final report

Project code: PRMS.067 and PRMS.070

Prepared by: Food Science Australia

Date published: January 2005

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Modelling transfer of E. coli and antimicrobial resistant organisms from animals to carcase during slaughter

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

Relationship between the concentration of generic *Escherichia coli*, *E. coli* O157:H7 and *Salmonella* in bovine faeces and on beef carcases

Introduction

Escherichia coli O157:H7 and *Salmonella* are both food-borne pathogens of humans that can be found in the gut of healthy livestock. Cattle are considered one of the main reservoirs of *E. coli* O157:H7 and consumption of under-cooked beef products has been identified as a cause of human disease due to this pathogen. A factor that determines the proportion of humans who become ill after exposure to *Salmonella* or *E. coli* O157:H7 in food is the number of viable cells ingested. Thus knowledge of the concentration of the pathogen at various stages of cattle production, beef processing, food preparation and consumption is of critical importance in assessing risk of human disease due to these pathogens.

Until recently it has been difficult to enumerate wild strains of *E. coli* O157:H7 in naturally contaminated samples. The enumeration of *Salmonella* has been possible for many years, although the methods used have been of questionable accuracy when only small numbers of the pathogen are present. As a result, inferences about the concentration or behaviour of *E. coli* O157:H7 and *Salmonella* in animals, faeces or foods or inferences about the suitability of conditions for growth of these pathogens have sometimes been based on the concentration of indicator organisms. Generic *E. coli* is an indicator organism that is attractive as a measure of risk because it is present in high concentrations in cattle faeces (and so are thought to be a good measure of faecal contamination on carcases), has biological similarities with *Salmonella* and *E. coli* O157:H7, and can be enumerated using simple and inexpensive methods. Despite the common use of generic *E. coli* for monitoring hygiene during meat production the extent to which this measure provides information on the risk of occurrence of either pathogen has not been well studied. If a relationship can be shown to exist between counts of generic *E. coli* in faeces and counts of pathogen in faeces then routinely

collected data on generic *E. coli* concentration on the surface of carcases becomes a valuable tool for studying the risk of contamination of carcases with pathogen.

New methods for accurately enumerating *E. coli* O157:H7 and *Salmonella* provide an opportunity to explore the above relationship. The technique involves combining the most probable number method (MPN) with immunomagnetic separation (IMS) [1,2]. A major advantage of this approach is the ability to quantify the amount of pathogen when only small numbers are present. A disadvantage of this approach is the accuracy of the MPN method itself.

In this work we therefore aim to describe the relationship between counts of generic *E. coli*, *E. coli* O157:H7 and *Salmonella* spp. in naturally contaminated cattle faeces and on beef carcases during a commercial slaughter process. Where useful relationships are found we attempt to express them in a probabilistic fashion for use in a quantitative risk model. The analysis is performed on existing data where the *Salmonella* and *E. coli* O157:H7 concentration component were generated by the combination of MPN and IMS.

Methods

Study 1: National survey of pathogens in bovine faeces

Background This study was based on a series of triplet observations describing the concentration of *E. coli* O157:H7, *Salmonella* and generic *E. coli* in cattle faeces. The observations were made on faecal samples obtained during a national survey of Australian beef cattle at slaughter[1,2]. Faecal specimens had been aseptically collected from faecal pats from abattoir holding pens and per-rectum. Of 310 specimens collected half were from grain-fed cattle and half were from grass-fed cattle. Faecal specimens were tested for the presence of *E. coli* O157:H7 and *Salmonella* using a protocol based on culture preceded by IMS and positive samples submitted for enumeration using MPN combined with IMS. The concentration of generic *E. coli* in each specimen was measured using Petrifilm™. In those specimens containing generic *E. coli* counts could be estimated down to 10 cfu/g, which is the limit of detection for the method used. The censoring point for *E. coli* O157:H7 and

Salmonella were both 3 cfu/g, that is, samples positive for either pathogen but containing less than 3 cfu/g could not be enumerated.

Data analysis The aim of data analysis was to identify whether a straight line or curve could be used to model the relationship between the concentration of generic *E. coli* and the concentration of either *Salmonella* or *E. coli* O157:H7 in individual samples of bovine faeces. We also aimed to identify if there was a relationship between the presence or concentration of *Salmonella* and the presence (or concentration) of *E. coli* O157:H7 and vice-versa.

Initially it was necessary to consider if there were any significant differences between the data for grass and grain fed cattle. Evidence of similarity of these groups would allow the data from each to be combined and treated as one thereby increasing the power of subsequent analysis. Grass and grain-fed cattle were compared using descriptive statistics for $\log_{10}$-transformed counts of generic *E. coli*, *E. coli* O157:H7 and *Salmonella*. Unpaired t-tests were used to assess the significance of differences between means of $\log_{10}$ transformed generic *E. coli* counts and non-parametric tests (Wilcoxon's rank-sum test) used for comparison of *Salmonella* counts and *E. coli* O157:H7 counts conditional on the pathogen being present. Fisher's exact test was used to test for differences in the prevalence of pathogens between the two groups.

