Listeria, Salmonella and the rest of the zoo!

Prof Pieter Gouws

5 March 2019
After Disney released “Princess and the Frog”, over 50 children were hospitalized with salmonella from kissing frogs.

- *Lancet 2016; 388: 1459–544*
Key Facts – Foodborne diseases

- Researchers have identified more than 250 foodborne diseases.
- Most of them are infections, caused by a variety of bacteria, viruses, and parasites.
- Harmful toxins and chemicals also can contaminate foods and cause foodborne illness.

CDC estimates that each year 48 million people get sick from a foodborne illness, 128 000 are hospitalized and 3 000 die

- Children under 5 years of age carry 40% of the foodborne disease burden, with 125 000 deaths every year.
- Diarrhoeal diseases are the most common illnesses resulting from the consumption of contaminated food, causing 550 million people to fall ill and 230 000 deaths every year.
- Food safety, nutrition and food security are inextricably linked.
- Foodborne diseases impede socioeconomic development by straining health care systems, and harming national economies, tourism and trade.
- Food supply chains now cross multiple national borders. Good collaboration between governments, producers and consumers helps ensure food safety.
Almost 1 in 3 are fatalities in South Africa’s listeriosis outbreak

BY DAN FLYNN | MAY 4, 2018

The deadly South African listeriosis outbreak now has a stunning 28.6 percent fatality rate, according to the latest report by the National Listeria Incident Management Team (IMT). The new IMT report says 200 of 1,024 confirmed cases of Listeria resulted in the deaths of the victims.

The outbreak is slowing down, but it’s not over. The National Institute for Communicable Diseases (NICD) has logged 55 new cases in the seven weeks after the source of the outbreak was announced on March 4.

That’s when Minister of Health Dr. Aaron Motsoaledi named ready-to-eat processed meat products from the Enterprise Foods’ production facility in Polokwane as the source of the Listeria monocytogenes and initiated recalls.

In the seven weeks prior the March 4 actions, 169 confirmed cases were added to the outbreak.

Dr. Motsoaledi declared the current outbreak last December after South Africa experienced a sharp increase in the number of listeriosis cases from an average of 60-to-80 cases a year. The 1,024 confirmed cases of listeriosis with 200 deaths brings the two-year total to 1,642 cases and 427 deaths.
Food safety

• Dealing with food safety problems is challenging and complex.

• **Food safety failure is not a commercial option.**

• Linking a product with a foodborne pathogen resulting in consumer illness is a catastrophic event for the food processor

• Contaminated food results in major health problems in the world and leads to reduced economic productivity.
Global challenges - WHO

1. **ERADICATE EXTREME POVERTY AND HUNGER**
   - Foodborne diseases and their resulting morbidity, disability, and mortality affect household incomes. Improvements in food availability for those living with hunger must be accompanied by corresponding reductions in foodborne diseases, otherwise the goal of “halving poverty by 2015” will be jeopardized.

2. **ACHIEVE UNIVERSAL PRIMARY EDUCATION**
   - Pregnant women are especially susceptible to infectious risks, including foodborne diseases. Infections with Listeria monocytogenes and Toxoplasma gondii are particular hazards in pregnancy. These infections can cause serious illness in the mother and fetus, as well as miscarriages, premature delivery and stillbirth, all of which increase the risk of maternal mortality.

3. **PROMOTE GENDER EQUALITY AND EMPOWER WOMEN**
   - Children are particularly vulnerable to the effects of foodborne diseases, often as a result of accompanying morbidity, including malnutrition or other infections. A reduction in child mortality may not be possible without substantial decrease in food and waterborne diseases in this age group.

4. **REDUCE CHILD MORTALITY**
   - People living with HIV/AIDS are prone to opportunistic infections, including those resulting from contaminated food. Serious complications and chronic symptoms as a result of foodborne diseases, including cerebral toxoplasmosis are common in immunocompromised people. Food safety interventions are, therefore, an important complement to achieve MDG 6.
Emerging foodborne pathogens

- Weakened or collapsed public health system
- Poverty, uncontrolled urbanisation and population displacements
- Environmental degradation and water and food sources contamination
- Ineffective disease control programmes
- Rise of antimicrobial resistance
- Diseases crossing from animals to humans
- Globalization of food supply
- Globalization of travel and trade
- Better methods for identification
Hazard vs. risk

Hazard
is the potential to cause harm

when crossing a road, cars are a hazard

Risk
is the likelihood of harm taking place based on exposure

when crossing a highway, the risk of an accident is high
when crossing a country road the risk of an accident is low

high exposure
low exposure
Foodborne diseases in Africa

- **Annual global death toll for foodborne diseases**
  - Rest of the world: 66.6%
  - Africa*: 33.3%

- **137,000 people die**

- **>91 million people fall ill**

**Diarrhoeal diseases** are responsible for **70%** of the burden of foodborne diseases

- Non-typhoidal *Salmonella*
- Foodborne cholera
- *E. coli*

* [www.who.int/about/regions/en](http://www.who.int/about/regions/en)
- **Salmonella**, a bacterium found in many foods, including raw and undercooked meat, poultry, dairy products, and seafood. *Salmonella* may also be present on egg shells and inside eggs.

- **Campylobacter jejuni** (*C. jejuni*), found in raw or undercooked chicken and unpasteurized milk.

- **Shigella**, a bacterium spread from person to person. These bacteria are present in the stools of people who are infected. If people who are infected do not wash their hands thoroughly after using the bathroom, they can contaminate food that they handle or prepare. Water contaminated with infected stools can also contaminate produce in the field.

