Objectives

- Food safety outbreaks linked to fresh produce
- Human pathogens linked to fresh produce
- Sources of contamination
- Interaction of human pathogens with growing plants
- Current and future initiatives

Fresh Produce Market

- Ready to eat Salads Market Growing at 10% per Year
- 6 Million Bags of fresh cut produce sold daily
- Current Market Value >US$70bn
- Greater Diversity of Produce Available (All Year Round)
- Centralized Production

Fresh Produce Industry

<table>
<thead>
<tr>
<th>Produce</th>
<th>Million $</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leafy Greens</td>
<td>2140</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>1637</td>
</tr>
<tr>
<td>Potato</td>
<td>1247</td>
</tr>
<tr>
<td>Onions</td>
<td>905</td>
</tr>
</tbody>
</table>

Production Regions

- California 74%
- Arizona 25%
- Other 1%

Outbreaks Associated with Salad Vegetables

<table>
<thead>
<tr>
<th>Year</th>
<th>Lettuce</th>
<th>Spinach</th>
<th>Sprouts</th>
<th>Tomatoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973-79</td>
<td>3.0</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1980-89</td>
<td>6.0</td>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1990-97</td>
<td>12.0</td>
<td>8.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Source: Centre for Disease Control & Prevention, USA
Fresh Produce and Human Pathogenicity
Prof. Keith Warriner, University of Guelph
A Webber Training Teleclass

2006 Annus horribilis (North America)
- Sprouts (Ontario) Feb Suspected Salmonella
- Sprouts (Australia) Feb Salmonella 100 cases
- Lettuce June E. coli O121:H19 4 cases
- Sprouts Aug Suspected Salmonella
- Spinach Sept E. coli O157:H7 202 cases
- Carrot Juice Sept Cl. botulinum 6 cases
- Lettuce (Ontario) Oct E. coli O157:H7 30 cases
- Lettuce Oct 8, 500 carton recall due to suspected E. coli

1996-2005

<table>
<thead>
<tr>
<th>Category</th>
<th>Outbreaks</th>
<th>Illnesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed</td>
<td>43</td>
<td>3,026</td>
</tr>
<tr>
<td>Produce</td>
<td>63</td>
<td>8,040</td>
</tr>
<tr>
<td>Sprouts</td>
<td>25</td>
<td>1,565</td>
</tr>
<tr>
<td>Seafood</td>
<td>120</td>
<td>2,567</td>
</tr>
<tr>
<td>Eggs</td>
<td>234</td>
<td>6,572</td>
</tr>
</tbody>
</table>

1998-2006 Produce Outbreaks by Commodity

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Outbreaks</th>
<th>Illnesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomatoes</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Cantaloupe</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Melons</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Honeydew melon</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Raspberries</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Romaine lettuce</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Lettuce</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Mixed lettuce</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cabbage</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spinach</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*as of August 7, 2006

1998-2006 Produce Outbreaks

- 5 commodity groups make up >75 percent of produce related outbreaks
  - Lettuce/leafy greens 30%
  - Tomatoes 17%
  - Cantaloupe 13%
  - Herbs (Basil, parsley) 11%
  - Green onions 5%
  - Total 5% of 5 top commodities 76%

Why the increase in Foodborne illness cases & Recalls

- Larger volume of product
- Increased awareness of food safety
- Better detection and investigation
- Lack of effective initiatives
- FDA: 2 letters to California growers
- Lack of urgency
- Lack of understanding of human Pathogen:Produce interactions

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Human Pathogens Linked to Produce

*Escherichia coli* O157:H7
Lettuce, Spinach, Sprouts

*Salmonella*
Tomatoes, Lettuce, Cantaloupe, Sprouts, Mangoes, Almonds

*Shigella sonnei*
Parsley, Lettuce, Green onions

*Listeria monocytogenes*
Cabbage

*Cyclospora*
Basil, Raspberries

Hepatitis A
Green onions, soft fruit

Why Pathogens Linked to Certain Produce?

- Unknown
- Pathogens within the environment?
- Pathogens adapted to produce?

*Escherichia coli* O157:H7

- Gram negative rod.
- Facultative anaerobic
- Temp 10–50°C (opt 37°C)
- Min $a_w$ 0.93
- Remain viable at low pH especially at low temps.