It was also necessary to consider how the inclusion of counts below the limit of detection affected the comparison between grass-fed and grain-fed animals. Thus each of the statistical comparisons mentioned in the previous paragraph were performed twice. In the first case all non-zero counts were used but with the censored observations replaced by a random uniform value between 0 and the censoring point (referred to as 'simulated replacement'). In the second case all observations below the censoring point were excluded from the analysis (referred to as 'excluded < censoring point'). Findings from these two approaches were compared to judge if the censoring of data would affect the validity of an analysis of the merged results for grass-fed and grain-fed cattle.

Analysis of the merged groups included transforming each estimate of concentration of generic *E. coli*, *Salmonella* and *E. coli* O157:H7 onto the scale of natural ($\log_e$) and base 10 logarithm ($\log_{10}$). A third transformation was applied to *E. coli* O157:H7 only

and consisted of a power transformation derived using the Box-Cox procedure [4]. In this latter technique the independent variable in a regression model (concentration of generic *E. coli*) is repeatedly regressed against the dependent variable (concentration of *E. coli* O157:H7) raised to some power (*lambda*) with *lambda* varying over a wide range (-4 to +4). The value of *lambda* that best explains the relationship between the two variables is that which maximises the value of the likelihood function. There were too few positive results for *Salmonella* to use the Box-Cox procedure.

Scatter plots were used to make pair-wise comparisons between each transformation of the two bacterial concentrations. The suitability of each such pair of transformations for prediction was judged by visual examination of scatter plots for possible linear or nonlinear relationships.

Predicting pathogen concentration from the concentration of generic E. coli

In risk models it is intuitively appealing to model the concentration of *E. coli* O157:H7 in a sample of faeces as a fixed fraction of the concentration of generic *E. coli* conditional on the presence of *E. coli* O157:H7 (the 'conditional fraction method'). This is equivalent to saying that in faeces where *E. coli* O157:H7 is present there is a random variable called *k* that describes the concentration of *E. coli* O157:H7 divided by the concentration of generic *E. coli*. From the earlier analysis we attempted to define a probability distribution for *k* that describes how good (or bad) the conditional fraction method is for modelling the concentration of *E. coli* O157:H7 in cattle faeces. The probability distribution for *k* would then provide a basis for modelling *E. coli* O157:H7 from the much more commonly available data on generic *E. coli*.

Probabilistic description of pathogen concentration

'Left censoring' occurs as a result of the inability of tests to accurately enumerate organisms when they are present at low concentrations (in this case 3 cfu per gram of faeces). In other words, data on bacterial concentration is left censored when the

pathogen is detected but there are too few bacteria present for enumeration. Use of standard 'distribution fitting' techniques (eg. maximum likelihood estimation) in the presence of this left censoring could deliver a biased estimate of the distribution of pathogen concentration (a necessary input for risk models that consider the transfer of contamination from animal to carcase). A technique for defining an appropriate log-normal distribution for left-censored data has been demonstrated elsewhere using the *Salmonella* concentration data described here combined with that from a separate survey by Food Science Australia of Queensland cattle at slaughter [3]. In this work a similar analysis was repeated for *E. coli* O157:H7. In short, observations that are censored and those not censored are individually identified as such before analysis. A censored regression routine in the Stata analysis package (release 8.1, Stata Corporation, College Station, Texas, USA) was then used to compute a parametric distribution of choice for the data. It was decided to fit a lognormal distribution to *E. coli* O157:H7 data because this is typically the underlying distribution used to describe the size of bacterial populations and because it is the theoretically correct distribution for outcomes resulting from the combination of many non-additive effects. Lognormal fitting can be conveniently achieved by fitting a normal distribution to the data after observations have undergone $\log_{10}$ transformation.

Study 2. Density of generic *E. coli* and pathogens in the alimentary tract of cattle at slaughter and on carcases

Data from a study on sources of contamination of *E. coli* O157 and *Salmonella* conducted by Food Science Australia (PRMS.030), consisted of multiple measurements made on cattle during the slaughter process. Carcases of 100 cattle at a commercial abattoir in south-eastern Queensland were followed through the slaughter and dressing process until after chilling. Observations were made on groups of 25 carcases per visit with one to three weeks between visits. Swab samples of the hide were collected from the hock, flank, back, brisket and neck (PRMS.030, Milestone 9), a total area of 500 cm^2 was sampled on each animal. Saliva samples were collected by inserting a Whirlpak sponge into the oral cavity after the animals had been stunned. The amount of saliva collected was estimated by weighing the sponges before and after collection. Rumen contents (20 g) and faecal (colonic) specimens (30 g) were

obtained following evisceration. Immediately before carcases were sent to be chilled surfaces were sponged following the procedure used in the ESAM program (AQIS Meat Notice 2000/09), a total are of 300 cm^2 was sampled for each carcase. Post-chill carcases were sampled in an identical manner to pre-chill carcases.

All samples were submitted for detection and enumeration of generic *E. coli*, *E. coli* O157:H7 and *Salmonella* spp. Generic *E. coli* was detected and enumerated using Petrifilm. The presence of *E. coli* O157:H7 and *Salmonella* was assessed using culture followed by IMS and enumerated using a combination of IMS and the MPN procedure[1,2]. Data from these measurement systems are also 'left censored'. For *E. col*i O157:H7 and *Salmonella* the smallest concentration that could be enumerated was 3 MPN/g for saliva, rumen contents and faeces, 0.06 MPN/cm^2 for hide swabs and 0.1 MPN/cm^2 for carcase swabs. For generic *E. coli* the limit of detection (LOD) of 0.33 cfu/cm^2 applied to carcase swabs and results reported at less than the LOD are all regarded as zero counts in this analysis (0 cfu/g or 0 cfu/cm^2).