- **Escherichia coli** (*E. coli*), which includes several different strains, only a few of which cause illness in humans. *E. coli O157:H7* (the shiga toxin producing one) is the strain that causes the most severe illness. Common sources of *E. coli* include raw or undercooked hamburger, unpasteurized fruit juices and milk, and fresh produce.

- **Listeria monocytogenes** (*L. monocytogenes*), which has been found in raw and undercooked meats, unpasteurized milk, soft cheeses, and ready-to-eat deli meats and hot dogs.

- **Vibrio**, a bacterium that may contaminate fish or shellfish.

- **Clostridium botulinum** (*C. botulinum*), a bacterium that may contaminate improperly canned foods and smoked and salted fish.
Foodborne Listeriosis

WHO

- 600 million people around the world contract foodborne diseases per year
- 420,000 dying

Listeriosis

- 20 - 30% mortality rate
- RTE products
  - Deli meats
  - Liver pate
  - Ice cream
  - Soft cheeses
  - Smoked chicken and fish
  - Vegetables
  - Cantaloupe / spanspek
New listeriosis cases down; newborns remain hardest hit

BY NEWS DESK | JULY 2, 2018

New information out of South Africa shows 1,053 people have been confirmed with Listeria infections in the past 18 months. One in five has died in what the World Health Organization has determined to be the largest listeriosis outbreak in recorded history.

Infants in their first month of life have been harder hit than any other age group in the outbreak. Of the infants who are 28 days or younger, for whom complete information is available, 91 have died. Overall, at least 212 people have died in the outbreak. Officials do not yet have final reports on all of the sick people, so there could easily be additional deaths confirmed.

Public health investigators traced the outbreak to Tiger Brand’s ready-to-eat polony, which is similar to baloney and hot dogs, that was produced at Enterprise Foods’ Polokwane production facility.

During the peak period of the outbreak, from late October 2017 through March this year, health officials often confirmed more than 40 new cases per week. Some weeks more than 100 new people were found to have infections.
Listeria Introduction

- *L. monocytogenes* is a Gram + bacterium responsible for Listeriosis (food-borne disease)
  - may result in severe illness and death
- Death toll is known to be the highest of all known food-borne pathogens, although Listeriosis is rare
- Listeriosis has always been regarded as an
  - invasive disease affecting susceptible groups but a
  - non-invasive form of Listeriosis in healthy adults has increased public awareness of *L. monocytogenes* due to the expanding vehicle of infection
Pathophysiology of Listeriosis
Most at risk..

- Food poisoning can happen to anyone but those most at risk for listeriosis...

Pregnant women, fetuses, and newborn infants

- Listeria can pass from pregnant women to their fetuses and newborns. It can cause miscarriages, stillbirths, and newborn deaths.

Chancy cheese

LISTERIA OUTBREAK: Queso fresco (a type of soft cheese) sickened 142 people, killed 10 newborns and 18 adults, and caused 20 miscarriages.

Adults 65 or older

- Listeria can spread through the bloodstream to cause meningitis, and often kills. The older you are, the greater the risk.

Tainted cantaloupes

LISTERIA OUTBREAK: Contaminated whole cantaloupes sickened 147 people in 28 states and caused one of the deadliest foodborne outbreaks in the US. There were 33 deaths, mostly in adults over 65, reported during the outbreak.

SOURCE: CDC, 2013

People with weakened immune systems

- Listeria can spread through the bloodstream to cause meningitis, and often kills. The weaker your immune system, the greater the risk.

Contaminated celery

LISTERIA OUTBREAK: Pre-cut celery in chicken salad served at hospitals sickened 10 people who had other serious health problems. Five of them died as a result.
Introduction – key points to remember

- **Environmental pathogen**
  - *Down to earth pathogen*
  - Contaminate food
  - Sewage
  - Water / Waste water
  - Animals
  - Decaying vegetation (silage)

- **Temperatures**
  - Below freezing temperature will prevent growth
  - Can multiply at refrigeration temperature

- **RTE**
  - *Listeria monocytogenes does not grow when*
    - pH less than or equal to 4.4
    - Water activity less than or equal to 0.92
Pathogenic *Listeria*

*Listeria* (red) forming actin comet tails (green) in an infected cell. Cell nucleus stained in blue.

Diagram showing the taxonomic relationships among different species of *Listeria*. The species are categorized into genera such as *Listeria*, *Murraya*, *Mesolisteria*, and *Paenilisteria*. Each node represents a species, with branches indicating evolutionary relationships. The diagram includes species like *Listeria monocytogenes*, *Listeria marthii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria grayi*, *Listeria fleischmannii*, *Listeria floridensis*, *Listeria aquatica*, *Listeria newyorkensis*, *Listeria cornellensis*, *Listeria rocourtiae*, *Listeria weihenstephanensis*, *Listeria grandensis*, *Listeria riparia*, and *Listeria booriae*. The root of the tree is labeled as 0.1 amino acid substitution/site.
Listeria ?

World wide

• Steady increase since 2000 - Why?

