final report

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E.coli O157 colonisation and shedding in cattle

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Executive Summary

Escherichia coli O157 is an important zoonotic organism with a global distribution. *E.coli* O157 can cause severe disease in humans and while the prevalence of human disease attributed to *E.coli* O157 is low in Australia, presence of this pathogen on meat exported to the USA can result in trade restrictions. Greater understanding of the distribution of *E.coli* O157 in cattle and the variation over time of shedding and 'super-shedding' of this organism from cattle was required within Australian production systems. In order to achieve this, after an initial period of laboratory methods validation and skill enhancement, two systems were investigated in a longitudinal manner – an extensive beef cattle production system (at Charles Sturt University) and a dairy replacement herd (at University of Sydney). Cattle within these herds were followed longintudinally for a number of months with repeated sampling twice a week during this time. In addition, more intensive sampling was also performed daily and twice daily over a period of weeks at both sites. An expert opinion elicitation exercise was also performed, where international experts in the area of *E.coli* O157 were questioned on multiple areas surrounding shedding of the pathogen, supershedding, risk factors and potential control measures. Finally, a number of simulation models were specified to identify the ability to pool samples in order to reduce the cost of future longitudinal studies, and also to identify key interventions for reduction of prevalence of carcass contamination with *E.coli* O157, in light of the findings of the current research.

This study found that shedding and super-shedding of *E.coli* O157 is highly variable both between and within animals. It also identified that synchronisation of shedding may occur within herds, where periods of time associated with high prevalence and concentration of *E.coli* O157 shed in faeces occurs across the herd. The only consistent risk factor identified for shedding *E.coli* O157 across the two production systems was rainfall, but a possible relationship between individual animal stress and shedding and concentration of *E.coli* O157 was also identified and further investigation of the relationships between these variables is required. From the research performed it does not appear that individual supershedding animals are able to be identified for implementation of pre-slaughter control measures. Indeed, all animals appear to have the capacity to supershed at some time. In addition, while the implementation of pre-slaughter control measures might be effective in reducing carcass contamination with *E.coli* O157, this is only likely in the presence of effective abattoir hygiene that results in a very low probability of transfer of this organism from both gastrointestinal tract and hide to the carcass. In the absence of such control measures at the level of the abattoir there appears to be little benefit to on-farm interventions.

This work provides a marked increase in the knowledge surrounding shedding and supershedding of *E.coli* O157 in Australian production systems.

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1 Background

A collaboration between The University of Sydney (USyd) and Charles Sturt University (CSU) was proposed in response to the Meat & Livestock Australia's terms of reference, *E.coli O157 colonisation and shedding in cattle*. The collaborative team proposed to review microbiological techniques available to detect the *E.coli* O157 supershedding state and identify effective and efficient methodology for use within the Australian red meat industry. We also proposed a study design to estimate the frequency of occurrence of supershedding and predictors of this phenomenon, and to use disease modelling studies to transform empirical field data to information that can be used by industry to develop a control program, should that decision be made by industry, customers and governments.

Escherichia coli O157 is an important zoonotic organism with a global distribution. Although it is shed asymptomatically in cattle faeces, direct or indirect exposure to *E.coli* O157 may result in severe gastrointestinal disease and possible mortality in humans (Locking et al., 2001). Cattle are a reservoir of *E.coli* O157. Recent research has identified marked variation in the quantity of *E.coli* O157 shed within the faeces of cattle that carry the organism (Fegan et al., 2005; Matthews et al., 2006a). The term 'supershedder' refers to those animals that shed pathogen at markedly higher levels (Cobbold et al., 2007) than others. The presence of a supershedder in a herd has been demonstrated as a risk factor for increased herd-level faecal prevalence, hide prevalence and hide load of *E.coli* O157 (Fegan et al., 2005; Matthews et al., 2006b; Arthur et al., 2009). A super-shedder can, on average, shed 3.0 log bacteria per gram of fecal output, sometimes for as long as two months (Davis et al., 2006). Given the fact that *E.coli* O157 are capable of long-term survival in manure, pasture and soil (Ogden et al., 2002) these super-shedders can therefore be a major source of environmental contamination.

Outbreaks of *E.coli* O157 infection associated with consumption of hamburger meat have occurred in the USA and other countries. Although *E.coli* O157 is not a major public health concern in Australia, it is in North America (a major market for Australian beef), particularly manufacturing beef sourced largely from cull beef and dairy cows. Focus in the USA is increasingly turning towards pre-harvest interventions. Supershedding has become an active area for pre-harvest research. Bayesian modelling has shown that most variation in shedding of *E.coli* O157 occurs at a within-animal, rather than between-animal, level (Robinson et al., 2004). However, this within-animal variation requires further elucidation, particularly for those animals that are proximal to food-chain entry.

The overarching aim of this project was to conduct a microbiologically and epidemiologically robust investigation of the occurrence and behaviour of *E.coli* O157 in cattle in Australia.

2 Projective Objectives

The objectives of this project were:

1) Reviewing the microbiological techniques available to detect the *E.coli* O157 supershedding state.
2) Identifying effective and efficient methodology for use within the Australian red meat industry
3) Proposing a study design to estimate the frequency of occurrence of the supershedding state and predictors of this phenomenon
4) Describe future disease modelling studies that could be undertaken to transform empirical field data to information that can be used by industry to develop a control program

3 Methodology

3.1 General methods

The study was divided into seven main parts:

Part A: Literature review

Part B: Technical training and pilot study

Part C: Laboratory skills validation

Part D: Longitudinal study

Part E: Expert Opinion exercise

Part F: Simulation modelling

Part G: National forum

3.1.1 Part A: Literature review methods

This review builds on the information about *E.coli* O157 supershedding in the review published by CSIRO in 2010. It focuses on methods of sampling, detection and enumeration of *E.coli* O157 and definition of the supershedder state. In particular, information regarding the accuracy and validity of currently available methods for detection and enumeration of *E.coli* O157 and methods of classifying the supershedder status in both dairy and beef herds are identified, critically appraised and summarised.

3.1.2 Part B: Technical training and pilot study methods

The research organisations conducted technical training with a research associate and technicians from both universities in order to ensure optimal ability for *E.coli* O157

isolation and enumeration techniques. Pilot studies were performed at both USyd and CSU using the university cattle herds between 30th April and 31st December 2011.

3.1.2.1 Technical training methods

a) CSU

The beef herd was sampled on a number of occasions to obtain faecal samples for laboratory training and preliminary assessment of *E.coli* O157 within the herd.

The herd was sampled on three occasions for this purpose as follows;

SB1. April 2011 – 233 adult cows were sampled using faecal grab, representing animals from most mobs on the property.

SB2. September 2011 – 184 adult cows and heifers were sampled using faecal grab (different animals to those sampled in April and with the ability to be utilised for the pilot study later in the year).

SB3. October 2011 – 90 heifers were sampled initially using faecal grab (same animals as sampled in September 2011) and then 24 selected and sampled on another 3 occasions over a 12 day period, using faecal grab and rectoanal mucosal swabs (RAMS).

These samples were used (n=40 from SB1; n=184 from SB2; n=90 from SB3), along with samples spiked with a known *E.coli* O157 strain, to ensure that laboratory methods are accurate and appropriate and to develop the protocol and SOPs for use throughout the project. The processes of freezing and appropriate recovery, plating on selective, differential media, latex agglutination, immumnomagnetic separation (IMS), polymerase chain reaction (PCR) and enumeration were all utilised and optimised.

b) USyd

The work at USyd commenced with the use of test cultures of *E.coli* O157 that were used to spike faecal samples.

In addition, the dairy herd was sampled on three occasions as follows;

SD1. August-September 2011 – 100 samples were collected from the floor of the milking bales, along with some faecal samples, on a trial basis

SD2. October 2011 – 220 cows in milk were sampled using faecal grab and RAMS

SD3. November 2011 – 31 young calves (1 – 12 weeks) were sampled using faecal grab and RAMS.

SD4. December 2011 – 92 older calves (3-8 months) were sampled using faecal grab and RAMS

These samples were used to practice and refine the protocol and SOPs developed with the use of the spiked samples.

3.1.2.2 Pilot study methods

A pilot study, including longitudinal data collection and comparison of sampling and isolation methods, was undertaken as a part of an honours project (BVBiol/BVSc (Hons)) that was performed from 17th October 2012 for 9 weeks at CSU.

This honours project used samples taken at SB3 to identify positive animals (n=4) and to sample these animals, along with a selection of negative cattle (n=20), for 12 days (sampled on four occasions over this period) to describe the pattern of shedding of *E.coli* O157 during this period of time. In addition, the agreement between faecal sampling procedures (faecal grab and RAMS) and isolation (direct plating and IMS) were also explored.

Full methods are presented in Appendix A.

3.1.3 Part C: Laboratory skills validation methods

Along with the use of known positive strains against which to test procedures 90 enriched and preserved cultures from CSU, which included four *E.coli* O157 positives, were shared with USyd. These samples were transported to the laboratory at Camden and cultured. The laboratory staff at USyd were unaware of the results of the tests performed on these samples at CSU.

3.1.4 Part D: Longitudinal study methods

The faecal shedding and super-shedding of the human pathogen *E.coli* O157 by cattle has been the focus of many previous studies with varied results observed. The heterogeneity of shedding is becoming more accepted, both in the numbers of animals shedding and the levels of pathogen in the faeces that animals shed. To clarify patterns in shedding and super-shedding we undertook longitudinal studies to investigate shedding within a cohort of replacement dairy heifers and a cohort of beef cows. Different intensities of sampling were investigated – weekly, twice weekly, daily and twice per day.

3.1.4.1 Longitudinal study in dairy cattle at USyd methods (reported in full in Appendix B)

This study was conducted in a cohort of 52 dairy heifers (aged 108–226 days on first sampling) being raised as replacement dairy animals. The cohort was sampled weekly from 8 October 2012 to 18 February 2013 (excluding the Christmas period). It was maintained as a typical dairy industry herd on pasture with supplementary feed (hay and high protein pellets) at the University of Sydney dairy farms, Camden, NSW. Each animal sampling point

(ASP) consisted of faecal samples and recto-anal mucosal swabs (RAMS) from an individual heifer at an individual time point.

Faecal samples were processed within two hours of collection and cultured on sorbitol maconkeys agar supplemented with cefixime and tellurite (CT-SMAC). After overnight incubation at 37°C, CT-SMAC plates were inspected for suspect *E.coli* O157 colonies. Samples which were not confirmed as positive by direct plating of faeces were tested by IMS of both faecal samples and RAMS.

All suspect colonies were counted and representative colonies were purified and tested with an O157 latex agglutination test and 16s RNA *E coli* and O157 specific PCRs. One isolate from each heifer on each sample date if tested positive for *E coli* O157 was tested for the presence of virulence genes by three rounds of multiplex PCRs. The first included primers for *Stx1, Stx2* and Rfb_{O157}; the second primer set included *Eae, HlyA, and fliC.*

Correlation coefficients were used to assess relationships between weekly results of prevalence and super-shedding events. Results were categorised for ordinal analysis: Not detected, Positive (IMS <100), High shedder (DFC ≥100), Super-shedder (DFC ≥10,000). Ordinal logistic regression was used to assess the effect of shedding levels prior to each sample date in GenStat (VSNI, 14th Edition). Runs tests were used to assess temporal clustering of results (GenStat).

Data were also collected on animal factors (hide cleanliness score, body condition score and faecal consistency) and data was derived on climate factors (rainfall, temperature, humidity, solar radiation), day length and pasture growth. Data was analysed using Generalised Linear Mixed Models, account for clustering by animal and sampling date, for host factors and for environmental factors. A final model was then built using significant host, climate and environmental factors.

3.1.4.2 Longitudinal study in beef cattle at CSU methods (Reported in full in Appendix C)

This study was conducted in a cohort of 23 primi- and multi-parous Hereford beef cows (aged between 1 and 10 years on first sampling) housed as a single herd. The cohort was sampled twice weekly from October 4th 2012 to March 28th 2013 (excluding the Christmas period) and from then once a week until June 20th 2013. The herd was maintained as a typical grass fed beef herd on pasture with supplementary feed (cereal and ryegrass silage) when required at the Charles Sturt University farm, Wagga Wagga, NSW. Each ASP consisted of faecal samples and recto-anal mucosal swabs (RAMS), and latterly (from January 7th 2013) a faecal sample only, due to consistently negative RAMS samples, from an individual animal at an individual point.

Faecal samples were processed identically to the method described above in the study performed at USyd.

Data were also collected on animal factors (hide cleanliness score, body condition score, faecal consistency, rectal temperature and weight) and data were derived on climate factors (rainfall, temperature, humidity, solar radiation), day length and pasture quality and quantity.

Data were analysed using graphical methods to assess relationships between the probability of animals shedding *E.coli* O157 and a range of animal and environmental variables. Individual data describing each animal's shedding status at each sampling event was summarised for the herd to produce the observed proportion of animals shedding. These proportions were then fitted to a cubic spline model in R to provide a smoothed curve (and accompanying 95% confidence interval) defining the probability of shedding over time and providing a basis for visually assessing the relationship between various factors (animal, environment and management) and the occurrence of shedding. Outcomes from the graphical analysis were used to guide the quantitative analysis.

