Ministry for Primary Industries
Manatū Ahu Matua

Source Attribution January – December 2014 of Human *Campylobacter Jejuni* Cases from the Manawatu

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Scientific Interpretive Summary

This SIS is prepared by MPI risk assessors to provide context to the following report for MPI risk managers and external readers

Source attribution January - December 2014 of human *Campylobacter jejuni* cases from the Manawatu.

Source attribution is the process of determining the proportions that various pathways and sources contribute to the total incidence of a specific disease in humans. This information is critical in creating targeted intervention strategies to reduce the human disease burden, and for monitoring progress in achievement of public health goals.

This report provides an update of the relative contribution of different reservoirs to the burden of human campylobacteriosis in the Manawatu sentinel site over the period 1 January 2014 – 31 December 2014.

Of the 204 primary samples, 157 were successfully cultured and 153 samples were sequence typed yielding 153 full MLST allelic profiles. Reservoir attribution modelling revealed a similar attribution of human cases to poultry (35%) and ruminants (45%). In both cases there were large confidence intervals.

A new sequence type ST-6964 was observed on poultry carcases and from three human cases. Antimicrobial sensitivity testing of two of the human isolates revealed resistance to ciprofloxacin, nalidixic acid, enrofloxacin and tetracycline.

Sampling of ruminant populations will take place to see if there has been a shift of ST distributions in cattle and sheep.

MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

Final Report: MPI Agreement 11777, Schedule 1A Source attribution January to December 2014 of human *Campylobacter jejuni* cases from the Manawatu

Completion of sequence typing of human and poultry isolates and source attribution modelling

June 2015

prepared for
the Ministry for Primary Industries

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Report: MPI Agreement 11777, Schedule 1A

Source attribution January - December 2014 of human *Campylobacter jejuni* cases from the Manawatu.

June 2015

Contents

1 Summary

This report provides an update of the relative contribution of different reservoirs to the burden of human campylobacteriosis in the Manawatu sentinel site. It summarises the results of multilocus sequence typing of isolates stored in the culture bank of *Campylobacter jejuni* samples from poultry and humans in the Manawatu sentinel site, and the epidemiological data linked to the human cases. The isolates were catalogued and stored in the Hopkirk mEpiLab between 1st January and 31st December 2014. A total of 239 samples were submitted to the mEpiLab in this period, of which 204 were considered to be primary samples (residing within the Manawatu region, and the first sample from an individual case acquired through the routine surveillance system). Of the 204 primary samples, 157 were successfully cultured and 153 samples were sequence typed (one was typed directly from the swab without culturing), yielding 153 MLST allelic profiles. Of these, 148 were *C. jejuni* that could be linked to EpiSurv data from 148 cases, the remainder were either *C. coli* (N=2) or could not be linked to EpiSurv data (N=3).

A new sequence type ST-6964 was observed on carcases from poultry suppliers B and C, as well as on chicken pieces from supplier A (collected as part of a separate study), and from three human cases. Antimicrobial sensitivity testing of two of the human isolates revealed resistance to ciprofloxacin, nalidixic acid, enrofloxacin and tetracycline.

Reservoir attribution modelling revealed a similar attribution of human cases to poultry (35%) and ruminants (45%) – a trend that has continued since 2010. In contrast to previous years, when cases were divided into urban and rural dwellers, the attribution for 2014 showed both urban and rural dwellers having a similar attribution to poultry and ruminants, however there were only 28 rural cases with MLST information, so there is significant uncertainty in the rural attribution estimates. Extended analysis of urban and rural dwellers over the 2005–2014 period showed little variation in the attribution of cases from rural dwellers over time, whereas there was much higher variation in the attribution of cases from urban dwellers, primarily due to variation in cases attributed to poultry.

A study of the number of urban and rural cases through time suggests increas-

ing case rates through time for urban and rural dwellers in the Manawatu before the poultry industry intervention of 2007/2008, with the intervention primarily affecting the number of cases among urban dwellers, where it reduced baseline case rates by 50%. In the period following the poultry intervention, the number of cases has continued to increase in the Manawatu, with the rate of increase being similar among urban and rural dwellers. This trend is not seen nation-wide, where total case numbers has been relatively constant since 2007/2008. The increasing trends in recent years may be due to an increasing submission rate of *Campylobacter* negative samples to mEpiLab, however, with 2013 and 2014 in particular having a lower number of samples that could be confirmed positive (77% and 66.1%) compared with earlier years (e.g. 2012 66.1%). A change in seasonality before and after the poultry intervention was also observed, with the large summer peaks of November and December in 2005–2007 being reduced, with relatively more cases appearing in August and September.

There is evidence of a differing age distribution over time, with the 65+ age group contributing to a higher proportion of cases in the last few years. As seen previously, young children in rural areas contribute a disproportionate number of rural cases compared to their urban counterparts.

2 Introduction

In 2006 the Ministry for Primary Industries (MPI, formally the New Zealand Food Safety Authority, NZFSA) set a public health goal of a 50% reduction in the foodborne proportion of campylobacteriosis over five years. Current surveillance data present a promising picture of having achieved this organisational goal. It is important to monitor any changes in the source attribution, especially from poultry, whether in response to a known intervention or from undetermined cause. However, continuing to genotype all human samples and those from a range of environmental sources, including food, was not financially tenable. MPI wished to establish a bank of appropriate *Campylobacter jejuni* samples to be catalogued and stored appropriately and to be available for immediate analysis in response to changes in either potential exposures or disease incidence.

This contract required the Hopkirk mEpiLab to:

1. Randomly select approximately 200 samples of human isolates (that have been collected and stored as part of agreement number 11424 between MPI and the Contractor for the period January 2014 to December 2014) for multilocus sequence typing;
2. Over the same time period select 100 samples of poultry carcase isolates (that have been collected and stored as part of the agreement number 11424 between MPI and the Contractor) for multilocus sequence typing;
3. Use the sequence typing to populate dynamic source attribution models developed by the Contractor as part of agreement number 11178 between the Contractor and MPI and detailed in the final report in Milestone 2 of Schedule 1 of that Agreement; and
4. Prepare and submit a draft report to MPI for comment detailing the outcomes of clause 3.3 of the schedule; and
5. Prepare and submit a final report to MPI's satisfaction detailing the outcomes of clause 3.3 of the schedule.

3 Methods

N.B. As the methods have generally remained consistent, this section is largely unchanged from the previous report. The only additions are the section on the method used to assign sequence types and impute missing alleles, and the section on the modelling of urban and rural case numbers through time.

3.1 Sampling and microbiology

3.1.1 Human faecal samples

Human specimens submitted to MedLab Central, Palmerston North that were positive for *Campylobacter* by ELISA (ProSpecT®, Remel, USA) were

sent to the Hopkirk mEpiLab. Faecal swabs were made using Amies Charcoal transport swabs (Copan, Italy). These were cultured on modified Cefoperazone Charcoal Deoxycholate agar (mCCDA) plates (Fort Richard, Auckland) and in Bolton Broth (Lab M, Bury, England) and incubated at 42°C in a microaerobic atmosphere (85% N_2, 10% CO_2, 5% O_2) for 2 days. A single colony resembling *Campylobacter* species was subcultured to Blood Agar (BA) (Fort Richard, Auckland) and incubated microaerobically at 42°C for 2 days before DNA preparations were made. Cultures were frozen at -80°C in Glycerol Broth (Difco, USA).

3.1.2 Poultry carcases

Whole chicken carcases were purchased from retail outlets in Palmerston North (six per month from different suppliers according to availability). These were washed and massaged in 200 ml of Buffered Peptone Water (BPW) (Difco, USA) in stomacher bags (Seward, England) or autoclave bags. The wash was centrifuged (10,000 rpm, 6°C, 35 mins, Sorvall RC5B) and the resultant pellet resuspended in 5 ml of BPW. Approximately 3 ml of the resuspended pellet was added to 90 ml of Bolton's broth which was incubated at 42°C microaerobically for 2 days. After incubation the broth was subcultured onto mCCDA agar and incubated microaerobically at 42°C for 2 days. Single colonies resembling *Campylobacter* species were subcultured to BA and incubated microaerobically at 42°C for 2 days before DNA preparations were made. Cultures were frozen at -80°C.

Presumptive *Campylobacter* spp. both in the wash and resuspended pellet were plated onto mCCDA using a Wasp Spiral Plater (Don Whitley Scientific, UK) for counting. Duplicate mCCDA plates were inoculated with 50μl (spiral plater) or 1ml (spread plate) aliquots of wash or 100μl (spiral plater) aliquots of resuspended wash pellet. The plates were incubated microaerobically at 42°C for 2 days. Colonies were counted manually or by using a plate reader (aCOLyte, Synbiosis, England).

3.1.3 Multilocus sequence typing

Multilocus sequence typing (MLST) of *C. jejuni* isolates was performed using seven house-keeping genes: *asp*A (aspartase A), *gln*A (glutamine synthase), *glt*A (citrate synthase), *gly*A (serine hydroxymethyltransferase), *pgm* (phosphoglucomutase), *tkt* (transketolase) and *unc*A (ATP synthase alpha subunit) based on the method outlined by Dingle et al., [1]. Alleles that did not give clear results were re-amplified and sequenced using primers sets published by Miller et al., (2005)[3]. Sequence data were collated by Dr Phil Carter at ESR, and alleles assigned using the Campylobacter PubMLST database (http://pubmlst.org/campylobacter).

3.1.4 Epidemiological data from human cases

Anonymised epidemiological human data were acquired from the national disease database (Episurv) by MidCentral Public Health Services (MCPHS), working with ESR Ltd. Specimen and isolate data (microbiological and molecular data) were linked to Episurv data via the unique Episurv and MedLab identification numbers (hospital ID number). Information gathered by MCPHS between January 1st and December 31st 2014 was acquired using both questionnaires and telephone interviews using the Episurv Case Report Form (CRF) format enhanced with additional questions relating to meat eaten and the consumption of unpasteurised milk during the case's incubation period.

3.2 Data analysis

3.2.1 Enumeration of *Campylobacter* on poultry carcases

Both the proportion of carcases that were positive, and the levels of *Campylobacter* present on positive carcases, were estimated using the technique described in Müllner et al [4]. The output from these models is presented as a series of graphs describing the probability of a carcase containing *Campylobacter*, by supplier and by quarter, and the estimated number of viable *Campylobacter* on positive carcases - again by supplier and quarter. This

method ensures that all the individual replicate counts for each sample are analysed appropriately.