Symptoms

VTED ingested

Abdominal cramps, non-bloody diarrhoea

Blood diarrhoea

5% Resolution

100% HUS

Low infective dose

100 cells

Heamolytic Uremic Syndrome

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Sources of *Escherichia coli* O157:H7

- Manure from cattle and other ruminants
- Farm effluent
- Person-to-Person contact
- Wild animals

**Shigella**

*Shigella dysenteriae*

*Shigella sonnei*

Fecal-Oral route or via contaminated foods

Very low tolerance to environmental stress and typically only recovered in GI tract or fresh sewage

- Similar symptoms to EHEC
- Very low dose required (10 – 100 cells)
- Typically pathogen transferred directly from fecal material or food handlers

**Salmonella**

- Gram negative non-sporulating rod
- Facultative anaerobic
- Temp for growth 6–46°C (opt 36–37°C).
- Min pH 4.5
- Min $a_w$ 0.97

**Taxonomy of Salmonella**

- *Salmonella* group composed of very closely related serovars.
- Only two species *Salmonella enteritica* and *Salmonella bongori*
- Serotyping (antibody reaction) used to differentiate types.
Most Frequent Serotypes Associated with Food

- S. Typhimurium
- S. Enteritidis
- S. Dublin

Salmonella Typhimurium DT 104

- Why is DT 104 of concern?
  - Multiple antibiotic resistance
    - Ampicillin
    - Chloramphenicol
    - Streptomycin
    - Sulfonamides
    - Tetracycline

Symptoms

- Typhoid or paratyphoid fever from S. typhi and S. paratyphi
- Salmonellosis
- Headache
- Fever
- Diarrhea
- Nausea
- Vomiting

- Infective dose $10^2$ – $10^6$
- Invasion of small intestine and colon by entering absorptive mucosal cells and mucosa associated macrophages.
- Grow inside fixed macrophages of liver and spleen.

Secondary Complications

- Arthritis
- Ms Reynard
- Salmonella from Chinese meal
- $2.5m$ Payout

Sources of Salmonella

- Poultry and pig manure
- Sewage
- Wild animals
- Insects

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Listeria monocytogenes

- Gram positive non-spore forming rod
- Facultative anaerobe
- Catalase positive
- Oxidase negative
- Hemolytic
- Psychrotrophic
- Growth range 1 - 44°C
- Opt temp 35-37°C
- pH 5.0 – 9.6 (opt 6 – 8) Survives at pH 4
- Min aw 0.93
- Can survive in 25-30% NaCl solutions

Listeria Infection Process

Illness

Healthy individuals: Mild flu

High risk groups (young, pregnant, old, immuno-compromised):
Stillbirth or abortion
Meningitis
Septicemia
Pneumonia

- Infective dose for high risk groups $10^9$
- 30% mortality rate
- Incubation period 1-4 weeks
- Illness can last 1-90 days

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Sources of *L. monocytogenes*
- Decaying plant material
- Manure
- Drains
- Endemic within processing facilities

**Route of infection—Faecal oral**

- Ingestion by new host, from food or water
- Replication in intestine
- Infectious dose is normally low $10^{-10}$

**Human Parasites**
- Protozoan
- Viruses

  The majority require human host to replicate.

  Infected handlers or human sewage

**Cyclospora**
- Increasing number of cases in Ontario
- Mexican basil
- Fecal contamination

  Person to Person less significant

**Viral Hepatitis: associated virus**

  Hepatitis A and E

  Most commonly associated with foods
  - Jaundice
  - Liver damage
  - Abdominal discomfort
  - Fever

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Viral Hepatitis: associated virus
Hepatitis A

- Hepatitis A is usually a mild illness characterized by sudden onset of fever, malaise, nausea, anorexia, and abdominal discomfort, followed in several days by jaundice.

- Hepatitis A represents about 1/3 of all cases of viral hepatitis.

- Transmitted via person-to-person contact, water and through food (fruit a specific problem).

- 150,000 cases a year in United States.

- In developing countries incidence in indigenous population is low, due to "childhood vaccination", Vaccine is recommended for travellers.