The quantitative analysis was run in two stages. By visualising the changes in the probability of shedding over time it was established that shedding occurred predominantly in three discrete events. Shedding events were defined to include all those time periods where the estimated probability of shedding exceeded 0.1, and non-shedding events were all other observation points. The second step was to screen whether the averages of a range of animal, environmental and management variables during shedding events were similar to the averages during non-shedding events. Screening first relied on the use of ANOVA to test for significance in the difference between the level of the variable in shedding and non-shedding events. Next, for animal-level variables (hide contamination, faecal consistency, body weight, body condition score, rectal temperature, pregnancy status, lactation status and calves at foot) a general linear mixed model was constructed with the animal variable as an outcome and with cow as a random effect. For example, the average temperature at shedding- and at non-shedding events was estimated by a linear model with temperature as the response, shedding class as a predictor and cows as random effect. Similarly for the environmental and management variables, a GLM was constructed with the environmental/management variable as outcome and sampling event as a random effect. In this way, it was possible to avoid the potential bias that arises due to chance associations that commonly occur when comparing two time series of events (Yule, 1926).

3.1.4.3 Intensive longitudinal study in beef cattle at CSU methods (reported in full in Appendix D)

In addition to the longitudinal study at CSU that lasted 8 months, a shorter term study intensively sampled 24 beef cattle that were managed in the same temperate grazing system over 14 days. The cattle were sampled twice daily for the first seven days and daily for the following seven days and the presence and concentration of *E.coli* O157 in faeces was elucidated.

Preliminary screening was performed to identify shedding of *E.coli* O157 in 196 cattle. Faeces were collected and transported as described previously. All samples were examined for the concentration of *E.coli* O157 by direct faecal culture. From those samples that were negative on direct culture, 100 were chosen at random for immunomagnetic separation (IMS). Twenty-four animals were selected as the study subjects (all of the direct culture positive animals, complemented with randomly selected IMS positive animals). These 24 animals were housed in a single paddock together and faecal samples were collected twice daily (8am and 3pm) during the first seven days, continuing with daily sampling (8am) for an

additional seven days. Data on rainfall (mm in 24 hours prior to sampling) and ambient temperature (°C in 24 hours prior to sampling) were recorded. Faecal samples were processed as described in 3.1.4.1.

3.1.4.4 The relationship between stress and E.coli O157 shedding methods (reported in full in Appendix E)

Individual animals within a herd exhibit marked variation in faecal shedding of *E.coli* O157 despite known environmental exposure to the bacteria. Furthermore, not all animals with simultaneous exposure to stressful stimuli will shed *E.coli* O157, which is speculated to be a result of animal variation in susceptibility to colonisation (Stoffregen, Pohlenz, & Dean-Nystrom, 2004). However, the host factors that predispose ruminants to *E.coli* O157 colonisation and the variation in individual animal shedding levels have not received necessary empirical attention. The aim of this study was to investigate if there is a direct relationship between the presence and concentration of *E.coli* O157 shedding and physiological stress in cattle.

This study utilised the samples obtained for the intensive longitudinal study in beef cattle at CSU, as reported in section 3.1.4.3. At the time of faecal collection, an additional faecal sample that was at least 10g in weight was collected, placed in a sterile Whirl-Pak plastic bag (Nasco, Melbourne, Australia) and frozen within 1 h of collection at -20^{o}C. A single sample per cow per day was assessed for faecal cortisol metabolite concentration. Concentration of faecal glucocorticoid metabolites was measured, as an indicator of acute adrenal activity, using the previously validated corticosterone I^{125} radioimmunoassay kit, as described by Morrow et al., (2002). Full methods are reported in Appendix E. The data were analysed using generalised linear mixed models and linear mixed models, depending on whether the outcome measure was dichotomous (presence of *E.coli* O157) or continuous (faecal corticosterone concentration).

3.1.5 Part E: Expert opinion exercise methods (reported in full in Appendix F)

National and international experts in the area of *E.coli* O157 in cattle were selected according to strict inclusion criteria applied to a literature search that identified them as experts in the field.

The questionnaire was developed applying principles described by Vose (2008) and Fink (2003). SurveyMonkey was used to create and distribute the survey. The questionnaire consisted of 10 questions, divided into seven sections: (1) About this survey; (2) demographics; (3) defining super shedding; (4) risk factors on farm; (5) risk factors during transport and at lairage; (6) intervention methods; (7) statements (previous research, future research, and super shedding).

In part 1 of the questionnaire a short overview of the aim of the survey, practical information and the ethics statement was given. Part 2 requested geographical details of the experts, a self-rating of their expertise in different areas and their experience with different cattle production systems. In part 3, participants had to identify a definition of super shedding they agreed with most. Subsequently in part 4, the experts had to select the concentration that indicates super shedding according to their opinion. Part 5 requested the opinion of the

experts on several potential risk factors on farm, which were based on a literature review. The experts were asked to indicate the magnitude of effect that variables have on the presence or absence of *E.coli* O157 in cattle faeces. An identical approach was used in part 6 and 7 in which first an opinion on risk factors during transport and at lairage was obtained, and subsequently on intervention methods. In part 7 the participants were requested to indicate to what extent they agreed with numerous statements about previous research, future research and super shedding.

The questionnaire was sent out on multiple occasions. Initially it was sent, via email, to all experts and subsequently at three and five weeks to the non-responders.

3.1.6 Part F: Simulation modelling methods

3.1.6.1 Model 1a: "Pool model" methods (reported in full in Appendix G)

The work that was performed in the longitudinal study (Part D) identified that marked variation in shedding was apparent both between and within cattle over time. In order to accurately represent this variation, a financially cumbersome sampling strategy is required which is likely to be prohibitive in most scenarios. A pooled sampling approach would avoid such financial burden, while permitting a more appropriate longitudinal (as opposed to cross-sectional) representation of herd shedding status. However, the trade-off between the accuracy of such method and the cost efficiency has not previously been addressed and is required in order to make an informed decision on sampling strategy.

This simulation model used the data acquired in the two field studies at CSU, as described above. The first study sampled 23 beef cows twice a week for a nine month period (58 sampling points), while the second study sampled 24 cattle twice a day or daily for 14 days (21 sampling points). At this stage the model includes data from the studies performed at Wagga Wagga but other data, including those obtained from USyd and described in previous milestones, can be included as desired for future simulations.

A dose-response curve was fitted to data obtained through a spiking experiment (performed during Part B) in order to model how the probability of obtaining a positive result varies with the concentration of pathogen in the sample.

The simulation model resampled from the longitudinal data to generate "pools" with a known concentration of *E.coli* O157. It then identified whether each pool was positive by the standard test, after accounting for the effect of dilution, using the dose-response curve. Each sampling event consisted of data from all available animals and each sampling event was treated as an independent herd. Within an infected herd the probability that a randomly chosen animal is shedding *E.coli* O157 was estimated from the true prevalence within the herd.

3.1.6.2 Model 1b: Financial model for estimating herd test costs methods (reported in full in Appendix G)

A complimentary model was created to estimate the financial cost of herd testing for *E.coli* O157 for the pooled testing protocols evaluated above. The costs of consumable items and

sample processing were estimated per pool and per sample. The financial model is represented in the following equation:

Cost herd test = $\sum_{i=1}^{np}(cpp + \sum_{j=1}^{nspp}(cps + cisc))$

In this equation, *np* is the number of pools per herd test (varies between 2 – 12), *cpp* is the cost per pool ($17.00), *nspp* is the number of samples per pool (varies between 1 – 12), *cps* is the cost per sample ($2.50) and *cisc* is the cost of individual sample collection ($1.75).

3.1.6.3 Model 2: "Chain model" methods (reported in full in Appendix H)

The aim of this simulation study was to identify the most effective intervention strategy to reduce the prevalence of beef carcass contamination with *E.coli* O157 in Australia, by using sensitivity analysis to determine points in the harvest chain that have the greatest influence on the range of prevalence. This model also facilitated identification of important data gaps for targeted future research.

A stochastic, individual-animal-based, discrete-time computational model was constructed and implemented in the Python programming language. The model simulated the presence of *E.coli* O157 in faeces, on hides and on carcasses of individual animals on a daily basis at the following stages in the harvest chain: origin (farm or feedlot), transport, lairage at the abattoir and slaughter up to stage of pre-chill carcass (Figure 1). Information from empirical studies was used to parameterise the model.

Each iteration of the model reflected the points and processes in the harvest chain for the cattle slaughtered on a single day in an abattoir by simulating each animal's status for faecal, hide and carcass presence of *E.coli* O157 on a daily basis at each stage of the model. Cattle were grouped by truck (truck capacity 2–65 cattle) from each point of origin. Duration of travel ranged from 1–2 days and duration of time spent in the lairage ranged from 1–5 days. Throughput at the abattoir was varied between 50 and 2500 cattle. Ranges used for parameters regarding prevalence of *E.coli* O157 in faeces, on hides and on carcasses and processes that occur throughout the model are shown in Appendix H. At the end of each iteration, the prevalence of *E.coli* O157 on carcasses was calculated and recorded.

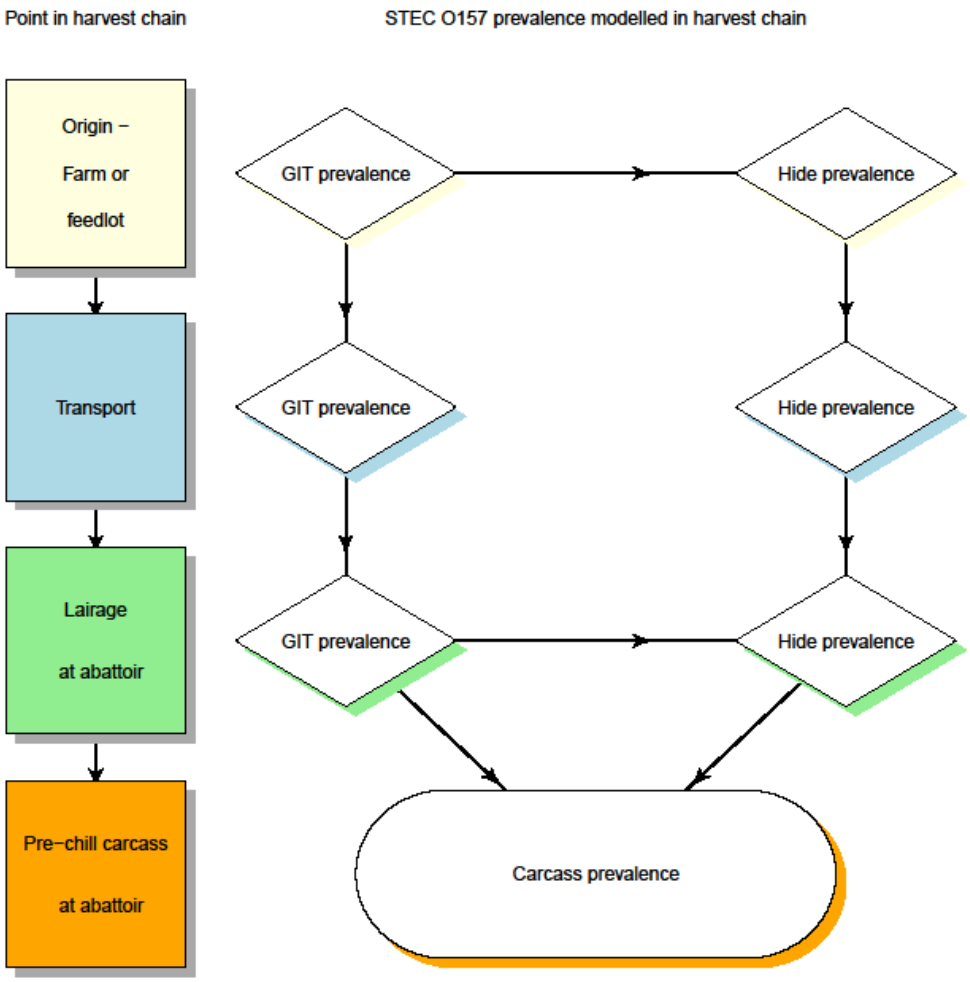

Figure 1. Structure of model to simulate beef carcass contamination with *E.coli* O157, following harvest of cattle from origin to abattoir (pre-chill carcass).

Variance-based global sensitivity analysis (GSA) using the Saltelli method identified risk factors with the greatest influence on the prevalence of *E.coli* O157 contaminated beef carcasses. Following an initial simulation with no interventions, a series of simulations systematically introduced interventions (Table 1) based on risk factors identified by repeated Saltelli GSA to determine the most effective intervention strategy.