Includes both pathogenic and non pathogenic

• Versatile - ecology in Agricultural systems not fully understood
• Resistance to both acids and alkalis
• Form biofilms
  • Matrix protect itself from chemicals
• Dormant, long-time survival
• Colonise factory environments

• Salmonella, E. coli and Campylobacter stops growing at below 20 °C
• Listeria able to grow at 4 °C
• Listeria use different types of nutrients – plants / animal gut !!
Virulence of *L. monocytogenes*

**Internalin (InlA + B)**
- Entry

**Listeriolysin O (hly)**
- Phospholipase C
- Lysis of the vacuole

**ActA (actA)**
- Intracellular movement and Cell-to-cell spread
Lifestyle of *Listeria monocytogenes*

- The bacterial pathogen *Listeria monocytogenes* is well adapted to both life in the soil and life in the cytosol of eukaryotic host cells.
- The **lifestyle switch** to intracellular pathogen includes increases in the expression of gene products that are known to promote cell-to-cell spread and bacterial replication in the host cytosol; these gene products are generally expressed at low levels outside of the host.
- How does *L. monocytogenes* implement the transition from life in the soil to life in the cell? **Bacteria must be capable of distinguishing the myriad of environmental cues encountered both inside and outside host cells and of correctly interpreting the signals so as to express gene products that promote survival in the appropriate location.**
- **We need to understanding how *L. monocytogenes* mediates the switch between its disparate lifestyles.**
From saprotroph to pathogen

PrfA and its role in the *L. monocytogenes* transition from the saprophytic stage to the virulent intracellular stage is important. *L. monocytogenes* is therefore clearly built to last in many different habitats.

**Thermoswitch**

- $\leq 30^\circ C$ – *pfrA* binding site unavailable due to RNA hairpin
- $37^\circ C$ - structure destabilize allowing translation of *pfrA* gene
Foods that pose a risk

High risk groups to practise precaution or avoid these foods

- **Dairy (milk raw/unpasteurised)**
  - Outbreaks linked to choc milk, butter, ice cream

- **Soft cheeses (made from unpasteurised milk) – matured and stored at refrigeration temperatures**
  - Camembert, brie, blue cheeses and pâté

- **Meat and meat products**
  - Outbreaks linked to frankfurters, luncheon meats and hotdogs
  - Raw and undercooked meat
  - Organism is relatively resistant to curing ingredients
    (has been found on salami, ham, corned beef etc)
Seafoods

- Outbreaks linked to **cold-smoked rainbow trout / fish / salmon**

Vegetables and fruit

- Outbreaks linked to **coleslaw / cabbage** (fertilizer prepared from manure infected with *Listeria*)
- Lettuce, celery, tomatoes, **raw sprouts, spanspek, watermelon**
- Avocado / **guacamole** (implicated in restaurant outbreaks)
RTE environment

- Due to pre-and post-production handling conditions, **RTE foods** are known for their risk of *Listeria monocytogenes* contamination.
- **Different lineages** of *Listeria monocytogenes*, display different adaptation mechanisms and resistance factors in response to processing factors in the RTE environment.
- *Listeria* loves **cool damp areas**
- *Listeria* loves **standing water**
- Increased **biofilm forming** ability under nutrient limited conditions

*Listeria* survive longer under adverse conditions than most other pathogens

- More **resistant to heat**
- Increase **resistance to sanitisers**
- **Movement of workers / actions of personnel / maintenance personnel**
- Tolerate **high salt / brine solutions**
# Lineages and serotypes

<table>
<thead>
<tr>
<th>Lineage</th>
<th>I</th>
<th>II</th>
<th>III/IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serotype</strong></td>
<td>1/2b, 3b, 3c, 4b&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1/2a, 1/2c, 3a&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4a, 4c&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Subgroups</strong></td>
<td>ECI, ECII, ECIV&lt;sup&gt;1&lt;/sup&gt;</td>
<td>ECIII&lt;sup&gt;1&lt;/sup&gt;</td>
<td>IIIA, IIIB, IIIC&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>High prevalence</strong></td>
<td>Human listeriosis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Food and environment&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Animal listeriosis&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Chemical resistance</strong></td>
<td>Increased resistance to antibiotics</td>
<td>Displays increased resistance to QAC</td>
<td>-</td>
</tr>
<tr>
<td><strong>Virulence</strong></td>
<td>Only lineage to carry listeriolysin S hemolysin. Contains sidophore, similar to other pathogens</td>
<td>Attenuated virulence due to premature stop codons in inlA and prfA</td>
<td>The lack of pfrA increases the disability to be virulent</td>
</tr>
</tbody>
</table>
Minimum spanning tree analysis of 360 L. monocytogenes and four L. innocua strains based on MLST data.

http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1000146
Novel listerial plasmid and an efflux transporter, *emrC*, to be associated with the emergence of meningitis caused by *L. monocytogenes* ST6 in the Netherlands, possibly through decreased susceptibility to disinfecting agents.

The *emrC* gene encodes an efflux protein that pumps quaternary ammonium compounds out of the cell and increases the capacity to form a biofilm.

Benzalkonium chloride is extensively used in the food-processing industry as a disinfectant and sterilization agent thus select for resistance mechanisms.
Drains

- The truth is, it's fairly easy for *Listeria* to spread from your drains to RTE food in your facility.
- **Employees** can spread *Listeria* to clean areas by walking over contaminated trench drain grates.
- **Fruit flies** and other insects can carry *Listeria* from a contaminated drain to production equipment.
- *Listeria* can spread easily **during drain cleaning**.
- **Flooded drains** spread *Listeria*. A clogged drain results in a pool of contaminated water on your production floor, which then spreads *Listeria* during clean-up.
Dirty !!!!