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Survival of Pathogens in Manure

- *E. coli* O157:H7  >100 days
- *Salmonella*  >200 days

- User interface
  - Cross-contamination (cutting boards)
  - Handling
  - Temperature abuse

- Processing Facility
  - Water
  - Food handlers
  - Processing environment

Source of Produce Related Outbreaks

- **Spinach *E. coli* O157**
  - Salinas Valley
  - 26 States and within Canada
  - 199 confirmed cases
  - 3 Deaths
  - >$100m in loss sales and production
  - Previous outbreak in California 2003
    - 16 cases (2 deaths)
Rapid Response

• Traceability

• Molecular typing

Rapid connection between cases and trace back

PFGE Banding Pattern

Salinas Valley CA

What they think happened

*E. coli* O157:H7 genotype

• Infected persons

• Product

• Processing facility

• Cattle ranch near spinach field.

Pre-Washed Spinach

How Effective is Washing?
Does Triple Wash Mean Anything?
Post-Harvest Washing

- Cross-contamination as opposed to decontamination.
- Hypochlorite rapidly sequestered
- Internalized populations protected
- Biofilms

Location of Bacteria Surviving Biocidal Washing

• BacLight Nucleic Acid Staining

Upper Side of washed Spinach Leaf
Human Pathogens as Endophytes

- Could human pathogens become integrated into plant endophytic microflora?
- Protected against UV and desiccation in the field
- Protected against post-harvest biocidal washing

Interaction of *E. coli* with Growing Spinach Plants

Potential routes:
- Seed
- Growth Matrix (soil/hydroponic solution)

Bioluminescent *E. coli*

<table>
<thead>
<tr>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 15</td>
<td>Day 15</td>
</tr>
</tbody>
</table>

*E. coli* on Spinach Roots

In Situ Gus Assay

<table>
<thead>
<tr>
<th>Spinach Plants</th>
<th>TAC Log cfu/g</th>
<th><em>E. coli</em> O157 Log cfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface</td>
<td>Internal</td>
</tr>
<tr>
<td>Day 9</td>
<td>5.97</td>
<td>2.31</td>
</tr>
<tr>
<td>Day 49</td>
<td>5.49</td>
<td>2.36</td>
</tr>
</tbody>
</table>
Conclusions from Inoculated Seeds

- Internalization of *E. coli* during early stages of germination
- *E. coli* established on/within roots and surface of leaves in mature plants

Inoculated Soil

Counts on Spinach

<table>
<thead>
<tr>
<th>Days after Planting</th>
<th>TVC</th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wash</td>
<td>Extract</td>
</tr>
<tr>
<td>12</td>
<td>6.3</td>
<td>6.0</td>
</tr>
<tr>
<td>14</td>
<td>5.4</td>
<td>5.7</td>
</tr>
<tr>
<td>16</td>
<td>6.6</td>
<td>3.9</td>
</tr>
<tr>
<td>20</td>
<td>5.9</td>
<td>4.7</td>
</tr>
<tr>
<td>23</td>
<td>6.4</td>
<td>5.2</td>
</tr>
<tr>
<td>25</td>
<td>6.8</td>
<td>5.1</td>
</tr>
<tr>
<td>32</td>
<td>6.4*</td>
<td>4.3</td>
</tr>
<tr>
<td>35</td>
<td>7.5</td>
<td>3.9</td>
</tr>
</tbody>
</table>

1 plant positive from a batch of three tested
ND: < 1 log cfu/g

Conclusions from Contaminated Soil

- *E. coli* proliferates in soil over the duration of plant cultivation.
- *E. coli* becomes established internally/externally on roots. Surface of leaves.
- Low level of internalization in mature plants

Spinach Cultivation

- Nutrient Film Technique (NFT) Hydroponic System
- Soil free cultivation
- Safer?
Fresh Produce and Human Pathogenicity
Prof. Keith Warriner, University of Guelph
A Webber Training Teleclass

Bacterial Counts From Spinach Plants

<table>
<thead>
<tr>
<th></th>
<th>Wash</th>
<th>Surface Sterilised</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TVC</strong> Root</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td><strong>E. coli</strong> Root</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TVC</strong> Leaves</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>E. coli</strong> Leaves</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND: <1 log cfu/g

Vacuum Cooling

Can pathogens be internalized into Spinach?
- Yes, in seedlings but not in mature plants.
- Pathogens can find entry via natural openings and cut edges
- Internalization only part of the problem
- Is vacuum cooling safe?