Table 1. Intervention strategies applied to the model and assessed through sensitivity analysis

Intervention strategy	
1	reduced prevalence of STEC O157 in faeces at origin
2	vaccination
3	reduced probability of hide to carcass transfer (probability = 0—0.03)
4	reduced probability of GIT to carcass transfer (0—0.03)
5	interventions 3 and 4: reduced probability of hide and GIT to carcass transfer (both 0—0.03)
6	reduced probability of hide (0—0.02) and GIT (0—0.03) to carcass transfer
7	reduced probability of hide (0—0.01) and GIT (0—0.03) to carcass transfer
8	interventions 5 and 1
9	interventions 5 and 2
10	interventions 7 and 1
11	interventions 7 and 2

3.1.7 Part G: National forum methods

A symposium was held on 9th/10th March 2015 at CSU in Wagga Wagga. The programme for the day was as follows:

STEC Symposium
9-10 March 2015
Charles Sturt University Convention Centre

Monday 9 March 2015

10.30 - 11am Arrive and Morning Tea

11.00 - 11.20am Overview of symposium
Ian Jenson, MLA

11.20 - 11.30am Introduction of facilitator and setting the scene
Facilitator – David Falepau

11.30 - 11.45am Importance of STEC to the Australian red meat industry: market access in an age of increasing specification
John Langbridge, AMIC

11.45 - 12.00am STEC human illness: public health concerns and product-pathogen pairs
Trish Desmarchelier, Food Safety Principles

12.00 - 12.15am *Questions and discussion*

12.15 - 1.15pm Lunch

1.15 - 3.15pm Research updates:

- Michael Ward, University of Sydney (25 minutes) – temporal dynamics of *E.coli* O157 in a dairy cattle system

Facilitated discussion (5 minutes)

- Jane Heller, Charles Sturt University (25 minutes) – temporal dynamics *E.coli* O157 in a beef cattle system

Facilitated discussion (5 minutes)

- Robert Barlow, CSIRO (25 minutes) – STEC in cattle faeces at slaughter – serotypes and virulence markers of public health concern

Facilitated discussion (5 minutes)

- Chawalit ("Jay") Kocharunchitt, University of Tasmania (25 minutes) – Opportunities for intervention in the slaughter/chilling process

Facilitated discussion (5 minutes)

3.15 - 3.45pm Afternoon Tea

3.45 - 4.10pm *E. coli* in manufacturing beef and the risks to human health: reducing risks post processing
Andreas Kiermeier (25 minutes)

4.10 - 4.45pm Discussion, Summary and scoping for Day 2
Facilitator – David Falepau

4.45 - 5.30pm Networking and drinks

Tuesday 10 March 2015

8.30 – 8.40am Re-cap from Day 1
Facilitator – David Falepau

8.40 - 9.30am STEC in beef - what is the issue/threat and can it be managed?
Facilitator – David Falepau
a) market access, issues and risks
b) can we further maximise the impact of current management practices?
c) risk management through chain – on farm, slaughter, post-processing

9.30 - 10.10am What does the future research agenda look like?
Research moving forward

- Views from University of Sydney (10 minutes)
- Views from Charles Sturt University (10 minutes)
- Views from CSIRO (10 minutes)
- Views from University of Tasmania (10 minutes)

10.10 - 10.55am How well do potential management and potential research agendas match up?
Facilitator – David Falepau and MLA

10.55 - 11.00am *Wrap up - thanks*
MLA

11am Morning Tea and depart

4 Results

4.1 *E.coli* O157 colonisation and shedding in cattle results

The results of this project are presented for each part, in line with the above methods.

4.1.1 Part A: Literature review results

Escherichia coli O157 is an important zoonotic organism with a global distribution. Although it is shed asymptomatically in cattle faeces, direct or indirect exposure to *E.coli* O157 may result in severe gastrointestinal disease and possible mortality in humans (Locking et al., 2001). Cattle are a reservoir of this pathogen and recent research has identified marked variation in the quantity of *E.coli* O157 shed within the faeces of cattle that carry the organism (Fegan et al., 2004; Matthews et al., 2006a).

The term 'super-shedder' refers to those animals that shed pathogen at a markedly higher level than others. The presence of high level shedders in a herd has been demonstrated as a risk factor for increased herd-level faecal prevalence, hide prevalence and hide load of *E.coli* O157 (Fegan et al., 2005; Matthews et al., 2006b; Arthur et al., 2009).

A review was performed that critically appraised the salient literature related to this topic, specifically covering the areas of pathobiology of *E.coli* O157 in cattle, sampling and detection methods and the definition of super-shedders. This review is presented in full in Appendix I.

4.1.2 Part B: Technical training and pilot study results

4.1.2.1 Technical training results

a) Laboratory training at CSU

All samples tested from SB1 (n=40) were negative for *E.coli* O157. However, after optimisation of the procedure, two positive isolates were found from SB2. In addition, *E.coli* O157 was recovered from all spiked samples plated from SB2. Four of the 90 samples from SB3 were found to be positive for *E.coli* O157.

b) Laboratory training at USyd

To start preliminary laboratory work and training, two *E.coli* cultures obtained from Elizabeth Macarthur Agricultural Institute, NSW DPI and one commercial strain from Invitrogen were used. Training in the following methods was undertaken: culture, Gram staining, selective media culture on CT-SMAC, Latex agglutination test for O157 and Stx1 PCR. One strain from EMAI was found to be O157. This was selected as a positive control for our laboratory tests on *E coli* culture samples. Spiking experiments with positive control on faeces collected from Camden dairy cows was completed, with different concentrations of bacteria. *E.coli* O157 was able to be successfully recovered from the spiked samples.

Table 2. Results from sampling of the beef herd at CSU

Source (No. of Animals sampled)	**Type of samples**	**CT- SMAC culture**	**Latex O157 Slide test**	**Stx1 and Stx2 PCR (only samples positive on Latex tested)**
SB1. Adult cows (n = 40)	Faecal grab	All negative	All negative	
SB2. Adult cows and heifers (n = 184)	Faecal grab	2 positive (after dilution)	2 positive	2 positive for stx1 and negative for stx2
SB3. Heifers (n = 90)	Faecal grab	4 positive	4 positive	4 positive for stx1 and negative for stx2

Table 3. Results from sampling of the dairy herd at USyd

Source (No. of Animals sampled)	**Type of samples**	**CT- SMAC culture**	**Latex O157 Slide test**	**Stx1 PCR**
SD1. Milking cows (n = 49)	Fresh faeces off the floor	28 colonies suspected O157	All negative	All negative
SD1. Milking cows (n = 50)	Faecal grab & RAJ swabs	18 colonies suspected O157	All negative	17 positive
SD2. Milking cows (n = 220)	Faecal grab & RAJ swabs	21 colonies suspected O157	All negative	10 tested (all negative)
SD3. Young dairy calves (n = 31)	Faecal grab & RAJ swabs	24 colonies suspected O157	All negative	Not tested

4.1.2.2 Pilot study results (reported in full in Appendix A)

A baseline prevalence of *E.coli* O157 was estimated to be 4.4% (95%CI 1.2 – 10.9%). Marked variation was identified in the quantity of *E.coli* O157 shed over a period of 12 days (Figure 1).

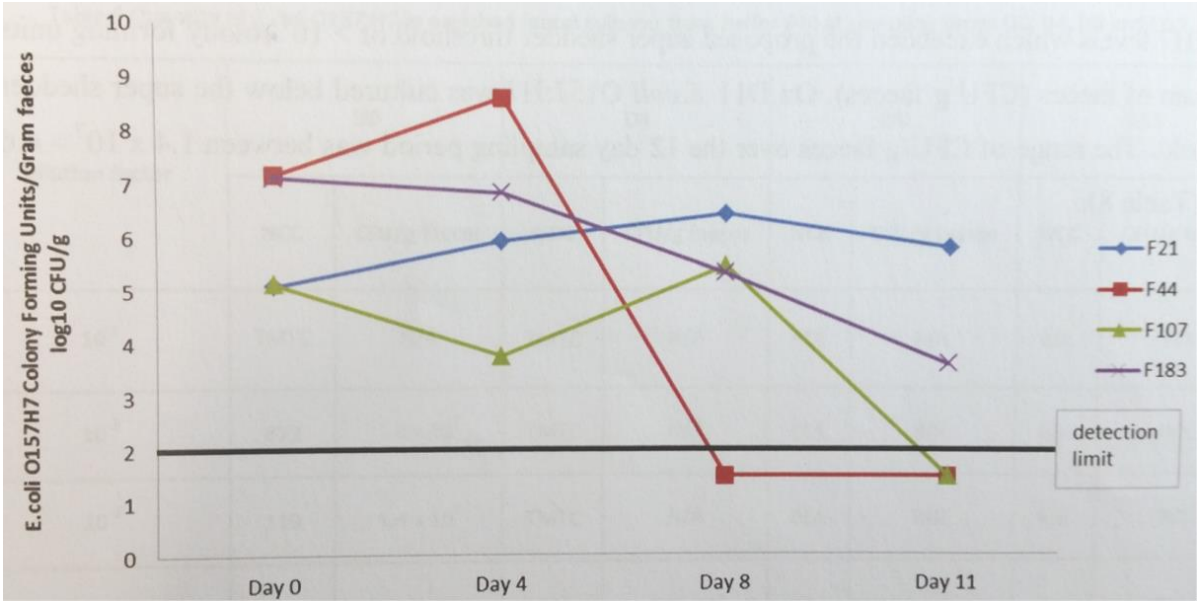

Figure 1. *E.coli* O157 shed by four positive heifers over a 12 day study period

4.1.3 Part C: Laboratory skills validation results

The samples (n = 90) obtained at SB3 were shared with USyd and the same four positive samples were identified at USyd that were identified at CSU (laboratory personnel at USyd were blinded to outcomes at CSU), thus providing validation of procedures. Spiked faecal samples were also used at each laboratory to independently validate the procedure.

Through collaboration and shared laboratory experience, a final protocol for identification of *E.coli* O157 was developed. This is shown in Figure 2.

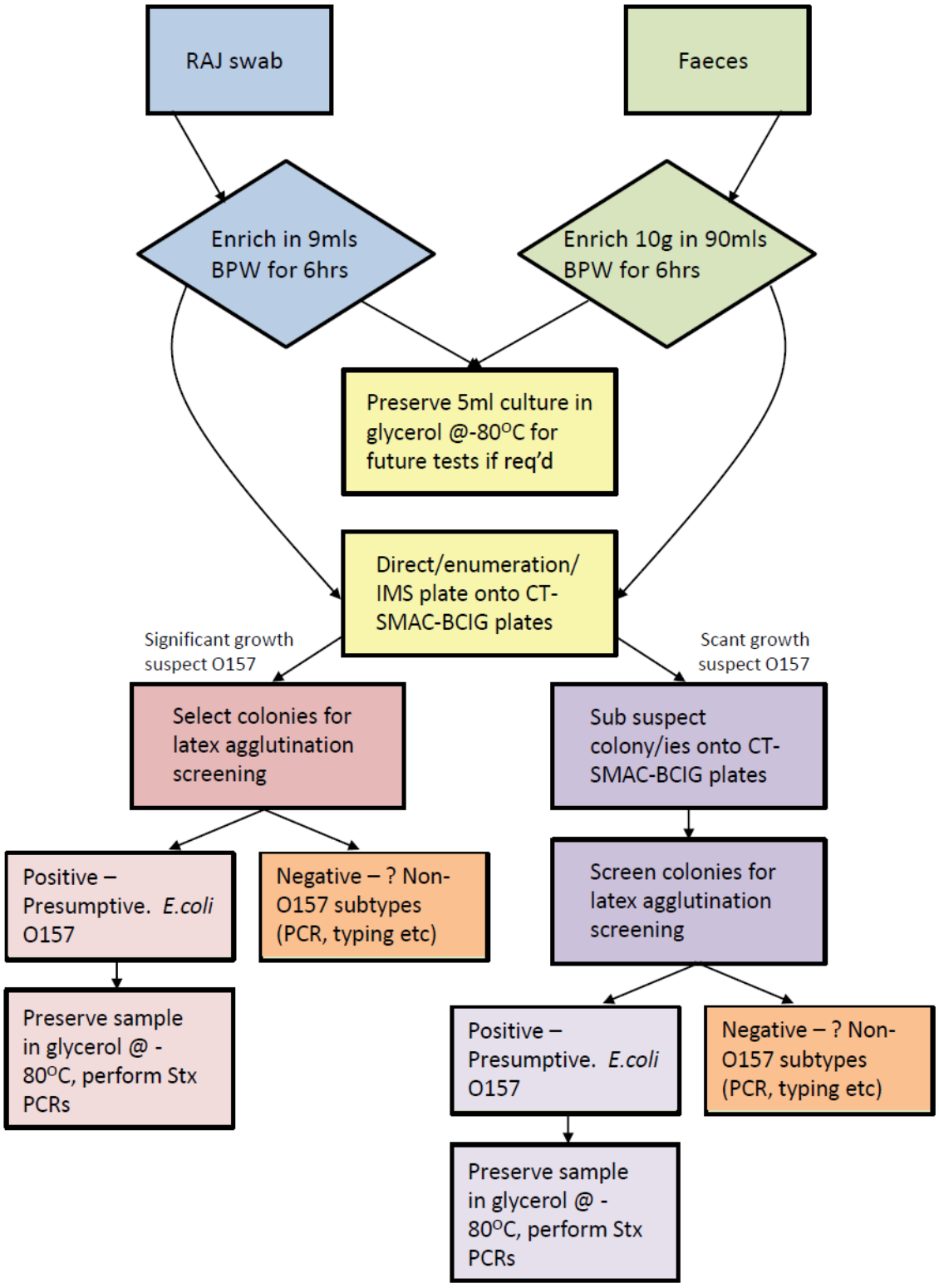

Figure 2. Flow chart for enrichment, culture and preservation of *E.coli* O157 from faecal samples and RAMS swabs.

4.1.4 Part D: Longitudinal study results

4.1.4.1 Longitudinal study in dairy cattle at USyd results (reported in full in Appendix B)

A total of 149 (16.0%) samples were identified as positive by direct culture (Table 3). Of these, 32 were enumerated as shedding >10,000 cfu/g and therefore classed as super-shedding. The 32 incidences of super-shedding detected were from 24 heifers, of which 19 were detected to be super-shedding once only. Three heifers were identified as super-shedding on three occasions and two on two occasions.

Of the total 347 samples detected positive by IMS, 266 (28.6%) were not detected positive by direct faecal culture therefore can be classified as shedding <100 cfu/g.

Over the duration of the study a slight upwards trend in prevalence was observed, with three larger peaks (Figure 3). The number of heifers super-shedding remained approximately constant throughout the duration of the trial, and the proportion of heifers shedding that were super-shedding varied from 0 to 40% as shown in Figure 4.

