Bacterial concentrations of poultry offal and in mechanically separated meat products at the processing plant

MAF Technical Paper No: 2011/59

Prepared for the Ministry of Agriculture and Forestry under project MFS/09/07 – Contamination of selected poultry products, as part of an overall contract for scientific services

by Dr Teck Lok Wong
Dr Beverley Horn
Christopher Graham
Dr Susan Paulin

ISBN 978-0-478-38469-7(online)
ISSN 2230-2794 (online)

May 2011

Ministry of Agriculture and Forestry
Te Manatū Ahuwhenua, Ngāherehere

BIOSECURITY NEW ZEALAND

NEW ZEALAND nzfsa FOOD SAFETY AUTHORITY

Disclaimer

Requests for further copies should be directed to:

Publication Adviser
MAF Information Bureau
P O Box 2526
WELLINGTON

Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the MAF website at
www.biosecurity.govt.nz/about-us/our-publications/technical-papers

Acknowledgements

The authors would like to thank:

- The PIANZ members who took part in this project, with special acknowledgement for technical assistance from the quality assurance staff at the processing plants.
- The staff of the Public Health Laboratory, ESR Christchurch, AsureQuality Auckland and Environmental Laboratory Services Ltd, Lower Hutt, for their technical support of the project.
- This project was funded by the Ministry of Agriculture and Forestry.

Client Report
FW10089

BACTERIAL COUNTS OF POULTRY OFFAL AND MECHANICALLY SEPARATED MEAT PRODUCTS AT THE PROCESSING PLANT

FINAL REPORT

Dr Stephen On
Food Safety Programme Leader

Dr Teck Lok Wong
Project Leader

Maurice Wilson
Peer Reviewer

TABLE OF CONTENTS

SUMMARY ..... **iv**

1. INTRODUCTION **1**

2. MATERIALS AND METHODS **3**

2.1. Product sampling 3
2.1.1. Whole Carcass Rinse Sampling 4
2.1.2. MSM sampling 4
2.1.3. Offal Sampling 4
2.2. Sample preparation and microbiological analyses 5
2.2.1. MSM 5
2.2.2. Offal – hearts, gizzards and necks 5
2.2.3. Offal – livers 5
2.3. Analysis 7
2.4. Interpretation of enumeration results 7

3. RESULTS AND DISCUSSION **8**

3.1. Mechanically separated meats 8
3.1.1. *Campylobacter* spp. and coagulase-positive staphylococci counts 8
3.1.2. Comparison of *Campylobacter* spp. and coagulase-positive staphylococci counts with indicator bacterial counts. 12
3.2. Offal Products: Heart, liver, gizzard and neck 16
3.2.1. *Campylobacter* spp. contamination of heart, liver, gizzard and neck products before washing 16
3.2.2. Comparison of *Campylobacter* spp. counts obtained from heart, liver, gizzard and neck rinsates with those from whole carcass rinsates. 19
3.2.3. Comparison of *Campylobacter* spp. counts with indicator bacterial *E. coli* counts in heart, liver, gizzard and neck rinsates. 22
3.3. Comparison of *Campylobacter* spp. counts from the internal matrix and external surfaces of livers 24

4. CONCLUSIONS **26**

4.1. Objective 26
4.2. Mechanically separated meat 26
4.3. Heart, liver, gizzard and neck products 27
4.4. Presence of *Campylobacter* spp. in the internal and on the external of livers 27

5. REFERENCES **29**

APPENDIX A: Whole carcass rinsate **31**

APPENDIX B: Mechanically seperated meat - summary statistics **33**

APPENDIX C: Heart, gizzard, neck and liver **34**

LIST OF TABLES

Table 1: Number of sets of five samples from a flock analysed for each sample type and processor. 3
Table 2: Microbiological testing requirements 6
Table 3: Lowest limit of detection 7
Table 4: Summary statistics for *Campylobacter* spp. in whole carcass rinsates taken from the three processors. 31
Table 5: Summary statistics for *E. coli* in whole carcass rinse samples taken from the three processors. 31
Table 6: Summary statistics for bacteria in mechanically separated meat products for three processors. 33
Table 7: Summary statistics for *Campylobacter* spp. counts from rinsates of poultry hearts, gizzards, livers and necks from Processors A and B. 34
Table 8: Heart, gizzard, liver and neck sampled product weights. 34

LIST OF FIGURES

Figure 1: Histogram of counts of *Campylobacter* spp. and coagulase-positive staphylococci (Log_{10} CFU/g) from samples of MSM. The first bar of each plot represents not detected results, which may be negative or below the limit of detection. 9
Figure 2: *Campylobacter* spp. counts from MSM samples taken on each day for the three processors. Not detected results (ND) are plotted in the grey band. 10
Figure 3: Coagulase-positive staphylococci counts from MSM samples taken on a given sampling day at processors A and C. Not detected (ND) results are plotted in grey band. 11
Figure 4: Correlation between *Campylobacter* spp. and *E. coli* or Aerobic Plate Count from samples of MSM from three processors; A, B and C. Solid diamonds represent counts for both microorganisms and the open circles represent counts for one of the microorganisms and not detected result for the other. The Spearman rank correlation coefficient, *r*, of the counts is provided for Processors A and B. 13
Figure 5: Correlation between coagulase-positive staphylococci counts and *E. coli* or APC counts from samples of MSM from Processors A and C. Solid diamonds represent counts for both microorganisms and the open circles represent counts for only one of the microorganisms. 15
Figure 6: Frequency of *Campylobacter* spp. counts (Log_{10} CFU/rinsate) from rinsates of poultry heart, gizzard, neck and liver samples from two processors. The first bar in each plot represents not detected results, which may be negative or below the limit of detection. 17
Figure 7: Correlation between *Campylobacter* spp. counts from rinsates of heart, liver and gizzard samples taken from the same bird. Solid diamonds represent samples with counts from both rinsates, the open circles represent samples with counts from only one of the rinsates, and the cross represents samples where neither rinsates produced counts. The Spearman rank correlation coefficient, *r*, and associated P value is given for each plot. 18
Figure 8: Comparison of *Campylobacter* spp. counts from five whole carcass rinsates (CFU/rinsate) with those obtained from five heart, gizzard, neck and liver

rinsates taken from samples from nine different cuts being processed at Processor A. 20
Figure 9: Comparison of *Campylobacter* spp. counts from five whole carcass rinsates (CFU/rinsate) with those obtained from five heart, gizzard, neck and liver rinsates taken from samples from 10 different cuts being processed at Processor B. 21
Figure 10: Comparison of *Campylobacter* spp. and *E. coli* counts from heart, gizzard, neck and liver rinsates from Processors A and B. Solid diamonds represent rinsates where both *Campylobacter* spp. and *E. coli* could be enumerated, open circles where only one bacterial species could be enumerated and a cross represents a rinsate where neither bacterial species could be enumerated. The correlation coefficient, *r*, and associated P value is given in each plot. 23
Figure11: Counts of *Campylobacter* spp. (Log_{10} CFU/rinsate) from external liver rinsates compared to counts from the internal tissues (Log_{10} CFU/liver). Solid diamonds represent liver samples with non-zero counts for external and internal sampling, circles representing the livers with no *Campylobacter* spp. detected from internal tissue and the cross representing a single liver where no *Campylobacter* spp. was detected in either the internal or external sample. 25
Figure 12: *Campylobacter* spp. and *E.coli* counts (Log_{10} CFU/rinsate) in whole carcass rinse samples taken from suspected positive flocks at three different processing plants. First column represents the frequency of not detected (ND) results. 32
Figure13: Correlation between sample weight and *Campylobacter* spp. concentration on heart, gizzard, neck and liver products. Open diamonds represent products with not detected *Campylobacter* spp. counts and *r* is the Spearman rank correlation coefficient. 35

SUMMARY

Campylobacteriosis is the most frequently reported bacterial foodborne illness in New Zealand, and a major route of infection with *Campylobacter* spp. is contaminated food consumption. The New Zealand Food Safety Authority (NZFSA) introduced a mandatory *Campylobacter* performance target (CPT) for poultry processing to reduce the reported incidence of foodborne campylobacteriosis by 50% by 2013.

Information is currently lacking on various high-risk poultry products at the processing plant, including offal (liver, heart, gizzard and neck), which are readily available at retail outlets, and mechanically separated meat (MSM) product.

This project has quantified the concentrations of *Campylobacter*, generic *Escherichia coli*, coagulase-positive staphylococci and Aerobic Plate Count (APC) in MSM, and *Campylobacter* and generic *E. coli* contamination on heart, liver, gizzard and neck samples. Samples were collected over the period February to mid-August 2010 from processing lines that were known to be, or anticipated as highly likely to be, positive for *Campylobacter*. The results are summarised below.

Mechanically separated meat

A total of 145 MSM samples were collected from three different processing plants. *Campylobacter* was countable in 87%, 66% and 33% of the three processors' samples, while coagulase-positive staphylococci were countable in 44%, 2% and 36% of the processors' samples. These values show that *Campylobacter* spp. can persist through processing and be detectable in the MSM product, and that coagulase-positive staphylococci can also be present in the MSM product.

The distribution of bacteria varied with the processor. The median counts (5^{th} to 95^{th} percentile) for *Campylobacter* in MSM at the three processors were 1.74 (Not detected (ND) to 3.17) Log_{10} CFU/g, 1.18 (ND to 2.55) Log_{10} CFU/g and ND (ND to 2.08) Log_{10} CFU/g. The median counts (5^{th} to 95^{th} percentile) for coagulase-positive staphylococci in MSM at the three processors were ND (ND to 3.52) Log_{10} CFU/g, ND (ND to 1) Log_{10} CFU/g and ND (ND to 2.72) Log_{10} CFU/g.

No significant correlation ($P>0.05$, $r \leq 0.24$) was evident between counts of *Campylobacter* from the MSM product and either *E. coli* or APC from the same sample. Similarly, no correlation was observed between coagulase-positive staphylococci and either *E. coli* or APC.

Heart, liver, gizzard and neck products

Ninety-five samples of heart, liver, gizzard and neck were sampled in total. *Campylobacter* was countable in 86% of heart rinsates, 99% of liver rinsates, 97% of gizzard rinsates and 99% of neck rinsates. The distribution of counts on these products differed between the two processors. This could be due to differences in the processing lines. The median (5^{th} to 95^{th} percentile) of the counts were:

- Heart: Processor A, 2.5 (ND to 4.7) and Processor B, 3.8 (2.1 to 4.9) Log_{10} CFU/rinsate.
- Liver: Processor A, 3.8 (2.2 to 5.5) and Processor B, 4.5 (3.7 to 5.4) Log_{10} CFU/rinsate.
- Gizzard: Processor A, 3.3 (ND to 4.8) and Processor B, 3.9 (3.0 to 5.0) Log_{10} CFU/rinsate.
- Neck: Processor A, 4.1 (2.2 to 5.0) and Processor B, 4.0 (2.7 to 4.8) Log_{10} CFU/rinsate.

The whole carcass rinsate results do not provide a consistent indicator of the presence of *Campylobacter* spp. on the heart, gizzard, neck and liver samples. There were some sampling days, where *Campylobacter* spp. were not detectable from the whole carcass rinsates, but were detected at high numbers in the heart, liver, gizzard and neck rinsates. No significant correlation ($P \geq 0.07$, $r \leq 0.28$) was evident between the *Campylobacter* and *E. coli* counts for the heart, liver and gizzard products. The neck samples taken from one processor show some positive correlation of the counts, with a correlation coefficient of 0.47 ($P < 0.05$). However, this observation was not repeated for the neck samples from Processor A ($P = 0.28$, $r = -0.16$).

Internal and external liver *Campylobacter* contamination

Forty-five liver samples were taken over the sampling period from a single processor. Of these livers, 22% had *Campylobacter* spp. only on the surface of the liver, 76% had the bacteria on the surface and in the internal tissues and 2% of the livers had no countable *Campylobacter* spp..

The distribution of the estimated count in internal liver tissue had median (5^{th} -95^{th} percentile) of 2.9 (ND to 4.5) Log_{10} CFU/ whole liver, compared to the counts obtained from the external liver rinsate; 3.8 (2.2 to 5.5) Log_{10} CFU/rinsate. A strong positive correlation was seen between the internal and external presence of *Campylobacter* spp. of the liver samples.

Washing the livers at the processors will not remove *Campylobacter* spp. internally from the organ. Any *Campylobacter* spp. remaining in the internal tissues of raw livers after chilling or freezing would need to be killed by appropriate cooking practices.