Foodborne Illness Outbreaks Linked to Tomatoes

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Year</th>
<th>Total cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Javiana</td>
<td>1990</td>
<td>174</td>
<td>0</td>
</tr>
<tr>
<td>Montevideo</td>
<td>1993</td>
<td>84</td>
<td>0</td>
</tr>
<tr>
<td>Baildon</td>
<td>1998</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>Javiana</td>
<td>2002</td>
<td>141</td>
<td>0</td>
</tr>
<tr>
<td>Newport</td>
<td>2002</td>
<td>297</td>
<td>0</td>
</tr>
<tr>
<td>Beranderup Javiana</td>
<td>2004</td>
<td>561</td>
<td>0</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>2006</td>
<td>181</td>
<td>0</td>
</tr>
</tbody>
</table>

Inoculate Flowers of Growing Plants

0.1 ml 7 log cfu/ml Screen for *Salmonella* External and Internal

Variety: Abigail VFFT

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Survey of Tomato Growing Regions in Florida and Mexico

- **Salmonella Montevideo**
- **Salmonella Javiana**

Soil  
Water  
Packing plant  
Feces of wild animals

Human pathogens adapted to environments outside the host?

### Sprouted Seeds

- **Ontario 2005**  
  > 600 cases of salmonellosis linked to mung bean sprouts

  34 outbreaks linked to alfalfa and other sprouted seeds since 1990

  Contaminated seed implicated in majority of cases

### Sprout Outbreaks 1996-2004

<table>
<thead>
<tr>
<th>Year</th>
<th>Alfalfa</th>
<th>Clover</th>
<th>Mung Bean</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>1</td>
<td>1</td>
<td></td>
<td>650</td>
</tr>
<tr>
<td>1997</td>
<td>3</td>
<td>1</td>
<td></td>
<td>277</td>
</tr>
<tr>
<td>1998</td>
<td>3</td>
<td>1</td>
<td></td>
<td>48</td>
</tr>
<tr>
<td>1999</td>
<td>5</td>
<td>2</td>
<td></td>
<td>389</td>
</tr>
<tr>
<td>2000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>2001</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>2002</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>2003</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>2004</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Total: 27 Outbreaks</td>
<td>1633 Cases</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Seeds are Main Source of Pathogens

- **Alfalfa Seed**  
- **Mung Bean**

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Growth of *E. coli* during mung bean sprouting

Apoplastic Fluid from Surface Sterilised Bean Sprouts

Seed Decontamination
- Calcium hypochlorite
- Acidified sodium chlorite
- Peroxyacetic acid + hydrogen peroxide
- Hot water pasteurization
- Heat treatment
- Irradiation
- Calcinated Calcium
- Organic acids

All have failed to successfully decontaminate seeds without adversely affecting seed germination.

Different Approach

Why are seeds so difficult to decontaminate?
- Protective sites on the seed coat
- Low number of survivors (<1 MPN/g) can proliferate during the first 24h of sprouting.
- Need to preserve seed viability
Calcium Hypochlorite (20,000 ppm) Vs Germin-8-or (200 ppm)

<table>
<thead>
<tr>
<th>Treatment of mung beans</th>
<th>E. coli O157:H7</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count Log cfu/g</td>
<td>Enrichment Log cfu/g</td>
</tr>
<tr>
<td>Calcium hypochlorite (20, 000 ppm, 20 mins)</td>
<td>8.59</td>
<td>NT</td>
</tr>
<tr>
<td>Germin-8-or (200 ppm)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Initial loading: 3-4 log cfu/g ND <1 cfu/25g

**Botulism Carrot Juice**

- 6 cases (4 USA; 2 Toronto)
- 28 cases per year
- Home canning
- Vegetables in oil

**Clostridium botulinum**

- Gram positive anaerobic rod.
- Forms resistant endospores
- Temperature range:
  - Proteolytic 10-48°C (opt 37°C)
  - Non-proteolytic 3.3-45°C (opt 30°C)
- aw min: 0.93
- pH min: 4.6

- Loss of muscle function
- Incubation period: 18-36 hours
- Symptoms:
  - Weakness in eye muscle
  - Slurred speech
  - Difficult swallowing, breathing and moving limbs.