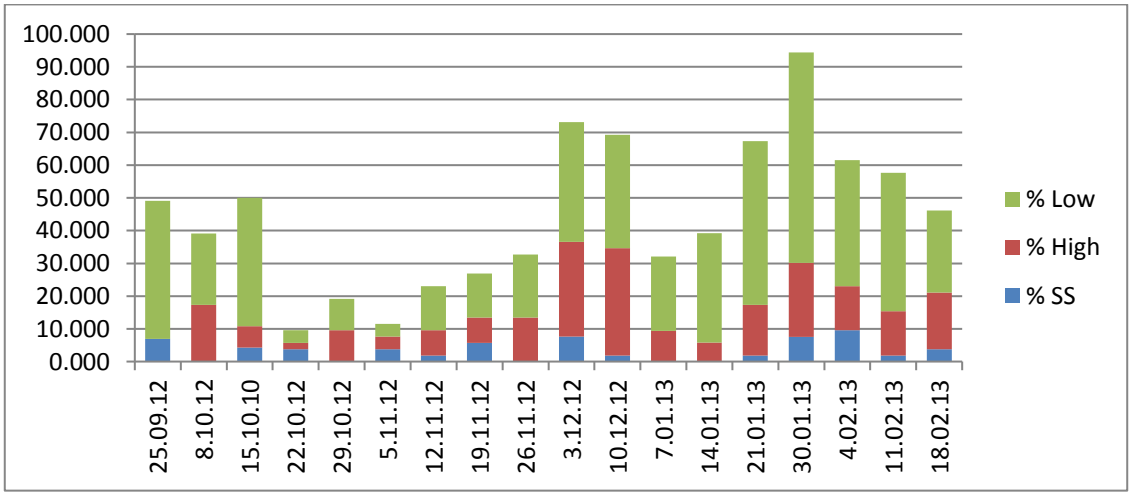

Figure 3. Percentage of heifers detected as positive and levels of detection by date, USyd dairy study.

Moderate correlations were found between prevalence and the number of heifers super shedding at the sample point in time (0.440) and the week preceding (0.356). However, the correlation between the observed prevalence and the number of heifers super-shedding the week prior was weak (0.064). Ordinal logistic regression of shedding levels at each point compared to shedding levels prior demonstrated significance only between a sample point and shedding one week earlier.

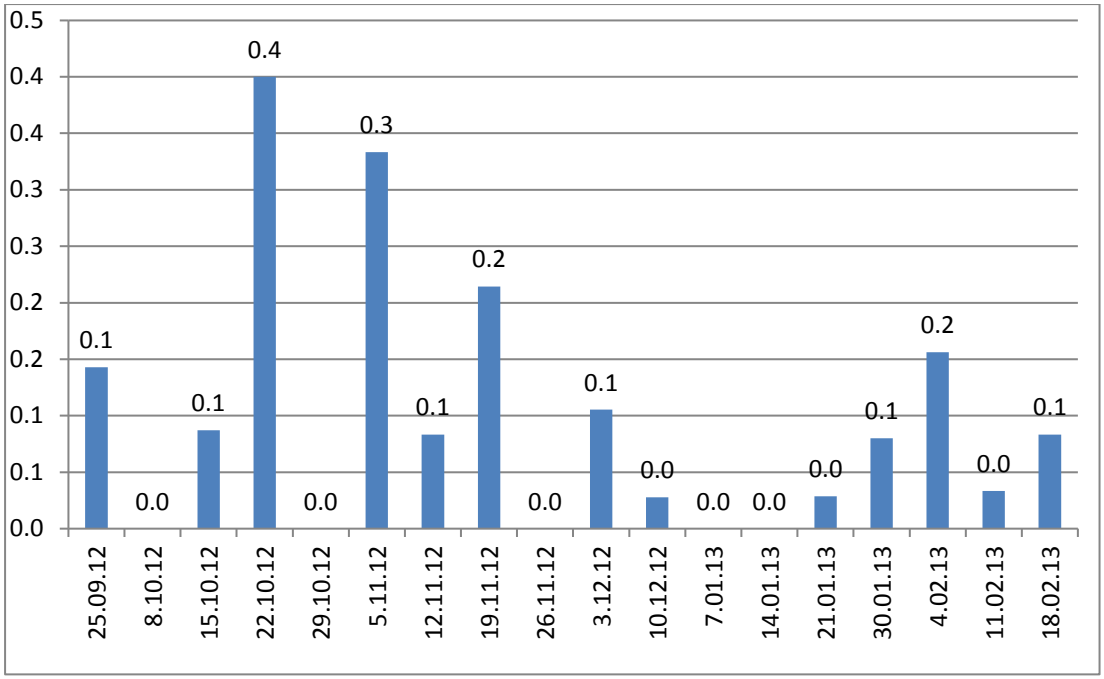

Figure 4. Proportion of heifers detected as positive identified as super-shedding, Camden dairy study.

The number of times a heifer was detected positive ranged from 3/15 to 16/18 samples; 94.2% of animals sampled yielded between four and eleven positive samples. The number of times a heifer tested positive over the duration of the trial was normally distributed (Figure 5). Two heifers were notably different, with 16 and 13 detections each, and only one was detected as super-shedding (once) during the longitudinal study.

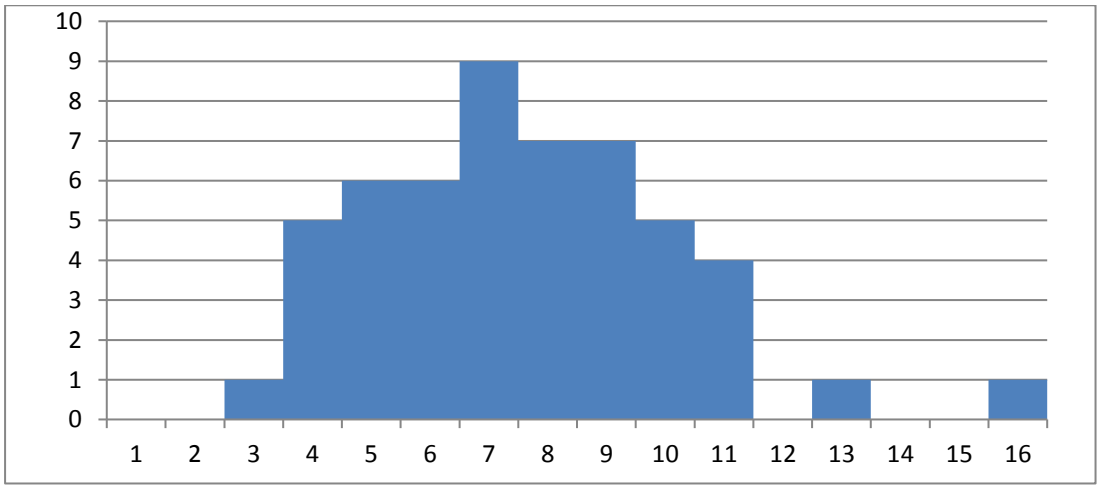

Figure 5. Total number of positives yielded by heifers, Camden dairy study.

Of 398 ASPs detected positive during the trial, 366 were tested by PCR, of which all but two were confirmed as *E coli* O157 by *rfbE-O157.* All samples confirmed as *E coli* O157 by PCR

were also positive for *Stx2* and all but four were positive for *Stx1*. Three of the four isolates *Stx1* positive and *Stx2* negative were isolated from the same sample point.

Table 4. Number of samples detected positive by direct faecal culture (DFC) and classification by enumeration, Camden dairy study.

Date sampled	Number sampled	$>10^4$ cfu/g	$>10^3$ cfu/g	$>10^2$ cfu/g	Not detected	Total DFC positive
25/09/2012	31	4	0	0	27	4
8/10/2012	46	0	3	5	38	8
15/10/2012	45	2	3	0	40	5
22/10/2012	50	2	1	0	47	3
29/10/2012	51	0	0	5	46	5
5/11/2012	52	2	1	1	48	4
12/11/2012	50	1	1	3	45	5
19/11/2012	52	3	1	3	45	7
26/11/2012	51	0	2	5	44	7
3/12/2012	51	4	3	12	32	19
10/12/2012	52	1	3	14	34	18
7/01/2013	53	0	1	4	48	5
14/01/2013	50	0	0	3	47	3
21/01/2013	52	1	2	6	43	9
30/01/2013	52	4	6	6	36	16
4/02/2013	52	5	3	4	40	12
11/02/2013	51	1	2	5	43	8
18/02/2013	52	2	3	6	41	11
Total	893	32	35	82	744	149

Body condition score ($P = 0{\cdot}029$) was positively associated with increased shedding, whereas hide contamination ($P = 0{\cdot}002$) and increased faecal consistency ($P = 0{\cdot}023$) were positively associated with super-shedding.

Higher temperature ($P < 0{\cdot}001$), rainfall ($P = 0{\cdot}02$), relative humidity ($P < 0{\cdot}001$) and pasture growth ($P = 0{\cdot}013$) (Figure 6) were positively associated with increased shedding. Higher rainfall ($P < 0{\cdot}001$) was positively associated with super-shedding. Increased solar exposure had a negative effect on both shedding and super-shedding within bivariate

analyses but in the final multivariate model for shedding demonstrated a positive effect (P = 0·017).

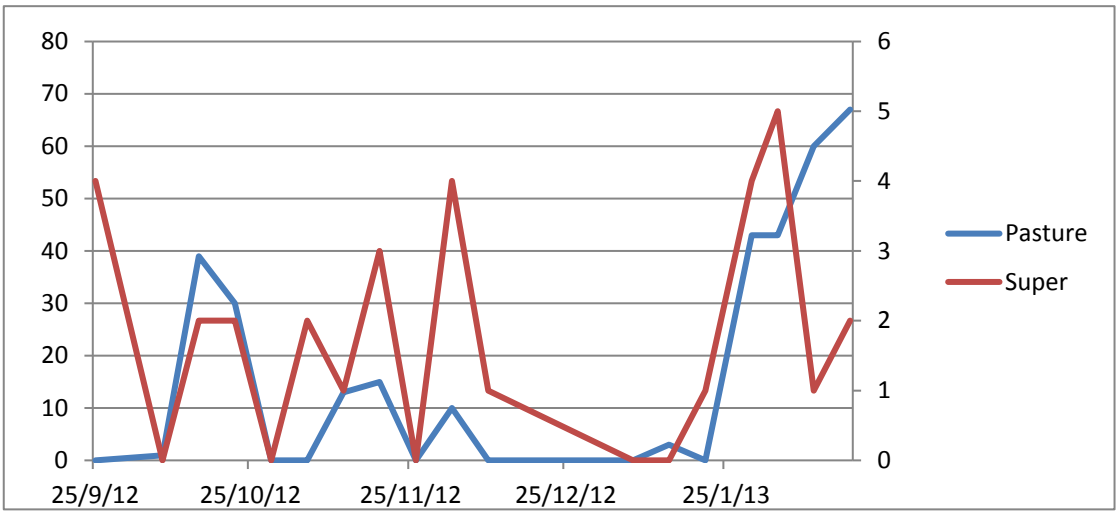

Figure 6. Pasture growth and *E.coli* O157 super-shedding, USyd dairy study.

The cohort of 52 heifers was sired by 16 bulls and 4 bull groups consisting of 2-4 bulls. No association between breed and *E.coli* O157 shedding (P=0.690) or super-shedding (P=0.545) was found.

Pasture growth and day length were included as categorical factors with three levels each. Climate factors were included at the levels successfully tested in the climate model but the model failed to resolve all effects. Rainfall and temperature were therefore collapsed from six to three levels each. Sequentially dropping non-significant terms from this GLMM resulted in a model that included all climate factors along with pasture growth and body score, with all factors categorised at three levels.

The final model of *E.coli* O157 shedding included rainfall, humidity and solar exposure during the previous 7 days, temperature during the previously 14 days, pasture growth and body score.

The final model of *E.coli* O157 super-shedding included rainfall during the previous 7 days, hide contamination and faecal consistency.

4.1.4.2 Longitudinal study beef cattle at CSU results (reported in full in Appendix C)

A total of 1323 faecal samples were collected, of which 170 (12.8%) samples were identified as positive, with 21 (1.6%) positive on direct enumeration plates and 149 (11.3%) only identified as positive using IMS. Only one animal shed *E.coli* O157 at a level of >10,000 CFU/g on one occasion and thus only a single sample (0.07%) could be classed as supershedding (if the definition of supershedder was relaxed to >1,000 CFU/g the numbers increased to 9 (0.6%)). The amount of *E.coli* O157 shed in the faeces ranged from <100 to

20,700 CFU per gram of faeces. All animals within the herd shed the pathogen at least once during this study (Figure 7). A large variation in the frequency of shedding among animals was seen. *E.coli* O157 was shed intermittently by all animals and the number of occasions on which an individual cow was identified as shedding *E.coli* O157 ranged between 2 and 15 (out of 58 sampling points in total). The maximum number of consecutive sampling points that an animal was found to be positive was seven (3.5 weeks). The prevalence per sampling day ranged from 0 to 56.5%.