INTENTIONALLY BLANK

1. INTRODUCTION

Campylobacteriosis is the most frequently reported bacterial foodborne illness in New Zealand and a major route of infection with *Campylobacter* spp. is contaminated food consumption. Presence of *Campylobacter* spp. on retail poultry products was reported as early as 1995 by Campbell and Gilbert (1995). This was followed by a landmark case-control study in 1996 when Eberhart-Phillips *et al.*, (1997) implicated broiler meats as the most important vehicle of human infection by *Campylobacter* spp. in New Zealand. Other retail studies have collectively shown high prevalence of *Campylobacter* spp. on New Zealand poultry carcasses and products (Wong *et al.*, 2007; Chrystal *et al.*, 2008; French, 2008 and 2009; Wong and Hudson, 2010). A recent attribution study conducted in the Manawatu district of New Zealand has identified poultry meat as a primary exposure pathway of campylobacteriosis (Mullner *et al.*, 2009).

Other useful information on the prevalences and concentrations *Campylobacter* spp. on New Zealand poultry products at slaughter has been collected via the National Microbiological Database (NMD[1]), on poultry products during secondary processing (Paulin, 2010) and on other poultry products after primary processing, including duck and turkey carcasses (Wong, 2010) and end-of-lay and breeder poultry carcasses (Wong and Chung, 2010).

The New Zealand Food Safety Authority (NZFSA) has introduced a mandatory *Campylobacter* performance target (CPT) for poultry processing in order to reduce the reported incidence of foodborne campylobacteriosis by 50% by 2013 (NZFSA, 2008). Since the introduction of the CPT and as improvements in primary poultry processing have been implemented, the poultry industry has reported a reduction of *Campylobacter* spp. counts in carcass rinsates at the end of processing (NZFSA, 2008). A significant decrease in the campylobacteriosis rate between 2007 and 2008 (Mantel Haenszel chi-square test, $P<0.05$) in the human population in New Zealand has also been reported (Anonymous, 2008).

Campylobacter colonises the gastrointestinal tract of poultry. Information is still required about poultry products that have potentially high counts because they are either part of, or can have cross contamination from the gastrointestinal tract. For example offal (liver, heart, gizzard and neck portions) and mechanically separated meats (MSM).

Offal is readily available at retail outlets. MSM is produced regularly from carcass frames following the removal of portions and breast meats from the carcasses. These products are used to manufacture low value chicken nuggets and chicken luncheons.

[1] Web site: http://www.foodsafety.govt.nz/industry/general/nmd (Note: Accessibility to some information on this website is restricted to NZFSA Laboratory Approval Scheme Administrator and approved NMD registered members).

This project has quantified the concentrations of *Campylobacter*, generic *Escherichia coli*, coagulase-positive staphylococci and Aerobic Plate Count (APC) in MSM, and *Campylobacter* and generic *E. coli* on heart, liver, gizzard and neck samples. Samples were collected over the period February to mid-August 2010 from flocks that were known to be, or anticipated as highly likely to be, positive for *Campylobacter*.

2. MATERIALS AND METHODS

2.1. Product sampling

Three processors (Processors A, B and C) identified flocks known to be *Campylobacter* positive or anticipated as highly likely to be positive (e.g. third or fourth cut) at fortnightly intervals over the course of the sampling period from February until mid-August 2010.

These flocks provided whole carcass and MSM samples. Processors A and B also took heart, liver and gizzard samples from each of five randomly selected birds from the identified flock. Five neck samples were also sampled, but from a different set of five birds.

MSM processing on a day uses the carcasses from multiple flock cuts. Therefore, MSM samples would include MSM produced from carcasses from the identified flock as well as other flocks processed on the same day. Processor B, manufactured MSM on the same day as the cut of birds from the flock was slaughtered, while Processors A and C aged their carcasses overnight in a chiller before manufacturing the MSM the following day.

Table 1 shows the number of sets of five samples that were analysed between February and mid-August 2010.

Table 1: Number of sets of five samples from a flock analysed for each sample type and processor.

Sample type	Processor A	Processor B	Processor C
Whole carcass rinsate	10	10	10
MSM homogenate	9*	10	10
Liver internal homogenate	9*	None[#]	None
Liver external rinsate	9*	10	None
Heart rinsate	9*	10	None
Neck rinsate	9*	10	None
Gizzard rinsate	9*	10	None
Total number of samples	**320**	**300**	**100**

*The results from one set of MSM or offal samples were disregarded due to temperature conditions outside the NMD specification.
[#]Sample type was not requested from the processor.

2.1.1. Whole Carcass Rinse Sampling

Five carcasses from each of the identified cuts in the poultry flocks at the end of processing were randomly chosen by NMD-approved samplers. The carcasses were rinsed in accordance to NMD protocol[2] and the rinsates were tested for *Campylobacter* spp. and *E. coli* either in a processors "in-house" laboratory or a sub-contracted approved commercial laboratory. The results were forwarded to ESR. A summary of the whole carcass rinse results is given in Appendix A.

2.1.2. MSM sampling

Five individual samples of MSM weighing about 50 g were collected by the processors. The first sample was collected about 30 min after the beginning of MSM processing, three samples collected at regular intervals throughout processing, and one sample was collected close to the end of the process run. Samples were separately bagged in sterile Whirlpak bags (BO1297WA, Nasco, Modesto, CA, USA) and stored chilled prior to delivery to the testing laboratory.

All of the samples from Processors A and C were sent via overnight courier to ESR's Christchurch Science Centre. The MSM samples from Processor B were sent to Asure Quality, Auckland via overnight courier. Each set of samples was transported in a chilly bin containing frozen pads to keep them below 10ºC. A temperature blank comprising a container of water was included in the chilly bin. The temperature of the water in this container equilibrates with the ambient temperature inside the sample container and gives an indication of the sample temperature on delivery. If the water temperature exceeded 10°C, sample temperatures were individually checked and confirmed as being outside specifications using a calibrated infra-red thermometer (RayTek MiniTemp, Santa Cruz, CA USA) before being discarded. Similarly, frozen samples were also discarded. Samples were tested within 24 h of being taken.

2.1.3. Offal Sampling

Five sets of each offal product (heart, liver, gizzard and neck) were collected by Processors A and B. Offal samples were collected at five-minute intervals at a position on the processing line before the spin chiller. The heart, liver and gizzard samples were collected from five individual birds. Due to reasons of impracticality, neck samples were not sampled from the same bird as the other offal samples.

[2] http://www.foodsafety.govt.nz/elibrary/industry/animal-products-national-nmd/schedule-2011.pdf

Samples were separately bagged in sterile Whirlpak bags (Nasco) and stored at 4°C prior to overnight courier delivery to the ESR's Christchurch Science Centre in chilly bins. Shipping conditions were as described above in 2.1.2.

2.2. Sample preparation and microbiological analyses

2.2.1. MSM

MSM samples weighing 25 g were placed in a filter stomacher bag to which 225 mL buffered peptone water (BPW, 218105, Becton Dickinson, Sparks, MD, USA) was added. The contents were stomached for 2 min. Two logarithmic dilutions (10^{-1} and 10^{-2}) were prepared using peptone diluent (0.1% peptone with 0.85% NaCl). Samples (and dilutions) were then plated as described in Table 2.

2.2.2. Offal – hearts, gizzards and necks

Hearts, gizzards and necks were analysed using a rinse method. Prior to testing, gizzards were placed individually on a sterile petri dish, dissected with sterile scalpels and forceps, and the contents removed by peeling away the thick inner lining. Each offal sample was placed individually in a sterile stomacher bag and weighed. For heart, neck and gizzard samples, 100 mL of BPW was added to the bag prior to stomaching for 2 min. The necks were hand massaged for 2 min. Serial dilutions of each rinsate were prepared as before and plated as described in Table 2.

2.2.3. Offal – livers

The external surface of the liver and the internal liver tissue were analysed using the method of Whyte *et al.* (2006). Briefly, each liver was placed in a sterile stomacher bag and weighed. A 100 mL volume of BPW was added and the liver was gently shaken for 2 min to re-suspend bacterial cells from the surface. The resulting rinsate was poured into another sterile stomacher bag and two logarithmic dilutions were prepared for the enumeration of *E. coli* and *Campylobacter* spp. The rinsed liver was removed from the bag and gently placed into boiling water for 15 s using a sterile spoon. The sample was then removed, placed on a sterile petri dish and cut in half using a sterile scalpel. A 10 g portion of internal uncooked tissue was aseptically removed and placed in a filter stomacher bag with 90 mL of BPW. The bag contents were then stomached for 2 min. If <10 g tissue was available, sufficient peptone diluent to make a 1/10 dilution was added. Two logarithmic dilutions were prepared, plated and enumerated for *Campylobacter* spp. only (Table 2).

Table 2: Microbiological testing requirements

Test Product	Media	Dilutions	Confirmation
Campylobacter External liver, heart, neck and gizzard	mCCDA[a] (Microaerobic, 42°C, 48 h)	2 mL (10^0) over 6 plates 0.1 mL (10^0) x2 0.1 mL (10^{-1}) x2 0.1 mL (10^{-2}) x2	Oxidase and latex agglutination on 5 suspect colonies
Internal liver tissue	mCCDA (Microaerobic, 42°C, 48 h)	2 mL (10^{-1}) over 6 plates 0.1 mL (10^{-1}) x2 0.1 mL (10^{-2}) x2	Oxidase and latex agglutination on 5 suspect colonies
MSM	mCCDA (Microaerobic, 42°C, 48 h)	2 mL (10^{-1}) over 6 plates 0.1 mL (10^{-1}) x2 0.1 mL (10^{-2}) x2 0.1 mL (10^{-3}) x2	Oxidase and latex agglutination on 5 suspect colonies
Generic *E. coli* External liver, heart, neck and gizzard	Petrifilm[b] (35/37°C, 18-24h)	1 mL (10^0) x2 1 mL (10^{-1}) x2 1 mL (10^{-2}) x2	Count all blue colonies +/- gas (according to MIMM[c])
MSM	Petrifilm (35°C, 18-24h)	1 mL (10^{-1}) x2 1 mL (10^{-2}) x2 1 mL (10^{-3}) x2	Count all blue colonies +/- gas (according to MIMM)
APC MSM	Plate Count Agar[d] (30°C, 48h)	Spiral plater (10^{-1} x2, 10^{-3} x2)	Not Applicable
Coagulase-positive staphylococci MSM	Baird Parker Agar[e] (35°C, 48h)	1 mL (10^{-1}) over 3 plates 0.1 mL (10^{-1}) x2 0.1 mL (10^{-2}) x2	Coagulase on 5 suspect colonies

a: mCCDA: modified Charcoal Cefoperazone Desoxycholate agar, made according to NMD procedure (http://www.nzfsa.govt.nz/animalproducts/legislation/notices/animal-material-product/nmd/nmd-09-schedule-1-technical-procedures.pdf).

b: *E. coli* Petrifilm by 3M (St. Paul, Mn, USA).

c: MIMM, Meat Industry Microbiological Methods (4th Edition).

d: Plate Count Agar, medium by Merck (1.05463, Darmstadt, Germany).

e: Baird Parker Agar, Base medium by Merck (1.05406) supplemented with egg yolk tellurite enrichment by BBL Benton Dickinson (212357 Sparks, MD, USA).

2.3. Analysis

Correlation of the bacterial CFU counts was examined graphically and using the Spearman Rank correlation, via the cor.test function in R. The cor.test function calculates the correlation coefficient, *r*, and provides an estimate of the probability, P, that the correlation between bacterial counts observed in the sample would occur given the null hypothesis that no correlation exists in counts from the product type.

The Spearman Rank method was chosen due to the non-normal nature of most of the count distributions. For correlation plots, the not detected (ND) results were set to half the lowest limit of detection for the sample. The lowest limit of detection for each microorganism or a group of microorganisms from each sample type are given in Table 3.

Table 3: Lowest limit of detection

	Campylobacter	***E. coli***	**Coagulase-positive staphylococci**	**APC**
Whole carcass rinse	200 CFU/rinsate	200 CFU/rinsate	NA	NA
MSM	5 CFU/g	5 CFU/g	10 CFU/g	100 CFU/g
Heart, gizzard, neck and external liver	50 CFU/rinsate	50 CFU/rinsate	NA	NA
Internal liver	5 CFU/g	NA	NA	NA

2.4. Interpretation of enumeration results

The prevalence of *Campylobacter* spp. detection described in this report should not be taken to reflect the prevalence of contamination in these poultry products produced by these processors at retail. The flocks were specifically chosen to be highly likely to be infected with *Campylobacter* spp.

The enumeration results indicate the likely concentrations to be found on products from infected poultry during processing. The heart, liver, gizzard and neck samples were taken before the product washing or chilling steps. These treatments are provided for products that are sent to retail outlets. Therefore, these results should not be taken as indicative of the presence of *Campylobacter* spp. at retail.

