Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
Epidemiological investigations of Shiga toxin-producing *Escherichia coli* (STEC) O157 and STEC O26 in New Zealand slaughter cattle, and the source attribution of human illness

A thesis presented in partial fulfilment of the requirements for the degree of

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in

Veterinary Science

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New Zealand.

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Abstract

Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) and related non-O157 STEC strains are enteric pathogens of significant public health concern worldwide, including New Zealand, causing clinical diseases ranging from diarrhoea and bloody diarrhoea to the life-threatening haemolytic uraemic syndrome. Cattle are considered the principal hosts and have been shown to be a source of STEC infection for both foodborne and environmental outbreaks of human diarrhoeal disease overseas. A series of observational studies were conducted to gain knowledge on the epidemiology of STEC O157 and STEC O26 in New Zealand slaughter cattle and assess the relative importance of cattle as a source of domestically-acquired STEC infections in humans.

A repeated cross-sectional study conducted on four selected New Zealand beef slaughter plants provided detailed data on the prevalence and concentration of faecal shedding of STEC O157 and STEC O26 in 695 very young calves (4–7 days-old) and 895 adult cattle post-slaughter, identifying calves as more prevalent carriers of STEC. Findings of a subsequent cohort study, the first of its kind, provided evidence that for the 60 calves examined, transportation and lairage was not associated with increase of faecal shedding of *E. coli* O157 and O26 (STEC and non-STEC) but increase of cross-contamination of hides and carcasses post-slaughter.

In a national prospective case-control study, 113 STEC cases and 506 random controls were interviewed for risk factor evaluation. The study findings implicate that environmental and animal contact, but not food, as significant exposure pathways for sporadic STEC infections in humans in New Zealand, and suggest ruminants as the most important source of infection. The molecular analysis of bovine and human STEC O157 isolates provided evidence for the historical introduction of a subset of the globally-circulating STEC O157 strains into New Zealand and ongoing localised transmission of STEC between cattle and humans.

These findings will contribute to the development of a risk management strategy for STEC, similar to those already implemented for *Campylobacter*, *Salmonella*, and *Listeria*, which
pose a high risk to public health and New Zealand’s access to international markets. Furthermore, risk factors identified in the case-control study will contribute to the design of public health interventions to reduce the incidence of STEC infections in New Zealand.
Acknowledgements

It was five years ago, April 2009, when I set sail to a long but adventurous journey, called a PhD. It was a life-changing journey loaded with numerous rewarding challenges, fulfilling experiences, and valuable friendships I have made. There are a number of people to whom I express my deep gratitude for their considerable help and contribution to this rather large research project and thesis.

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Most of all I would like to express my special thanks and love to my parents in Switzerland and Bryce for continued moral support, encouragement and love throughout my PhD.
Declaration

Studies presented in this thesis are written in manuscript style prepared for publication in peer-reviewed journals, hence there is some repetition, particularly in the introductions and methodologies. Two chapters have been formatted in style required of the journals, to which they have been submitted. Co-authors of the papers have made their contributions to the research and/or manuscripts, however, my input was the greatest as I designed the studies, conducted all the fieldwork; entered and analysed all data, and wrote the manuscripts. Angie Reynolds, a designated laboratory technician from the "EpiLab, helped me with the processing of samples.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>A/E lesions</td>
<td>Attachment and effacement lesions</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike information criterion</td>
</tr>
<tr>
<td>AMOVA</td>
<td>Analysis of molecular variance</td>
</tr>
<tr>
<td>BPW</td>
<td>Buffered peptone water</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CT-RMAC</td>
<td>Cefixime-tellurite rhamnose MacConkey agar</td>
</tr>
<tr>
<td>CT-SMAC</td>
<td>Cefixime-tellurite sorbitol MacConkey agar</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EHEC</td>
<td>Enterohaemorrhagic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>ERL</td>
<td>Enteric Reference Laboratory</td>
</tr>
<tr>
<td>ESR</td>
<td>Institute of Environmental Science &amp; Research Ltd</td>
</tr>
<tr>
<td>HUS</td>
<td>Haemolytic uraemic syndrome</td>
</tr>
<tr>
<td>IMS</td>
<td>Immunomagnetic separation</td>
</tr>
<tr>
<td>MPN</td>
<td>Most probable number</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed-field gel electrophoresis</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>SBI</td>
<td>Shiga toxin-encoding bacteriophage insertion</td>
</tr>
<tr>
<td>STEC</td>
<td>Shiga toxin-producing <em>Escherichia coli</em></td>
</tr>
<tr>
<td>Stx</td>
<td>Shiga toxin</td>
</tr>
<tr>
<td>TSB</td>
<td>Tryptic (or tryptone) soy broth</td>
</tr>
<tr>
<td>UPGMA</td>
<td>Unweighted pair group method with arithmetic mean</td>
</tr>
<tr>
<td>US or USA</td>
<td>United States of America</td>
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</tbody>
</table>
Pastoral farming
New Zealand’s beef and dairy production systems are pasture-based with year-round grazing (supplemented with hay, baleage, maize silage, or palm kernel during winter), outdoor housing, and seasonal calving in late winter/early spring (July/August to September/October). In 2013, 3.7 million beef cattle and 6.6 million dairy cattle (including bobby calves) were recorded [1].