60% of dirty dishcloths contain life threatening bacteria

Listeria was found to be present on around 14% of 200 household dishcloths in Ireland
33% of *L. monocytogenes* linked to domestic behaviour

- European Food Safety Authority (EFSA) study 2013 - 2014
- Due to growth of *Listeria monocytogenes* in
  - Food prepared and stored at home in refrigerator
  - Poor personnel hygiene
  - Refrigerator management
  - Raw vs cooked
  - Sanitation / cleaning

The same is true for all the other foodborne pathogens
The food production chain from the farm to the table
Classification of Foodborne Diseases

Food-Borne Diseases

Poisonings

Chemical Poisoning

Poisonous plant tissues

Mycotoxins

Algal toxins

Intoxication

Poisonous animal tissues

Microbial intoxications

Bacterial

Enteric viruses

Protozoan

Helminthic

Bacterial

E.coli

Salmonellosis

Bacillus cereus

C. perfringens

Listeriosis

Shigellosis

Yersiniosis

Neurotoxins

Clostridium botulinum

Bacterial toxins

Entero toxins

Staphylococcus aureus

Fig. 2: Classification of food borne diseases
Control of pathogenic organisms

- Prevent contamination
  - (Keep them out)
- Destroy foodborne disease agents
  - (Kill them)
- Prevent multiplication of foodborne disease agents
  - (Control them)
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Total 2015</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of deaths</td>
<td>Total # of cases</td>
<td>CFR</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>20,107</td>
<td>0.38</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>11</td>
<td>6,309</td>
<td>0.17</td>
</tr>
<tr>
<td>Listeria†</td>
<td>15</td>
<td>116</td>
<td>12.93</td>
</tr>
<tr>
<td>Salmonella</td>
<td>32</td>
<td>7,728</td>
<td>0.41</td>
</tr>
<tr>
<td>Shigella</td>
<td>1</td>
<td>2,688</td>
<td>0.04</td>
</tr>
<tr>
<td>STEC§ O157</td>
<td>3</td>
<td>463</td>
<td>0.65</td>
</tr>
<tr>
<td>STEC non-O157</td>
<td>1</td>
<td>796</td>
<td>0.13</td>
</tr>
<tr>
<td>Vibrio</td>
<td>5</td>
<td>192</td>
<td>2.60</td>
</tr>
<tr>
<td>Yersinia</td>
<td>1</td>
<td>139</td>
<td>0.72</td>
</tr>
</tbody>
</table>
### Percentage change in incidence of confirmed or CIDT-positive* bacterial, confirmed parasitic, and hemolytic uremic syndrome (HUS) in 2016† compared with 2013–2015 average annual incidence, by pathogen, FoodNet

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Confirmed % Change‡</th>
<th>(95% CI)</th>
<th>Confirmed or CIDT Positive % Change‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>11%↓</td>
<td>(18%↓ – 3%↓)</td>
<td>3%↑</td>
</tr>
<tr>
<td>Listeria</td>
<td>4%↑</td>
<td>(18%↓ – 30%↑)</td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td>2%↑</td>
<td>(4%↓ – 8%↑)</td>
<td>6%↑</td>
</tr>
<tr>
<td>Shigella</td>
<td>7%↑</td>
<td>(17%↓ – 38%↑)</td>
<td>25%↑</td>
</tr>
<tr>
<td>STEC</td>
<td>21%↑</td>
<td>(3%↑ – 42%↑)</td>
<td>43%↑</td>
</tr>
<tr>
<td>Vibrio</td>
<td>2%↑</td>
<td>(18%↓ – 26%↑)</td>
<td>16%↑</td>
</tr>
<tr>
<td>Yersinia</td>
<td>29%↑</td>
<td>(2%↑ – 64%↑)</td>
<td>91%↑</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>45%↑</td>
<td>(11%↑ – 89%↑)</td>
<td></td>
</tr>
<tr>
<td>Cyclospora</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HUS</td>
<td>15%↓</td>
<td>(42%↓ – 25%↑)</td>
<td></td>
</tr>
</tbody>
</table>
Salmonella was discovered in 1885
Is one of the most important foodborne pathogens worldwide
Gram negative, facultative anaerobic, rod shaped bacilli
Consists of two species, *Salmonella enterica* and *Salmonella bongori*

2600 serovars:
**Host restricted serovars** – typhoid-like disease in single host
  - *S. Typhi*, *Paratyphi* in humans and *S. Gallinarum* and *Pullorum* in poultry
**Host adapted serovars** – able to cause disease in other hosts
  - *S. Choleraesius* and *Typhisius*
**Broad host serovars** – wide range of animals
  - *S. Typhimurium* and *Enteritidis* (also known as nontyphoidal serovars)
Salmonella

- **Taxonomy**
- **CDC – 50% of all infections**
  - Salmonella serovar – 100 000 – 1 000 000 cfu/g needed for infection
    - Typhimurium
    - Enteritidis
    - Newport
- **Environment**
  - Water, soil, plants,
  - Do not multiply significantly in natural environment, but what about the fermentation step?
  - They can survive if conditions of temperature, humidity and pH are favorable.
- **Management**
  - Process control, proactive approach
  - Handling distribution, storage
  - Complete food chain
What are they doing in dry products?

- *Salmonella* in baked products and cereal?
- *Salmonella* is one hell of a resilient bacteria
- Dry heat actually makes *Salmonella* more persistent in a food or ingredient
- *Salmonella* is extremely adaptable. Strains will often adapt to whatever stress they are exposed to
- **If *Salmonella* is exposed to dry environments, they are better able to resist heat treatment**
Heat resistance

- *Salmonella* spp. are bacteria that ordinarily are sensitive to heat and high acidity. This sensitivity is often the basis for food processing used to control the presence of the organism. For example, it takes only **3 seconds to achieve a 5-log** reduction in *Salmonella* at **71 °C** in fruit juices.

- While considered heat sensitive, *Salmonella* spp. can become heat resistant in dry food products such as powdered milk or in low water activity products such as chocolate syrup and peanut butter.

- The relationship of *Salmonella* heat resistance to water activity has been well-studied at water activities between 0.99 and 0.85.

- Generally, *Salmonella* becomes more heat resistant as the water activity of a food becomes lower. For example, it takes less than **5 minutes to achieve a 5-log** reduction of *Salmonella* at **60 °C** in a food with a **water activity of 0.99**.