3. RESULTS AND DISCUSSION

3.1. Mechanically separated meats

3.1.1. *Campylobacter* spp. and coagulase-positive staphylococci counts

Five MSM samples were taken throughout the processing period each sampling day. The samples were tested for *Campylobacter* spp., coagulase-positive staphylococci, *E. coli* and APC. Appendix B gives the summary statistics for all the bacterial counts for MSM samples taken from the three processors.

The *Campylobacter* spp. and coagulase-positive staphylococci counts obtained from the MSM samples from the three processors are presented as a histogram in Figure 1.

Campylobacter spp. was countable in 87% of Processor A samples, 66% of Processor B samples and 33% of Processor C samples. Processor C tended to have lower counts compared with Processors A and B when countable *Campylobacter* spp. colonies were present. The maximum counts detected in MSM samples from the three processors were 3.27, 2.98 and 2.37 Log_{10} CFU/g for Processors A, B and C, respectively.

Coagulase-positive staphylococci was countable in 44% of Processor A's samples, 2% (1 sample) of Processor B samples and 36% of Processor C's samples. The highest counts were observed in Processor A's samples, but both processors A and C product had highly variable counts of coagulase-positive staphylococci.

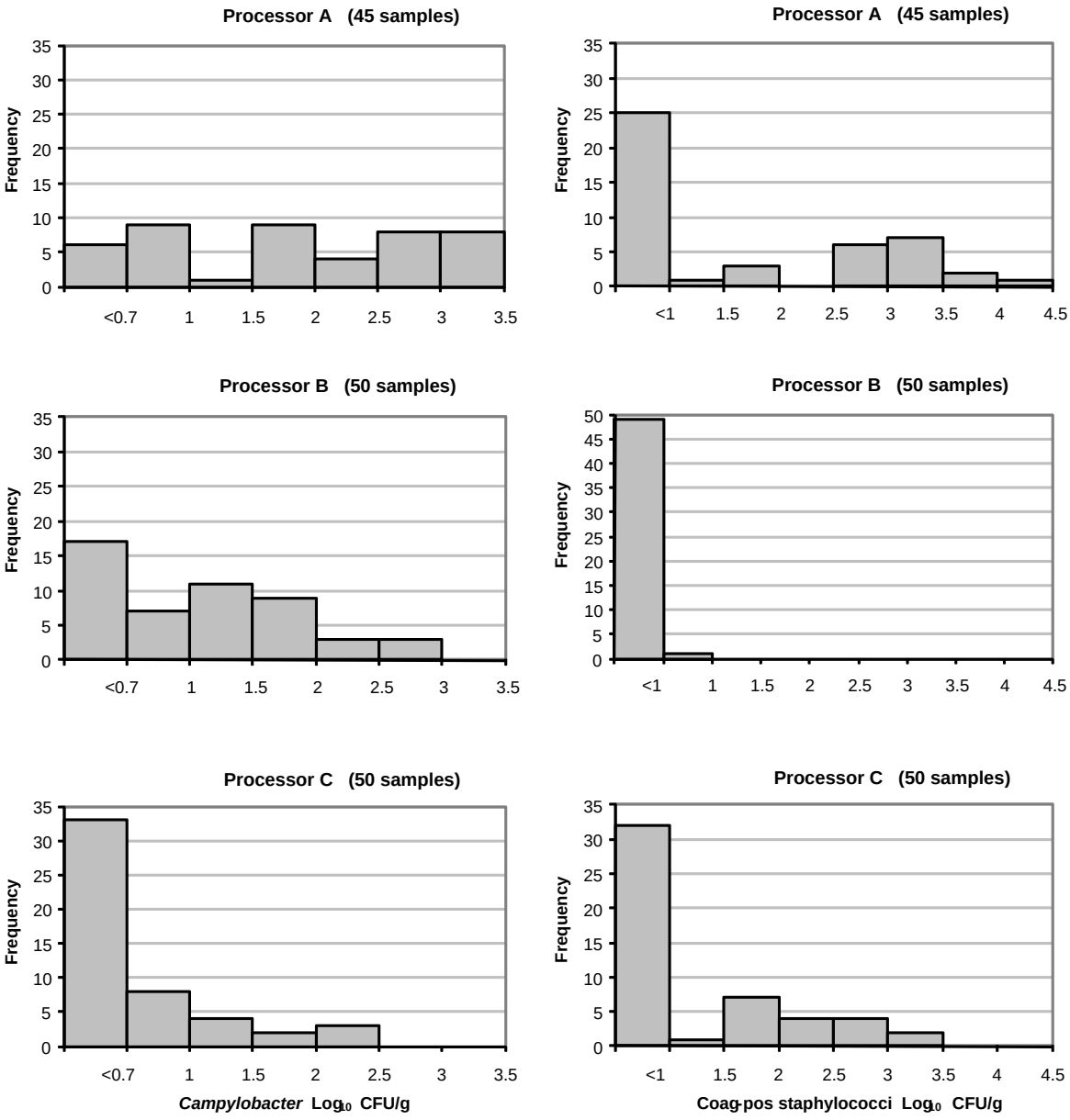


Figure 1: Histogram of counts of *Campylobacter* spp. and coagulase-positive staphylococci (Log_{10} CFU/g) from samples of MSM. The first bar of each plot represents not detected results, which may be negative or below the limit of detection.

Figure 2 plots the *Campylobacter* spp. counts from each of the processors on each sampling day. These plots show that the counts of *Campylobacter* spp. in MSM product can vary throughout the duration of processing on a day. For example, sampling day 7 at Processor A produced MSM samples with *Campylobacter* spp. counts that ranged from 0.7–3 Log_{10} CFU/g (Fig. 2).

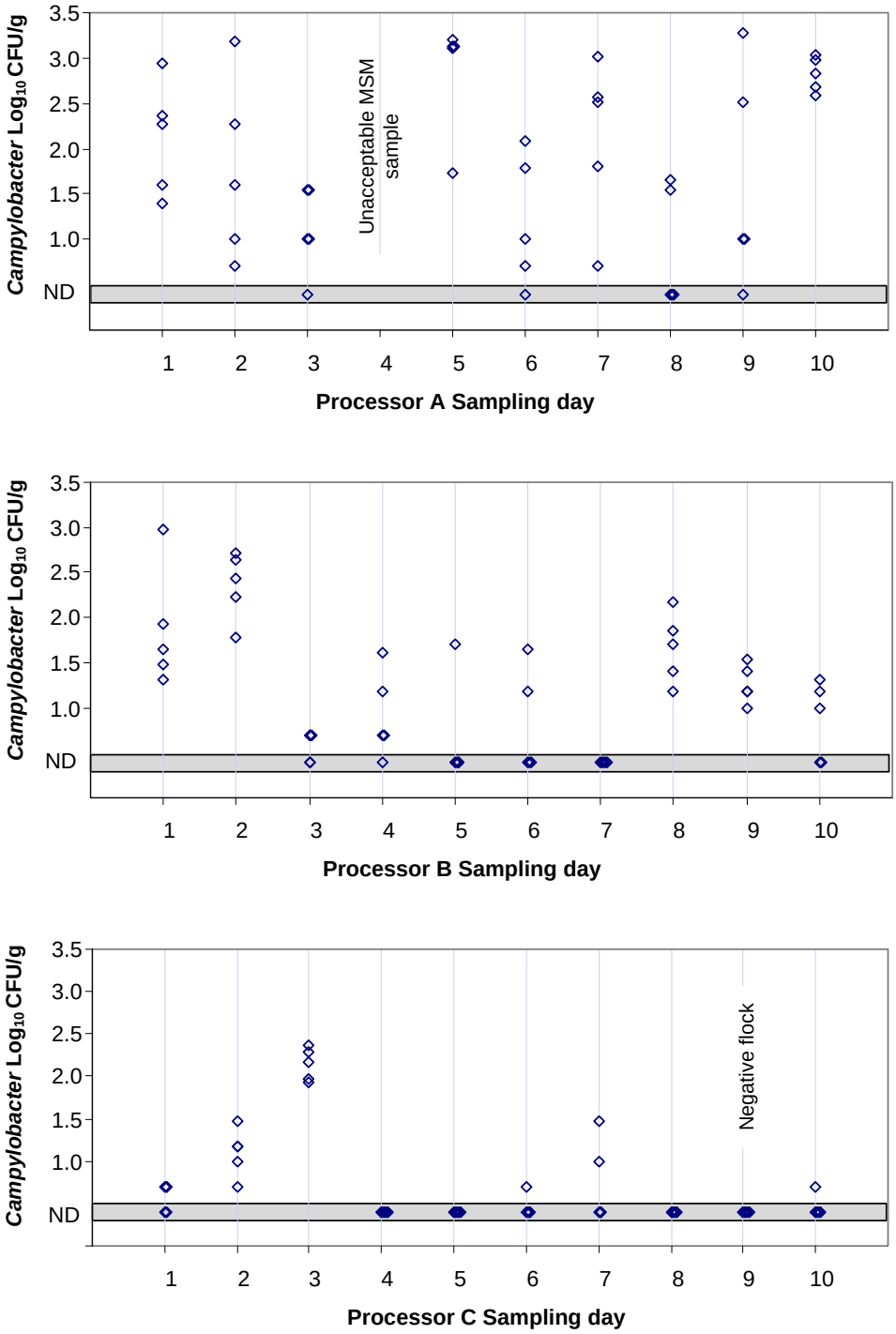


Figure 2: *Campylobacter* spp. counts from MSM samples taken on each day for the three processors. Not detected results (ND) are plotted in the grey band.

Figure 3 plots the coagulase-positive staphylococci counts from MSM samples taken by Processors A and C on each sampling day. Like the C*ampylobacter* spp. counts, the coagulase-positive staphylococci counts can vary throughout the time the product is processed on a single day.

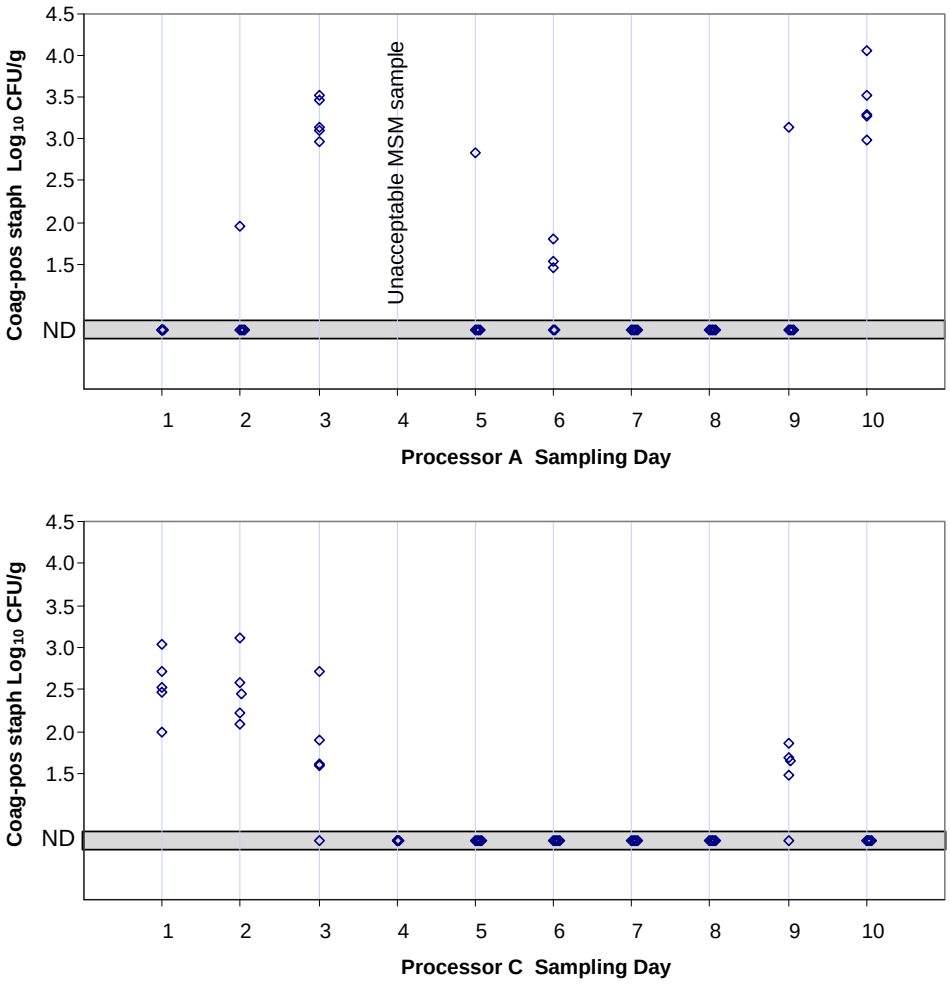


Figure 3: Coagulase-positive staphylococci counts from MSM samples taken on a given sampling day at processors A and C. Not detected (ND) results are plotted in grey band.