A simple descriptive analysis of this data was performed. Where possible, associations between the occurrences of organisms in saliva, on hides, in rumen content and in faeces were explored using two-way plots (continuous data). Calculation of crude risk-ratios and corresponding confidence intervals (from dichotomous data formed into two-way tables) were produced where possible. Risk ratios are a ratio of the prevalence of the outcome (i.e. carcase contamination with a specific organism) in groups with the 'risk factors' (prevalence of a specific organism in saliva, hide swabs, rumen contents or faeces) and in groups without the risk factor. All analysis was performed in Stata release 8.1.

Results

Study 1

An extensive comparison of the data describing faecal microbiology of grass-fed and grain fed cattle did not reveal a substantial difference between the two groups (Tables 1 to 4). Comparison of the descriptive data also suggested that the method of handling censored data would not impact on the decision to pool data from grass-fed and grain-

fed groups. One exception was with *E. coli* O157:H7 where the manner in which censoring was handled did impact on comparison between grass-fed and grain-fed cattle (Table 4), although the medians only differed by 6 cfu/g. It was decided to merge the data from grass-fed and grain-fed cattle because the medians were not too dissimilar and merger would provide a much stronger basis for addressing the aims of the study.

The Box-Cox procedure when applied to the *E. coli* O157:H7 data identified that 0.27 was the best power transformation for defining the statistical relationship between the concentration of this pathogen in bovine faeces and that of generic *E. coli* This does not imply that the 0.27 power transformation is a good basis for modelling the relationship under question. Rather, it is the best of the power transformations for this purpose.

Plots of paired observations on transformed and untransformed concentrations of generic *E. coli* and *E. coli* O157:H7 in bovine faeces are shown in Figure 1. None of the pair-wise plots reveals any suggestion of a linear or curvilinear relationship that could possibly be exploited in a predictive fashion. A similar analysis performed to describe the relationship between concentration of generic *E. coli* and *Salmonella* is shown in Figure 2. There were fewer positive counts of *Salmonella* to use in this analysis and there was no discernable relationship between the two concentrations.

It was found that $\log_e$ transformation of k (equivalent $\log_e$ of the ratio of the concentration of *E. coli* O157:H7 to the concentration of generic *E. coli*) yielded a quantity (k') that was approximately normally distributed (mean -11.19., sd = 3.95). Back-transformation from the distribution of k' provided a mean value of 1.3×10^{-5} with 95% confidence limits for k of 3.1×10^{-2} to 6.0×10^{-9} (the ratio of the concentration of *E. coli* O157:H7 and the concentration of generic *E. coli*)

Normal probability densities for the log concentration of *E. coli* O157:H7 generated from censored regression (mean = 0.865, sd = 1.40) and that from the standard distribution fitting technique (mean = 1.02, sd = 1.20) are shown in Figure 3.

The relationship between occurrences of *E. coli* O157:H7 and *Salmonella* in bovine faeces is shown in Table 5. There were too few specimens containing both pathogens to assess the correlation in their concentrations and so analysis was confined to

comparing the probability of occurrence of *E. coli* O157:H7 in samples that were *Salmonella* positive and those that were *Salmonella* negative. A relative prevalence statistic of 2.50 was obtained for this relationship indicating that samples containing *Salmonella* were 2.5 times more likely to contain *E. coli* O157:H7 than samples not containing *Salmonella*.

Study 2

The key feature of this study was the general decline in the prevalence of pathogens (or our ability to detect them) as processing progressed (Table 6) and the absence of *E. coli* O157:H7 in rumen contents despite it being common in saliva and faeces. As well, although generic *E. coli* was found more frequently and at higher concentrations on hides, in saliva, rumen contents and faeces it was only infrequently found in swabs of pre-chill and post-chill carcases (Table 6) and in positive carcase swabs it was only present at a low density per unit area (Figure 4). Despite the substantial variation in the occurrence of *Salmonella* and *E. coli* O157:H7 in saliva, hide swabs and faeces, both pathogens were detected too infrequently on pre-chill carcases and on post-chill carcases to warrant any further analysis. For example, only a small number of risk ratios could be calculated for associations between dichotomous carcase outcomes. Similarly, the lack of variation in the presence or absence of generic *E. coli* on hides, in saliva, in rumen contents and in faeces precluded further analysis. Some additional descriptive analysis of generic *E. coli* data provided little evidence that counts of organisms in swabs of hide, saliva, rumen contents or faeces could be used to predict counts on carcases (pre-chill or post-chill) (Figure 5). Due to zero values in two-by-two tables, calculation of relative risk could only be performed for the occurrence of *E. coli* O157:H7 in faeces as a risk factor for *E. coli* O157:H7 in pre-chill carcase swabs (RR = 8.9, 95% CI: 2.19 to 35.8) and for the occurrence of *Salmonella* in saliva as a risk factor for *Salmonella* on pre-chill carcase swabs (RR = 2.4, 95% CI: 0.2 to 37)[1]. In the analysis it was not possible to take into account the day of sampling or the impact of possible confounders due to the sparseness of the data.