- However, it takes **50 minutes** to achieve the same reduction of *Salmonella* at **60 °C** in a food with a **water activity of 0.85**.
**FDA-BAM Salmonella Protocol**

**Sample**

Buffered Peptone Water

<table>
<thead>
<tr>
<th>Pre-enrichment</th>
<th>34–38°C 18 ± 2 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rappaport Vassiliadis Soya</strong> or <strong>MSRV</strong></td>
<td>and</td>
</tr>
<tr>
<td>Selective enrichment</td>
<td>34–38°C 18 ± 2 hr</td>
</tr>
<tr>
<td><strong>MKTTn</strong></td>
<td>41.5 ± 1°C 24 ± 3 hr</td>
</tr>
</tbody>
</table>

**Plating out**

- **XLD**
- **RAPID'Salmonella** or **XLT 4**
- **or Kristensen** or **Hektoen**

**Selective isolation**

Nutrient 2.1%

**Confirmation**

Biochemical and serological* confirmation

* For details on serotyping, review ISO 6579-3.

**Enrichment broth dependent on matrix being tested**

- **Non-selective pre-enrichment**
- **Rappaport Vassiliadis** and **Tetrathionate**

**Selective enrichment**

- **37°C 24 ± 2 hr**
- **37 ± 1°C 24 ± 3 hr**

**Plating out**

- **42°C 24 ± 2 hr**
- **37°C 24 ± 2 hr**

**Confirmation**

- **TSI and LIA**
- Biochemical and serological confirmation
Peanut Corporation of America’s then-president Stewart Parnell arrives at federal court in 2009. Parnell was sentenced Monday to 28 years in prison for his role in a deadly salmonella outbreak from tainted peanut butter products. **Don Petersen/AP hide caption**

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**How does *Salmonella* get into peanut butter?**

Faeces from some animal is a strong possibility. A leak in the roof, for example, caused one of the early outbreaks. How salmonella got into the water that was on the roof, no one knows for sure. Maybe birds, for instance, which accumulate around peanut butter processing plants.

The roasting of peanuts is the only step that will kill the *Salmonella*. If contamination occurs after the roasting process, the game is over and *Salmonella* is going to survive. Can survive for many months in peanut butter once it's present. Fatty foods are also more protective of *Salmonella*, so when it gets into the acid of the stomach -- which is our first line of defence -- it may not get destroyed. Peanut butter, being a highly fatty food, could survive better.
U.K. sees rise in Campylobacter, Salmonella cases

By Joe Whitworth on January 19, 2019

Campylobacter, Salmonella and non-O157 Shiga toxin producing E. coli (STEC) cases increased in the United Kingdom in 2017, according to a report.

The U.K. Zoonoses Report found Listeria and STEC O157 cases declined. Zoonoses are diseases that can be transmitted from animals to humans. Campylobacter remained the most commonly reported human gastrointestinal pathogen and cases increased in 2017 after a decline over the previous two years.
Child hospitalized in Salmonella outbreak; source unknown

By News Desk on January 21, 2019

A child has been hospitalized in Southern Australia as part of a Salmonella outbreak.

The outbreak has been traced to an early learning center and seven children aged up to four years are affected.
Random amplified polymorphic DNA PCR (RAPD-PCR) DNA fingerprints of Salmonella isolates with (A) primer OPP-16 and (B) primer OPS-11. Lanes 1–12: non-human isolates; lanes 13–18: human isolates and lane M contains a 1-Kb molecular weight DNA ladder.
Salmonella?

ID the Salmonella on XLD
Salmonella Agona outbreak associated with infant formula milk

On 6 December 2017, France reported an outbreak of Salmonella Agona in infants <1 years of age linked to consumption of infant milk formula based on an epidemiological investigation. Different brands of infant formulas from the same producer in France and distributed to different countries inside and outside the EU were implicated as the vehicle of infection in this outbreak.

An outbreak of *Salmonella Agona* linked to the consumption of ..... of *Salmonella* populations, this strain did not produce *H2S* and gas
Detection of *Salmonella*
To minimize the risk of *Salmonella* contamination the following seven elements should be applied to control *Salmonella* in low-moisture products:
1. Prevent spread of *Salmonella* in the processing facility.

- Conduct a hazard analysis to determine potential sources of *Salmonella*, including those associated with facility integrity, air flow, personnel and traffic movement, equipment design and incoming raw materials.
- Segregate ingredients known to be contaminated with *Salmonella* and establish a program to minimize the risk from water usage.
- Educate employees on the potential sources of contamination, adherence to traffic patterns, and proper hygienic practices to follow in order to minimize the spread of *Salmonella* in the processing area.
2. **Enhance the stringency of hygiene practices and controls in the Primary Salmonella Control Area.**

- The Primary *Salmonella* Control Area (PSCA) in a low-moisture product facility is the area where handling of ingredients and product requires the highest level of hygiene control.
- Establish barriers to separate the PSCA from the rest of the facility.
- Control all traffic between the PSCA and the rest of the facility, including the movement of personnel and materials.
- Avoid activities that may lead to contamination of the PSCA.
3. Apply hygienic design principles to building and equipment design.

- Building design and layout should be based on hygienic principles, using common practices such as those outlined in the literature.
- Particular attention should be given to sanitary design, layout and maintenance of equipment located in the Primary Salmonella Control Area (PSCA) to ensure that moisture can be excluded from the processing environment, including the utilization of dry cleaning procedures.
4. Prevent or minimize growth of *Salmonella* within the facility.