The within-day variation in bacterial counts may be due to a combination of inter-carcass variation and the MSM samples coming from different flocks during the processing day, some of which may not have been infected with *Campylobacter* spp. or coagulase-positive staphylococci.

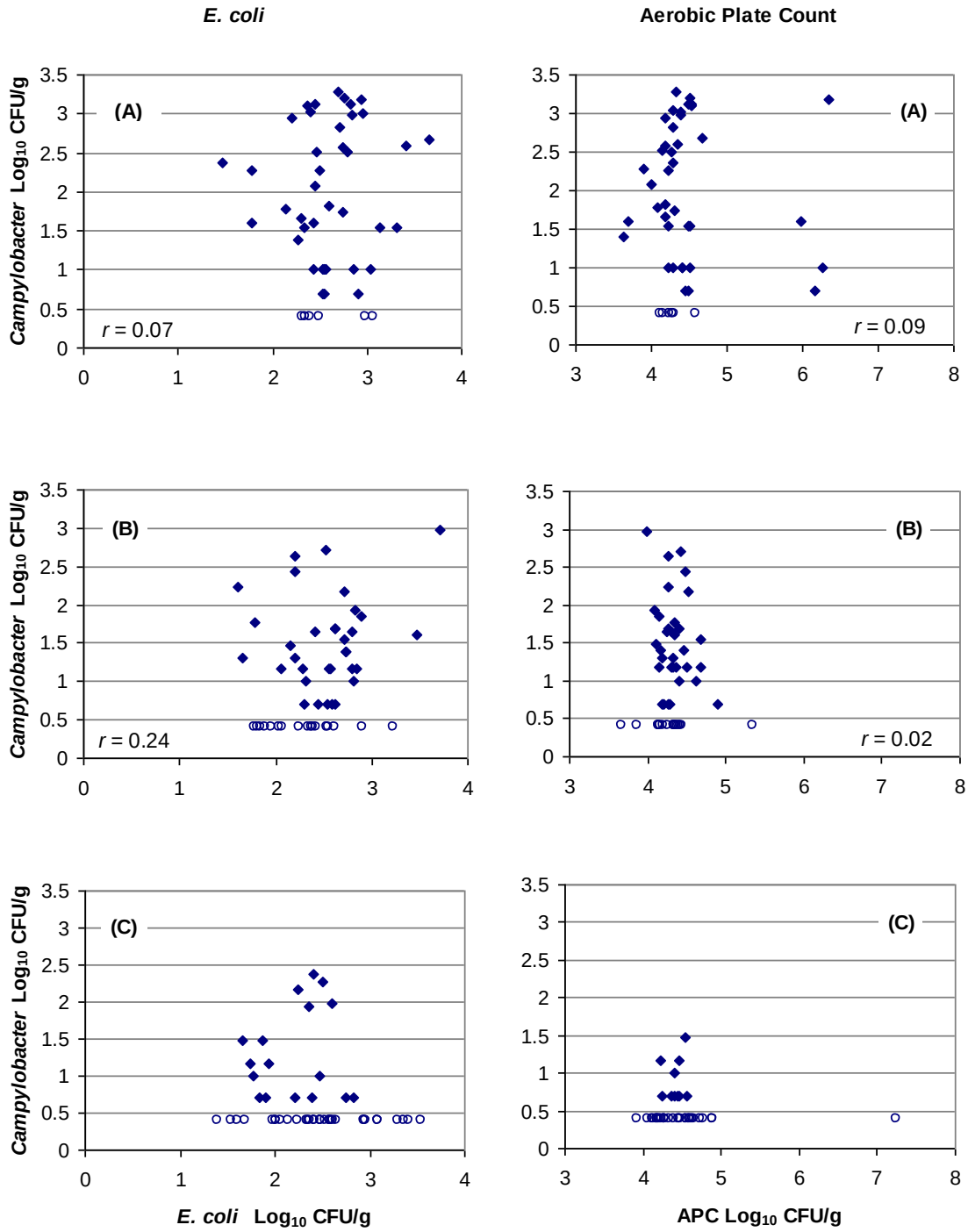
3.1.2. Comparison of *Campylobacter* spp. and coagulase-positive staphylococci counts with indicator bacterial counts.

This section evaluates *E. coli* counts and APC as possible indicators for *Campylobacter* spp. and coagulase-positive staphylococci levels in MSM. *E. coli* is an indicator organism for faecal contamination since it is found in high numbers in the faeces of broiler poultry. Likewise, APC is a measurement of mesophilic aerobic organisms that are present on chicken carcasses. Coagulase-positive staphylococci are a subset of the APC organisms. Both the *E. coli* and APC groups of organisms are used to measure the performance of dressing processes in a poultry plant.

Figure 4 plots the counts obtained from MSM samples for *Campylobacter* spp. against the counts for *E. coli* and APC. Each point on the plot represents the results from one MSM sample, or multiple samples which have resulted in the same combination of counts. If the two sets of bacterial counts given on a plot are correlated, the plot will indicate this relationship between the two bacteria. There is no correlation evident from a visual examination of the plots in Figure 4.

Figure 4 also provides the Spearman rank correlation coefficient, *r*, for the data from Processors A and B. The correlation coefficient was not calculated for Processor C due to the large number of not detected results. The value of *r* can range from minus one to one, where a value of zero suggests there is no correlation between the counts of the two bacteria in a plot. The closeness of the *r* value to one or minus one indicates a greater correlation between the two sets of bacterial counts. A positive correlation implies the counts of one bacteria increase with increasing counts of the other bacteria, and a negative correlation implies the counts of one bacteria decrease as the counts of the other bacteria increase.

Figure 4: Correlation between *Campylobacter* spp. and *E. coli* or Aerobic Plate Count from samples of MSM from three processors; A, B and C. Solid diamonds represent



counts for both microorganisms and the open circles represent counts for one of the microorganisms and not detected result for the other. The Spearman rank correlation coefficient, *r*, of the counts is provided for Processors A and B.

The *Campylobacter* spp. count against APC correlation coefficients in Figure 4 are close to zero, which concurs with the visual inspection of the plots. The correlation coefficients suggest a weak correlation between *Campylobacter* spp. and *E. coli* for Processor B, however, the data were not significantly correlated (P=0.09).

Figure 5 is a plot of coagulase-positive staphylococci counts against *E. coli* counts or APC from MSM samples from two of the processors, A and C. Processor B was not plotted, as only one sample was positive for coagulase-positive staphylococci. The correlation coefficient was not calculated for correlations between coagulase-positive staphylococci counts and *E. coli* counts or APC due to the large proportion of ND results observed in MSM samples from all three processors.

There is no correlation between coagulase-positive staphylococci counts and either *E. coli* or APC when considering all the samples. Over half the data points in these plots relate to ND results for coagulase-positive staphylococci, with the plots showing that higher counts of *E. coli* do not necessarily imply the presence of coagulase-positive staphylococci at levels that can be enumerated. This is also seen in the results from Processor B, where the *E. coli* results are similar to those of Processors A and C (Figure 4), but the level of coagulase-positive staphylococci in the MSM is very low. Processor A shows a slightly positive correlation between *E. coli* and coagulase-positive staphylococci counts.

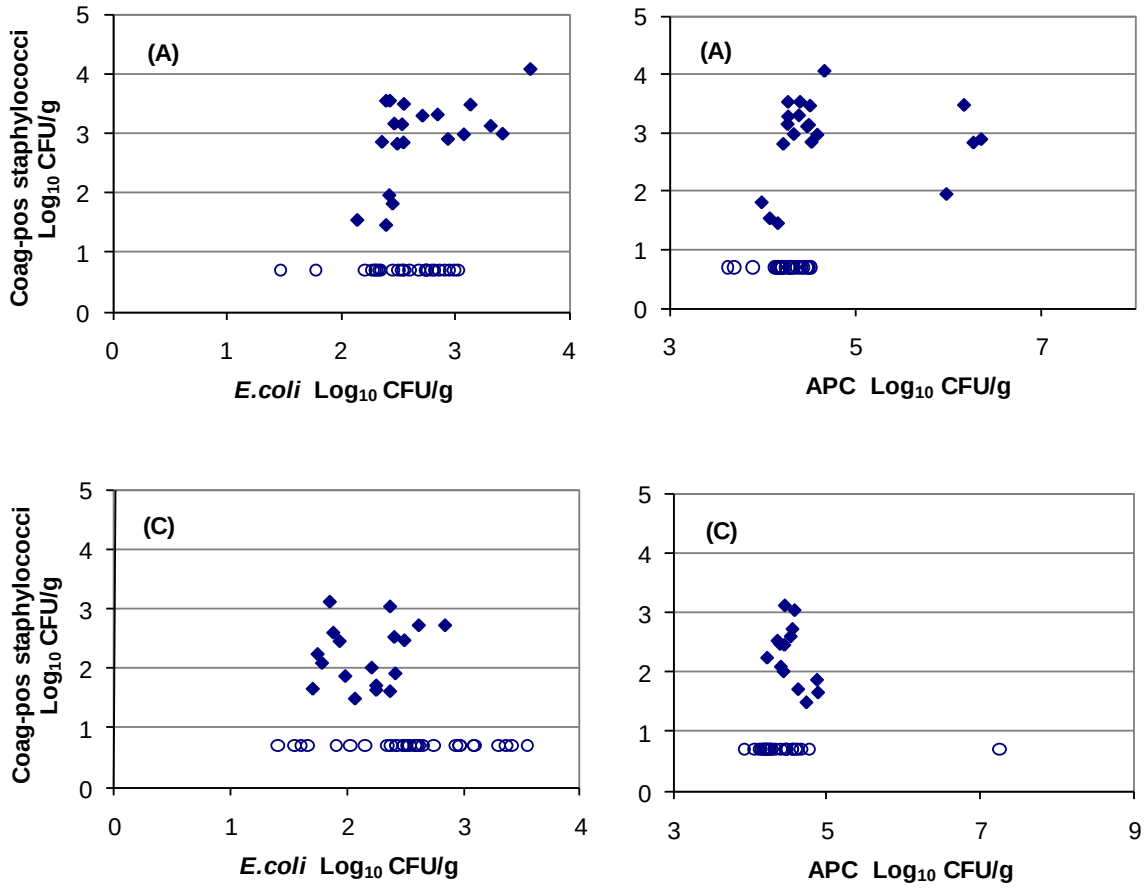


Figure 5: Correlation between coagulase-positive staphylococci counts and *E. coli* or APC counts from samples of MSM from Processors A and C. Solid diamonds represent counts for both microorganisms and the open circles represent counts for only one of the microorganisms.

3.2. Offal Products: Heart, liver, gizzard and neck

3.2.1. *Campylobacter* spp. contamination of heart, liver, gizzard and neck products before washing

The second half of the project was to test offal products (heart, liver, gizzard and neck) from Processors A and B as these are thought to pose a higher risk of foodborne campylobacteriosis than poultry meat to the customer. Figure 6 shows a histogram of *Campylobacter* spp. counts obtained from the rinsates of the four products over the six-month sampling period. The summary statistics for *Campylobacter* spp. counts on the heart, liver, gizzard and neck are given in Appendix C, Table 7.

Campylobacter spp. was enumerated from most of the rinsates of the heart, liver, gizzard and neck samples from the Processors A and B. For Processor B, only one of the heart rinsates resulted in a ND result and for Processor A, 27% of heart, 7% of gizzard, 2% of liver and 2% of neck rinses resulted in ND results.

Comparison of *Campylobacter* spp. counts from the 45 offal rinsates obtained from Processor A and the 50 rinsates obtained from Processor B shows an apparent difference in the frequency distribution of the counts (Figure 6). Processor B has a higher proportion of samples yeilding counts of 4 Log_{10} CFU/rinsate or more.

The mean weight of the heart, neck and liver product samples from Processor B were slightly heavier than the mean weights observed from Processor A (Appendix C; Table 8), which may explain some of the difference between counts observed between the two processors. However, the *Campylobacter* spp. counts from the rinsates of products of similar weights are highly variable, with up to a 5 Log_{10} CFU/rinsate difference observed between samples (Appendix C; Figure 13).

Without further investigation, including knowledge of the relationship between weight and surface area of the offal samples, it is not possible to determine how much of the difference in count frequencies between processors as observed in Figure 6 can be attributed to the difference in sample weights. It is likely that differences in processing equipment also affects the cross contamination of these samples.

