Guzewich
  - Thomas Hill
  - Mike Mahovic