- Moisture control is critically important in preventing *Salmonella* contamination in low moisture products.
- Dry conditions must be maintained at all times in the PSCA, except for the occasions when controlled wet cleaning is deemed essential, e.g., in response to a product contamination incident.
- Efforts must be made to remove water immediately from the PSCA in the event of water, for example, leaking water or steam valves, infiltration of water following heavy rains (e.g., leaky roofs), etc. in order to keep the plant environment as dry as possible.
5. Establish a raw materials/ingredients control program.

- “Salmonella-sensitive” ingredients are ingredients that have been historically associated with *Salmonella* (tested positive for the pathogen), have been implicated in past outbreaks, or are used to make products that are intended for at-risk individuals.

- Obtain sensitive ingredients from an approved supplier (one that can provide a high degree of assurance that *Salmonella* is not likely to occur in the ingredient through the implementation of appropriate process controls).

- Evaluate the supplier’s food safety program with respect to a pathogen environmental monitoring program, sanitation practices, raw materials/ingredients storage, a finished product hold and release testing program, process validation, and a corrective action plan if positive *Salmonella* results are found (with evaluation of the potential significance for other products or ingredients manufactured in the processing facility or on the line being evaluated).
6. Validate control measures to inactivate *Salmonella*.

- Determine the target level of *Salmonella* reduction in the product and process under consideration.
- Determine the adequacy of the selected control measure and associated critical limits for processing, keeping in mind the increased heat resistance reported for *Salmonella* at low water activities.
- Challenge studies may be warranted.
- Once the lethality of the process is validated by scientific data, ensure the operation can deliver the critical limits and that the parameters are consistently met through in-plant validation, which is an integral part of the validation process.
- Non-thermal control measures can also be used, with validation, to eliminate *Salmonella*. 
7. Establish procedures for verification of *Salmonella* controls and corrective actions.

- Verification should focus on implementing a robust environmental monitoring program that has been designed to identify transient and/or resident *Salmonella* in the processing areas.
- Environmental monitoring for *Salmonella* is generally conducted on non-product contact surfaces, with samples taken primarily in the Primary *Salmonella* Control Area under normal operating conditions. Product contact surface testing may be done as part of corrective actions for an environmental positive.
- Manufacturers should decide whether or not to conduct finished product testing based on an evaluation of risk.
Campylobacter jejuni

- Mostly linked to raw meat, undercooked poultry, and unpasteurized milk
- Infective dose = about 400-500 bacteria
- Symptoms
  - 2 – 5 days after ingestion
  - Gastroenteritis, nausea, headache, abdominal cramps, diarrhoea
Other infections

♦ Bacteremia
♦ Pancreatitis, nephritis
♦ Abortion & perinatal infection
♦ Myocarditis, endocarditis, aneurysms
♦ Meningoencephalitis, hepatitis
♦ Chronic osteomyelitis
♦ Abscesses: lung, brain, liver, breast
Post infective

♦ Reactive arthritis
♦ Guillain-Barré Syndrome
  ▪ Acute neuromuscular paralysis
  ▪ One third of GBS cases have a *Campylobacter* infection
  ▪ Guillain-Barré syndrome in South Africa associated with *Campylobacter jejuni* O:41 strains
Reservoirs

- Naturally occurring in intestinal tracts of wild & domesticated birds & animals
- Fresh and salt water, sewerage
- Person to person — family contact, nosocomial
- Pets — cats, dogs, hamsters, rabbits
- Unpasteurized milk
- Uncooked or undercooked beef, lamb, poultry
- Uncooked or undercooked liver, offal
- Shellfish
- Butter, cake icing, lettuce & other produce
Cape Town Protocol – filtration

- Mixed ester 0,6 μm, 47 mm diameter filter
- H$_2$-enhanced microaerophilic atmosphere

Results

All species of *Campylobacter*, *Helicobacter* & *Arcobacter* can be isolated from clinical and veterinary samples.
Campylobacter on isolation plate
Escherichia coli

- Inhabits intestinal tract of humans and animals
- Indicator organism of possible faecal contamination of enteric pathogens in foods and water
  - **Enterohemorrhagic E. coli** (*E. coli* O157:H7): Infective dose can be as few as 10 organisms
- Undercooked hamburgers – most common meat source
- Raw milk, fresh fruit and vegetables, yogurt
- **Symptoms**
  - Severe abdominal cramps, vomiting, diarrhoea
E coli as normal flora

- *E coli* colonizes GI tract with hours of birth
- Adheres to mucus of large intestine
- Very common in the mouths and GI tracts of humans & animals
- Benign commensals, usually
- If acquire genetic elements encoding for virulence factors by conjugation, transduction or transformation, can become pathogenic
Important groups

- Enteropathogenic *E. coli* (EPEC)
- Enteroinvasive *E. coli* (EIEC)
- Enterotoxigenic *E. coli* (ETEC)
- Diffuse adhering *E. coli* (DAEC)
- Enterohaemoragic *E. coli* (EHEC)
- Enteroaggregative *E. coli* (EAEC)
Reservoir

- Healthy cattle are the major reservoir for human infection
  - Deer, sheep, goats, horses, dogs, birds and flies
- Bacterial cells can survive in manure and water
- Infection is more common during the summer in both the northern and southern hemisphere
Figure 3—Scanning electron micrographs of stomata on baby spinach leaves inoculated with *E. coli* O157:H7 and stored at 4°C for 0 h (A), 24 h (B), 48 h (C), or 72 h (D). Intrusion and development of cell populations peripheral to and within the stomatal wells are indicated by arrows. (Note magnification change of Figure 3C, enhanced to show detail.)
• Transmitted via food
  • Ground beef
  • Raw milk
  • Lamb meat
  • Venison biltong
  • Salami and other fermented dried meat products
  • Lettuce, spinach, alfalfa sprouts
  • Unpasteurized apple cider

• Transmitted via water
  • Drinking and swimming in unchlorinated water

• Direct person to person contact
  • Diaper changing
  • Improper sanitation
  • Day care & chronic adult care facilities
The Burger That Shattered Her Life
Trail of E. Coli Shows Flaws in Ground Beef Inspection System

By MICHAEL MOSS

Stephanie Smith, a children's dance instructor, thought she had a stomach virus. The aches and cramping were tolerable that first day, and she finished her classes. Then her diarrhea turned bloody. Her kidneys shut down. Seizures knocked her unconscious. The convulsions grew so relentless that doctors had to put her in a coma for nine weeks. When she emerged, she could no longer walk. The affliction had ravaged her nervous system and left her paralyzed.