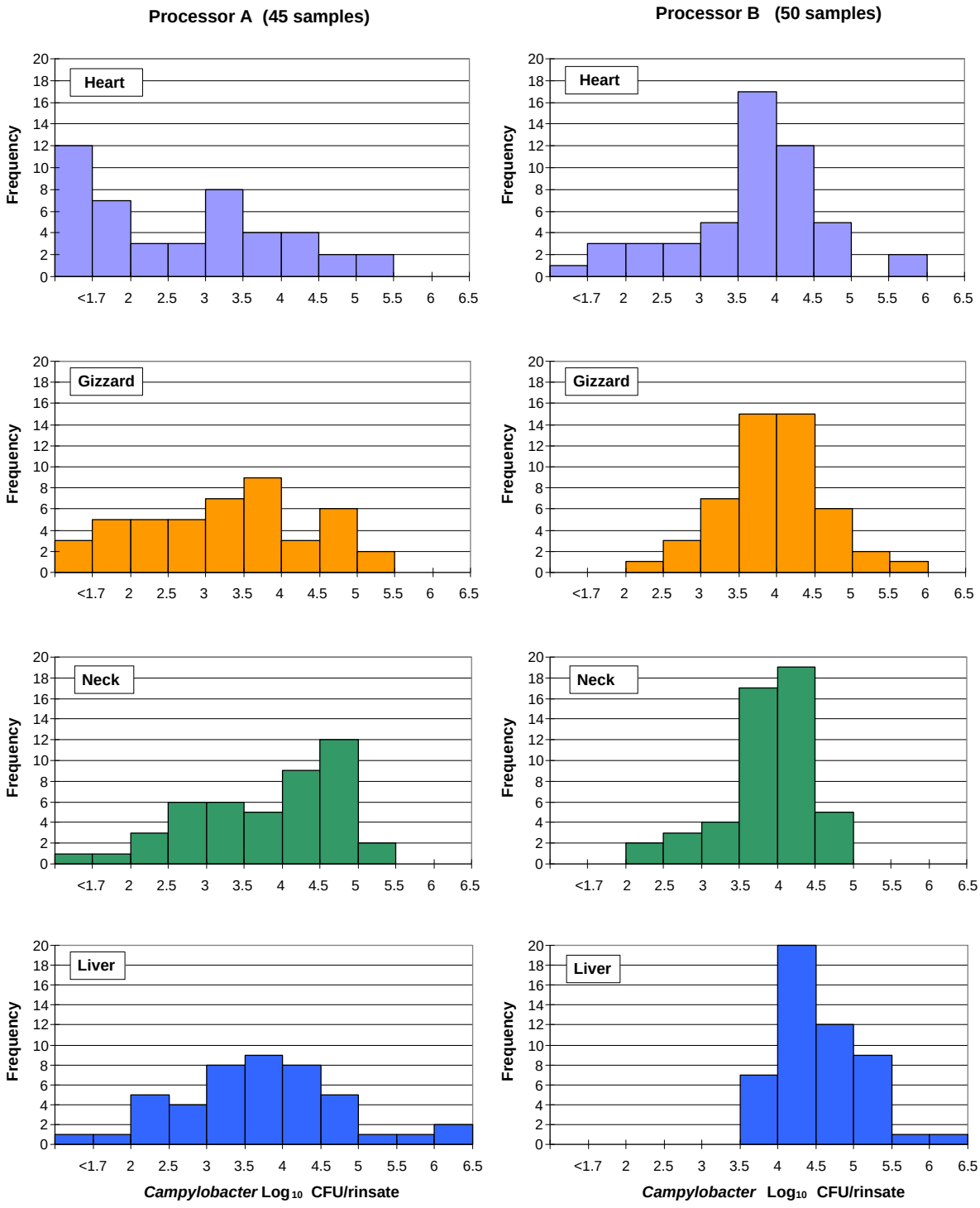


Figure 6: Frequency of *Campylobacter* spp. counts (Log_{10} CFU/rinsate) from rinsates of poultry heart, gizzard, neck and liver samples from two processors. The first bar in each plot represents not detected results, which may be negative or below the limit of detection.

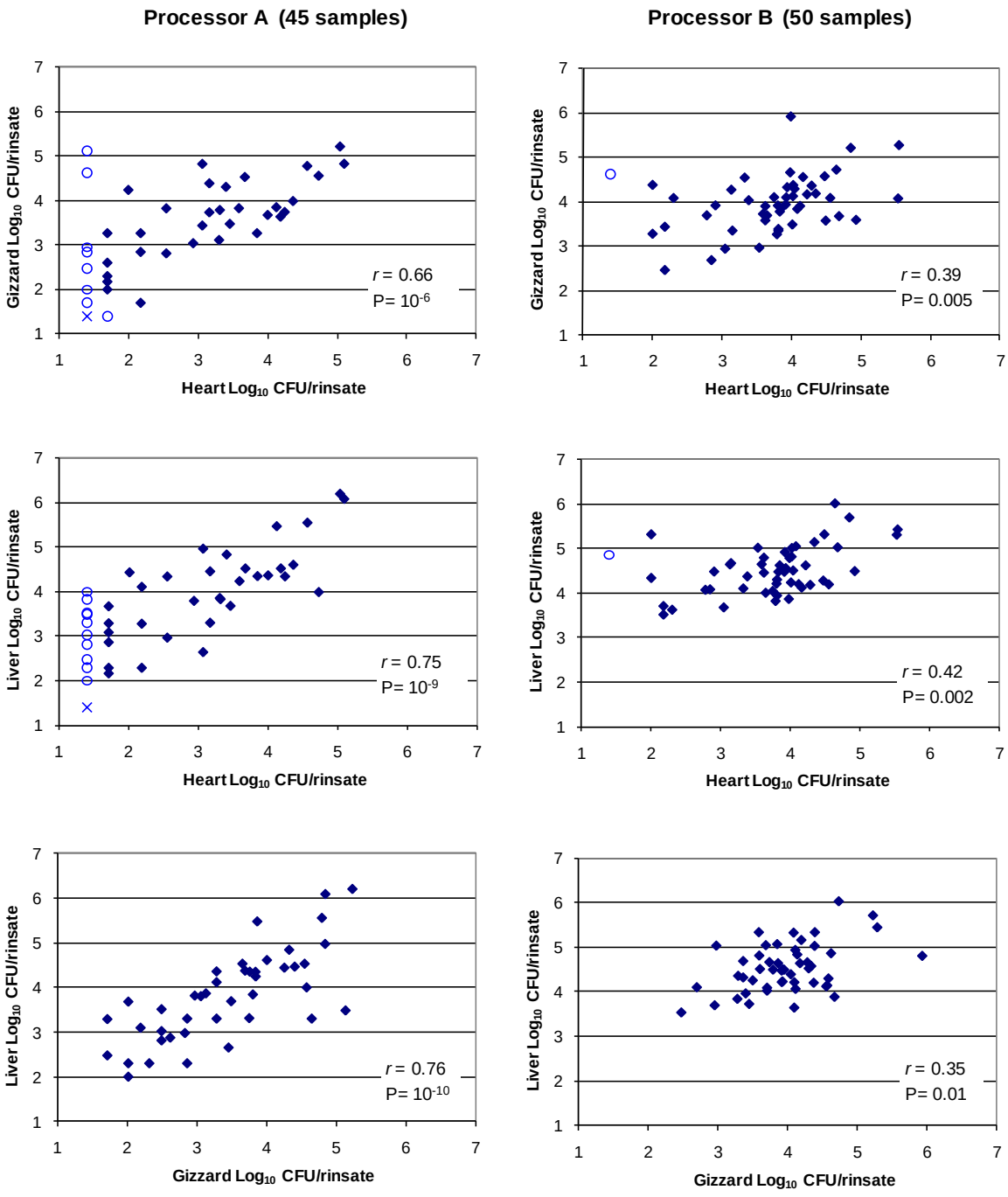


Figure 7: Correlation between *Campylobacter* spp. counts from rinsates of heart, liver and gizzard samples taken from the same bird. Solid diamonds represent samples with counts from both rinsates, the open circles represent samples with counts from only one of the rinsates, and the cross represents samples where neither rinsates produced counts. The Spearman rank correlation coefficient, *r*, and associated P value is given for each plot.

Figure 7 shows the correlation between *Campylobacter* spp. counts in rinsates ofthe heart, liver and gizzard rinsates taken from the same bird. The neck samples are not included in this figure, as neck samples were taken from different birds to the heart, liver and gizzard samples. For Processor A and B there is positive correlation between the three products, with stronger correlation observed in Processor A (r = 0.66 to 0.76, $P < 10^{-5}$) than Processor B (r = 0.35 to 0.42, $P<0.02$). However, not detected results for the heart rinsate corresponded to liver rinsate counts in the range ND to 4 Log_{10} CFU/rinsate and gizzard counts of ND to 5.12 Log_{10} CFU/rinsate. Therefore, not detected results from heart rinsates are not a good indicator of the absence of *Campylobacter* spp. in the liver or gizzard rinsates.

3.2.2. Comparison of *Campylobacter* spp. counts obtained from heart, liver, gizzard and neck rinsates with those from whole carcass rinsates.

Figure 8 and Figure 9 compare the *Campylobacter* spp. counts from whole carcass rinsates with those obtained from heart, gizzard, neck and liver rinsates taken from a flock on each sampling day, at processors A and B, respectively. A summary of the *Campylobacter* spp. counts from whole carcass rinsates is given in Appendix A. The whole carcass rinsates are obtained from carcasses which have been through a spin chiller, however the offal rinsates are obtained from the offal before the product underwent any washing or chilling.

These figures show variability in the counts of *Campylobacter* spp. on the five heart, gizzard, neck and liver samples taken from the flocks on each sampling day. Based on these figures, the whole carcass rinsate results do not provide a consistent indicator of the presence of *Campylobacter* spp. on the heart, gizzard, neck and liver samples.

For both processors there were sampling days when there were no detected counts from the whole carcass rinsates, but *Campylobacter* spp. were detected at high counts in the liver, gizzard and neck rinsates. For example, on sampling day 7, Processor A had ND counts for the five whole carcass rinses, while the gizzard rinsate counts ranged from 2.5–4.6 Log_{10} CFU/rinsate, the neck rinsate counts ranged from 3.0–3.4 Log_{10} CFU/rinsate and the liver rinsate counts ranged from 3.0–3.8 Log_{10} CFU/rinsate. A similar pattern can be seen for sampling days 1 and 9 at Processor B. However on these occasions, high counts were also observed in the heart rinsate samples.