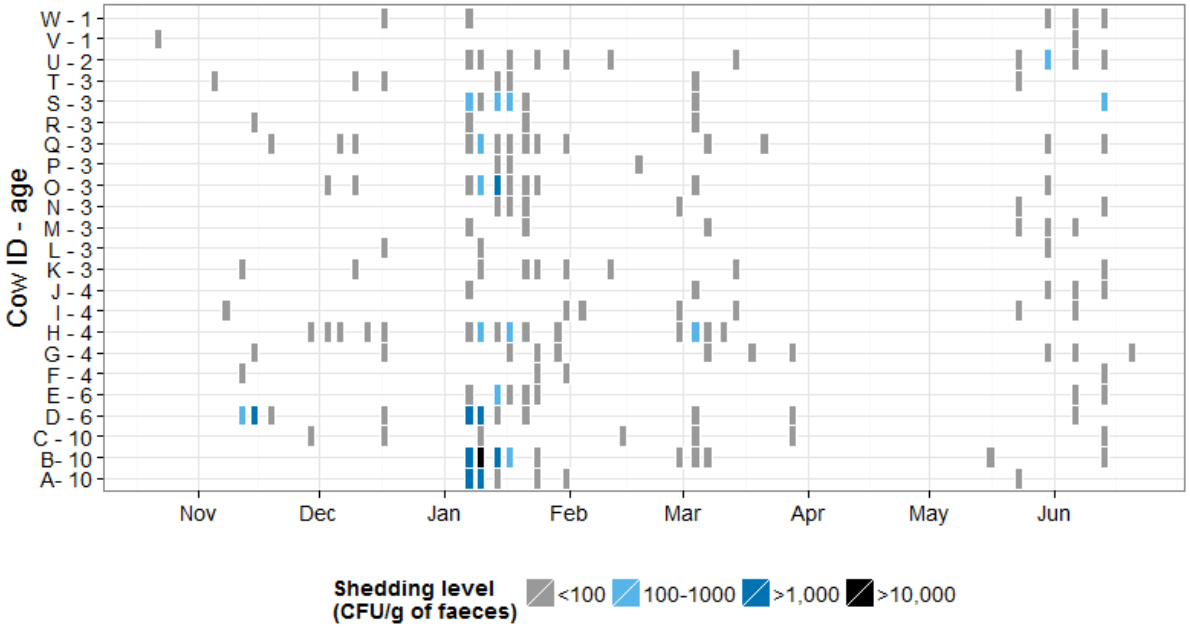

Figure 7. Shedding pattern of *E.coli* O157 per cow over time, CSU beef study. This figure shows shedding levels of each individual cow at each samling event, with no square/white area indicating that the pathogen was not detected.

Temporal data on shedding events are shown in Figure 8. This figure shows that there are three distinctive peaks surrounding the probability of shedding. There is a major peak in mid-summer (late December to early February), a minor peak in autumn (March to April) and a second major peak in early winter (late May to late June).

All 170 isolates were tested by PCR of which all were confirmed as *E.coli* O157 by *rfbE-O157.* All isolates were also positive for *Stx1* and all but two were positive for *Stx2*.

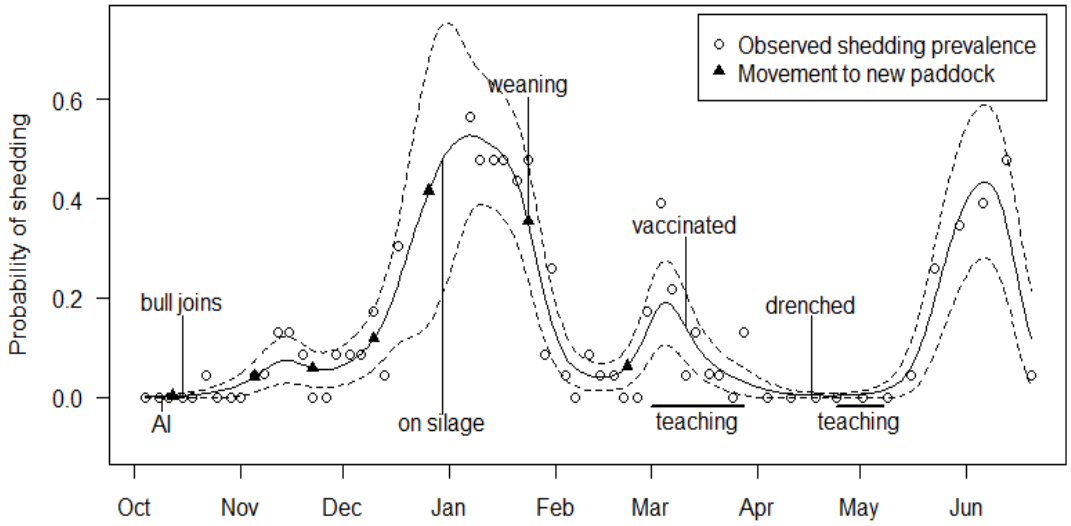

Figure 8. Temporal change in probability of animals shedding *E.coli* O157 (and 95% confidence intervals, dashed lines), the timing of management variables and the timing of movement of the animals between paddocks, CSU beef study.

Temporal data on shedding events and management events are shown in Figure 8. None of the management variables appear to be associated with any of the peaks in shedding. Time-based change in the probability of shedding is also shown in Figure 9 accompanied by the temporal patterns of rainfall and humidity. Figure 9 shows that although all of the peaks in shedding are immediately preceded by rainfall this can also be said for many periods where shedding was not detected. Thus, there is scant evidence for an association between rainfall and shedding of *E.coli* O157. Visually, there is no apparent association between humidity and shedding.

Temporal change in the probability of shedding in Figure 9 is accompanied by the temporal patterns of temperature, hours of bright sunshine and day length. All three variables show a similar temporal curve. Based on this figure there is no association between any of the three environmental variables with the probability of *E.coli* O157 shedding.

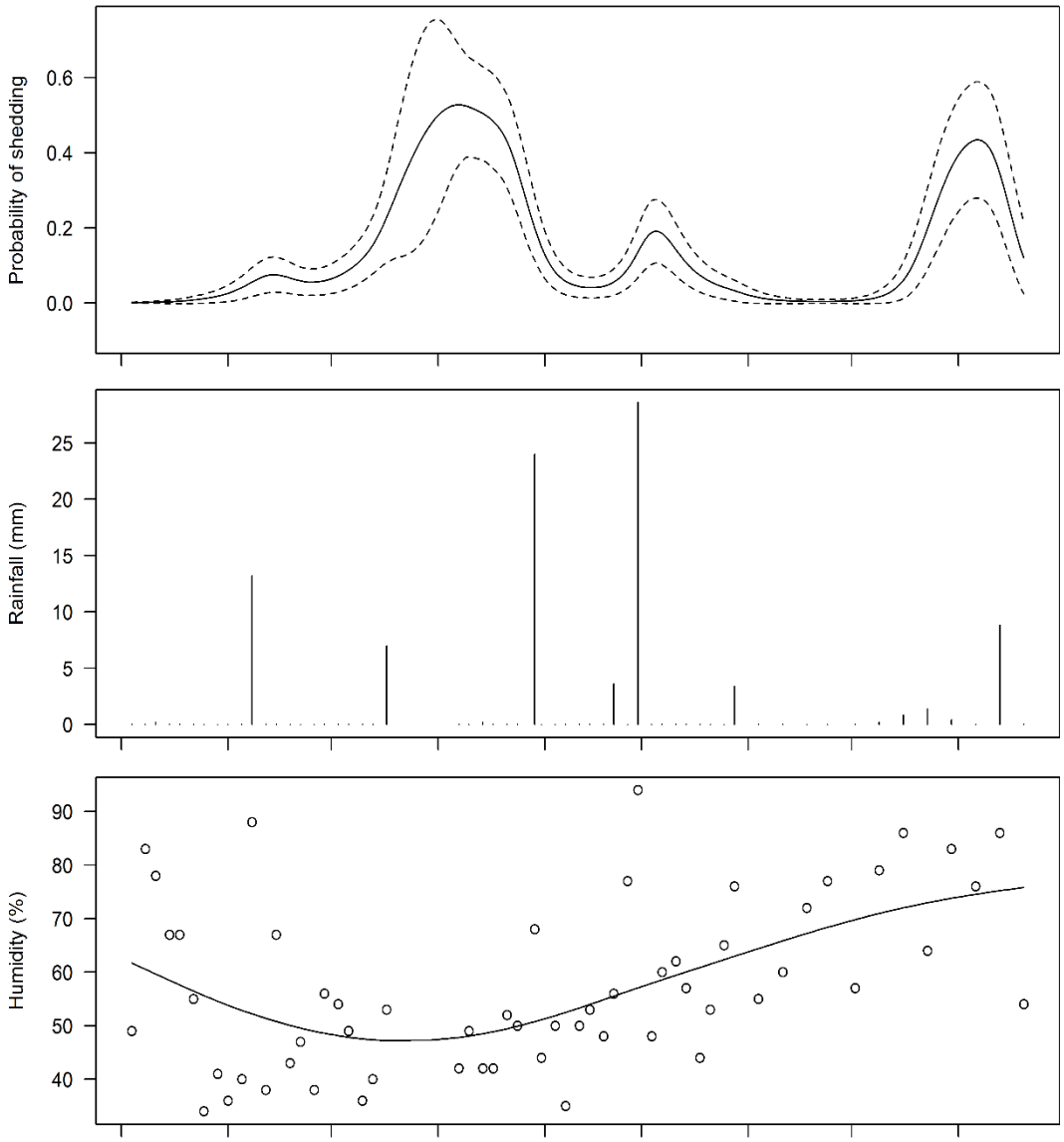

Figure 9. Temporal change in probability of animals shedding *E.coli* O157, rainfall (mm) in the 24 hours prior to sampling, and relative humidity (%) at 9am at the day of sampling, Wagga Wagga beef study.

Time-based change in the probability of shedding is shown in Figure 10 together with the temporal patterns of the individual animal variables: body weight, faecal consistency and hide contamination. Visually, there is no apparent association between hide contamination or faecal consistency and the likelihood of shedding *E.coli* O157. Body weight shows two peaks in weight gain coinciding with the two largest peaks in shedding probability; however, during the lower peak of shedding no similar association is seen. Whether there is an association between weight of the animal and the probability of shedding is inconclusive from this plot.

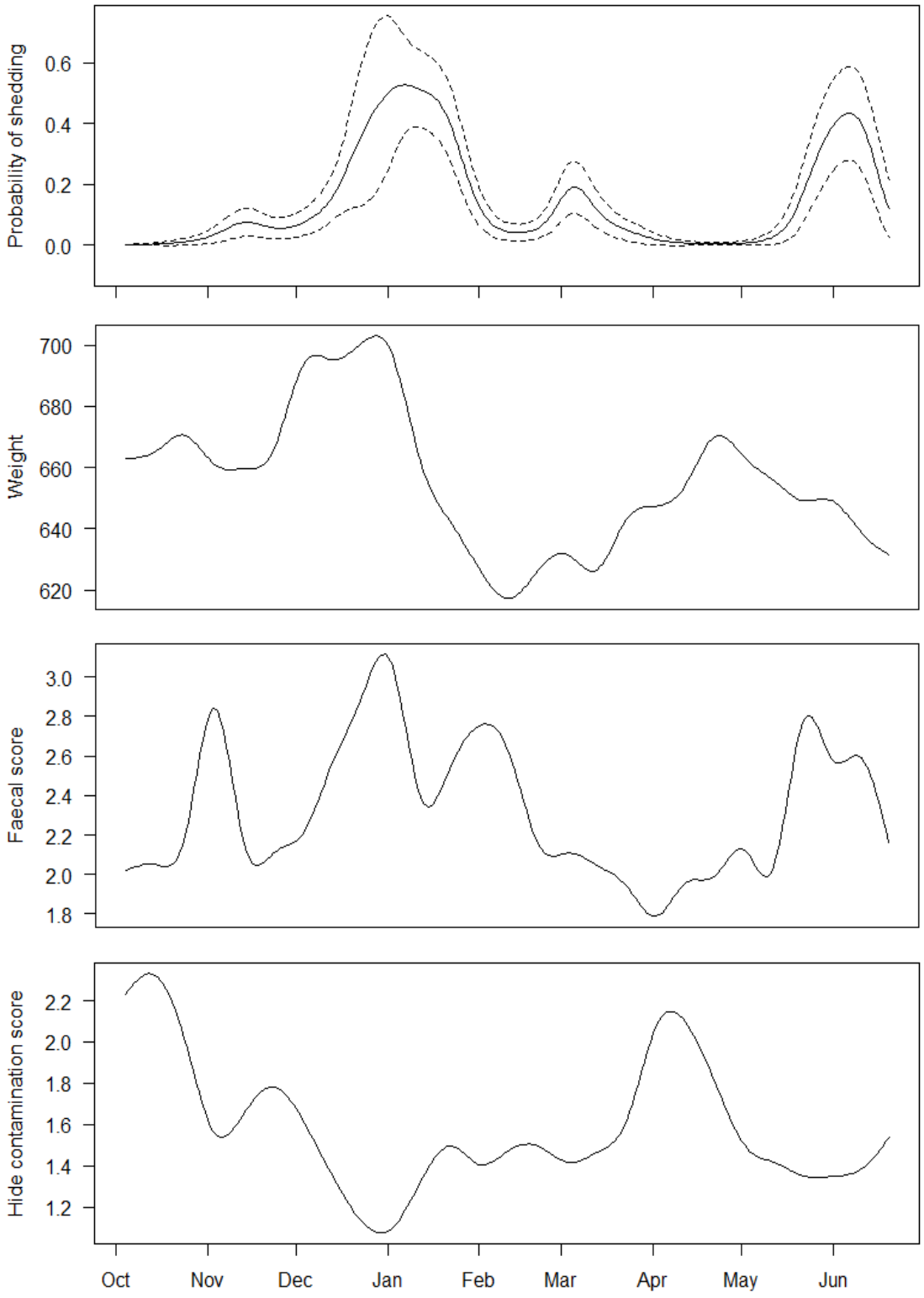

Figure 10. Temporal change in probability of animals shedding *E.coli* O157, body weight, faecal consistency and hide contamination, CSU beef study.

Analysis of all the animal, management and environmental variables by ANOVA and GLM identified two significant risk factors associated with the probability of *E.coli* O157 shedding: hide contamination (P = 0.04) and rainfall in the 24 hours prior to sampling (P = 0.03). The average level of hide contamination score during shedding (1.4) differed from non-shedding (1.7). In addition, the mean of rainfall during shedding events (3.4) differed significantly from non-shedding events (0.6).