[1] As an example of interpretation of relative risk, in this work we estimated that animals with *E. coli* O157:H7 in their faeces at slaughter were 8.9 times more likely to have *E. coli* O157:H7 detected on swabs of their pre-chill carcases compared to animals without *E. coli* O157:H7 in their faeces. The 95% confidence interval (2.19 to 35.8) does not include 1. However, these are crude estimates of risk (no control of confounders) and they do not take into account the effect of day of sampling in the calculation of confidence limits.

Discussion

In Australia and other developed countries counts of generic *E. coli* are often monitored during meat production. Because of the large volume of accumulated data it is intuitively appealing to use the information on counts of generic *E. coli* to model the probability of occurrence of *E. coli* O157:H7 and *Salmonella*. For modelling to occur the relationship between the indicator and each pathogen (if any exists) needs to be described. Although the conditional fraction method is a simple technique for this purpose the findings of analysis of Study 1 indicates that there is very poor association between measurements of bacterial density of these organisms in faeces (generic *E. coli* vs *E. coli* O157:H7) which is regarded as the primary source of pathogens that contaminate the surface of beef carcases, albeit indirectly by transfer from the integument. Essentially all these microbial densities appear to be quantities that are statistically independent. Thus, if the conditional fraction method were to be used to model *E. coli* O157:H7 concentration in faeces from generic *E. coli* then extremely broad bounds of uncertainty would need to be used (10^7 order of magnitude) as indicated by the wide confidence interval for the mean value of the ratio between *E. coli* O157:H7 and generic *E. coli* (*k*).

The analysis of Study 2 highlighted the difficulty of extrapolating from carcase microbiology results to generate a 'risk of occurrence' of either *E. coli* O157:H7 or *Salmonella*. This is largely due to the very small amount of variation in the presence of either pathogen in swabs of carcases which constrains the options for data analysis. Further, generic *E. coli* counts in saliva, rumen contents, hide swabs and faeces all appear to be poor predictors of counts on carcases owing to the lack of any hint of a monotonic relationship between any of these data. In fact the data analysis conducted both for Study 1 and Study 2 was more extensive than what would normally be justified. We felt this was necessary so to comprehensively assess and where possible exclude the possibility of relationships between the measurements. Also the data is unique, addressing issues of critical importance in the meat industry, which have been the subject of speculation in the scientific literature.

From our analysis it is clear that progressing the understanding of these relationships in the future will probably require an alternative measurement system for assessing

the microbiology of carcases. While the laboratory methods available appear adequate, the current method for sampling from carcases in commercial establishments involves swabbing a 300 cm^2 area. While this appears to be acceptable for quality assurance purposes it does not always meet the needs of research. Under Australian commercial conditions where levels of contamination with gut-derived bacteria are very low (MLA, 2005) sampling from a 300 cm^2 area has only a small chance of detecting pathogens because, in the rare instances that they are detected, they only occur at an extremely low density. The ideal measurement system of the future would allow rapid sampling from a much larger surface of the carcase and be able to be conveniently deployed in a commercial setting. The feasibility of developing such a measurement system is presently unknown, although research is currently underway in PRMS.030 looking at the effect of large area sampling on pathogen isolation rates from carcases.

Importantly the analysis of Study 1 also showed little difference between grass and grain-fed cattle with respect to concentration of generic *E. coli*, *E. coli* O157:H7 and *Salmonella* in the faeces of slaughtered cattle. Previously there has been intense speculation in the literature on the effect that type of ration has on shedding of pathogen in faeces. We believe the present data and analysis provides one of the best available descriptions of the true relationship between ration and shedding of pathogens in faeces because it was obtained from naturally challenged cattle in a commercial setting, it was measured at the time of slaughter making it most relevant to food-safety, grass and grain rations do contrast in terms of the balance of fibre, energy and protein components, and because this data was based on accurate enumeration of each pathogen rather than being restricted to a presence-absence result. We have also demonstrated that a statistical correction of the left-censoring in the data on concentration of *E. coli* O157:H7 in faeces provides a more appropriate distribution for use in risk models compared to that available from standard distribution-fitting techniques. Effectively the distribution generated from censored regression guards against overestimation of the number of *E. coli* O157:H7 present in faeces. Although this overestimation is not large in this instance (Figure 3) such differences could have a meaningful impact on the output of risk models because of the small number of pathogen cells sometimes required to cause disease in humans. The practical benefit of having derived this distribution is that it can be used as input

in quantitative risk assessments. This particular distribution (derived from censored regression) is more correct for describing the very low end of the concentration distribution which cannot be estimated by distributions derived by conventional methods.

Recommendations

Based on the information collected to date it will be difficult to build any model that represents an advance on knowledge or a better organisation of existing knowledge. The outputs of the work completed to date are better estimates for some of the inputs for use in risk assessments. There is still a large amount of uncertainty about the process of carcase contamination that acts as a barrier to generating an improved model.

While quantitative modelling is not yet feasible, it would be possible to qualitatively go through the issues around carcase contamination and say what is known, the extent to which it is known and what is not known. However, there will be a rather large amount of 'expert opinion' entering into such a process due to the degree of uncertainty. Nevertheless, this might be of help in identifying the key issues, so that the research priorities can be better addressed by MLA i.e. large area sampling, more focused microbiological surveys.

However, there is the concern that what will be produced if we move on to the next stage might not meet the needs of MLA or the scientific community or be used by either.

