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**A food chain approach to control of  
Shiga toxin-producing  
*Escherichia coli* in New Zealand**

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

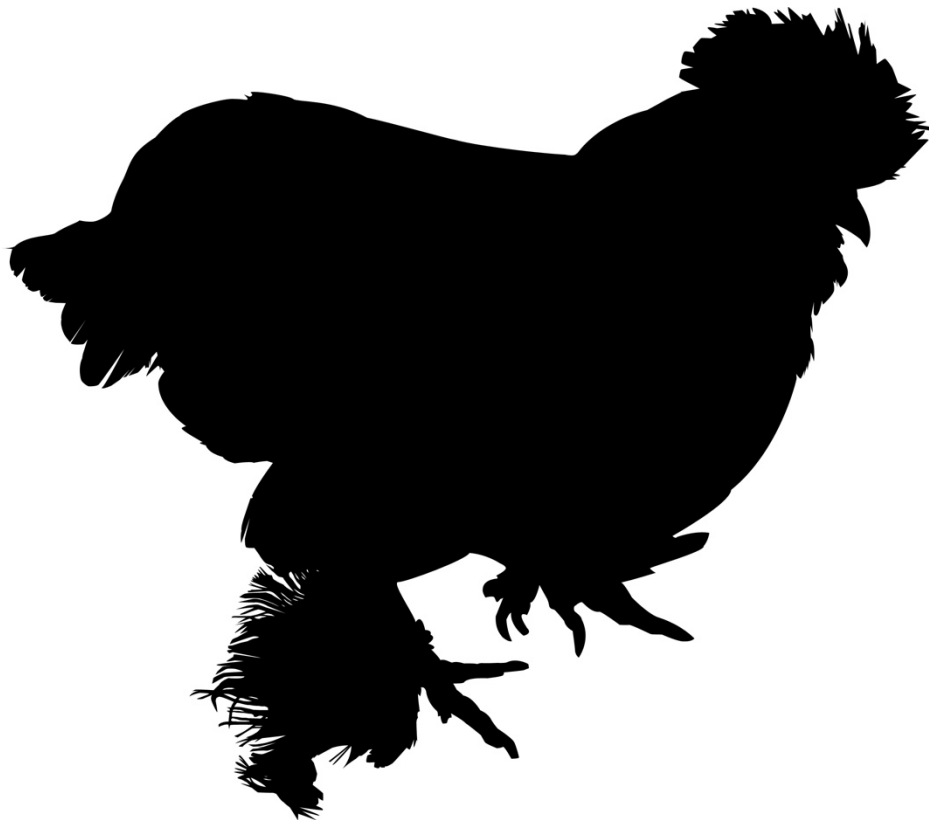
of Doctor of Philosophy in Veterinary Science

at Massey University, Palmerston North, New Zealand

**Andrew Springer Browne**

**2018**

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# Abstract

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This thesis describes the prevalence and molecular epidemiology of Shiga toxin-producing *Escherichia coli* (STEC) in New Zealand using microbiological, genomic, molecular, and statistical methods. STEC are a zoonotic pathogen that can cause bloody diarrhoea and acute kidney failure. Cattle are a well-recognized STEC reservoir, and previous research has identified living near cattle and contact with their faeces as an increased risk for human infection. Seven STEC serogroups (O157, O26, O45, O103, O111, O121, O145), known as the ‘Top 7’ STEC, have been identified as an increased risk to human health, with the New Zealand meat industry undertaking testing to ensure that veal beef exports to some international markets are free of these ‘Top 7’ serogroups.

A random stratified cross-sectional study of ‘Top 7’ STEC prevalence of young dairy calves (n=1,508) on New Zealand dairy farms (n=102) found that approximately 20% of calves and 75% of farms were positive for one or more of the ‘Top 7’ STEC. ‘Top 7’ STEC prevalence was positively associated with increased number of calves in a calf pen, and prevalence significantly varied by region. This study utilized a new culture-independent diagnostic test, NeoSEEK (PCR/MALDI-TOF method), and used statistical and microbiological techniques to evaluate the sensitivity and specificity of the method for this and further studies.

A longitudinal study evaluating prevalence and transmission of ‘Top 7’ STEC in animals and the dairy farm environment found evidence of calf-to-calf, dam-to-calf, and environment-to-calf transmission. Whole genome sequencing analysis and

prevalence data revealed cross-contamination of young veal calf hides occurs during transport and lairage to processing plants.

Analysis of New Zealand serogroup O26 bacterial isolates (n=152), in comparison to publicly available genome sequence data (n=252) from other countries (n=14), suggested introduction of STEC and non-STEC O26 into New Zealand during few periods in the 20<sup>th</sup> and early 21<sup>st</sup> century. Populations of New Zealand serogroup O26 *E. coli* are monophyletic, possibly due to minimal live cattle importations into the country.

Further research in this area should focus on effective interventions at the farm and meat processing level to decrease the risk of veal beef contamination, while protecting public health.