4.1.4.3 Intensive longitudinal study in beef cattle at CSU results (reported in full in Appendix D)

A total of 4 (2.0%) of the 196 Angus cattle tested at the time of screening were identified as shedding *E.coli* O157 by direct plating. Concentration of the pathogen in these samples ranged from 200 to 500 CFU/g of faeces. Of the 100 randomly chosen samples that were tested by IMS, a total of 40 (40%) were positive for *E.coli* O157. Out of these 40 positive cattle, 20 were randomly selected to be part of the study group.

A total of 504 faecal samples were collected at 21 sampling points over the 14 day study period. Only one of the 24 animals in the study group did not shed the pathogen during the period of the study and no common pattern was observed in the shedding of *E.coli* O157. Variation in the frequency of shedding was also observed, with the proportion of sampling occasions in which an animal shed varying between 23.8 – 85.7%. In addition, the stx genes were not consistent over the 14 day study period and these varied within cows and between and within days.

The only significant association observed was between shedding concentration from one day to the next (P < 0.001) and within a day (P < 0.001).

4.1.4.4 The relationship of cortisol metabolites to E.coli O157 shedding results (reported in full in Appendix E)

The overall concentration of bovine faecal GM across the 14 days was low, ranging from 3.15 to 26.45 ng/g (mean, 11.2 ± 3.8 ng/g). There was a high level of inter-animal and intra-animal variability in the concentration of faecal GM.

Over the period of study, a daily trend of increasing prevalence of *E.coli* O157 shedding was identified that peaked on Day 9, which is consistent with the peak in mean faecal GM that occurred two days prior (Figure 11). These peaks were followed by a concurrent decline in the two variables. A similar graphical pattern was also observed between faecal concentration of *E.coli* O157 and faecal GM (Figure 12).

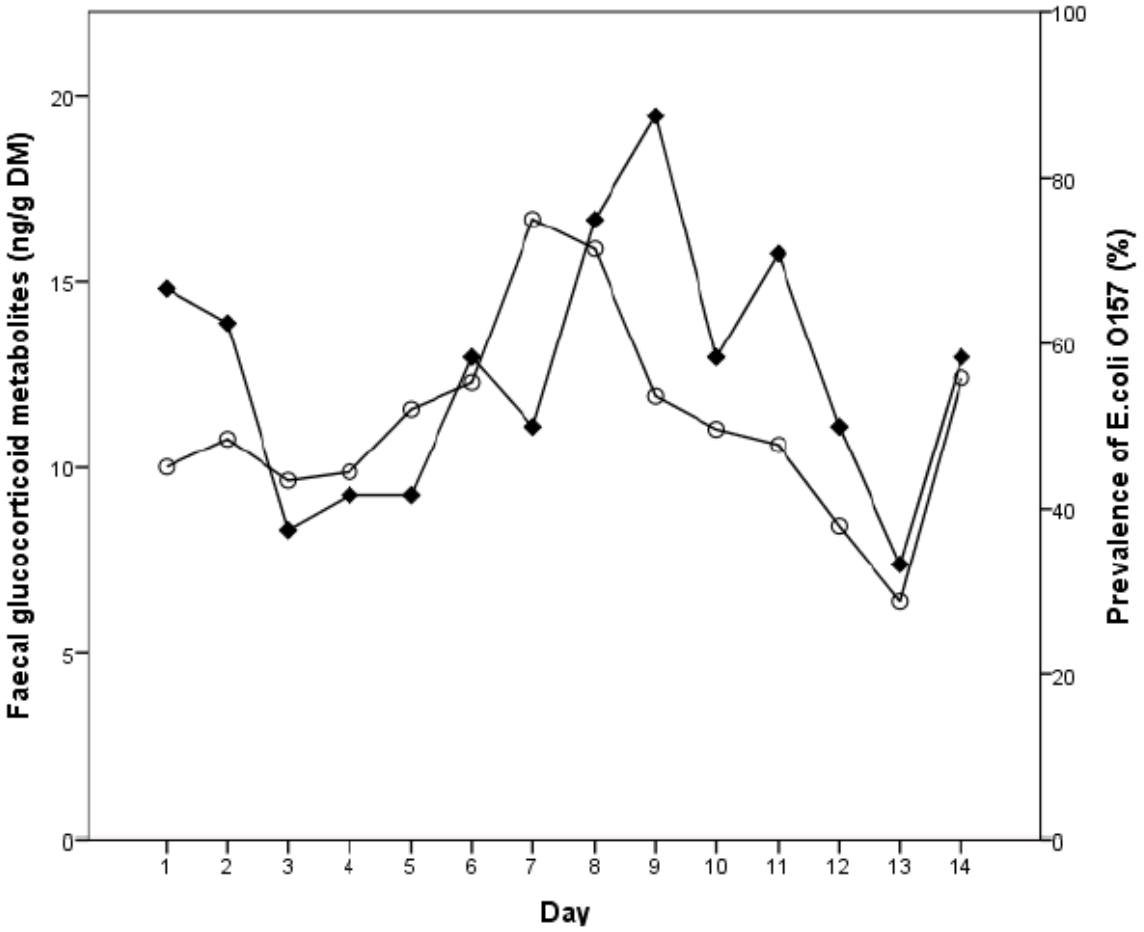

Figure 11. Daily prevalence of *E.coli* O157 shedding in faeces (closed diamond) and mean faecal GM excreted (open circle).

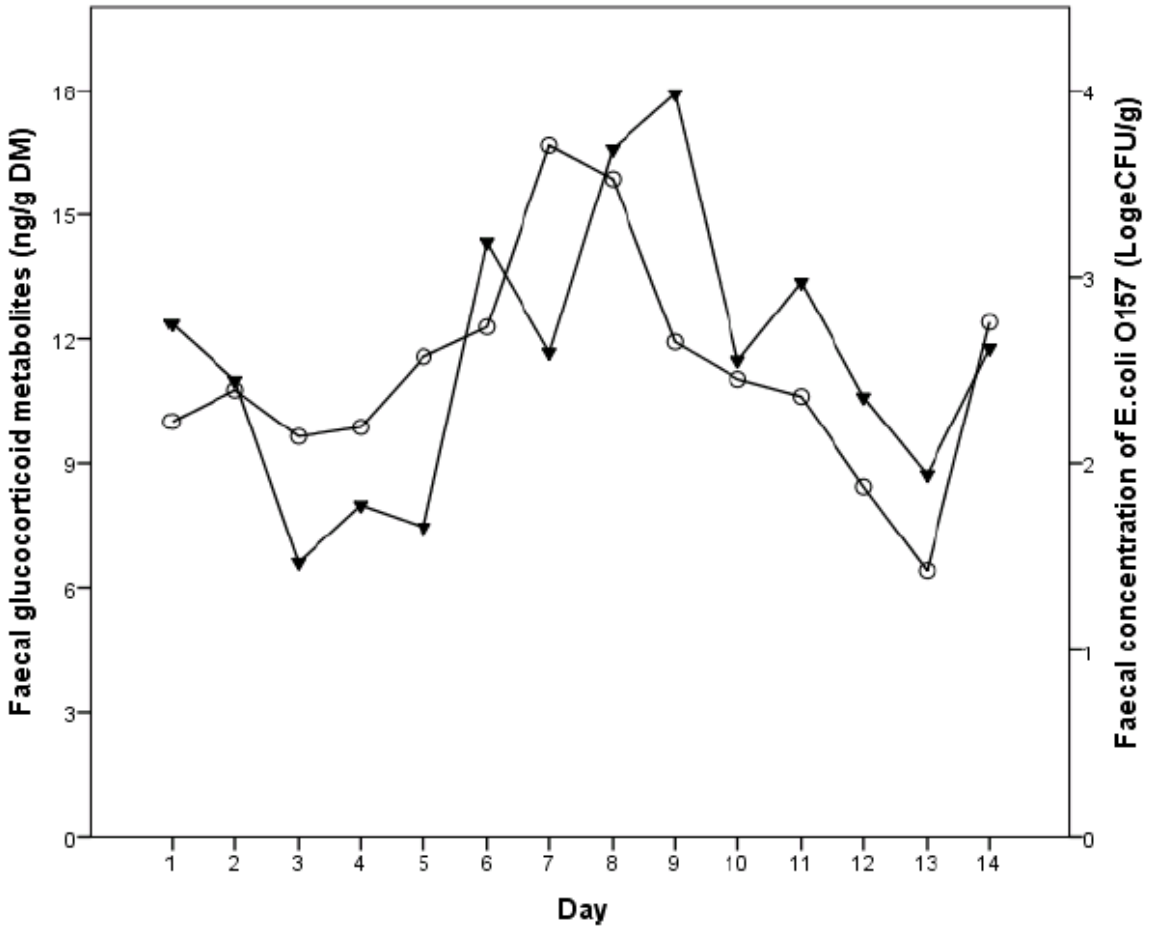

Figure 12. Mean counts of *E.coli* O157 in faeces (Log*e* CFU/g, closed triangle) and mean faecal GM excreted (open circle) across the 14 days.

Linear mixed model analysis revealed that animal weight (P = 0.023) and relative humidity (P = 0.002) were both associated with the concentration of faecal GM. Increased excretion of faecal GM was found to be associated with increased presence and concentration of *E.coli* O157 in bovine faeces (P = 0.018 and P = 0.012, respectively). An association was also found between the interaction between humidity and faecal GM, and both presence and concentration of *E.coli* O157 (P = 0.030 and P = 0.028, respectively).

4.1.5 Part E: Expert Opinion exercise results (reported in full in Appendix F)

Two hundred and eighty participants were selected to be approached for their expert opinion, of which 189 could be contacted by email. A total response rate of 102/189 (54.0%) was obtained. Experts were geographically dispersed and emanated from North America, South America, UK/Ireland, Europe, Africa, Asia, Australia and New Zealand. There was also a range of expertise with *E.coli* O157 research; food safety, zoonotic disease and public health were the main areas of reported expertise.

While some similarities existed between some expert opinion, marked differences of opinion were found for almost all questions. Variation in belief of influential risk factors for shedding

E.coli O157 in cattle faeces was found, along with the effect of differing intervention methods.

4.1.6 Part F: Simulation modelling results

4.1.6.1 Model 1a: "Pool model" results (reported in full in Appendix G)

The dose response data showed that the limit of detection for *E.coli* O157 in bovine faeces ranges between 0.2 and 1 CFU per gram of faeces (Figure 13).

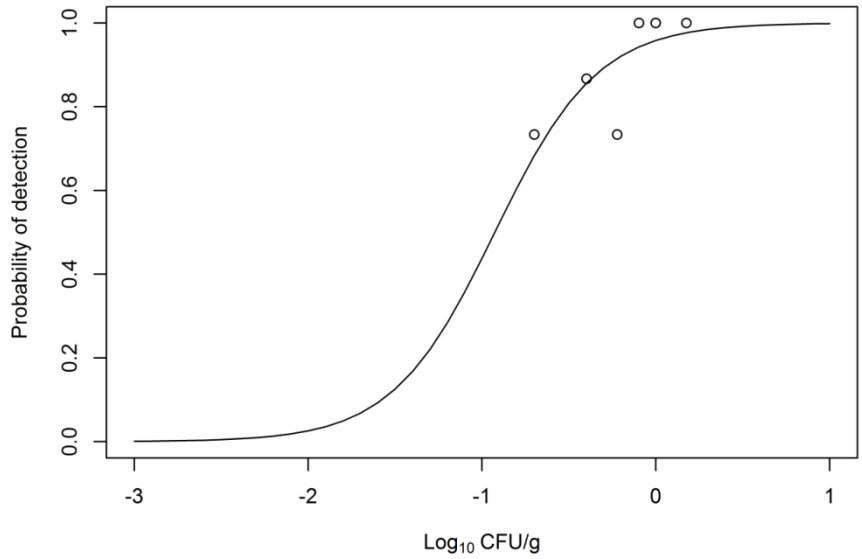

Figure 13. Probability of detection of E.coli O157. Dots represent the observed proportion of detections of *E.coli* O157 vs the $\log_{10}$ concentration in faeces.

Analysis of the data describing the concentration of *E.coli* O157 in cattle faeces by logistic regression provided the intercept and concentration coefficients for the $\log_{10}$ transformed CFU per gram of 3.1347 and 3.3852 respectively, used to describe the dose-response relationship within the simulation model.

The sensitivity of detection of herds shedding *E.coli* O157 using pooled faecal culture relative to individual sample testing increases in a non-linear fashion with increasing number of pools and number of samples per pool (Figure 14).

A larger improvement in sensitivity of detection was seen for each increment in the number of samples per pool (*nspp*) with a smaller number of pools per herd (*np*) than occurs with a larger number of pools. Effectively this demonstrates that for small values of *nspp* the benefit of increasing the number of pools is greatest. The sensitivity of detecting herds shedding *E.coli* O157 ranged from 0.647 using two pools and two samples, to 0.998 using 12 pools and 12 samples. Above an arbitrary detection target of 95% numerous combinations of *np*

and *nspp* exist that might be suitable, for example four pools and nine samples (0.954), six pools and six samples (0.954) or eight pools and four samples (0.950) (Figure 12).