3.2.2 Assigning sequence types and imputing missing alleles

We assign the sequence type by utilising the PubMLST database[1] to look up the allelic profile and note down the corresponding sequence type. This allows us to also note down whether the assigned sequence type is a *C. jejuni* or *C. coli* strain, allowing isolates to be speciated reliably.

We can further utilise the PubMLST database to assign STs to those isolates for which we have incomplete allelic profiles. We match on the alleles we do have for each isolate, and produce a list of potential STs. In the case where only one ST from PubMLST matches, we can impute the ST (and thus the unknown loci) allowing the use of those isolates for attribution purposes. In the case where more than one ST (or no STs) from PubMLST match, we remove the isolate from the dataset prior to performing attribution.

3.2.3 Annual source attribution estimates

Source attribution estimates for the 12 months between 1st January and the 31st December 2014 were calculated using the Assymetric Island model as described elsewhere [7, 2, 6]. This was repeated for each of the preceding 8 years. If more than one isolate was typed from a source sample, only unique STs were included in the analysis.

3.2.4 Dynamic source attribution modelling: Island model

The Island model [7] is a bayesian source attribution model, where each source is represented by an island. It is assumed that the sequences we observe have arisen through a process of mutation (where we observe a novel allele at a particular locus), recombination (where we observe a sequence that represents alleles from two previously observed sequences), and migration (where sequences may move between source islands). Probabilities are

[1] http://pubmlst.org/campylobacter

assigned to each of these processes on each island source, and the sampling distribution $\phi(y|k, Y)$ is derived, which gives the likelihood of observing sequence y from source k, given previously observed sequences Y.

Given this, we can estimate the probability that a particular human isolate h comes from source k using

$$p(h|k, Y) = \sum_k F_k \phi(h|k, Y) p(F_k),$$

where F_k represents the probability that a random human isolate comes from source k, ϕ is the sampling distribution described above, and $p(F_k)$ is the prior distribution on F_k, where we assume each source is equally likely.

This model may be extended to allow attribution to change through time by modelling the F_k probabilities through time. Let

$$F_{kt} = \begin{cases} \dfrac{e^{-f_{kt}}}{1 + \sum_{k=1}^{K-1} e^{-f_{kt}}} & k = 1 \dots K-1, \\ \dfrac{1}{1 + \sum_{k=1}^{K-1} e^{-f_{kt}}} & k = K. \end{cases}$$

where

$$\begin{aligned} f_{kt} &= X_t \beta_k + \epsilon_{kt}, \\ \epsilon_{kt} &\sim \text{Normal}(\rho_k \epsilon_{k(t-1)}, \sigma_k^2). \end{aligned}$$

Here X_t represents the design matrix for covariates in time, and β_k are the source-specific coefficients of those covariates.

Thus, f_{kt} is modelled using a time series model with autoregression, and we may add covariates in time to assess temporal effects. For now, we assume $X_t = 1$, thus modelling a constant mean attribution for each source, where the residuals ϵ_{kt} will soak up variation in time.

3.2.5 Modelling cases through time by rurality

As part of the epidemiological investigation for this report, we include a model of monthly urban and rural cases from 2005–2014. We model the number of cases Y_{ijt} per month t in urban ($i = 1$) and rural ($i = 2$) areas before ($j = 1$) and after ($j = 2$) the poultry intervention using

$$Y_{ijt} \sim \mathsf{Poisson}(\lambda_{ijt})$$
$$\log(\lambda_{ijt}) = \alpha_{ij} + \beta_{ij}t + s_{ij}(t)$$

where α and β describe the long-term trend through time, and s is a seasonal term. We can then statistically test for differences in baseline rates (α) and changes in rates through time (β) between urban and rural areas as well as assessing the effects (if any) of the poultry intervention on those rates. Further, we can assess whether the seasonality of cases ($s(t)$) differs between rural and urban areas or has changed following the poultry intervention.

4 Results

4.1 Human samples

4.1.1 Human sample information

A total of 239 samples were submitted to the mEpiLab in this period, of which 204 were considered to be primary samples (residing within the Manawatu region, and the first sample from an individual case acquired through the routine surveillance system). Of the 204 primary samples, 157 were successfully cultured and 153 samples were sequence typed (one was typed directly from the swab without culturing), yielding 153 full MLST allelic profiles. Of these, 148 were *C. jejuni* that could be linked to EpiSurv data from 148 cases, the remainder were either *C. coli* (N=2) or could not be linked to EpiSurv data (N=3). Note that the number of EpiSurv reports in Table 1 are obtained from the Monthly Notified Disease Surveillance Reports published by ESR[2], and that these differ from the number of EpiSurv data available to be linked to primary samples. This discrepancy is under investigation.

As highlighted in the previous interim report, the proportion of submitted samples that were confirmed as positive for *Campylobacter* spp. by culture and PCR was lower than in previous years, most notably in the months of April and May. This is a pattern that has occurred over previous years as well, as shown in the Tables 2 and 3. It is unclear at this stage why this pattern is occurring.

[2] https://surv.esr.cri.nz/surveillance/monthly_surveillance.php

2014					
Month	No. samples	Growth	%positive	EpiSurv reports	%coverage
January	16	15	93.8	22	72.7
February	18	15	83.3	25	72.0
March	21	16	76.2	26	80.8
April	4	3	75.0	11	36.4
May	15	5	33.3	27	55.6
June	17	9	52.9	18	94.4
July	20	13	65.0	20	100.0
August	21	18	85.7	28	75.0
September	14	13	92.9	15	93.3
October	16	14	87.5	22	72.7
November	23	20	87.0	27	85.2
December	19	16	84.2	21	90.5
Total	204	157	77.0	262	77.9

Table 1: Details of *Campylobacter* spp. ELISA positive human samples submitted by MedLab Central in 2014. The number of EpiSurv notifications for the same period from MidCentral DHB are also provided, in addition to the proportion of samples that grew presumptive *Campylobacter* spp. colonies, and the coverage of samples per EpiSurv report.

2013					
Month	No. samples	Growth	%positive	EpiSurv reports	%coverage
January	24	16	66.7	28	85.7
February	8	4	50.0	13	61.5
March	10	8	80.0	14	71.4
April	4	1	25.0	10	40.0
May	23	4	17.4	29	79.3
June	12	9	75.0	17	70.6
July	26	16	61.5	31	83.9
August	16	11	68.8	19	84.2
September	26	17	65.4	27	96.3
October	17	14	82.4	25	68.0
November	29	23	79.3	27	107.4
December	35	29	82.9	49	71.4
Total	230	152	66.1	289	79.6

Table 2: Details of *Campylobacter* spp. ELISA positive human samples submitted by MedLab Central in 2013. The number of EpiSurv notifications for the same period from MidCentral DHB are also provided, in addition to the proportion of samples that grew presumptive *Campylobacter* spp. colonies, and the coverage of samples per EpiSurv report.

	2012				
Month	No. samples	Growth	%positive	EpiSurv reports	%coverage
January	15	13	86.7	23	65.2
February	18	15	83.3	19	94.7
March	12	11	91.7	13	92.3
April	5	3	60.0	12	41.7
May	7	7	100.0	11	63.6
June	8	8	100.0	9	88.9
July	6	6	100.0	8	75.0
August	10	9	90.0	14	71.4
September	17	16	94.1	22	77.3
October	20	17	85.0	26	76.9
November	16	12	75.0	21	76.2
December	18	11	61.1	18	100.0
Total	152	128	84.2	196	77.6

Table 3: Details of *Campylobacter* spp. ELISA positive human samples submitted by MedLab Central in 2012. The number of EpiSurv notifications for the same period from MidCentral DHB are also provided, in addition to the proportion of samples that grew presumptive *Campylobacter* spp. colonies, and the coverage of samples per EpiSurv report.

4.1.2 Distribution of MLST genotypes of human cases

The proportion of human cases with each ST in 2014 is compared with previous years in Table 4. The previously dominant ST-474 accounted for just 2.6% of cases in 2014. This year the most common ST was ST-50 accounting for 10.5% of cases, with ST-45 and ST-53 each accounting for 7.8% of cases also being high as in the previous two years. A total of 45 different STs were isolated from human cases in 2014.

ST	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
21	0.9	1.6	1.1	2.5	1.0	0.9	4.7		4.0	1.3
25		0.5					0.9	1.1		0.7
38	2.6	2.1	3.3	4.9	2.1			1.1	1.6	1.3
42	3.5	3.2	4.4	4.9	4.2	3.4	5.7	6.5	4.8	5.2
45	7.8	8.5	12.7	7.4	11.5	12.9	14.2	18.3	15.2	7.8
48	7.0	12.8	6.6	3.7	6.2	5.2	7.5	8.6	4.8	0.7
50	4.3	2.7	9.4	8.6	7.3	15.5	9.4	9.7	6.4	10.5
51	0.9				1.0		3.8			2.6
53	11.3	4.8	4.4	16.0	5.2	9.5	8.5	9.7	8.8	7.8
61	4.3	2.7	3.3	6.2	5.2	6.9	5.7	7.5	6.4	7.2
190	9.6	4.8	1.7	8.6	1.0	2.6	4.7	7.5	8.0	0.7
257	4.3	1.6	2.2		2.1	5.2	0.9	2.2	3.2	3.3
320										0.7
354	1.7	3.7	8.3	4.9					1.6	2.6
422			1.7	1.2		3.4	0.9	1.1	1.6	3.3
436	0.9	0.5	1.1	6.2	1.0	2.6	1.9	2.2	1.6	2.0
474	29.6	39.9	27.1	19.8	32.3	12.9	6.6	4.3	3.2	2.6
508										3.9
520	3.5	1.6	1.1		1.0	3.4	9.4	3.2	1.6	3.9
538						0.9	0.9	1.1	2.4	1.3
583	0.9	1.1	3.9		6.2	4.3	2.8	1.1	6.4	1.3
677	0.9	0.5	2.2		1.0	1.7		1.1	4.0	2.0
704										0.7
991										0.7
1457	0.9									0.7
1517	0.9	1.1	0.6		1.0	0.9	1.9	1.1	2.4	2.0
1919										0.7
2026	2.6	2.1	1.7	2.5	3.1	0.9	2.8	1.1	2.4	2.6
2076										0.7
2343	0.9					0.9				1.3
2345	0.9	1.6			1.0		0.9		4.8	5.9
2350		1.1			1.0		0.9			1.3
3072		0.5	1.1	1.2		0.9		1.1		0.7
3676		0.5	1.1	1.2	2.1	0.9		2.2	0.8	1.3
3711			1.1		1.0	4.3	1.9	1.1	0.8	0.7
3717		0.5						1.1		0.7
3798							0.9		0.8	1.3
4009										0.7
6964										2.0
NEW					2.1		1.9	6.5	2.4	3.9
No. samples	115	188	181	81	96	116	106	93	125	153

Table 4: The distribution of *C.jejuni* and *C.coli* multilocus sequence types in human cases in 2014 compared with the distribution of the same STs in human cases in the preceding years.

