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

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Patricia Jaros

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[m]EpiLab, Hopkirk Research Institute
Institute of Veterinary, Animal and Biomedical Sciences
Massey University
Palmerston North, New Zealand

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.

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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 ^{m}EpiLab, helped me with the processing of samples.

Nomenclature

A/E lesions	Attachment and effacement lesions
AIC	Akaike information criterion
AMOVA	Analysis of molecular variance
BPW	Buffered peptone water
CFU	Colony forming unit
CI	Confidence interval
CT-RMAC	Cefixime-tellurite rhamnose MacConkey agar
CT-SMAC	Cefixime-tellurite sorbitol MacConkey agar
DNA	Deoxyribonucleic acid
EHEC	Enterohaemorrhagic *Escherichia coli*
ERL	Enteric Reference Laboratory
ESR	Institute of Environmental Science & Research Ltd
HUS	Haemolytic uraemic syndrome
IMS	Immunomagnetic separation
MPN	Most probable number
OR	Odds ratio
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
rRNA	Ribosomal ribonucleic acid
SBI	Shiga toxin-encoding bacteriophage insertion
STEC	Shiga toxin-producing *Escherichia coli*
Stx	Shiga toxin
TSB	Tryptic (or tryptone) soy broth
UPGMA	Unweighted pair group method with arithmetic mean
US or USA	United States of America

Glossary – New Zealand specific descriptions and terminology

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).

*m*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 (*m*EpiLab), which is located within the Hopkirk Research Institute at Massey University in Palmerston North, New Zealand.

List of Publications

Jaros P, *et al*. (2014) Geographic divergence of bovine and human Shiga toxin-producing *Escherichia coli* O157:H7 genotypes, New Zealand. *Emerging Infectious Diseases*, doi: 10.3201/eid2012.140281 (e-published ahead of print).

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Jaros P, *et al*. (2014) Between- and within-island divergence of bovine and human Shiga toxin-producing *Escherichia coli* O157:H7 genotypes in New Zealand. In: *Proceedings of the Food Safety, Animal Welfare & Biosecurity Branch of the New Zealand Veterinary Association (NZVA)* (16–20 Jun 2014; Hamilton, New Zealand), pp 127-128.

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Jaros P, *et al*. (2012) Population dynamics of *E. coli* O157 and O26 – the effect of transport and lairage on faecal shedding and carcase contamination of very young calves in New Zealand. In: *Proceedings of the 8th International Symposium on Shiga Toxin (Verocytotoxin) Producing Escherichia coli Infections, VTEC 2012* (6–9 May 2012; Amsterdam, The Netherlands), pp 70-71.

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Jaros P, *et al*. (2011) Shedding of *Escherichia coli* O157:H7 and O26 STEC by slaughter cattle in New Zealand. In: *Book of Abstracts of the Annual meeting of NZMS* (23–25 Nov 2011; Palmerston North, New Zealand), p 86.

Jaros P, *et al*. (2010) Prevalence of faecal shedding of *E. coli* O157:H7 and non-O157 STEC in New Zealand slaughter cattle. In: *Book of Abstracts of the Annual meeting of NZMS* (30 Nov–3 Dec; Auckland, New Zealand), p 129.

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Preface

"Learn from yesterday, live for today, hope for tomorrow.
The important thing is not to stop questioning."

Albert Einstein