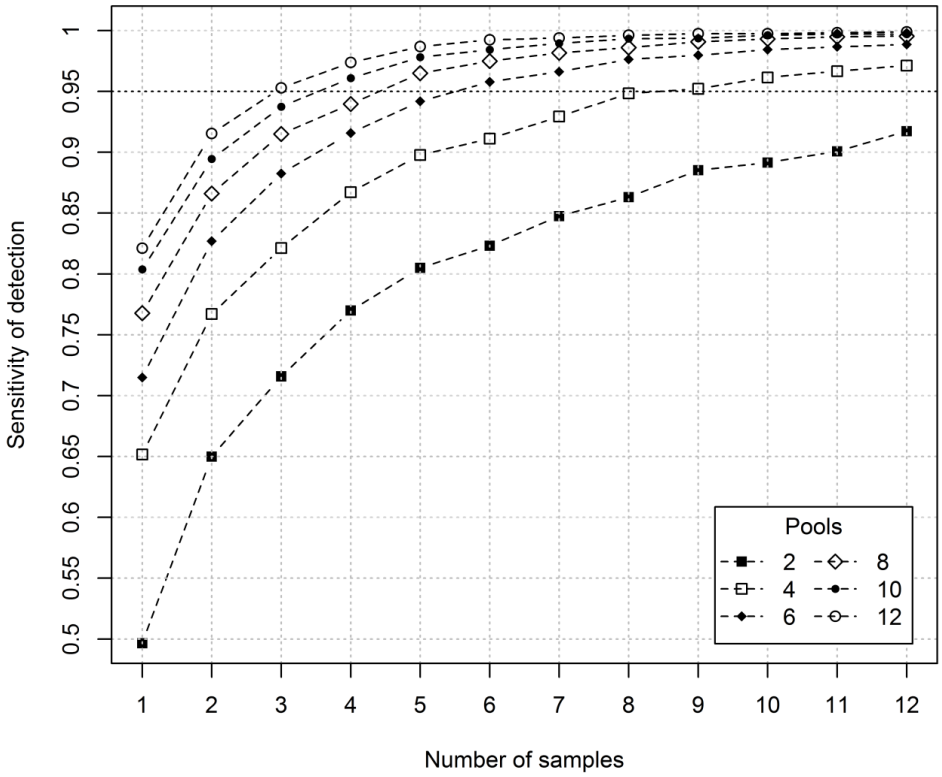

Figure 14. Simulated proportion of pooled faecal samples positive for *E.coli* O157 for various combinations of number of samples per pool and number of pools per herd.

4.1.6.2 Model 1b: Financial model for estimating herd test costs results(reported in full in Appendix G)

The simulated per-herd cost for classifying the *E.coli* O157 shedding status of a herd for various pooled test protocols is shown in Figure 15. At a sensitivity of detection of 95% or higher the total herd test costs range from 211 AUD (four pools and nine samples) to 779 AUD (12 pools and 12 samples).

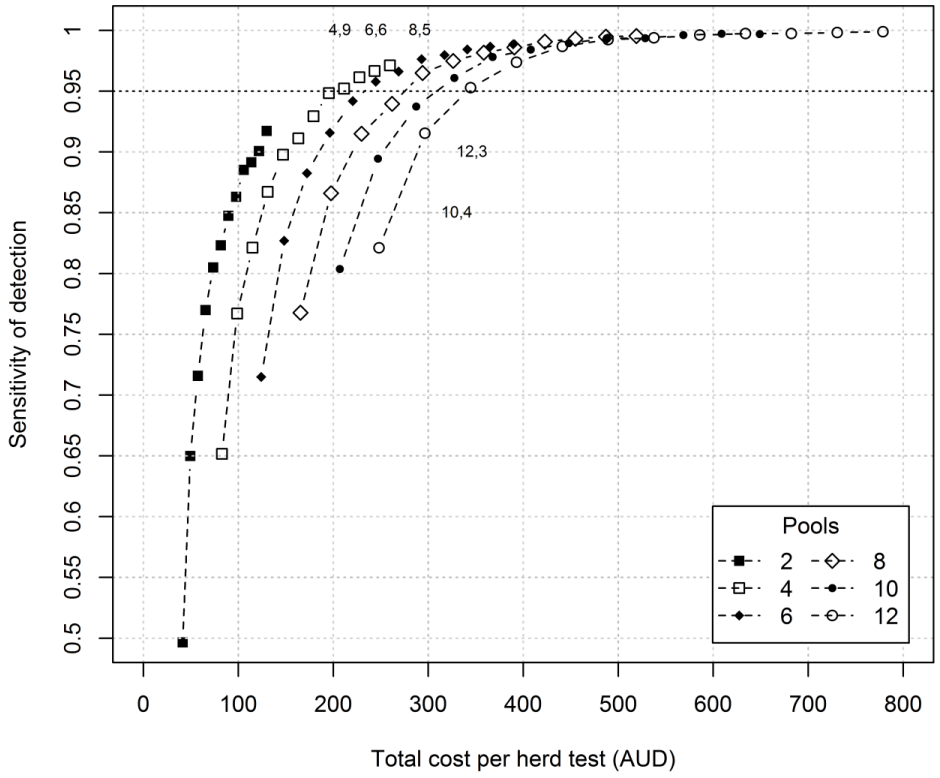

Figure 15. Cost comparison in Australian dollars for various combinations of number of samples per pool (*nspp*) and number of pools (*np*) per herd versus the simulated proportion of pooled faecal samples positive for *E.coli* O157. For a few points the *np* and *nspp* are given.

4.1.6.3 Model 2: "Chain model" results (reported in full in Appendix H)

As the process modelled a rare event, the outcome of interest is reported as the upper limit of the range of prevalence of *E.coli* O157.

When the model is run with parameters set to their full range, the total effect of prevalence of *E.coli* O157 in faeces of origin, transmission between hide and carcass and between GIT and carcass are found to have the greatest influence on the variation in prevalence of *E.coli* O157 contaminated carcasses (Figure 14).

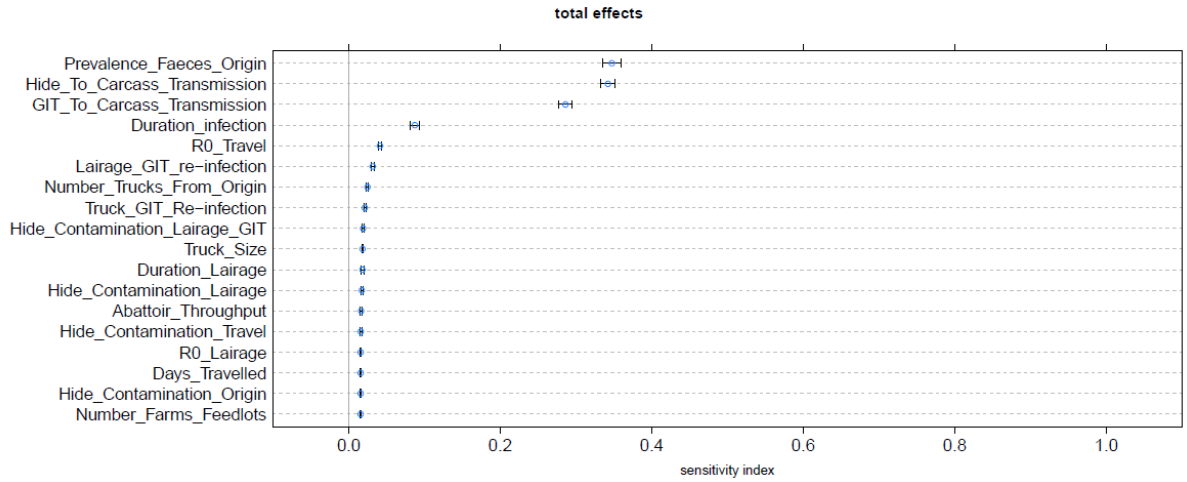

Figure 14. Sensitivity indices (total effect) of parameters importance to carcass prevalence of *E.coli O157* in a model to simulate beef carcass contamination, with no mitigation measures implemented. Bars = 95% confidence intervals.

Interventions, modelled by reducing the range of the parameters where intervention strategies are targeted, were introduced sequentially. These interventions are described in Table 1 and their results in Figure 15.

Due to interactions between inputs (identified by Saltelli GSA), combinations of interventions based on reduced transfer of STEC O157 from hide or gastro-intestinal tract to carcass (improved abattoir hygiene) achieved a greater reduction of range of prevalence than that expected due to an additive effect of single interventions. The most effective intervention was improved abattoir hygiene with vaccination, which achieved a greater than ten times reduction in contaminated carcass prevalence than the initial simulation.

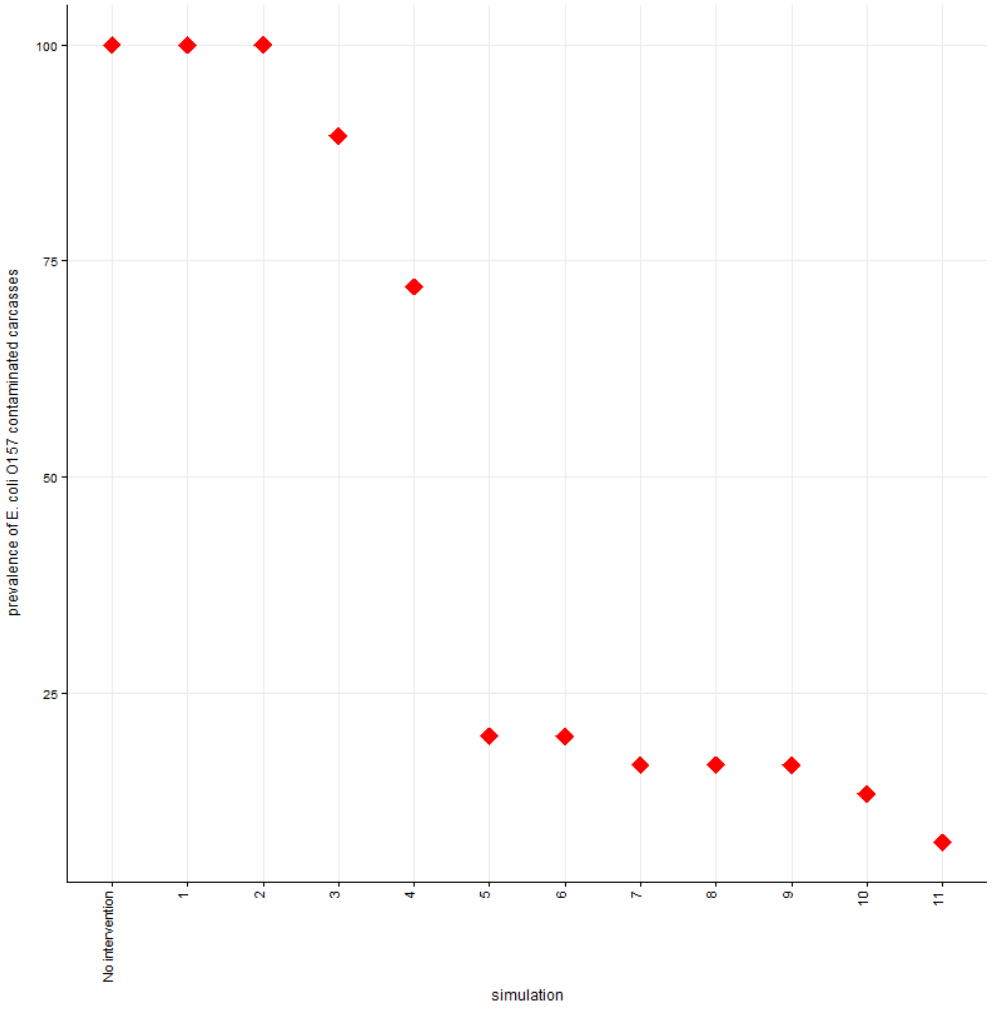

Figure 15: Carcass prevalence of *E.coli* O157 from a model to simulate beef carcass contamination, with increased levels of intervention.

4.1.7 Part G: National forum results

Summaries of work were given by teams at the University of Sydney, the University of Tasmania, CSIRO and Charles Sturt University. Discussions about future directions for research were guided by processor concern and questions, and researchers were able to respond with suggestions and specific possibilities for potential projects and strategies to address the issues raised.

A fact sheet was produced directly from the symposium (Appendix **) which summarises the background to the symposium, the talks that were presented and the key messages.

5 Discussion

The objectives of this project were:

1) Reviewing the microbiological techniques available to detect the *E.coli* O157 supershedding state.
2) Identifying effective and efficient methodology for use within the Australian red meat industry
3) Proposing a study design to estimate the frequency of occurrence of the supershedding state and predictors of this phenomenon
4) Describe future disease modelling studies that could be undertaken to transform empirical field data to information that can be used by industry to develop a control program

All objectives of this study were met within established milestone dates and within budget.

Part A - Literature review

A comprehensive review of the published literature on *Escherichia coli* O157 was undertaken. This review covered the geographical distribution, pathobiology, sample collection and testing methods, estimates of prevalence, definition of the super-shedding phenomenon and risk factors for shedding and super-shedding.

An important outcome of this review was documenting the reports of super-shedding. Early work on *E.coli* O157 described supershedding as an animal persistently infected with this bacteria and shedding it in faeces at > log 4. In a minority of studies, super-shedding was defined as shedding *E.coli* O157 at > log 3. This review helped to formulate a definition of super-shedding and distinguish between super-shedding animals and a super-shedding event.

The concept of persistant infection and colonisation of the recto-anal junction have become accepted as a prerequisite for supershedding. In addition, early modelling work introducted the concept of supershedding animals being responsible for maintaining and transmitting infection within a herd and through the production chain. The previous research conducted in Australia has focused mostly on sampling meat and meat products. No Australian studies using a longitudinal study design in the field – either in beef or dairy herds – were identified. Thus, no estimates of incidence of shedding and super-shedding of *E.coli* O157 under Australian field conditions could be identified.