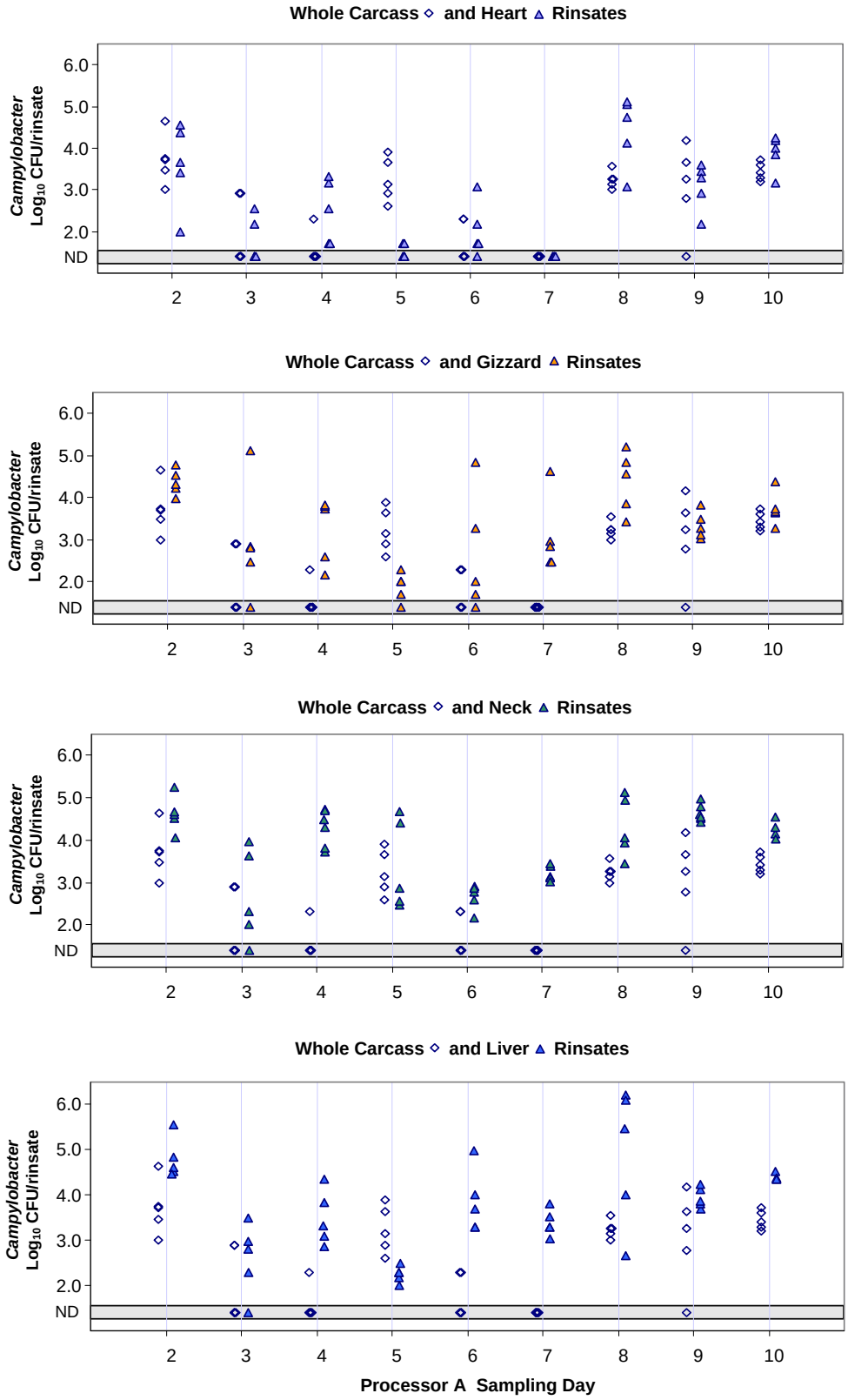


Figure 8: Comparison of *Campylobacter* spp. counts from five whole carcass rinsates (CFU/rinsate) with those obtained from five heart, gizzard, neck and liver rinsates taken from samples from nine different cuts being processed at Processor A.

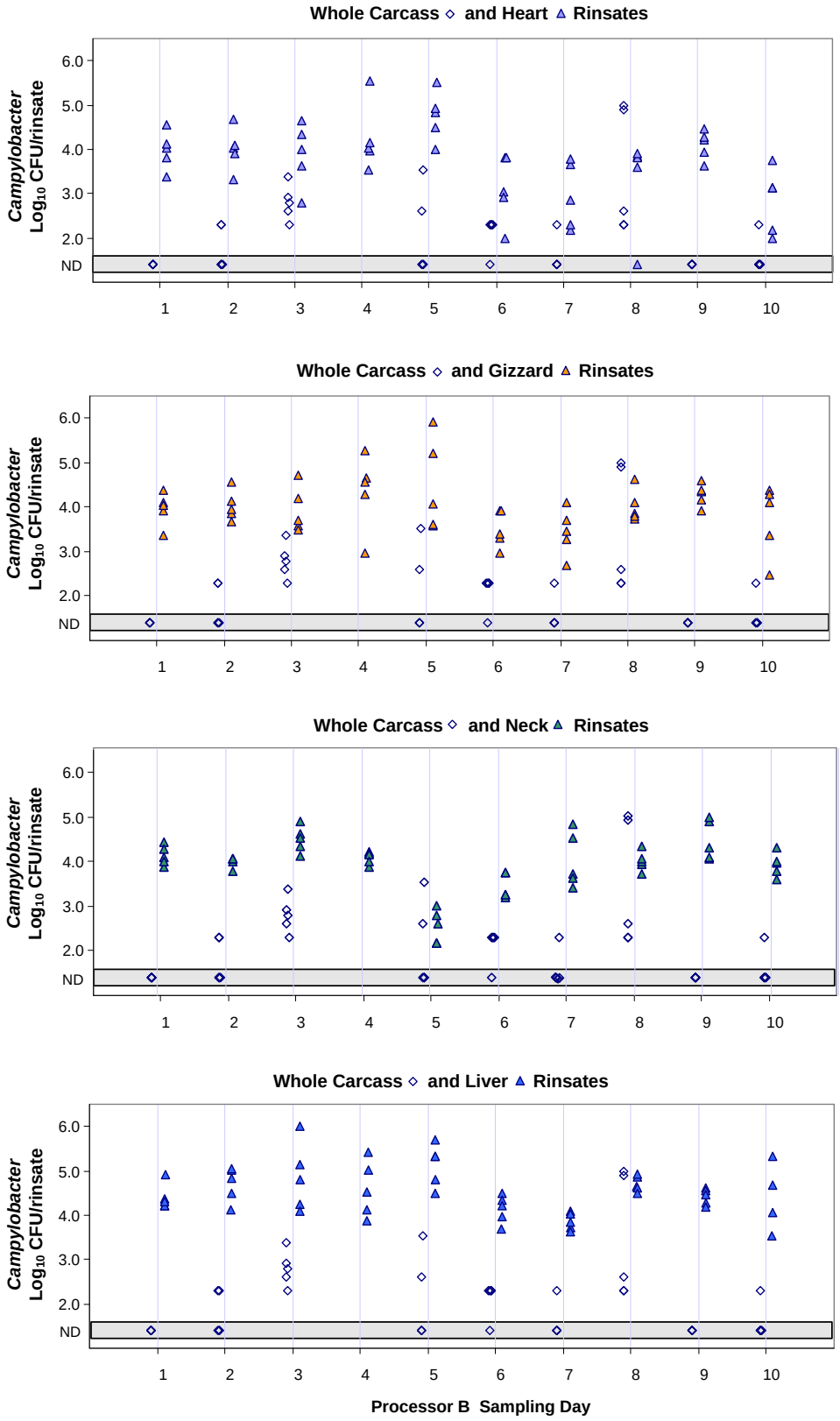


Figure 9: Comparison of *Campylobacter* spp. counts from five whole carcass rinsates (CFU/rinsate) with those obtained from five heart, gizzard, neck and liver rinsates taken from samples from 10 different cuts being processed at Processor B.

3.2.3. Comparison of *Campylobacter* spp. counts with indicator bacterial *E. coli* counts in heart, liver, gizzard and neck rinsates.

Figure 10 plots *Campylobacter* spp. counts against *E. coli* counts obtained from heart, gizzard, neck and liver sample rinsates. Each point on the plot represents the results from one sample, or multiple samples with identical pairs of counts for *Campylobacter* spp. and *E. coli*.

Little correlation is evident from visual inspection of the plots in Figure 10 or the correlation coefficient values, apart from counts from the neck samples taken from processor B which show some positive correlation (r = 0.47, P = 0.0005). However, this correlation was not seen for the neck samples from Processor A (r = -0.16, P = 0.28).

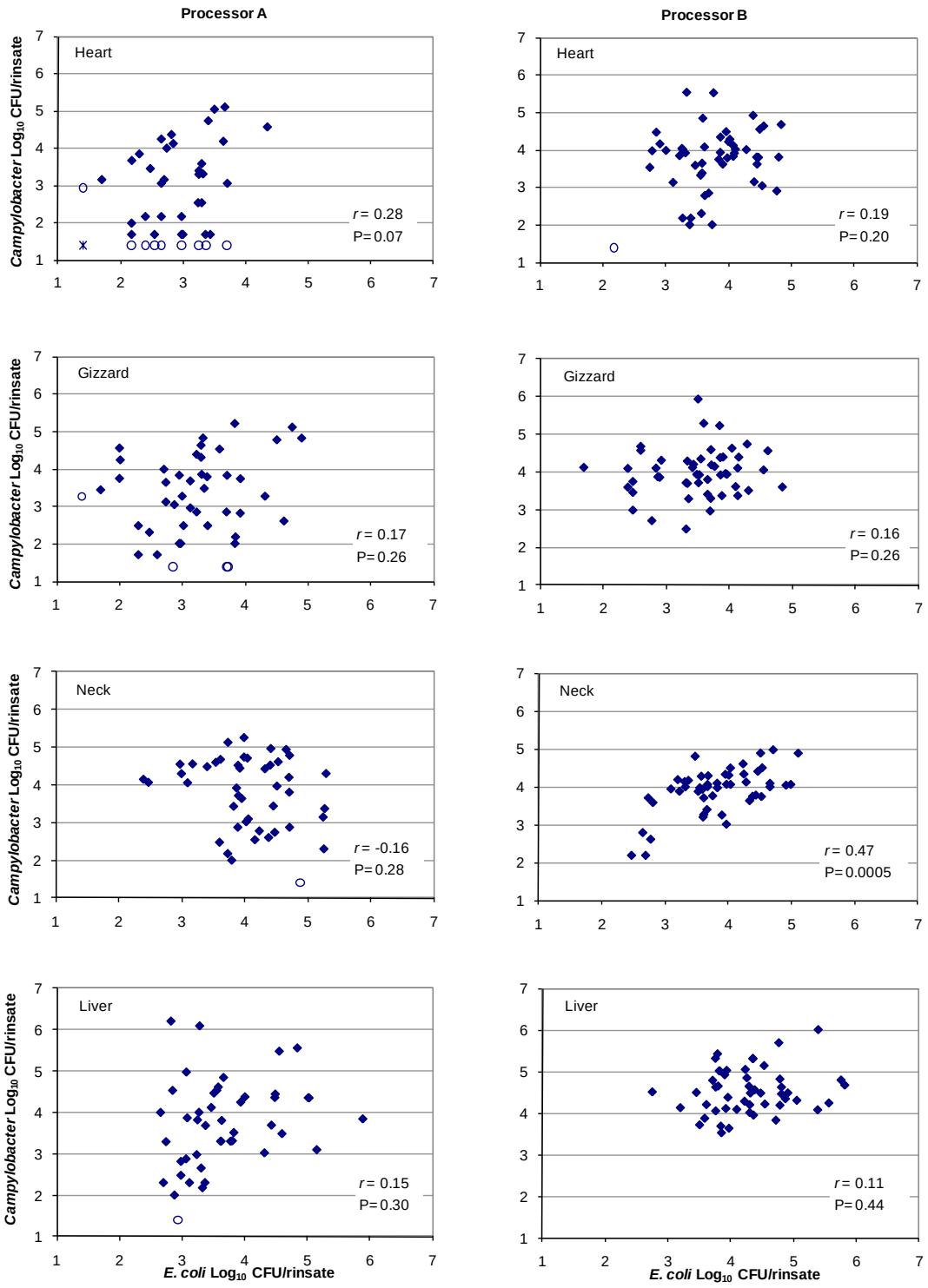


Figure 10: Comparison of *Campylobacter* spp. and *E. coli* counts from heart, gizzard, neck and liver rinsates from Processors A and B. Solid diamonds represent rinsates where both *Campylobacter* spp. and *E. coli* could be enumerated, open circles where only one bacterial species could be enumerated and a cross represents a rinsate where neither bacterial species could be enumerated. The correlation coefficient, *r*, and associated P value is given in each plot.

3.3. Comparison of *Campylobacter* spp. counts from the internal matrix and external surfaces of livers

The presence of *Campylobacter* spp. on/in poultry livers is possible through two different pathways (Barot, 1983):

1. Cross contamination of the external liver surface during poultry processing.
2. Colonisation of *Campylobacter* spp. outside the gastrointestinal track, via the bile duct.

Some papers have attributed *Campylobacter* spp. counts obtained from liver samples to be due to cross-contamination of the surface of the livers during processing rather than from *Campylobacter*-infected livers (Barot, 1983). However, recent papers by Meade *et al.* (2009), Kenar *et al.* (2009) and previous work at ESR (Whyte *et al.*, 2006) suggest surface contamination by *Campylobacter* is widespread but internalisation in poultry liver tissue is also common.

To further investigate the location of *Campylobacter* spp. on poultry livers, 45 livers from Processor A were tested externally and internally. Of the 45 livers; one (2%) had ND counts both internally and externally, 10 (22%) had *Campylobacter* spp. counts in the external rinsates, but ND counts internally, and 34 (76%) had *Campylobacter* spp. counts from both the external rinsates and internal samples. The *Campylobacter* spp. counts obtained from the 45 livers are displayed in Figure11.

The counts from the internal liver tissue samples ranged from ND to 5.6 Log_{10} CFU/liver. The external rinsate counts ranged from ND to 6.2 Log_{10} CFU/rinsate. There is a positive correlation between the Log_{10} CFU counts from internal and external samples ($r = 0.78$).

The highest external rinsate count for livers that had ND internal counts was 3.7 Log_{10} CFU/rinsate. All of the external rinsate counts above 3.7 Log_{10} CFU/rinsate corresponded to countable levels of *Campylobacter* spp. internally. However, *Campylobacter* spp. was also countable from internal samples, when the external rinsate contained *Campylobacter* spp. counts as low as 2.2 Log_{10} CFU/rinsate.