4.2 Poultry samples

4.2.1 Poultry sample information

As planned, 6 poultry samples were taken per month (N=72), of which 61 (84.7%) were *Campylobacter* positive. Of the 61, 78.7% (48) were confirmed as containing *C. jejuni*, 7 of which were mixes of two STs, and 21.3% (13) were confirmed as *C. coli*. Of these isolates, three were mixes containing both *C. coli* and *C. jejuni* STs. All suppliers yielded positive samples. The proportion of carcases positive for *Campylobacter* was 95% for supplier A, 80% for supplier B, and 81.5% for supplier C. Suppliers A and C had higher prevalence than the previous period (January–December 2013), while supplier B had lower prevalence.

Company	Positive	Total	C.jejuni	C.coli	%positive	%2013
Supplier A	19	20	17	2	95.0	86.4
Supplier B	20	25	13	7	80.0	96.3
Supplier C	22	27	18	4	81.5	56.5
Total	61	72	48	13	84.7	80.6

Table 5: The number of samples positive for presumptive *Campylobacter*, *C. jejuni*, and *C. coli* from each poultry supplier, and the percentage positive in 2014 compared to 2013.

4.2.2 MLST genotypes of poultry isolates

A total of 107 isolates from poultry were successfully MLST typed from 58 of the 61 positive samples, with the most prevalent ST being ST-45 (found in suppliers B and C this year, and normally found in all suppliers), followed by ST-3105 (found only in supplier A in 2014, but has been isolated from the other two suppliers previously) and ST-1581 (A *C. coli* type found in all suppliers). ST-2345 was found again this year only in supplier A, as it has been the last 3 years, which is a change from 2005–2008, where this ST was mainly recovered from supplier B.

With the third most prevalent type being a *C. coli* strain, 2014 is the year with the second highest prevalance (18.1%) of *C. coli*, behind 2013 (25%). These are considerably higher than previous years, with the next highest

	2012			**2013**			**2014**		
ST	A	B	C	A	B	C	A	B	C
21			42.9						
45	12.5	37.5	14.3	11.5	5.7	16.7		9.5	38.5
48		6.2			22.9			19.0	
50	33.3	6.2			8.6			14.3	
53	8.3		7.1		2.9				
61	4.2								
190			7.1			8.3			
227						8.3			
257				7.7			4.5		3.8
354				3.8			22.7		3.8
356							4.5		
486		31.2							
520	12.5				2.9	25.0			
535		6.2		15.4			4.5		
583	16.7	12.5		15.4	2.9		13.6		7.7
585									3.8
825						8.3			
854					2.9	8.3			
1033	4.2		7.1						
1581					17.1		9.1	19.0	3.8
1590					14.3			9.5	
1900					5.7				
2256						8.3			
2345	4.2			23.1			4.5		
2350					2.9				
2584			7.1						
3105	4.2		14.3	3.8	2.9		36.4		
3230									11.5
3792								4.8	
4009				7.7		8.3		4.8	
4337					5.7				
6964								19.0	15.4
NEW				11.5	2.9	8.3			11.5
No. samples	24	16	14	26	35	12	22	21	26

Table 6: The distribution of *C.jejuni* and *C.coli* multilocus sequence types in poultry in 2014 compared with the distribution of the same STs in poultry in the preceding two years (2012, 2013). No. samples refers to the total number of samples examined in each year. Note that the previous reporting period for 2012 covered October 2010 through December 2012, so percentages for this year will differ from the April 2012 report. Furthermore, some duplicate isolates (multiple isolates from the same sample) were excluded in this analyses that were included in previous reports.

years being 2006 and 2007 (Table 7).

Year	Total	C.jejuni	%jejuni	C.coli	%coli
2005	202	127	62.9	7	3.5
2006	138	62	44.9	9	6.5
2007	186	93	50.0	17	9.1
2008	216	92	42.6	6	2.8
2009	123	41	33.3	0	0.0
2010	72	40	55.6	3	4.2
2011	72	59	81.9	3	4.2
2012	72	42	58.3	0	0.0
2013	72	43	59.7	18	25.0
2014	72	48	66.7	13	18.1
Total	1225	647	52.8	76	6.2

Table 7: The number and prevalance of *C. jejuni* and *C.coli* over the years 2005–2014. Totals are the number of poultry carcass samples. A carcass is a positive if one or more isolates from the carcass has been sequence typed as *C.jejuni* or *C.coli*.

Of interest is the emergence of a new type ST-6964 in suppliers B and C, which has not been observed previously. This sequence type was also found in three human cases in 2014, as well as in chicken pieces from supplier A (as part of a separate study). Table 8 lists the isolates by date and source.

Month	Source	Isolate
May 2014	Supplier C	P1416
May 2014	Supplier C	P1417
Jun 2014	Supplier C	P1426
Jul 2014	Supplier B	P1433
Jul 2014	Supplier A	P1439
Aug 2014	Supplier C	P1451
Aug 2014	Human	H2042
Aug 2014	Human	H2029
Oct 2014	Supplier B	P1466
Oct 2014	Supplier B	P1467
Oct 2014	Supplier C	P1471
Nov 2014	Supplier C	P1472
Nov 2014	Human	H2090
Dec 2014	Supplier B	P1483

Table 8: Isolates typed as ST-6964 by source and month. Note that not all poultry samples are from poultry carcases, with some being collected as part of a separate PhD project *Molecular epidemiological studies of human campylobacteriosis in New Zealand between 2005 and 2014*.

4.2.3 Enumeration of *Campylobacter* spp. on poultry carcases

The spiral and spread plating of carcase rinsates were used to update the estimates of proportion of carcases positive from each supplier (Figure 1) and the estimated number of *Campylobacter* spp. given the carcase is positive (Figure 2). While there is evidence of a marked reduction in counts in supplier A since the fourth quarter of 2010, there is no evidence to suggest that either supplier B or C are markedly reduced compared with the pre-intervention period, though it should be noted that the levels on these suppliers were lower on average than supplier A. In particular, although the credible intervals are wide, the levels of contamination in carcases from supplier B were higher in the second half of 2013 (Figure 2), but have reduced again in 2014.

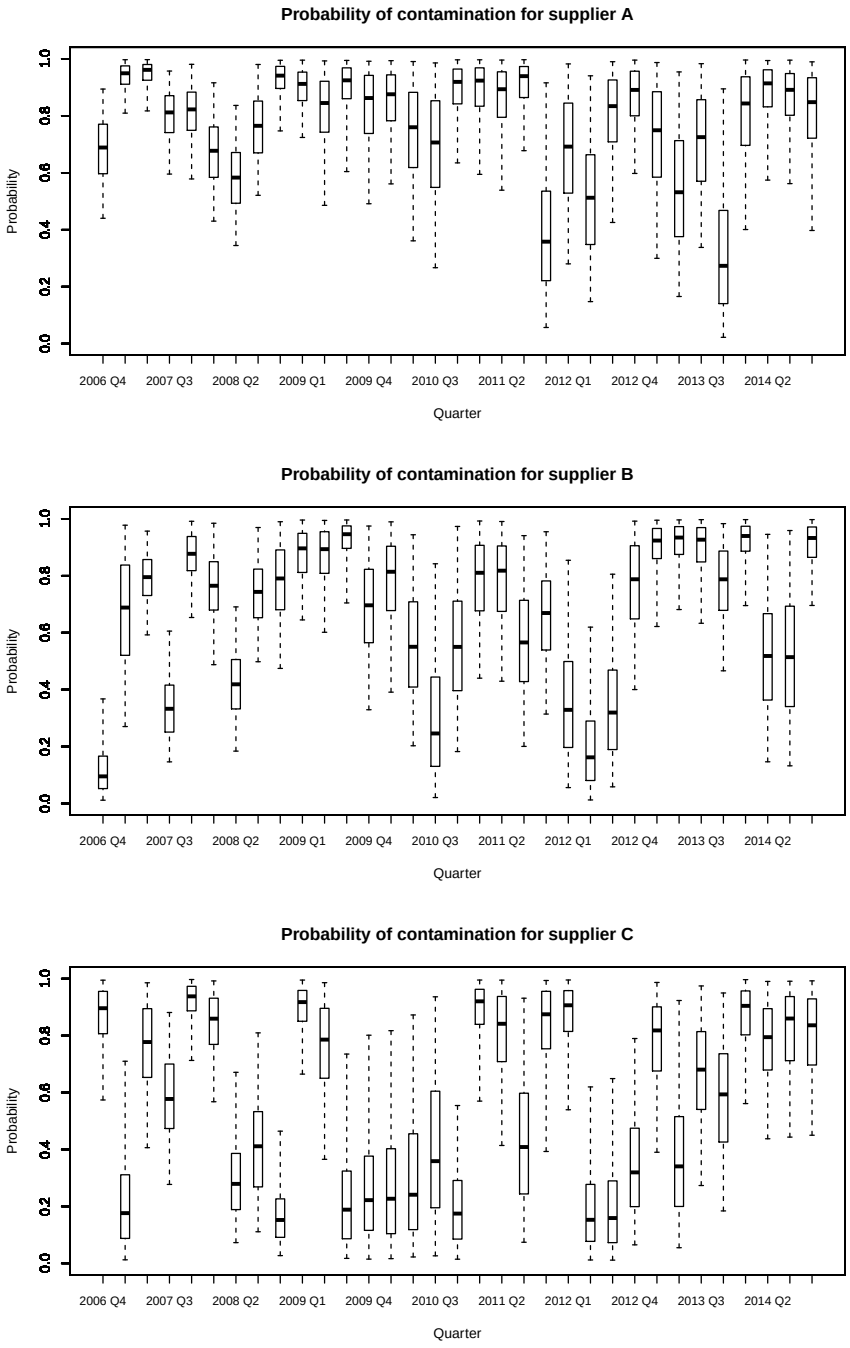

Figure 1: *Campylobacter* on chicken carcases by quarter: probability of contamination for each supplier, showing the median (thick horizontal line), interquartile range (box) and 95% (credible) intervals (dashed lines) of the posterior distribution.