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## List of Publications

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1. Browne, AS, Midwinter, AC, Withers, H, Cookson, AL, Biggs, PJ, Marshall JC, Benschop, J, Hathaway, S, Haack, N, Akhter, R, and French, NP. Molecular epidemiology of Shiga toxin-producing *Escherichia coli* (STEC) on New Zealand dairy farms: application of a culture-independent assay and whole genome sequencing. *Applied and Environmental Microbiology*, accepted for publication.
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## List of Presentations

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February 22<sup>nd</sup>, 2018

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# Glossary and Abbreviations

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Adulterant	A poisonous or deleterious substance on a carcass or meat product that can be injurious to human health
Allele	An alternate form of a gene that arises due to a fixed substitution in a nucleotide
Antibiotic	A medicine that inhibits the growth or destroys bacteria
Beef trim	Smaller pieces of beef muscle used in the production of ground beef products
Bobby calf	In New Zealand, a calf between the ages of four and ten days that is slaughtered for veal meat. The calf usually is born in a dairy herd, where the calf is surplus to requirements for replacement animals in the herd and is not viable for meat production.
CIDT	Culture independent diagnostic test; in comparison to methods where bacteria are isolated on nutrient agar
Clade	A group of descendants of a common evolutionary ancestor
Dam	The bovine mother of a calf
eae	intimin; a virulence gene that facilitates attachment of <i>E. coli</i> to the epithelial cells in the intestine
Enrichment broth	A nutrient broth that is mixed with bacteria and incubated at a specific temperature over a specific time in



	order to increase the number of bacteria present
HUS	Haemolytic uremic syndrome; a clinical presentation of haemolytic anemia (low red blood cell count due to destruction of red blood cells), acute kidney failure (anuria, lack of urine production), and thrombocytopenia (low platelet count); associated with severe clinical cases of STEC
MLST	Multilocus sequence typing; a method of differentiating organisms based on the variations (alleles) in seven housekeeping genes, in order to assign a sequence type (ST)
MPI	Ministry of Primary Industries; a public service department of New Zealand, in charge of overseeing, managing, and regulating the farming, food, and biosecurity sectors in New Zealand
PCR	Polymerase chain reaction; a molecular detection method where a pair of primers, sequences of DNA that are specific markers for a gene or number of genes, are amplified and detected in an agarose gel by the length of the sequence
PCR/MALDI-TOF	Polymerase chain reaction / Matrix Assisted Laser Desorption/Ionization – Time of Flight; a culture independent diagnostic test where a sample is ionized and then molecules

	are detected using time of flight mass spectrometry, with specific molecular mass indicating specific targets for detection; this method is used by the NeoSEEK assay
PFGE	Pulse field gel electrophoresis; a DNA fragmentation technique to produce a “DNA fingerprint” of particular bacteria
Phylogenetic tree	A branching diagram to illustrate evolutionary relationships of organisms based on similarities or differences of genetic characteristics
Potential STEC	In this thesis, this refers to an enrichment sample that tests positive for a <i>stx</i> gene as well as the <i>eae</i> gene, but may or may not have an STEC bacterium ( <i>stx</i> and <i>eae</i> present) present in the sample
Prebiotic	In animals, a non-digestible carbohydrate that promotes the growth of microorganisms in the intestines which may benefit health
Probiotic	A mixture of microorganisms that are ingested by animals that may promote intestinal health
$R_0$	Basic reproduction number; in epidemiology, this refers to the number of cases of disease caused by one infective individual
RAMS	Recto-anal mucosal swab; a sterile cotton tipped swab is inserted into

	<p>the rectum of a cow; this sample is then enriched in liquid media to increase detection of STEC</p>
RT-PCR	<p>Real time polymerase chain reaction; similar to PCR where a specific DNA sequence between primers is amplified, but a colour based probe reacts to binding in the region and is detected by a machine, leading to real time recognition of the amplification of the DNA sequence</p>
SNP	<p>Single nucleotide polymorphism; Single nucleotide differences between genes that are shared between organisms</p>
Spring calving season	<p>For dairy farms in New Zealand, this usually begins in late June to early July, and ends in September to October. Dairy farming in New Zealand typically follows an annual cycle, although some farms may allow for an Autumn calving season.</p>
ST	<p>Sequence type; a number assigned through the MLST method to differentiate groups of bacteria</p>
STEC	<p>Shiga toxin-producing <i>Escherichia coli</i>; <i>E. coli</i> bacteria that contain the <i>stx</i> gene and therefore may be able to produce Shiga toxin; also called verocytotoxigenic <i>E. coli</i> (VTEC), due to its pathogenicity to vero (kidney) cells</p>
Strain	<p>A genetic variant of an organism</p>

<i>stx</i>	A virulence gene that leads to the production of Shiga toxin
'Top 7' STEC	The seven O serogroups (O157, O26, O45, O103, O111, O121, O145) of STEC declared adulterants of beef by the USDA-FSIS, and recognized as a significant risk to human health
UK	United Kingdom
USA	United States of America
USDA-FSIS	United States Department of Agriculture–Food Safety and Inspection Service; in charge of protecting public health by ensuring the safety of meat, poultry, and processed egg products in the USA
Zoonoses	Pathogens (bacterial, viral, fungal, prion) that are transmissible between animals and humans