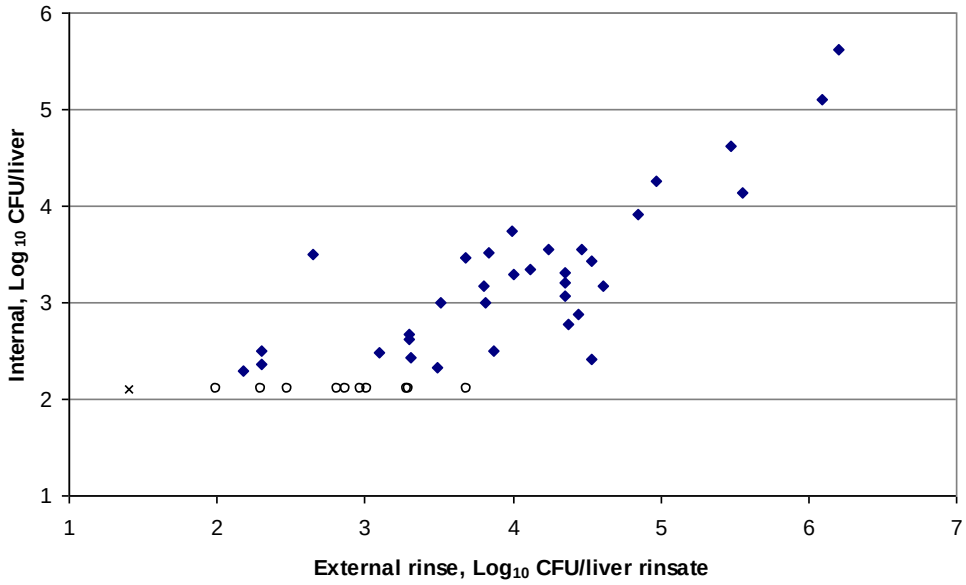


Figure11: Counts of *Campylobacter* spp. (Log_{10} CFU/rinsate) from external liver rinsates compared to counts from the internal tissues (Log_{10} CFU/liver). Solid diamonds represent liver samples with non-zero counts for external and internal sampling, circles representing the livers with no *Campylobacter* spp. detected from internal tissue and the cross representing a single liver where no *Campylobacter* spp. was detected in either the internal or external sample.

The results obtained in this survey support the theory that livers can become infected with *Campylobacter* spp. through internal infiltration of the liver from the gastrointestinal tract prior to slaughter, as well as, external cross-contamination during poultry processing.

These samples were taken before any washes of the liver that would normally be performed prior to supplying the product to retail outlets were carried out. Such washes would reduce the surface contamination of the livers with *Campylobacter* spp. However, any washes at the processors will not reduce the *Campylobacter* spp. located inside the livers.

4. CONCLUSIONS

4.1. Objective

The objective of this project was to quantify the concentrations of *Campylobacter* spp., generic *E. coli*, coagulase-positive staphylococci and APC in poultry MSM and *Campylobacter* spp. and generic *E. coli* in poultry heart, liver, gizzard and neck samples. Testing of these poultry products produced the following results.

4.2. Mechanically separated meat

A total of 145 MSM samples were collected from three different processing plants, on days when it was suspected that at least one *Campylobacter*-positive flock would be contributing to the production of the MSM.

Campylobacter spp. were countable in 87%, 66% and 33% of the three processors' samples, while coagulase-positive staphylococci were countable in 44%, 2% and 36% of the processors' samples. MSM on a given day may be made from multiple flocks, not all of which are contaminated with *Campylobacter* spp. at slaughter. Therefore, these values show that *Campylobacter* spp. can persist through processing to contaminate MSM products and that coagulase-positive staphylococci can also be found in MSM products.

The distribution of *Campylobacter* spp. in MSM also varied with the processor. The median (5^{th} to 95^{th} percentile) counts for *Campylobacter* spp. in MSM at the three processors were 1.74 (ND to 3.17) Log_{10} CFU/g, 1.18 (ND to 2.55) Log_{10} CFU/g and ND (ND to 2.08) Log_{10} CFU/g.

The median (5^{th} to 95^{th} percentile) counts for coagulase-positive staphylococci in MSM at the three processors were ND (ND to 3.52) Log_{10} CFU/g, ND (ND to 1) Log_{10} CFU/g and ND (ND to 2.72) Log_{10} CFU/g.

No significant correlation ($P > 0.05$ and $r \leq 0.24$) was evident between *Campylobacter* spp. counts from MSM and either *E. coli* counts or APC from the same sample. Similarly, no correlation was observed between coagulase-positive staphylococci and either *E. coli* or APC by visual inspection of Figure 5.

4.3. Heart, liver, gizzard and neck products

A total of 95 samples of heart, liver, gizzard and neck were analysed. Samples were taken from two processors from flocks that were positive for *Campylobacter* spp. Sample rinses were used to detect *Campylobacter* spp. on the products.

Campylobacter spp. was countable in 86% of heart rinsates, 99% of liver rinsates, 97% of gizzard rinsates and 99% of neck rinsates. The distribution of counts on these products differed between the two processors. This could be due to differences in the processing lines as well as differences in offal size. The median (5^{th} to 95^{th} percentile) of the counts were:

- Heart: Processor A, 2.5 (ND to 4.7) and Processor B, 3.8 (2.1 to 4.9) Log_{10} CFU/rinsate.
- Liver: Processor A, 3.8 (2.2 to 5.5) and Processor B, 4.5 (3.7 to 5.4) Log_{10} CFU/rinsate.
- Gizzard: Processor A, 3.3 (ND to 4.8) and Processor B, 3.9 (3.0 to 5.0) Log_{10} CFU/rinsate.
- Neck: Processor A, 4.1 (2.2 to 5.0) and Processor B, 4.0 (2.7 to 4.8) Log_{10} CFU/rinsate.

Results from whole carcass rinsates did not provide a consistent indicator of the presence of *Campylobacter* spp. on the heart, gizzard, neck and liver samples. There were sampling days when there were no detectable counts from the whole carcass rinsates, but *Campylobacter* spp. were detected at high counts in the heart, liver, gizzard and neck rinsates

No significant correlation ($P \geq 0.07$, $r \leq 0.28$) was evident between the *Campylobacter* spp. and *E. coli* counts for the heart, liver and gizzard products. The neck samples taken from processor B showed some positive correlation of the counts with a correlation coefficient of 0.47 ($P < 0.05$). However, this observation was not seen in the neck samples from Processor A ($P = 0.28$, $r = -0.16$).

4.4. Presence of *Campylobacter* spp. in the internal and on the external of livers

Forty-five liver samples were taken over the sampling period from a single processor. Of these livers, 22% was positive for *Campylobacter* spp. only on the surface of the liver, 76% was positive on the liver surface and in the internal tissues and 2% had no countable *Campylobacter* spp..

The distribution of the estimated count in internal liver tissue had a median (5^{th} to 95^{th} percentile) of 2.9 (ND to 4.5) Log_{10} CFU/whole liver, compared to the counts obtained from the external liver rinsate; 3.8 (2.2 to 5.5) Log_{10} CFU/rinsate. The high

proportion of samples showing internal contamination of the liver suggests that it is common for *Campylobacter* spp. to colonise the liver in infected birds before slaughter. A strong positive correlation was seen between the internal and external presence of *Campylobacter* spp. of the liver samples (r=0.78).

Washing of livers at the processors will not remove internal contamination. *Campylobacter* spp. in the internal tissues of raw livers following any chilling or freezing processes would need to be killed by cooking practices that could sufficiently heat the centre of the liver, as advocated by Whyte *et al.* (2006).

5. REFERENCES

Anonymous (2008). Annual New Zealand Notifiable Disease Report 2008. Available at: http://www.surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2008AnnualSurvRptTables.pdf (last accessed on the 19 February 2010).

Barot, M.S., Mosenthal, A.C. and Bokkenheuser, V.D. (1983). Location of *Campylobacter jejuni* in infected livers. Journal of Clinical Microbiology, May: 921-922.

Campbell, K.W. and Gilbert, S. (1995). National Food Project – Poultry Quality Assessment. Public Health Commission Report, February 1995. Ministry of Health, Wellington, New Zealand..

Chrystal, N.D., Hargraves, S.J., Boa, A.C., and Ironside, C.J. (2008). Counts of *Campylobacter* spp. and prevalence of *Salmonella* associated with New Zealand broiler carcasses. Journal of Food Protection 71(12), 2526-2532.

Eberhardt-Phillips, J., Walker, N., Garrett, N., Bell, D., Sinclair, D., Rainger, W. and Bates, M. (1997). Campylobacteriosis in New Zealand: results of a case-control study. Journal of Epidemiology and Communicable Health 51:686-691.

French, N. (2008). Enhancing surveillance of potentially foodborne enteric diseases in New Zealand: Human campylobacteriosis in the Manawatu. Available from: http://www.foodsafety.govt.nz/elibrary/industry/enhancing-surveillance-potentially-research-projects-2/Campy_Attribution_Manawatu.pdf Hopkirk Institute, Massey University, NZ.

French, N. (2009). Enhancing surveillance of potentially foodborne enteric diseases in New Zealand: Human campylobacteriosis in the Manawatu: Project extension incorporating additional poultry sources. Available from : http://www.foodsafety.govt.nz/elibrary/industry/enhancing-surveillance-potentially-research-projects/finalreportducketc2009.pdf. Hopkirk Institute, Massey University, NZ.

ICMSF (1998). Micro-organisms in food 6, microbial ecology of food commodities. ISBN: 0751404306, Blackie Academic and Professional: 102.

Kenar, B. Akkaya, L. and Birdane, Y.O. (2009). Prevalence of thermotolerant *Campylobacter* in chicken livers in Turkey and antimicrobial resistance amoung the *Campylobacter* strain. Journal of Animal and Veterinary Advances 8 (5): 853-856.

Meade, G.M., Narciandi, F., Cahalane, S., Reiman, C., Allan, B. and O'Farrelly, C. (2009). Comparative *in vivo* infection models yield insights on early host immune response to *Campylobacter* in chickens. Immunogenetics 61: 101-110.

Mullner P., Spencer S.E, Wilson, D.J, Jones, G., Noble, A.D, Midwinter, A.C, Collins-Emerson, J.M, Carter, P., Hathaway, S. and French, N.P. (2009). Assigning the source of human campylobacteriosis in New Zealand: a comparative genetic and epidemiological approach. Infect Genet Evol; 9: 1311-9.

NZFSA. (2008). New Zealand Food Safety Authority *Campylobacter* Risk Management Strategy 2008-2011. Available from: http://www.foodsafety.govt.nz/elibrary/industry/Campylobacter_Risk-Comprehensive_Aimed.pdf. (Last accessed on 15 February 2011).

Paulin, S. (2010). Longitudinal Carcass Mapping. Client report FW09103 to the New Zealand Food Safety Authority, Wellington, New Zealand.

Whyte R, Hudson J A and Graham C (2006) *Campylobacter* in chicken livers and their destruction by pan frying. Letters in Applied Microbiology; 43: 591-595.

Wong T L, Hollis L, Cornelius A, Nicol C, Cook R C and Hudson J A (2007) Prevalence, numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. Journal of Food Protection; 70: 566-573.

Wong, T.L. (2010). Enumeration of *Campylobacter* spp. from turkey and duck carcasses in New Zealand. Client report FW09108 to the New Zealand Food Safety Authority, Wellington, New Zealand.

Wong, T.L. and Hudson, A.J. (2010). *Campylobacter* spp. in uncooked retail chicken meats: the 2009 survey. Client report FW10009 to the New Zealand Food Safety Authority, Wellington, New Zealand. (Draft report subject to NZFSA approval).

Wong , T.L. and Chung, P. (2010). *Campylobacter* and *Salmonella* from end-of lay and breeder poultry carcasses in New Zealand. Client report FW10068 to the New Zealand Food Safety Authority, Wellington, New Zealand.

APPENDIX A: WHOLE CARCASS RINSATE

Whole carcasses where taken at the NMD testing point and rinsed according to NMD protocol. The concentrations of *Campylobacter* spp. and *E. coli* in the rinsate is summarised in Table 4, Table 5 and Figure 12.

Table 4: Summary statistics for *Campylobacter* spp. in whole carcass rinsates taken from the three processors.