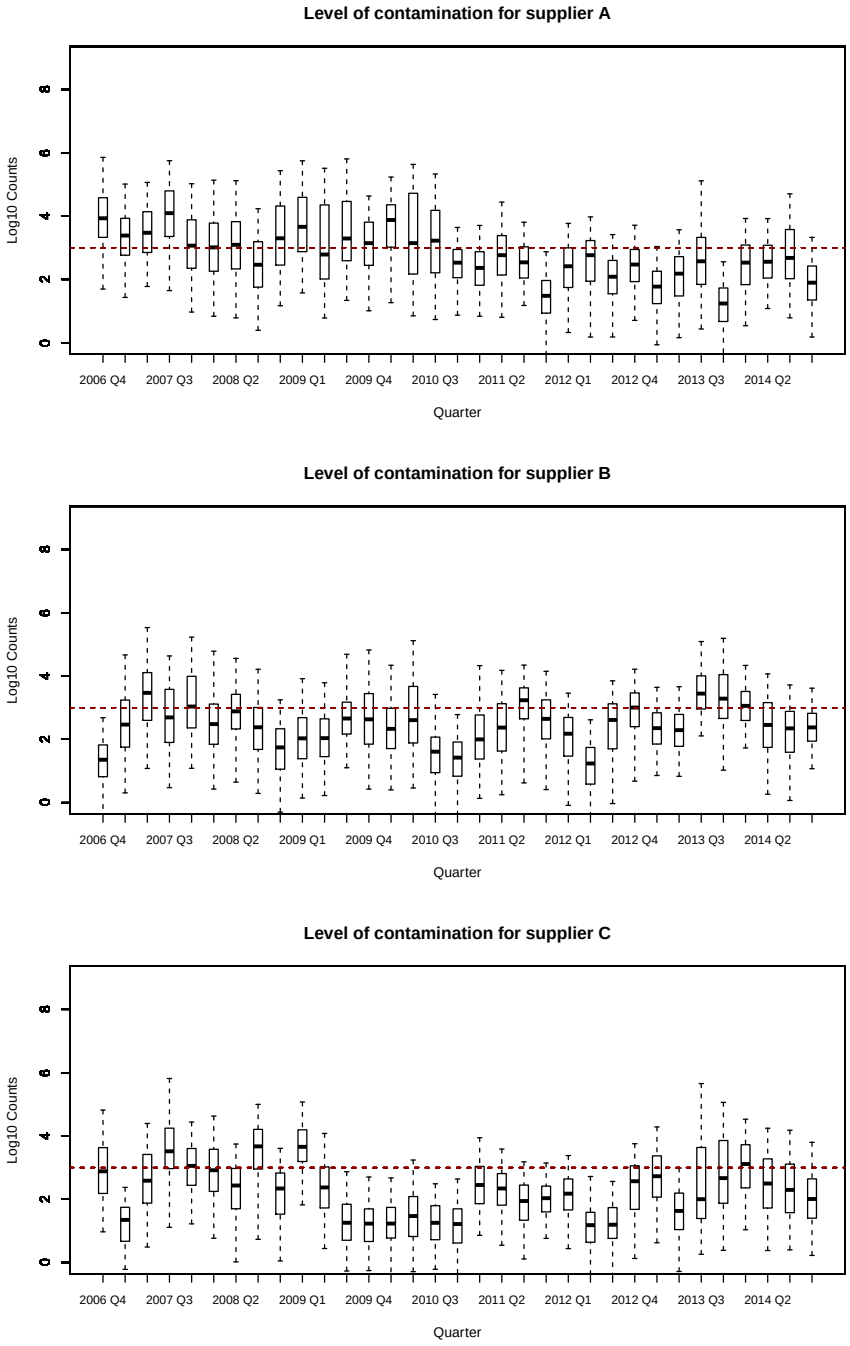

Figure 2: *Campylobacter* on chicken carcases by quarter: estimated level of contamination on positive carcases for each supplier, showing the median (thick horizontal line), interquartile range (box) and 95% (credible) intervals (dashed lines) of the posterior distribution. The dashed horizontal line marks 1000 cfu in the carcase rinsate.

4.2.4 Packaging of poultry carcases

The proportion of carcases from each poultry supplier with leaking wrappers was examined for the the whole 10 year period (1225 carcases). Differences between suppliers was observed, with supplier C generally having a higher proportion of leaking wrappers than the other two suppliers. In general, however, it can be seen that all suppliers have improved in recent years (Figure 3).

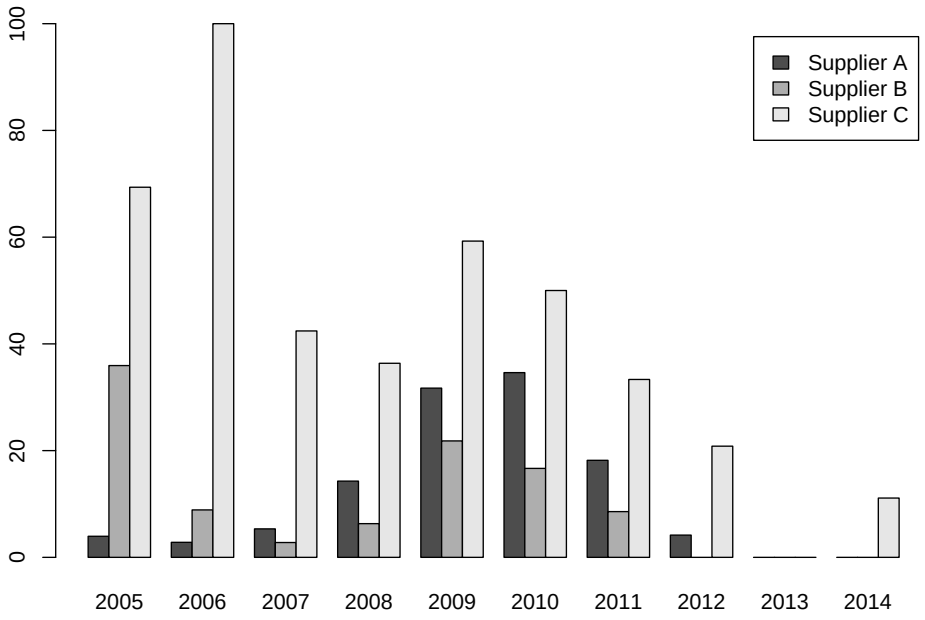

Figure 3: The percentage of chicken carcases with leaking packaging from each supplier from 2005–2014, N=1225.

4.3 Samples available for attribution

There are a total of 3410 isolates with complete *C. jejuni*or *C. coli* allelic profiles available for source attribution, with an additional 70 isolates with partial profiles that could be uniquely assigned to a sequence type from PubMLST, giving 3480 total isolates available for attribution across the period 2005–2014. Source-specific totals are given in Table 9.

	Complete	Imputed	Total
Human	1391	9	1400
Supplier A	329	5	334
Supplier B	328	3	331
Supplier C	222	3	225
Duck	49	1	50
Turkey	20	4	24
Spent Hen	26	1	27
Cattle	283	10	293
Sheep	243	11	254
Dog/Cat	33	1	34
Wild Water Bird	143	12	155
Other Wild Bird	74	3	77
Water	269	7	276
Total	3410	70	3480

Table 9: Number of complete and imputed *C. jejuni* isolates available for attribution for the years 2005–2014. Imputed isolates are those that have partial allelic profiles that match a unique sequence type in the PubMLST database.

4.4 Source attribution estimates for human cases in 2014 compared to previous years

4.4.1 Source attribution by year

Figure 4 shows the source attribution estimates for the pre-intervention period 1st July 2005 to 30th June 2006 compared to the most recent period between 1st January and 31st December 2014. The marked decline in the proportion of cases attributable to poultry continues to be strongly evident. The increase in relative contribution from ruminants, particularly cattle, is also evident.

Figures 5 and 6 summarise all sources over the ten years (N.B. There were fewer samples available for the first 4 months of 2005). The attribution estimates for the combined sources of all chicken (suppliers A through C), all ruminants (cattle and sheep) and other sources are presented in Figure 6, with the same information presented as a stacked bar plot in Figure 18 in the appendix). The complete table of estimates, including credible intervals is shown in Table 10.

