Introduction

- New Zealand is a geographically isolated country, similar in size to Great Britain, with a population of 4 million
- The economy has a strong agricultural and pastoral focus

Background

- Foodborne disease in NZ estimated at $80 billion, 90% attributed to the loss of productivity due to the absence from work
- Salmonellosis is a worldwide public health problem
- National surveillance of Salmonella is carried out at ESR’s NCBID Wallaceville Centre

NCBID

- Joint initiative from:
  - MAF Biosecurity New Zealand (Ministry of Agriculture and Forestry)
  - Environment, flora, fauna, marine life, Māori resources
  - AgResearch’s Infectious Disease diagnostic team (Animal Health)
  - ESR (People and their Environment)
  - AsureQuality (Food safety)
- Approximately 90 microbiologists, molecular biologists, disease modellers, epidemiologists and researchers
- Access to the only infectious disease laboratory that provides a PC3+ level of containment in NZ
- Increases NZ’s capability to protect human and animal health
Microbiology Laboratory
- Enteric Reference Laboratory
  - Provides national reference and surveillance laboratory services
  - Human, animal, food and environmental enteric bacterial pathogens,
  - Salmonella, Shigella, Yersinia, toxigenic Escherichia coli (VTEC), Vibriobacteri.
  - Identification and characterisation using standard methods: Biochemical profiling (metabolic activity), serology, phage typing, biotyping and molecular typing (PCR and PFGE).
  - Reference Laboratory: Only lab in NZ with the capability to identify and type enteric bacterial pathogens
  - Surveillance:
    * Incidence and distribution
    * Introduction of new species/strain in NZ
    * Outbreak detection and investigation
- Leptospira Reference Laboratory
  - Screening and confirmatory tests for common Leptospira serovars found in NZ.

Salmonella
- Salmonella genus has two species:
  - S. enterica divided into 6 subspecies (> 2579 serovars)
    * Human infections predominantly caused by subspecies I (enterica) and subspecies II (salmae)
      * S. bongori
    - Salmonella enterica subspecies enterica
      - serovar Typhimurium
      - Salmonella Typhimurium
    - Salmonella Typhimurium is our most common serotype

Serotyping of Salmonella
- Serotyping: useful tool for surveillance
- Robust and comparable worldwide
- Based on identifying the O antigen on bacterial cell wall (lipopolysaccharide) and H antigen on flagella (protein)
  - 99% of human infections caused by O Groups A, B, C, D, and E
- Based on Kauffman White scheme
  - S. Typhimurium (Group B)
    - 4,5,12:i,1,2
      - (O antigen): (H antigen)

Phage typing of Salmonella
- S. Typhi
- S. Typhimurium
- S. Enteritidis
  - Selective ability of bacteriophages to infect certain strains of Salmonella
  - Subsequent bacterial lysis
  - Formation of plaques
Molecular typing: PFGE and MLVA

Pulsed-field Gel Electrophoresis (PFGE)
- Molecular “fingerprinting” technique reflecting DNA sequence of the entire bacterial genome (Degree of genetic diversity)
- “Gold standard”: CDC has developed and standardized protocols
  (E. coli O157:H7, Salmonella, Campylobacter jejuni, Staphylococcus aureus, Yersinia pestis, Listeria monocytogenes)
- Rare-cutting enzymes are used to cut DNA
- Separated by pulsed-field electrophoresis
- Computer software to analyse and compare PFGE patterns
- Stored in database for future reference (PulseNet)
- More discriminatory than phenotypic methods, accurate, reproducible, allows comparison between laboratories
  - To track spread of foodborne pathogens
  - Some bacteria are highly clonal (limited number of PFGE profiles)

Analysis of PFGE using Bionumerics software: Dendogram

MLVA
- Discrimination between isolates is based on loci distributed throughout the bacterial genome harboring variable numbers of short repetitive DNA sequences (tandem repeats) (VNTR)
- The number of tandem repeats for each locus varies between isolates
- This variability was exploited by the development of MLVA (Multiple Locus VNTR Analysis)
- MLVA is used to determine the relatedness of isolates
- Amenable to standardization and high-throughput analysis
- Methods have now been developed for large number of bacterial species and are being used internationally
- S. Typhimurium: (Lindstedt et al. 2003) Salmonella Typhimurium DT104
  - 5 VNTR (STTR3, STTR5, STTR6, STTR9, STTR10)
PCR amplification

Genetic analyzer is used to determine the size of the amplicon

Once the size is known, the number of repeat sequences is calculated

This is repeated for multiple loci and results for all loci are represented as a string of numbers.

Emergence of DT RDNC-May 06

- First human isolate 11th May 2006 from a 3 year old male in Auckland.
- No history of overseas travel.
- No source of infection determined.
- Next 2 years: Auckland, Hawke’s Bay, Northland, Whanganui, Waikato
- June 2008: First human case in the South Island (2Y male, Canterbury)
- Dec 2008: Southern (5Y male)
- Spread more in the North Island: Mid Central, Lakes, Capital Coast, Bay of Plenty
**Human S. Typhimurium DT**

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<th>Number of Isolates</th>
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<td>2006</td>
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<tr>
<td>2010</td>
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**Epidemiology of DT RNDC-May 06**

- Total number of cases 2006-2010 = 250 confirmed by ERL
  - 55% are children aged 6 years or under (50% are male)
  - 50% are Male
  - Ethnicity: European (59%), Māori (13%), Asian (4%), Pacific Island (2%), Other (22%)
- No deaths has been reported
- 12% of cases have been hospitalised
- Risk factors: Food premises (16%), contact with farm animals (14%), drinking untreated water (10%), recreational water (5%), contact with symptomatic people (3.5%), recent overseas travel (0.4%)

**Non-human isolates of DT RDNC-May 06**

**Non-Human isolates (2006-2010)**
- May 2006: Equine
- Aug 2006: Porcine
- September 2006: Feline, Bovine
- December 2006: Avian, Poultry feed
- Since then has been confirmed in Lapine, Canine, Food (lettuce), Alpaca, Caprine, but NOT in Ovine

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<td>2009</td>
<td>22</td>
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<tr>
<td>2010</td>
<td>39</td>
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**Conclusion**

- Spread from a single location to entire country
- Peaked at 85 (2010) of total human *Salmonella* isolates (n = 1195).
- % increase in 4 years: 1.1% (2006) to 7.1% (2010)
  - Jan-Jun 2007: 18 human isolates
  - Jan-Jun 2011: 42 human isolates
- Established as a pathogen in animals, particularly cats, cattle and horses
- DT RDNC-May 06 is isolated most of the year and has the typical spring/summer peak of *Salmonella* species
- DT RDNC-May 06 increased at expense of other DTs and is becoming our predominate phage type
- This phage type has not yet been seen by the Australian *Salmonella* Reference Centre (Dianne Davos - Personal communication)
- Source of New Zealand strain remains unknown

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