References

1. Fegan N, Higgs G, Vanderlinde P, Desmarchelier P. Enumeration ofEscherichia coli O157 in catle faeces using most probable number technique and automated immunomagnetic separation. *Letters in Applied Microbiology* 2004; 38: 56-59.

2. Fegan N, Vanderlinde P, Higgs G, Desmarchelier P. Quantitation and prevalence of *Salmonella* in beef cattle presenting at slaughter. *J Appl Microbiol* 2004; 97: 892-898.

3. Jordan D. Simulating the sensitivity of pooled-sample herd tests for fecal *Salmonella* in cattle. *Prev Vet Med* 2005; in press.

4. MLA (2005). Microbiological quality of Australian beef and sheepmeat – results of the industry's third national abattoir survey. Meat & Livestock Australia Ltd, Sydney.

5. Myers RH. Classical and modern regression with applications. Myers RH. Classical and modern regression with applications. Belmont: Duxbury, 1990.

Table 1. Descriptive analysis of generic *E. coli* counts in faeces (log_{10} CFU/g) from grass-fed and grain-fed cattle conditional on the count exceeding zero and with data below the censoring point either excluded or simulated.

	Censored data excluded (exclude specimens < 3 cfu/g)		Censored data replaced by simulated data (all positive specimens)	
	Grass-fed	Grain-fed	Grass-fed	Grain-fed
number of tests	155	155	155	155
number of samples analysed	154	154	155	155
mean	5.72	5.72	5.40	5.68
sd	1.12	1.19	1.64	1.26
min	2.20	1.00	0.00	0.30
5%	4.00	3.48	0.90	3.48
10%	4.25	4.28	3.90	4.28
25%	5.11	5.19	4.58	5.18
50%	5.83	5.88	5.78	5.88
75%	6.49	6.41	6.44	6.41
90%	7.04	7.04	7.00	7.04
95%	7.37	7.29	7.37	7.29
max	8.58	7.61	8.58	7.61

Table 2. Descriptive analysis of counts of *E. coli* O157:H7 in faeces (log_{10} CFU/g) from grass-fed and grain-fed cattle conditional on the pathogen being present and with data below the censoring point exclude and with data below the censoring point simulated.

	Censored data excluded (exclude specimens < 3 cfu/g)		Censored data replaced by simulated data (all positive specimens)	
	Grass-fed	Grain-fed	Grass-fed	Grain-fed
number of tests	155	155	155	155
number of obs analysed	8	19	16	23
mean	1.29	1.56	0.77	1.35
sd	1.14	1.22	0.95	1.20
min	0.48	0.48	0	0.30
5%	0.48	0.48	0	0.30
10%	0.48	0.56	0.30	0.30
25%	0.56	0.56	0.30	0.56
50%	0.76	1.18	0.48	0.96
75%	1.78	2.63	0.76	1.97
90%	3.63	2.63	2.38	2.63
95%	3.63	3.63	3.63	3.63
max	3.63	5.04	3.63	5.04

Table 3. Descriptive analysis of counts of *Salmonella* in faeces (log_{10} CFU/g) from grass-fed and grain-fed cattle.

	Censored data excluded (exclude specimens < 3 cfu/g)		Censored data replaced by simulated data (all positive specimens)	
	Grass-fed	Grain-fed	Grass-fed	Grain-fed
number of tests	155	155	155	155
number of obs analysed	3	10	7	14
mean	1.16	1.66	0.70	1.28
sd	1.05	1.12	0.76	1.17
min	0.56	0.56	0	0
5%	0.56	0.56	0	0
10%	0.56	0.56	0	0
25%	0.56	0.56	0.48	0.30
50%	0.56	1.30	0.48	0.76
75%	2.38	2.97	0.56	1.97
90%	2.38	3.21	2.38	2.97
95%	2.38	3.45	2.38	3.44
max	2.38	3.45	2.38	3.44

Table 4. Summary of analyses of data from study 1 comparing faecal microbiology of grass-fed and grain-fed groups of cattle

Measurement	Procedure	Treatment of censored data	Hypothesis under test	Differences (grass, grain)	P-value
$\log_e$ generic *E. coli* cfu/g	unpaired t-test	simulated	equality of means	12.4, 13.1 (means)	0.09
		exclude < l0	equality of means	13.2, 13.2 (means)	0.97
Salmonella cfu/g	rank sum test	simulated	equality of medians	3, 6.4 (medians)	0.17
		exclude < 3	equality of medians	3.6, 26.1 (medians)	0.38
E. coli O157:H7 cfu/g	rank sum test	simulated	equality of medians	3, 9.2 (medians)	0.03
		exclude < 3	equality of medians	6.4, 15.0 (medians)	0.78
Salmonella present/absent	Fisher's exact test	included	equality of prevalence	0.05, 0.09 (medians)	0.17
E. coli O157:H7 present/absent	Fisher's exact test	included	equality of prevalanec	0.10, 0.15 (medians)	0.30

Table 5. Relationship between the occurrence of *Salmonella* and *E. coli* O157:H7 in bovine faeces in Study 1 (relative risk = (6/21)/(33/289) = 2.50[1], 95% CI: 1.2 to 9.3).

		E. coli O157:H7 status		
		+ve	-ve	total
Salmonella status	+ve	6	15	21
	-ve	33	256	289
	total	39	271	310

[1] Interpreted as *E. coli* O157:H7 is 2.5 times more likely to be present in faecal samples that are *Salmonella* positive compared to faecal samples that are *Salmonella* negative.