Bobby calves
Bobbies are milk-fed calves, which are not reared for dairy replacement stock or the dairy beef market, but are slaughtered at 4–7 days of age and exported as veal to overseas markets (mainly the US).

Cattle slaughter system
Slaughter cattle in New Zealand comprise bobby calves and adult cattle. Adult slaughter cattle consist of dairy and beef animals, which are classified according to sex and maturity as bulls, steers, heifers, and cows [2]. Bulls are entire male cattle, compared to steers, which were castrated at a young age; heifers and cows are female cattle with ≤4 and >4 permanent incisors, respectively.

Beef slaughter plant
In 2013, there were 55 beef slaughter plants actively processing adult cattle (and bobby calves) including slaughter and dressing and/or further processing of carcasses at primary industry level. Of those 55 beef plants, 42 were listed on the National Microbiological Database (NMD) as undertaking STEC-testing of beef as required for overseas market access.

Transportation and lairage of slaughter cattle
Adult cattle are usually transported as a mob in a livestock transporter from a farm to a slaughter plant where the animals are kept in separate holding pens in the lairage area until
slaughter. Adult cattle of the same mob are slaughtered consecutively as a line. In contrast, bobby calves are collected by a regional livestock transporter, picking-up calves from local dairy farms on a pre-determined route, and transported collectively to a slaughter plant where the calves are kept in large holding pens until slaughter. Bobby calves are slaughtered as large groups from different regions.

**National Microbiological Database (NMD)**

NMD is a mandatory food safety programme for New Zealand primary processors of meat, poultry, game and ratites, which receive live animals for slaughter, dressing or other processing to produce food suitable for human consumption [3]. The microbiological monitoring programme is controlled by the Ministry for Primary Industries (MPI, Wellington, New Zealand) and aims to minimise the incidence of foodborne pathogens. It ensures common microbiological standards for food sold on the domestic market and to meet the export requirements set by destination countries.

**Human disease notification system**

General practitioners/medical centres/hospitals notify cases of STEC infections to regional Public Health Units. Any notified case is then investigated by a Public Health Officer/Medical Officer, who completes a case report form and enters epidemiological data into a national surveillance database (EpiSurv) used for notified cases of communicable and other diseases. EpiSurv is held by the Institute of Environmental Science & Research Ltd (ESR). Most of STEC cases are confirmed by culture isolation of STEC from clinical specimen submitted to medical laboratories or the Enteric Reference Laboratory (ERL).

**EpiLab**

Unless stated otherwise, laboratory testing of samples and isolates used in studies presented in this thesis was conducted at the Molecular Epidemiology and Public Health Laboratory (”EpiLab), which is located within the Hopkirk Research Institute at Massey University in Palmerston North, New Zealand.
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“Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning.”

*Albert Einstein*