**Possible Sequence of Events**

Botulism spores in soil
Pasteurization:
- Inactivated natural anti-microbial constituents
- Reduction in competitive microflora
- Activation of spores
- Outgrowth of **Cl. botulinum** with product held at elevated temperatures.

**Future Directions**

- Possible sequence of events
- Clostridium botulinum
- Botulism carrot juice
- Future directions
Lettuce Safety Initiative (Aug 2006)
- Review current procedures
- Rapid response to outbreaks
- Documentation (on-farm HACCP)
- Introduce regulations

Post-harvest Intervention
- Can contamination in the field be prevented? No
- Is testing the answer? Yes, but cannot provide total assurance.
- Washing is ineffective

Decontamination of Fresh Produce
- Pre Wash: Potable water to remove visible soil

Biocidal wash
- 200ppm sodium hypochlorite
- Organic acids
- Peroxyacetic acid
- Acidified Sodium Chlorite
- Ozonated water

Synergistic Action of UV and Hydrogen Peroxide

Decontamination of Fresh Produce

Inactivation of Different Bacteria on Agar Plates

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Log Count Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV&lt;sub&gt;324&lt;/sub&gt;</td>
</tr>
<tr>
<td>Pectobacterium carotovora</td>
<td>2.25</td>
</tr>
<tr>
<td>Escherichia coli O157</td>
<td>2.83</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>2.50</td>
</tr>
<tr>
<td>Salmonella Montavideo</td>
<td>0.52</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>2.32</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>ND</td>
</tr>
</tbody>
</table>

Initial loading 6 log cfu
ND Not Detected

Prototype System

Lamp
LP 12W
254nm
Spray Heads
Sample Tray

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Spinach  Log Count Reductions

<table>
<thead>
<tr>
<th></th>
<th>External</th>
<th>Internal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli O157:H7</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV:H₂O₂</td>
<td>4.75 ± 0.85</td>
<td>0.63 ± 0.15</td>
</tr>
<tr>
<td>Hypochlorite</td>
<td>0.46 ± 0.07</td>
<td>- 005 ± 0.01</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV:H₂O₂</td>
<td>3.65 ± 0.12</td>
<td>0.89 ± 0.18</td>
</tr>
<tr>
<td>Hypochlorite</td>
<td>0.46 ± 0.08</td>
<td>- 004 ± 0.34</td>
</tr>
</tbody>
</table>

**Electrolyzed Water**
- Generates chlorous acid
- 2 log cfu reduction in lettuce inoculated with Salmonella

**Irradiation**
- Cobalt 60
- E beam
- Consumer acceptance?

**Reduction of E. coli O157:H7 on spinach leaves as affected by e-beam irradiation**

**Final Thoughts**
• Fresh produce represents a continuing food safety issue.

• One outbreak is one too many.

• Greater understanding on routes by which human pathogens enter and disseminate through produce chain.

• Focus on interventions.

• Regulation (Industry or Government led)?

---

The Next Few Teleclasses

<table>
<thead>
<tr>
<th>Date</th>
<th>Topic</th>
<th>Speaker/Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 21</td>
<td>Infection Control in the Endoscopy Clinic</td>
<td>Dr. Richard Everts, Nelson Marlborough Health Service</td>
</tr>
<tr>
<td>February 22</td>
<td>Best Practice for Hospital Construction Management</td>
<td>Andrew Streifel, University of Minnesota</td>
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<tr>
<td>March 6</td>
<td>Tuberculosis in the Modern Age</td>
<td>Faculty to be announced</td>
</tr>
<tr>
<td>March 8</td>
<td>Voices of CHICA</td>
<td>CHICA-Canada Board Members &amp; Guests</td>
</tr>
<tr>
<td>March 22</td>
<td>A Year of Cleaner, Safer Care – A Worldwide Experience</td>
<td>Dr. Didier Pittet, World Health Organization, Geneva</td>
</tr>
</tbody>
</table>

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