Table 6. Prevalence of samples (%) yielding test positive results for different organisms and sampling sites from Study 2.

	specimen or site sampled					
organism	hide	saliva	rumen	faeces	pre-chill	post-chill
generic *E. coli*	100	100	100	100	26	5
E. coli O157:H7	44.0	26.3	0	10.0	6.0	0
Salmonella	68.0	29.3	25.3	15.9	2.0	3.0

.

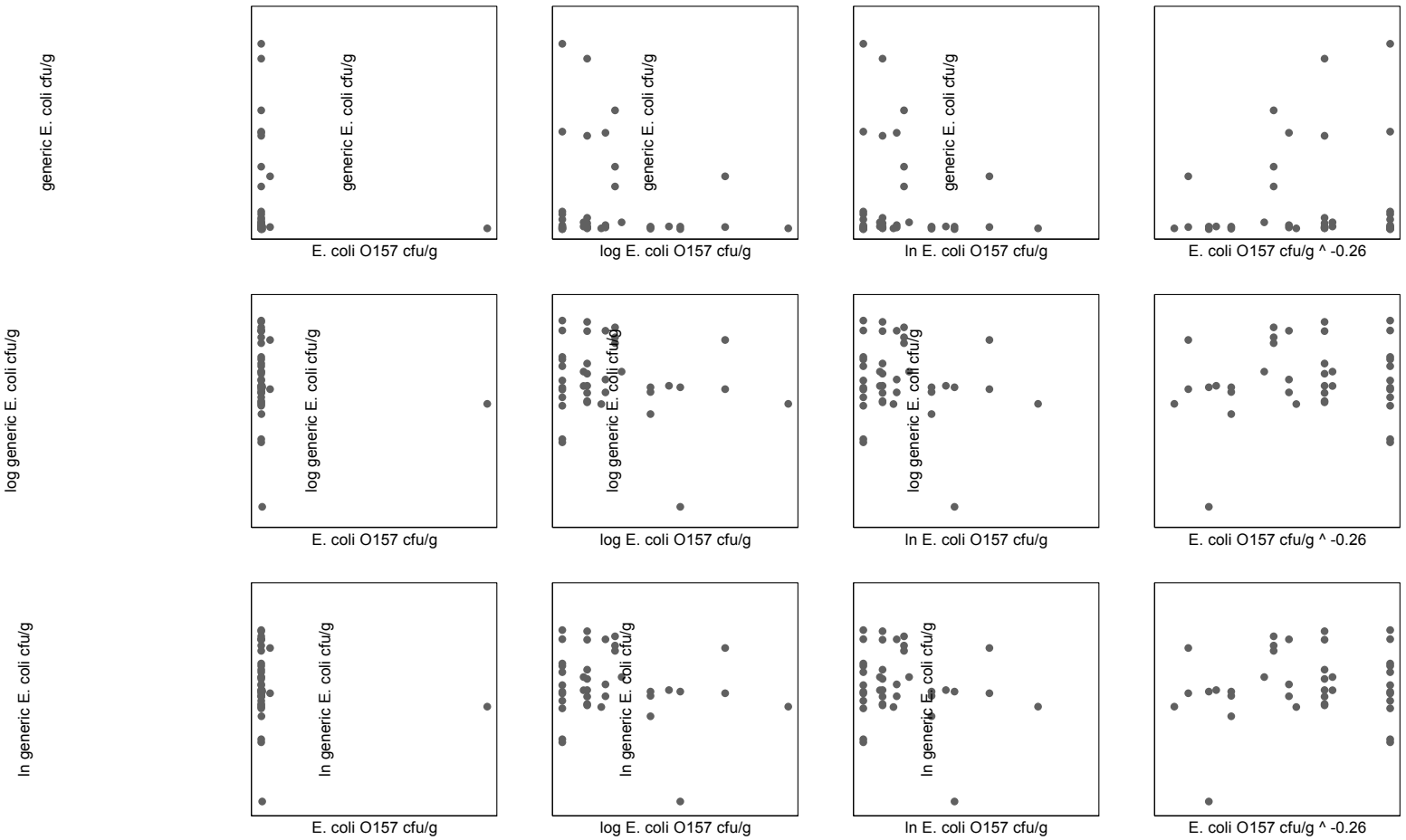

Figure 1. Scatter plots of pairwise transformation of generic *E. coli* and *E. coli* O157:H7 concentrations in bovine faeces (Study 1).