	Processor		
	A	B	C
Number of samples	50	50	50
Number of ND[a] (%)	16 (32%)	25 (50%)	38 (76%)
5th percentile (Log_{10} CFU/rinsate)	ND[a]	ND	ND
25th percentile (Log_{10} CFU/rinsate)	ND	ND	ND
Median (Log_{10} CFU/rinsate)	2.90	ND-2.3 [b]	ND
75th percentile (Log_{10} CFU/rinsate)	3.46	2.6	ND
95th percentile (Log_{10} CFU/rinsate)	4.07	4.26	3.31
Maximum (Log_{10} CFU/rinsate)	4.64	5.00	4.49

a ND: Counts were recorded as <200 CFU/carcass and recorded as "Not Detected" in NMD protocol.
b The median value lies between a ND result and 2.3 Log_{10} CFU/rinsate.

Table 5: Summary statistics for *E. coli* in whole carcass rinse samples taken from the three processors.

	Processor		
	A	B	C
Number of samples	40	50	50
Number of ND (%)	7 (18%)	11 (22%)	0 (0%)
5th percentile (Log_{10} CFU/rinsate)	ND	ND	3.38
25th percentile (Log_{10} CFU/rinsate)	2.60	2.90	3.68
Median (Log_{10} CFU/rinsate)	3.51	3.48	4.06
75th percentile (Log_{10} CFU/rinsate)	4.04	4.26	4.45
95th percentile (Log_{10} CFU/rinsate)	5.12	5.14	4.79
Maximum (Log_{10} CFU/rinsate)	6.81	5.25	5.42

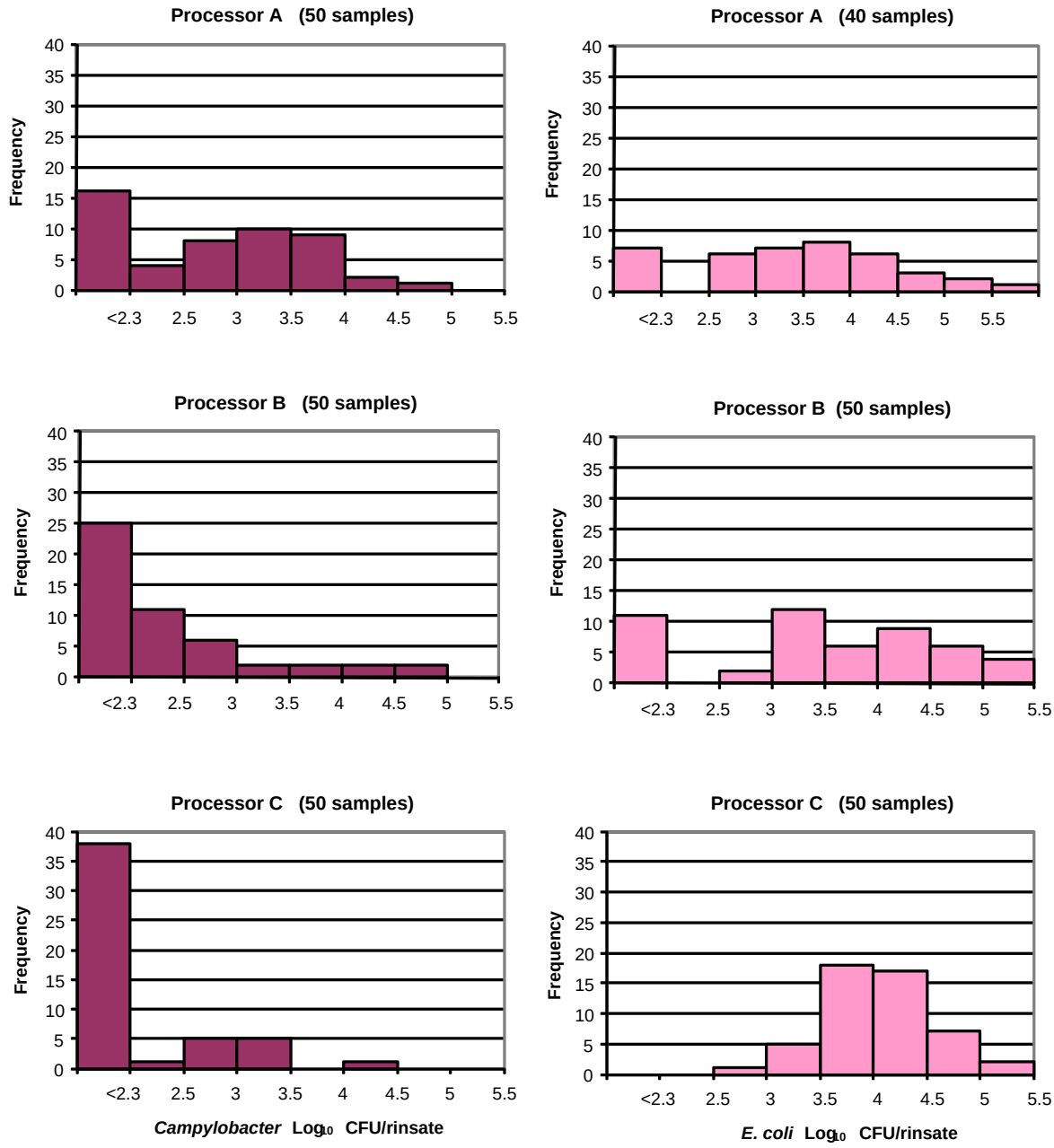


Figure 12: *Campylobacter* spp. and *E.coli* counts (Log_{10} CFU/rinsate) in whole carcass rinse samples taken from suspected positive flocks at three different processing plants. First column represents the frequency of not detected (ND) results.

APPENDIX B: MECHANICALLY SEPERATED MEAT - SUMMARY STATISTICS

Table 6: Summary statistics for bacteria in mechanically separated meat products for three processors.

	Campylobacter			Coagulase-positive Staphylococci			*E.coli*			APC		
Processer	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Number of samples	45	50	50	45	50	50	45	50	50	45	50	40
Number of ND (%)	6 (13%)	17 (34%)	33 (66%)	25 (56%)	49 (98%)	32 (64%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
5th percentile (Log_{10} CFU/g)	ND	ND	ND	ND	ND	ND	1.88	1.78	1.63	3.92	4.03	4.12
25th percentile (Log_{10} CFU/g)	1.00	ND	ND	ND	ND	ND	2.40	2.20	2.02	4.19	4.18	4.23
Median	1.74	1.18	ND	ND	ND	ND	2.55	2.48	2.41	4.30	4.32	4.43
75th percentile (Log_{10} CFU/g)	2.67	1.64	0.70	2.97	ND	1.82	2.85	2.72	2.62	4.48	4.41	4.57
95th percentile (Log_{10} CFU/g)	3.17	2.55	2.08	3.52	ND	2.72	3.28	3.11	3.34	6.14	4.68	4.89
Maximum (Log_{10} CFU/g)	3.27	2.98	2.37	4.06	1.00	3.11	3.66	3.72	3.54	6.35	5.34	7.26

APC: Aerobic plate count.
ND: Not detected count.

APPENDIX C: HEART, GIZZARD, NECK AND LIVER

Table 7: Summary statistics for *Campylobacter* spp. counts from rinsates of poultry hearts, gizzards, livers and necks from Processors A and B.

	Processor A				Processor B			
Product	**Heart**	**Gizzard**	**Ext. Liver**	**Neck**	**Heart**	**Gizzard**	**Ext. Liver**	**Neck**
Number of samples	45	45	45	45	50	50	50	50
Number of ND (%)	12 (27%)	3 (7%)	1 (2%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)
5th percentile (Log_{10} CFU/rinsate)	ND	ND	2.20	2.20	2.08	2.96	3.70	2.68
25th percentile (Log_{10} CFU/rinsate)	ND	2.48	3.02	3.02	3.34	3.59	4.15	3.71
Median (Log_{10} CFU/rinsate)	2.54	3.27	3.80	4.06	3.84	3.94	4.49	3.99
75th percentile (Log_{10} CFU/rinsate)	3.59	3.99	4.37	4.55	4.15	4.33	4.84	4.25
95th percentile (Log_{10} CFU/rinsate)	4.70	4.83	5.54	4.96	4.89	5.00	5.38	4.84
Maximum (Log_{10} CFU/rinsate)	5.10	5.22	6.20	5.26	5.54	5.92	6.01	4.97

Table 8: Heart, gizzard, liver and neck sampled product weights.

	Processor A				Processor B			
Product	**Heart**	**Gizzard**	**Liver**	**Neck**	**Heart**	**Gizzard**	**Liver**	**Neck**
Mean weight (g)	16.0	28.4	54.2	49.9	18.7	27.9	67.4	58.0
Standard deviation (g)	3.6	7.9	9.7	11.9	3.7	5.4	11.6	11.6

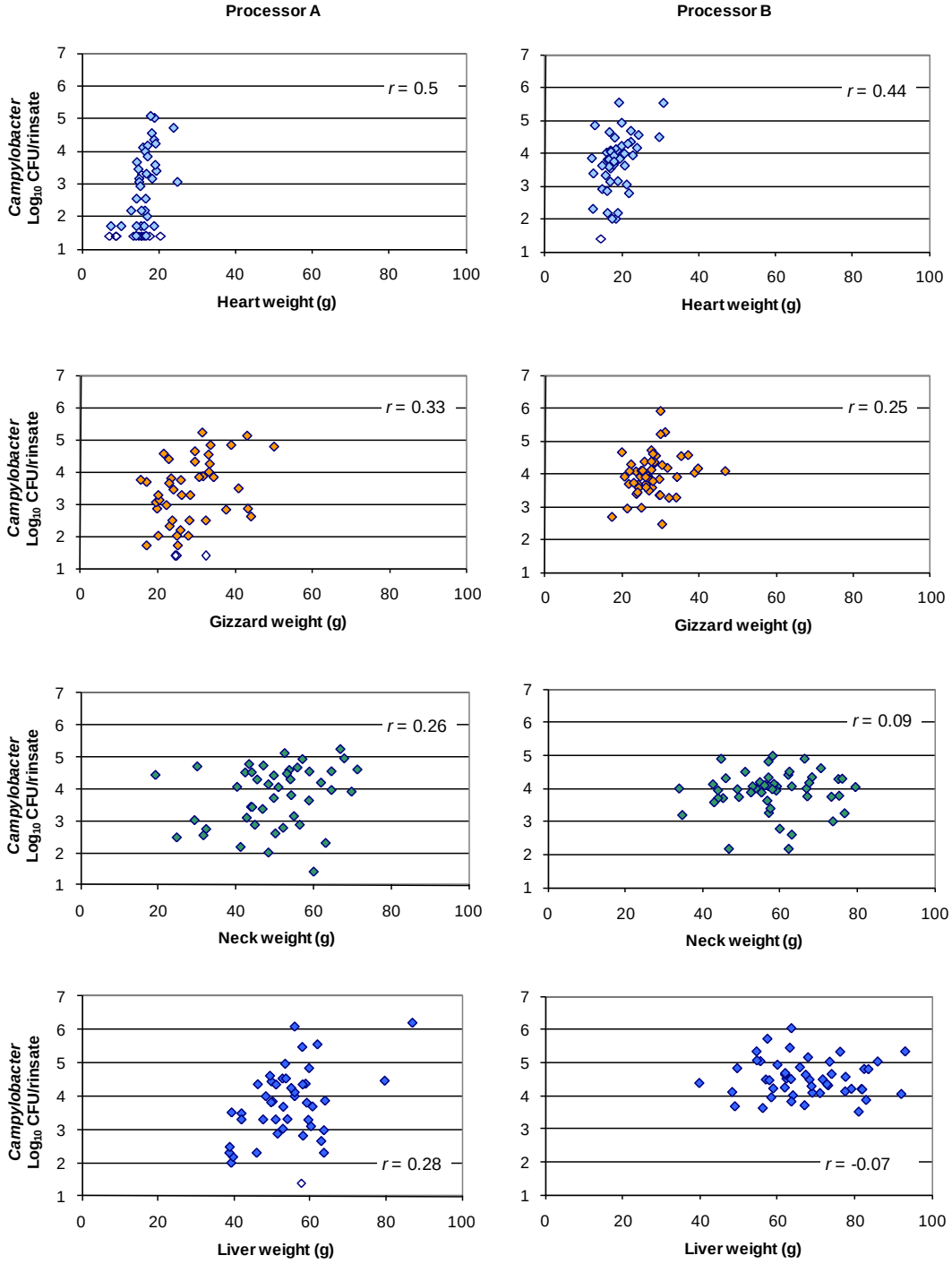


Figure13: Correlation between sample weight and *Campylobacter* spp. concentration on heart, gizzard, neck and liver products. Open diamonds represent products with not detected *Campylobacter* spp. counts and *r* is the Spearman rank correlation coefficient.

INTENTIONALLY BLANK