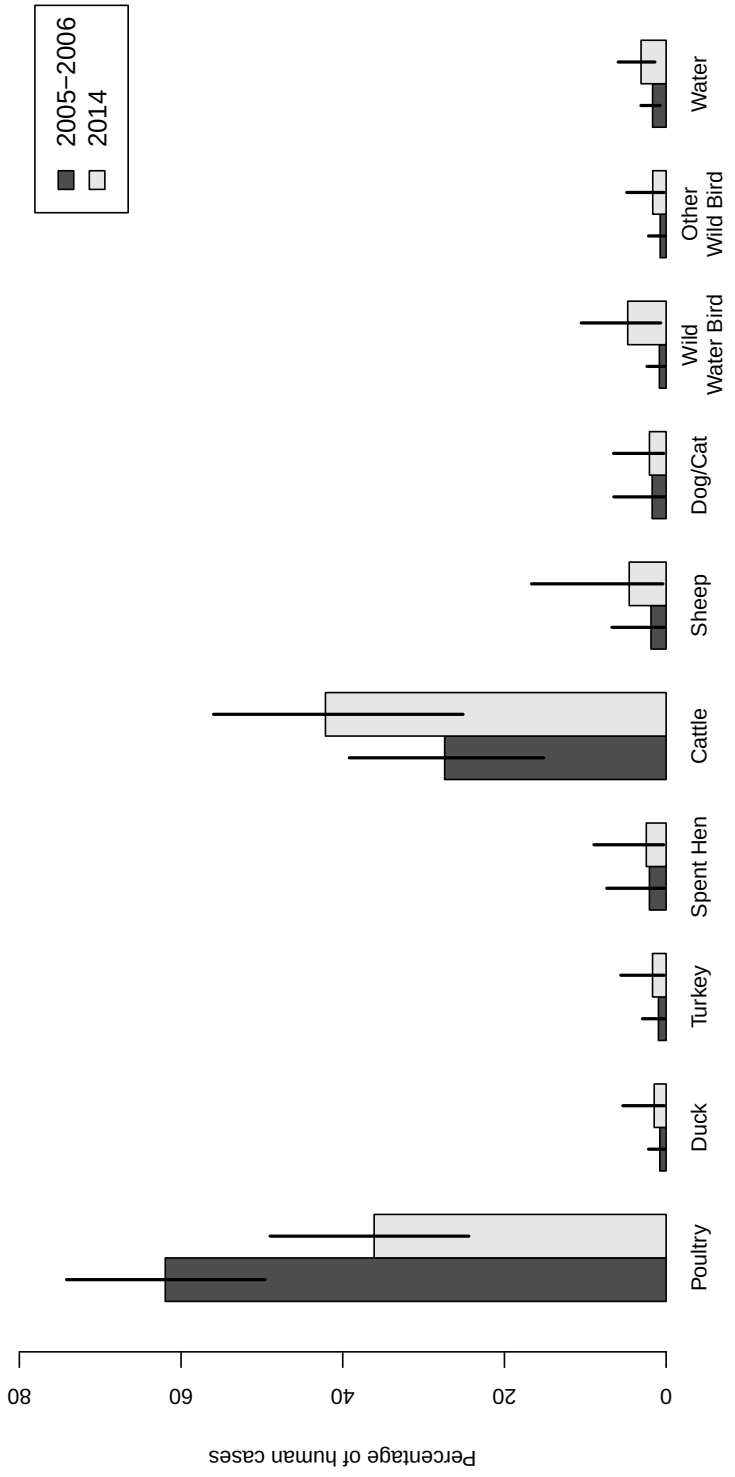

Figure 4: Source attribution for human cases in the Manawatu for cases reported between July 1st 2005 and June 30th 2006 compared to cases reported between January 1st and December 31st 2014. Error bars represent 95% confidence or credible intervals

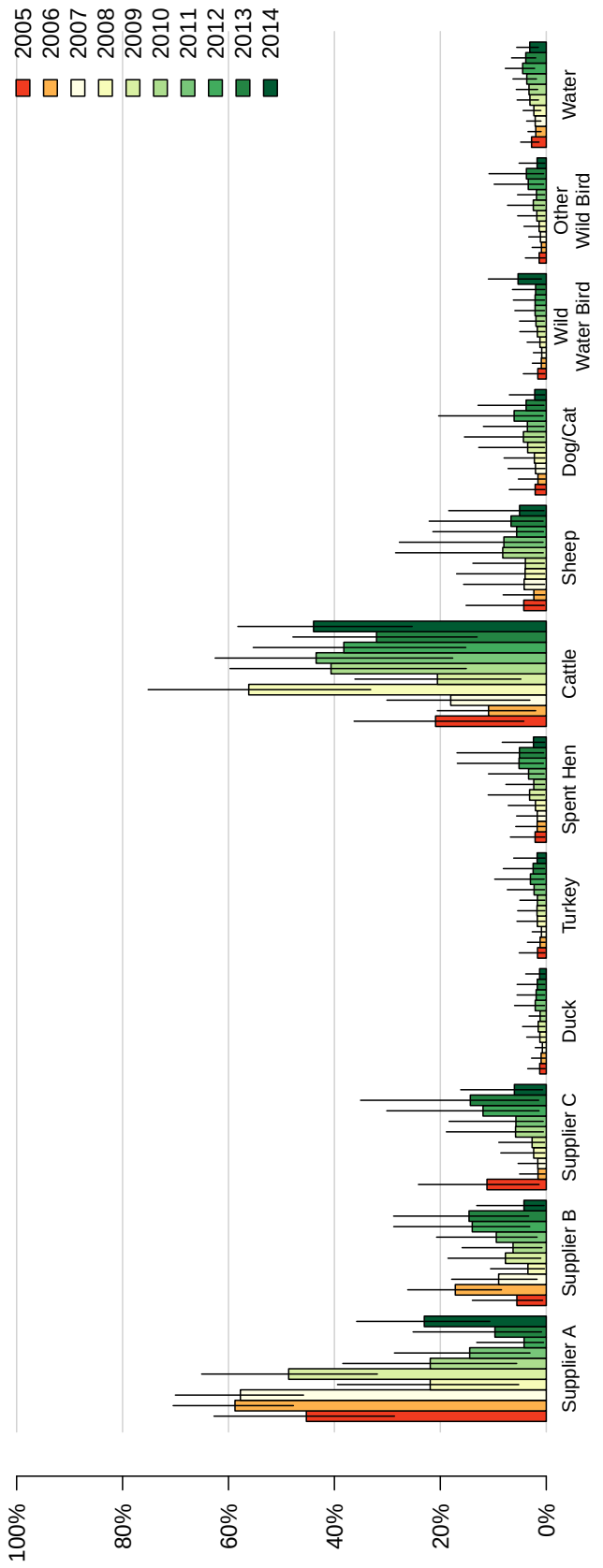

Figure 5: Source attribution estimates for human cases in the Manawatu between 2005 and 2014. Years 2005 and 2006 represent the pre-intervention period.

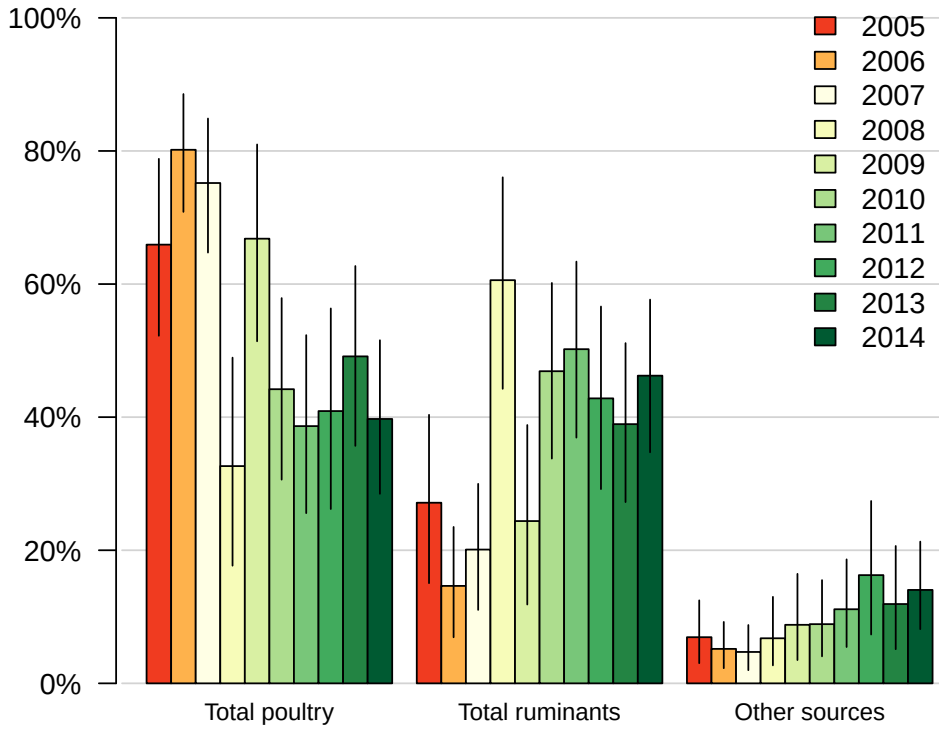

Figure 6: Poultry, ruminant and other source attribution estimates for human cases in the Manawatu between 2005 and 2014. Years 2005 and 2006 represent the pre-intervention period.

	2005	**2006**	**2007**	**2008**	**2009**
Supplier A	45.3 (28.7, 62.7)	58.8 (47.7, 70.4)	57.8 (45.8, 70.0)	21.9 (5.2, 39.4)	48.6 (31.9, 65.1)
Supplier B	5.6 (0.7, 14.0)	17.2 (8.5, 26.2)	9.0 (1.7, 17.9)	3.5 (0.3, 10.5)	7.7 (1.0, 18.6)
Supplier C	11.2 (1.4, 24.1)	1.6 (0.2, 5.0)	1.6 (0.2, 5.3)	2.4 (0.2, 8.6)	2.7 (0.3, 9.0)
Duck	1.2 (0.2, 3.5)	1.0 (0.2, 2.8)	0.7 (0.1, 2.1)	1.2 (0.2, 3.7)	1.5 (0.2, 4.5)
Turkey	1.6 (0.2, 5.1)	1.2 (0.2, 3.6)	0.9 (0.1, 2.7)	1.7 (0.2, 5.5)	1.7 (0.2, 5.4)
Spent Hen	2.1 (0.3, 6.8)	1.7 (0.2, 5.8)	1.7 (0.2, 5.6)	2.0 (0.2, 7.2)	3.2 (0.3, 11.0)
Cattle	20.9 (4.2, 36.3)	10.9 (2.0, 20.6)	18.0 (3.1, 30.1)	56.2 (33.1, 75.2)	20.5 (4.8, 36.1)
Sheep	4.2 (0.4, 15.1)	2.3 (0.2, 8.1)	4.2 (0.3, 15.6)	4.0 (0.3, 16.9)	3.9 (0.4, 13.9)
Dog/Cat	2.1 (0.3, 7.0)	1.6 (0.2, 5.3)	2.0 (0.2, 7.2)	2.2 (0.2, 8.0)	3.5 (0.4, 12.8)
Wild Water Bird	1.6 (0.3, 4.3)	0.9 (0.2, 2.6)	0.9 (0.1, 2.4)	1.2 (0.2, 3.6)	1.7 (0.3, 5.0)
Other Wild Bird	1.3 (0.2, 3.9)	0.9 (0.1, 2.7)	1.1 (0.2, 3.3)	1.4 (0.2, 4.2)	1.8 (0.3, 5.4)
Water	2.8 (1.4, 4.8)	2.0 (1.0, 3.5)	2.1 (1.0, 3.7)	2.3 (1.0, 4.4)	3.1 (1.5, 5.5)
	2010	**2011**	**2012**	**2013**	**2014**
Supplier A	21.9 (5.6, 38.4)	14.5 (3.1, 28.7)	4.2 (0.5, 13.1)	9.7 (0.9, 25.2)	23.0 (10.7, 35.8)
Supplier B	6.3 (0.8, 15.9)	9.4 (1.7, 20.7)	14.0 (3.1, 28.8)	14.6 (3.3, 28.8)	4.2 (0.4, 13.1)
Supplier C	5.8 (0.5, 18.9)	5.7 (0.6, 18.3)	11.9 (1.4, 30.1)	14.3 (1.4, 35.1)	6.0 (0.7, 16.2)
Duck	1.2 (0.2, 3.2)	2.1 (0.3, 6.0)	1.9 (0.3, 5.5)	1.7 (0.3, 5.5)	1.3 (0.2, 3.9)
Turkey	1.7 (0.3, 5.0)	2.3 (0.3, 7.4)	3.0 (0.4, 9.7)	2.5 (0.3, 8.1)	1.7 (0.2, 6.1)
Spent Hen	2.4 (0.3, 7.6)	3.3 (0.4, 10.9)	5.1 (0.5, 16.8)	5.1 (0.5, 16.9)	2.4 (0.3, 8.3)
Cattle	40.6 (15.1, 59.7)	43.5 (17.7, 62.5)	38.2 (15.2, 55.3)	32.1 (13.1, 47.8)	43.9 (25.3, 58.2)
Sheep	8.2 (0.6, 28.5)	8.0 (0.6, 27.7)	5.6 (0.5, 21.4)	6.7 (0.6, 22.1)	5.0 (0.4, 18.4)
Dog/Cat	4.3 (0.4, 15.5)	3.6 (0.4, 11.9)	6.1 (0.6, 20.3)	3.8 (0.4, 12.9)	2.2 (0.3, 7.0)
Wild Water Bird	1.9 (0.4, 5.0)	2.1 (0.3, 5.9)	2.1 (0.3, 6.2)	2.0 (0.3, 6.4)	5.3 (0.9, 10.9)
Other Wild Bird	2.4 (0.3, 7.3)	1.8 (0.3, 5.4)	3.4 (0.5, 9.8)	3.7 (0.5, 10.8)	1.7 (0.3, 5.1)
Water	3.3 (1.6, 5.7)	3.7 (1.8, 6.3)	4.5 (2.2, 7.7)	3.9 (2.0, 6.5)	3.1 (1.5, 5.6)