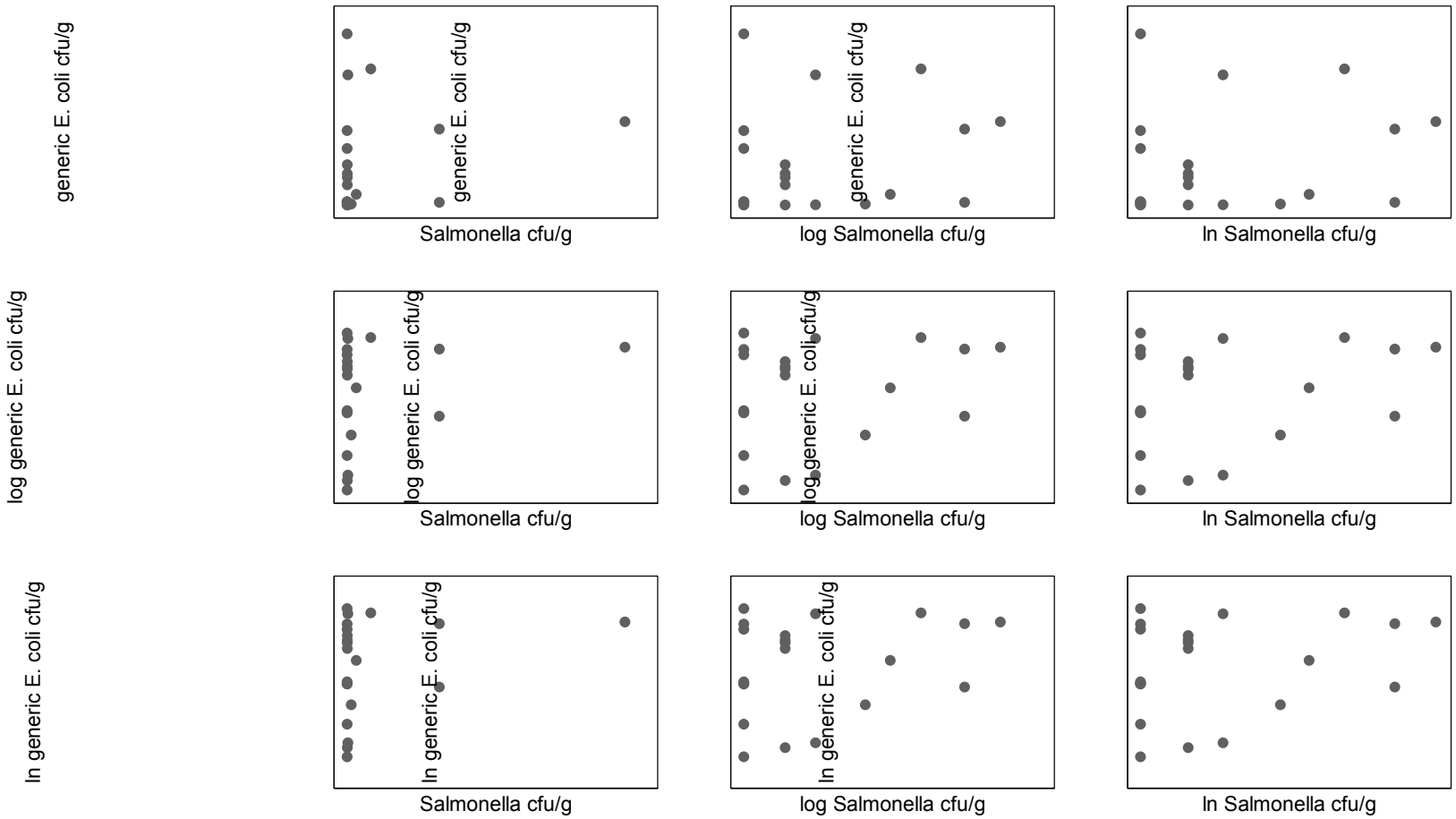

Figure 2. Scatter plots of pairwise transformation of generic *E. coli* and *Salmonella* concentrations in bovine faeces (Study 1)..

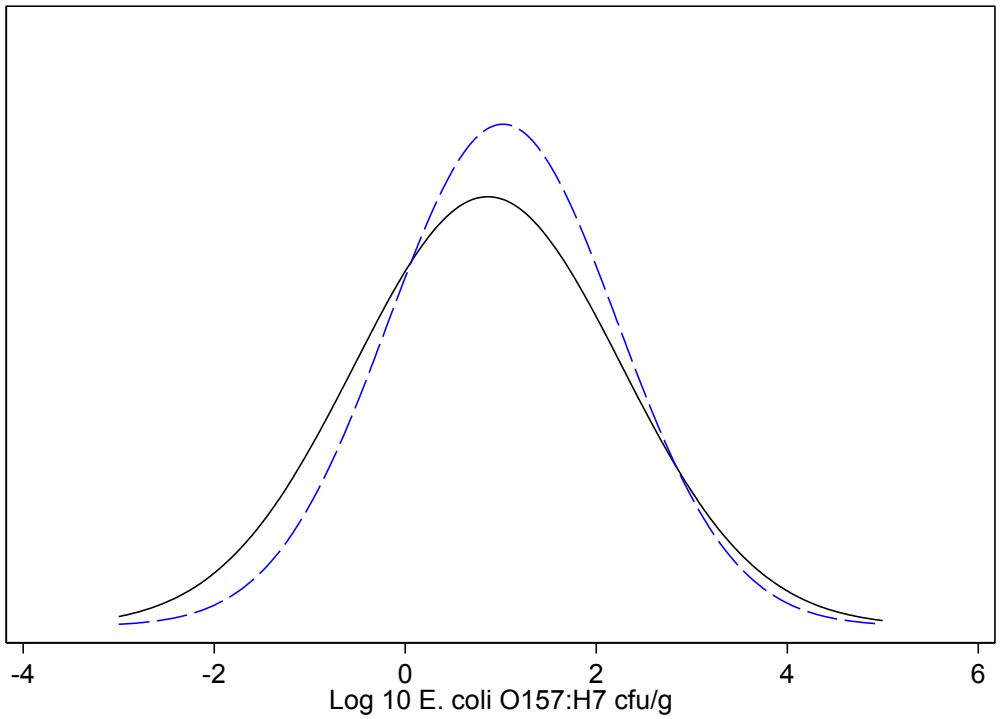

Figure 3. Probability density plots showing normal distribution of $\log_{10}$ *E. coli* O157:H7 cfu/g in bovine faeces estimated by censored regression (solid line) and standard maximum likelihood techniques (broken line). Distribution parameters noted in text.