Ms. Smith, 22, was found to have a severe form of food-borne illness caused by E. coli, which Minnesota officials traced to the hamburger that her mother had grilled for their Sunday dinner in early fall 2007.

"I asked myself every day, "Why me?" and "Why from a hamburger?" Ms. Smith said. In the simplest terms, she ran out of luck in a food-safety game of chance whose rules and risks are not widely known.

Meat companies and grocers have been barred from selling ground beef tainted by the virulent strain of E. coli known as O157:H7 since 1994, after an outbreak at Jack in the Box restaurants left four children dead. Yet tens of thousands of people are still sickened annually by this pathogen, federal health officials estimate, with hamburger being the biggest culprit.
Anatomy of a burger

- Typical burger sold by fast food restaurants
- Made by food giant – Cargill
- 844812 pounds of patties recalled (422 tons)
- 940 people were sickened
- Including Stephanie Smith (22)
Combinations of the O & H antigens identify the serotype

ETEC
Enterotoxigenic *E. coli*

EPEC
Enteropathogenic *E. coli*

EHEC
Enterohemorrhagic *E. coli*

EAEC
Enteroaggregative *E. coli*

EIEC
Enteroinvasive *E. coli*

---

**Clinical Microbiology Reviews 1998 11:142-201**
Clinical Features

- Average interval between exposure & illness is 3 days
- Most patients recover with 7 days
- 70% of patients report bloody stools
- 30-60% of patients report vomiting
- Approx 5% of patients develop HUS

Figure 2: Natural history of post-diarrhoeal HUS
**E. coli O104:H4 - Germany**

As of June 18\textsuperscript{th}
3222 outbreak cases
39 deaths
810 Hemolitic uremic syndrome – 27 deaths
2412 Shiga toxin producing *E coli* – 12 deaths

<table>
<thead>
<tr>
<th>Date</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 May</td>
<td><strong>Start of outbreak</strong></td>
</tr>
<tr>
<td>8 May</td>
<td>Outbreak grew dramatically</td>
</tr>
<tr>
<td>21 May</td>
<td>HUS peak</td>
</tr>
<tr>
<td>23 May</td>
<td>Shiga Toxin peak</td>
</tr>
<tr>
<td>24 May</td>
<td>Hospital peak, strain ID O104:H4</td>
</tr>
<tr>
<td>26 May</td>
<td>Isolated bacteria from Cucumbers - Germany</td>
</tr>
<tr>
<td>28 May</td>
<td>UK – people had fallen ill</td>
</tr>
<tr>
<td>31 May</td>
<td>First person outside Germany has passed away</td>
</tr>
<tr>
<td>1 June</td>
<td>Germany admitted strain on Cucumber not the disease strain</td>
</tr>
<tr>
<td>2 June</td>
<td>Identified farm south of Hamburg – Bean Sprouts</td>
</tr>
<tr>
<td>3 June</td>
<td>Advised people not to eat sprouts</td>
</tr>
<tr>
<td>4 June</td>
<td>Imported seeds, two farm employee's had the infection</td>
</tr>
</tbody>
</table>
|            | Search reached restaurant were on 13 May people have dined, by now 18 had become ill,
|            | one died, two other had HUS                                                       |
| 10 June    | Scientific evidence showed that cause was contaminated seeds from farm           |

News Flash
27 July 2011
53 people have died
Points to remember – *Staph aureus*

- Heat stable toxin (D$_{98.9}$-2h)
- Toxin dose – less than 1 $\mu$g/kg
- Amount of toxin produced by $10^5$ cfu and more
- Toxin produced between 10°C and 46°C, with the optimum between 40°C and 45°C.
- Present in nasal passages, throats, skin, hair of 50% or more healthy individuals
- Lack of hygiene standards – transmission via hands
- Keep hot foods hot (above 60°C)
- Keep cold foods cold (below 8°C)
- Antibiotic resistant strains in food chain
Key Practices in Control and Tracing

• Key Practices in Control
  • HACCP
  • Maintain and clean the processing environment
  • Establish good personal hygiene and clean working practices
    • Training of personnel
  • Clean food contact surfaces
  • Prevent cross contamination
  • Control Water

• Tracing Pathogens
  • Determine hotspots
  • Further information from molecular typing
  • Finally analyse the results

• Food Safety Management revolution !!!!!
This can only be achievable if HACCP become mandatory for sectors of the food industry. The relevant industries should adopt HACCP as an urgent priority. The detection of *L. monocytogenes* in the food-processing environment should be considered evidence that the pathogen is "reasonably likely to occur" and therefore must be addressed in the hazard analysis critical control point (HACCP) plan. Therefore, HACCP becomes an integral part of controlling the organism in the food industry.

To summarize, make HACCP mandatory in the industry, use Codex Alimentarius Commission as scientific reference point for decision making related to new regulations.