Parts B and C - Testing methodology

Training was performed initially based on published methods. Spiking experiments were used to verifiy that *E.coli* O157 could be recovered reliably. Over a period of several weeks, the ability to detect known *E.coli* O157 was rapidly improved. An important part of this project was to ensure consistency of laboratory methods at both CSU and USyd. To ensure consistency, samples were shared between the two sites, and staff also visited both sites. Exchanged sampes were tested blindly. A result of this training and sample exchange was the production of a Standard Operation Procedure for testing of samples collected as part of this project (Figure 2). This was a key achievement ensuring that subsequent studies were

conducted with validity in terms of detection of *E.coli* O157. The methods developed were to ensure high sensitivity and specificity within this project. These might not be the same methods used in commercial laboratories or for testing relatively "clean" samples such as meat carcass swabs or core meat samples. Wherever possible, samples in the current study were cultured rather than relying solely on molecular detection methods such as IMS. Although costly, study design required this approach to be used.

Part D – Longitudinal study - Occurrence and predictors of shedding and super-shedding

The incidence of super-shedding of *E.coli* O157 in this project was found to be substantially different between dairy and beef cattle, and also was found to vary greatly within herd in terms of the animal super-shedding at a particular point-in-time.

One super-shedding event was detected in the CSU beef herd. In contrast, 32 such events were detected in the USyd dairy herd. In the latter herd, approximately 20% of detections were classified as super-shedding. Nearly half of the heifers monitored experienced supershedding events, but most of these only experienced one event. These observations suggest that supershedding might be rare in beef cattle production, but more common in dairy production. When supershedding occurs, it can be relatively uniformly distributed within a herd over time (Table 4). Little correlation between the proportion of a herd supershedding over time was found – at the most, there was some correlation up to a period of a week. These results do not support the previous theory of a small number of persistently infected, super-shedding animals mainitaining *E.coli* O157 within a herd.

In contrast, *E.coli* O157 shedding (<log 3) was common in both beef and dairy herds. For example, in the dairy herd the median number of times an animal shed was 7 or 8 and some animals shed up to 13 or 16 times. In the beef herd, all animals shed the pathogen at least once and similarly individuals shed *E.coli* O157 between 2 and 15 occassions. The estimated prevalence within herds had a wide range, effectively between 10 and 90%. Based on this research, with sufficient sampling most animals can be detected to shed *E.coli* O157 at some point in time.

In both herds, evidence of peaks in shedding over time were observed. Such peaks in shedding ("synchronicity") appeared more pronounced in the beef than the dairy herd. Synchronisation of shedding amongst cattle was observed, accompanied by a marked elevation in the concentration of the pathogen in faeces during times of increased probability of shedding.

In the dairy herd, *E.coli* O157 shedding was associated with rainfall, humidity and solar exposure during the previous 7 days, temperature during the previous 14 days, and pasture growth and body score. Super-shedding was associated with rainfall during the previous 7 days, hide contamination and faecal consistency. In the beef herd, hide contamination and rainfall in the preceding 24 hours were associated with shedding. Based on the combined results of this project, rainfall appears to be one of the driviers of *E.coli* O157 shedding. Rainfall might be particulary important for super-shedding. If rainfall is one of the principle drivers of *E.coli* O157 shedding, other factors – such as hide contamination and faecal consistency – could be explained via their association with rainfall events.

Intensive studies performed over a shorter period of time (sampling once or twice daily for 14 days) showed similar variation in frequency and concentration of *E.coli* O157 shed between days, within days and within and between animals. This further supports the difficulty in accurately identifying shedding or supershedding animals or events from a single cross-sectional sample of a herd.

In addition, an important and convincing association between faecal GM and increased presence and concentration of *E.coli* O157 in bovine faeces was found. This finding might provide a foundation for explaining the shedding of *E.coli* O157: many factors – such as rainfall – can act as stressors, eventually leading to shedding. Studies found that nearly all isolates in boths herds are potentially pathogenic: *Stx1* and *Stx2* positive.

Parts E and F - Disease modelling studies to develop a control program

Expert Opinion

In a survey of experts, a response rate of 54% was achieved. Experts were geographically dispersed, with a broad range of expertise in *E.coli* O157 research, food safety, zoonotic disease and public health. Marked differences of opinion were found for almost all questions posed to these experts. This indicates that the current status of knowledge regarding *E.coli* O157 is inconsistent. Either this reflects very different and specific local effects in different countries, or generally a great deal of uncertainty in the scientific community. Such uncertainty requires targeted research to address. Without reseach addressing such key knowledge gaps, the development and uptake of control and risk mitigation programs is problematic.

Simulation modelling

A model describing *E.coli* O157 through the market chain was developed. It was parameterised with information from the literature, expert opinion and the current research program. This model suggests that *E.coli* O157 transmission between hide and carcass and between gastrointestinal tract and carcass have the greatest influence on the variation in prevalence of *E.coli* O157 contaminated carcasses (Figure 14). This model indicated that interventions resuting in improved abattoir hygiene have the most impact on reducing prevalence. Assuming improved abattoir hygiene combined with vaccination, >10-times reduction in contaminated carcass prevalence can be achieved (compared to baseline simulations). These results reinforce the importance of abattoir systems in the routine control of *E.coli* O157; however, once these standards are achieved, addtional interventions to reduce the risk of high challenge periods or events offer further control options.

All project objectives were met as follows:

1) Reviewing the microbiological techniques available to detect the *E.coli* O157 supershedding state.
    - This objective was met through the literature review, technical training and pilot studies, performed in Parts A and B and reported in sections 3.1.1, 3.1.2, 4.1.1, 4.1.2 Appendix A and Appendix I.

2) Identifying effective and efficient methodology for use within the Australian red meat industry
    - This objective was met through the technical training and laboratory skills validation, performed in Parts B and C and reported in sections 3.1.2.1, 3.1.3, 4.1.2.1 and 4.1.3.
3) Proposing a study design to estimate the frequency of occurrence of the supershedding state and predictors of this phenomenon
    - This objective was met through the longitudinal studies performed in Part D and reported in sections 3.1.4, 4.1.4 and Appendices B, C, D and E.
4) Describe future disease modelling studies that could be undertaken to transform empirical field data to information that can be used by industry to develop a control program
    - This objective was met through the Expert Opinion and simulation models performed in Parts E and F and reported in sections 3.1.5, 3.1.6, 4.1.5, 4.1.6 and Appendices F, G and H.

6 Conclusions/Recommendations

This work (Part D) identifies that shedding of *E.coli* O157 by cattle is highly variable, in both duration and concentration. Cattle shedding in an uninterrupted manner were very rare, as were animals that did not shed the pathogen at all. In the longitudinal studies that sampled individual animals on a weekly basis, no animals were detected that did not shed the pathogen at least once (Part D). It is likely that a similar heterogeneous shedding pattern will be found in all animals if they are exposed to a combination of intensive and longitudinal sampling. Several studies on super shedding claim that with the identification of super shedding cattle, strategies could be specifically targeted at this subpopulation, thereby reducing *E.coli* O157 transmission at the production and lairage environment (Arthur et al., 2010; Munns et al., 2015). However, this research did not observe cattle that shed at super shedding levels ($>10^3$ or $>10^4$ CFU/g of faeces) without interruption, or identify risk factors to enable prediction of super-shedding in individual animals (Munns et al., 2014; Williams et al., 2015). This indicates that super-shedding is a transient phenomenon. These studies are the first to define and measure the frequency of super-shedding and shedding in the field under typical Australian cattle management systems. Results indicate that the strategy of identifying individual super-shedding cattle within herds and removing these cattle is unlikely to affect the prevalence of infection or the risk of contamination during processing.

In this research, shedding at both the individual- and herd-levels was unpredictable. No obvious patterns within days were found. Within one day, populations of *E.coli* O157 shed by some cattle varied between undetectable to 1000 CFU/g of faeces. The highly variable nature of the excretion of *E.coli* O157 in cattle makes it difficult to predict. Furthermore, the unpredictability of shedding at the herd level was reflected by the lack of correlation between the number of cattle detected positive one day and the number of cattle shedding the previous day. While rainfall was identified as a risk factor for shedding at both sites, further study is required in order to elucidate the precise effect of such an environmental risk factor. Whilst it might be possible to predict periods and conditions in which *E.coli* O157 shedding

could be elevated, prediction of shedding animals within a herd or at a particular point-in-time appears unlikely.

In the current research program, the data were predominantly analysed at a herd level, across two sites. While two individual herds cannot be fully representative of any production systems or geographical environments, inclusion of multiple herds was precluded due to obvious financial and labour restrictions associated with the high sampling intensity applied within this study. One dairy herd and one beef herd were choosen to reflect the two sectors which influence risk of *E.coli* O157 through the production chain. High intensity sampling was considered a better approach than prevalecne sampling of a large number of herds. The results support this decision, showing how dynamic shedding within herds can be.

Modelling (Part F) has identified that pooled sample analysis is appropriate for future studies where longitudinal sampling is required. The reduction in cost associated with such pooling of samples will allow larger numbers of animals and herds to be included in future studies.

The simulation study of the beef harvest chain in this project (Part F) highlighted that implementation of interventions in the chain prior to the abattoir (for example, vaccination of cattle on farm) do not reduce the potential range of prevalence of *E.coli* O157 contaminated carcasses without effective interventions to reduce transfer from hide and the gastro-intestinal tract to carcasses in the abattoir.

Although there is already considerable research into abattoir interventions to reduce carcass coliform contamination, particularly from hide, the current study identified the following key points as targets for future research:

1. Regarding the efficacy of interventions to reduce prevalence of contaminated carcasses:

   Which interventions are most effective to reduce the probability of transfer from hide or GIT to carcasses? How effective are these interventions in the field, and is this enough for implementation of pre-abattoir interventions to be worthwhile?

2. Regarding the efficacy of interventions to reduce the incidence of detection of *E.coli* O157 in manufacturing beef and the incidence of *E.coli* O157 in humans:

   How does the distribution of *E.coli* O157 on carcasses change as prevalence of contamination is reduced? For example, if fewer carcasses are contaminated but they all have a high level of contamination, will the incidence of detection of *E.coli* O157 in manufacturing beef and the incidence of *E.coli* O157 in humans be reduced? This links with previous work regarding processing interventions that are applied as a series of hurdles to reduce the amount of *E.coli* O157, and also to a recent risk assessment by (Kiermeier et al., 2015) which determined that the dispersion of organisms throughout ground mince was an important factor in determining the incidence of human illness.

In addition, further studies to unpack the relationship between synchronised shedding and risk factors such as stress are clearly indicated and, with the methods identified within this report, are now able be performed across numerous establishments in a longitudinal manner. Building on this, research that enables pre-emptive identification of high risk periods (through modelling environmental conditions and mapping to coliform counts across multiple

establishments) would enhance the knowledge of both the synchronisation of shedding and further elucidate the effect of the relationship between stress and shedding of *E.coli* O157.

7 Key Messages

A national symposium (Part G) was held in March 2015, where summaries of work surrounding *E.coli* O157 in Australia were presented by teams at the University of Sydney, Charles Sturt University, the University of Tasmania and CSIRO. Discussions about future directions for research were guided by processor concern and questions, and researchers were able to respond with suggestions and specific possibilities for potential projects and strategies to address the issues raised. A fact sheet was produced directly from the symposium (Appendix J) which summarises the background to the symposium, the talks that were presented and the key messages.

The key messages relating to this project are that;

1) Shedding and super-shedding of *E.coli* O157 is highly variable both between and within animals.

   Message*: identifying individual "super-shedders" is not feasible.*

2) Synchronisation of shedding may occur within herds, where there are periods of time that are associated with a high prevalence and concentration of *E.coli* O157 shed in faeces across the herd.

   Message*: it might be feasible to predict periods and conditions of increased E.coli O157 risk*

3) The only consistent risk factor identified for shedding *E.coli* O157 across the two production systems studied was rainfall. There also appears to be a relationship between individual animal stress and shedding and concentration of *E.coli* O157. Further investigation into the effect of these two variables is required to fully elucidate the nature of these relationships.

   Message*: environmental factors likely directly or indirectly – via a stress pathway – increase the likelihood of E.coli O157* shedding *and should be considered as a potential risk forecasting tool.*

4) It does not appear that supershedding animals can be identified for implementation of pre-slaughter control measures and that, indeed, all animals may have the capacity to super-shed at some time.

   Message*: E.coli O157 is an endemic infection and most herds are likely infected, with the prevalence probably fluctuating considerably over time. Maintaining an E.coli O157 free herd is unlikely to be feasible.*

5) Pre-slaughter control measures may be effective in reducing carcass contamination with *E.coli* O157, but only in the presence of effective abattoir hygiene that results in a very low probability of transfer of this organism from both GIT and hide to the carcass. In the absence of such control measures at the level of the abattoir there is little benefit of on-farm interventions.

***Message**: abattoir interventions to improve the general microbiological quality of meat products remain critical. Additonal interventions can have an impact, but their cost-effectiveness needs to be determined.*

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9 Appendix list

9.1 Appendix A – Pilot study

9.2 Appendix B – Longitudinal study in dairy cattle at the University of Sydney

9.3 Appendix C – Longitudinal study in beef cattle at Charles Sturt University

9.4 Appendix D – Intensive longitudinal study in beef cattle at Charles Sturt University

9.5 Appendix E – The relationship between stress and *E.coli* O157 shedding in cattle

9.6 Appendix F – Expert Opinion Exercise regarding *E.coli* O157 in cattle

9.7 Appendix G – Model to identify the utility of pooled sampling in detecting E.coli O157 in longitudinal studies

9.8 Appendix H – *E.coli* O157 contamination of beef carcasses: Saltelli global sensitivity analysis to identify risk factors and develop mitigation strategies in the beef harvest chain.

9.9 Appendix I - Literature review of *E.coli* O157 colonisation and shedding in cattle

9.10 Appendix J – MLA fact sheet – Shiga toxin-producing *E.coli* and beef production