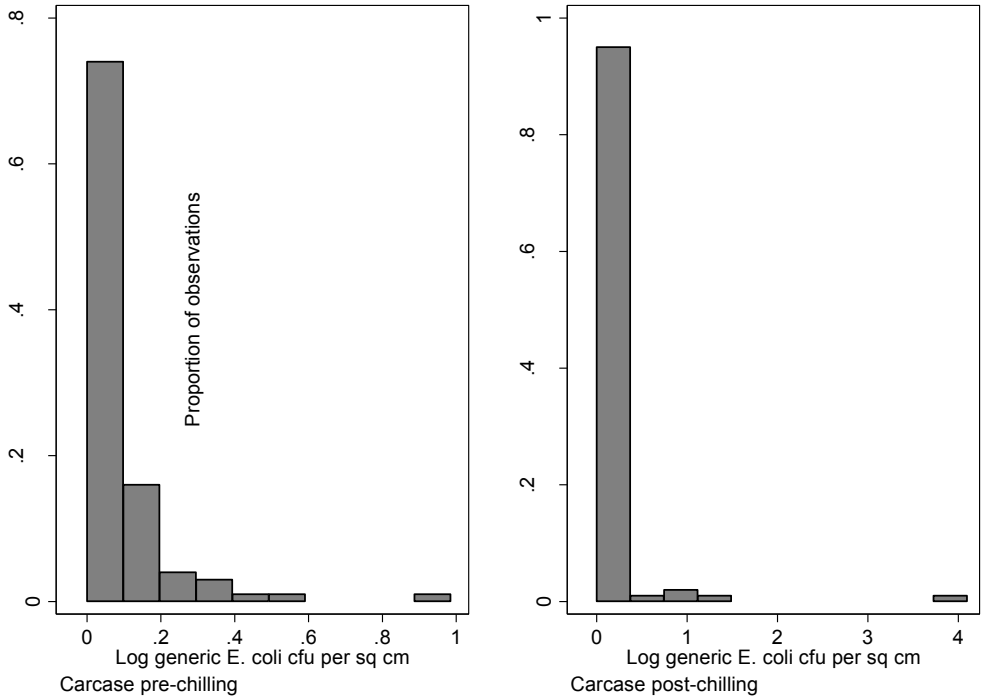

Figure 4. Histograms of $\log_{10}$ transformed counts of generic *E. coli* (cfu/cm^2) on the surface of beef carcases prior to chilling and after chilling.

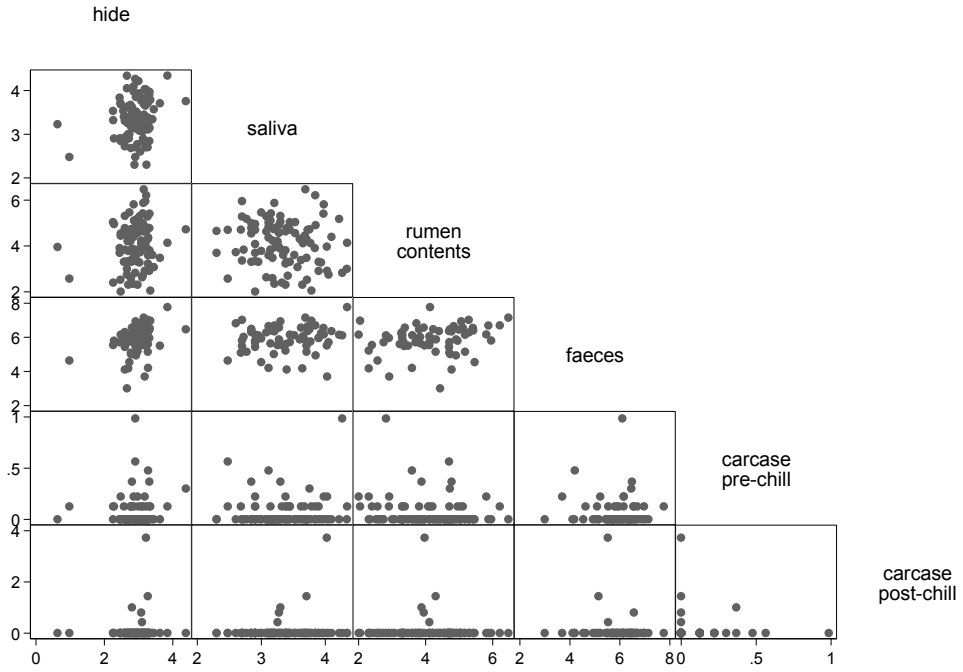

Figure 5. Relationship between density of generic *E. coli* (log_{10} cfu/cm^2 for hide, pre-chill and post-chilled carcases; log_{10} cfu/g for saliva, rumen and faeces) at different sampling sites and from identical animals/carcases throughout processing (data from Study 2).