Table 10: The estimated percentage of human cases of campylobacteriosis in the Manawatu attributable to each source for each year from 2005–2014. Means and 95% credible intervals were estimated using the Island model, fitted for each year individually. All available source data from the Manawatu were used to fit the models. Supplier A, B and C are the poultry suppliers.

4.5 Dynamic modelling

4.5.1 Dynamic Island model

Figure 7 shows the output from the dynamic Island model, displaying the attribution to poultry, ruminant, water, and other sources over the ten year period. The recent increase in cases at the ends of 2013 and 2014 are largely attributed to poultry. It is also worth noting that the number of *Campylobacter* on carcases, particularly in Supplier B, increased in the latter half of 2013 (Figure 2), though this was not seen in 2014. Figure 8 shows the updated number of cases attributed to poultry and ruminants including 95% credible envelopes.

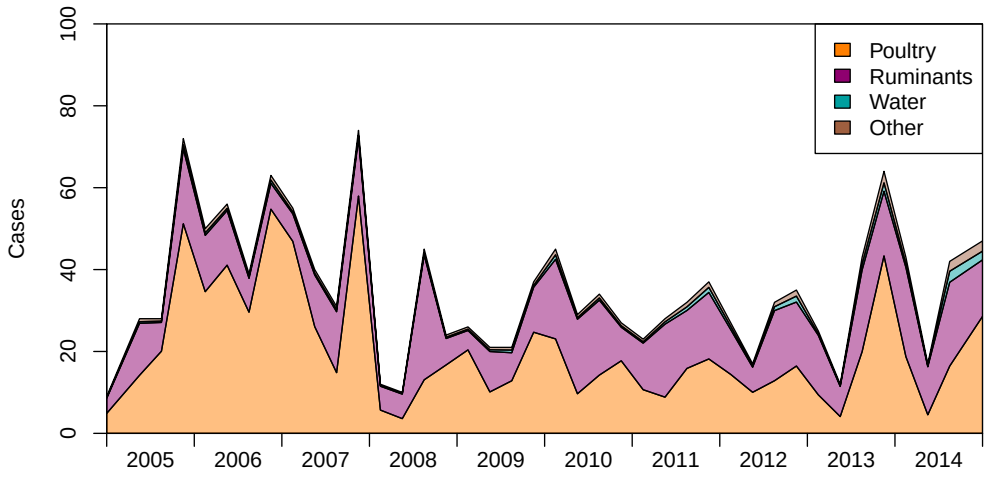

Figure 7: Estimated number of human cases per month attributed to each source by the dynamic Island model using three-monthly intervals from 1st March 2005 to 31st December 2014. Colours indicate the source the cases are attributed to: poultry (orange), ruminants (purple), water/environmental (blue) and other (brown).

4.5.2 Reservoir attribution by rural/urban status

The reservoir attribution estimates were stratified by rurality and show evidence that ruminants are the most important reservoir for cases residing

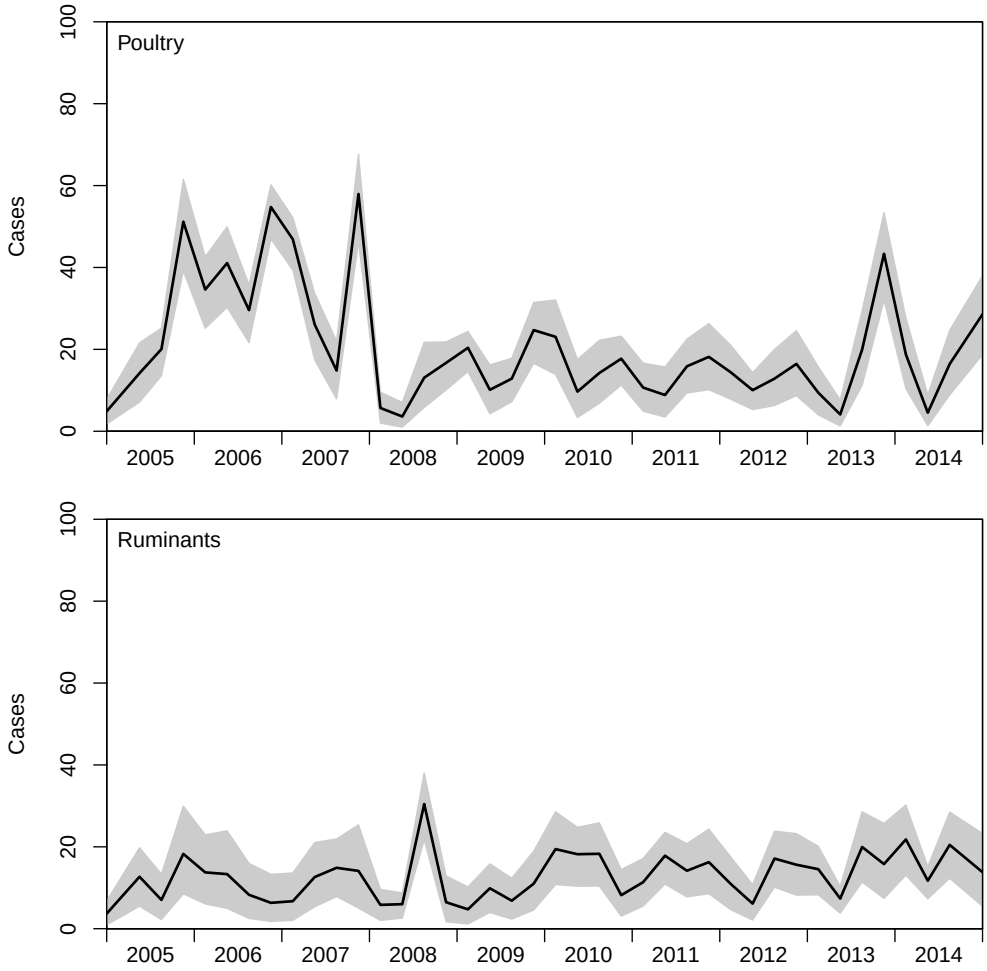

Figure 8: Estimated number of human cases per month attributed to poultry and ruminants by the dynamic Island model with 95% credible intervals using three-monthly intervals from 2005 – 2014.

in rural areas, whereas poultry are the most important reservoir for cases residing in urban areas (Figure 9).

Further, we see clear evidence that the intervention in the poultry industry in 2007/2008 had very little effect on rural cases, whereas it had quite a large effect on urban cases, with lower total cases and a higher proportion of ruminant associated cases.

Interestingly, the attribution of rural and urban cases in 2014 is very similar in terms of the proportions attributed to poultry and ruminants, as seen in Figure 10, with the main difference being the larger credible intervals in

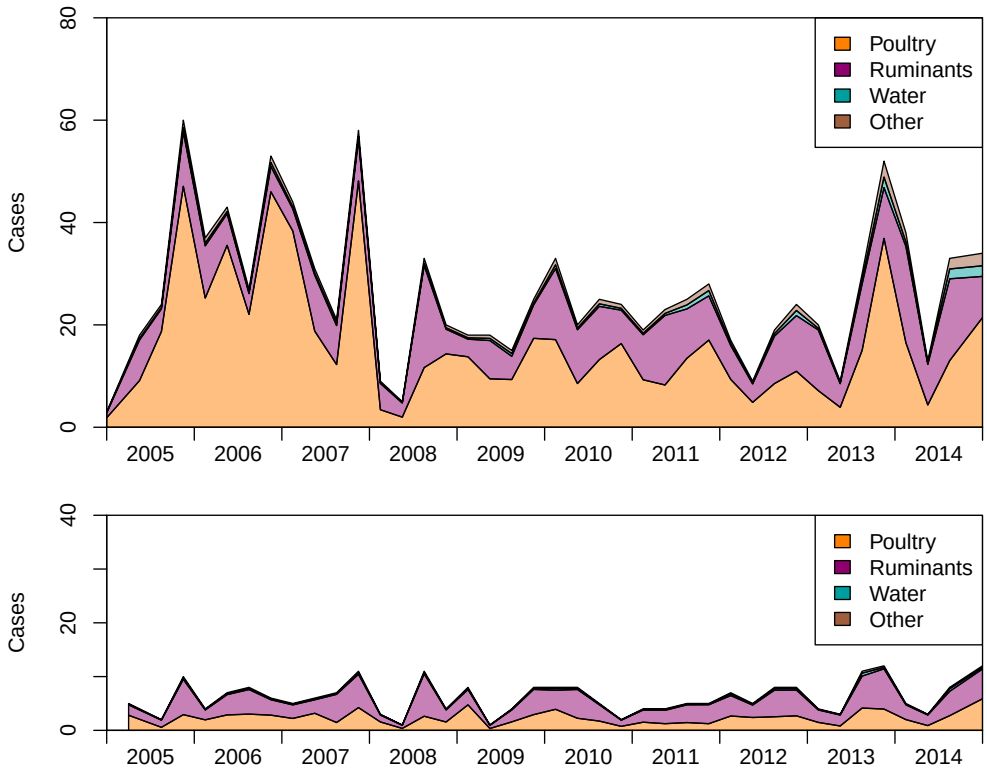

Figure 9: Urban and rural cases 2005–2014: Estimated number of human cases attributed to each reservoir determined by the Island model.

the rural estimates due to fewer cases (26 rural versus 112 urban). This is somewhat different to what was seen in 2013, where rural cases were more likely to be associated with ruminant sources, while urban cases were more likely to be associated with poultry sources. This was largely attributed to the large number of poultry-associated cases at the end of 2013, as seen in Figure 9.