Food Safety Management Team

Understanding the risk (*Listeria, Salmonella* etc)
Food Safety Trends

• New and novel Pathogens
• Antibiotic resistance bacteria
• Incorrect use of sanitisers
• Polluted irrigation water
• Global warming – microorganism will adapt
• Survival of pathogens in dry products
Control the risks

- Hurdle concept
  - Heat
  - Water activity
  - pH
  - Packaging
  - Preservative
  - Additives
  - Etc.
- Know your enemy
- Correctly identified the risks
- Scientific research
  - Peer reviewed articles

ISO 22000
HACCP

FOOD SHOULDN'T BE DANGEROUS.
"Messieurs, c’est les microbes qui auront le dernier mot" (Gentlemen, it is the microbes that will have the last word)
Questions and comments
Centre for Food Safety - HUB

- Innovation through collaboration
- Multidisciplinary approach
- Partnered approach to strengthening the food safety system in SA
- It will support and encourage research partnerships and alliances with other entities, both nationally and internationally
Centre for Food Safety

Scientific research and training in the all aspects of food safety

• To consolidate and integrate current thinking on food safety
• To perform cutting edge research in the area of food safety by means of collaborative and multidisciplinary teams
• To make available the results of research by means of publications and research papers.
• To communicate results to industry by means of workshops and technical reports

Consumer engagement and awareness

• To protect people’s health through provision of information that enables them to make informed food safety decisions
• Provide objective information continuously, develop communication material that will be beneficial for effective consumer education
• To make available the results of research by means of seminars, short courses and the dissemination of findings to relevant stakeholders, including the broader community by means of effective industry and consumer education

Food Safety Policy

• The Centre will use a science based approached
• The Centre will actively translate research into relevant, constructive and realistic policy options for consideration by relevant authorities
• The Centre will be proactive members on the relevant committees and provide leadership
Innovation through collaboration

“Our vision is to become Africa's leading research-intensive university, globally recognised as excellent, inclusive and innovative, where we advance knowledge in service of society,” Prof W de Villiers
Value Proposition to the Food industry

• Enhanced consumer safety within the South African food system

Value of the CFS@SU to its members

• Research that makes a tangible difference to South Africa’s food systems
• Enhanced international reputation for safe and high quality food
• Access to trusted, independent and credible food safety research, knowledge and advice
• Contributing to critical mass and enhanced capability in the science of food safety
• Access to work of collaborative networks in South Africa and internationally
• Identification of emerging issues and guidance on addressing identified risks
• Enhanced knowledge dissemination at the science/academia/industry interface

What is of importance is that the Centre of Food Safety should deliver credible high quality food safety research that is led by experts in the field.
Strategic Objectives

**High Quality Science**
- Deliver credible high quality food safety research that is led by experts in the field

**Respond to emerging risks and opportunities**
- Use the Centre’s extensive collaborative networks to develop systems for identifying and responding to emerging food safety risks

**Outputs that matter**
- Translate food safety science and research to ensure tangible impact throughout the food industry, government and consumers
This will be achieved by:

• **Develop an improved food safety culture** – Educate and raise awareness to support an increase in food safety culture in South Africa

• **Growing capability in food safety** – in science and government to collectively future proof South Africa’s food safety ecosystem

• **Innovating for food safety** – provide the science base for safety in food innovation

• **Design programmes that support collaboration** – design programmes that build critical mass and collaboration to minimise fragmentation and duplication

• **Being the food safety partner of choice** – be recognised as the preferred food safety partner

• **Providing trusted food safety advice** – provide trusted, independent, timely and credible food safety advice
The Centre of Food Safety (CFS) will be part of the Department of Food Science

Industry memberships

International networks

“Our vision is to become Africa’s leading research-intensive university, globally recognised as excellent, inclusive and innovative, where we **advance knowledge in service of society**,“ Prof W de Villiers
Scientific Advisory Board

**Prof Wilhelm Holtzapfel**
President of the International Commission on Food Microbiology and Hygiene

**Prof Mieke Uyttendaele**
Department of Food Safety and Food Quality, University of Ghent

**Prof Stephen Forsythe**
Retired Professor of Microbiology at Nottingham Trent University. Author of “The Microbiology of Safe Food”.
Pier Sandro Cocconcelli, is Chair Professor of Food Microbiology at the Università Cattolica del Sacro Cuore (UCSC). He is Rector’s delegate for internationalization projects of the same university and the president of CHEI, the Centre for Higher Education Internationalisation at UCSC, which promotes and conducts research, training, and policy analysis to strengthen the international dimensions of higher education.

- Since 2003, he is scientific expert of the European Authority of Food Safety (EFSA) as Panel and Working Group member focusing on the microbiological risk assessment.
- From 2006 to 2010 he has chaired the Standing Working Group on Microorganisms of FEEDAP and now he chairs the Standing Working Group of Genetically Modified Microorganisms.
- He is also member of the BIOHAZARD Working Group on Qualified Presumption of Safety of Microorganisms.
- He is member of the EFSA-EMA (European Medicine Agency) groups on the Alternatives to antibiotics in animal nutrition.
Centre for Food Safety - Team

Scientific research and training in all aspects of food safety

Consumer education and awareness

Food Safety Standards and Policy
Centre for Food Safety at Stellenbosch University

- Food Safety is an essential public health issue for all
- Science-based food controls are essential for the protection of food products
- Provide expert opinion and academic support to the industry
- Contribute to the knowledge of food safety
- Food Safety Revolution
- Innovate through collaboration
- Develop and exchange knowledge, experience, and expertise in the areas of food safety, food defence and food processing
Prof Pieter Gouws
pgouws@sun.ac.za