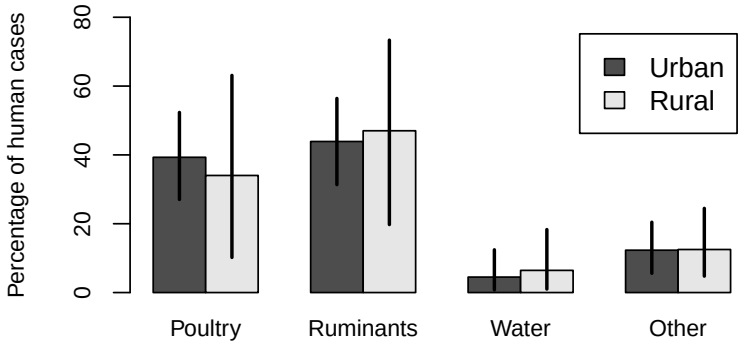

Figure 10: Urban and Rural cases 2014: Estimated proportion of human cases attributed to each reservoir determined by the Island model.

4.6 Epidemiological data

The relationships between campylobacteriosis notifications and the variables: rurality, age, gender, the effect of the intervention in the poultry industry, and time of year were examined in this report.

4.6.1 Urban-rural status, and time of year

A total of 1866 cases had urban-rural classification information available from 2005–2014, which were divided into urban dwellers (living in main, independent and satellite urban areas, or rural areas with high urban influence) and rural dwellers (living in remote rural areas or rural areas with moderate and low urban influence). The number of rural cases does not vary as much as the number of urban cases per year (Table 11).

Year	Urban	Rural	Total
2005	154	25	179
2006	224	35	259
2007	214	42	256
2008	110	27	137
2009	124	32	156
2010	129	33	162
2011	133	30	163
2012	94	34	128
2013	183	53	236
2014	157	33	190
Total	1522	344	1866

Table 11: Number of cases by urban-rural categorisation from 2005–2014. Note that there were fewer samples available for the first 4 months of 2005.

The number of cases through time in each category is presented in Figure 11. We see that there is some evidence of a decrease in urban cases from 2008 onwards, but that the number of cases appears to be increasing in later years.

We note that the increase is mostly found in 2013 and 2014, and that this appears to be Manawatu-specific, in that the same trend is not found in the National case data, seen in Figure 12.

The trends for the Manawatu as fitted by the generalised linear model out-

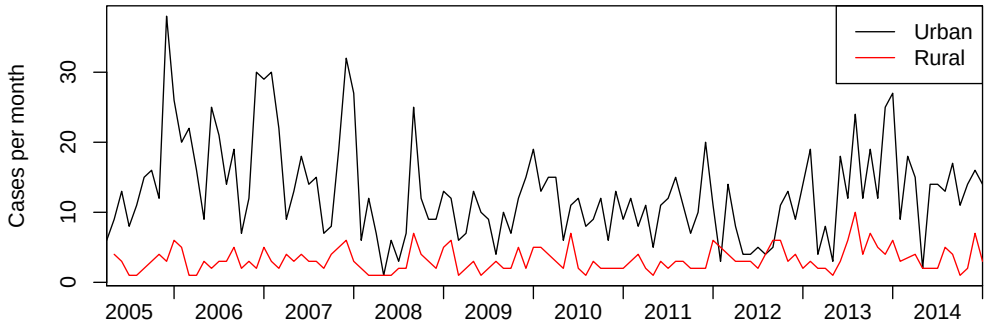

Figure 11: The number of cases per month in urban and rural areas for 2005–2014.

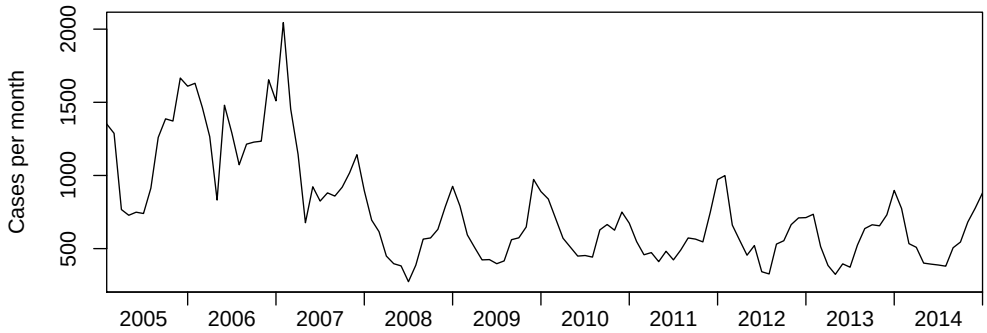

Figure 12: The number of cases per month for all of New Zealand.

lined in section 3.2.5 are summarised in Figure 13. Notice that both before and after the poultry intervention, the number of both urban and rural cases are increasing at approximately the same rate of 7% per annum.

This is confirmed by the model, where we see no evidence of a difference in the slope of the trend through time between urban and rural areas, either pre-intervention ($P = 0.93$) or post intervention ($P = 0.63$). Further, there was no evidence of a difference in the slope of the trend through time pre- and post-intervention across urban and rural cases combined ($P = 0.96$). There were, however, significant differences between the baseline of urban and rural cases pre-intervention ($P < 0.0001$) and post-intervention ($P = 0.0001$), which can be seen clearly in Figure 13.

The effect of the poultry intervention is a marked reduction in urban cases

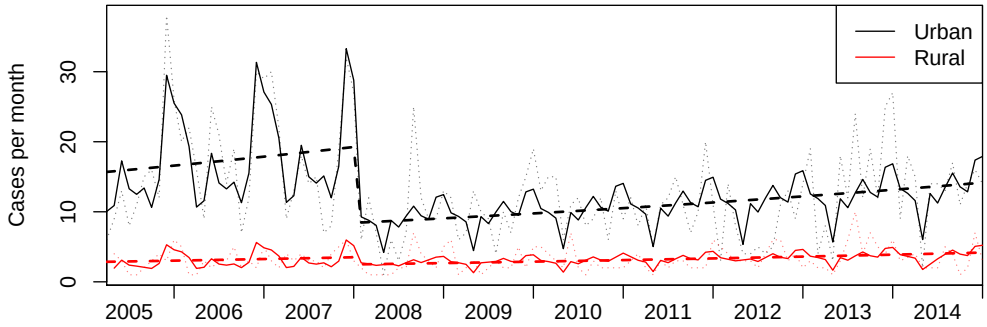

Figure 13: The number of cases per month in urban and rural areas for 2005–2014 (dotted) with fitted trends (dashed) incorporating seasonality (solid).

($P < 0.0001$), but is less marked ($P = 0.02$) in rural cases.

There was no evidence of a difference in seasonality between urban and rural areas either pre-intervention ($P = 0.27$) or post-intervention ($P = 0.85$). However, there was evidence of a common difference in seasonality across both urban and rural cases following the poultry intervention ($P = 0.0012$). This is most easily seen in Figure 14 where we see that following the poultry intervention, the proportion of cases in November and December are lower, with comparatively more cases in August and September.

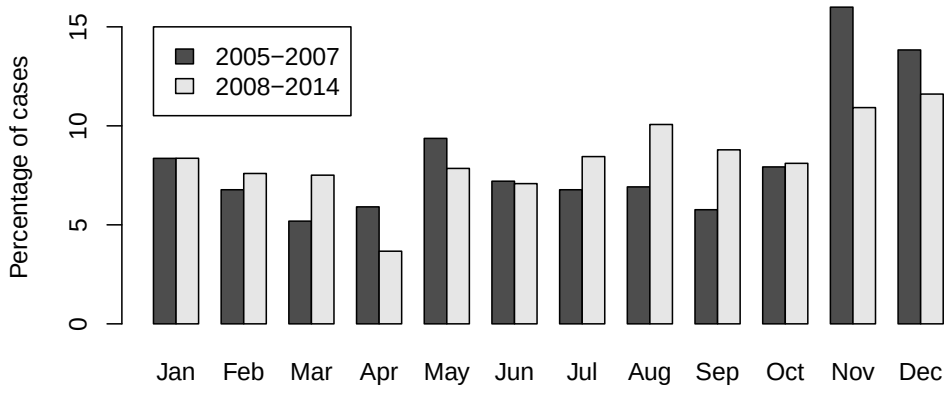

Figure 14: Percentage of cases per month before and after the poultry intervention in 2007–2008.

4.6.2 Age, time of year, and gender

The cases were divided into age categories 0–4, 5–19, 20–64 and 65+ years of age. The number of cases per year in each category is presented in Figure 15.

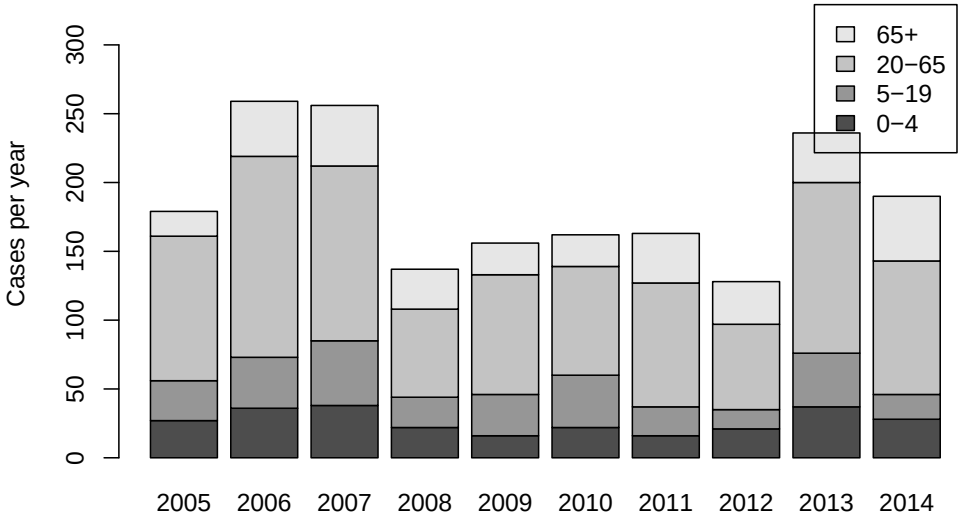

Figure 15: Number of cases per year by age category from 2005–2014.

The distribution of cases across the age groups through time differs, with the 65+ age group having a larger proportion of the cases in later years ($P = 0.01$). However, there is no evidence for a difference in the proportion of cases in each age group seasonally ($P = 0.22$, Figure 16).

There is an interesting relationship between urban-rural status and age, with rural cases being more likely to be young children (0–4 years old) while urban cases are more frequently adults ($P < 0.0001$, Figure 17).

There is also some suggestion ($P = 0.07$) of a difference in the age distribution of cases by gender. Table 12 summarizes the number of cases in each age group by gender, where it can be seen that those of school age (5–19 years) are more likely male than in other age groups, with the elderly (65+ years) being the most balanced group by gender. Overall, males are more likely (56%) than females to be a case.

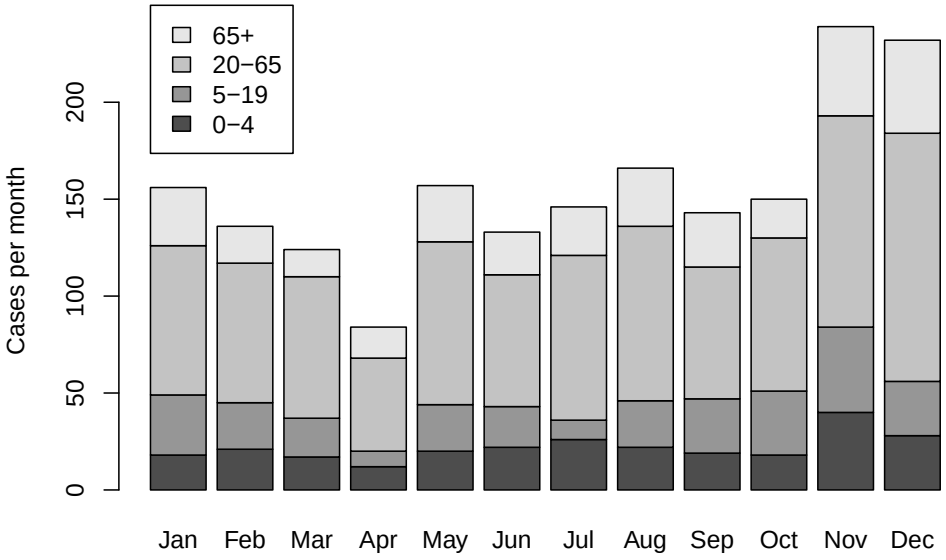

Figure 16: Number of cases per month by age category from 2005–2014.

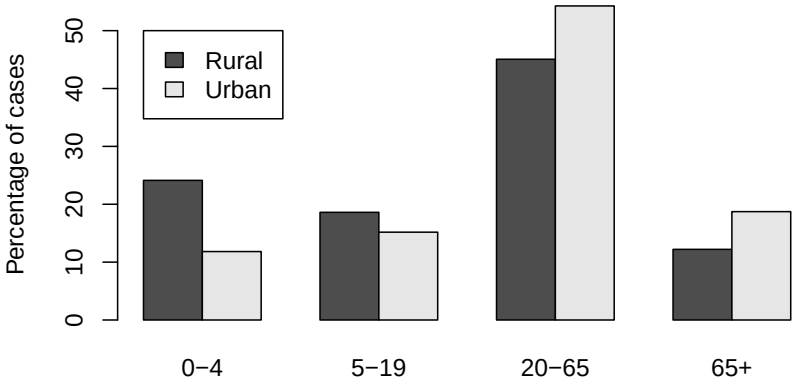

Figure 17: Percentage of cases by age category from 2005–2014.

Age	Male	Female	Total	%male
0-4	127	106	233	54.5
5-19	170	100	270	63.0
20-65	500	404	904	55.3
65+	151	136	287	52.6
Total	948	746	1694	56.0

Table 12: Number of cases by gender and age categorisation from 2005–2014.

5 Discussion and Conclusions

This report provides an update of the molecular epidemiology of campylobacteriosis in the Manawatu in 2014 in comparison with previous years, both pre- and post-intervention in the poultry industry. With the exception of an increase in poultry-associated cases in the last quarter of 2013 (mainly in December 2013), the pattern of similar attribution to poultry and ruminants observed since 2010 has continued.

In contrast with recent years, the attribution of urban and rural cases in 2014 are broadly similar with ruminants and poultry contributing approximately equally to the attribution in both cases. An examination of the attribution through time reveals that the number of rural cases (and the attribution to ruminants and poultry) is relatively stable through time, whereas the number of urban cases is more variable, with most of the variability being attributed to poultry.

The analysis of all urban and rural cases for the full 10 year period shows strong seasonal behaviour in case numbers, particularly in urban cases, along with an increasing trend in the number of cases through time. The intervention in the poultry industry contributed to a significant reduction of approximately 10 urban cases and 1-2 rural cases per month. However, the increasing trend in case numbers in the last 7 years has negated over half this gain in the Manawatu. As this analysis is based on the number of samples submitted to mEpiLab that have epidemiological information (urban/rural status) linked from EpiSurv, these findings may be biased by increasing submission rates to the laboratory, which was seen particularly in 2013 and 2014. The national trend in cases is relatively stable over the period 2007/2008 onwards. The seasonality in cases has changed since the poultry intervention, with the large peaks in November and December being replaced by higher case numbers in August and September.

The age distribution of cases has also changed over this period, with the 65+ age group accounting for a larger proportion of cases in the last few years. The age distribution may also vary by gender, with males being particularly over-represented in the school age group (5-19 years). As noted in the past [5], the age distribution of cases differs based on rurality, with younger children

accounting for 25% of rural cases, considerably higher than the 12% of cases from young children in urban areas.

There has been an increased proportion of *C. coli* isolates from poultry in 2013 and 2014 compared to previous years. This may be in part due to a more rigorous investigation of non-*jejuni* isolates, as *C. coli* is the subject of an ongoing Ph.D programme 'Molecular epidemiological studies of human campylobacteriosis in New Zealand between 2005 and 2014'. We expect to be able to describe the results from this study more fully in future reports.

In 2014 a new sequence type, ST-6964, emerged in the Manawatu, causing three human clinical cases. This ST was also recovered from whole carcases from poultry suppliers B and C and from chicken pieces form supplier A (as part of separate study). The only other ST-6964 in the PubMLST databases was from China in September 2014. Antimicrobial sensitivity testing revealed the two human isolates in August were resistant to ciprofloxacin, nalidixic acid, enrofloxacin and tetracycline. This finding requires further investigation.

As raised in previous years, it may be timely to consider re-sampling ruminant populations, to see if there has been a shift of ST distributions in cattle and sheep. It is also strongly recommended that a source-assigned case control study be conducted to identify more precise pathways for human infection. Future modelling work could also consider allowing covariates of human cases (rurality, age, gender, consumption of raw milk) to be incorporated into the dynamic modelling. This should be possible by introducing a heirarchical structure to the source effects in the dynamic island model, with overall attribution to a source being made up from individual attribution information from each case. Further refinement of the source attribution models has been underway to refine the Hald model which allowed for differing type effects (representing virulence or ability to survive in the food chain) by introducing clustering of the type effects, and improving the structure of uncertainty in the source populations. We hope to be able to include attribution estimates using this model in the next report.

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7 Appendix

Figure 18 displays the same data as presented in Figure 6 as a stacked bar plot.

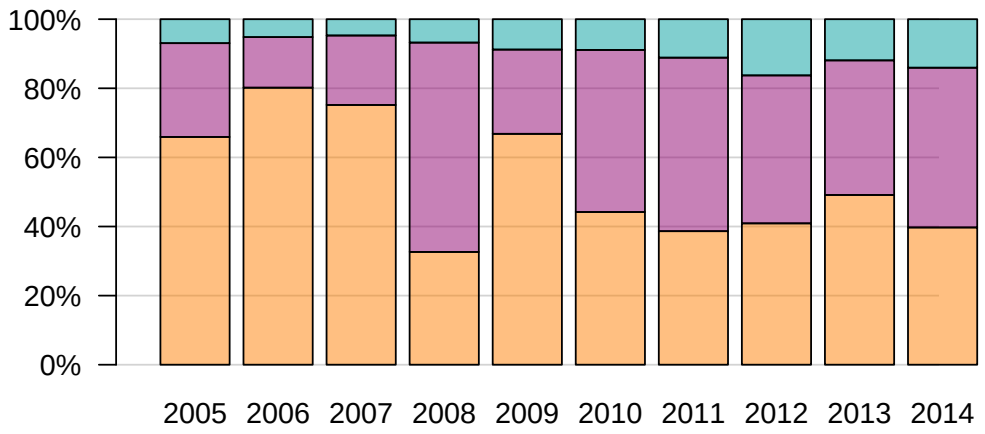

Figure 18: A stacked bar plot showing poultry (orange), ruminant (purple) and other (blue) reservoir attribution estimates for human cases in the Manawatu for each year from 2005 to 2014.

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